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Regulation of Blood Pressure and Salt Homeostasis by Endothelin

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Abstract

Endothelin (ET) peptides and their receptors are intimately involved in the physiological control of systemic blood pressure and body Na homeostasis, exerting these effects through alterations in a host of circulating and local factors. Hormonal systems affected by ET include natriuretic peptides, aldosterone, catecholamines, and angiotensin. ET also directly regulates cardiac output, central and peripheral nervous system activity, renal Na and water excretion, systemic vascular resistance, and venous capacitance. ET regulation of these systems is often complex, sometimes involving opposing actions depending on which receptor isoform is activated, which cells are affected, and what other prevailing factors exist. A detailed understanding of this system is important; disordered regulation of the ET system is strongly associated with hypertension and dysregulated extracellular fluid volume homeostasis. In addition, ET receptor antagonists are being increasingly used for the treatment of a variety of diseases; while demonstrating benefit, these agents also have adverse effects on fluid retention that may substantially limit their clinical utility. This review provides a detailed analysis of how the ET system is involved in the control of blood pressure and Na homeostasis, focusing primarily on physiological regulation with some discussion of the role of the ET system in hypertension.

I. INTRODUCTION

Since the discovery in 1988 of endothelin (ET)-1 as an endothelial cell-derived peptide with greater vasoconstrictive potency than any known substance (857), there have been over 22,000 publications dealing with the ET system in the physiological and pathological control of almost every organ system. It is now evident that the ET system is particularly important in the control of systemic blood pressure (BP) and Na homeostasis; the current review is devoted to this subject. The review focuses primarily on the role of the ET system in normal physiological processes, using disease states mainly to illustrate physiological principals. Consequently, while the ET system is involved in the pathogenesis and maintenance of disorders of BP regulation and Na homeostasis, such as congestive heart failure, chronic kidney disease, and others (with the exception of hypertension), these will not be emphasized. The review focuses on ET biology in those systems that are primarily involved in BP and salt balance regulation, including the vasculature, kidney, nervous system, adrenal

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gland, circulating hormones and, to a lesser extent, the heart. As will be evident, the ET system is intimately involved in virtually every aspect of BP regulation and Na homeostasis.

II. GENERAL BIOLOGY OF ENDOTHELIN

A. ET Synthesis and Degradation

1. ET genes, mRNA, and prepropeptide—There are three members of the mammalian ET gene family: ET-1, ET-2, and ET-3. All three mature ET peptides contain 21 amino acids as well as 2 intrachain disulfide bonds and vary by not more than 6 amino acids (Fig. 1). The sequence for each iso peptide is preserved across mammalian species and is closely related to the snake venom sarafotoxins. Each iso peptide is encoded by a separate gene that does not undergo alternate splicing, although gene sequence varies between species. The vast majority of studies involving ET regulation of BP and salt homeostasis have focused on ET-1, hence the ensuing discussion on ET metabolism will primarily address ET-1. The human ET-1 gene consists of 5 exons distributed over 6,838 base pairs (323), is located on chromosome 6 (24), and encodes a 2,026-base pair mRNA (323). The majority of studies indicate that ET-1 production and secretion are largely controlled at the gene transcription level. Cooperation between a large host of tissue-specific transcription factors permits tissue-selective ET-1 gene transcription and helps ensure that ET-1 is appropriately activated (854). Such cooperation is facilitated by the presence of multiple regulatory elements in the ET-1 promoter, including activator protein 1 (AP-1), nuclear factor of activated T-cells (NFAT)-binding domains, GATA binding protein 2 (GATA-2), CAAT-binding nuclear factor-1 (NF-1), and many others (718).

ET-1 mRNA has a short half-life (~15 min) which likely relates to the presence of three destabilizing AUUUA motifs in the 3'-untranslated region (323) (Fig. 1). Indeed, some studies have found that alterations in ET-1 synthesis can be affected by modification of ET-1 mRNA stability (159, 475, 623). Human ET-1 mRNA encodes a 212-amino acid prepropeptide that undergoes removal of a short signal sequence by a signal peptidase to yield proET-1. ProET-1 is cleaved by dibasic-pair-specific endopeptidases to yield the 38-amino acid Big ET-1 (464, 857). In endothelial cells, the convertases furin and PC7 have been shown to proteolyze proET-1 to Big ET-1 (55).

2. Conversion of Big ET-1 to ET-1—Big ET-1 is present in the circulation; however, it has at least two orders of magnitude less vasoconstrictor potency than the mature peptide (131). The physiologically relevant conversion of Big ET-1 to ET-1 occurs primarily through the action of ET converting enzymes (ECE) (Fig. 1). ECE are integral membrane zinc peptidases of which three isoforms (ECE-1, -2, and -3) have been identified (131, 785). ECE-1 is present mainly in endothelial cells but can be found elsewhere. It has the greatest affinity for Big ET-1, although it can proteolyze other peptides. ECE-1 has a pH optimum of 6.8 with a narrow pH profile (842). There are four ECE-1 isoforms (ECE-1a, -1b, -1c, and -1d) that are derived from alternative splicing of a single gene (682, 694, 793). The ECE-1 isoforms differ only at the amino terminus, resulting in different cellular localization. ECE-1a is found in intracellular vesicles and on the cell surface; ECE-1b is located in the endosomal compartment near the *trans*-Golgi network, while ECE-1c and -1d are on the extracellular face of the plasma membrane (ECE-1d may also be located in endosomes) (131, 463). The different ECE-1 locations mean that mature ET-1 can be formed from Big ET-1 both intracellularly and extracellularly. Big ET-1 can be present in the plasma in concentrations similar to or greater than ET-1, suggesting the extracellular conversion is physiologically relevant. With regard to intracellular ECE localization, the above findings suggest that, since ECE is also localized to vesicles, the potential exists for ET-1 to be stored and secreted upon demand. Such a possibility has been confirmed in endothelial cells,

wherein ET-1 can be found in Weibel-Palade bodies and released from vesicles upon stimulation (264, 652, 654).

ECE-2 has ~60% homology with ECE-1, hydrolyzes ET-1, and also consists of four isoforms with varying amino termini that may confer different intracellular (not extracellular) localization (176, 317). Its pH optimum is 5.5 with virtually no activity at pH 7.0 (176). This pH optimum indicates that ECE-2 is involved in intracellular processes and particularly in association with the *trans*-Golgi network. Mice with combined knockout of ECE-1 and ECE-2 have appreciable ET-1 levels, suggesting that other proteases could be involved in ET-1 formation (856). An ECE-3 has also been identified; however, this preferentially cleaves ET-3 (266). ET-1 may also be generated by other enzymes, although their physiological relevance is uncertain (131). Finally, there is no clear evidence to date that any ECE exert a rate-limiting effect on mature ET-1 synthesis.

Big ET-1 can be alternatively processed by chymase to yield ET-1-(1–31) (131). ET-1-(1–31) can potently vaso-constrict (among other properties). In addition, ET-1-(1–31) can be converted to ET-1 by neutral endopeptidase or ECE. This raises the interesting possibility that alternative processing of Big ET-1 is of biologic relevance.

3. ET catabolism—ET-1 is degraded, at least in part, by neutral endopeptidase (131, 801). Another enzyme, deamidase (also called lysosomal protective protein), that is mutated in galactosialidosis, can proteolyze ET-1 (329, 334). Notably, deamidase has an acid pH optimum in contrast to the optimal neutral pH of neutral endopeptidase. Both of these enzymes are widely distributed throughout the body. While ET-1 can likely be catabolized by most, if not all, tissues, the kidney may be of particular importance. ET-1 causes prolonged BP increases in bilaterally nephrectomized rats (378). Since circulating ET-1 does not appear in the urine (3), this suggests that the kidney catabolizes the protein to a substantial degree.

B. ET Receptors

1. Molecular biology of ET receptors—All mammalian ET receptors derive from two separate genes. The human ET_A receptor (ET_A) contains 427 amino acids, its gene is located on chromosome 4, and the receptor binds ET-1 ≥ ET-2 >> ET-3 (303). Recently, splice variants of ET_A have been reported in the rat anterior pituitary (268). One of these variants had reduced efficacy in mobilizing intracellular Ca²⁺ ([Ca²⁺]_i) and in stimulating adenylyl cyclase activity; however, it is unknown whether this or other splice variants exist in other tissues and whether they play a significant physiological role. The human ET_B receptor (ET_B) contains 442 amino acids, its gene is located on chromosome 13, and the receptor binds all ETs with equal affinity (22, 659). Alternatively spliced human ET_B with an additional 10 amino acids has been described (699); the splice variant was not seen in other species and had no apparent differences in evoked cell signaling. Another human ET_B splice variant was identified that markedly differed in the cytoplasm domain and 3'-untranslated region (172); this variant had minimal signaling activity, leading to the suggestion that it functioned as a clearance receptor. A rat ET_B splice variant has been described, although functional effects were not evaluated (103). As for the ET_A variant, the cellular distribution and physiological significance of these ET_B splice variants remains to be determined. Variable ET glycosylation has been described in rats, but no functional significance has been identified (66). Finally, a third ET receptor has been identified in *Xenopus laevis*, but does not appear to exist in mammals (407).

2. ET receptor localization and signaling—Almost every cell in the body expresses one or more ET receptors. ET_A and ET_B can be expressed individually or together by

individual cell types. ET_A are particularly predominant in vascular smooth muscle and myocytes, while ET_B is the major ET receptor found in endothelial cells and renal tubules. ET receptors activate a host of signaling systems that vary depending on the cell type. ET receptors couple to members of the G_i, G_q, G_s, and G_{α12/13} G protein families (161, 320, 363) with resultant regulation of a variety of signaling cascades, including adenylyl cyclases, cyclooxygenases (COX), cytochrome *P*-450, nitric oxide synthase (NOS), the nuclear helix-loop-helix protein p8 (251), serine/threonine kinases, tyrosine kinases, and others (718). ET_A and ET_B often have opposing actions, i.e., in the vasculature, ET_A activation causes vasoconstriction, while ET_B activation, at least initially, causes vasodilation. Additionally, ET_A stimulation tends to promote cell proliferation and fibrosis, while ET_B typically does the opposite. However, many exceptions exist wherein ET_A and ET_B can elicit similar biologic responses. Given this complexity and cell-specific responses, detailed discussion in this review of ET receptor signaling will be done in the context of each organ system.

A variety of pharmacological agents have been used to define ET receptor isoform function. Initially, agonists and antagonists were peptides with high ET receptor isoform selectivity. For example, BQ123 and BQ788 are very specific peptide antagonists of ET_A and ET_B, respectively, while sarafotoxin 6c is a highly selective peptide ET_B agonist (41). Subsequently, numerous orally active antagonists (obviously preferable to peptides) with varying ET_{A/B} binding were developed, some of which are now used clinically. In general, these agents range from those with combined ET_{A/B} blockade (e.g., bosentan) to those that selectively inhibit ET_A (e.g., sitaxsentan) (41). One caveat is that the receptor selectivity of these compounds is almost entirely based on *in vitro* studies; hence, their *in vivo* specificity remains to be fully confirmed. In addition, substantial uncertainty exists about individual ET receptor isoform function. Part of this problem may be due to ET receptor dimerization (58). Heterologous expression of ET receptors revealed the presence of ET_A and ET_B homodimers (254). Of greater potential biologic impact, this same group found evidence for ET_A/ET_B heterodimerization based on ligand-dependent differences in receptor internalization (253). Using HEK293 cells, Evans and Walker found that ET_A and ET_B heterodimerized through a PDZ finger; mutation of the PDZ domain caused delayed ET receptor internalization and a prolonged increase in [Ca²⁺]_i in response to ET-1, raising the possibility that ET_A/ET_B heterodimerization affects receptor function (187). In addition, ET_B may heterodimerize with receptors other than ET_A, including the dopamine D3 and the angiotensin II (ANG II) AT₁ receptors (871, 872). No definitive evidence exists for a physiological role of such heterodimers or whether they actually exist *in vivo*; resolution of this issue has obvious biologic relevance as well as being important in interpreting studies using purportedly specific ET receptor isoform agonist or antagonists.

ET-1 binding to its receptors, and particularly to ET_A, causes unusually prolonged biologic effects. This is partly due to the almost irreversible binding of the peptide. Furthermore, ET-1 can remain associated with ET_A up to 2 h after endocytosis, suggesting that the peptide continues to activate signal transduction long after internalization (113). The persistent ET_A signaling relates, at least in part, to its intracellular trafficking. Epitope-tagged, heterologously expressed ET_A enters the recycling pathway, an effect that is mediated by an internal PDZ ligand in the receptor's cytoplasmic tail (71, 563, 564). In contrast, ET_B were targeted to lysosomes (71), perhaps explaining the more transient response to ET_B as well as how ET_B can serve as a clearance receptor. ET receptors may also exert biologic effects independent of extracellular ET-1; both ET_A and ET_B have been reported to be present in the nucleus of fetal human endocardial endothelial cells (336).

C. ET in Tissues

Throughout the ensuing considerations, it is important to keep in mind the concept that ET biology is best understood in the context of local production and actions of the peptides. Plasma ET peptide levels are quite low (see discussion in following section) and likely do not physiologically regulate cell function. Rather, local tissue ET concentrations are likely to be many times greater than that found in the circulation and are most likely to have physiological significance. Most polarized cells secrete ET-1 towards the abluminal side. Thus, in the vasculature, endothelial cells secrete ET-1 predominantly towards smooth muscle or the interstitium. Similarly, renal tubule cells secrete ET-1 mainly toward the interstitium and much less into the urine. ET receptors may also be primarily located on the abluminal side of epithelial cells (372), thereby setting the stage for autocrine and paracrine regulation. In essence, ET peptides should be viewed primarily as autocrine and paracrine regulators of cell function, and not as endocrine factors. This conceptualization helps explain how ET can exert such a tremendous variety of effects, some of which can diametrically oppose each other.

III. ENDOTHELIN AND THE VASCULATURE

A. Overview

The first evidence suggesting the existence of ET comes from early studies by Hickey et al. (286) who demonstrated that conditioned media taken from primary endothelial cell cultures produces constriction in the classic muscle bath preparation. Yanagisawa et al. (857) went on to purify the peptide and named it endothelin due to its origin from vascular endothelium. In this paper, Yanagisawa et al. (857) first described the effects of ET-1 on BP and hemodynamics. A bolus intravenous injection results in transient hypotension followed by prolonged elevations in BP. These effects are known to involve ET_B and ET_A, respectively, although ET_B-dependent vasoconstriction does contribute to the hypertensive effects as well.

Injection of an ET_B agonist produces a similar biphasic effect on BP. It is interesting that both ET_B agonists and antagonists produce vasoconstriction. The response to exogenous agonist is due to ET_B present on vascular smooth muscle. The response to ET_B blockade is due to displacement of ET-1 from ET_B to ET_A as well as reducing endothelial-derived relaxing factor production. ET_B-dependent vasoconstriction varies from one vascular bed to another. Veins appear to possess a more potent ET_B-dependent constriction compared with arteries (773). The functional relevance of this observation is yet unknown.

The signaling pathways by which ET-1 produces vasoconstriction are typical for G protein-coupled receptors, and as one might expect, involve the influx of Ca²⁺ from both extracellular and intracellular stores through rather standard mechanisms (98, 331, 584, 648); they will be discussed in more detail in following sections. Most of these mechanisms have been worked out for ET_A activation, but little has been done to specifically confirm that ET_B operate in the same manner, although all indications suggest that they do. The vasorelaxing actions of ET_B also involve increases in intracellular Ca²⁺ that results in NOS activation and release of nitric oxide (NO). Like other endothelial-dependent vasodilators, ET_B also stimulate the release of vasodilator prostanoids. An overview is shown in Figure 2 and is discussed in more detail in the renal vasculature section.

There have been a great many review articles written on the ET system in general and within specific vascular beds, especially under pathological conditions. The reader is referred to those for more details of nonrenal vascular actions (648, 671). However, it is important to provide some general information and highlight some of the unique aspects related to

physiological control of the ET system in the vasculature that apply to both renal and nonrenal beds.

B. Endothelial Production and Activity of ET-1

Despite more than 20 years of research, the mechanisms responsible for the physiological regulation of ET-1 synthesis in endothelium are not well understood (142). Weibel-Palade bodies store a range of vasoactive mediators, including ET-1 (560, 661). These endothelial storage granules also appear to be important storage sites for the precursor peptide Big ET-1 and the isoforms of ECE-1 (653, 654). Given that Big ET-1 can be found in plasma at concentrations at or above that of ET-1 itself (687), it is generally believed that both Big ET-1 and ET-1 are released together.

There are a great many factors that can stimulate ET-1 production in vitro such as cytokines, thrombin, shear stress, hypoxia, and others. These mechanisms have been thoroughly reviewed elsewhere (648, 652), but the important point is that the physiological conditions that regulate ET-1 release from the vascular endothelium in vivo under nonpathological conditions are poorly understood. Factors that increase intracellular Ca^{2+} , including ANG II and thrombin, appear to function through a common pathway to stimulate regulated release from Weibel-Palade bodies (652). There also appears to be a constitutive pathway for ET-1 synthesis that accounts for continuous basal production of ET-1 from secretory vesicles. Interestingly, these vesicles also stain positive for ECE and Big ET-1 immunoreactivity, suggesting additional synthesis of the mature peptide even after cellular release (35).

It is important to note that ET production in the vascular wall is not always limited to the endothelium. ET-1 synthesis by vascular smooth muscle cells can be demonstrated using cell culture preparations as well as in vivo (352, 754, 837). Under normal physiological conditions, ET-1 is not produced by vascular smooth muscle, but inflammatory cytokines are known to be potent inducers of ET-1 synthesis. This is thought to become particularly relevant in cardiovascular disease.

C. Plasma ET-1 Concentrations

A major complication in studying the synthesis of ET-1 is the very efficient system for clearing ET-1 from the circulation. A complicating feature of understanding plasma ET-1 levels comes from the range of specificities for ET-1 versus Big ET-1 and the other isopeptides. Many of the early commercially available RIA and ELISA kits had wide ranges of specificity and most had a low range of sensitivity that was at or even above the level of circulating peptide concentrations. It is now established with highly reliable assays that plasma levels of ET-1 are typically in the low picomolar range, e.g., 0.1–0.4 pM (783), which is below the EC_{50} for many of the biological actions of ET-1. Intravenous infusion of ET-1, even at doses that produce significant vascular effects, do not change plasma ET-1 immunoreactivity (D. Pollock, unpublished observations). This efficient clearance mechanism is due to the fact that ET receptors have unusual binding characteristics to their ligand. Described often as “irreversible,” the binding of ET-1 to either ET_A or ET_B has an extremely slow dissociation rate (806, 840).

Evidence that ET_B functions to clear ET-1 from the circulation originates from pharmacological studies that demonstrate increases in plasma ET-1 when ET_B is blocked (552, 727). In most studies, ET_A blockade has little effect on plasma ET-1, yet administration of a mixed ET_A/ET_B antagonist or a selective ET_B antagonist will produce significant increases in plasma ET-1 (214, 444). One cannot conclude, however, that ET_A does not clear ET-1 from plasma. Practically by definition, if both ET_A and ET_B display this so-called “irreversible” nature, any binding would remove ET-1 from the circulation. Also,

in specific disease models where there is thought to be some degree of ET_B dysfunction, such as the ET_B-deficient rat and the DOCA-salt hypertensive rat, ET_A blockade produces further increases in circulating ET-1 (11, 169).

Opgenorth et al. (552) determined the effect of a highly selective ET_A antagonist and a highly selective ET_B antagonist on plasma ET-1 immunoreactivity in both normal Sprague-Dawley rats and healthy human volunteers. Chronic receptor blockade with both types of antagonists increased plasma ET-1, but the increase produced by the ET_B antagonist was much larger. Addition of ET_A blockade on top of ET_B blockade produced a further increase in plasma ET-1, confirming that both receptor subtypes function to clear ET-1. However, given the more dominant effect of ET_B blockade to influence circulating ET-1, we hypothesize that ET_B predominate in terms of the total number of receptors within the vasculature. At the very least, a significant factor is the immediate access of endothelium to the plasma such that circulating ET-1 has to traverse the endothelial layer before reaching the majority of ET_A on vascular smooth muscle. One could ask then, Why does vasoconstriction predominate when exogenous ET-1 is infused? The answer most likely comes from the fact that ET-1-induced vasoconstriction is prolonged even after the ligand is cleared, while the vasodilator influence is more transient in nature.

D. Functional Heterogeneity of the ET System in Organ Systems

Virtually every vascular bed contains the primary components of the ET-1 system. Again, this has been reviewed in thorough detail elsewhere, so the reader is referred to previously published reviews for nonrenal circulations (648, 671). Of note relative to the current review, however, one of the first studies to investigate the actions of ET-1 in terms of hemodynamic studies was by Clozel and Clozel (121) who observed a preferential effect of ET-1 to produce a powerful vasoconstriction in the renal vascular bed of squirrel monkeys compared with the iliac and mesenteric circulations. This study has been the basis of the argument that the renal circulation is more sensitive to the constrictor actions of ET-1, but unfortunately, the influence of endogenous ET-1 cannot be inferred from this observation.

Another tenet of ET-1 physiology, but perhaps still an assumption, is that the site of conversion from Big ET-1 to ET-1 is the site of functional significance and is supported by evidence for a differential distribution of ECE activity within vascular beds. Support for this idea comes from comparing hemodynamic responses to ET-1 and the precursor Big ET-1. In intact rats, intravenous infusion of Big ET-1 at a dose of 100 pmol·kg⁻¹·min⁻¹ produces a larger increase in mean arterial pressure than ET-1 at 12 pmol·kg⁻¹·min⁻¹, yet ET-1 produces much larger decreases in renal blood flow (RBF) and glomerular filtration rate (GFR) (586). In a separate study, comparison of Big ET-1 and ET-1 at doses of 100 and 20 pmol·kg⁻¹·min⁻¹ produced nearly identical decreases in cardiac output and increases in total peripheral resistance, yet Big ET-1 produced a significantly greater increase in mean arterial pressure (585). Systematic studies comparing functional ECE activity in different vascular beds are needed to more fully understand this apparent disconnect between ECE activity and ET-1 vasoconstriction.

While many investigators have demonstrated that the renal circulation is particularly sensitive to the vasoconstrictor actions of exogenously applied ET-1, because of the paracrine nature of the ET-1 system, this standard should not be applied to assign functional significance. The observations that Big ET-1 is less potent than ET-1 in terms of reducing RBF in vivo could allow one to conclude that the renal circulation is less dependent on ET-1 vasoconstriction to regulate vascular tone and blood flow. Although ET-1 infusion studies have taught us a great deal about the ET system, drawing conclusions about the physiological significance based on exogenously applied ET-1 is risky. Determining the actions of endogenous ET-1 would be the preferred approach, but there have been few

studies investigating endogenous ET-1 effects on vascular function. Studies using receptor specific antagonists have provided useful guidance on this subject, but experiments comparing specific vascular beds are extremely limited such that a clear understanding is not apparent.

Acute administration of ET_A or ET_A/ET_B antagonists generally has either no effect or decreases total peripheral resistance and arterial pressure, but these effects vary according to species, the level of baseline BP, and dietary salt intake (272, 273, 671). However, administration of ET_B-selective antagonists produces vasoconstriction in a very short time frame, suggesting that the more important physiological role of ET-1 is actually through ET_B actions in promoting vasodilation and preventing ET_A constriction (583, 588).

E. Vascular Permeability

Early in vivo studies examining ET-1 infusion reported increases in hematocrit that could reflect a shift in fluid from plasma to interstitial space (202, 794). This effect of ET-1 can be blocked by an ET_A antagonist (201, 410) or a mixed ET_A/ET_B antagonist (476). ET_B-selective agonists can reduce capillary filtration coefficient, but this was associated with precapillary constriction (168). In contrast, Brändli et al. (69) observed that plasma extravasation in the rat dura mater produced by ET-1 was not blocked by an ET_A-selective antagonist, but rather a combined ET_A/ET_B antagonist. These authors concluded that ET_B may mediate ET-dependent increases in permeability. There have been a number of studies in animal models demonstrating that ET_A blockade will reduce capillary leakage (167, 200, 490).

Victorino et al. (800) used a direct measure of ET-1 actions on postcapillary hydraulic permeability (L_p) to determine ET-1 influence independent of hemodynamic or indirect hormonal influence that can occur in the in vivo models. These investigators showed that 8 pM ET-1 had no effect on L_p in mesenteric vessels while 80 pM ET-1 actually decreased L_p . This effect to reduce L_p can be attributed to ET_B (799). Although one cannot be certain of local ET-1 concentrations in intact tissues, these findings suggest that ET receptor blockade, in particular, ET_B blockade, could contribute to fluid shifts into the interstitial space. This may be especially relevant under pathophysiological conditions when ET-1 levels are increased. Whether there are differences in ET-1 effects on permeability in different vascular beds has yet to be addressed.

With all the preclinical studies taken into account, it will be important for clinical studies to determine specificity of receptor blockade under conditions where fluid retention becomes a problem. At present, it appears that the fluid retention problems associated with ET_A blockade in humans (see more detailed discussion in section IVI) is not related to a direct action to increase permeability, but rather, may be the result of a different mechanism related to changes in capillary pressure or blockade of ET_B effects on fluid handling. However, specific studies that verify receptor specificity in humans are needed.

IV. ENDOTHELIN AND THE KIDNEY

A. Overview of ET in the Kidney

The exciting discovery of ET-1 catalyzed a rapid response by the renal research community. Initial studies revealed that the kidney was likely to be of particular importance in the biology of the ET system. ET-1 was found to be produced by the kidney in relatively high amounts; indeed, Kitamura et al. (364) reported that, of all tissues in the body (using the pig as a model), the inner medulla of the kidney had by far the greatest concentration of immunoreactive ET-1. Subsequent studies determined that almost every cell type within the kidney synthesized ET-1. Similarly, the kidney was found to contain abundant ET receptors,

particularly in the vasculature and the medulla. Insofar as could be determined, every cell type within the kidney expresses ET receptors. The kidney was also exquisitely sensitive to ET-1, having up to 10-fold greater sensitivity to the vascular effects of the peptide compared with other organ beds (455, 574). Given such high ET production and receptor expression, it was not surprising that the ET system was found to be capable of regulating kidney function and that multiple renal functional parameters were affected. It is now evident that within the kidney, the ET system can modulate total and regional blood flow, GFR, Na and water excretion, acid/ base handling, drug transporters, cell proliferation, extra-cellular matrix accumulation, inflammation, and other functions. In addition, the ET system has emerged as being of substantial importance in mediating renal injury and/or disease progression in a variety of pathological conditions. The ensuing discussion on ET and the kidney will primarily focus on the physiological role of the renal ET system as it relates to Na and BP homeostasis.

Almost every cell type within the kidney is potentially involved in ET regulation of Na excretion. It is likely that ET derived from individual renal cell types acts primarily in an autocrine or paracrine fashion; thus the renal ET system must be viewed within the context of the local microenvironment. Such complex regional actions of ET have generally confounded interpretation of studies examining the effects of systemically or intrarenally administered ET agonist or antagonists, as well as understanding the significance of changes in renal ET-1 levels or urinary ET-1 excretion in response to stimuli. Nonetheless, a brief review of some of these studies will provide some insight into the role of renal ET in the regulation of BP and Na homeostasis, as well as the problems encountered with using these types of approaches.

Exogenously administered ET-1, when given at a sufficient dose, consistently reduced RBF and GFR in experimental animals and humans (7, 300, 353, 586, 602, 717). In general, such renal hemodynamic effects have been associated with reduced urinary Na and water excretion, particularly when RBF is reduced by at least 25% (115, 353). Consequently, a number of renal clearance studies were performed in which ET receptor agonists were given at doses with only modest or no effects on RBF or GFR. Under these circumstances, intravenously administered Big ET-1, ET-1, or ET-3 increased Na excretion in some studies (151, 263, 297, 678); however, such a natriuretic effect was not consistently observed (209, 248, 344, 674). One group reported that the natriuretic effect of exogenous ET-1 was due solely to increased BP since renal decapsulation or maintaining renal perfusion pressure at baseline values (ET agonists often increase arterial pressure) with an aortic clamp prevented ET-1-induced natriuresis (792). Another explanation for the inconsistent findings about ET agonist-induced natriuresis may be due, at least partly, to differential activation of ET_A and ET_B. For example, when ET_A, but not ET_B, are blocked, a natriuretic effect of ET-1 was detected (73, 116). However, the major lesson from these studies is that the role of the renal ET system in controlling urinary Na excretion cannot be ascertained from maneuvers that exert generalized renal effects.

In contrast to the studies on Na excretion, there is much clearer evidence that systemically administered ET increases urinary water excretion (248, 344, 674). Even when given at doses that markedly decreased RBF, renal artery infusion of ET-1 increased urine volume and free water clearance (344). This effect may be mediated by ET_B, since infusion of ET_B-specific agonists [IRL1620 or sarafotoxin 6c (S6c)] increased urine flow (116, 473, 867). These agents typically increased RBF, albeit modestly and transiently; hence, it was not possible to rule out a hemodynamic component to the diuretic response. Again, these studies underscore the need to examine renal ET biology in the context of local actions, not global renal effects.

Changes in renal ET levels or urinary ET-1 excretion have been measured in response to alterations in fluid volume status. While a few studies have seen either decreased (in humans) (495, 607, 685) or unchanged (in humans) (16, 199, 299) urinary ET-1 excretion following Na loading, the majority of studies have found increases in urinary ET-1 excretion (in humans and experimental animals) (5, 127, 307, 313, 335, 374, 457, 657, 672). In addition, water loading typically increases urinary ET-1 excretion (in humans and experimental animals) (374, 474, 838, 870). Thus, taken together, the bulk of evidence indicates that volume expansion is associated with increased renal ET-1 production. This conclusion suggests that endogenous renal ET-1 exerts a net natriuretic effect; as will be seen from the discussion below, this does indeed appear to be the case.

B. Renal Vasculature

1. Renal vascular ET receptor expression—As in other tissues, the renal vascular effects of ET are mediated by activation of ET_A and/or ET_B. The relative proportion of receptor expression seems to vary between animal models and humans and may reflect regional differences within the kidney. For example, in the canine renal cortex, the ET_A/ET_B proportion is reported to be ~22/78 based on binding studies with cortical membranes (74). Medullary and papillary ratios were determined as 40/60 and 50/50, respectively (74). In the rat kidney, the ET_A/ET_B distribution approaches 30/70 in the renal cortex and medulla alike (530). Descending vasa recta in the outer medulla express both ET_A and ET_B. In the porcine kidney, the relative ET_A/ET_B proportion averaged ~60/40 in glomerular membranes, but only ET_B binding could be identified in papillary membrane preparations (25). Similar studies conducted using human tissues suggest that ~30% of the receptors expressed in both the renal cortex and medulla are of the ET_A subtype (529). Thus it is important to note that renal ET receptor expression varies in a species-specific manner.

How much of this expression is ascribable to renal microvascular ET receptor expression is not well understood. The physiological regulation of renal vascular ET receptor expression has not been extensively investigated; however, a potential schema is shown in Figure 3. Functional data clearly indicate the expression of both receptor subtypes by preglomerular vessels and efferent arterioles (165, 178, 325, 418, 449), but the relative distribution of receptors in these microvascular elements has only been studied to a limited extent. Edwards and Trizna (164) performed binding studies on preglomerular microvascular membranes from rat and rabbit kidney to assess relative ET_A and ET_B expression. Membranes prepared from preglomerular microvessels of both the rat and rabbit species express ET_A/ET_B in approximately a 40/60 proportion, while ¹²⁵I-ET-1 bound in a manner consistent with a single binding site. Calculated dissociation constants were nearly identical and averaged ~20–21 pmol/mg protein in the rat and rabbit, respectively, suggesting that preglomerular microvascular membranes from both species exhibit nearly identical affinities for ET-1. In the rat, ET_A expression (determined by ¹²⁵I-ET-1 binding) was lower in glomeruli and inner medullary collecting duct than in preglomerular microvessels, whereas in the rabbit, the ET_A/ET_B proportion was similar for the microvasculature and inner medullary collecting duct, but rabbit glomeruli exhibited primarily (80%) ET_B binding. Using an ET_A selective radioligand, Davenport et al. (141) noted that ET_A were expressed along human arcuate, interlobular arteries and veins, and arterioles and glomeruli. In a more recent report, Wendel et al. (823) used immunofluorescence to survey renal ET_A and ET_B expression in the rat kidney. ET_A were detected on vascular smooth muscle cells of the large interlobar, arcuate, and interlobular arteries and in veins; ET_A immunoreactivity was also detected on smooth muscle cells of afferent and efferent arterioles, but not on the endothelial cells of these arteriolar segments. They reported weak immunostaining for ET_B on endothelial cells of interlobular arteries, but much stronger ET_B immunoreactivity on endothelial cells lining

peritubular capillaries. Endothelial cells of afferent or efferent arterioles did not exhibit detectable ET_B immunostaining.

ET receptor expression in many vascular beds is influenced by changes in physiological status. For example, chronic elevation of ET-1 suppresses ET_A, with little effect on ET_B, expression (405, 766). Surface flow and NO enhance vascular smooth muscle ET_A expression and affinity (614, 615). Direct assessment of changes in renal microvascular receptor expression is limited; however, in a recent study, Schneider et al. demonstrated that chronic salt feeding increases renal vascular ET_B expression, while ET_A expression remained unchanged (673). The prospect that physiological challenges will alter renal microvascular ET receptor expression is exciting because it provides a new level of regulation/compensation, which may provide important clues on novel roles for ET in the kidney.

2. Renal vascular ET production—Relatively little is known regarding which specific renal vascular cells produce ET peptides and under what conditions production can be modulated. ECE-1 in human kidney was detected on the endothelial surface of arcuate and interlobular arteries as well as glomerular arterioles and endothelial cells (599). In the medulla, ECE-1 was detected in vasa recta bundles and tubular elements. Endothelial staining was confirmed by immunohistochemical detection with von Willebrand factor, which paralleled ECE-1 mRNA distribution (599). Subsequent immunocytochemistry studies confirmed that endothelial cells of human interlobular and arcuate arteries and adjacent veins produce mature ET-1 (348). Big ET-1 colocalized with ET-1 in those vascular segments (348). Positive staining was also detected in glomerular capillary endothelial cells but not endothelia from other intrarenal capillaries. Interestingly, no immunostaining was evident in the vascular smooth muscle cells of these endothelium-positive arterial segments.

Generally speaking, ET-1 release occurs soon after it is generated by ECE-1 from pre-pro ET-1. Therefore, secreting cells do not contain stores of biologically active ET-1 to be released in response to a secretory stimulus. Endothelial cells produce biologically active ET-1 from pre-pro ET-1, mainly through the catalytic actions of ECE; however, other mechanisms are implicated under physiological and pathophysiological conditions, and in tissue specific manners (131, 386). ET-1 production can come from many sources and under a number of conditions. Renal ET-1 production is facilitated by shear stress, inflammatory conditions/mediators, oxidative stress, the renin/ANG II system, and others (58, 171, 234, 533). Most of this is probably generated by tubular epithelial and interstitial cells; how much renal ET is derived directly from renal microvascular endothelial and smooth muscle cells, and how this ET might influence renal microvascular or tubular function, is not known.

3. Effect of ET on the renal circulation

A) OVERVIEW: As in other tissues, the renal vascular effects of ET are mediated by activation of ET_A and ET_B. ET produces a powerful and prolonged renal vasoconstriction following either luminal or adventitial peptide delivery (56, 150, 178, 237, 325, 418, 449, 583, 586, 587, 702). This vasoconstriction arises from activation of either ET_A or ET_B and occurs in a segment-specific manner (178, 325, 418, 449) but does not appear to exert a direct influence on tubuloglomerular feedback (355, 744). ET also influences mesangial cell signaling, migration, and proliferation (see discussion below and Ref. 718). Therefore, the relationship between ET and regulation of renal hemodynamics or renal microvascular function is complex and highly varied. In this section, we will try to highlight how ET influences renal microvascular function and how these actions translate into modulation of renal hemodynamics.

Probably the best information on the direct, segment-specific actions of ET on the renal microcirculation has come from in vitro studies. Vascular-specific data on ET's effects, without any confounding influences of tubular events or autocrine/paracrine modulators, arise from studies using isolated arteries and arterioles from rat and rabbit and in vivo or in vitro hydronephrotic kidney models. Some of the earliest work using isolated arterioles was that of Edwards et al. (165) to assess microvascular reactivity to ET-1, ET-2, and ET-3. They reported that ET-1 produced a concentration-dependent and long-lasting vasoconstriction of afferent and efferent arterioles with an ED₅₀ of ~1.4 and 0.9 nM for afferent and efferent arterioles, respectively. ET-2-mediated vasoconstriction of these arterioles was similar to that of ET-1, but ET-3 was significantly less potent than either ET-1 or ET-2. In similar work, using isolated rat microvessels, efferent arterioles were ~10-fold more sensitive to ET-1 compared with afferent arterioles (418, 562). It is important to note that ET-1 was more potent on efferent arterioles compared with identically prepared afferent arterioles. This carries important implications for ET as a paracrine regulator of glomerular hemodynamics by virtue of its potential ability to influence glomerular filtration pressure.

B) STUDIES IN THE HYDRONEPHROTIC KIDNEY: Much of our knowledge of the renal microcirculation has benefitted from using the in vitro and in vivo hydronephrotic kidney. This renal model that is devoid of renal tubules while most of the vascular architecture is retained and is readily visualized for study (540). Studies using this model provided the first in situ resolution of ET's actions on intra-renal microvascular elements. Initial reports indicated that ET is a potent vasoconstrictor of afferent arterioles of hydronephrotic kidneys in vitro (449, 758, 759) and in vivo (210, 256, 724). While ET-1 is a very potent vasoconstrictor of preglomerular arteries and arterioles, it appears to exert more modest, and perhaps more variable effects on the efferent arterioles of hydronephrotic kidneys; the variability may depend on whether the data are collected in vitro or in vivo. For example, ET-1 produced a modest efferent arteriole vasoconstriction in the in vitro hydronephrotic kidney setting (449, 759), whereas the magnitude of the efferent response was much greater in the in vivo hydronephrotic kidney setting (178, 210). Investigators reported that the afferent vasoconstriction in the hydronephrotic kidney in vivo model reflected activation of both ET_A and ET_B (88, 178), whereas efferent vasoconstrictor responses appeared to be exclusively ET_B mediated (178). Blockade of ET_A reduced afferent vasoconstriction to ET-1, but had no effect on efferent arteriolar responses. In contrast, ET_B blockade or ET_B agonists were effective modulators of arteriolar diameter in both afferent and efferent arteriolar segments. Using a different in vivo approach, Gulbins et al. (256) infused antibodies directed at ET-1 and ET-3 to scavenge endogenous ET peptides, and then monitored changes in renal microvascular diameter. They noted that anti-ET-1/ET-3 antibody infusion evoked vasorelaxation from arcuate and interlobular arteries and the proximal portion of afferent arterioles, while the diameter of efferent and distal afferent arterioles did not change. These data suggest that ET-1 may produce a more prolonged vasoconstriction of distal afferent arterioles and efferent arterioles than more upstream preglomerular segments.

C) STUDIES IN THE BLOOD-PERFUSED JUXTAMEDULLARY NEPHRON

PREPARATION: The blood-perfused juxtamedullary nephron preparation was developed in the mid-1980s by Daniel Casellas to evaluate inner cortical nephron function and microvascular reactivity (85, 86); the major advantage of this approach is that the vascular-tubular associations remain intact. Application of this approach to the question of the ET system has clearly revealed that ET-1, ET-2, and ET-3 vasoconstrict both afferent and efferent arterioles (322, 325, 673). The magnitude of the vasoconstriction to ET-1 and ET-3 was greater for afferent arterioles than for efferent arterioles, whereas afferent and efferent responses to ET-2 were similar between the two microvascular segments (325). ET-1 was significantly more potent than ET-2 or ET-3 as a renal microvascular vasoconstrictor with

significant reductions in afferent and efferent arteriolar diameter being evoked by 1.0 and 10 pM ET-1, respectively, whereas higher concentrations were required for ET-3 (322, 325). These results suggest that much of the vasoconstriction at lower concentrations of ET-1 is ET_A dependent and is consistent with earlier in vivo studies showing that ET_A blockade could completely block the ET-1-mediated decline in RBF and GFR (586, 587).

The above studies indicate that afferent vasoconstriction reflects activation of both ET_A and ET_B. ET-1-mediated vasoconstriction of afferent arterioles is blunted by ET_A blockade and is completely abolished when both receptor subtypes are blocked (325). Efferent vasoconstriction reflects activation of both ET_A and ET_B as well, but a more complex interaction appears to exist. Acute blockade of ET_A with A-127722 (racemic mixture of ABT-627 or atrasentan) converts the pronounced efferent vasoconstriction to a modest vasodilation at lower ET-1 concentrations (10–100 pM) before a stronger vasoconstriction appears when ET-1 concentrations reach 1 and 10 nM. Interestingly, blockade of ET_B (A-192621) shifts the ET-1 concentration-response curve slightly to the left, suggesting enhanced ET-1 potency. The ET_B agonist S6c also vasodilates efferent arterioles, and this vasodilation reverts to a slight vasoconstriction when ET_B are blocked (325). These findings suggest that vasodilatory ET_B present on vascular endothelium may have a more dominant role on the efferent arteriole and that ET_B-dependent constriction is only observed at higher agonist concentrations. This would also suggest that vascular smooth muscle ET_B might have a lower affinity for ET-1 than endothelial ET_B.

The accumulated data from the juxtamedullary nephron model suggest that ET_B provide a vasodilatory influence on normal efferent arteriolar vascular tone, whereas it is mainly a vasoconstrictor of afferent arterioles. These data may explain the variability noted in efferent arteriolar responses in the hydronephrotic kidney model in that the efferent arterioles can respond to ET with either a vasoconstriction or vasodilation, depending on the ambient conditions. It is interesting to note that a high-salt diet attenuates afferent arteriolar vasoconstrictor responses to ET-1, and this appears to be mediated by enhanced glomerular microvascular expression of ET_B (673).

D) RENAL HEMODYNAMIC STUDIES USING IN VIVO AND ISOLATED

PERFUSED KIDNEYS: Whole kidney responses to systemic or intrarenal infusion of ET uniformly demonstrate profound vasoconstriction (27, 80, 186, 585, 658, 815, 822). Early in vitro and in vivo work established that intrarenal ET-1 infusion into rabbit or rat kidneys reduced RBF, increased renal vascular resistance, and reduced GFR, all variables consistent with significant vasoconstriction of the preglomerular vasculature. However, whole kidney studies do not yield specific information regarding which intrarenal microvascular elements respond to ET-1, and in what way they respond. Using a unique intravital video-microscopy system, Saito et al. (658) examined surface glomeruli and related arterioles in isolated perfused kidneys from Munich-Wistar rats. They reported that ET-1 potently constricted surface afferent arterioles beginning at a concentration of ~30 fM with the maximum response yielding a 25% decline in afferent diameter at an ET-1 concentration of 30 pM. In these studies, efferent diameter decreased by a maximum of ~3% with 30 pM ET-1 (658). These findings in intact kidneys are similar to in vitro studies suggesting more active vasoconstriction in preglomerular versus postglomerular vessels.

Micropuncture studies are uniformly supportive of ET-1 as a preglomerular vasoconstrictor, but results on efferent arteriolar involvement are equivocal with studies suggesting more, less, or no difference in the vasoconstrictor response to ET-1 in the efferent arteriole. Badr et al. (27) showed that ET-1 reduced rat RBF and increased afferent and efferent arteriolar resistance by 65 and 82%, respectively. Calculated ultra filtration coefficient (K_f) declined by ~68%, and single-nephron GFR declined by ~54%. Similarly, King et al. (360) observed

that intrarenal ET-1 reduced rat renal plasma flow and K_f and increased afferent and efferent arteriolar resistance by ~23 and 73%, respectively (360). In contrast, Kon and co-workers (386, 387) observed maintenance of a stable K_f during ET-1 infusion, but they found proportionally greater increases in afferent arteriolar resistance compared with efferent changes. Furthermore, Denton and Anderson (151) found that intrarenal ET-1 infusion reduced RBF and increased afferent and efferent resistances similarly.

Antagonist studies indicate that the renal microcirculation is under the influence of endogenous ET-1. Infusion of the combined ET_A/ET_B antagonist bosentan reduced BP slightly along with significant decrease in glomerular capillary pressure (601). In the same setting, selective ET_A blockade had no effect on mean arterial pressure or glomerular hemodynamics. These data suggest that endogenous ET exerts a tonic vasodilatory influence on the renal microcirculation that is of ET_B origin. Endogenous NO appears to contribute to ET-modulation of renal vascular resistance similar to the early investigations into the ET system (147, 600, 601).

In the canine kidney, intrarenal infusion of ET-1 reduces RBF and GFR (275). In another study, both ET-1 and ET-3 reduced RBF in the dog kidney, and this effect was enhanced by NOS inhibition, suggesting that NO buffers the renal vasoconstrictor actions of ET (106). In the same study, inhibition of COX augmented ET-1-mediated renal vasoconstriction, but abolished the ET-3-mediated renal vasoconstriction. More specific renal cortical mechanisms of action in the canine kidney were identified using micropuncture techniques. Those data reveal that intrarenal infusion of ET-1 reduces RBF and GFR by activating ET_A , thereby increasing afferent and efferent arteriolar resistance and decreasing K_f (275). In this study, efferent resistance increased two times more than afferent resistance. The renal vasoconstriction appears to reflect a combination of the direct effects of ET-1 on microvascular resistance and indirect actions of ET-1 to stimulate production of other vasoactive mediators, such as thromboxane, adrenergic influences, or ANG II (275). Thus regulation of renal vascular resistance by ET peptides involves direct interaction of the agonist with ET_A and ET_B to modulate afferent and efferent arteriolar resistance, while at the same time, these ET-dependent effects are modulated by other vasodilator and vasoconstrictor agents, apparently in a receptor-specific manner.

4. Effect of ET on medullary blood flow—It is clear that ET exerts a powerful vasoconstrictor influence in the renal microcirculation (Fig. 3). Up to this point, discussion has focused on the effects of ET on afferent and efferent arteriolar resistance. These resistance changes will have a profound impact on whole kidney and cortical blood flow, but how does ET influence medullary blood flow? The findings that ET-1 potently vasoconstricts both afferent and efferent arterioles of the juxtamedullary nephrons found at the inner cortical surface provide some clues about how ET might influence medullary blood flow (325). The juxtamedullary microvasculature provides blood flow to the vasa recta that perfuse the renal medulla (565); thus one would predict that ET reduces medullary blood flow in concert with reducing cortical blood flow. However, ET's influence on medullary blood flow seems more complex (189). ET-1, ET-2, and ET-3 potently vasoconstrict isolated rat descending vasa recta with threshold effects visible at concentrations of $\sim 10^{-16}$ M, 10^{-14} M, and 10^{-9} M for the three peptides, respectively (565). The constrictor effects of ET-1 and ET-2, but not ET-3, were attenuated during ET_A blockade (BQ-123 or BQ-610). In contrast, ET-3-mediated constriction was inhibited by ET_B , but not ET_A , blockade. Interestingly, PGE_2 could abrogate ET-induced (ET-1 or ET-3) constriction of descending vasa recta (700). ET also reportedly increases medullary blood flow in rabbit kidneys in vivo, while at the same time reducing whole kidney and renal cortical blood flow (188). ET_A blockade decreased BP and increased RBF by increasing both cortical and medullary blood flow. ET_B blockade increased BP and reduced renal and cortical blood flow while

having no significant effect on medullary blood flow. ET_B blockade also potentiated ET-1-mediated reduction in renal and cortical blood flow and abolished ET-1-mediated increases in medullary blood flow. Qualitatively similar results were observed in rats and mice (72, 527, 737, 797). These data argue that ET_B are important regulators of the medullary blood flow response to ET, while ET_A play a more important role in regulating ET's influence on renal cortical blood flow.

The role of prostanoid metabolites in modulating ET's influence on intrarenal perfusion is unclear. Similar to vasa recta, prostaglandins or NO appear to modulate ET's effects on RBF (106, 256, 279, 280, 605), but this effect on regional blood flow in the kidney is not well defined. While thromboxane A₂ was implicated in the dog kidney, it does not appear to play a major role in effecting ET's effects on renal perfusion in the rat kidney (106). Blockade of prostanoid TP receptors did not alter ET's ability to reduce cortical or medullary blood flow (829).

The impact of ET on medullary perfusion is influenced by gender and environmental conditions. For example, infusion of ET-1 directly into the renal medulla had no detectable effect on medullary blood flow in normal or ET_B-deficient female rats, whereas similar infusion led to a marked reduction in medullary blood flow in male rats of both strains (527); ovariectomy eliminated the gender difference of ET on medullary blood flow. Big ET-1 decreases cortical and medullary blood flow in rats fed a normal-salt diet, but when fed a high-salt diet, the medullary vasoconstriction is abrogated (797). It is interesting to note that ECE-1 expression is significantly enhanced in the renal medulla of rats fed a high-salt diet. In addition, the sensitivity of rat juxtamedullary afferent arterioles to ET-1 was shifted significantly to the right in rats fed a high-salt diet. This shift was accompanied by an increase in ET_B expression by the preglomerular microvasculature (673). These data support involvement of medullary ECE-1 and ET_B activation in the medullary perfusion response to ET precursors and peptides.

5. ET signaling pathways in the renal circulation—This section presents what is known about the intracellular signaling mechanisms responsible for ET-mediated renal microvascular vasoconstriction (Fig. 2). Modulation of $[Ca^{2+}]_i$ is a major mechanism by which pre- and postglomerular microvascular resistance is regulated (23, 533). Studies have clearly shown that renal microvascular vasoconstriction is strongly linked to elevation of $[Ca^{2+}]_i$ and that changes in $[Ca^{2+}]_i$ involve both Ca^{2+} influx and mobilization signaling cascades. Interestingly, afferent and efferent arterioles often use different Ca^{2+} signaling mechanisms to produce vasoconstriction evoked by the same agonist (82, 83, 270, 447, 448, 450, 533). Therefore, studies were performed to determine the role of intracellular Ca^{2+} in ET-mediated renal microvascular vasoconstriction.

At the vascular smooth muscle cell level, ET rapidly increases $[Ca^{2+}]_i$. ET-1, ET-2, and ET-3 stimulated peak increases in cytosolic Ca^{2+} concentration in freshly isolated preglomerular smooth muscle cells with a rank order potency of ET-1 > ET-2 >> ET-3 (680). The peak response to ET-1 and ET-2 was followed by a smaller, but sustained plateau elevation of $[Ca^{2+}]_i$. The peak Ca^{2+} responses were unaffected by depletion of extracellular Ca^{2+} , but the sustained increases in Ca^{2+} were abolished. This pharmacological profile suggests that ET-mediated elevations in $[Ca^{2+}]_i$ arise primarily through activation of ET_A, with perhaps a smaller component arising through ET_B stimulation. The peak responses to ET-1 are primarily generated by mobilization of Ca^{2+} from intracellular stores, whereas the sustained increases in Ca^{2+} reflect influx of extracellular Ca^{2+} . ET_A- and ET_B-stimulated Ca^{2+} responses were corroborated by Fellner and Arendshorst (194), who noted that ET-1 and the ET_B agonist IRL-1620 stimulated increases in $[Ca^{2+}]_i$ in preglomerular smooth muscle cells and isolated rat afferent arterioles. The relative ET_A- and ET_B-dependent

responses were attenuated by their respective receptor antagonists, and combined ET_A/ET_B blockade virtually eliminated the response, demonstrating select receptor-dependency.

Initial detailed studies performed in hydronephrotic kidneys provide the first data using intact, pressurized arterioles. Loutzenhiser et al. (449) reported that ET-mediated vasoconstriction of afferent arterioles was completely inhibited in vitro by blockade of L-type voltage-gated Ca²⁺ channels with the dihydropyridine antagonist nifedipine; nifedipine had a more modest effect on efferent arterioles. Activation of membrane chloride channels may contribute to the depolarization necessary to activate voltage-dependent Ca²⁺ channels. Indeed, Takenaka showed that inhibition of voltage-gated Ca²⁺ channels with isradipine reversed ET-mediated vasoconstriction of afferent arterioles in hydronephrotic kidneys, and went on to show that similar effects could be obtained during chloride channel blockade (758, 759). In contrast, Fretschner et al. (210) found no effect of Ca²⁺ channel blockade on the afferent or efferent response to ET using a different dihydropyridine, nitrendipine, in vivo. More recently, Pollock et al. (583) showed that nifedipine significantly attenuated the decline in RBF evoked by ET-1, or the ET_B agonist S6c. In the same study, Ca²⁺ channel blockade attenuated intracellular Ca²⁺ signaling events in preglomerular smooth muscle cells and blunted afferent arteriolar vasoconstriction in response to lower (1 and 10 pM), but not higher (100 pM), ET-1 concentrations.

Taken together, these data indicate that ET peptides activate ET_A and ET_B to increase [Ca²⁺]_i in preglomerular smooth muscle cells through the contribution of both Ca²⁺ influx and Ca²⁺ release mechanisms. Voltage-dependent Ca²⁺ influx may occur through ET receptor-mediated opening of chloride channels leading to membrane depolarization and subsequent activation of L-type, voltage-dependent Ca²⁺ channels. The reasons for slight discrepancies between in vitro and in vivo preparations pertaining to the efficacy of Ca²⁺ channel blockers to blunt or eliminate ET-1 mediated signaling is unclear but may reflect differences in physiological or hemodynamic status among preparations.

Calcium mobilization from intracellular stores involves activation of several receptor-specific Ca²⁺ signaling cascades (533). ET-mediated Ca²⁺ release in the renal microcirculation appears to involve activation of Ca²⁺-induced Ca²⁺ release/cADP-ribose mechanisms, reactive oxygen species, Rho-kinase, protein kinase C (PKC), chloride channels, and cytochrome P-450 metabolites (23, 89, 193, 322, 343, 362, 533, 759, 771). As predicted based on preglomerular smooth muscle Ca²⁺ signaling experiments, release of Ca²⁺ from intracellular stores is an important signaling mechanism employed by ET. Activation of renal microvascular ET_A or ET_B presumably stimulates ADP-ribosyl cyclase to produce cyclic ADP-ribose (cADP-ribose) and nicotinic acid adenine dinucleotide phosphate (NAADP) leading to Ca²⁺ release (193, 771, 772). cADP ribose activates ryanodine receptor-dependent Ca²⁺ release, and NAADP may stimulate Ca²⁺ release through a lysosome-dependent pathway. ET-1 or S6c-stimulated Ca²⁺ signaling events and reductions in RBF were attenuated during inhibition of ADP-ribosylcyclase activity and during ryanodine receptor blockade (193, 772). Mice with defective ADP-ribosyl cyclase exhibit markedly reduced renal hemodynamic sensitivity to ET-1 infusion compared with wild-type controls.

More recent work has implicated a novel lysosomal Ca²⁺ signaling mechanism (771). Interventions such as inhibition of NAADP actions, or disruption of lysosomal pH and Ca²⁺ regulation simultaneously blunt ET-1 and norepinephrine-mediated increases in [Ca²⁺]_i in rat afferent arteriolar smooth muscle. These data indicate that ET-1 employs a host of mechanisms to stimulate ET receptor-dependent Ca²⁺ signaling events in renal microvascular smooth muscle.

Reactive oxygen species are important modulators of renal microvascular function and appear to influence preglomerular responsiveness to ET (193, 343). ET-1 increases $[Ca^{2+}]_i$ and superoxide accumulation in preglomerular smooth muscle cells, and this effect was markedly diminished in the presence of the superoxide dismutase mimetic tempol (193). ET_B activation with S6c also increases $[Ca^{2+}]_i$, but had only a slight effect on superoxide accumulation. When examined at the whole kidney level, the NADPH oxidase inhibitor apocyanin attenuated the ability of ET-1 or S6c to reduce RBF (343). Thus the oxidative status present in the renal microvascular environment can have a significant influence on the renal microcirculatory response to ET receptor activation.

ET-1 binding to ET_A and ET_B also stimulates phospholipase A₂ activation, arachidonic acid release, and production of COX and cytochrome *P*-450 metabolites from renal tubular and vascular cells (158, 321, 322, 558, 670, 841). Cytochrome *P*-450 and COX metabolites contribute to ET's ability to reduce RBF, but comparatively little is known about the vascular sites of action and cellular signaling mechanisms involved. In some pioneering studies, Imig et al. (321, 322) determined that COX and cytochrome *P*-450 metabolites contribute directly to ET-1-mediated afferent arteriolar vasoconstrictor responses through modulating Ca^{2+} signaling events in preglomerular smooth muscle cells (321, 322). They noted that inhibition of COX or cytochrome *P*-450 hydroxylase activity attenuated ET-1 mediated afferent arteriolar vasoconstriction. In contrast, inhibition of cytochrome *P*-450 epoxygenase activity enhanced ET-1 mediated afferent arteriolar vasoconstriction. They also examined the impact of these inhibitors on ET-1 mediated Ca^{2+} signaling events. As predicted from the vasoconstrictor studies, inhibition of COX or cytochrome *P*-450 hydroxylase activity attenuated ET-1 mediated increases in $[Ca^{2+}]_i$ in preglomerular smooth muscle cells, but cytochrome *P*-450 epoxygenase inhibition had no effect. These data establish that COX and cytochrome *P*-450 hydroxylase metabolites that contribute to the ET-1-mediated vasoconstriction are generated by renal microvascular smooth muscle cells to a significant degree. In contrast, inhibition of the CYP-450 epoxygenase pathway enhanced the vasoconstrictor response to ET-1. Thus ET-1-mediated production of vasodilatory epoxyeicosatrienoic acids (EETs) may counteract the vasoconstrictor actions of ET-1. This conclusion conflicts with an in vivo study indicating that cytochrome *P*-450 epoxygenase inhibition with clotrimazole had no effect on the ET-1-mediated decreases in RBF and GFR (559). An important difference between the two studies is that the work of Oyekan and McGiff (559) was conducted in vivo at the whole kidney level while the work of Imig et al. (322) was in vitro and focused on juxtamedullary afferent arterioles. Nevertheless, the vascular smooth muscle cell signaling data were collected using cells from the entire preglomerular microvascular tree, and thus reflect Ca^{2+} signaling events for vascular smooth muscle cells throughout the renal cortex (322).

While ET-1-mediated increases in $[Ca^{2+}]_i$ are important signaling mechanisms by which ET influences renal microvascular resistance, changes in Ca^{2+} sensitivity can also play an important role in regulating vascular function (714). One of the major mechanisms by which Ca^{2+} sensitivity is thought to be regulated involves receptor-mediated activation of the RhoA/Rho-kinase pathway, which inhibits myosin light-chain phosphatase or PKC (13, 454, 575). While there are few studies specifically addressing the role of changes in microvascular smooth muscle Ca^{2+} sensitivity on renal microvascular function, there are a few studies that provide a glimpse of these systems. In two separate studies, different PKC inhibitors had no significant effect on ET_A- or ET_B-mediated vasoconstriction of afferent arterioles (89, 759). In contrast, Rhokinase may be involved in the ET response in renal vasculature. Rat afferent arterioles express elements of the Rho-kinase signaling pathway, while arteriolar reactivity is influenced by inhibitors of Rho-kinase activity (89, 324). ET_B activation reportedly activates RhoA of the Rhokinase signaling pathway (363). Acute exposure to Rhokinase inhibitors rapidly reduces microvascular resistance in normal and

hydronephrotic kidneys. Furthermore, pretreatment with Rho-kinase inhibitors virtually eliminates the preglomerular vasoconstriction induced by the ET_B agonist IRL-1620 and attenuates IRL-1620-mediated vasoconstriction of the efferent arteriole. Unfortunately, ET_A -dependent effects have not been examined, so the contributions of the Rho-kinase system on ET_A -mediated renal microvascular responses remain to be determined. Nevertheless, the limited data available thus far support a role for Rho-kinase activity in ET-1-mediated renal microvascular reactivity.

Integration of the above data argues that ET is a potent vasoconstrictor of the renal microcirculation and may act in an important, segment-specific manner. These vasoconstrictor effects involve both ET_A and ET_B and may be altered under differing environmental conditions, such as high dietary salt or hypertension. The physiological implications of such regional influences along the renal vascular tree remain to be determined. There is general agreement that ET regulates renal microvascular tone by modulating $[Ca^{2+}]_i$ and may also modulate Ca^{2+} sensitivity, thus providing a remarkably sensitive mechanism for ET-1-dependent regulation of renal cortical and medullary resistance.

C. Glomerulus

This discussion is limited to mesangial cells and podocytes; endothelial cells are reviewed in the context of the renal vasculature. ET exerts several effects on glomerular cells, although most of the identified actions are primarily relevant to pathophysiological conditions associated with glomerular sclerosis, cell proliferation, and/or proteinuria (37). However, there are data that suggest the ET system can affect GFR through modification of glomerular cell function; this section will focus on this topic.

D. Podocytes

1. Podocyte ET production—Glomerular podocytes can synthesize ET peptides. A rat glomerular epithelial cell line had positive immuno-staining for ET-1, Big ET-1, and ET-3 (350). These cells also secreted ET-1 and contained ET-1 mRNA. Primary cultures of rat glomerular epithelial cells released ET-1 and expressed ET-1 mRNA (128). ET-1 production by these cells was augmented by activation of PKC; thrombin and phorbol 12-myristate 13-acetate (PMA) enhancement of ET-1 release was markedly reduced by PKC inhibition or depletion of PKC. ET-1 immunostaining was detected in podocytes in human kidney sections (208), while cultured human podocytes released ET-1 (123). Mouse podocytes also produce ET-1 production; interestingly, these studies suggested that reorganization of the actin cytoskeleton via Rho-kinase-dependent focal adhesion kinase (FAK) activation of nuclear factor- κ B (NF- κ B) and activator protein-1 (AP-1) led to increased ET-1 generation (502). Taken together, these data indicate that podocytes synthesize ET-1 and that such production is regulated, at least partly, by factors that are modified by changes in podocyte conformation.

2. Podocyte ET receptor expression—Glomerular epithelial cells express ET receptors. Cultured human glomerular epithelial cells bind ET-1 (609). Electron microscopic autoradiography localized ET-1 binding to podocytes in rat kidney (215). Glomerular epithelial cells may express both ET_A and ET_B . Immunoelectron microscopy localized ET_B to podocytes in rat glomeruli (850), while ET-1 induced thrombin receptor internalization in cultured human glomerular epithelial cells via activation of ET_A (99).

3. ET actions in the podocyte—ET-1 can stimulate nephrin shedding from glomerular epithelial cells, in part due to cytoskeleton redistribution, raising the possibility that ET-1 may affect podocyte conformation. This notion is supported by the finding that ET-1

induced cytoskeletal rearrangement leading to increased protein permeability in cultured mouse podocyte monolayers (503); this effect was mediated via ET_A activation and involved phosphatidylinositol 3-kinase (PI3K) and Rho-kinase pathways. An interesting finding in this latter study was that shigatoxin-stimulated podocyte cytoskeletal changes were mediated by ET-1, indicating that ET-1 can act in an autocrine manner to cause actin remodeling. ET-1 also increases [Ca²⁺]_i in podocytes (609), an effect that may lead to cytoskeletal changes. Notably, ET-1 increased [Ca²⁺]_i in the parietal cells of Bowman's capsule of rat glomeruli (460); this was associated with contraction of the parietal sheet, an effect that may lead to changes in GFR.

Taken together, the above studies indicate that glomerular podocytes release, bind, and respond to ET-1. ET-1 can induce cytoskeletal remodeling in podocytes; how such changes would affect GFR remains speculative. While ET-1 may increase podocyte protein permeability, this does not necessarily mean that water and small solute filtration will be increased (and may even decrease). ET-1 can contract glomerular parietal epithelial cells, an effect that would presumably decrease GFR and even RBF. ET_A may mediate ET-1 effects on both podocyte permeability and parietal cell contraction; whether this participates in ET_A-mediated reductions in GFR and RBF remains to be determined.

E. Mesangial Cells

1. ET production by mesangial cells

A) BASELINE ET PRODUCTION BY MESANGIAL CELLS: Mesangial cell ET-1 production has been relatively extensively studied; the impetus for such analysis stems from the pleiotropic effects of the peptide on mesangial cell function, including hypertrophy, proliferation, extracellular matrix production, and contraction. This review is primarily concerned with regulation of Na homeostasis and BP; hence, mesangial cell contraction, which can affect GFR, will be the ET-1 action relevant to these considerations. However, if a given factor stimulates ET-1 release by mesangial cells, it is certainly conceivable that, despite that particular agent's known effects on noncontractile behavior, any mesangial cell ET-1 synthesis it modifies could potentially impact cell contraction. Hence, we will review factors known to regulate mesangial cell ET-1 release as well as the mechanisms by which such release is modulated.

B) REGULATION OF MESANGIAL CELL ET PRODUCTION: Numerous factors can affect mesangial cell ET-1 gene transcription, including vasoactive substances, growth factors, cytokines, reactive oxygen species, and others. The major vasoactive regulators identified to date that increase ET-1 synthesis include ET-1 itself (332), ANG II (316, 377), arginine vasopressin (AVP) (316, 660, 743), and thromboxane A₂ (884), while atrial and brain natriuretic peptides decrease ET-1 production (381). The autoinduction of ET-1 in rat mesangial cells was shown to be mediated by activation of ET_B and to occur through augmented ET-1 gene transcription rate as well as increased ET-1 mRNA stability (332).

The 5'-flanking region of the ET-1 gene contains positive regulatory elements (e.g., modulated by thrombin), while negative regulation is exerted by the distal 5' domain (206). Increased preproET-1 expression requires p38 mitogen-activated protein kinase (MAPK) and PKC. Thrombin, tumor necrosis factor (TNF), and interleukin-1 (IL-1) synergistically increase ET-1 expression in mesangial cells, an effect that requires activation of p38 MAPK and PKC. Extracellular signal-regulated kinase (ERK), c-Jun NH₂-terminal kinases/stress-activated protein kinase (JNK/SAPK), or intracellular Ca²⁺ release are uninvolved in this process (206). The events upstream of p38 MAPK activation involve TAK1 kinase, TAK1 binding protein-1 (TAB1), TNF receptor-associated factor 2 (TRAF2), and several MAPK/

extracellular signal regulated kinase kinases (MEKKs). PMA or ectopic expression of PKC- β 1 also stimulate ET-1 expression by mesangial cells (282).

Mesangial cell ET-1 production seems to be primarily regulated at the level of gene transcription. As discussed previously, this is a common theme for control of ET-1 synthesis; while alterations in mRNA stability, preproET-1 processing, ECE activity, or even vesicular trafficking have been variably described for ET-1, they have been infrequently identified as playing a significant role in the control of mesangial cell ET-1 release. Such focused regulation of ET-1 gene transcription involves a host of factors activating a complex of intracellular signaling pathways. While the net effect on mesangial cell ET-1 release depends on the interplay between these varied systems, certain general statements can be made. In particular, vasoconstrictors tend to increase mesangial cell ET-1 release, while vasodilators decrease ET-1 production. Such mesangial ET-1 release acts to enhance the action of the given vasoactive substance. In addition, since mesangial cell ET-1 release is largely dependent on gene transcription, its regulation can lead to not only potentiation of the magnitude of vasoactive factor's physiological effect, but could substantially prolong the given factor's duration of action. This latter concept may be of particular importance in that ET-1, as discussed earlier, exerts long-lasting effects. Thus the combination of stimulated gene transcription (with sustained ET-1 production) in combination with prolonged ET-1 actions once bound to its cognate receptor(s), provide a potentially powerful combination to substantially lengthen a stimulus (e.g., mesangial cell contraction) exerted by a given physiological agent. Such considerations may apply to essentially all cell types that produce and respond to ET-1; once activated, this regulatory system exerts powerful and long-acting effects as opposed to being involved in minute to minute regulation.

2. ET receptor expression by mesangial cells—Mesangial cells express both ET_A and ET_B (Fig. 3). Initial studies, using cross-linking, found two ET receptors in cultured rat mesangial cells with molecular masses of 58 and 34 kDa (735). Shortly thereafter, binding studies, based on heterologous competition for ET-3 and sarafotoxin, were consistent with the presence of two ET binding sites in cultured rat mesangial cells (31). Subsequent competition binding studies by this group, using the same cells, found evidence for classical ET_A binding (competed off by BQ123) as well as a second ET receptor whose ET-1 binding was not displaced by S6C (i.e., was not classical ET_B binding) (705). In addition, only ET_A mRNA was detected by PCR. Similarly, competition binding analysis and PCR of mRNA in rat mesangial cell cultures revealed predominant ET_A expression (8), as did binding analysis studies in cultured rat mesangial cells by another group (122). In contrast, Yokokawa et al. (862) detected both ET_A and ET_B mRNA in rat mesangial cells using Northern analysis, while they also found evidence for ET_A- and ET_B-mediated Ca²⁺ signaling in these cells. In addition, mRNA for both receptor isoforms has been reported in cultured rat mesangial cells (742, 753). ET-1 also stimulated cultured rat mesangial cell cGMP production via activation of ET_B (557). The reasons for these discrepant results are uncertain but likely relate, at least in part, to studies done relatively early after the ET field was established and before anti-ET receptor antibodies and a large variety of receptor-specific agonists and antagonists were available. Subsequent to these studies, investigators have found evidence for ET_A- and ET_B-mediated actions in mesangial cells. Several studies have identified both ET receptor subtypes in human mesangial cells. ET_A and ET_B expression has been reported in cultured human mesangial cells (282) and confirmed by others (553). Strong mesangial cell ET_A immunostaining was observed in human kidney sections (823). In most of the binding studies using cultured cells, ET-1 binds to mesangial cell ET_A and ET_B with high affinity (K_d in the 100 pM range) and, as alluded to above, exerts effects lasting for several hours. Since normal plasma levels of ET-1 average 1–5 pM, this suggests that ET-1 primarily functions as an autocrine or paracrine factor. Thus, as for virtually all cell systems involving

ET-1, it is critical to view mesangial cell ET-1 action in the context of the local microenvironment.

An interesting feature of mesangial cell ET receptors is that their binding and signaling are markedly down-regulated by preincubation with ET-1 (receptor desensitization). Exposure of cultured rat mesangial cells to ET-1 greatly reduced ET-1 binding capacity without affecting its affinity (31). Similar adaptive desensitization, in terms of evoked $[Ca^{2+}]_i$ responses, was observed in cultured rat mesangial cells preincubated with ET peptides (703).

3. ET actions in mesangial cells

A) MECHANISMS OF ET-INDUCED MESANGIAL CELL CONTRACTION: ET-1 activates a wide variety of signaling systems in mesangial cells with resultant alterations in cell contraction, hypertrophy, proliferation, and extracellular matrix accumulation (718). This discussion will focus on those systems most likely to affect cell contraction; however, it is possible that activation of some pathways may lead to more than one biologic effect.

ET-1 is a potent stimulator of mesangial cell contraction; this effect is independent of dihydropyridine-sensitive Ca^{2+} channels and is likely mediated through ET_A activation (27, 701, 752). ET-1 activates phospholipase C (PLC) (337, 706, 707) and PKC (703). The increased inositol trisphosphate levels are associated with cell alkalization due to increased Na^+/H^+ exchange and elevated $[Ca^{2+}]_i$ (27, 703, 705, 706). The increase in $[Ca^{2+}]_i$ is caused by release from intracellular stores in addition to influx from dihydropyridine-insensitive pathways (703, 862). Relatively low ET-1 concentrations (0.1–10 pM) evoke slow sustained increases in $[Ca^{2+}]_i$ that are dependent on Ca^{2+} influx through a voltage channel-independent mechanism, while higher ET-1 concentrations (≥ 100 pM) cause a rapid and transient increase that is dependent on Ca^{2+} release from intracellular stores through activation of PLC and PKC (706). The increase in $[Ca^{2+}]_i$ appears to be primarily due to ET_A activation since ET-1-induced increases in $[Ca^{2+}]_i$ in rat mesangial cells are almost completely blocked by BQ123 (862). ET_B activation by ET-3 in rat mesangial cells can also raise $[Ca^{2+}]_i$; however, this response is markedly less than that seen with ET-1 (862).

Several kinases play an important role in ET-1-mediated contraction of mesangial cells. One such kinase, proline-rich tyrosine kinase-2 (Pyk2), is the only cytoplasmic tyrosine kinase activated by mobilization of $[Ca^{2+}]_i$ (431); tyrosine phosphorylation is essential for the contractile effects of many G protein-coupled receptor ligands (462, 779, 818, 886). ET-1 stimulates Pyk2 autophosphorylation in a Ca^{2+} -dependent manner in mesangial cells (719). Notably, Pyk2 mediates p38 MAPK activation in mesangial cells; the latter factor has been implicated in contraction of this cell type (719). Other MAPKs may also be involved; ERK5 is stimulated by ET-1 in cultured human mesangial cells, while dominant-negative ERK5 inhibits mesangial cell contraction (157). ET-1-mediated contraction may also involve stimulation of the GTPase Rap1 (649). This latter effect was shown to be associated with Pyk2 related phosphorylation of p130Cas, which then increasingly interacts with BCAR3, a protein with a GDP exchange-factor-like domain; this potentially leads to BCAR3-mediated GTP-loading of Rap1 with resultant modulation of the actin cytoskeleton and possibly alterations in cell contraction (though definitive proof of this is lacking). ET-1 can also increase the adapter protein CrkII association with BCAR3 in human mesangial cells, although it is unknown if this is involved in ET-1-induced mesangial cell contraction (650). ET-1-mediated mesangial cell contraction may also involve Src tyrosine kinases, possibly via β -arrestin-1-mediated recruitment of Src to a molecular complex with the ET receptor (320) and/or adhesion-dependent activation of Src through interaction with FAK (90). ET-1 may activate Src, at least in part, through activation of Ca^{2+}/CaM -dependent protein kinase II (CaMKII) in mesangial cells (813). Finally, platelet activating factor (PAF) may mediate,

at least in part, ET-1-induced rat mesangial cell contraction (446, 499); ET-1 stimulates mesangial cell PAF release, while blockade of PAF reduces ET-1-induced cell contraction.

B) REGULATION OF ET-INDUCED MESANGIAL CELL CONTRACTION: ET-1-stimulated mesangial cell contraction may be modified by vasorelaxant factors. ET-1 stimulates COX activity with resultant increases in PGE₂ and small increases in thromboxane A₂ (704). This occurs through ET_A-mediated induction of phospholipase A₂ (213) and COX2, but not COX1 (311). COX2 activation is dependent on intracellular Ca²⁺ release, CaMKII, and non-receptor-linked protein tyrosine kinase activity (124) and is independent of PKC (213). ET-1 activated COX2 in mesangial cell is due, at least in part, to nuclear factor of activated T cell 2 (NFAT2) translocation to the nucleus and binding to the COX2 promoter (734). ET-1 may also stimulate NO production by mesangial cells; however, the evidence for this is equivocal. ET-3 increases NO-dependent cGMP production by isolated rat glomeruli through activation of ET_B (162, 742); however, ET-3 did not alter cGMP content in mesangial cells cultured from these preparations (742). In contrast, Owada et al. (557) reported that ET-3 increases NO production and cGMP in cultured rat mesangial cells, an effect that was dependent on activation of ET_B, release of Ca²⁺ from intracellular stores, and calmodulin. This induction of NO and cGMP occurred within a period of a few minutes, suggesting activation of either NOS1 or NOS3. In contrast, ET-1 inhibits induction of cytokine-stimulated NOS2 activity in rat mesangial cells, an effect that was mediated by ET_A (43, 291).

In summary, ET-1 contracts mesangial cells through activation of ET_A. This effect may be mitigated by ET_A-mediated PGE₂ accumulation and ET_B-induced NO formation. It must be noted that these conclusions are based entirely on in vitro studies; we are lacking conclusive demonstration of a physiological role for ET-1-mediated mesangial cell contraction. Such confirmation would be quite problematic, so it is likely that the functional or pathological importance of mesangial cell-derived ET-1 in regulating renal responses to alterations in Na intake or BP will remain speculative. Nonetheless, it is interesting to note that alterations in mesangial cell ET-1 production have been reported in hypertension. Specifically, Ikeda et al. (316) found that phorbol ester-, ANG II-, and AVP-stimulated ET-1 release was greater in mesangial cells isolated from spontaneously hypertensive (SHR) compared with Wistar-Kyoto (WKY) normotensive rats (316). These findings raise the interesting possibility that heightened ET-1-mediated mesangial cell contraction, leading to reduced GFR, could contribute to impaired renal Na excretion in the setting of some forms of essential hypertension.

F. Proximal Tubule

1. ET production by the proximal tubule

A) BASELINE PROXIMAL TUBULE ET PRODUCTION: The proximal tubule (PT) synthesizes ET-1, although in much smaller amounts than more distal nephron segments (368). LLC-PK₁ cells, a porcine PT cell line, release ET-1 (690). Cultured rabbit PT cells made much less ET-1 compared with the thick ascending limb (TAL) and collecting duct (CD) (368). Two groups detected ET-1 mRNA in rat proximal convoluted tubules using PCR or in situ RT-PCR (97, 501); however, two other groups failed to detect a PCR signal for ET-1 mRNA in PTs (787, 790). ET-1 synthesis and mRNA has been detected in cultured human PT cells (549, 859). ET-1 immunostaining was observed in the most proximal regions of the rat PT (828). The PT may also produce ET-3; cultured rabbit PTs secreted ET-3, albeit in amounts that were 5- to 10-fold less than ET-1 (368). Terada et al. (769) reported that microdissected rat PTs express ET-3 mRNA. Finally, ECE-1 immunostaining was detected in human PTs (599). Thus PTs can synthesize ET peptides, but the magnitude of such production is relatively small compared with other renal cell types.

B) REGULATION OF PROXIMAL TUBULE ET PRODUCTION: PT ET-1 production is regulated by a variety of factors (Fig. 4); however, the identified modulators appear to be primarily activated under pathophysiological conditions. For example, following renal injury, epidermal growth factor (EGF) and hepatocyte growth factor (HGF), most likely via activation of receptor tyrosine kinase activity, inhibit basal and calcineurin inhibitor-stimulated cultured rabbit PT ET-1 release (269). Acute hypoxia increases PT ET-1 immunostaining (537). Glomerular disease associated with excessive proteinuria and lipiduria may augment PT ET-1 synthesis; high-density lipoproteins stimulate ET-1 release by cultured human proximal tubular cells via PKC- and cAMP-independent pathways (549), while proteins contained in plasma (e.g., albumin, immunoglobulin G) increase ET-1 release by a rabbit PT cell line (883). A number of factors associated with inflammatory conditions, including thrombin, transforming growth factor- β (TGF- β), TNF- α , and IL-1 β induce ET-1 release from LLC-PK₁ cells (543). Phorbol ester also increases ET-1 release by LLC-PK₁ cells, indicating that PKC is involved in stimulation of ET-1 synthesis in PT cells (543). Metabolic acidosis increases ET-1 mRNA expression in rat PTs (440). While some of these systems could conceivably be involved in regulation of PT ET-1 production in response to physiological or pathophysiological changes in extra-cellular fluid volume (ECFV) or BP, they have not been well studied in this context. One group observed an increased in ET-1 mRNA, using in situ hybridization, as well as ET-1 immunostaining, in PTs of uninephrectomized SHR rats compared with WKY controls (420).

2. ET receptor expression by the proximal tubule

A) BASELINE PROXIMAL TUBULE ET RECEPTOR EXPRESSION: Binding studies, using microdissected rat nephron segments, indicate that the PT expresses ET receptors, although in very small amounts compared with more distal nephron segments (757) (Fig. 3). In vitro autoradiography of rat kidney localized ET-1 binding to PTs (383), while ET-1 binds to LLC-PK₁ cells (536). Additionally, numerous studies, as described below, have reported direct modulation of PT function by ET. The ET receptor subtypes expressed by PT remains somewhat uncertain. Terada et al. (767) were unable to detect ET_B mRNA in PT (767); however, several groups have observed ET_B-mediated functional effects of ET-1 in this nephron segment. ET-1 and ET-3 were equipotent in decreasing Na⁺-K⁺-ATPase activity in microdissected rat proximal convoluted tubules (548). While ET-1 and ET-3 had a similar IC₅₀ for competition with radiolabeled ET-1 binding to LLC-PK₁ cells (561), PT ET_B immunoreactivity was detected in sections of rat kidney (853). Immunostaining studies identified ET_B, but not ET_A, in rat PT (823). In contrast, based on competition binding and RNA analysis, human PT cells have been reported to contain both ET_A and ET_B (550). ET_A immunostaining has also been detected in the basal infoldings of rat PTs (852). Thus, albeit expression is low, the PT most likely expresses both ET_A and ET_B.

B) REGULATION OF PROXIMAL TUBULE ET RECEPTORS: PT ET receptors appear to be subject to regulation. Rat WKY PT cell ET_B protein and mRNA content was increased by dopamine D3 receptor activation, an effect that was dependent on extracellular Ca²⁺ or dihydropyridine-sensitive Ca²⁺ channels (865). D3 and ET_B coimmunoprecipitated, raising the possibility of a physical interaction. In contrast, rat SHR PT cell ET_B expression was much lower than in WKY cells and was further decreased by D3 agonism, suggesting that D3 and ET_B interaction may be abnormal in this hypertensive model. The same group has reported that ANG II, via AT1 receptors, increases immortalized rat WKY PT cell ET_B protein, but does not affect ET_B expression in rat SHR PT cells (872). They also noted that AT1 and ET_B colocalized and coimmunoprecipitated. Short-term (15 min) ANG II exposure decreased ET_B phosphorylation in WKY and SHR cells, but increased ET_B cell surface expression only in WKY cells. This group also reported that ET_B activation decreased AT1

receptor expression in WKY, but not SHR, PT cells (873). Taken together, these studies raise the interesting possibility that ET_B physically associate with dopamine and ANG II receptors. Furthermore, decreased ET_B expression may be associated with some forms of hypertension; as will be discussed below, such downregulation of ET_B could conceivably contribute to enhanced renal Na retention.

3. ET actions in the proximal tubule

A) ET REGULATION OF PROXIMAL TUBULE SODIUM AND FLUID

TRANSPORT: Initial studies on ET-1 regulation of PT Na and water reabsorption found widely divergent results. Studies based on lithium clearance suggested that low-dose (minimal effects on GFR) intravenous ET-1 reduced rat PT fluid reabsorption (263, 573). More direct evidence for such an effect was found when basolateral administration of ET-1 (1 nM) inhibited fluid and bicarbonate absorption by the microperfused isolated rat proximal straight tubule, an effect that was ascribed to suppression of Na⁺-K⁺-ATPase activity (230). ET-1 reduced rat PT brush-border membrane Na⁺-glucose cotransporter activity (456). In contrast, ET-1 did not change human PT Na reabsorption as evidence by lithium clearance (717) nor did it alter Na⁺-K⁺-ATPase activity in rabbit proximal convoluted tubule suspensions (869). Other studies have found that ET-1 stimulates PT Na reabsorption. Apically administered ET-1, given at a relatively high dose (100 nM), increased PT fluid reabsorption in micropuncture studies (624); single-nephron GFR was markedly enhanced by luminal ET-1, albeit fractional fluid reabsorption actually increased. The significance of these latter studies remains an open question, particularly since high doses of ET-1 were used, the bulk of endogenous ET-1 is likely to be present on the basolateral side of the cell, and since the coincident change in SNGFR raises the possibility of confounding influences associated with changes in tubuloglomerular feedback. ET-1 stimulated Na⁺/H⁺ antiporter and Na⁺/HCO₃⁻ cotransporter activity in plasma membrane vesicles from rabbit renal cortex (although Na⁺-glucose and Na⁺-succinate transporter activities were unaffected) (166). ET-1 also increased Na⁺/H⁺ exchange and Na⁺/P_i cotransport by rat renal cortical slices, effects that were mediated by activation of protein kinase A (PKA) and PKC (258). Similarly, ET-1 stimulated Na⁺/H⁺ exchange activity in OKP cells, an opossum PT-like cell line, and this effect was prevented by blockade of PKC (809).

The apparent discrepancies in ET-1 effects on the PT were studied by Garcia and Garvin (218); they observed a biphasic effect of ET-1 on fluid absorption by the rat proximal straight tubule. ET-1 (0.1 pM) stimulated fluid absorption through activation of PKC, while ET-1 (1 nM) decreased fluid transport through PKC-, COX-, and lipoxygenase-dependent mechanisms. These findings were supported by the observation that 5-lipoxygenase or leukotriene C₄/D₄ antagonism prevented ET-1-induced natriuresis in rats (ET-1 natriuresis was partly ascribed, based on lithium clearance, to actions on the PT) (573). In addition, ET-1 inhibition of rat PT Na⁺-K⁺-ATPase activity was abolished by blockade of ω-hydroxylase; in this same study, ET-1 increased PT 20-hydroxyeicosatetraenoic acid (20-HETE) production while both arachidonate and 20-HETE inhibited Na⁺-K⁺-ATPase activity (185). Based on these considerations, it is tempting to speculate that, if the concentration of ET-1 is low enough to only activate PKC, PT Na reabsorption is increased. If ET-1 levels rise to the point where arachidonate metabolites accumulate, PT Na reabsorption is reduced. The ET-1 concentration around the PT is unknown; however, any increase in local ET-1 levels would likely exceed “background” plasma levels of 1–10 pM ET-1, concentrations that may already be above those that stimulate PT fluid reabsorption (218). This speculation must be balanced, however, by consideration of the possibility that local proteases could reduce the ET-1 concentration in the immediate region of the PT.

The simplest explanation for biphasic concentration-dependent effects of ET-1 on the PT relates to differential sensitivity of ET receptors and ensuing opposing actions. However,

such evidence is lacking. Indeed, ET_B activation has been associated with both stimulation and inhibition of PT Na transport processes. ET-1 activates the Na⁺/H⁺ antiporter in OKP cells transfected with ET_B, but not ET_A (110). Several subsequent studies have confirmed that ET_B mediates ET-1 activation of Na⁺/H⁺ exchange in the PT and have partly worked out the relevant mechanisms. Laghmani et al. (416) found that ET-1 activation of the Na⁺/H⁺ exchanger 3 (NHE3) in OKP cells transfected with ET_B was due to the COOH-terminal tail and the second intracellular loop of ET_B (with a requisite KXXX-VPKXXXV consensus sequence). Using the same cells, this group found that ET_B-induced NHE3 activity was partly dependent on tyrosine kinase pathways (111). In addition, ET_B stimulation of NHE3 activity is Ca²⁺ and Rho kinase dependent and involves phosphorylation and exocytosis of NHE3 into the apical membrane (593). ET-1 stimulated Na⁺/H⁺ (and Na/P_i) exchange in PT may also depend on G_i-mediated inhibition of cAMP accumulation (258, 561). In contrast to activation of NHE3, stimulation of ET_B in PTs from WKY rats, using an ET_B specific agonist, inhibited Na⁺-K⁺-ATPase activity, and this effect was blocked by ET_B antagonism (865). Interestingly, this group observed that ET_B stimulation did not alter Na⁺-K⁺-ATPase activity in PTs derived from SHR rats, suggesting a possible role for ET_B-related signaling pathway dysfunction in enhanced Na retention associated with hypertension. ET-1, via stimulation of ET_B, enhances cGMP accumulation in LLC-PK₁ cells (and independent of G_i) (561). ET-1 stimulation of cGMP in this cell type is most likely due to Ca²⁺-dependent NO production (327). Since NO, via cGMP, inhibits Na transport by the PT (622, 812), it seems probable, albeit unproven, that ET_B stimulated PT NO production leads to inhibition of Na reabsorption by this nephron segment. Taken together, the above studies indicate that PT ET_B activation can lead to either stimulation or inhibition of Na transport processes. While it is possible that this somehow relates to different concentrations of ET-1 (presumably activating different pools of ET_B), it seems likely that other factors are involved. For example, PT ET-1-dependent NHE3 activation is clearly associated with metabolic acidosis, serving as an important mechanism for increased urinary acid excretion (593). It may be, therefore, that acidosis stimulates PT ET-1 release, accumulation and receptor activation in specific microdomains and amounts that lead to selective activation of NHE3, while ECFV expansion may affect different regions of the cell leading to ET-1 inhibition of Na⁺-K⁺-ATPase. Such spatial segregation of ET production and ET receptor-mediated actions has never been conclusively demonstrated and clearly deserves further evaluation.

The above considerations pertain to PT ET_B; however, there are data, albeit quite limited, on a potential role for ET_A in the PT. In particular, one group found that BMS182874, a relatively ET_A-selective antagonist, blocked ET-1 stimulation of 20-HETE in rat PTs (185). In addition, ET-1 induced human PT NF-κB via ET_A activation (238).

G. Thin Limb of Henle's Loop

Very little is known about the biology of ET in this nephron segment. ET-1 mRNA was detected in the thin descending limb of rat kidney using in situ RT-PCR, albeit at lower levels than elsewhere in the nephron (501). Similarly, ET-1 was detected in human thin descending limb using immunohistochemistry (550). Small signals (relative to other nephron regions) for ET-3 mRNA were observed in microdissected rat inner medullary thin limb (769). In contrast, no ET-1 was found in thin descending limbs from rat using immunohistochemistry (828) or in situ hybridization (77), nor was any ET-1 found in human thin descending limbs using in situ hybridization (598). In essence, if the thin descending (ascending has not been reported) limb makes ET peptides, it is in very small amounts that are of questionable physiological importance.

ET receptor expression and activation have not been well studied in thin limbs. The one study to address this issue examined ET receptors and signaling in acutely isolated rat thin

descending limbs (28). RT-PCR detected both ET_A and ET_B mRNA, while ET_A, but not ET_B, activation induced increases in [Ca²⁺]_i. This Ca²⁺ signal was detected only in thin limbs from long-looped nephrons, but not from short loops or in thin ascending limbs. Interestingly, the ET-1-induced Ca²⁺ signal was substantially diminished in descending limbs from hypertensive rat strains (SHR and Lyon hypertensive) compared with normotensive WKY and Lyon strains.

In summary, there is very limited evidence that ET-1 modulates thin descending limb function and no data that this is related to ECFV status. That some aspect of ET-1 responsiveness in this nephron segment may somehow affect, or be impacted by, hypertension is intriguing but remains speculative.

H. Thick Ascending Limb

1. ET production by the thick ascending limb

A) BASELINE ET PRODUCTION BY THE THICK ASCENDING LIMB: The TAL synthesizes ET peptides. Primary cultures of rabbit medullary TAL (MTAL) released more ET-1 than PT (368, 369). Primary cultures of rat MTAL also produce ET-1 (284). ET-1 mRNA was observed in microdissected rat TAL (97). MTAL can synthesize ET-3, albeit at much lower levels than ET-1 (368). ET-3 mRNA was also detected in microdissected rat MTAL (769). ECE-1 mRNA was observed in microdissected rat cortical and MTAL (155). In contrast, ET-1 mRNA was not detected in microdissected cortical or MTAL (787). ET-1 mRNA was also not found in microdissected rat MTAL, although ET-1 release, albeit quite low, was observed by this same nephron segment (790). Other groups did not detect ET-1 expression in TAL, using immunohistochemistry of rat kidney (828), in situ hybridization in human kidney (598), or in situ hybridization in rat kidney (77). Given that most of these latter negative studies were done using either relatively insensitive or technically demanding techniques, we believe that the bulk of evidence strongly favors the notion that TAL produce ET-1 and possibly, although at much lower levels, ET-3. Such production is smaller than by the CD (see sect. *IVI*), but probably greater than that by the PT.

B) REGULATION OF THICK ASCENDING LIMB ET PRODUCTION: Regulation of TAL ET-1 production has not been extensively studied; however, a relatively recent study has provided a very nice demonstration of how salt intake may be coupled to TAL ET-1 synthesis (Fig. 5) (284). This group made the following observations: 1) high salt intake increased NOS3 expression in rat TAL, 2) high salt intake increased outer medullary osmolality, 3) nonselective ET receptor inhibition prevented high salt intake-induced increases in TAL NOS3, 4) raising media osmolality in primary cultures of rat TAL increased NOS3 expression and ET-1 release, and 5) blockade of ET_B prevented hyperosmolality induction of NOS3 expression. Taken together, these observations indicate that high salt intake increases medullary tonicity, leading to increased TAL ET-1 release. Such ET-1 release can then act in an autocrine manner to stimulate NO production that, in turn, may inhibit TAL NaCl transport (see sect. *IVH3*). Thus ET-1 production by TAL may exert a natriuretic response to high salt intake.

2. ET receptor expression by the thick ascending limb—As described above, there is clear evidence that TAL express functional ET receptors (Fig. 3). However, other modes for detection of TAL ET receptor expression have not yielded consistent results. ET_B, but not ET_A, immunostaining was detected in rat TAL (207). Similarly, ET_B, but not ET_A, mRNA was found in microdissected rat MTAL (768). Several other studies failed to observe evidence for ET receptors in TAL. These include immunofluorescent staining of rat kidney sections (823) and electron microscopic autoradiography of radiolabeled ET-1 binding to rat kidney (148). Very faint ET-1 binding was detected in microdissected rat MTAL, while no

binding was observed in cortical TAL (757). Taken together, it appears that TAL primarily express ET_B, but not ET_A, and ET_B levels are relatively low.

3. ET actions in the TAL

A) ET REGULATION OF THICK ASCENDING LIMB SODIUM CHLORIDE

TRANSPORT: ET-1 inhibits chloride transport by acutely isolated rat cortical TAL (580). This effect occurred when ET-1 was administered on the basolateral side of the tubule, was prevented by ET_B, but not ET_A, blockade, and was mimicked by S6c, an ET_B-specific agonist. *N*^G-nitro-L-arginine methyl ester (L-NAME) prevented ET-1 inhibition of Cl flux, indicating that this effect is mediated by NO. ET-1 also increased [Ca²⁺]_i. In another study, ET-1 inhibited mouse cortical and medullary TAL chloride reabsorption (146). Both luminal and basolateral administration of ET-1 reduced Cl transport; however, a direct comparison of the dose-responsiveness between the two sides of the tubule was not assessed. Blockade of ET_B with BQ788 prevented ET-1 inhibition of Cl flux when both ET-1 and BQ788 were added to either the apical or basolateral side (an interesting experiment would be to see whether BQ788 blocked the effect of ET-1 when added on the opposite side of the tubule to help determine if ET-1 was diffusing between the cells to reach the opposite side). The ET-1 effect was not affected by blockade of prostaglandin production and was not associated with an increase in cAMP content (the failure to see ET-1 modulation of TAL cAMP is supported by studies failing to see an effect of ET-1 on AVP-stimulated cAMP accumulation in microdissected rat cortical or medullary TAL; Ref. 781). PKC blockade prevented ET-1 inhibition of Cl flux, however, surprisingly, and in contrast to the rat studies above, no increase in [Ca²⁺]_i was detected (regardless of the side of the tubule to which ET-1 was added). In a follow-up study by this group, ET-1 unexpectedly reduced luminal atrial natriuretic peptide (ANP) inhibition of mouse cortical and medullary TAL Cl flux (29). Notably, ET-1 decreased ANP-stimulated cGMP accumulation in TAL, an effect that would not be anticipated if ET-1 inhibition of Cl transport in mouse TAL was mediated, at least in part, by NO. The reasons for the different results between the mouse and rat studies are unknown, but could conceivably relate to species-specific differences or differential ET-1 sensitivity. Another possibility is that ET-1-induced signaling, insofar as NOS activation is concerned, could depend on which ET_B are activated. The rat study found that basolateral ET-1 stimulated NOS activation, while the latter mouse study only examined the effect of apical ET-1 administration. Yet another possibility is that ET-1 increases TAL sensitivity to NO (with regard to inhibition of Cl transport) in TAL. Such a scenario is suggested by studies showing that a high-salt diet increased spermine NONOate inhibition of Cl absorption by acutely isolated rat TAL, but did not clearly increase NO production (554). That NO does have a role in mediating, at least partially, ET-1 inhibition of Cl flux in TAL seems very likely in light of several additional pieces of evidence. First, NO has been well shown to inhibit Na-K-2Cl cotransport in TAL (reviewed in Ref. 555). Second, ET-1 increased NOS3 expression and NO production by primary cultures of rat MTAL (283). This NO stimulatory effect of ET-1 was prevented by ET_B blockade and mimicked by ET_B-selective activation with S6c, while wortmannin, a PI3K inhibitor, blocked the ET-1 effect. Third, as described above, L-NAME abolished ET-1 inhibition of Cl transport in acutely isolated rat MTAL (580). Fourth, ET-1 markedly stimulated acutely isolated rat MTAL ET-1 production in wild-type mice, but had no effect on NO levels in MTAL from NOS3 knockout mice (285). This latter study showed that ET-1 induction of NO in MTAL was Akt dependent; in particular, dominant-negative Akt blocked ET-1 stimulated NO production and phosphorylation of NOS3 at Ser¹¹⁷⁷, while NOS blockade or dominant-negative Akt prevented ET-1 inhibition of NKCC2 activity.

Taken together, the considerations above suggest that ET-1 inhibition of TAL transport occurs, at least in part, through NO-, and possibly PKC-, mediated decreases in NKCC2

activity. However, other mechanisms may also be operative. For example, ET-1 and ET-3 can stimulate the MAPK cascade in rat MTAL, effects which may or may not be related to PKC activation and which could conceivably affect transport processes (770). Another intriguing possibility is that *P*-450 arachidonate metabolites, and particularly 20-HETE, may be involved. As discussed earlier, ET-1 stimulates PT 20-HETE formation, which in turn can inhibit Na⁺-K⁺-ATPase activity (185). 20-HETE is produced by the TAL where it can inhibit NKCC2 activity, in part by blocking a 70-pS apical potassium channel as well as inhibiting Na⁺-K⁺-ATPase activity (reviewed in Refs. 664, 879). Studies on 20-HETE regulation of ET-1 actions in the TAL are clearly needed.

I. Collecting Duct

1. ET production by the collecting duct

A) BASELINE COLLECTING DUCT ET PRODUCTION: The CD produces more ET-1 than any other cell type in the kidney and possibly in the entire body (367). In addition, there are regional differences in ET-1 synthesis within the CD. Metabolic labeling, immunohistochemical and radioimmunoassay analysis of ET levels, as well as RT-PCR of mRNA expression in microdissected nephron segments have repeatedly shown that the inner medullary CD (IMCD) produces more ET (primarily ET-1) than any other cell type in the nephron, at least in the rat and rabbit (97, 368, 787, 790). Similarly, porcine IMCD synthesize relatively high levels of ET-1 (25). These findings were further supported by in situ hybridization studies of human kidneys (598); in addition, ET-1 release and mRNA expression have been detected in cultured human IMCD cells (367). The outer medullary CD (OMCD) produces more ET-1 than any other cell type in this region of the kidney (97, 370, 598, 787, 790). OMCDs and IMCDs had greater ECE-1 immunostaining than other nephron segments (599). The cortical CD (CCD) synthesizes about half as much ET-1 as the IMCD, but more than any other cell type in the cortex (about 5 times as much as the PT) (368). In situ RT-PCR localized relatively high ET-1 mRNA to the CCD and OMCD (501). Finally, relatively large signals for ET-3 mRNA were observed in microdissected rat CCD, OMCD, and IMCD (769). In summary, the CD is the predominant nephron site of ET production, with the IMCD being the region producing the greatest amount of these peptides. It is also important to note that no published studies have specifically examined intercalated cell ET-1 production; the majority of studies described in this section focus on the IMCD, a region where only principal cells are located.

The CD, like the rest of the nephron, consists of polarized epithelium. Consequently, studies have examined the polarity of CD ET-1 secretion. Cultured rat IMCD cells predominantly release ET-1 on the basolateral side (372). Similarly, predominant basolateral ET-1 release was observed in a mouse CCT cell line (776) and in MDCK cells (677, 786). Overall, these studies found between 2- and 10-fold greater basolateral than apical ET-1 secretion by CD cells. Since, as will be described below, the majority of CD ET receptors are expressed on the basolateral side, the potential exists for ET-1 to function in an autocrine manner in these cells.

B) REGULATION OF COLLECTING DUCT ET PRODUCTION: The factors that regulate CD ET synthesis have been relatively extensively studied (Fig. 6). Such focus on the CD stems from numerous studies (described in section IV/3), indicating that ET-1 is an important modulator of CD salt and water transport. When considering such regulation, it must be acknowledged that one cannot necessarily generalize findings made in one region to the entire CD. Hence, while the following discussion, for the sake of clarity and organization, will generally look at the CD as a whole, clear demonstration that such regulation is common to all regions of the CD is lacking.

CD ET production appears to be regulated by ECFV status. As discussed earlier, renal ET-1 production is increased during ECFV expansion; it now appears that at least part of such augmented ET-1 production is due to alterations in CD ET-1 synthesis. Na loading increases medullary ET-1 mRNA in rats and raises urinary ET-1 excretion (5). A high-Na diet increases medullary ET-1 mRNA and medullary ECE-1 mRNA and protein (192). More specifically, urinary ET-1 excretion is reduced in mice with CD-specific knockout of the ET-1 gene, while ECFV expansion-associated increases in urinary ET-1 excretion are markedly blunted in these animals (5).

One possibility is that circulating hormones involved in salt and water homeostasis are responsible for ECFV expansion-induced increases in CD ET-1 production. To date, however, there is no compelling evidence for this hypothesis. One might predict that aldosterone, which is increased during ECFV contraction, would decrease renal ET-1 production (to reduce the natriuretic effect of ET-1). However, aldosterone rapidly (within 1 h) increases renal ET-1 mRNA in adrenalectomized rats (832). Gumz et al. (257) observed an increase in ET-1 gene expression after a 1-h exposure of mouse mIMCD cells to aldosterone. In addition, medullary ET-1 content and IMCD ET-1 levels are elevated in the rat DOCA/salt model (307). The aldosterone-stimulated increase in ET-1 synthesis may be mediated by Sgk1 via a pathway that involves derepression of ET-1 gene expression through inhibition of Dot1a-Af9-induced histone hypomethylation (877). Thus aldosterone induction of CD ET-1 may provide negative feedback for the Na-retaining effects of the steroid or may even be involved in aldosterone's extracellular matrix-stimulating effects (597), but clearly does not explain how ECFV expansion stimulates CD ET-1 production.

Other hormones involved in volume and BP homeostasis do not affect CD ET-1 synthesis. AVP does not alter ET-1 production by mouse IMCD cells lines (777), primary rat IMCD cultures (370), or cultured porcine IMCD cells (489). As mentioned above, AVP, together with hypertonic NaCl, but neither factor alone, stimulates IMCD cell line ET-1 release (776); the meaning of this observation remains speculative. Epinephrine did not change rat IMCD cell ET-1 release (370). ANP, cGMP, or NO very modestly reduced cultured rat IMCD ET-1 release (373), while ANP did not change ET-1 synthesis by cultured porcine IMCD cells (489). Bradykinin also did not affect ET-1 release by porcine IMCD cells (489). In summary, there is no clear evidence that hormones mediate ECFV-induced changes in CD ET-1 production.

If hormones are not the link between ECFV and CD ET-1 production, then local renal factors may be involved. A high Na diet is associated with increased outer medullary osmolality (284), raising the possibility that osmolality could be a signal for changes in ECFV. Acute renal intramedullary infusion of hypertonic, but not isotonic, NaCl or mannitol into rats increased urinary ET-1 excretion (59), suggesting that renal ET-1 production could be stimulated by increased medullary tonicity. A high-Na diet increases renal medullary ECE-1 expression (192). Furthermore, as discussed above, hyperosmolar (as compared with normal plasma osmolality) media stimulated TAL ET-1 production (284). With regard to the distal nephron, Kramer et al. (401) reported that media made hypertonic with betaine or urea increased ET-1 synthesis by MDCK cells, a canine distal nephron-like cell line. They also noted that this effect was dependent on p38 MAPK and ERK activation. An interesting aspect of these studies was that hypertonic urea increased ET-1 synthesis; since urea can readily enter cells, the stimulatory effect could not be ascribed to media hypertonicity (i.e., an effect of relatively impermeant solutes). Similarly, Migas et al. (489) noted that media made hyperosmolar with NaCl and/or urea increased ET-1 release by cultured porcine IMCD cells. Yang et al. (858) found that hypertonic NaCl increased ET-1 accumulation in rat and rabbit IMCD suspensions and raised ET-1 mRNA content in microdissected rat IMCD. In contrast to the above studies, hypertonic urea decreased ET-1 accumulation by the

tubule suspensions. Furthermore, hypertonic mannitol had no effect on ET-1 protein or mRNA levels. Thus there is disagreement about which solutes regulate CD ET-1 production as well as the nature of their effects. The only common theme from these studies seems to be that hypertonic NaCl increases CD ET-1 production. It also seems likely that such hypertonic NaCl stimulation of CD ET-1 production is under complex regulation; one group found that short-term (up to 6 h) hypertonic NaCl stimulated CD cell line (mouse M-1 and mIMCD-K2) ET-1 synthesis only in the presence of AVP (777). Unfortunately, even NaCl-stimulated CD ET-1 production is controversial. Schramek et al. (677) observed that media made hyperosmolar with NaCl or raffinose decreased ET-1 secretion by MDCK cells; to make matters even more confusing, they found that hyperosmolar urea increased ET-1 release. Similarly, Todd-Turla et al. (777), the same group that saw short-term stimulation of ET-1 release by hypertonic NaCl, noted that longer term (24 h) exposure to hypertonic NaCl or mannitol reduced M-1 cell ET-1 secretion. Finally, Kohan et al. (374) observed that increasing media tonicity with NaCl or mannitol, but not urea, caused a dose- and time-dependent decrease in cultured rat IMCD cell ET-1 release and mRNA levels, while these conditions had no effect on mesangial cell ET-1 production (done as a control). How do we reconcile these disparate findings? Unfortunately, there are not obvious differences in study design or cell models that permit such determination. Virtually all of the studies were done in vitro, so it is possible that the process of cell isolation and/or culture affects the outcome. In addition, as yet undefined physiological conditions may be lacking in vitro that allow detection of the true effect of osmolality on CD ET-1 production, including circulating plasma hormones, paracrine substances released by neighboring cells, or even physical factors. Thus, if and how osmolality regulates CD ET-1 production remains very much an open question.

Another local factor that might regulate CD ET-1 production is tubule fluid flow. This intriguing possibility is suggested by the findings that salt, salt plus water, or water loading alone increases urinary ET-1 excretion in humans and experimental animals (112, 127, 313, 457, 473, 474, 494, 495, 657, 761, 838, 870). Since physiological urinary ET-1 excretion reflects, at least in large part, CD ET-1 production (3, 5), some factor common to salt and/or water loading could be involved in augmenting CD ET-1 synthesis. One such factor is CD luminal fluid flow; this is increased during both salt and water expansion. While no studies have shown that increased tubule fluid flow, via shear stress or some other mechanism, augments CD ET-1 production, there is evidence from other systems to suggest that this possibility exists. For example, shear stress can increase ET-1 release by endothelial or mesothelial cells (133, 139, 500, 808). Furthermore, CD cells can sense alterations in flow; for example, mouse CCD change K secretion in response to varying flow (762, 839), while tubule fluid flow increases epithelial Na channel (ENaC) open channel probability (P_o) in rabbit CCD (504). Furthermore, CD primary cilia can mediate luminal fluid flow-induced changes in cell signaling (306, 443, 591, 592, 708). Studies are needed to determine if and how tubule luminal fluid flow regulates CD ET-1 production.

A number of factors can modify CD ET-1 synthesis; however, these are not obviously associated with salt balance. These regulators include inhibitors of cultured rat IMCD ET-1 release, such as pH (lower pH inhibits) (731), interferon- γ (373), and ATP via P2Y receptors (312). Similarly, a variety of other factors can induce CD ET-1 production, including hypoxia (in cultured rat IMCD cells) (491), IL-1 β partly via NF- κ B (in mouse IMCD-3 cells), and TGF- β (in several CD cell preparations) (301, 489, 675).

The intracellular signaling pathways through which CD ET-1 synthesis is regulated are just beginning to be elucidated. One pathway that has emerged as being of potential importance relates to $[Ca^{2+}]_i$. The Ca^{2+} ionophore A23187 increased ET-1 production by porcine IMCD cells (489). Cultured rat IMCD ET-1 protein and mRNA content were greatly decreased

after chelation of intracellular Ca^{2+} or inhibition of calmodulin (CaM) or CaMKII (729). CaM inhibition did not alter ET-1 mRNA stability. Transfection with rat ET-1 promoter-luciferase constructs showed maximal activity within 1.9 kb 5' to the transcription start site with only one-third of this activity in the region 0.56 kb 5' to the transcription start site. CaM inhibition markedly reduced activity of the 1.9-kb, but not 0.56-kb, promoter regions. Interestingly, CaM inhibition did not affect ET-1 release by rat aortic endothelial cells, nor did it alter activity of transfected ET-1 promoter regions. These findings indicate that IMCD ET-1 synthesis is regulated by Ca^{2+} /CaM/CaMKII-dependent pathways that are not universally active in all cell types.

CD ET-1 production may be altered in hypertension. Hughes et al. (310) reported that acutely isolated or cultured IMCD cells obtained from 8-wk-old hypertensive SHR rats released less ET-1 than did IMCD cells from normotensive WKY animals. In addition, SHR rats had less ET-1 in the outer and inner medulla compared with WKY rats. Similar reductions in papillary and/or medullary ET-1 content in SHR rats were made by others (243, 365). In addition, Prague hypertensive rats have less ET-1 protein and mRNA in the renal papilla, but not in the rest of the medulla or in the cortex, compared with normotensive controls (803). Medullary ET-1 levels are also reduced in Dahl S. It is interesting to note that, in albeit limited studies, patients with essential hypertension (especially those with salt-sensitive hypertension) have decreased urinary ET-1 excretion (298, 313, 882). Taken together, these studies suggest that renal medullary, and in particular CD, ET-1 production may be reduced in the setting of genetic hypertension. As will be discussed, such a decrease in CD ET-1 production may contribute to renal Na retention and elevated BP.

2. ET receptor expression by the collecting duct

A) BASELINE COLLECTING DUCT ET RECEPTOR EXPRESSION: The CD is the major renal site of ET receptor expression (Figs. 3 and 6). Initial studies, using ET binding, identified ET receptor expression in the CD. Autoradiographic studies found predominant ET-1 binding to renal medulla in rat, pig, and human (143, 805, 828). Using electron microscopic autoradiography, the high medullary binding in rats was localized, at least in part, to CDs (866). More detailed binding studies, using microdissected rat nephron segments, demonstrated the highest ET-1 and ET-3 binding to IMCD, moderate binding to OMCD and CCD, and relatively little binding to more proximal nephron segments (757). Cultured rat IMCD cells bound ET-1 with a B_{max} of 206 fmol/mg and a K_d of 218 pM, i.e., ET receptors were present with high affinity and in relatively high density (371).

ET_B is the predominant ET receptor isoform expressed by the renal medulla in general, and the CD in particular. Porcine renal papillary membranes contained a single class of receptors with a K_d of 137 pM; ET-1 binding was unaffected by BQ123, but completely blocked by bosentan (combined $\text{ET}_{A/B}$ blocker), suggesting that only ET_B was present (25). Binding studies in microdissected rat CCD showed a single class of receptors with similar ET-1 and ET-1 binding, while specific ET_B , but not ET_A , antagonism blocked ET-1 binding (757). Autoradiographic studies in human kidney, using specific competitors for the ET receptor isoforms, revealed predominant ET_B expression in the collecting system (349). A strong signal for ET_B mRNA was observed in microdissected rat IMCD, with relatively moderate signals in the OMCD and CCD (768). In situ RT-PCR of rat renal medulla detected ET_B mRNA in the OMCD and IMCD (107). Immunofluorescence of rat kidney revealed ET_B expression in the IMCD (823), while similar studies in human kidney showed relatively intense CD ET_B localization (404). ET binding analysis and PCR of cultured rat IMCD showed the presence of ET_B (371). Finally, as will be described below, numerous studies indicate the presence of functional ET_B in the CD. Taken together, these studies demonstrate that ET_B is strongly expressed by the CD, with the greatest levels in the IMCD.

In contrast to ET_B , ET_A expression by the CD has been more difficult to demonstrate. Autoradiography studies using whole kidneys or RT-PCR of microdissected nephron segments detected no ET_A message in rat or human CD (107, 348, 757, 768). However, acutely isolated canine IMCD bound ET peptides in a pattern consistent with ET_A binding (91), while immunostaining of rat kidney revealed CD ET_A expression (547). Binding assays of suspensions of rat and rabbit IMCD revealed ET_A (164), and autoradiography of rat renal medulla found that ET_A antagonism reduced ET-1 binding (866). Cultured rat IMCD ET binding indicated the presence of two types of ET receptors, one of which had a characteristic ET_A binding pattern; in addition, ET_A mRNA was detected (371). More recent studies, using immunostaining, detected ET_A in the rat CCD (823). In addition, using a combination of light and electron microscopy, Yamamoto et al. (852) most recently demonstrated ET_A immunoreactivity in the basal infoldings of the CD, with the strongest staining in the IMCD. Most importantly, evidence exists for functional CD ET_A (see below). Thus, while CD ET_A expression is substantially less than ET_B expression, the bulk of evidence supports the presence of ET_A in this nephron segment.

As described above, CD cells have preferential basolateral ET-1 secretion (372, 677, 776, 786). It is, therefore, relevant to consider the sidedness of ET receptor expression. While this has not been examined in detail, Kohan et al. (372) found that cultured rat IMCD cells bind ET-1 predominantly on the basolateral side. Thus it seems likely that ET peptides can exert an autocrine effect on CD tubule function. As will be described below, this does indeed appear to be the case.

B) REGULATION OF COLLECTING DUCT ET RECEPTORS: CD ET receptor expression can be modified, although there is limited information on this topic (Fig. 6). Exposure of microdissected rat CCD to AVP, forskolin, or dibutyryl cAMP for 20 min decreased ET-1 binding affinity (756). The inhibitory effect of AVP was abolished by PKA blockade; this finding is interesting in that ET_B , but not ET_A , contains a consensus amino acid sequence for PKA-mediated phosphorylation (756). Overnight incubation of rat IMCD cell suspensions with AVP caused a cAMP-dependent reduction in the B_{max} for ET-1 binding; this was associated with a decrease in ET_B mRNA (831). Thus AVP may cause an acute decrease in ET_B affinity and a long-term decrease in ET_B number. ANG II may also regulate IMCD ET receptors: overnight exposure to ANG II down-regulated ET_A and ET_B binding and mRNA content in rat IMCD cells via a PKC-mediated pathway (830). These latter findings are interesting in light of previous studies by this group showing that hamster IMCD ET_B expression is decreased in a cardiomyopathic model and that this downregulation of ET_B is prevented by angiotensin receptor blockade (833). Similarly, CD ET_B is markedly decreased in rats with decompensated congestive heart failure (207). Taken together, the above observations raise the possibility that AVP and ANG II, which stimulate nephron Na reabsorption, reduce IMCD ET receptor expression to limit the opposing natriuretic effects of the ET system.

3. ET actions in the collecting duct

A) OVERVIEW OF ET REGULATION OF COLLECTING DUCT SODIUM

TRANSPORT: Shortly after the discovery of ET-1, the peptide was found to inhibit Na^+ - K^+ -ATPase activity in suspensions of rabbit IMCD (869). ET-1 decreases Na reabsorption in isolated rat CCD (780), while it reduces Na uptake by amphibian distal nephron A6 cells through an increase in mean Na channel closed time (217). ET-1 also reduces Na transport by isolated perfused rabbit CCD (409). ET-1 reduces amiloride-sensitive currents in NIH 3T3 cells stably expressing all three ENaC subunits (242). Recently, Bugaj et al. (78) found that ET-1 decreases ENaC P_o in isolated split-open rat CCD. Direct physiological evidence for the importance of the CD ET system in regulating Na excretion and BP was obtained

from studies in which the ET-1 gene was disrupted specifically within principal cells of the CD. These mice, termed CD ET-1 knockout (CD ET-1 KO), had no CD ET-1 mRNA and had reduced urinary ET-1 excretion (5). CD ET-1 KO mice on a normal-Na diet were hypertensive (systolic BP ~15 mmHg greater than controls), while a high-Na diet worsened hypertension (systolic BP ~35 mmHg greater than controls) and caused excessive weight gain. Both acute and chronic Na loading were associated with impaired ability to excrete a Na load by CD ET-1 KO animals. Amiloride reduced BP in CD ET-1 KO mice on a normal- or high-Na diet and prevented excessive Na retention, suggesting that CD Na channels may be involved. Finally, graded increases in renal perfusion pressure were associated with reduced Na excretion in CD ET-1 KO mice (672). Taken together with the in vitro studies, the CD ET-1 KO mice provide compelling evidence that the CD ET system is an important physiological inhibitor of CD Na reabsorption, thereby exerting a potent antihypertensive effect.

B) ET RECEPTOR ISOFORMS MEDIATING COLLECTING DUCT SODIUM

TRANSPORT: Most studies to date have primarily implicated ET_B in mediating ET-1 inhibition of CD Na transport (Fig. 6). Indirect evidence supporting a role for CD ET_B in eliciting natriuresis comes from studies involving direct renal medullary infusion. Intramedullary infusion of A192621, an ET_B antagonist, reduced the natriuretic response to volume expansion in rats without detectable changes in renal or medullary blood flow (259). Similarly, intramedullary infusion of S6c, an ET_B-specific agonist, increased Na excretion in rats; this effect was absent in ET_B-deficient rats (528). This latter model is based on the spotting lethal (*sl/sl*) rat that has a naturally occurring null mutation in ET_B (227). These animals, when rescued from lethal intestinal aganglionosis by directed ET_B transgene expression in the enteric nervous system (using a dopamine β-hydroxylase promoter-ET_B transgene), are highly sensitive to DOCA- and salt-induced hypertension (225, 227). In particular, the hypertension in these rats is partially corrected by amiloride, suggesting a role for CD ET_B inhibition of ENaC. In vitro studies also point to CD ET_B inhibition of ENaC. In A6 cells, basolaterally administered picomolar concentrations of ET-1 inhibit Na channel activity; this effect is mimicked by ET_B agonism and is blocked by ET_B antagonism (217). In agreement, basolaterally administered ET_B, but not ET_A, blockers abolish the inhibitory effect of ET-1 on ENaC P_o in rat CCD (78). The most direct evidence for a role of CD ET_B in modulating BP and Na excretion derives from studies using mice with principal cell-specific knockout of the ET_B gene (CD ET_B KO) (233). CD ET_B KO mice had elevated systolic BP (~8 mmHg greater than controls) on a normal-Na diet, which further increased during high salt intake (systolic BP ~20 mmHg greater than controls). CD ET_B KO mice also had reduced Na excretion after acute Na loading compared with control animals. These studies provide definite evidence of a role for CD ET_B in facilitating renal Na excretion and maintaining normal BP. However, the magnitude of BP elevation in CD ET_B KO mice was about one-half of that observed in CD ET-1 KO mice. This finding suggests that CD-derived ET-1 exerts antihypertensive and natriuretic effects that are partly dependent on autocrine activation of ET_B, but also involve activation of ET_A and/or paracrine regulation of neighboring cell function.

There is limited evidence for ET_A exerting a natriuretic effect. In the presence of an ET_B antagonist, nano-molar concentrations of ET-1 increased Na channel activity in A6 cells (217). Nakano and Pollock (527) made the intriguing observation that intramedullary infusion of ET-1 increased urinary Na excretion in female, but not male, *sl/sl* rats and that this effect was prevented by ABT-627, an ET_A antagonist. Notably, ET-1 reduced medullary blood flow in male, but not female, *sl/sl* rats, suggesting that the failure of males to manifest a natriuretic response to intramedullary ET-1 infusion was due to decreased medullary blood flow. No gender-related differences in BP or Na excretion were observed in any of the CD-specific knockout models, so the meaning of these findings remains to be fully determined.

More direct examination of the role of CD ET_A was obtained from studies involving principal cell-specific disruption of the ET_A gene (CD ET_A KO) (236). CD ET_A KO mice had no differences in BP or urinary Na excretion, compared with controls, whether given a normal- or high-Na diet. This suggested that CD ET_A are not involved in the physiological regulation of BP or renal Na excretion.

Taken together, the CD ET-1 KO, CD ET_A KO, and ET_B KO studies indicated that collecting-derived ET-1 exerts natriuretic and antihypertensive effects partly through autocrine activation of ET_B and partly through paracrine actions. To confirm this, mice were generated with principal cell-specific disruption of both the ET_A and ET_B genes (CD ET_{A/B} KO) (672). It was expected that the CD ET_B KO and CD ET_{A/B} KO would have similar BP and Na excretory phenotypes since the CD ET_A KO mice manifested no changes in BP or renal Na handling; however, this was not the case. On a normal-Na diet, CD ET_{A/B} KO mice had increased BP, which was exacerbated by high salt intake; the magnitude of BP elevation on either diet was substantially higher than in CD ET_B KO animals. On a normal-Na diet, systolic BP was similar between CD ET-1 KO and CD ET_{A/B} KO mice. During the first 4 days of Na loading, CD ET_{A/B} KO mice had lower systolic BP than CD ET-1 KO animals, but after 5 days of Na loading, BP was similar between these two knockout lines. In addition, CD ET_{A/B} KO mice had reduced Na excretion after either acute or chronic Na loading compared with controls; the degree of Na retention was greater in the double ET receptor KO mice compared with CD ET_B KO mice and was similar to that observed in CD ET-1 KO mice. How can these findings with CD ET_{A/B} mice be explained in light of the prior observations in CD ET_A KO or CD ET_B KO mice? One possibility is that, because ET_B predominate in the CD, deficiency of ET_A causes no apparent Na excretory phenotype. However, when ET_B are absent, ET_A, which could be natriuretic, may assume a more significant role than they normally provide. Such adaptation may be due to upregulation of ET_A number or enhanced receptor sensitivity, although these possibilities have not been investigated. Another possibility involves ET receptor dimerization. As described earlier in this review, ET receptors are capable of homo- and heterodimerization, potentially leading to functional differences depending on which dimer pairs predominate. While entirely speculative, since ET_B are most abundant in the CD, then ET_B homodimers and ET_A/ET_B heterodimers may predominate. In the CD ET_B KO model, ET_A homodimers, which may not normally exist in sufficient amounts, might potentially exert a natriuretic effect. This area of investigation, while having substantial technical challenges, is one in need of further investigation.

C) MECHANISM OF ET REGULATION OF COLLECTING DUCT SODIUM

TRANSPORT: ET-1 regulates CD Na transport through a variety of signaling pathways (Fig. 6). ET-1 inhibition of isolated rat CCD AVP-stimulated chloride transport was found to be dependent on PKC activation (780); however, PKC or PLC blockade did not affect ET-1 inhibition of ENaC activity in isolated rat CCD (78). In NIH 3T3 cells stably transfected with all ENaC isoforms, ET-1 reduced Na channel activity through a Src kinase-dependent pathway, although Src kinases did not directly phosphorylate any of the ENaC subunits (242). In the split-open rat CCD preparation, ET-1, via ET_B, rapidly (within 5 min) inhibited ENaC P_o and activated Src kinase and MAPK1/2; blockade of these kinases prevented ET-1 regulation of the Na channel (78). ET-1 has also been shown to stimulate MAPK activity in rat OMCD and IMCD (770). Thus ET-1 inhibition of CD ENaC is likely mediated, at least in part, by ET_B stimulation of Src kinase, leading to MAPK activation. Notably, phosphorylation of ENaC in response to MAPK signaling decreases both ENaC P_o and channel number (N) (63, 104, 191, 688).

NO is also likely to be involved in mediating ET-1 inhibition of CD Na reabsorption. CD NO production is stimulated by ET. Taylor et al. (763) found indirect evidence for this based

on the observation that acutely isolated IMCD from *sl/sl* (ET_B-deficient) rats had reduced NOS activity compared with heterozygous (*sl/wild-type*) animals (763). Subsequent studies found that ET-1, through ET_B activation, stimulates NOS1-dependent NO production and cGMP accumulation in cultured rat IMCD cells (730). ET-1 increased NOS1 protein expression in IMCD3 cells, an effect that may be ET_A-dependent, at least at the high concentration (50 nM) of ET-1 tested (736). ET_A or ET_B antagonism decreased NOS3 mRNA and protein expression in cultured rat IMCD cells (861). CD ET-1 KO mice had reduced urinary nitrate/nitrite excretion on normal or high Na intakes compared with control animals (672). Furthermore, graded increases in renal perfusion pressure were associated with reduced NO metabolite excretion in CD ET-1 KO mice. In this same study, inner medullary NOS1 and NOS3 activities were lower in CD ET-1 KO than in control mice given normal- or high-Na diets (although NOS1 or NOS3 protein expression was not different). ET-1-induced CD NO production could lead to inhibition of Na transport. As discussed earlier, ET-1 inhibition of Cl flux in TAL is NO dependent (580). NO has been shown to reduce Na flux in CCD (555), although this has not been consistently demonstrated (294). NO reduces ENaC P_o in A6 cells and M1 mouse CCD cells (276). Intramedullary infusion of S6c, an ET_B agonist, elicited a natriuresis that was inhibited by blockade of NOS1 or protein kinase G (528). The most direct evidence for a role of NO in mediating the physiological effects of CD-derived ET-1 come from the CD ET-1 KO model. Generalized NOS inhibition markedly increased BP in control mice but caused a much smaller increase in BP in CD ET-1 KO mice (essentially abolishing the difference in BP between control and knockout mice), suggesting that NO deficiency was at least partly responsible for the hypertension in CD ET-1 KO animals (672). Taken together, the above studies provide compelling evidence that NO is an important mediator of ET-1 inhibition of CD Na transport. The mechanisms by which NO exerts this effect, including changes in ENaC P_o and N , require further investigation.

ET-1 also stimulates accumulation of COX metabolites in the CD. As mentioned earlier, initial studies found that ET-1 inhibited Na⁺-K⁺-ATPase activity in rabbit IMCD suspensions (869). These authors found that COX blockade prevented the inhibitory effect of ET-1. In addition, ET-1 increases rabbit and mouse IMCD PGE₂ production, an effect that is mediated by activation of ET_B and is COX2 dependent (375, 869). However, global COX or COX2-specific inhibition did not affect Na retention or BP in CD ET-1 KO mice, while IMCD PGE₂ levels were actually elevated (235). Thus there is not convincing evidence that COX-dependent pathways substantially mediate the natriuretic effects of ET in the CD. Finally, ET-1 does not alter basal or ANP-induced cGMP accumulation, indicating that ANP-related pathways operate separately from those of ET (91).

D) PARACRINE EFFECTS OF COLLECTING DUCT-DERIVED ET: It is conceivable that ET-1 secreted by any renal cell type could affect neighboring cell function. However, no studies have clearly identified such paracrine action. Because the CD releases very high amounts of ET-1, it stands to reason that paracrine effects might be most evident for ET-1 derived from this nephron segment. Obvious paracrine effects include regulation of medullary blood flow or inhibition of Na transport by other nephron segments; however, these have not been clearly demonstrated. One potential and unanticipated paracrine effect of CD-derived ET-1 relates to the renin-angiotensin-aldosterone axis. An interesting observation in the CD ET-1 KO mice was that, despite their being hypertensive and retaining Na, plasma renin activity and aldosterone levels were not different compared with control mice (5). This was examined in more detail in a follow-up study (234). Renal renin content and plasma renin concentration were similar in CD ET-1 KO and control mice during normal Na intake, while a high Na intake suppressed renin levels to a similar degree in both groups of mice. Administration of an angiotensin receptor blocker normalized BP in CD ET-1 KO mice during normal Na intake and partly corrected the hypertension during

high Na intake. Spironolactone, an aldosterone antagonist, had similar effects on BP in CD ET-1 KO animals. These findings suggested that the hypertension in CD ET-1 KO is partly due to failure to suppress renin production. While the reasons for this are speculative, it is pertinent to note that ET-1 potently inhibits renal renin production (4, 177, 411, 441, 496, 518, 619, 620, 655, 676, 679, 746). Given that the CCD and connecting segment are anatomically adjacent to the juxtaglomerular apparatus and afferent arteriole (616, 802), one might envision how CD-derived ET-1 could directly influence renal renin release. Clearly, definitive proof of such a novel intrarenal regulatory system is lacking and will be challenging to obtain; however, the concept of CD feedback is highly intriguing and merits further investigation.

E) ET REGULATION OF COLLECTING DUCT WATER TRANSPORT: ET also inhibits water reabsorption by the CD. As discussed earlier, ET-1 or ET-3 increases urinary water excretion in the absence of obvious renal hemodynamic changes (248, 634, 674) or even at doses that decreased RBF (344). In vitro studies have consistently demonstrated that ET-1 inhibits AVP-stimulated osmotic water permeability in rat CCD and IMCD (163, 517, 544, 780). ET-1 inhibition of water transport is dose-dependent, unlike its biphasic affect on PT fluid reabsorption (780). CD ET-1 KO mice have impaired ability to excrete an acute water load, while 1-desamino-8-D-arginine AVP infusion increased urine osmolality, aquaporin 2 (AQP2) mRNA, and AQP2 phosphorylation to a greater extent in CD ET-1 KO compared with control mice (232). In addition, plasma AVP levels were reduced in CD ET-1 KO mice. These data indicated that CD ET-1 KO increases renal sensitivity to the urinary concentrating effects of AVP and suggest that ET-1 functions as a physiological autocrine regulator of AVP action in the CD. Finally, ET-1 does not globally inhibit AVP action in that ET-1 does not reduce AVP-stimulated CD urea permeability (544, 782).

F) MECHANISM OF ET INHIBITION OF COLLECTING DUCT WATER TRANSPORT: ET reduces AVP-stimulated water transport in the CD, at least in part, through inhibition of adenylyl cyclase-dependent cAMP accumulation (Fig. 6). This ET effect has been demonstrated in rat CCD, OMCD, and IMCD as well as in porcine IMCD and is independent of phosphodiesterase activity (163, 375, 489, 517, 544, 757, 780, 781). That blockade of cAMP formation is critical to the inhibitory effect of ET on water transport was shown by studies demonstrating that ET-1 does not reduce dibutyryl cAMP-stimulated osmotic water permeability in the IMCD (544). CD suspensions from CD ET-1 KO mice had enhanced AVP- and forskolin-stimulated cAMP accumulation, suggesting that deficiency of endogenous ET-1 augmented adenylyl cyclase activity (232). The increased adenylyl cyclase activity in CD ET-1 KO mice was associated with increased levels of adenylyl cyclases 5 and/or 6, suggesting that ET-1 may have long-term effects on adenylyl cyclase content in addition to short-term actions on activity (728). Curiously, exposure of rat IMCD to ET-1 increased AVP V2 receptor mRNA and protein; however, since AVP-induced cAMP responsiveness was not assessed in these preparations, it is not possible to know whether this was a primary or compensatory effect (716). Notably, anti-ET-1 antibodies increased AVP-stimulated cAMP accumulation by IMCD cells, supporting the notion that ET-1 can function as autocrine inhibitor of AVP action (372).

ET-1 acts through an inhibitory G protein since pertussis toxin blocks the effects of ET-1 on AVP-stimulated cAMP accumulation and water flux in the IMCD (375, 517). ET-1-induced decreases in AVP-stimulated osmotic water permeability and cAMP content in rat CD are mediated by PKC (375, 517, 781, 782, 835). Relevant to PKC activation, ET peptides have been shown to stimulate accumulation of inositol phosphates in rat IMCD (834). Such accumulation of inositol phosphates and PKC activation may be related, at least in part, to ET-stimulated increases in $[Ca^{2+}]_i$. In acutely isolated superfused mouse CCT or cultured porcine IMCD cells, ET-1 elicited an initial rapid rise, followed by a sustained elevation, in

$[Ca^{2+}]_i$ (489, 532). In the isolated CCD studies, elimination of extracellular Ca^{2+} reduced the initial $[Ca^{2+}]_i$ transient and almost abolished the sustained increase, suggesting a major dependence on extracellular Ca^{2+} entry (532). Inhibition of dihydropyridine-sensitive Ca^{2+} channels did not affect ET-1-induced changes in $[Ca^{2+}]_i$. Similar $[Ca^{2+}]_i$ responses to ET-1, as well as dependence on extracellular Ca^{2+} entry, were observed by this same group using isolated perfused rabbit CCD (409). However, in these latter studies, Ca^{2+} influx was found to depend on dihydropyridine-sensitive Ca^{2+} channels. Korbmacher et al. (396), using mouse M-1 CCD cells, found that the initial ET-1-induced $[Ca^{2+}]_i$ increase was independent of extra-cellular Ca^{2+} , while the sustained elevation of $[Ca^{2+}]_i$ required extracellular Ca^{2+} and was abolished by nifedipine (396). Nadler et al. (517) found that ET-1 caused a transient increase in perfused IMCD $[Ca^{2+}]_i$ that did not require extracellular Ca^{2+} . Taken together, these studies suggest that ET-1 causes a biphasic increase in $[Ca^{2+}]_i$ wherein the initial transient peak is primarily caused by release of Ca^{2+} from cell stores, while the second sustained rise is primarily due to Ca^{2+} entry via dihydropyridine-sensitive channels. The ET-1-stimulated dihydropyridine-sensitive component of Ca^{2+} entry may not be involved in regulation of adenylyl cyclase activity since nicardipine did not alter AVP-induced cAMP accumulation in rat CCD, OMCD, or IMCD (781, 782). However, increased $[Ca^{2+}]_i$ has been shown to inhibit AVP-stimulated adenylyl cyclase activity in rat IMCD through PLC-mediated activation of PKC (765), raising the possibility that the transient increase in $[Ca^{2+}]_i$ may be involved in ET-1 inhibition of CD water transport.

Prostaglandins and NO could potentially be involved in ET regulation of renal water transport because, as described earlier, ET-1 stimulates production of these mediators by the CD. However, COX blockade has no effect on ET-1 inhibition of AVP-stimulated cAMP accumulation in cultured rat IMCD or acutely isolated rat CCD, OMCD, or IMCD (375, 781, 782). NO can inhibit AVP-stimulated water permeability and cAMP accumulation in the rat CCD (219); however, NOS blockade had no effect on ET-1 inhibition of AVP-stimulated cAMP content in IMCD suspensions (730). Notably, NO donors, L-arginine, NADPH, or tetrahydrobiopterin did not alter AVP-stimulated cAMP accumulation in the IMCD cell suspensions. It is possible that the role of NO in mediating ET-1 inhibition of adenylyl cyclase activity is different between the CCD and IMCD, but given that no differences have been seen between these different regions of the CD with regard to other aspects of ET-1 regulation of water transport, it seems likely that neither COX metabolites nor NO play a significant role.

G) ET RECEPTOR ISOFORMS MEDIATING COLLECTING DUCT WATER

TRANSPORT: ET_B likely mediates the predominant diuretic effects of ET-1 in the CD. ET-1, ET-3, and S6c were equipotent in inhibiting AVP-induced cAMP accumulation in rat IMCD, while BQ123, an ET_A -specific antagonist, did not alter the degree of inhibition (163, 375, 835). That ET_B is the main inhibitor of CD water transport is supported by findings in CD ET_A KO mice. These knockout mice have an increased plasma AVP levels and an enhanced ability to excrete a water load, while IMCD suspensions from CD ET_A KO mice have reduced AVP- and forskolin-stimulated cAMP accumulation (236). These data suggest that CD ET_A may actually exert an antidiuretic effect; however, caution must be used before making this conclusion. Similar studies are needed in the CD ET_B KO and CD $ET_{A/B}$ KO animals since, as demonstrated for ET regulation of Na transport, the full picture of ET receptor action in the CD cannot necessarily be inferred from findings from a single CD ET receptor knockout line. Finally, CD ET receptors may, in turn, be regulated by AVP. AVP decreased ET-1 binding to rat CCD (by reducing K_d of ET_B) through a PKA-dependent pathway (756). Thus ET-1 inhibition of AVP action may be limited, at least partly, by AVP downregulation of ET-1 activity.

V. ENDOTHELIN AND HUMORAL SYSTEMS

This section focuses on ET interaction with humoral systems whose major purpose is the regulation of BP and/or ECFV. These include aldosterone, ANG II, adrenal-derived catecholamines, AVP, and natriuretic peptides. While other hormones, such as corticosteroids, thyroid, and others can impact BP, particularly when their production is abnormal, they are not primarily involved in physiological modulation and will not be discussed herein. The ensuing discussion will primarily focus on ET regulation of humoral factor production. ET modification of hormonal actions can also be found in sections addressing ET actions within specific tissues.

A. Adrenal Cortex: Aldosterone

1. ET production by the zona glomerulosa—ET-1 production by the adrenal gland has been demonstrated in animals and humans. ET-1 mRNA is present in rat (290, 663) and human (318, 626, 627) adrenal gland. ECE-1 mRNA was also found in human and bovine adrenal gland (629), while ECE immunostaining was observed in rat adrenal gland (750). Immunoreactive ET-1 is present in the human adrenal gland (874).

The zona glomerulosa is a source of adrenal ET-1. Cultured human adrenal cells enriched for glomerulosa cells, as well as a human adrenocortical cell line (H295R), contain ET-1 and ECE-1 mRNA (465). Immunostaining studies detected ET-1 and Big ET-1 in human zona glomerulosa (292). The ET-1 was initially localized to cytoplasmic vacuoles as well as grains within the cytoplasm of human zona glomerulosa cells (438), and in subsequent studies was found in vacuoles, mitochondria, rough endoplasmic reticulum, and plasma membranes (439).

The factors regulating adrenal ET-1 production are not well understood. ET-1 was shown to autoinduce its secretion in rat zona glomerulosa cells, an effect that was due to ET_B activation (46). In perfused rat adrenal gland (the precise source of ET-1 within the adrenal was not determined), ACTH increased adrenal vein ET-1 content (290); this effect has been ascribed to ACTH-induced increases in adrenal blood flow (81). In the same experimental model, NOS inhibition increased, while L-arginine decreased, ET-1 release through an apparently cGMP-independent mechanism (288).

Taken together, the above data indicate that adrenal zona glomerulosa cells produce ET-1, however, the conditions under which its production are modified are poorly understood. Surprisingly, the effect of obvious regulators on aldosterone production, such as serum potassium concentration or circulating ANG II levels, on adrenal ET-1 production is unknown. Despite this, it is interesting to note that clinical trials in heart failure (RALES and EPHESUS) showed that aldosterone secretion persists despite blockade of the renin-angiotensin system; while speculative, it has been proposed that adrenal-derived ET-1, independent of the renin-angiotensin system, may be involved (628). Clearly, further studies are needed to address this important issue.

2. ET receptors in the zona glomerulosa—ET receptors have been localized to the adrenal gland in general and the zona glomerulosa in particular. Early studies noted ET-1 binding in rat (398, 535, 723) and human (318) adrenal glands. ET-1 binds to rat zona glomerulosa (384) and to a human aldosterone-producing adenoma (874); the latter study found a single class of binding sites. Similarly, in human adrenal cortex, a single class of ET-1 binding sites with higher affinity for ET-1 than ET-2 or ET-3 was observed (318). Subsequent studies have demonstrated the presence of both ET_A and ET_B in the zona glomerulosa, although there is controversy as to which receptor isoform predominates. Originally, ET_B immunostaining was identified in rat (239) and bovine (260) adrenal gland;

the latter study localized ET_B to the endothelium. In rat, autoradiography showed both ET_A and ET_B in the zona glomerulosa (46). Binding studies in rat detected both ET_A and ET_B; however, ET_A predominated (346). Both ET_A and ET_B have been repeatedly observed in human zona glomerulosa. Autoradiography showed ET_A and ET_B in human zona glomerulosa, as well as ET_A and ET_B mRNA in adrenal cortex (140, 318, 626, 627). While both ET_A and ET_B immunostaining were present in human zona glomerulosa, ET_B strongly predominated (292). Cultured human zona glomerulosa cells express ET_A and ET_B mRNA (590). Thus it appears that the zona glomerulosa expresses both ET receptor isoforms; however, due to potential technical issues as well as possible species-specific differences, which isoform, if any, predominates remains an open question.

3. ET regulation of zona glomerulosa function

A) OVERVIEW OF ET REGULATION OF ZONA GLOMERULOSA FUNCTION:

Numerous studies have demonstrated that ET-1 and/or ET-3 stimulate experimental animal and human whole adrenal and isolated zona glomerulosa cell aldosterone production in vitro and in vivo (Fig. 7) (17, 126, 290, 479, 567, 611, 814). This stimulatory effect of ET peptides is relatively small compared with that of ANG II, based on studies in which ET-1 was systemically administered in vivo (480), in dispersed rat zona glomerulosa cells (478, 836), or using human adrenal slices (875). In addition, ET-1 may potentiate ANG II actions in the adrenal. While ET-1 did not affect basal or ANG II-stimulated aldosterone release from isolated bovine or rat zona glomerulosa cells (478, 625), systemic ET-1 in rats did increase basal and ANG II-stimulated aldosterone levels (480).

ET-1 can also stimulate zona glomerulosa cell growth and proliferation. Sustained (1 wk) infusion of ET-1 in rats raised plasma aldosterone concentration associated with hypertrophy of the zona glomerulosa, an increase in mitochondrial volume, and proliferation of smooth endoplasmic reticulum (481). This same group noted that ET-1 infusion in rats increased the mitotic index in the zona glomerulosa, although the effect was not additive with that of ANG II (477). Endogenous adrenal ET-1 appears to be involved in that nonselective ET receptor blockade decreased plasma aldosterone concentration, zona glomerulosa proliferation, and aldosterone release by zona glomerulosa preparations in rats transgenic for the renin gene (18). Furthermore, in humans with high to normal renin hypertension, ET_A or combined ET_{A/B} blockade reduced plasma aldosterone (although plasma renin activity was also decreased) (632).

Taken together, the above data indicate that ET, via autocrine pathways, stimulates adrenal zona glomerulosa cell steroidogenesis, growth, and proliferation. This effect seems to be physiologically relevant, although the relative importance of the endogenous adrenal ET system in regulating aldosterone production under different physiological conditions characterized by changes in the reninangiotensin system (e.g., variations in plasma K⁺ concentration, ECFV, or salt intake) is largely undetermined.

B) ET RECEPTOR ISOFORMS MEDIATING ET REGULATION OF

ALDOSTERONE PRODUCTION: There is some controversy, as well as species-specific differences, with regard to the ET receptor isoform(s) mediating stimulation of aldosterone secretion. In cultured calf zona glomerulosa cells, ET-1, much greater than ET-3, stimulated aldosterone secretion, suggestive of ET_A activation (126). Similarly, in frog adrenal gland, ET-1 augmented aldosterone production via ET_A (84, 814). ET-1 increased aldosterone production by rat zona glomerulosa cells primarily through ET_A with a small ET_B-mediated component (346). ET-1 stimulated aldosterone secretion by the rat adrenal gland via ET_A activation (287). In contrast, several studies have shown ET-1 induces aldosterone production primarily through ET_B activation. All three ET peptides were equipotent in stimulating aldosterone secretion by rat zona glomerulosa cells (289). Only ET_B activation

increased aldosterone secretion by rat zona glomerulosa cells as well as capsule-zona glomerulosa strips (19, 47, 479, 611, 612). Intravenous infusion of ET-1 increased rat zona glomerulosa aldosterone content via ET_B (566). While not fully explanatory, some of these differences observed in the rat may be due to the nature of the adrenal preparation; ET-1 increases aldosterone secretion by dispersed rat zona glomerulosa cells via ET_B, while both ET_A and ET_B mediate ET-1 stimulated aldosterone release by adrenal slices (613). This suggests that ET-1 may exert both autocrine and paracrine regulation of aldosterone production. Autocrine regulation may be ET_A and/or ET_B mediated, depending on the species. Paracrine regulation may be due to ET-1 activation of ET_A on neighboring capsular cells (possibly stromal) with subsequent modulation of factors that can exert an effect on zona glomerulosa cells. This raises the interesting possibility that ET-1 modulation of aldosterone production could be differentially regulated, at least in rat, depending on which region of the adrenal gland is stimulated. In human, ET-1 induces aldosterone secretion by zona glomerulosa or adrenocortical cells through stimulation of ET_A and ET_B (17, 630, 631). Interestingly, human adrenal gland synthesizes ET-1-(1–31) (which can be converted to mature ET-1 by chymase); while ET-1 increased aldosterone secretion through ET_A and ET_B activation, ET-(1–31) appears to directly stimulate aldosterone production via ET_A (631). Thus ET-1 increases adrenal aldosterone secretion, and this can occur through activation of either receptor; which receptor(s) that mediate(s) the response seems to depend on species and possibly the location within the adrenal gland. Finally, ET-1 induction of aldosterone production may depend on the stimulus; in rats *in vivo*, ET-1-stimulated basal aldosterone secretion was ET_A and ET_B dependent, while ET-1 augmentation of ANG II-stimulated aldosterone release was only ET_B dependent (480).

In contrast to the complex and unsettled issue of which ET receptor isoform(s) mediate(s) induction of aldosterone secretion, ET-1 stimulation of zona glomerulosa cell proliferation has consistently been shown to be due to ET_A activation (482, 483, 612). ET-1, via ET_A, increased zona glomerulosa cell proliferation in *in situ* perfused rat adrenal gland (483), while ET_A also mediated ET-1 enhanced proliferation of cultured rat zona glomerulosa cells (482).

C) MECHANISM OF ET REGULATION OF ZONA GLOMERULOSA FUNCTION:

It is challenging to arrive at a cohesive picture of how ET modifies zona glomerulosa cell function given the apparent species differences in ET receptor expression and potential activation of signaling pathways. Rather than attempting to dissect out what happens within each species, this section will attempt to integrate commonalities wherever possible.

ET stimulation of aldosterone secretion partially depends on increases in $[Ca^{2+}]_i$ in zona glomerulosa cells. In rat adrenal glomerulosa cells, ET-1 increased $[Ca^{2+}]_i$ (836). ET_B activation increased $[Ca^{2+}]_i$ in rat zona glomerulosa cells, at least in part, through intracellular release; the response was independent of dihydropyridine-sensitive Ca^{2+} channels (19). In contrast, ET-1-stimulated increases in aldosterone production and $[Ca^{2+}]_i$ in dispersed rabbit adrenocapsular cells were blocked by inhibition of dihydropyridine-sensitive Ca^{2+} channels (505). In frog adrenal slices, ET-1 induction of aldosterone production was dependent on extracellular Ca^{2+} (149). In cultured bovine zona glomerulosa cells, ET-1-stimulated aldosterone secretion depends on extracellular Ca^{2+} and verapamil-sensitive Ca^{2+} channels (125). In cultured human zona glomerulosa cells, ET-1 increased $[Ca^{2+}]_i$ via ET_A and ET_B activation (590). In human adrenal tissue slices, ET-1-stimulated aldosterone secretion was mediated in part by dihydropyridine Ca^{2+} channels (875). Finally, ET-1 stimulation of aldosterone secretion by dispersed human zona glomerulosa cells was reduced by inhibition of CaM (17). Taken together, the bulk of evidence supports the notion that ET-stimulated aldosterone production is Ca^{2+} dependent and that increases in $[Ca^{2+}]_i$ are due to extracellular Ca^{2+} entry and release from intracellular stores.

ET-1-induced increases in $[Ca^{2+}]_i$ in zona glomerulosa cells are associated with increases in inositol phosphates (IP) and activation of PLC. In frog adrenal explants, ET-1 increased IP₃ via ET_A (84). In rat adrenal glomerulosa cells, ET-1 increased IP levels (836). In dispersed human zona glomerulosa cells, ET_A and ET_B activation increases IP₃ (17). In cultured human zona glomerulosa cells, ET-1, via ET_A, increases IP levels (590). ET_B activation in rat zona glomerulosa cells stimulated aldosterone secretion and was partly PLC dependent (19). ET-1 stimulated aldosterone secretion by frog adrenal explants was reduced by PLC inhibition (84). In dispersed human zona glomerulosa cells, ET_A-dependent aldosterone secretion was completely, while the ET_B response was partially PLC dependent (17).

PKC is involved in ET regulation of zona glomerulosa cell function. ET_B-dependent induction of aldosterone secretion by dispersed rat zona glomerulosa cells and capsule-zona glomerulosa strips was reduced by PKC blockade (19, 611). ET_A- and ET_B-mediated aldosterone release by human zona glomerulosa cells was partly PKC dependent (17). In bovine cultured zona glomerulosa cells, ET-1-potentiated ANG II-stimulated aldosterone secretion was PKC dependent (125). PKC is also important in ET regulation of zona glomerulosa cell proliferation. In the in situ perfused rat adrenal gland, ET-1 increased cell proliferation in zona glomerulosa cells via ET_A stimulation of PKC (483). In cultured rat zona glomerulosa cells, ET-1-stimulated cell proliferation was reduced by PKC inhibition (482).

PKA or adenylylate cyclase does not appear to have a prominent role in mediating ET-1 actions in the zona glomerulosa. ET_B-mediated aldosterone secretion by dispersed rat zona glomerulosa cells and capsule-zona glomerulosa strips was independent of PKA (611). In addition, ET_B activation of rat zona glomerulosa cells stimulated aldosterone secretion and was independent of adenylylate cyclase and PKA (19). Frog adrenal gland may be different in that ET-1 increased cAMP content in frog adrenal explants, while ET-1-stimulated aldosterone secretion was partly PKA dependent (84). ET_A-mediated increases in zona glomerulosa cell proliferation in rat adrenal gland have also been shown to be PKA independent (483).

ET-1 can activate several other kinases in zona glomerulosa cells; this has been primarily implicated in stimulation of cell proliferation. In rat zona glomerulosa, ET-1 stimulated tyrosine kinase activity, and this was PKC dependent (347). ET-stimulated cultured rat zona glomerulosa cell proliferation was prevented by inhibition of tyrosine kinase or MAPK (482). ET_A-mediated cell proliferation in zona glomerulosa cells within the perfused rat adrenal gland was dependent on tyrosine kinase (483). In bovine zona glomerulosa cells, ET-1 stimulates phosphorylation of src, Pyk2, ERK1/2, p90 ribosomal S6 kinase (RSK-1), and CREB (686). This occurred primarily through activation of a G_i and to a lesser extent via G_q; PKC and matrix metalloproteinase-dependent EGF-R transactivation were also involved. With regard to aldosterone production, ET_B-mediated induction of aldosterone secretion by rat zona glomerulosa cells was independent of MAPK or tyrosine kinases, but was reduced by inhibition of PI3K (19). In addition, ET-1 stimulation of dispersed human zona glomerulosa cells and aldosterone secretion was partly PI3K dependent (17).

The role of arachidonate metabolites in modulating ET-1 effects on zona glomerulosa cells has been examined. In isolated rat zona glomerulosa cells or rat capsule-zona glomerulosa strips, ET_B-stimulated aldosterone secretion was independent of COX or lipoxygenase activity (19, 611). In contrast, in frog adrenal slices, ET-1 increased PGE₂ and PGI₂ production, while indomethacin suppressed ET-1-stimulated aldosterone secretion (149). In dispersed human zona glomerulosa cells, ET_B, but not ET_A, activation increased PGE₂ production, while COX inhibition reduced ET_B-induced aldosterone secretion (17). ET-1-stimulated zona glomerulosa cell proliferation in perfused rat adrenal gland was not affected

by inhibition of COX or lipoxygenase (483). Thus, while still unresolved, the balance of evidence points to a possible role for ET_B-stimulated COX metabolites in partially mediating zona glomerulosa steroidogenesis, but not in modulating ET_A signaling.

Other factors may modulate ET-stimulated aldosterone production. Intravenous infusion of ET-1 increased Na⁺-K⁺-ATPase activity in the rat zona glomerulosa via ET_B (566), while ouabain potentiated the effect of ET-1-enhanced rat adrenal aldosterone production, suggesting that ET-1 stimulation of Na⁺-K⁺-ATPase might provide negative-feedback regulation of ET-1-induced aldosterone production (568). NOS inhibition augmented ET_B-induced aldosterone secretion by dispersed rat zona glomerulosa cells; this was reported to be independent of cGMP (479). In contrast, ET_B-stimulated aldosterone release by dispersed rat zona glomerulosa cells was not affected by NOS inhibition, while NOS inhibition decreased aldosterone secretion by capsule-zona glomerulosa strips (611). Interestingly, catecholamines have been suggested to modify ET-1 actions in the zona glomerulosa. Adrenoreceptor antagonism reduced ET_A- and ET_B-stimulated aldosterone secretion by rat zona glomerulosa cells, but only when they were present in adrenal slices that contained medullary tissue (as opposed to dispersed zona glomerulosa cell preparations) (613). Since ET-1 can increase adrenal medullary catecholamine release (see following section), this raises the possibility that ET-1 can also indirectly enhance aldosterone production through modulation of paracrine pathways within the adrenal gland.

D) SUMMARY OF ET AND THE ZONA GLOMERULOSA: ET can serve as an autocrine and paracrine stimulus of adrenal aldosterone production. While some of the mechanisms involved in such regulation have been established, due to species-specific differences and possible cross-talk between different regions of the adrenal gland, much remains to be resolved. Most importantly, we do not have a good understanding of the role of adrenal ET in the physiological modulation of aldosterone production with respect to maintaining Na and BP homeostasis. Part of the challenge relates to difficulties in interpreting studies using ET receptor antagonists in which multiple potentially confounding variables are affected, including changes in BP, adrenal blood flow, and plasma renin activity. Nonetheless, some limited insight into the relative importance of the endogenous adrenal ET system might be gleaned from clinical studies using ET receptor antagonists in which plasma aldosterone levels have been assessed. Administration of ET_A-selective or combined ET_{A/B} antagonists is associated with fluid retention (41, 824). Might this be associated with alterations in adrenal aldosterone production? Administration of atrasentan, a relatively ET_A-selective antagonist, to healthy individuals for 1 wk at doses that significantly reduced BP did not alter plasma aldosterone concentration (300). Similarly, acute ET_A blockade with BQ123 did not change plasma aldosterone levels in healthy humans (247). While dietary Na intake was not adjusted in these studies, and aldosterone was measured as an end point as opposed to serially assessing its levels, the available data do not suggest that endogenous ET is a key regulator of aldosterone production, at least under normal physiological circumstances. However, studies are needed in which aldosterone levels are monitored during ET receptor blockade under varying ECFV conditions.

B. Adrenal Medulla: Catecholamines

1. ET production by adrenal medulla—Early studies did not detect ET-1 immunostaining in human adrenal medulla (438). In contrast, Davenport et al. (140) have provided evidence using high-performance liquid chromatography and radioimmunoassay showing the presence of ET-1 and ET-3 in the adrenal medulla. ECE-1 mRNA and protein have been identified in secretory cells from human adrenal adenoma tissue. Studies using in situ hybridization and immunohistochemistry reveal ECE-1 protein in adrenal medulla (308,

397). ECE has also been identified in bovine adrenal medulla (666). Thus the bulk of evidence indicates that adrenal medulla does produce ET-1.

2. ET receptors and signaling in adrenal medulla—Autoradiographic studies of selective displacement of ^{125}I -ET-1 and ^{125}I -ET-3 have identified ET_A and ET_B in the adrenal medulla of the rat (46, 383, 384) as well as that of pig and human adrenal gland (144). Binding occurs not only on blood vessels (46, 54), but on the secretory cells (140); mRNA encoding both receptor subtypes mirrors the distribution of binding sites.

In tissue from rat adrenal medulla, ET_A activation increases phosphatidylinositol hydrolysis by 30% (20, 228) and depends on the presence of extracellular Ca^{2+} (228, 608). ET-3 and the selective ET_B agonist IRL 1620 increase cGMP in rat adrenal medulla in a dose-dependent manner that can be inhibited by blockers of NOS, soluble guanylyl cyclase, or selective ET_B antagonism with BQ 788 (467). Thus, in whole rat adrenal medulla, ET peptides stimulate NO-induced cGMP generation via an ET_B -dependent mechanism.

In cultured bovine adrenal chromaffin cells, ET-1 (305), but not ET-3 (422), increases $[\text{Ca}^{2+}]_\text{i}$ via uptake of extracellular Ca^{2+} and evokes catecholamine release. This response to ET-1 is abolished by BQ123, but not BQ788, consistent with an ET_A -mediated effect. Furthermore, nifedipine only partially inhibits the response that is completely abrogated by further addition of agents that block Ca^{2+} entry via voltage-independent cation channels (422). ET_B agonists also stimulate Ca^{2+} efflux from cells preloaded with $^{45}\text{Ca}^{2+}$ and increase NO generation (305). NOS blockade prevents both the rise in NO and Ca^{2+} efflux. Removal of extracellular Na also inhibits Ca^{2+} efflux. Together, these observations suggest that activation of ET_B in adrenal chromaffin cells stimulates extra-cellular Na-dependent Ca^{2+} efflux via activation of NOS.

ET also affects other signaling systems in the adrenal medulla. Tyrosine hydroxylase, a key enzyme in the formation of catecholamines, possesses four phosphorylation sites: Ser-8, Ser-19, Ser-31, and Ser 40. Like ANG II, ET-3 selectively increases phosphorylation of Ser-31 via the ERK-MAPK pathway in bovine adrenal chromaffin cells (271). Interestingly, ET-1 attenuates C-type natriuretic peptide-induced cGMP production by bovine adrenal chromaffin cells (784).

Taken together, the above findings indicate that adrenal medullary cells express both ET_A and ET_B . Activation of both receptors can lead to increases in $[\text{Ca}^{2+}]_\text{i}$, while ET_B stimulation increases NO production.

3. ET regulation of adrenal catecholamine secretion—ET-1 ($\text{EC}_{50} \sim 1 \text{ nM}$) increases both norepinephrine and epinephrine secretion by bovine adrenal chromaffin cells (57, 596) and rat adrenal medullary fragments (46); the latter effect can be partially blocked by either ET_A or ET_B inhibitors. Injection of an ET_B agonist into frog chromaffin cells stimulates synthesis of dopamine β -hydroxylase, an enzyme in the catecholamine biosynthetic pathway (87). In cultured adrenal chromaffin cells, ET-1 activation of ET_B increases $[\text{Ca}^{2+}]_\text{i}$ via dihydropyridine-sensitive Ca^{2+} influx leading to release of catecholamines. Moreover, ET-1 potentiates catecholamine release evoked by acetylcholine (541). In contrast, ET receptors do not mediate catecholamine release and subsequent pressor response evoked by adrenomedullin in the rat (92). Thus ET-1 can enhance adrenal medullary catecholamine release under basal conditions, and this effect may be mediated by both ET_A and ET_B . ET-1 may also increase catecholamine secretion in response to some, but not all, stimulatory factors.

In the dog, infusion of ET-1 into the adrenal gland via the adrenolumbar artery evokes a dose-dependent increase in epinephrine and norepinephrine that persists for ~30 min without a change in systemic BP. The rise in catecholamines could be prevented by pretreatment with nifedipine (846), but not pentolinium or atropine (845). Thus neither nicotinic nor muscarinic cholinergic mechanisms mediate the effect of ET-1. ET_A blockade inhibits the response, whereas ET_B antagonism was ineffective except at very high doses (847), suggesting that there may be species differences in which ET receptor isoform mediates ET-1-enhanced catecholamine secretion.

Adrenal catecholamine output increases in response to increases in splanchnic nerve stimulation in a frequency-dependent manner (304, 760). Exogenous ET-1 augments catecholamine release induced by 3-Hz nerve stimulation. If the dogs are pretreated with an ET_A antagonist, ET-1 actually suppresses catecholamine output to either 1- or 3-Hz nerve stimulation. When ET_B antagonism is added to the ET_A inhibitor, ET-1 evokes neither facilitation nor inhibition of nerve-stimulated catecholamine release. In rat adrenal glands, transmural electrical stimulation of catecholamine release was blocked by ET_A antagonism (519). Treatment with an ET_B antagonist ameliorated the inhibitory effect of concurrent ET_A blockade in this model, suggesting that ET_B reduces neuronally stimulated catecholamine release. These findings indicate that ET_A mechanisms facilitate and ET_B mechanisms likely inhibit adrenal catecholamine secretion during nerve stimulation of the gland.

Zaretsky et al. (868) studied the effect of restraint stress on ET-induced adrenal catecholamine release in conscious male Wistar rats chronically instrumented with vascular catheters and a microdialysis probe placed into the adrenal gland. Norepinephrine and epinephrine levels in adrenal perfusate were stable at baseline and increased significantly with restraint stress, associated with concomitant elevation in BP and heart rate. Infusion of a mixed ET_{A/B} antagonist did not change either the stress-induced catecholamine release or the pressor and tachycardic responses. Thus ET does not mediate the heightened catecholaminergic and hemodynamic responses to restraint stress.

Infusion of exogenous ET-1 into isolated perfused adrenal glands evokes an increase in the basal release of norepinephrine and epinephrine in both control and DOCA-salt hypertensive rats (419). Interestingly, nerve-stimulated catecholamine release is inhibited by ET-1 in DOCA-salt, but not in control, rats. ET_A blockade enhances nerve-stimulated epinephrine, but not norepinephrine, release by DOCA-salt rats. ET_A antagonism has no effect on nerve-stimulated catecholamine release in control rats, and ET_B inhibition affects neither DOCA-salt nor control rats. Thus activation of ET_A by endogenously generated ET can inhibit epinephrine release by the nerve-stimulated adrenal gland in DOCA-salt hypertensive rats. This action may further contribute to the elevation in BP by limiting β -adrenergic vasodilation.

It has been suggested that ET-1-induced constriction of adrenal veins is another mechanism to effect increased discharge of catecholamines from the adrenal gland (445). In this regard, it is noteworthy that at least in the porcine adrenal vein, ET-1 is a potent vasoconstrictor and facilitates contractions evoked by neuropeptide Y (NPY) (54).

Plasma ET-1 is at least twofold higher in patients with pheochromocytoma compared with normotensive individuals or patients with aldosterone-secreting adenomas or essential hypertension (429, 545), although this finding is not observed in all pheochromocytomas (715). The ET-1 content of pheochromocytoma tissues is more than threefold higher than in normal adrenal tissue, and plasma levels of ET-1 return to normal after resection of the pheochromocytoma (545).

C. Natriuretic Peptides

1. Overview of ET and natriuretic peptides—ET interactions with natriuretic peptides are potentially complex; there are three mammalian natriuretic peptides [ANP, brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP)] as well as three natriuretic peptide receptors (NPR-A, NPR-B, and NPR-C) (261). ANP is mainly synthesized by the cardiac atria, BNP by ventricle, and CNP by endothelial cells, although other cell types can produce these peptides. NPR-A predominates in the vasculature, NPR-B predominates in the brain, and NPR-C, the ANP clearance receptor with no cGMP signaling activity, is found in several tissues. Thus ET peptides can potentially modulate natriuretic peptide levels, receptors, and actions in a wide variety of cell types, while natriuretic peptides can similarly affect ET biology. This discussion will primarily address ET interaction with ANP and BNP, focusing on regulation of their cardiac production with relatively lesser mention of modification of their actions. Further discussion of ET and ANP interactions can be found within sections dealing with specific organ systems.

As the following discussion will show, ET-1 stimulates natriuretic peptide production. Despite this, ET-1 and natriuretic peptides frequently have opposing biologic effects. For example, ANP prevents ET-induced renal vasoconstriction (739), while in rat aortic smooth muscle cells, ET-1 inhibits ANP vasorelaxation through reduction of cGMP (338). ET-1 inhibits ANP-dependent cGMP accumulation in pulmonary artery endothelial cells (459). In cardiac myocytes, CNP decreased contractility and increased cGMP, while ET-1 inhibited these effects (205, 778). ET-1 can downregulate ANP-C receptors in A-10 smooth muscle cells (65). ANP can also reduce ET-1 production. ANP decreased basal as well as ANG II- and thrombin-stimulated ET-1 production by human endothelial cells (379, 656), while ANP and BNP inhibited thrombin- or ANG II-induced ET-1 synthesis in cultured rat or porcine aortic endothelial cells (173, 380). In cocultures of cardiac myocyte and fibroblasts, ANP inhibited ET-1 promoter activity (245). Thus local cardiac ET-1 stimulates natriuretic peptide production; however, ANP can then act to downregulate aspects of cardiac and vascular ET-1 action.

2. ET regulation of natriuretic peptide production by the heart—ET stimulates natriuretic peptide production and release by the myocardium (Fig. 8). ET-3 infusion increased plasma ANP levels in rats (795). ET-1 infusion in rats increased plasma ANP and caused a natriuresis; the latter was blunted by administration of an anti-ANP antibody (514). In conscious rats, intravenous ET-1 increased plasma ANP levels at doses that reduced central venous pressure (i.e., the hemodynamic changes should tend to reduce ANP secretion) (220). That ET-1 can directly stimulate myocyte ANP production, independent of hemodynamic effects or comodulators, has been extensively demonstrated. ET-1 increases ANP peptide and mRNA production in acutely isolated, perfused, and cultured rat adult and neonatal atrial myocytes (211, 212, 224, 302, 309, 326, 425, 513, 577, 610, 667, 668, 684, 696, 709, 738, 791) and natriuretic peptide release from chick myocytes (51). In fact, on a molar basis, ET-1 is the most potent ANP secretagogue (651). ET-1 also increases ANP secretion by cultured rat ventricular myocytes (224, 302, 424, 485, 610, 698, 791). ET-1 increases BNP peptide and mRNA in cultured rat atrial myocytes (738) and cultured rat ventricular myocytes (302). In the latter study, atrial cells primarily secreted BNP, while ventricular cells released ANP and BNP.

ET may also indirectly increase cardiac natriuretic peptide secretion. ET-1 injection into the third cerebral ventricle in conscious rats increased plasma ANP levels and BP; both of these effects were blocked with an AVP V1 receptor antagonist (851). Similarly, ET-3 administration into the third cerebral ventricle of water-loaded rats caused a natriuresis and a rapid increase in plasma ANP (21).

There is evidence that ET plays a physiological role in control of natriuretic peptide secretion. In isolated rat atria, ET augmented stretch-induced ANP secretion (458, 668, 709). Notably, stretch-induced ANP release in these preparations was reduced by BQ123, indicating a role for endogenous ET (710). In addition, ET_A-selective blockade with BQ123 or nonselective ET receptor blockade with bosentan abrogated volume load-induced increases in NH₂-terminal ANP secretion in conscious rats (651), while anti-ET antiserum reduced volume-stimulated ANP secretion in rats (216). Evidence, albeit indirect, also exists for endogenous ET regulation of BNP production. In the two-kidney, one-clip Goldblatt, as well as the DOCA-salt, hypertension models, 6 wk of treatment with an ET_A antagonist decreased plasma BNP levels and reduced ventricular gene expression (52, 53) (admittedly, the reductions in BNP could have been secondary to the cardioprotective effects of ET_A blockade in these models). That such physiological regulation of cardiac natriuretic peptide secretion by ET could occur is supported by findings that ET-1 is produced by endocardial cells and myocytes in the heart (75, 569, 880). Modulation of ET-1 or its receptors during conditions of differing ventricular pressures has not been assessed; however, one group reported that there was no difference between left and right ventricular ET-1 or ET receptor expression in normal rats, suggesting that myocardial ET expression is not affected by physiological pressure load (789). However, such analysis would clearly need to be done for each cardiac chamber under varying physiological pressure loads to make this determination. Taken together, and particularly in view of the studies using ET antagonists, the above findings suggest that ET, most likely via an autocrine or paracrine mechanism, partly mediates the cardiac natriuretic peptide response to volume loading.

3. Mechanism of ET regulation of natriuretic peptide production

A) ET RECEPTORS MODULATING NATRIURETIC PEPTIDE PRODUCTION:

ET_A appears to be the major regulator of cardiac natriuretic peptide production. This ET receptor isoform predominates in atrial myocardium (651). In cultured adult rat atrial myocytes, ET-1 increased ANP release via ET_A activation; these cells did not express ET_B (425). As mentioned above, ET_A blockade reduced stretch-induced ANP release in isolated perfused atria (710). In cultured neonatal rat atrial myocytes, ET-1 was much more effective than ET-3 in stimulating ANP release; these cells contained little ET_B mRNA (326). Finally, ET-1 increased ANP release by neonatal rat ventricular myocytes that expressed only ET_A (610).

B) SIGNALING PATHWAYS INVOLVED IN ET-STIMULATED NATRIURETIC PEPTIDE PRODUCTION:

No clear difference has been discovered between ET-stimulated ANP secretion by ventricular or atrial myocytes; findings in these cell types will be combined for the purposes of discussion. The bulk of evidence suggests that ET-stimulated ANP release depends on increases in [Ca²⁺]_i. In cultured rat atrial myocytes, the ANP response to ET-1 was partially nifedipine sensitive (212). In the same cell type, ET augmented ANP release occurred in a rapid and sustained phase; the former was associated with release of Ca²⁺ from intracellular stores, and the latter required extracellular Ca²⁺ entry (684). Nifedipine markedly decreased ET-1 stimulated ANP release in rat ventricular myocytes (610). In superfused rat atria, ET-induced ANP secretion was nitrendipine sensitive but did not depend on intracellular Ca²⁺ release (667). Furthermore, in cultured neonatal rat atrial and ventricular myocytes, ET-1-stimulated ANP release was unaffected by inhibition of the rise in [Ca²⁺]_i using diltiazem treatment (791). This same group found that PKC blockade further increased the rise in [Ca²⁺]_i, but reduced the ANP response. Taken together, the above data suggest that ET-1-induced elevations in atrial myocyte ANP require extracellular Ca²⁺ entry through dihydropyridine-sensitive Ca²⁺ channels.

In keeping with the Ca^{2+} dependence, ET-1-enhanced ANP release by atrial and ventricular cells is CaM dependent (224) and is associated with phosphatidylinositol hydrolysis, diacylglycerol generation, and PKC activation (326, 577, 698). While PKC may be involved in the ANP response (326, 577, 698), not all studies have demonstrated this (610). cAMP may be involved in the ET induction of ANP release (424, 610). There are varying data on a role for arachidonate metabolites. ET-stimulated ANP release from rat atria was partly dependent on cytochrome *P*-450 metabolites (424); COX inhibition decreased ET-1-induced ANP release from perfused rat heart (696), but not from cultured rat ventricular myocytes (610). ET-1-stimulated ANP secretion by stretched isolated atria was augmented by inhibition of NO production; exogenous NO alone reduces ANP release from these preparations (709). Finally, in a recent study, the molecular mechanism of ET-1-stimulated BNP production by ventricular rat myocytes was examined (246). The authors reported that the increase in BNP expression was mediated by the transcription factor Yin Yang 1 which underwent an ET-1-induced reduction in acetylation accompanied by increased nuclear localization; this process required an association with histone deacetylase.

D. Vasopressin

1. CNS sites of ET production related to vasopressin—All components of the ET system have been identified within central nervous system (CNS) sites that are integrally involved in AVP synthesis and secretion. The vasopressinergic magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus contain both ET-1 and ET-3 isoforms in roughly equivalent amounts (423, 526), along with their respective mRNAs (453) and ECE activity (711, 816, 817). This distribution of ET components in hypothalamic neurons as well as endothelial cells has been verified in human brain as well (240). Soon after their discovery, ET immunoreactive peptides corresponding to the ET-1 isoform were identified in posterior pituitary (864). Immunogold studies showed that ET-1 colocalized with AVP in the neurosecretory granules more often than oxytocin granules (526).

Despite these observations, there is scant direct evidence about the regulation of neural ET production or release within these hypothalamic loci during conditions that influence AVP secretion. Immunohistochemical evidence indicates that the magnocellular neuron cell bodies contain both Big ET-1 and ET-1, whereas the axonal projections were immunopositive only for Big ET-1 (843). This has led to speculation as to whether mature ET-1 is involved in hypothalamic neurotransmission and Big ET-1 is released from the nerve terminals. Neural lobe ET immunoreactivity decreases with water deprivation (864), although plasma concentrations of ET-1 remain unchanged despite clear elevations in circulating AVP (788). In contrast, during hemorrhage, which is a powerful stimulus for AVP release, ET-1 immunoreactivity increased up to threefold in hypothalamic tissue, but remained unchanged in the axon terminals (788). Although these findings suggest that the ET system itself is modulated under conditions of osmotic and/or hypovolemic stress when BP is threatened, this has not been further explored. For example, it is not known whether ET-1 coreleased with AVP influences subsequent AVP and/or ET-1 secretion by the neurohypophysis. Whether dendritic release of ET peptides by magnocellular neurons occurs and is regulated under these physiological stressors also remains unexplored.

2. ET receptors, signaling, and regulation of AVP secretion—The supraoptic and paraventricular nuclei are richly endowed with ET binding sites (Fig. 9). Importantly, ET receptors are also located on circumventricular organs such as the subfornical organ and the region anteroventral to the third ventricle (Av3V) that encompasses the organum vasculosum of the lamina terminalis, and the neural lobe itself (399). ET_A exist on the magnocellular neuronal cell bodies (843) as well as the neural lobe itself (689). ET_B are more widely distributed within the hypothalamus, most prominently in the organum

vasculosum of the lamina terminalis and the median eminence (853). In situ hybridization studies also support the existence of ET_B on astrocytes and ependymal cells lining the ventricles (523). Since the circumventricular loci lie outside the blood-brain barrier, they are at the interface of the CNS and the peripheral circulation. Thus the hypothalamoneurohypophyseal system is poised to respond to ET signaling from either the CNS, cerebrospinal fluid, or the peripheral blood to influence AVP secretion.

The effects of ET on the hypothalamo-neurohypophyseal system and AVP secretion are complex and critically depend on the locus of action and the receptor subtype. Studies in explants of hypothalamus (691, 860) or of the hypothalamo-neurohypophyseal system (634) demonstrated that ET peptides are potent secretagogues for AVP secretion. Central administration of ET into the lateral ventricles also elicits an increase in plasma AVP (356, 471, 538, 851). These in vivo studies proved to be complicated by the concurrent pressor response elicited by central ET administration that can reflexly inhibit AVP release and mask the direct effect of ET receptor activation (644) (see also sect. **VIB**). Direct application of ET-3 onto the soma of phasically firing, presumptive vasopressinergic magnocellular neurons inhibits 61% of the neurons; continuously firing oxytocinergic neurons are largely unaffected. In contrast, ET-3 stimulates up to 21% and inhibits only 4% of the cells within the Av3V region (848). In sinoaortic denervated rats wherein baroreflex mechanisms are interrupted, electrolytic lesioning of the Av3V region prevents the increase in plasma AVP induced by intracerebroventricular administration of ET-1 (644). In vitro studies using the compartmentalized hypothalamo-neurohypophyseal explants have shed some light on the receptor sub-types responsible for these effects on AVP neurons and secretion. These explants contain the Av3V region, supraoptic nucleus, and neural lobe, but not the subfornical organ or paraventricular nucleus. Selective ET_B activation directed exclusively to hypothalamus increases both somatodendritic and neurohypophyseal AVP release by explants in vitro. This effect is blocked by tetrodotoxin consistent with at least one intervening synapse. In contrast, hypothalamic ET_A inhibit and neurohypophyseal ET_A stimulate AVP secretion (635). Together with the in vivo data and electrophysiological studies, these findings support the concept that ET_B within the lamina terminalis whose neurons project to the supraoptic nucleus stimulate AVP release from axon terminals in the neural lobe as well as from dendritic processes. ET_A on the soma of the magnocellular neuron itself inhibits neurohypophyseal AVP secretion, whereas ET_A on the neural lobe stimulates release.

The subfornical organ sends efferent projections to both the supraoptic and paraventricular nuclei. Early studies by Wall and Ferguson (807) demonstrated that ET-1 in the systemic circulation can act at the subfornical organ to increase excitability of antidromically identified AVP neurons; ablation of the subfornical organ prevents this response. Microinjection of ET-1 into subfornical organ results in an increase in plasma AVP levels that could be blocked by preinjection with an ET_A blocker (511). Direct injection into paraventricular nucleus did not elicit a change in plasma AVP (511). Lesioning either the Av3V region, which destroys fibers of passage from the subfornical organ to the supraoptic nucleus (644), or the paraventricular nucleus bilaterally (640) also prevent the rise in plasma AVP. That the increase in plasma AVP does not require the elimination of both inputs may at first seem paradoxical. The supraoptic neurons receive excitatory afferent projections from the ipsilateral paraventricular nucleus. Likewise, most of the magnocellular neurons within the paraventricular nucleus receive inputs from the supraoptic nucleus; there is evidence that burst generation and synchronization occur between supraoptic and paraventricular nucleus that may serve to maximize AVP release. ET_A activation at the neural lobe by ET-1 coreleased with AVP could further enhance AVP output from the neurohypophysis during osmotic or hypovolemic stimuli.

ET actions within the hypothalamus appear to be mediated via glutamate, an excitatory amino acid neuro-transmitter. In vitro studies in hypothalamo-neurohypophyseal explants indicate that ET_B-induced AVP release is stimulated by the *N*-methyl-D-aspartate (NMDA) receptor and attenuated by γ -aminobutyric acid (GABA) receptor mechanisms (639). The NMDA receptor subtype involved is not known. In contrast, plasma AVP resulting from stimulation of subfornical organ ET_A in vivo appears to be mediated by an AMPA (non-NMDA) receptor sub-type within the paraventricular nucleus (639). This apparent disparity may be due to the ET receptor subtype stimulated, the site where ET-1 is acting (Av3V alone vs. Av3V plus subfornical organ) and the glutamatergic receptor subtypes on the target cells of their respective axonal projections (supraoptic vs. paraventricular nucleus), differences in the preparations, the unique properties of the antagonists used, or other factors. These findings are, however, consistent with glutamate acting primarily on NMDA receptors in supraoptic nucleus to drive basal NO generation. NO, in turn, restrains firing of putative vasopressinergic neurons (725). In organotypic cell cultures of the paraventricular nucleus, AMPA but not NMDA receptor stimulation induces a Ca²⁺ transient that is also modulated by NO (647). Thus it is not only feasible, but likely that effects of ET on AVP are mediated via different ionotropic glutamatergic receptors at different neural loci.

Notably, the ET_B agonism increases NOS activity in the anterior hypothalamus, an effect that can be blocked by inhibition of NOS1 (neuronal NOS) (340). Several studies have shown that ET_B signals via Ca²⁺-dependent activation of PLC/PKC pathway leading to formation of IP₃ and subsequent increases in NOS activity and cGMP accumulation (229, 466, 468). Chelation of extracellular Ca²⁺, but not inhibition of mobilization of intracellular Ca²⁺ stores with 3,4,5-trimethoxybenzoate hydrochloride, prevents ET-3 stimulated AVP secretion (633). PKA and CaMKII have also been implicated (340); so far the evidence suggests these are primarily involved in catecholaminergic signaling in the posterior rather than the anterior hypothalamus (154, 572).

ET_B agonism increases AVP secretion, and the effect of submaximal doses of ET_B agonists is potentiated by L-NAME. Explants from NOS1 knockout mice display an augmented AVP secretory response to ET_B activation that is not further enhanced by L-NAME (636), suggesting that NOS1 rather than NOS3 is the primary source of NO responsible for the suppression of AVP. In experiments where the dominant negative construct of NOS1 was transfected into the paraventricular nucleus of baroreceptor-intact Long Evan rats, the pressor effect of ET-1 microinjected into the subfornical organ was potentiated and had shorter latency consistent with nitrergic suppression of inputs to the paraventricular nucleus (637). However, plasma AVP was not increased in these animals presumably due to reflex inhibition of AVP release by the greater increase in BP. Collectively, the evidence from pharmacological and genetic manipulations of the NO system in vitro strongly support a role for NO modulation of ET-induced AVP secretion independent of the effects of central ET on cerebral blood flow and/or systemic hemodynamics.

The magnocellular neurons possess voltage-dependent, Ca²⁺-activated potassium channels (also known as maxi K or BK channels). Opening BK channels results in cell membrane hyperpolarization and a decrease in excitability. Thus BK channels contribute to the hyperpolarizing after potentials that follow propagation of an action potential. This leads to spike-frequency adaptation and modulation of phasic firing characteristic of AVP neurons. In the neurohypophysis, ET-3 initiates Ca²⁺ entry, leading to depolarization (633). This mechanism is opposed by hyperpolarizing forces linked to Ca²⁺ accumulation, namely, activation of the BK channels. Charybdotoxin blocks BK channels and should, therefore, augment neuronal excitability. Consistent with such a mechanism, charybdotoxin potentiates ET-3 stimulated AVP secretion.

In summary, ET signaling via ET_A and ET_B is poised to play a pivotal role in controlling AVP secretion (Fig. 9). ET system components are located in neural loci involved with osmosensation as well as sites at the interface of the CNS with the peripheral circulation. Thus changes in cerebrospinal fluid and plasma ET during physiological or pathological conditions modulate the AVP response to osmotic and/or hypovolemic stress. These mechanisms permit ET to influence Na and water homeostasis and ultimately volume and BP.

3. Physiological effects of ET on vasopressin secretion—Shortly after the discovery of ET-1, several investigators demonstrated that intravenous infusion (249, 492, 525), intracerebroventricular injection (356, 849), or direct microinjection into the subfornical organ (509) with ET peptides results not only in profound systemic hemodynamic effects but also in higher plasma AVP levels. One notable exception is the study by Nakamoto et al. (524) in conscious dogs where a low dose (40 fmol·kg⁻¹·min⁻¹) intravenous infusion of ET elicits a small but significant decrease in arterial pressure as well as a significant reduction in plasma AVP. However, a higher infusion rate (400 fmol·kg⁻¹·min⁻¹) of ET increases both BP and plasma AVP. Initial studies suggested that AVP release mediates the systemic pressor response that occurs after ET-1 injection into the lateral ventricles (356, 471, 538, 851) or the subfornical organ (509). Indeed, intravenous infusion of a V1a receptor blocker partially attenuates the pressor response to central ET-1. Importantly, the α_1 -adrenergic antagonist prazosin completely blocked the rise in BP (849, 851). It was later shown that central ET-1 evokes a virtually identical pressor response in Long Evans and Brattleboro rats (644), but is abolished by ganglionic blockade (471, 644) (the Brattleboro rat lacks circulating AVP). Moreover, circulating AVP is not required for the systemic hemodynamic effects of intravenous ET-1 or ET-3 (222), nor those of ET-2 or sarafotoxin S6b (221). The increase in BP as well as changes in heart rate and vascular resistances of renal, mesenteric, and hindquarter vascular beds are equivalent in Long Evans and Brattleboro rats. Collectively, these data support the notion that the increase in BP response to central ET peptide administration is due to mechanisms that enhance sympathetic outflow rather than plasma AVP. The attenuation of this pressor response by AVP antagonism in some studies (849) but not others (356, 644) is likely to have been due to the potentiation of AVP secretion by concurrent hypovolemia due to the number and volume of blood samples taken for the AVP assay without equivalent restitution of blood volume. Studies that meticulously replaced blood volume have not observed increases in plasma AVP in conscious baroreceptor intact rats. It is thought that the pressor response that accompanies ET administration reflexly inhibits AVP secretion, since sinoaortic denervation removes the restraint permitting plasma AVP levels to rise significantly (640, 643, 644).

The same appears to be true in the SHR and the normotensive WKY rats, where AVP increased to pressor levels only after baroreceptor inputs were interrupted. In sinoaortic denervated SHR rats that have systemic pressure normalized prior to intracerebroventricular injection ET-1, the increase in plasma AVP levels is further potentiated (645). Similar to Long Evans rats (643), peripheral V1a antagonism does not alter the pressor response to central ET-1 in baroreflex intact SHR or WKY rats. However, in sinoaortic denervated SHR, V1a blockade decreases the pressor response by ~50%. A similar but smaller effect is seen in the WKY rat strain (645). Intravenous infusion of ET-1 in rats that have been osmotically stimulated with hypertonic saline results in a robust increase in plasma AVP that does not appear to be dampened by afferent arterial baroreceptor inputs (726). One mechanism that may account for the increase in plasma AVP despite the increase in BP when ET is given intravenously is that ET-1 itself suppresses the activity of the peripheral baroreceptors especially at high arterial pressures (94). This effect would not occur when ET increases in the CNS or cerebrospinal fluid (see sect. **VIB**). Chronic intracerebroventricular infusions of ET-1 over 7–10 days increase urinary AVP excretion by days 5 to 7 (538), but

not plasma AVP levels on day 9. Notably, BP rose to similar levels and was maintained throughout the period of infusion. Thus in vivo central ET mechanisms are more likely to contribute to AVP secretion under conditions where baroreflex inhibition of neurohormone release is impaired. When the baroreflex influences are removed, plasma AVP levels can achieve pressor levels and then may contribute to increase systemic BP along with increases in sympathetic outflow. This does not preclude the possibility that central release of AVP from dendritic sources within the blood-brain barrier contributes to the enhancement of sympathetic outflow. Studies on the direct impact of cardiopulmonary baroreceptors on ET-induced AVP secretion are needed. As is often the case, the response of different species and strains may vary.

E. Renin-Angiotensin System

1. ET and renin release—Given the importance of ET and renin in BP regulation, one would expect that a direct interaction would exist between these highly active vasoactive systems. Early studies suggested a clear effect of ET-1 to inhibit renin release from isolated juxtaglomerular apparatus (472, 606, 745). These actions depend on increases in intracellular Ca^{2+} . In vivo, low doses of ET-1 that do not produce any significant changes in total peripheral resistance also reduce renin release as observed in anesthetized dogs (441, 556). Higher doses that increase arterial pressure and, perhaps more importantly, increase renal vascular resistance actually increase plasma renin activity (556). The effect of the higher doses is most likely due to reductions in hydrostatic pressure reaching the juxtaglomerular cells and stimulation of the intrarenal baroreceptor. The actions of ET-1 on renin release extend to inhibiting the effect of other factors that increase renin release such as isoproterenol and cAMP (411, 496).

Several laboratories have investigated whether the actions of ET-1 to modulate renin secretion are influenced by other endothelial-derived factors, primarily NO, since the inhibitory effects of ET-1 on renin release most likely are mediated by ET_B (4, 620, 676). There does not appear to be any clear consensus in terms of defining the interactions between ET-1 and NO on renin release. Much of this confusion is because there has been considerable controversy as to the effects of NO itself, which appears to directly stimulate renin release in juxtaglomerular cells, but may inhibit renin release in response to afferent arteriolar vasodilation (681). In renal cortical slices, Beierwaltes and Carretero (44) showed that NOS inhibition did not affect the ability of ET-1 to reduce renin release. Work from Kurtz' laboratory showed that endothelial cells cocultured with juxtaglomerular cells inhibit renin release (400, 411, 620). More specifically, this group showed that ET-1 inhibited cAMP-stimulated renin release, and this effect was not changed during coculture with endothelial cells or treatment with NOS or COX inhibitors (411). These studies suggest that endothelial-derived ET-1 may serve to attenuate renin release in response to a more traditional stimulus such as increased sympathetic nerve activity, but this effect would be independent of prostanoids or NO. Tharaux et al. (774) used isolated perfused afferent arterioles and observed that NOS inhibition with L-NAME inhibited renin release in a Ca^{2+} -dependent fashion similar to ET-1. They went on to demonstrate that the dual receptor antagonist bosentan inhibited the effects of L-NAME and thus concluded that NO functions to inhibit the influence of endogenous ET-1 on renin secretion.

Despite wide agreement that ET-1 inhibits renin release, the physiological significance of these observations is unknown. One can speculate that ET-dependent inhibition of renin release fits with other physiological functions of ET-1 to reduce BP and promote excretion of Na. Unfortunately, there has been little follow-up to these initial studies, so we do not yet know under what conditions ET-1-dependent regulation of renin release becomes important. However, a relevant study from Berthold et al. (48) in conscious dogs revealed that systemic

administration of an ET_A-selective antagonist, LU 135252, increased plasma renin activity along with lowering arterial pressure and increasing blood flow. Unfortunately, one cannot separate direct ET_A-dependent inhibition from the renal baroreceptor-dependent stimulation when renal perfusion pressure is decreased in these studies. However, these investigators went on to measure pressure-dependent changes in renin release and observed that ET_A blockade exaggerated pressure-dependent renin release. These data clearly indicate that endogenous ET-1 can modulate pressure-dependent control of renin release.

Given all the evidence we have reviewed that renal ET-1 functions to promote Na excretion, we can hypothesize that stimulation of endogenous ET-1 during high salt may promote Na excretion by attenuating the renal baroreceptor responsible for renin release. This idea has yet to be explored, and in addition, we are not certain which ET receptor may be most important. The Berthold study certainly indicated that ET_A was responsible for attenuating renin release in dogs, but in vitro studies have indicated that the effect of ET-1 to inhibit cAMP-dependent renin release can be mimicked by ET_B-selective agonists (620). Thus an apparent inconsistency exists between the in vitro and in vivo findings in terms of which receptor is more important in control of renin release. Since ET_B stimulate NO production and NO increases renin release, it is unclear how ET_B can inhibit renin release. Obviously more work is needed to resolve this issue.

It is important to note that the influence of ET receptor blockade to increase plasma renin activity has not been observed in more chronic studies in hypertensive rats or humans (403, 679). In addition, there may be species differences in this pathway since ET antagonists, when given to isolated perfused rat kidneys, had no effect on renin release (676), although pressure-dependent renin release has yet to be investigated in rodents or humans.

2. Angiotensin and ET interactions—Another important relationship between ET-1 and the renin-angiotensin system comes from a large variety of studies that suggest ET-1 is increased by ANG II and that ET-1 mediates some of the vascular actions of ANG II. ANG II stimulates ET-1 release and increases mRNA expression in cultured endothelial cells (174, 175, 319). ANG II can also induce ET-1 production in cultured smooth muscle cells (617) and cardiomyocytes (328). The consequences of ANG II-dependent increases in ET-1 would appear to contribute to the vasoconstrictor effects of ANG II. ET-1 mediates vasoconstrictor effects of ANG II in isolated vascular preparations (96, 819). ET_A blockade inhibited the vasoconstrictor response to ET-1 in rat mesenteric artery, but not in rat aorta (96) or rat tail artery (341). ET-1 increases the sensitivity of isolated vascular preparations to other vasoconstrictors, and in fact, ET-1 stimulated by ANG II has the same effect to potentiate vasoconstriction (156, 863).

ET receptor blockade can inhibit the acute vasoconstrictor responses to ANG II in vivo including within the renal circulation (30, 618). A similar effect has been observed in the skin microcirculation in healthy human volunteers (825). The simplest explanation for both the in vivo and previously mentioned in vitro observations is that ANG II stimulates ET-1 release and thus results in ET_A-dependent vasoconstriction and increased renal vascular resistance. However, the speed at which ANG II produces contractions is much faster than ET-1, plus ET-1 contractions are slow to subside while ANG II effects wear off quite rapidly.

The extent to which ANG II directly stimulates production of ET-1 within the kidney has not been fully elucidated. Chronic ANG II administration increases renal mRNA and protein expression in both the cortex and medulla (9, 39). ANG II also increases urinary ET-1 excretion, a measure of intrarenal production, but this increase is not nearly as large as that seen with high salt intake (665).

There have also been a number of studies demonstrating that selective ET_A or dual ET receptor antagonists can attenuate the hypertension produced by chronic ANG II infusion (32, 132, 281, 604). The degree to which this is due to ET-1 effects on peripheral vascular resistance is not clear. The development of ANG II hypertension is dependent on the level of Na intake. Thus there would appear to be an interaction between ANG II and ET-1 to influence renal control of Na excretion, but this has not been investigated. More discussion of ET-1 in ANG II hypertension will be addressed in a later section. From a renal injury perspective, the relationship between ANG II and ET-1 in the kidney may be important in profibrotic processes, especially within the vascular system (see Chatziantoniou and Dussault for a more specific review of this topic, Ref. 95).

VI. ENDOTHELIN AND THE NERVOUS SYSTEM

A. Ganglia and Peripheral Nerves

In addition to the well-known role of ET in the development of neural crest and enteric neurons (160, 231, 621, 804), ET peptides affect peripheral sympathetic ganglia that modulate systemic hemodynamics. The superior cervical ganglion (SCG) has been the focus of studies on ET in sympathetic ganglia; the SCG lies adjacent to the bifurcation of the internal and external carotid arteries and supplies sympathetic innervation to the head and neck. In addition, some studies have examined ET in the stellate ganglion (innervating cardiac and pulmonary structures), the celiac ganglion (innervating the kidney and mesentery), and the dorsal root ganglia (DRG) (primary locus of afferent inputs to the spinal cord).

1. Sites of ET production and regulation in ganglia and peripheral nerves—

Sympathetic neurons cultured from neonatal rat SCG produce ET-1 mRNA and protein (138). Both ET-1 and ET-3 are released by adult rat SCG cultures and extracts (138); ET-1 immunoreactivity is present in the rat SCG, albeit in relatively small amounts. Interestingly, SHR rats appear to have increased ET-1 in the SCG compared with WKY rats; whether this is a cause or consequence of hypertension is uncertain (493). ET-1 mRNA and protein are also present in DRG (241). PC12 cells, an immortalized pheochromocytoma cell line, assume characteristics of sympathetic neurons when cultured in the presence of nerve growth factor (NGF) (101, 252, 798); these cells express both ET-1 and ET-2. ECE-1 has been identified in human paragangliomas (308). In vivo, ET-2 has only been identified in parasympathetic ganglia of the cat (539) and pig (277), but not in sympathetic ganglia. Taken together, these studies suggest that ET isoforms exist in neurons within the sympathetic ganglia.

2. ET receptor localization and signaling in ganglia and peripheral nerves—

In general, ET_B immunoreactivity is present only on nonneuronal cells in ganglia. Robust ET_B immunostaining is present in DRG; however, this appears to localize to satellite cells (394). ET_B also are on or near unmyelinated Schwann cells surrounding afferent sensory nerves and nerve bundles close to the renal pelvic wall. There are high-affinity binding sites for ET-3 (461) and selective ET_B-mediated effects on NGF-differentiated PC12 cells (223, 826). In contrast, ET_A can be present on nerves associated with the sympathetic nervous system. Immunohistochemistry revealed ET_A primarily on a distinct group of small-diameter primary afferent sensory neurons; about two-thirds were associated with C fibers and one-quarter were associated with A fibers (589). Kopp et al. (394) have made similar observations in thoracolumbar spinal cord (T₉ to L₁) that contain the majority of the cell bodies of renal afferent nerves. ET_A has also been detected in NGF-differentiated PC12 cells (755). Note that the above studies on ET receptor isoform localization are largely based on relatively insensitive immunostaining; more useful information can be gleaned from

functional studies as described below. Nonetheless, the existing studies indicate that ET and ET receptors exist in sympathetic ganglia, particularly on preganglionic fibers and afferent inputs.

ET peptides can modulate the release of classic neurotransmitters and influence the generation of action potentials. Studies in NGF-differentiated PC12 cells have helped to shed light on how ET exerts these effects. With the use of these cells, ET_B has been implicated in the modulation of ATP release (223, 826) and ET_A in catecholamine biosynthesis (755). ET-1 and S6c inhibit K⁺ depolarization-induced release of ATP, but not NPY or dopamine (826). The inhibition of ATP release could be blocked by either ET_{A/B} or ET_B antagonism, but not selective ET_A antagonism (223). Others have shown that ET-3 elicits an increase in [Ca²⁺]_i and dopamine release. The ET receptor subtype was not evaluated in these experiments; however, both pertussis toxin and blockers of voltage-gated Ca²⁺ channels attenuate the response (385). ET-induced dopamine release is totally abolished in the absence of extracellular Ca²⁺ (385). Likewise, ET-1-induced inhibition of K⁺-evoked ATP release does not occur in PC12 cells pretreated with pertussis toxin or in the presence of nifedipine (223). The latter finding is consistent with the observation that ET-1 evokes an increase in [Ca²⁺]_i in cultured SCG (136). Taken together, these data support the notion that ET-1, via ET_B and its associated G_i protein, attenuates ATP release by decreasing Ca²⁺ influx through L-type Ca²⁺ channels. G_i protein signaling and Ca²⁺ entry are also implicated in ET-3-induced dopamine release.

ET_B activation inhibits cardiac sympathetic ganglionic transmission by reducing acetylcholine release from preganglionic nerve terminals. In canine stellate ganglion, ET-3 (412, 413, 844) or the selective ET_B agonist IRL1620 (844) reduces acetylcholine release and inhibits the positive chronotropic response to preganglionic stimulation (844). The effect of ET-3 on acetylcholine could be blocked by selective inhibitors of NOS1 or soluble guanylyl cyclase, or mimicked by an NO donor or 8-bromocGMP; ET_B activation in the stellate ganglion increases NO production (844). In addition, in cultured sympathetic neurons from chick embryo, ET-1 evokes a rapid but transient increase in Ca²⁺ influx, followed by NO release over the ensuing 30 min (359). Antagonism of ET_B-mediated Ca²⁺ entry or CaM also prevents ET-3-induced increases in NO (844). Furthermore, the decrease in acetylcholine release is dependent on PLA₂ and COX, but not PLC or PKC.

Thromboxane A₂ analogs elicit similar reductions in acetylcholine, and the effect of ET-3 could be blocked by either inhibition of thromboxane A₂ synthesis or by a thromboxane A₂ receptor antagonist (412). Although PGE₂ also diminishes acetylcholine release by the cardiac sympathetic ganglion, blockade of the PGE₂ receptor does not prevent the decrease in acetylcholine due to ET-3 (413). Thus ET_B activation inhibits cardiac sympathetic ganglionic transmission via ET_B-mediated Ca²⁺/CaM-dependent activation of endogenous NO generation as well as PLA₂-dependent production of thromboxane A₂.

ET-1 can modulate neurotransmission at the vascular neuroeffector junction where norepinephrine is released with other neuromodulators and/or neurotransmitters. ET-1 acts prejunctionally to attenuate sympathetic neurotransmission by decreasing norepinephrine release in response to electrical stimulation of sympathetic nerves in femoral artery of the guinea pig (827) and dog coronary artery (1). Similar findings have been reported in rat mesenteric artery (741). Others found that ET-1, ET-3, or S6c do not change norepinephrine, but decrease release of NPY in the mesenteric vascular bed via ET_B activation (295).

ET-1 promotes the development and differentiation of postganglionic sympathetic neurons (136, 184). In the presence of ET-1, cultured SCG neurons extend 58% more processes (136). Both ET-1 and ET-3 increase neurite outgrowth, but only in the presence of NGF (136, 876). Moreover, combined ET_{A/B} antagonism decreases neuronal survival when SCG

neurons are cocultured with vascular smooth muscle cells isolated from rat aorta (137). Ieda et al. (314) have shown that ET_A activation regulates cardiac sympathetic innervation by modulating NGF expression, whereas neither ANG II nor insulin-like growth factor (IGF)-I elicits this effect (314). This signaling by ET-1 involves G_iβγ, PKC, the Src family, and EGFR. Both AP-1 and CCAAT/enhancer-binding protein δ elements on the NGF promoter are required in this signal transduction pathway. This is consistent with *ET-1*^{-/-} mice displaying a lower number of stellate ganglion neurons and decreased staining for tyrosine hydroxylase and GAP43 which serve as markers for adrenergic sympathetic nerves and nerve sprouting, respectively. Total cardiac norepinephrine is also lower in *ET-1*^{-/-} compared with their *ET-1*^{+/+} littermates (314). Taken together, these findings indicate that ET-1 plays a crucial role in modulating NGF-driven sympathetic innervation of blood vessels and cardiac muscle, thereby providing the neuroanatomic basis for sympathetic regulation of systemic hemodynamics.

In summary, ET and ET receptors are present in sympathetic ganglia and are involved in neuronal plasticity, cardiac and vascular innervation, release of neurotransmitters, and generation of action potentials. ET_B activation, through a variety of mechanisms, generally inhibits sympathetic nerve activity. Thus the ET system in the peripheral sympathetic nervous system has the potential to participate either directly or indirectly in BP regulation.

3. Physiological effects of ET in ganglia and peripheral nerves—ET effects on peripheral sympathetic nervous system BP control are complex; these can be depressor or pressor and involve multiple organ systems. In general, the field is relatively poorly understood, with a number of conflicting results. Despite these problems, the following discussion will attempt to synthesize our current understanding of this system as it relates to BP control and Na homeostasis.

A) RENAL AFFERENT NERVES: The ET system may modulate afferent inputs from renal mechanosensory nerves that influence renorenal reflexes (389, 392, 394). Afferent renal nerves located in the renal pelvic wall are activated by increases in renal pelvic pressure (393); an increase in afferent renal nerve activity elicits a reflex decrease in efferent renal sympathetic nerve activity resulting in diuresis and natriuresis (395). This mechanism may be important in regulating Na excretion in that salt-sensitive hypertension develops in rats after interruption of renal afferent inputs (391, 392). Recent studies suggest that ET_B activation on Schwann cells surrounding renal afferent nerves plays a role in noradrenergic mediated release of PGE₂; PGE₂, in turn, induces the release of substance P which stimulates the renal afferent nerves, thereby activating the renorenal reflex (392, 394). In keeping with this proposed mechanism, ET_B blockade within the renal pelvis in rats fed a high-Na diet attenuates renal afferent nerve activity in response to increased pelvic pressure (392). Thus, under conditions of high dietary Na, ET-1 stimulation of ET_B on renal mechanosensory nerves may augment the renorenal reflex response and facilitate Na excretion.

The role of renal pelvic wall ET_A is complex. ET_A is located on the smooth muscle of the pelvic wall rather than directly on neuron fibers or nerve bundles (394). During normal or high Na intake, ET-1 exerts minimal influence on activation of renal mechanosensory nerves; however, during a low-Na diet, ET_A suppresses renal afferent nerve activity in response to increased pelvic pressure (389, 394). Kopp et al. (394) have reasoned that it is unlikely that ET_A-induced pelvic wall contraction is involved, since this would lead to increased, rather than decreased, afferent renal nerve activity. The data further suggest that the effect of ET_A is downstream of PGE₂ release.

There is evidence that ET-1 and ANG II interact in modulating the renorenal reflex. Like ET-1, ANG II suppresses the renorenal reflex (390, 392). Dual blockade of AT₁ and ET_A does not enhance renal afferent activity more than antagonism of either receptor alone, suggesting that ANG II and ET-1 impair the renorenal reflex via a common pathway (389). This notion is supported by the finding that ANG II suppression of PGE₂-induced substance P release is completely reversed by ET_A blockade. Although it has been shown that ANG II suppression of renal afferent nerve activity occurs via inhibition of PGE₂-mediated activation of adenylyl cyclase and substance P release (390), there are no studies definitively showing whether ET_A in the renal pelvis signal via this pathway. How these two pathways interact remains speculative and could involve ANG II stimulation of ET release, crosstalk between AT₁ and ET_A, or other mechanisms.

B) POSTJUNCTIONAL ACTIONS: Postjunctionally, ET-1 augments noradrenergic contractile responses in rabbit aorta (278); this response is independent of the endothelium or Ca²⁺ influx. PKC inhibition and PMA prevent this potentiation. In contrast, in the rabbit saphenous artery, postjunctional ET_A activation potentiates the contractile response to exogenous ATP, but does not influence the response to exogenous norepinephrine (515). Electrical field stimulation studies also suggest an effect of nerve-related ET on vascular tone. Short bursts of stimulation optimize the purinergic component, whereas longer duration of stimulation favors the noradrenergic component of the contractile response. In the saphenous artery, ET-1 increases the contractile response to both short-lasting and long-lasting electrical field stimulation (515), supporting postjunctional ET-1 potentiation of contractile responses to α_2 -adrenergic and P_{2x}-purinergic receptor activation. The same investigators showed that in rat tail artery, ET-1 diminishes electrical field-stimulated release of norepinephrine and ATP via ET_B activation. In contrast, ET-3 increased the release of both norepinephrine and ATP, but only at very high concentrations of the peptide (516); this ET-3 effect was inhibited by both ET_A- and ET_B-selective antagonists. Overall, the effects of ET peptides on neurogenically induced vascular contractile responses appear to vary depending on the ET peptide and ET receptors, the vascular bed, the species, and the neurotransmitters involved. Nonetheless, the available evidence suggests that the ET system can regulate vascular resistance, not only by direct action on the vascular smooth muscle and endothelium, but also by modulating neural inputs at the neuroeffector junction.

C) SYMPATHETIC GANGLIA: Several studies indicate that ET in sympathetic ganglia may participate in BP regulation in health and hypertension. One common theme for such regulation relates to ET induction of oxidative stress. Several models of hypertension are associated with increased formation of reactive oxygen species (49, 64, 255, 274, 417, 498, 603, 713), while reducing superoxide decreases BP (49, 100). Induction of reactive oxygen species by ET receptor activation in the sympathetic ganglia may cause sympathoactivation and elevated BP. Dai et al. (135) found that superoxide levels in prevertebral sympathetic ganglia were elevated in DOCA-salt hypertensive rats. Exposure of celiac ganglia from the normotensive rats to ET-1 increased superoxide production via ET_B activation. Although ET-1 levels were similar in ganglia from DOCA and control rats, ET_B mRNA and protein were higher in the DOCA-salt animals. In vivo infusion of S6c increases superoxide levels in the inferior mesenteric ganglion by two mechanisms: directly by ET_B activation and indirectly by the pressor effect itself (421). Since BP declines to a greater extent after ganglionic blockade in DOCA-salt hypertensive rats than in control rats (204), these investigators suggested that, in the context of up-regulated ET_B in DOCA-salt hypertensive rats, ET-1 may augment reactive oxygen species in the sympathetic ganglia, thereby potentially leading to heightened sympathetic excitability that contributes to the hypertension.

Other studies on the role of ET system in sympathetic ganglia have drawn attention to regulation of venous capacitance. The increase in venomotor tone in DOCA-salt

hypertension is due to sympathetically mediated venoconstriction (204, 342). Continuous infusion of S6c over 5 days into rats causes a ~15 mmHg rise in BP associated with venoconstriction and reduced venous capacitance (203, 437); there is also an ET_B-mediated increase in superoxide levels in sympathetic ganglia, but not in arteries or veins. Either surgical ablation of the celiac ganglionic plexus or antioxidant treatment with tempol attenuates the pressor response (437). These findings suggest that hypertension associated with infusion of an ET_B agonist is associated with an enhanced sympathetic outflow to the splanchnic venous circulation and that the sympathoexcitation is provoked by oxidative stress.

These observations with infusion of ET_B agonist infusion are particularly noteworthy in the context of the rescued ET_B-deficient rat (226, 296, 542, 764). Since the ET_B-deficient rat was rescued from its lethal phenotype by a transgene harboring the wild-type ET_B cDNA driven by the dopamine β -hydroxylase promoter, ET_B-deficient rats express ET_B in nervous tissue such as adrenal medulla and sympathetic ganglia (227, 402, 542). Initial studies found that the ET_B-deficient rats manifested salt-sensitive hypertension that was prevented by amiloride, suggesting a role for renal ENaC (227). However, Ohhita et al. (542) have performed renal cross-transplantation experiments that unmasked an extrarenal neurogenic component of the hypertension. Specifically, resting tachycardia and elevated BP on high-salt diet segregated with the ET_B-deficient phenotype of the recipient rather than the kidney. Furthermore, ganglionic blockade caused a greater depressor response in the ET_B-deficient rats, suggesting augmented sympathetic tone. While it would have been highly informative to know the effect of ganglionic blockade in the cross-transplanted rats, these studies suggest that ganglionic ET_B exert a tonic vasodepressor effect.

In summary, ET and its receptors have been implicated in the enhanced sympathetic excitability observed in models of salt-sensitive hypertension such as the DOCA-salt and ET_B-deficient rats. Venoconstriction in the splanchnic circulation driven by ET_B-induced sympathoexcitation also appears to play a role. At least one mechanism that is involved is ET-induced production of reactive oxygen species within the sympathetic ganglia. ET impairment of afferent renal nerve inputs and attenuation of renorenal reflexes may also contribute to salt sensitivity and hypertension.

B. Central and Baroreceptor Control of Baroreflex Function

This section focuses on the mechanisms involved with CNS function (primarily baroreflex) and BP regulation. Due to the complexity of this system, a brief overview of the relevant pathways may facilitate understanding of the ensuing discussion about ET interactions (Fig. 10). High-pressure baroreceptors are located in the aortic arch and the carotid sinus. These regions, via the vagus and glossopharyngeal nerves, respectively, send nervous inputs to the nucleus tractus solitarius (NTS) in the brain stem. From the NTS, outputs project to the caudal, and from there to the rostral, ventrolateral medulla (CVLM and RVLM, respectively) and nucleus ambiguus from which emerge sympathetic and parasympathetic outputs to the periphery. Projections from the paraventricular nucleus and area postrema also modulate the NTS which, in turn, sends inputs to the paraventricular nucleus. Collectively, these regions function to regulate sympathetic and parasympathetic outflow to the heart and sympathetic output to the vasculature, kidney, and elsewhere. Interactions between these regions are complex, involving both excitatory and inhibitory signals. In our discussion, we will indicate whenever possible how ET-induced alterations in nerve activity in a given region could potentially impact the baroreflex.

Please note that much is uncertain about the role of ET-1 in regulating baroreflex and other CNS functions that impact BP. Part of the problem relates to the presence of anesthesia as well as the type of anesthesia; these can profoundly influence baroreflex function (45, 354,

695). In addition, salt balance and volume status can modify baroreflexes (551); these are not typically well controlled.

1. Sites of ET production and receptors in the CNS

A) ET PRODUCTION BY THE CNS: ET peptides and mRNA are found in hypothalamic and brain stem areas known to regulate cardiovascular function. ET-1 protein and mRNA are present in human paraventricular nuclei and the dorsal motor nucleus of the vagus (240, 241, 522, 749). ET-1 mRNA is located in human medulla oblongata (520). ECE-1 has been mapped to these same areas (522, 816). ET-3 mRNA and peptide are found in more rostral brain regions (469, 748, 749). ET-1 protein and mRNA are also abundant in choroid plexus (521) from which ET-1 may easily enter the cerebrospinal fluid (CSF). ET-1 is detectable in the CSF, and its levels may change with alterations in BP. During phenylephrine-induced hypertension, CSF ET-1 decreased (510); in contrast, in sinoaortic-denervated rats, CSF ET-1 did not decrease after phenylephrine, suggesting that afferent baroreceptor inputs regulate CNS ET-1 levels.

The area postrema, which lies outside the blood-brain barrier and has projections to cardiovascular regulatory nuclei, can respond to changes in plasma ET-1 (34, 195–197, 382, 433). In addition, ET-1 released from endothelial cells overlying the carotid sinus baroreceptors act in a paracrine manner on baroreceptor nerve endings (94). Thus mechanisms that influence endothelial production of ET-1 and circulating ET-1 may also influence the baroreflexes and neural control of cardiovascular function.

B) ET RECEPTORS IN THE CNS: ET binds to carotid sinus, vagus nodose ganglion, area postrema, ventrolateral medulla, and the NTS (382, 486, 689, 721, 796). ET-1 binds primarily to ET_A in the carotid body (486). In general, the specific ET receptor isoforms bound by ET-1 throughout the CNS are not definitively characterized.

2. ET regulation of baroreflex activity—The heterozygous ET-1-deficient mouse has elevated BP, a rather unexpected finding given the known hypertensive effects of ET-1 (408). Resting renal sympathetic nerve activity (RSNA) is higher in ET-1-deficient mice; the maximum response of RSNA to a decrease in BP is potentiated, suggesting that resetting of the baroreflex may account for part of the hypertension. Thus endogenous ET-1 may play a role in reflex regulation of RSNA (442).

Studies employing intravenous ET-1 administration suggest that the ET system may participate in regulation of baroreflex activity. The precise nature of such regulation cannot be determined from systemically administered ET or ET antagonists, since multiple hemodynamic variables are potentially impacted. Indeed, these studies have yielded conflicting results whereby both ET-1 (524) and nonspecific ET receptor antagonism (720) have been reported to reduced baroreflex sensitivity.

The effect of intracerebroventricularly (ICV) administered ET has also been determined. Several studies have shown that ICV ET-3 or ET-1 increase BP and decrease heart rate and RSNA in conscious rats (345, 641, 645, 747). Sinoaortic denervation potentiates the pressor response in normotensive rats (641, 645), abrogates the reflex bradycardia, and attenuates the decrease in RSNA (345), consistent with interruption of baroreflex buffering of the central pressor effect of ET. The increases in BP and sympathetic output induced by ICV ET-1 are mediated by a non-NMDA glutamatergic mechanism (638) and are suppressed by NO within the paraventricular nucleus (637). Intravenous naloxone potentiates the rise in BP and RSNA that occurs with ICV ET-1, most likely by attenuating baroreflex sensitivity (470). Of note, ICV ET_A blockade decreases the upper plateau and range of the baroreflex responses of both heart rate and RSNA in rats with heart failure, but has no effect on arterial

baroreflex parameters in control animals (642). Taken together, these studies indicate that centrally administered ET has a hypertensive effect that is influenced by baroreflex activity; however, they do not clearly demonstrate a direct effect of ET to modulate the baroreflex itself.

ICV ET-1 also elicits a pressor response in SHR and WKY rats; however, the response is shifted to the right in SHR (645). Centrally administered ET_A blockade decreases BP in conscious SHR, but not WKY, rats, suggesting that endogenous CNS ET-1, via ET_A, exerts a tonic hypertensive effect, at least in the SHR rat. The baroreflex buffers the pressor effect of centrally administered ET-1 in both strains, but the effect is blunted in SHR.

ET has also been administered into the cisterna magna; however, these studies have yielded conflicting results. Intracisternal ET-1 has been reported to increase BP, heart rate, and RSNA in rats; however, higher doses (>1 pmol) of ET-1 led to a delayed reduction in these parameters, in addition to suppressing the arterial baroreflex (415, 510). Furthermore, intracisternal ET-3 elicits only depressor and bradycardic responses (510). Intracisternal ET-1 or ET-3 did not change BP or heart rate in rats given intravenous phenylephrine or nitroprusside; however, baroreflex sensitivity increased (330). In addition, intracisternal ET sensitizes the cardiac baroreflex response in SHR and WKY rats (796).

In summary, central ET may modulate baroreflex activity; however, direct evidence for this is lacking from studies involving ET-1 knockout or exogenously administered ET into the circulation, the cerebral ventricle, or the cisterna magna. ICV administration of ET can increase BP, indicating that CNS ET peptides have the potential to impact systemic hemodynamics.

3. ET regulation of baroreceptor activity—ET-1 may directly regulate baroreceptor activity; however, the nature of such regulation is incompletely understood. Direct exposure to ET-1 suppresses baroreceptor activity in the dog (94). Injection of ET-1 or ET-3 into feline carotid artery depresses baroreceptor discharge (721). Perfusing the isolated rat carotid sinus with lower concentrations of ET-1 (1 nM) increases baroreceptor activity, while higher concentrations of ET-1 (10–100 nM) suppress activity. This inhibitory effect of ET-1 was mediated by ET_A activation of ATP-sensitive K⁺ channels (432). Notably, ET suppression of baroreceptor activity is most evident at high levels of BP (93). Taken together, these studies suggest that ET, possibly through paracrine mechanisms acting on baroreceptors, may interfere with baroreflex buffering of BP.

Few studies have evaluated the role of ET on cardiopulmonary baroreceptor inputs. Systemic ET_{A/B} antagonism does not alter cardiopulmonary baroreflex sensitivity in rats (244).

4. ET regulation of brain stem and spinal cord activity

A) AREA POSTREMA: The effects of ET-1 on the area postrema have received limited attention. Part of the difficulty in interpreting these studies lies in the complex structure of the region, the doses of ET-1 employed, the state of anesthesia, and other experimental factors. ET-1 can increase neuronal discharge in the area postrema (195–197) resulting in increased BP (<1 pmol ET-1) or decreased BP (>2 pmol ET-1) (196, 510). The reasons for these differing responses, including which ET receptors are involved, are unknown. The key point, however, is that low concentrations of ET-1 in the area postrema can alter BP. Since the area postrema is exposed to the circulation, and plasma ET-1 concentration is within the range of concentrations that affect area postrema activity, it may be that circulating ET-1 can modify BP through modulation of CNS activity.

B) NTS: As for other regions of the brain, ET actions on the NTS are complex and controversial. Indeed, opposing effects of ET-1 on the NTS have been obtained by different laboratories using varying experimental preparations and designs. ET-1, either given into the rat fourth ventricle or directly into the dorsal strip and commissural area of the NTS, reduced BP and heart rate (267, 510). Bilateral microinjections of ET_A antagonists into the NTS of rats elicited an initial pressor response (albeit followed by hypotension) (508). In agreement with this, ET-1 or glutamate applied to brain stem slices stimulated NTS neuronal discharge from both WKY and SHR rats (293, 693); notably, ET-1 enhanced the response to glutamate in WKY, but not in SHR, rats. Increased NTS neuronal activity would be expected to reduce sympathetic efferent activity and lower BP, thereby augmenting baroreflex buffering of BP. That ET-1 does not enhance the response to glutamate in SHR rats suggests that the NTS component of the reflex is impaired in SHR. ET_A antagonism attenuates NTS neuronal activity and blocks the ET-1-induced increase in glutamatergic neuronal discharge (693). Further studies indicate that a non-NMDA (AMPA-like) receptor likely mediates the facilitatory effect of ET-1 on glutamatergic transmission in NTS (692).

In contrast to the above studies, others have reported that ET-1 inhibited neuronal activity in the commissural NTS (197). Furthermore, unilateral microinjection of ET-1 into the NTS increased BP (134). Prior injection of the ET_A inhibitor blocked this effect, although the antagonist alone elicited no change in these hemodynamic parameters. Pretreatment with hexamethonium abrogated the pressor response consistent with a sympathetically mediated mechanism. The reasons for these opposing effects of ET-1 are speculative; however, it could be relevant to note that the NTS is a large nucleus consisting of several subnuclei (36); it may be that individual subnuclei exhibit different responses to ET-1 and/or that ET receptors exist on either excitatory or inhibitory neurons.

C) VENTROLATERAL MEDULLA: Few studies have evaluated the response to ET-1 in the ventrolateral medulla. Micro-injection of ET-1 into the RVLM evokes a pressor and bradycardic response with increased RSNA, followed by a prolonged decrease in BP (510, 512); these effects appear to be ET_A mediated. Similarly, transient pressor responses followed by hypotension were observed with low doses of ET-1 (<2 pmol) given bilaterally to RVLM, while higher doses (>8 pmol) caused a sustained fall in BP (451); again, these responses appeared to be ET_A mediated. In the CVLM, ET-1 decreases BP and RSNA, but increases heart rate, suggesting suppression of both sympathetic and parasympathetic activity (512). In contrast, intracisternal or topical application of ET-1 to the ventral surface of the medulla in an area subjacent to the RVLM in rats excited vasomotor neurons without altering BP or heart rate (414). ET_A blockade within the RVLM in rats prevented vasomotor neuron excitation by exogenous ET-1, but did not alter their basal firing rate (406). Thus, as for other regions in the brain stem, ET-1 effects on the ventrolateral medulla appear to be complex, potentially involving different responses depending on which neurons are affected.

D) SPINAL CORD: Very limited and conflicting data are available on ET in the spinal cord. ET-3 in superfusates of spinal cord directly correlates with resting BP (366); maneuvers that increase sympathetic activation, such as hypotensive hemorrhage, increase ET-3. However, at doses that do not alter spinal blood flow, intrathecal application of ET-1 or ET-3 reduces BP and heart rate (262). This depressor response is associated with sustained hind-quarter vasodilation and transient mesenteric vasoconstriction with no change in renal blood flow.

E) SUMMARY: Overall, the CNS and baroreceptors can synthesize and bind ET-1. CNS ET can modulate systemic hemodynamics; however, the mechanisms responsible for this are poorly understood. ET may modulate baroreflex activity; in general, although the studies are not consistent, it appears that ET tends to attenuate baroreflex sensitivity. This would impair

baroreflex buffering of increases in BP and conceivably predispose to hypertension. In addition, the ability of the baroreflex to buffer the pressor actions of centrally administered ET may be attenuated in hypertensive subjects; this could also facilitate higher BP.

VII. ENDOTHELIN AND THE HEART

A. Overview of ET and the Heart

The role of the ET system in regulating cardiac function has been extensively studied. Particular emphasis has been placed on the pathophysiological role of ET in coronary ischemia/reperfusion injury, cardiac hypertrophy, congestive heart failure, and arrhythmias. It is evident that the ET system is involved in all of these pathological processes, and this continues to be an exciting area of ongoing research. The current review, however, is primarily focused on the role of the ET system in the physiological control of BP and Na homeostasis; hence, the quite extensive literature on ET in cardiac pathology will not be discussed. Rather, we will examine whether endogenous cardiac ET is involved in normal changes in cardiac output related to alterations in plasma volume and/or BP. Relatively little is understood about direct ET chronotropic effects (i.e., directly modifying action potential); the ensuing discussion will, therefore, focus on the inotropic effects of ET peptides. For more detailed reviews of ET actions in the heart, please see the cited references (75, 179, 733). In addition, please see the section on the nervous system for discussion of the role of ET in mediating nervous reflex responses to alterations in plasma volume and BP, including those effects leading to changes in cardiac inotropy and chronotropy.

B. ET Production by the Heart

While ET-1 seems to be made by all endothelial cells, within the heart or not, there has been some controversy over ET-1 production by cardiac myocytes. Early studies, using neonatal cardiomyocytes from rat in culture, detected ET-1 mRNA and mature peptide release into the media (328, 740). Other groups have also reported ET-1 production by cardiomyocytes, including acutely isolated adult porcine (775) and rat neonatal cardiomyocytes (576). Chick embryonic cardiomyocytes contained ECE mRNA (683). In contrast, Preisig-Müller et al. (594) reported that PCR of carefully isolated cells from adult guinea pig hearts revealed that ET-1 mRNA was expressed by endothelial cells, but not by cardiomyocytes. A different group found that acutely isolated rat adult cardiomyocytes did not contain ET-1 mRNA (487); furthermore, they noted that cardiomyocytes released a factor, most likely ANG II, that stimulated vascular ET-1 production. On the basis of these studies, it has been speculated that healthy adult, as opposed to neonatal or embryonic, cardiomyocytes do not produce ET-1. This assertion has been challenged, however, by studies in which cardiomyocyte-specific disruption of the ET-1 gene caused mice to be relatively resistant to hyperthyroid-induced cardiac hypertrophy (697); this study also found that adult mouse cardiomyocytes contained ET-1 mRNA. Taken together, it seems most likely that adult cardiomyocytes produce relatively little ET-1 under normal circumstances; however, this does not necessarily preclude such ET-1 from serving a physiological role.

It is possible that ET-1 derived from endothelial or vascular smooth muscle cells in the heart can directly or indirectly regulate cardiomyocyte function (76, 484). As such, ET-1 release by these cell types is subject to modification by multiple factors known to influence vascular ET-1 production, including vasoactive compounds, inflammatory substances, cytokines, growth factors, and others. Whether this has physiological impact is uncertain. In contrast, the factors regulating ET-1 production by cardiomyocytes, albeit perhaps in relatively (as compared with vasculature) small amounts, are not well ascertained. Furthermore, such regulation has been examined primarily in the context of pathophysiological conditions, such as cardiomyocyte growth, proliferation, and/or apoptosis. However, one study has

directly examined cardiomyocyte ET-1 biosynthetic pathways in response to varying mechanical load, i.e., under conditions that may mimic physiological states (576). These investigators found that neonatal rat ventricular myocytes exposed to cyclic mechanical stretch in vitro for as little as 2 h had increased ET-1 secretion, mRNA levels, and activity of a transfected ET-1 promoter-reporter construct; curiously ET-1 synthesis was reduced in stretched cardiac fibroblasts. Inhibition of stretch-activated ERK prevented the increases in transcriptional activity in the myocytes. In addition, Yamazaki et al. (855) showed that mechanical stretch stimulated ET-1 protein release and mRNA accumulation in rat neonatal cardiomyocytes after only 10–20 min of stimulation. Another group found that stretching cat heart papillary muscle for as little as 15 min increased ET-3, but not ET-1, mRNA and that this effect was reduced by ANG II receptor blockade (181). As will be seen, these findings are relevant to studies suggesting that ET is involved in the myocardial response to preload.

C. Inotropic Effects of ET

1. Inotropic effects of ET: physiological relevance—In general, ET exerts a positive inotropic effect (increases cardiac contractility); however, results have varied widely depending on species, developmental stage, preparation, ET dose and duration of exposure, nerve activity, cardiac chamber, hormonal milieu, and underlying cardiac pathology. A detailed discussion of ET effects on cardiac contractility can be found elsewhere (75, 569, 733); we will focus on the key aspects that potentially bear on BP and volume regulation. ET-1 enhances cardiomyocyte contractility in several species, including guinea pig, mouse, rat, rabbit, ferret, cat, dog, and human (109, 145, 376, 488, 531, 751, 810). However, others have either failed to detect an effect of ET on myocardial contractility (50) or have observed a negative inotropic effect (108, 109, 333, 662, 881). The reasons for the discrepant results are likely multifactorial and partly relate to the factors mentioned above. For example, ET-1 produced a negative inotropic effect in embryonic chick and neonatal rat ventricular myocytes, while, in the same study, ET-1 augmented cell contraction in adult rabbit ventricular myocytes (376); this suggests that ET-1's effects vary by species and/or developmental stage. In addition, while ET-1 did not contract dog ventricular myocardium, it had a positive inotropic effect in the presence of low (0.1–1 nM) concentrations of norepinephrine and a negative inotropic effect in the presence of high (0.1–1 μ M) concentrations of norepinephrine (109). This biphasic response was attributed to evoked differences in $[Ca^{2+}]_i$. However, another group found that, in the presence of β -adrenergic blockade, ET-1 decreased isolated mouse cardiomyocyte shortening without affecting Ca^{2+} transients (662). An additional factor is that ET-1 can cause coronary vasoconstriction, an effect that potentially opposes the peptide's positive inotropic action (50). Finally, Namekata et al. (531) reported that ET-1 effects on isolated mouse cardiomyocytes depended on which receptor was activated, wherein ET_A -mediated enhanced contractility and Ca^{2+} sensitivity, while ET_B did the opposite. Taken together, the above studies indicate that ET has a complex effect on myocardial contractility that may involve opposing actions depending on the prevailing conditions.

While the above studies examined ET effects on inotropy, they did not assess the true physiological inotropic effect of endogenous cardiac ET. Knockout studies in mice, in which the ET-1 gene was disrupted specifically in cardiomyocytes, revealed that the animals developed a dilated cardiomyopathy associated with increased cardiac cell apoptosis (880). However, up until 7 mo of age, there was no apparent difference in left ventricular function, suggesting that while cardiomyocyte-derived ET-1 is necessary for cardiomyocyte survival, it may not be crucial for normal heart function during much of the animal's life. Similarly, mice with cardiomyocyte-specific deletion of the ET_A gene exhibited normal baseline and ANG II-stimulated cardiac contractility (357); since ET_A likely mediates the inotropic effects of ET (please see discussion below), this suggests that ET modulation of

cardiomyocyte contractility is not of physiological relevance. In contrast, direct infusion of BQ123, an ET_A-selective antagonist, into the left coronary artery of patients with atypical chest pain decreased contractility, suggesting the ET, via ET_A, exerts a tonic positive inotropic effect (452). In vitro studies also suggest that endogenous ET may affect cardiac contractility. In response to stretch, cardiac muscle rapidly (Frank-Starling mechanism) and slowly over several minutes (slow force response) increases muscle shortening and/or developed force; ET may be involved in the slow force response (114). Pérez et al. (570) demonstrated that ET_A or nonselective ET receptor blockade prevented the slow force response in cat cardiac papillary muscle after mechanical stretch (570). Curiously, this same group reported that ET_A antagonism in the same preparation did not reduce exogenous ET-1-stimulated cardiomyocyte contraction and that cardiomyocyte stretch or ANG II stimulation increased ET-3, but not ET-1 or ET-2, mRNA (181). Strangely, ET_A blockade inhibited the contractile response to ET-3; since ET-3 has very low affinity for ET_A, it remains an open question as to whether ET-3 is really the relevant ET isopeptide mediating the autocrine ET slow force response to myocardial stretch. This latter group also found, as noted earlier, that stretch-induced ET-3 mRNA induction was prevented by ANG II receptor blockade, suggesting that ET induction under these circumstances is secondary to increased ANG II levels. Furthermore, the positive inotropic effects of low-dose ANG II were prevented by nonselective ET receptor blockade, while ANG II receptor blockade did not prevent the positive inotropic effect of ET-1 in cat papillary muscle (571). Finally, ET receptor blockade did not affect the Frank-Starling responses in intact hearts from normal rats (579). Taken together, the above studies suggest that endogenous cardiac ET-1 (and perhaps ET-3), possibly derived from cardiomyocytes, can partly mediate the slow force response to mechanical stretch. This auto-crine/paracrine ET pathway may be of importance in cardiac adjustments to altered preload in humans, thereby potentially playing a physiological role in the response to alterations in ECFV.

2. Receptors mediating inotropic effects of ET—The bulk of evidence indicates that cardiomyocyte ET_A mediate the positive inotropic effect of ET-1. Several studies indicate that ET_A is the predominant ET receptor isoform on cardiac myocytes in rat, dog, and human (42, 497, 733), although ET_B are present. As discussed above, ET_A mediates the slow force response to mechanical stretch of cat papillary muscle (570). In a number of preparations, including the isolated perfused mouse heart, ET-1 is substantially more potent than ET-3 in stimulating cardiac contractility, and the effect is inhibited by ET_A blockade (358). A number of studies have shown that while ET_A enhances cardiac contractility, ET_B can exert a negative inotropic effect. In the isolated perfused mouse heart, ET_A elicited enhanced force generation, while ET_B activation mitigated the response (578). Similarly, ET-1 increased tension in rat papillary muscle via ET_A, while ET_B agonism opposed this effect (427). In pig intact hearts, the positive inotropic effect of ET-1 was mediated by ET_A, while ET_B effected negative inotropy (388). There is not, however, uniform consensus on ET receptor isoform effects on cardiac contractility. Before ET receptor antagonists were available, it was noted that ET-1 and ET-3 had similar contractile potency in rabbit papillary muscle (751). Beyer et al. (50) found that ET-1 increased contractility in exposed rat hearts in the presence of ET_A blockade (although it was not possible to entirely rule out a positive inotropic effect due to coronary vasodilation). Another group made the rather confusing finding that, in isolated human cardiac tissue, S6c was more potent than ET-1 in enhancing contractility; however, ET_B blockade had no effect on the response to either agonist (79). ET_A blockade had a modest preventative effect, while combined ET_A/ET_B antagonism was significantly inhibitory. These latter studies raise the possibility, therefore, that some cooperativity may exist between ET_A and ET_B; furthermore, these effects may be due to activation of ET receptor isoforms on the same or different cell types. Overall, these studies point to cardiomyocyte ET_A as the predominate mediator of the positive inotropic effect of

ET peptides; cardiomyocyte ET_B may effect positive or negative inotropy depending on as yet incompletely understood factors. Finally, these considerations on ET receptor isoform function relate to cardiomyocytes; however, there are other potential mechanisms by which cardiac ET receptors could modulate contractility. For example, ET receptors on neurons in the heart may modulate cardiac norepinephrine levels (26). Such complexity of ET receptor function and location within the heart may help explain some of the varying results seen in different preparations and is an area requiring further investigation.

3. Mechanisms of ET regulation of cardiomyocyte contractility—ET-induced signaling in cardiomyocytes can lead to changes in contractility, relaxation, growth, hypertrophy, and other effects. In addition, pathways that affect physiological processes (e.g., contractility) may also impact pathophysiological processes (e.g., hypertrophy); which effects occur obviously depends on a large variety of factors. Consequently, while this discussion is confined to signaling processes most likely involved in mediating inotropic effects of ET, it is recognized that some of these pathways, under the right circumstances, can also be involved in the development of cardiac dysfunction. Excellent reviews on ET-induced cardiomyocyte signaling in health and/or disease can be found elsewhere (114, 733).

As detailed elsewhere (114), myocardial stretch leads to release of preformed ANG II from myocardial cytosolic granules which, in turn, stimulates ET-1 synthesis and release (Fig. 8). The initial event upon ET binding to cardiomyocyte ET_A is activation of PLC with resultant formation of inositol trisphosphate (IP₃) and diacylglycerol (DAG) (120). It is unclear if IP₃ plays a substantial role in alterations in [Ca²⁺]_i; however, ET-1 induces PKC activation and translocation in cardiomyocytes, most likely via DAG, and this can lead to increases in [Ca²⁺]_i (117). PKC can directly stimulate Na⁺/H⁺ exchange (NHE) in myocytes, leading to increased intracellular Na concentration and alkalization (180); inhibition of ECE in myocardium suppresses both the slow force response to stretch and the elevation in intracellular Na (570). That PKC and NHE are important in mediating ET-1-induced contraction was confirmed in studies demonstrating that the positive inotropic effects of ET-1 were inhibited by blockade of PKC or NHE (885). ET-1 stimulation of NHE, by virtue of increasing intracellular Na concentration, leads to elevated Na/Ca²⁺ exchange (NCX) and increased [Ca²⁺]_i. ET-1 increases NCX activity in reverse mode (Na out of the cell and Ca²⁺ in) in isolated cat cardiomyocytes (6). Similarly, Zhang et al. (878) found that ET-1 increases NCX activity in isolated guinea pig ventricular myocytes and that this was PKC dependent. Perez et al. (571) showed that ET-1 increased NCX activity in cat papillary muscles and that this effect was prevented by NHE blockade. The increase in NCX activity leads to increases in [Ca²⁺]_i and enhanced contractility. It is also possible that intracellular alkalization, if this does in fact occur, could increase contractility by augmenting the sensitivity of contractile proteins to Ca²⁺ (114). Taken together, the above findings suggest the following schema for stretch-induced slow force response: stretch increases ANG II, leading to increased ET-1 which, via ET_A, stimulates sequentially PLC, PKC, NHE, and NCX, causing elevations in [Ca²⁺]_i and augmented contractility.

ET-1 may modulate cardiomyocyte contractility through other mechanisms. ET-1, likely via PKC, activates several small G proteins in cardiomyocytes, including Ras, RhoA, and Rac1 (105, 119). Subsequently, ET-1 activates the cascade of protein kinases leading to ERK1/2 activation in neonatal rat ventricular myocytes (60, 62). In addition, ET-1 activates JNK and p38 MAPK in the same neonatal rat cardiomyocyte cultures (61, 118). ERK1/2 activation by ET-1 can lead to activation of the 90-kDa ribosomal S6 kinase (712) which, as noted by Sugden and Clerk (733), can increase NHE activity. In addition, ERK1 and ERK2 can directly phosphorylate and activate NHE (646). ROS may also be involved in that ET-1 can increase ROS in the heart; ROS can trigger MAPK signaling and NHE activation (114, 646).

Indeed, De Giusti et al. (145) demonstrated in isolated cat ventricular myocytes that the positive inotropic effect of ET-1 was associated with ROS generation and was reduced by blocking ROS. Thus the final common pathway of ET-1-enhanced myocardial contractility seems to be through NHE; however, NHE phosphorylation may be mediated directly by PKC, MAPKs, the 90-kDa ribosomal S6 kinase, and potentially other pathways.

D. Effects of ET on Diastolic Function

It is unknown whether cardiac-derived ET plays a physiological role in modifying diastolic function, i.e., regulation of myocardial relaxation (lusitropy). Under pathological conditions, ET may affect diastolic function through changes in chamber size, stiffness, or thickness, but this will not be addressed herein. Relatively few studies have addressed ET effects on lusitropy. To avoid the potentially confounding effects of coronary artery vasoconstriction, Leite-Moreira et al. (426, 427) examined the effect of exogenous ET-1 on diastolic function in rat papillary muscle preparations. ET-1 increased the rate of myocardial relaxation (positive lusitropic effect) through an ET_A-dependent pathway. The same studies found that ET-1 increased diastolic distensibility in stretched papillary muscles, again via ET_A activation. More recently, this same group noted that ET_B on the endocardial endothelium were involved in the positive lusitropic effect of ET-1 (70). The authors speculated that, even though the cardiac muscles were stretched beyond normal maximal limits, no decrease in contractility was observed (75), suggesting that ET-1 might facilitate the ventricle achieving higher volumes without undue increases in filling pressure or impaired contractile function.

VIII. ENDOTHELIN AND THE PATHOPHYSIOLOGY OF HYPERTENSION

Since Yanagisawa's description of the potent and prolonged hypertensive response to ET-1 infusion in a ganglion blocked rat (857), investigators have been trying to understand the role of ET in the development and maintenance of hypertension. This section briefly examines some of these studies, primarily focusing on those that illustrate how alterations in the physiological processes we have discussed above may lead to impaired BP regulation. In addition, emphasis will be placed on those studies that have examined the renal ET system in hypertension, since this organ has been a major area of investigation into the physiological role of ET in the regulation of BP and Na homeostasis. More extensive reviews of ET in hypertension, including those examining clinical trials of ET antagonists, can be found elsewhere (2, 152, 153, 581).

A. ET in Animal Models of Hypertension

Several animal models of hypertension are associated with increased vascular ET-1 synthesis (669). Since vascular injury per se augments ET-1 production, it has been difficult to determine whether these changes in the ET system are a cause or consequence of hypertension (669). Certainly, overactivity of vascular ET-1 under special circumstances has the potential to elevate BP; however, chronic infusion or genetic overexpression of ET-1 in an otherwise normal animal does not cause hypertension presumably due to the efficient clearance of ET-1 from the circulation (15, 507). However, chronic infusion of ET-1 can cause hypertension when animals are maintained on a high-salt diet (15, 507, 588). Similarly, mice overexpressing ET-1 become hypertensive when given a high-salt diet (14). Increased endogenous ET-1 levels caused by genetic or pharmacological disruption of ET_B results in hypertension (227, 588) due, at least in part, to reduced ET_B clearance of ET-1 and enhanced ET_A activation since ET_A blockade prevents the development of hypertension (169, 588). ET receptor blockers also reduce BP and improve vascular function in a variety of animal models of hypertension, suggesting that endogenous ET-1 contributes to increased vasomotor tone, reduced endothelial function, and vascular remodeling (315, 669).

However, increased vascular ET-1 activity is not the full explanation for the hypertension in experimental hypertension, since the increased BP produced by chronic ET-1 infusion or ET_B deficiency is salt dependent, suggesting that renal Na excretion plays a significant role (170, 227, 506, 588). Thus, while vascular ET-1 may be of pathophysiological relevance in hypertension, it is not the full explanation nor is it necessarily the primary event.

Numerous studies support the hypothesis that renal ET-1 participates in the pathogenesis of hypertension (152, 315, 669). However, the nature of such pathophysiological regulation is complex and, similar to the vasculature, likely depends on the degree of renal damage; any form of renal injury, regardless of the underlying cause, increases renal ET-1 production (367). Indirect evidence for a role of renal ET-1 derives from experiments in which both ET_A and combined ET_{A/B} antagonists lower BP in salt-sensitive models such as DOCA-salt, chronic ANG II infusion, SHR-stroke prone, and Dahl salt-sensitive rats (11, 33, 38, 351, 436, 546, 604, 722). However, such evidence is clearly not conclusive, particularly since some hypertensive models, such as the SHR and the two-kidney, one-clip Goldblatt hypertensive rats, appear unresponsive to ET receptor blockade (434, 435). Furthermore, other models not associated with high salt intake respond to ET receptor blockade with lower BP, including the eucapnic intermittent hypoxia rat (10).

More direct evidence for a role of intrarenal ET-1 comes from studies examining renal ET-1 production in hypertension. Curiously, investigators have found that intrarenal ET-1 may be either increased or decreased in hypertension. Such differences may relate to the region of the kidney that was analyzed as well as the patho-physiological state of the kidney. For example, in many of the studies, renal fibrosis and/or inflammation was not extensively examined; since, as alluded to earlier, renal injury increases ET-1 production, it could be that changes in renal ET-1, at least in some experimental models, are a consequence and not a cause of hypertension. Bearing these caveats in mind, it is notable that several animal models of high salt intake-induced hypertension are associated with increased expression of preproET-1 mRNA in renal cortical tissue along with greater sensitivity to ET receptor blockade (2, 534, 581); presumably, the increased cortical ET-1 production could exacerbate renal vasoconstriction. The increase in intrarenal production of ET-1 in animal models of hypertension may be related to dietary salt intake as much as hypertension per se. For example, urinary excretion of ET-1, an index of intrarenal production, is elevated in chronic ANG II hypertension; however, the increase associated with salt intake is at least 10-fold greater in magnitude (665). In contrast, renal medullary ET-1 content has been reported to be decreased in several experimental models of hypertension, including in SHR, Dahl S, and Prague hypertensive rats (2, 244, 250, 310, 803). Such decreased medullary ET-1 content may be due, at least in part, to an intrinsic reduction in IMCD ET-1 production, since cultured SHR IMCD cells synthesize less ET-1 than do WKY IMCD cells (310). Since collecting duct-derived ET-1 exerts a tonic natriuretic and diuretic effect, it is possible that decreased IMCD ET-1 production contributes to the hypertension.

Changes in renal ET receptor expression and/or activity may occur in hypertension. In the DOCA-salt model of hypertension, the increase in renal ET-1 excretion is also associated with an increase in renal medullary ET_B-mediated ET-1 binding (582). Furthermore, the ratio of ET_A to ET_B is significantly reduced in SHR compared with normotensive Sprague-Dawley rats (237). Such a relative increase in renal ET_B expression would favor a natriuretic effect, potentially providing a compensatory response to the increase in salt load and/or hypertension.

B. ET in Human Hypertension

Initial efforts to determine a role for ET in human hypertension came from measurements of immunoreactive ET in plasma. Several reports suggested that patients with hypertension

have elevated plasma ET levels (12, 339, 430, 545). However, many other studies reported no difference in plasma ET-1 levels between normotensive and hypertensive subjects (68, 299, 428, 732). Even within the same group of investigators, there are reports that plasma ET-1 levels are either unchanged or decreased in subjects with hypertension when placed on a high-salt diet (67, 68). The reasons for these disparate results most likely relate to a variety of factors, including specificity of the antibodies used in the immunoassay, degree of vascular injury (671), dietary salt intake (335, 457), obesity (198), diabetes (430), and race (182, 183, 190, 783). Another key factor in measuring plasma ET-1 in humans relates to the effect of stress at the time the blood sample was taken (428, 783). This has been observed in both humans (190, 783) and animals (129, 130). Notably, plasma ET-1 levels are elevated as a result of venopuncture but stabilize once vascular access has been achieved (783); most studies do not describe whether blood samples are taken by immediate venopuncture or from established vascular access. It is not clear whether stress-dependent changes are the result of sudden release or reduced clearance, nor is it clear whether this is endothelial or neuronally derived. After stress is accounted for, plasma ET-1 levels are elevated in African Americans compared with age-matched Caucasians (183, 190, 783); such findings may be of pathophysiological relevance given the relatively high prevalence of salt-dependent hypertension in African Americans (265, 821). Nonetheless, taken together, the available evidence does not strongly support a role for circulating ET-1 in the pathogenesis of human hypertension.

A strong correlation between urinary ET-1 (which derives entirely from the kidney) and Na excretion has been reported in humans (199, 457) that appears particularly strong in African American youths (335). In both rats and humans, renal ET production appears closely related to salt intake and is not influenced by changes in systemic BP (335, 457, 588). Furthermore, unlike plasma ET-1, urinary ET excretion is increased by a high-salt diet but is unaffected by either ET_A or ET_B antagonists (588). Interestingly, and analogous to findings in animals with experimental hypertension, urinary ET excretion has been reported by some investigators to be reduced in subjects with hypertension. Hoffman et al. (299) noted that increased dietary salt intake augmented urinary ET excretion in salt-resistant, but not salt-sensitive, hypertensive subjects, suggesting that a defect in the pro-natriuretic capacity of the ET system could contribute to salt-dependent hypertension. Similarly, decreases in urinary ET-1 excretion have been observed in patients with essential hypertension (313). In addition, urinary ET excretion is increased in normal pregnancy, but not in individuals who develop preeclampsia (811). Renal hypertension is associated with higher rates of urinary ET excretion compared with subjects that are normotensive or have essential hypertension (882). In contrast, others have reported that urinary ET excretion is elevated in hypertension (199). Unfortunately, all of these studies are based on small numbers of patients; thus it remains uncertain if and what changes in renal ET-1 production occur in human hypertension.

A wide range of ET receptor antagonists have been investigated for therapeutic utility in human hypertension and other cardiovascular diseases (152, 315, 361). Kirkby et al. (361) have recently published a thorough review of these studies from a pharmacological perspective. Several ET antagonists are available for the treatment of pulmonary hypertension. The rationale for their use has been reviewed elsewhere and is beyond the scope of the present review (40, 102, 595). In systemic human hypertension, ET antagonists have also been shown to be effective at reducing BP (152, 315, 361). Preliminary data from an on-going phase III trial for resistant hypertension have recently suggested that darusentan, an ET antagonist with a moderate degree of selectivity for ET_A, may be effective at producing significant reductions in BP beyond ANG II antagonism and other therapies (820). Additional clinical studies will also be necessary to discern ET receptor-specific actions in human hypertension.

IX. SUMMARY

ET affects virtually every system that regulates BP and Na homeostasis. In general, ET_B activation reduces BP and promotes urinary Na excretion since ET_B causes 1) endothelial cell NO production with resultant vasodilation; 2) inhibition of Na transport in the proximal tubule, thick ascending limb, and collecting duct; 3) reduced water reabsorption in the collecting duct; 4) inhibition of nerve-stimulated adrenal catecholamine release; 5) inhibition of renin release; and 6) possibly negative inotropy (Fig. 11). Furthermore, intravenous administration of ET_B-selective agonists typically reduces BP and increases urine volume, while ET_B-deficient rodents are hypertensive. In contrast, ET_A generally increases BP since activation of this receptor causes 1) vasoconstriction, 2) enhancement of nerve-stimulated adrenal catecholamine release, and 3) positive inotropy (Fig. 12). Administration of ET_A-selective antagonists typically reduces BP. However, this simple paradigm does not hold up for all organ systems, since both ET_A and ET_B can enhance basal adrenal catecholamine release, aldosterone production, and AVP secretion. Furthermore, ET_A activation increases cardiac ANP secretion, while ET_B can cause vasoconstriction under some circumstances. Finally, both ET_A and ET_B are involved in the neurologic control of BP, although their precise effects are uncertain. In essence, the ET system must be viewed as an autocrine and paracrine system; understanding how ET regulates BP and Na homeostasis will only be achieved by examining this system in the context of the local environment.

This complexity has greatly confounded clinical studies in which ET_A or nonselective ET receptor blockers have been administered (ET_B-selective blockers are not used clinically since they can increase BP and promote fibrosis). These agents are now used for treating pulmonary hypertension and are in clinical trials for use in diabetic nephropathy, resistant hypertension, systemic sclerosis, multiple forms of cancer, and other diseases (41). However, treatment with either ET_A or nonselective ET receptor blockers is associated with a high incidence of hemodilution and edema formation, strongly suggestive of renal fluid retention (41). This adverse effect has had significant clinical consequences, possibly being responsible for the failure of these drugs to improve cardiovascular outcomes in patients with congestive heart failure. As another example, a recent phase III trial in patients with diabetic nephropathy found that ET_A blockade caused a 50% reduction in proteinuria; however, the trial was discontinued due to problems associated with fluid retention (824). Thus continued studies are clearly needed that define how these ET receptor antagonists exert their effects on Na homeostasis. While much work has been done, we still need to better define how individual organ systems involved in the control of BP and Na balance are regulated by ET.

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Fig. 1.

Biosynthetic and degradation pathways for endothelin (ET)-1. ET-1 mRNA encodes preproET-1. The short signal peptide is cleaved to yield proET-1 which, in turn, is cleaved by furin or PC7 convertases at dibasic amino acids to yield Big ET-1. Big ET-1 is cleaved by different ET converting enzymes (ECE) to mature ET-1. ET-1 is degraded by neutral endopeptidase and deamidase.

Fig. 2.

Schema of ET in vasculature. Endothelial cells express ET_B exclusively and are the predominant vascular source of ET-1. ET-1 and nitric oxide synthase 3 (NOS3) can increase ET_B activity or amount, respectively, leading to nitric oxide (NO) and PGE₂ production with resulting vasorelaxation. Activation of vascular smooth muscle ET_A or ET_B leads to a signaling cascade involving G proteins, phospholipase C (PLC), and inositol trisphosphate (IP₃) that activate voltage-operated Ca²⁺ channel (VOC) and sarcoplasmic reticulum (SR)-mediated increases in [Ca²⁺]_i and calmodulin (CaM) activation. CaM, together with activation of protein kinase C (PKC) by diacylglycerol (DAG) and Ras/Raf/ERK1/2 activation, causes myosin light-chain kinase (MLCK) activation and cell contraction.

Fig. 3. Schema of functional ET receptors in the glomerulus and renal arterioles (A), nephron (B), and vasa recta (C). The amount of ET receptor shown in a given area is representative of the level of ET receptor activity in that region. Afferent arteriolar smooth muscle has more vasoconstrictive ET receptors than do efferent arterioles, while efferent arteriole endothelium has more vasodilatory ET_B than does afferent arteriole (A). Podocytes and mesangial cells contain primarily contractile ET_A (A). The inner medullary collecting duct (IMCD) has the greatest density of natriuretic ET receptors, although natriuretic ET receptors exist in the cortical collecting duct (CCD), thick ascending limb (TAL), and proximal tubule (PT) (B). Vasa recta express contractile ET_A on pericytes and vasodilatory ET_B on endothelial cells (C).

Fig. 4.

Synthesis and actions of ET-1 in the proximal tubule. ET-1 production is enhanced during inflammation, hypoxia, glomerular injury, and acidemia. Most studies implicate ET_B in mediating ET effects on the proximal tubule, although ET_A activation may result in inhibition of Na reabsorption. ET_B effects appear to depend on the concentration of ET-1, with lower concentrations stimulating Na transport processes and higher concentrations having the opposite effect. It is likely that ET-1 exerts primarily a natriuretic effect on the proximal tubule under physiological conditions. See text for definitions.

Fig. 5.

Synthesis and actions of ET-1 in the thick ascending limb. ET-1 production is stimulated by increased medullary osmolality which occurs during high Na intake. ET-1 can then act in an autocrine manner, via ET_B, to stimulate NOS3 activity and inhibit NKCC2. ET_B may also increase 20-HETE with possible inhibition of Na⁺-K⁺-ATPase activity. See text for definitions.

Fig. 6. Synthesis and actions of ET-1 in the collecting duct. ET-1 gene transcription is under complex control, involving transactivators binding to *cis*-elements in the ET-1 promoter, as well as histone methylation. The latter effect mediates aldosterone stimulation of collecting duct ET-1 production; this may serve as a negative-feedback regulator of aldosterone-stimulated Na transport in this nephron segment. ET_B mediates ET-1 inhibition of water transport, primarily through inhibition of AVP-stimulated adenylyl cyclase (AC) activity. ET_B also mediates ET-1 inhibition of ENaC activity; this involves both NO and MAPK. V2 and AT₁ receptors have been reported to inhibit ET_B expression in this nephron segment. The role of ET_A in regulating collecting duct Na and water transport is uncertain. See text for definitions.

Fig. 7. Synthesis and actions of ET-1 in adrenal cortical zona glomerulosa cells. These cells can synthesize ET-1. ET-1 increases aldosterone production via activation of both ET_A and ET_B, although there is uncertainty as to whether these effects are autocrine and/or paracrine mediated. ET_A activation also leads to zona glomerulosa cell growth and proliferation, increasing the potential to synthesize aldosterone. See text for definitions.

Fig. 8.

Synthesis and actions of ET-1 in cardiomyocytes. Stretch, via angiotensin II (ANG II), stimulates ET-1 production. ET-1 can act in an autocrine manner to activate ET_A. This leads through several signaling pathways to increased Na⁺/H⁺ exchange activity which, in turn, activates Na⁺/Ca²⁺ exchange, thereby elevating [Ca²⁺]_i, leading to enhanced cell contraction. ET_B activation opposes this effect. Stretch, through as yet undefined mechanisms, stimulates ET-1 activation of ET_A which leads to increased ANP release. While components of the indicated pathways can regulate ANP production, there remains much uncertainty as to which signaling systems are predominantly involved in the ET-1 effect. See text for definitions.

Fig. 9.

Schematic representation of ET-induced AVP secretion. Circulating ET-1 can stimulate neuronal activity in circumventricular organs via ET_A in the subfornical organ (SFO) or ET_B in the area anteroventral to the third ventricle (Av3V). These areas send projections to the supraoptic nucleus (SON) and paraventricular nucleus (PVN) which contain the vasopressinergic magnocellular neurons that release AVP from axon terminals in the posterior pituitary (PP). Stimulation of ET_A or ET_B within the SON leads to inhibition or stimulation, respectively, of AVP secretion. In addition, inputs to the PVN are further integrated; projections from the PVN to brain stem loci lead to increases in sympathetic efferent output and blood pressure. A-, inhibitory ET_A effect; A+, stimulatory ET_A effect; B+, stimulatory ET_B effect.

Fig. 10.

Overall scheme of ET receptors in the baroreflex arc. Input from arterial baroreceptors enters the CNS via excitatory synapses with the nucleus tractus solitarius (NTS). NTS excitatory outputs project to the caudal ventrolateral medulla (CVLM), which sends inhibitory outputs to the rostral ventrolateral medulla (RVLM) from which emerge the preganglionic sympathetic neurons that synapse with the post-ganglionic sympathetic neurons within the paravertebral ganglia (G). The NTS and CVLM also send projections to the nucleus ambiguus (NA) and its vagal motor neurons from which emerge the parasympathetic outputs to the heart. Although not technically part of the baroreflex arc itself, the area postrema (AP) is a circumventricular organ outside the blood-brain barrier and receives input from substances within the plasma circulation. Inputs from the AP can modulate NTS neurons. The heart receives both postganglionic efferent sympathetic innervation (tachycardia) and parasympathetic innervation (bradycardia). ET influences the baroreflex arc via ET_A or ET_B receptors (see text for details). Within the central sites, most data support a role for ET_A receptors.

Fig. 11.

Integrated systemic effects of ET_B . In general, ET_A activation leads to decreased arterial pressure and natriuresis through effects on the nervous system, heart, adrenal gland, kidney, and vasculature. ET_A -stimulated AVP and aldosterone release may mitigate its anti-hypertensive and natriuretic effects.

Fig. 12.

Integrated systemic effects of ET_A . In general, ET_A activation leads to increased arterial pressure and Na retention through effects on the nervous system, heart, adrenal gland, kidney, and vasculature. ET_A -stimulated atrial natriuretic peptide (ANP) release and possibly Na excretion (at least in females) may mitigate its hypertensive and Na-retaining effects.