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Relative contributions of Ca^{2+} mobilization and influx in renal arteriolar contractile responses to arginine vasopressin

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Abstract

Experiments addressed the hypothesis that afferent and efferent arterioles differentially rely on Ca^{2+} influx and/or release from intracellular stores in generating contractile responses to AVP. The effect of Ca^{2+} store depletion or voltage-gated Ca^{2+} channel (VGCC) blockade on contractile responsiveness to AVP (0.01–1.0 nM) was assessed in blood-perfused juxtamedullary nephrons from rat kidney. Depletion of intracellular Ca^{2+} stores by 100 μM cyclopiazonic acid (CPA) or 1 μM thapsigargin (THAPS) treatment increased afferent arteriolar baseline diameter by 14% and 21%, respectively, but did not significantly alter efferent arteriolar diameter. CPA attenuated the contractile response to 1.0 nM AVP by 34% and 55% in afferent and efferent arterioles, respectively ($P=0.013$). The impact of THAPS on AVP-induced afferent arteriolar contraction (52% inhibition) was also less than its effect on the efferent arteriolar response (88% inhibition; $P=0.046$). In experiments probing the role of the Ca^{2+} influx through VGCCs, 10 μM diltiazem evoked a 34% increase in baseline afferent arteriolar diameter and attenuated the contractile response to 1.0 nM AVP by 45%, without significantly altering efferent arteriolar baseline diameter or responsiveness to AVP. Combined treatment with both DILT and THAPS prevented AVP-induced contraction of both vascular segments. We conclude that Ca^{2+} release from the intracellular stores contributes to the contractile response to AVP in both afferent and efferent arterioles, but is more prominent in the efferent arteriole. Moreover, the VGCC contribution to AVP-induced renal arteriolar contraction resides primarily in the afferent arteriole.

Keywords

cyclopiazonic acid; diltiazem; thapsigargin; vasoconstriction; Ca^{2+} signaling

INTRODUCTION

Arginine vasopressin (AVP) plays a critical role in cardiovascular and renal physiology by virtue of its direct effects on vascular resistance and renal water reabsorption. Vasoconstrictor responses to AVP arise via its interaction with Ca^{2+} concentration V_1 receptors and consequent alterations in intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) that have been unveiled primarily through studies using cultured aortic myocytes (38). The signaling process involves phospholipase C-dependent production of 1,4,5-inositol trisphosphate (IP_3), which triggers Ca^{2+} mobilization from intracellular storage site(s) and a phasic increase in $[\text{Ca}^{2+}]_i$ (44). A subsequent sustained elevation in $[\text{Ca}^{2+}]_i$ reflects Ca^{2+} influx that has been attributed to opening of either voltage-gated Ca^{2+} channels (VGCCs) or receptor-operated channels

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(28;46). Although this scheme involving both Ca^{2+} mobilization and Ca^{2+} influx represents the general mechanism through which AVP induces vasoconstriction, the processes utilized by vascular smooth muscle (VSM) cells for accessing Ca^{2+} vary both quantitatively and qualitatively at different sites within the vasculature (8;29), including a distinction between large and small arteries (29).

In the kidney, agents that influence Ca^{2+} signaling have been shown to alter vasoconstrictor responsiveness in a manner consistent with regulation of renal vascular resistance through both Ca^{2+} mobilization and Ca^{2+} influx (35). For example, VGCC antagonists reduce the renal blood flow response to AVP by ~35%, while agents that interfere with Ca^{2+} release decrease the response by ~65% (14). It seems likely that specific intrarenal vascular segments differ with regard to the relative importance of Ca^{2+} influx and Ca^{2+} mobilization events in eliciting vasoconstriction. Indeed, although intracellular Ca^{2+} store depletion attenuates contractile responses to ANG II and norepinephrine in both afferent and efferent arterioles, the role of Ca^{2+} mobilization in the response to these agonists appears more prominent in the efferent arteriole than in the afferent arteriole (22;24). These observations could reflect the relative capacity of resident VSM cells to achieve Ca^{2+} mobilization, in concert with the differences exhibited by afferent and efferent arterioles concerning the functional role of VGCCs in eliciting vasoconstriction (35). The role of Ca^{2+} mobilization in eliciting constriction of afferent and efferent arterioles might also reflect disparities in agonist-specific signaling events within these cells, although few agonists have been studied in this regard. In particular, although Ca^{2+} influx plays a role in the $[\text{Ca}^{2+}]_i$ response to AVP in VSM cells isolated from the preglomerular microvasculature (12;27), the relative roles of Ca^{2+} influx and release from intracellular stores in eliciting AVP-induced responses of afferent and efferent arterioles remains largely speculative. Accordingly, the present study addressed the hypothesis that afferent and efferent arterioles differentially rely on Ca^{2+} influx and/or release from intracellular stores in generating contractile responses to AVP. The experimental approach was to quantify AVP-induced contractile responses in the absence and presence of pharmacological agents that either a) deplete the intracellular Ca^{2+} store by inhibiting the sarcoplasmic-endoplasmic reticulum Ca^{2+} -ATPase (SERCA), or b) block Ca^{2+} influx via voltage-gated channels. The results reveal diverse effects of these maneuvers on afferent and efferent arteriolar AVP responsiveness.

METHODS

Animals

The procedures used in this study were approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee and conducted according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Fifty-five male Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA) and provided ad libitum access to food and water prior to study.

In vitro blood-perfused juxtamedullary technique

Arteriolar contractile function was assessed in experiments performed using the rat *in vitro* blood-perfused juxtamedullary nephron technique (7). Each rat was anesthetized with pentobarbital sodium (50 mg/kg ip). The right renal artery was cannulated via the superior mesenteric artery, initiating *in situ* perfusion of the kidney with Tyrode solution containing 52 g/L dialyzed BSA. The rat was then exsanguinated via a carotid arterial cannula into a heparinized syringe and the kidney was harvested for *in vitro* study. Renal perfusion was maintained throughout the dissection procedure needed to reveal the tubules, glomeruli, and vasculature of juxtamedullary nephrons. Ligatures were placed around the distal segments of the large arterial branches that supplied the exposed microvasculature. The collected blood

was processed to remove leukocytes and platelets, as detailed previously (21). No pharmacological inhibitors were added to the resulting perfusate, which had a hematocrit of 0.33. The perfusate was stirred continuously in a closed reservoir that was pressurized under 95% O₂–5% CO₂, thus providing both oxygenation and the driving force for perfusion of the dissected kidney at a renal arterial pressure of 110 mmHg. The renal perfusion chamber was warmed and the tissue surface was superfused with Tyrode solution containing 10 g/L BSA at 37°C. All pharmacological and vasoactive agents were presented to the tissue via this superfusate bath. The tissue was transilluminated on the stage of a compound microscope (Nikon Optiphot). Prior to any experimental manipulations (thus, before exposure to AVP or imposition of a change in perfusion pressure), a single afferent or efferent arteriole was selected for study based on adequate visibility and acceptable blood flow (inability to discern the passage of individual erythrocytes). Arteriolar diameter was monitored at this measurement site throughout each experimental protocol. Afferent arteriolar responses were monitored at “mid-afferent” locations, defined as $\geq 100\ \mu\text{m}$ from the glomerulus (to avoid the renin-containing granular cells) or the parent interlobular artery (as these branch points may be hyper-reactive to vasoactive stimuli due to their unusually high expression of voltage-gated Ca²⁺ channels (16). Efferent arteriolar responses were measured at sites $\leq 100\ \mu\text{m}$ from the glomerulus, as the initial portion of this vessel is widely considered the primary site of postglomerular resistance alterations. Video images of each microvessel were generated continuously and stored on videotape for later analysis. In one experiment, two arterioles could be visualized clearly within the same field of view, a situation that allowed responses of both vessels to be recorded simultaneously and analyzed separately during videotape playback.

Experiment Protocols

The impact of various pharmacological agents on AVP-induced arteriolar contractile responses was assessed with a standard protocol. After a stabilization period, afferent or efferent arteriolar lumen diameter was monitored under baseline conditions (5–10 min) and during sequential exposure to increasing concentrations of AVP (0.01, 0.1 and 1.0 nM; 3 min at each concentration). After allowing a 10 min recovery period (no AVP), a pharmacological agent known to alter Ca²⁺ mobilization or influx was added to the bath. Following 10 min of this treatment, and in the continued presence of the pharmacological agent, the AVP exposure sequence was repeated, followed by a recovery period (no AVP). The efficacy of SERCA inhibitors (thapsigargin, THAPS; cyclopiazonic acid, CPA) in our experimental setting was evaluated based on their ability to attenuate afferent arteriolar contractile responses to an increment in renal perfusion pressure. This was accomplished by expanding the basic protocol to include a brief (2 min) period during which perfusion pressure was held at 135 mmHg, followed by a return to the basal pressure (110 mmHg). This perfusion pressure increment was imposed in both the absence and presence of the SERCA inhibitor.

Solutions and drugs

All chemicals were purchased from Sigma (St. Louis, MO). AVP (0.25 mM stock) was diluted in Tyrode solution on the day of the experiment. CPA was dissolved in DMSO at a concentration of 50 mM, stored at –20°C, and diluted on the day of each experiment in Tyrode solution to achieve a final concentration of 100 μM . THAPS was dissolved in DMSO at a concentration of 500 μM , stored at –20°C, and diluted in Tyrode solution on the day of the experiment to achieve a final concentration of 1 μM . Diltiazem HCl (DILT; 10 μM in Tyrode solution) was also prepared fresh daily.

Data analysis

Arteriolar lumen diameter was measured from videotaped images at 5-sec intervals from a single point along the length of the vessel. The average diameter (in μm) during the final minute of each treatment period was utilized for statistical analysis. Statistical analysis was performed by ANOVA for repeated measures, followed by Newman-Keuls multiple range test. Statistical computations were performed utilizing the SigmaStat software package (SPSS Inc, Chicago, IL), with statistical significance defined as $P < 0.05$. All data are reported as means $\pm$ SE (n = number of arterioles).

RESULTS

Effect of SERCA inhibition on pressure-induced afferent arteriolar constriction

In untreated kidneys (prior to CPA or THAPS exposure), afferent arteriolar lumen diameter averaged $21.8 \pm 1.1 \mu\text{m}$ at a renal perfusion pressure of 110 mmHg, and an increase pressure to 135 mmHg evoked a significant decline in diameter ($-2.0 \pm 0.3 \mu\text{m}$; $n = 15$ arterioles; $P < 0.001$). Restoration of perfusion pressure to 110 mmHg was associated with a return of afferent arteriolar diameter to baseline levels ($21.0 \pm 1.4 \mu\text{m}$). Ten of these arterioles were subsequently exposed to 100 μM CPA, which increased baseline diameter by $4.3 \pm 0.8 \mu\text{m}$ ($P = 0.040$) and prevented a vasoconstrictor response to the 110-to-135 mmHg pressure increment ($\Delta = 0.7 \pm 1.0 \mu\text{m}$; $P = 0.002$ vs response before CPA). The remaining 5 arterioles received treatment with 1 μM THAPS, which increased diameter by $4.3 \pm 1.4 \mu\text{m}$ ($P = 0.041$ vs response before THAPS), with no significant change in diameter evident upon subsequent imposition of the pressure increment ($\Delta = -0.2 \pm 0.6 \mu\text{m}$; $P = 0.045$ vs untreated). Thus, SERCA inhibition by treatment with either CPA or THAPS evoked afferent arteriolar dilation and abrogated pressure-induced constriction of this vascular segment.

Effect of CPA on AVP-induced arteriolar constriction

Figure 1A summarizes the effect of CPA on afferent arteriolar lumen diameter responses to exogenous AVP. Afferent arteriolar lumen diameter averaged $22.5 \pm 1.5 \mu\text{m}$ ($n = 10$) under untreated baseline conditions and decreased by 0.4 ± 0.3 , 2.3 ± 0.7 and $13.3 \pm 2.0 \mu\text{m}$ upon exposure to 0.01, 0.1 and 1.0 nM AVP, respectively. Upon removal of AVP from the bathing solution, arteriolar diameter was restored to $97 \pm 2\%$ of baseline. Addition of 100 μM CPA to the bath caused a $15 \pm 5\%$ increase in afferent arteriolar diameter. During continued CPA exposure, the afferent diameter response to 1.0 nM AVP ($\Delta = -10.3 \pm 1.8 \mu\text{m}$) was significantly less than responses of the same vessels prior to CPA treatment ($P = 0.028$). Subsequent exposure to CPA alone (no AVP) allowed restoration of afferent diameter to a value averaging $98 \pm 3\%$ of the CPA baseline ($P > 0.05$). Moreover, removal of CPA from the bath resulted in recovery of arteriolar diameter to $107 \pm 5\%$ of untreated baseline ($P > 0.05$). Thus, CPA treatment evoked afferent arteriolar dilation and inhibited 1.0 nM AVP-induced constriction of this vascular segment by $34 \pm 4\%$.

Figure 1B summarizes efferent arteriolar diameter responses to exogenous AVP before and during CPA treatment. Under untreated baseline conditions, efferent arteriolar lumen diameter averaged $26.0 \pm 2.0 \mu\text{m}$ ($n = 7$). When exposed to increasing concentrations of AVP (0.01, 0.1 and 1.0 nM), efferent diameter declined by 0.1 ± 0.5 , 0.8 ± 0.6 and $8.0 \pm 1.5 \mu\text{m}$, respectively. Removal of AVP from the bath restored diameter to $25.8 \pm 2.0 \mu\text{m}$, a value not significantly different from baseline. Addition of 100 μM CPA to the bath failed to alter efferent arteriolar baseline diameter ($26.8 \pm 2.2 \mu\text{m}$). During continued CPA treatment, efferent arteriolar diameter declined to levels averaging 0.5 ± 0.2 , 0.9 ± 0.4 and $3.4 \pm 0.6 \mu\text{m}$ below baseline during exposure to 0.01, 0.1 and 1.0 nM AVP, respectively. The response to 1.0 nM AVP during CPA treatment was diminished significantly compared to the response observed in the same arterioles prior to CPA treatment. Removal of AVP from the CPA-

containing bath restored efferent arteriolar lumen diameter to $96 \pm 1\%$ of the CPA baseline value ($P > 0.05$). Moreover, efferent diameter averaged $98 \pm 1\%$ of the untreated baseline value ($P > 0.05$) upon removal of CPA from the bath. Thus, while not altering baseline diameter, pharmacological depletion of the intracellular Ca^{2+} store by CPA exposure suppressed efferent arteriolar responsiveness to 1.0 nM AVP by $55 \pm 7\%$. CPA inhibition of responsiveness to 1.0 nM AVP was significantly greater in the efferent arteriole than in the afferent arteriole ($P = 0.013$).

Effect of THAPS on AVP-induced arteriolar constriction

Figure 2A depicts the impact of THAPS on AVP-induced afferent arteriolar constriction. Afferent lumen diameter averaged $20.4 \pm 1.4 \mu\text{m}$ ($n = 5$) under untreated baseline conditions. AVP (0.01, 0.1 and 1.0 nM) reduced afferent diameter by 0.2 ± 0.3 , 3.3 ± 1.2 and $12.8 \pm 1.4 \mu\text{m}$, respectively. Removal of AVP from the bath allowed restoration of arteriolar diameter to $19.6 \pm 1.4 \mu\text{m}$. THAPS (1 μM) significantly increased afferent arteriolar diameter (to $24.0 \pm 2.7 \mu\text{m}$) and suppressed contractile responses to 0.1 and 1.0 nM AVP (both $P < 0.05$ vs untreated). Removal of AVP from the bath allowed restoration of afferent arteriolar diameter to $23.6 \pm 3.1 \mu\text{m}$ ($P > 0.05$ vs THAPS baseline). Thus, SERCA inhibition with THAPS evoked afferent arteriolar dilation and inhibited the afferent arteriolar response to 1.0 nM AVP by $52 \pm 14\%$.

The effect of 1 μM THAPS on efferent arteriolar contractile responses to AVP is illustrated in Figure 2B. Efferent arteriolar diameter averaged $20.1 \pm 1.7 \mu\text{m}$ ($n = 7$) under baseline conditions, and increasing concentrations of AVP evoked progressive reductions in diameter such that values averaged $8.8 \pm 2.3 \mu\text{m}$ below baseline in the presence of 1.0 nM AVP. Removal of AVP from the bath restored efferent diameter to $20.6 \pm 1.8 \mu\text{m}$ ($P > 0.05$ vs baseline) and subsequent THAPS treatment failed to significantly alter efferent diameter ($21.0 \pm 1.7 \mu\text{m}$). However, responses to 0.1 and 1.0 nM AVP were attenuated markedly by THAPS ($\Delta = -1.8 \pm 0.9 \mu\text{m}$ during exposure to 1.0 nM AVP) such that 2-way repeated measures ANOVA failed to indicate a significant effect of AVP on efferent arteriolar diameter in the presence of THAPS. Thus, although SERCA inhibition by THAPS treatment failed to alter basal efferent arteriolar diameter, this treatment prevented significant AVP-induced efferent contraction. The impact of THAPS on the response to 1.0 nM AVP in the efferent arteriole ($88 \pm 9\%$ inhibition) significantly exceeded its effect on the afferent arteriolar response ($P = 0.046$).

Effect of DILT on AVP-induced arteriolar constriction

Figure 3A summarizes the effect of VGCC blockade with DILT on afferent arteriolar contractile responses to AVP. In these experiments, afferent arteriolar baseline diameter averaged $21.9 \pm 0.9 \mu\text{m}$ ($n = 5$) and progressive increases in bath AVP levels to a final concentration of 1.0 nM ultimately reduced lumen diameter by $15.0 \pm 1.4 \mu\text{m}$. After recovery from AVP challenge, addition of 10 μM DILT to the bath increased afferent diameter to a value averaging $28.4 \pm 2.5 \mu\text{m}$ ($P = 0.038$). During continued DILT exposure, 1.0 nM AVP reduced afferent arteriolar diameter by $10.7 \pm 1.8 \mu\text{m}$ ($P < 0.05$ vs. response prior to DILT treatment). Removal of AVP from the bath allowed recovery of afferent diameter to $27.0 \pm 3.0 \mu\text{m}$ ($P > 0.05$ vs DILT baseline). Thus, DILT evoked afferent arteriolar dilation and attenuated the response to 1.0 nM AVP by $45 \pm 8\%$.

The effect of DILT on efferent arteriolar responses to AVP is shown in Figure 3B. Efferent diameter averaged $20.6 \pm 1.9 \mu\text{m}$ ($n = 6$) and responded to increasing concentrations of AVP such that lumen diameter averaged $8.1 \pm 2.2 \mu\text{m}$ below baseline during exposure to 1.0 nM AVP. Removal of AVP from the bath allowed recovery of efferent diameter to baseline values and subsequent exposure to 10 μM DILT did not significantly alter efferent arteriolar

diameter. Moreover, in the continued presence of DILT, AVP-induced reductions in efferent diameter did not differ significantly from those observed prior to DILT treatment (1.0 nM AVP: $\Delta = -5.5 \pm 2.0 \mu\text{m}$; $P = 0.107$ vs response in the absence of DILT). Thus, in contrast with the afferent arteriolar data, the results of these experiments failed to reveal a statistically significant effect of DILT on efferent arteriolar diameter or contractile responsiveness to AVP.

Effect of THAPS+DILT on AVP-induced arteriolar constriction

Figure 4 summarizes the impact of combined DILT and THAPS treatment on AVP-induced afferent and efferent arteriolar contractile responses to AVP. In untreated afferent arterioles with baseline diameter averaging $19.1 \pm 1.2 \mu\text{m}$ ($n = 6$), AVP evoked the typical concentration-dependent reduction in lumen diameter (1 nM AVP: $\Delta = -11.0 \pm 1.7 \mu\text{m}$). After recovery from the AVP challenge, simultaneous exposure to 10 μM DILT and 1 μM THAPS increased afferent diameter by 27% ($23.2 \pm 2.0 \mu\text{m}$). Subsequent exposure to increasing AVP concentrations yielded no change in afferent arteriolar diameter ($102 \pm 3\%$ inhibition of the response to 1 nM AVP). Similar effects were observed in the efferent arteriole, where baseline diameter averaged $24.5 \pm 1.8 \mu\text{m}$ ($n = 7$) and 1.0 nM AVP decreased diameter by $5.7 \pm 0.7 \mu\text{m}$ under untreated conditions. Combined DILT and THAPS treatment did not significantly alter efferent diameter ($26.4 \pm 2.4 \mu\text{m}$; $P = 0.115$), but the constrictor response to AVP was attenuated by $96 \pm 7\%$. Thus, pharmacological inhibition of both VGCCs and SERCA prevented any discernible afferent or efferent arteriolar contractile response to AVP.

DISCUSSION

Renal vasoconstrictor responses to AVP arise via interaction of the peptide with V_1 receptors, with little or no apparent involvement of V_2 receptors (13). Afferent and efferent arterioles express V_{1a} receptor mRNA and protein (37), and the juxtamedullary afferent arteriolar contractile response to AVP is prevented by V_1 receptor blockade (19). Previous studies indicate that the renal vasoconstrictor response to AVP is attenuated by pharmacological agents that block Ca^{2+} influx through VGCCs (10;14;39;40;42) or interfere with Ca^{2+} release from intracellular stores (10;14;42). These observations indicate that both Ca^{2+} influx through VGCCs and Ca^{2+} release from intracellular stores contribute to the renal vasoconstrictor response to AVP. In light of the suggestion that Ca^{2+} influx through VGCCs might play a predominant role in the preglomerular microvascular response to AVP (26), we postulated that the Ca^{2+} mobilization-dependent component of the renal vasoconstrictor response to AVP might reside primarily in the efferent arteriole.

To quantify the role of Ca^{2+} release from the intracellular store in afferent and efferent arteriolar contractile response to AVP, experiments were performed using the *in vitro* blood perfused juxtamedullary nephron technique in concert with pharmacological agents known to inhibit the SERCA. Two structurally different SERCA inhibitors were employed — CPA and THAPS (15;33;45). Both agents have been used extensively to deplete intracellular Ca^{2+} stores in a wide range of cell types. CPA was utilized at a concentration previously reported to be maximally effective in depleting intracellular Ca^{2+} stores (34), and the concentration of THAPS used in these studies exceeds by at least 20-fold the IC_{50} for binding to SERCA and inhibition of Ca^{2+} release from intracellular stores in a variety of cell types (47). The specificity of these agents was not validated in the present study, and some evidence indicates that thapsigargin can influence Ca^{2+} influx via VGCCs (36;41). However, investigations performed in the same experimental setting have documented the ability of afferent arterioles to respond to BAY K 8644 in the presence CPA (22) and the retention of contractile responses to K^+ -induced depolarization during CPA or THAPS treatment (22;24). The efficacy of these agents in our experimental setting has been

established on the basis of reports that treatment with THAPS or CPA prevents or markedly attenuates renal arteriolar contractile responses to ATP, AngII and norepinephrine (22;24;25). Moreover, THAPS and CPA have identical effects to prevent pressure-induced afferent arteriolar constriction (23), a phenomenon confirmed in the present study. Previous reports indicate that SERCA inhibition causes no change or an increase in juxtamedullary afferent arteriolar diameter (22–24), and the afferent arteriolar dilator responses to CPA and THAPS documented in the present study are consistent with inhibition of the pressure-dependent component of basal tone that is evident in this experimental setting (4). In accord with this reasoning, the lack of a pressure-dependent component of efferent arteriolar tone (11) may explain the failure of CPA and THAPS to evoke an increase in efferent arteriolar diameter under basal conditions.

CPA treatment attenuated constrictor responses to AVP in both afferent and efferent arterioles; however, the effect of CPA was significantly greater in the efferent arteriole (55% inhibition) than in the afferent arteriole (34% inhibition). Follow-up experiments revealed that THAPS also reduced afferent and efferent arteriolar contractile responses to AVP. Similar to CPA, the inhibitory effect of THAPS was significantly greater in the efferent arteriole than in the afferent arteriole. The tendency for THAPS to be more effective than CPA in attenuating agonist-induced contraction of juxtamedullary arterioles has been noted previously (22), but the mechanism remains unexplained. Nevertheless, the overall effects of CPA and THAPS indicate that Ca^{2+} mobilization plays a significant role in the contractile response to AVP at both afferent and efferent arteriolar sites, with a more prominent role evident in the efferent arteriole. The involvement of Ca^{2+} release from intracellular stores in AVP-induced juxtamedullary arteriolar signal transduction exemplifies the events described in most VSM populations studied to date, as well as glomerular mesangial cells (2;29;38;44;46). Moreover, our observations add to the mounting evidence that Ca^{2+} mobilization is of particular importance in eliciting efferent arteriolar contraction in response to a variety of agonists, including not only AVP but also AngII and norepinephrine (9;22;23;48).

The failure of SERCA inhibition to abrogate AVP-induced contraction, especially in the afferent arteriole, provoked studies investigating the contribution of Ca^{2+} influx in the contractile response. VGCCs play a central role in evoking ANG II-induced activation of the afferent arteriole (5;30), and studies at the whole kidney level indicate their involvement in AVP-induced alterations in renal blood flow (14). Indeed, AVP provokes depolarization of rat afferent arteriolar VSM (1), which expresses genes encoding the α subunits of L-, P-/Q-, and T-type VGCCs (17;18). Given the widely reported depolarization-induced $[\text{Ca}^{2+}]_i$ and contractile responses of this vascular segment, the ability of DILT to attenuate AVP-induced afferent arteriolar contraction is predictable. Indeed, AVP-induced $[\text{Ca}^{2+}]_i$ responses of freshly isolated preglomerular VSM cells have been attributed either partially (12) or almost entirely (26;27) to Ca^{2+} entry through L-type VGCCs. However, DILT only reduced the contractile response to AVP by ~50%, a modest effect compared with the ability of DILT to prevent the juxtamedullary afferent arteriolar contractile response to ANG II (5). The failure of DILT to more markedly attenuate AVP-induced afferent arteriolar contraction in the present study cannot be ascribed readily to an inadequate concentration of the agent, as previous studies performed in our laboratory demonstrated that 10 μM DILT fully reverses the rat juxtamedullary afferent arteriolar contractile response to 80 mM K^+ (6). Rather, a comparison of the effects of THAPS and DILT administered separately suggests that Ca^{2+} release and Ca^{2+} influx each contribute ~50% to the afferent arteriolar contractile response to AVP. In accord with this contention, combined THAPS + DILT fully prevented AVP-induced afferent arteriolar contraction. In contrast, the failure of DILT to significantly alter the efferent arteriolar response to AVP suggests minimal involvement of VGCCs in this response. Similarly, VGCC blockade does not alter efferent arteriolar contractile response to

ANG II in this experimental setting (5) or in the isolated perfused hydronephrotic kidney (30;43). Recent studies have demonstrated expression of L- and T-type VGCCs in juxtamedullary efferent arterioles (18), although the literature contains conflicting evidence concerning their functional significance (3;18;20;32;43). Results of the present study indicate that, at least in the rat *in vitro* blood perfused juxtamedullary nephron, the efferent arteriolar contractile response to AVP does not display a significant dependence on VGCCs. Rather, the efferent arteriolar contractile response to this agonist seems mainly dependent on Ca^{2+} mobilization from the intracellular store. It is possible that AVP, like ANG II (31), does not depolarize efferent arteriolar VSM, thereby failing to present the usual stimulus for increasing the open probability of VGCCs; however, further studies are required to assess the validity of this scenario.

In summary, the results of the present study reveal that SERCA inhibition to deplete the intracellular Ca^{2+} store significantly attenuated juxtamedullary arteriolar contractile responses to AVP, implicating Ca^{2+} mobilization from intracellular stores as an important component of the AVP-induced Ca^{2+} signaling cascade in the renal microvasculature. This phenomenon was most prominent in the efferent arteriole. Blockade of Ca^{2+} influx through VGCCs significantly reduced afferent, but not efferent, arteriolar contractile responses to AVP. Combined inhibition of both SERCA- and VGCC-dependent processes abrogated AVP-induced contraction at both arteriolar sites. Taken together, these data support the contention that the afferent arteriolar contractile response to AVP involves both Ca^{2+} mobilization from intracellular stores and Ca^{2+} influx through VGCCs, while Ca^{2+} mobilization is the prominent process in the efferent arteriolar response. These observations lend further credence to the concept that pre- and postglomerular resistance vessels rely on disparate Ca^{2+} signaling processes in responding to vasoconstrictor agonists.

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Figure 1.

Influence of the CPA on renal arteriolar lumen diameter responses to AVP. Lumen diameter responses to AVP were monitored before (Untreated) and during exposure to 100 μ M CPA. Panel A: Afferent Arteriole. Panel B: Efferent Arteriole. * P <0.05 vs. baseline (0 nM AVP); † P <0.05 untreated vs. CPA.

Figure 2.

Influence of the THAPS on renal arteriolar lumen diameter responses to AVP. Lumen diameter responses to AVP were monitored before (Untreated) and during exposure to 1 μ M THAPS. Panel A: Afferent Arteriole. Panel B: Efferent Arteriole. * $P < 0.05$ vs. baseline (0 nM AVP); † $P < 0.05$ untreated vs. THAPS.

Figure 3.

Influence of the DILT on renal arteriolar lumen diameter responses to AVP. Lumen diameter responses to AVP were monitored before (Untreated) and during exposure to 10 μ M DILT. Panel A: Afferent Arteriole. Panel B: Efferent Arteriole. * $P < 0.05$ vs. baseline (0 nM AVP); † $P < 0.05$ untreated vs. DILT.

Figure 4.

Influence of the combined DILT and THAPS treatment on renal arteriolar lumen diameter responses to AVP. Lumen diameter responses to AVP were monitored before (Untreated) and during exposure to both 10 μ M DILT and 1 μ M THAPS. Panel A: Afferent Arteriole. Panel B: Efferent Arteriole. * $P < 0.05$ vs. baseline (0 nM AVP); † $P < 0.05$ untreated vs. DILT + THAPS.