The subtype 2 of angiotensin II receptors and pressure-natriuresis in adult rat kidneys

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1 The present work examined the effects of the subtype 2 of angiotensin II (AT₂) receptors on the pressure-natriuresis using a new peptide agonist, and the possible involvement of cyclic guanosine 3', 5' monophosphate (cyclic GMP) in these effects.

2 In adult anaesthetized rats (Inactin, 100 mg kg⁻¹, i.p.) deprived of endogenous angiotensin II by angiotensin converting enzyme inhibition (quinapril, 10 mg kg⁻¹, i.v.), T₂-(Ang II 4-8)₂ (TA), a highly specific AT₂ receptor agonist (5, 10 and 30 µg kg⁻¹ min⁻¹, i.v.) or its solvent was infused in four groups. Renal functions were studied at renal perfusion pressures (RPP) of 90, 110 and 130 mmHg and urinary cyclic GMP excretion when RPP was at 130 mmHg. The effects of TA (10 µg kg⁻¹ min⁻¹) were reassessed in animals pretreated with PD 123319 (PD, 50 µg kg⁻¹ min⁻¹, i.v.), an AT₂ receptor antagonist and the action of the same dose of PD alone was also determined.

3 Increases in RPP from 90 to 130 mmHg did not change renal blood flow (RBF) but induced 8 and 15 fold increases in urinary flow and sodium excretion respectively. The 5 µg kg⁻¹ min⁻¹ dose of TA was devoid of action. The 10 and 30 µg kg⁻¹ min⁻¹ doses did not alter total RBF and glomerular filtration rate, but blunted pressure-diuresis and natriuresis relationships. These effects were abolished by PD.

4 TA decreased urinary cyclic GMP excretion. After pretreatment with PD, this decrease was reversed to an increase which was also observed in animals receiving PD alone.

5 In conclusion, renal AT₂ receptors oppose the sodium and water excretion induced by acute increases in blood pressure and this action cannot be directly explained by changes in cyclic GMP.

Keywords: Angiotensin II; AT₂ receptors; cyclic GMP; pressure-natriuresis

Abbreviations: ACE, angiotensin converting enzyme; AI, angiotensin I; AII, angiotensin II; AT₁, subtype 1 of angiotensin II receptor; AT₂, subtype 2 of angiotensin II receptor; cyclic GMP, cyclic guanosine 3', 5' monophosphate; NO, nitric oxide; RPP, renal perfusion pressure; TA, T₂-(Ang II 4-8)₂

Introduction

At the renal level, angiotensin II (AII) exerts potent vasoconstrictor effects and enhances the tubular sodium reabsorption, thus lowering the pressure-natriuresis (Mattson *et al.*, 1991; Van Der Mark & Kline, 1994) which is a major determinant of the long-term blood pressure level (Guyton, 1990). Most of the renal actions of AII are mediated through subtype 1 receptors (AT₁). The role of the receptors belonging to the subtype 2 (AT₂) remains poorly defined. In the whole organism, mice lacking the AT₂ receptor gene exhibit increases in the blood pressure response to AII (Hein *et al.*, 1995; Ichiki *et al.*, 1995) thus suggesting that AT₂ receptors oppose the AT₁-mediated vasoconstriction. In the kidneys where AT₂ receptors represent 5–10% of the total AII receptors (Chang & Lotti, 1991; Zhuo *et al.*, 1992; 1993; Ozono *et al.*, 1997), the reported vascular effects are controversial. PD 123319, an AT₂ receptor antagonist was found to block the AII-induced renal vasoconstriction (Chatziantoniou & Arendshorst, 1993) and intracellular calcium mobilization in vascular smooth muscle cells (Zhu & Arendshorst, 1996). In contrast, other studies

showed that PD 123319 augmented the AII-induced vasoconstriction in both pre- and post-glomerular arterioles (Arima *et al.*, 1996; Endo *et al.*, 1997). Concerning the excretory functions, AT₂ receptor blockade with PD 123319 increased the urine volume, chloride and bicarbonate excretion in anaesthetized rats (Cogan *et al.*, 1991) and increased free water formation in anaesthetized dogs (Keiser *et al.*, 1992). We previously observed that, in rats infused with AII and the AT₁ receptor antagonist losartan, the blockade of AT₂ receptors with PD 123319 increased the pressure-natriuresis while CGP 42112B, an AT₂ receptor ligand with agonistic properties, lowered the pressure-natriuresis (Lo *et al.*, 1995). Since the interpretation of the above reported data relies upon the selectivity of the various ligands, our attention was drawn by a new compound T₂-(Ang II 4-8)₂ (TA), which is the most specific peptide agonist available for AT₂ receptors (Grouzmann *et al.*, 1995). We therefore thought it is of interest to examine its effects on the pressure-natriuresis. In addition, since it has been suggested that AT₂ receptors may interact with the formation of cyclic guanosine 3', 5' monophosphate (cyclic GMP) which mediates the renal vasodilatation and natriuresis induced by nitric oxide (NO) (Siragy *et al.*, 1992) and atrial natriuretic peptide (Margulies & Burnett, 1994), we measured urinary excretion of cyclic GMP so as to approach the intracellular mechanisms of the effects of AT₂ receptor stimulation.

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Methods

Animals

Ten week-old male Sprague-Dawley rats (Iffa-Credo, Les Oncins, France) were used. They were housed in controlled conditions (temperature: $21 \pm 1^\circ\text{C}$; humidity: $60 \pm 10\%$; lighting: 8–20 h), and fed a standard rat chow containing 0.3% sodium (Elevage UAR, Villemoisson sur Orge, France) and tap water *ad libitum*. The studies were conducted in agreement with our institutional guidelines for animal care.

AT₂ receptor ligands

TA (T₂-(Ang II 4–8)₂) is a template-assembled peptide agonist for AT₂ receptors (Institute of Organic Chemistry, Lausanne, Switzerland). It is made of two angiotensin II 4–8 pentapeptide fragments (Ang II 4–8)₂, attached to a carrier molecule (T₂) which alone did not bind to either AT₁ or AT₂ receptors (Grouzmann *et al.*, 1995). Binding assays showed that in the presence of an AT₁ antagonist, TA completely inhibited the specific binding of ¹²⁵I-AII to the AT₂ receptors of a rat adrenal membrane preparation and that half-maximal inhibition (IC₅₀) occurred at the concentration of 2×10^{-7} M. In contrast, TA at the concentration of 10^{-5} M did not bind to AT₁ receptors of rat aortic smooth muscle cells (Grouzmann *et al.*, 1995). PD 123319 (Parke-Davis, Ann Arbor, MI, U.S.A.), a non-peptide AT₂ receptor antagonist, exhibits an IC₅₀ of 0.5×10^{-7} M for AT₂ receptors and did not bind to AT₁ receptors of rat aortic smooth muscle cells at the concentration of 10^{-5} M (Grouzmann *et al.*, 1995). PD 123319 was dissolved in saline and infused at the dose of $50 \mu\text{g kg}^{-1} \text{ min}^{-1}$ which, according to Macari *et al.* (1993), yields plasma concentrations close to 3×10^{-6} M, i.e. a value which remains highly specific for AT₂ receptors. TA was dissolved in a 12% solution of dimethyl sulphoxide in saline and three doses (5, 10 and $30 \mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v.) were chosen according to preliminary experiments.

Surgical preparation

Pressure-natriuresis was studied using the method of Roman & Cowley (1985). The right kidney and adrenal gland were removed and the rats allowed 7–10 days to recover. On the day of experiment, the rats were anaesthetized with Inactin (100 mg kg^{-1} , i.p., Research Biochemicals, Natick, MA, U.S.A.) and placed on a heating blanket (Model 50-6980, Harvard Apparatus, Edenbridge, KY, U.S.A.) to maintain the rectal temperature at $37 \pm 0.5^\circ\text{C}$. After tracheotomy, the left jugular vein was cannulated for infusion. Catheters were placed into the left carotid and femoral arteries to sample blood and to record the mean arterial blood pressure through a pressure transducer (Model P23ID, Statham Instrument Division, Gould Inc., Cleveland, OH, U.S.A.). After an abdominal incision, the left kidney was exposed and denervated by stripping all the visible renal nerves and coating the renal artery with a 10% solution of phenol in ethanol. The remaining adrenal gland was then removed and the left ureter cannulated for urine collection. Two adjustable silastic balloon cuffs were placed around the aorta, one above the renal artery between the superior mesenteric and celiac arteries, the other below the left renal artery so that the renal perfusion pressure (RPP) could be fixed at different levels. Silk ligatures were placed loosely around the superior mesenteric and celiac arteries and tighten to further elevate the RPP. An ultrasonic flow probe (1RB) was placed around the left renal artery so as

to continuously record the total renal blood flow using a transonic transit-time flowmeter (Model T106, Transonic Systems Inc., Ithaca, NY, U.S.A.). After a priming dose (250 mg kg^{-1} , i.v.) of polyfructosan (Inutest, Laevosan, Linz, Austria), a hormone-cocktail (Mattson *et al.*, 1991; Liu *et al.*, 1996) designed to fix the circulating levels of the most important sodium- and water-retaining hormones was infused at a rate of $330 \mu\text{L kg}^{-1} \text{ min}^{-1}$ (Pump Model 2400-001, Harvard Apparatus, South Natick, MA, U.S.A.). It contained d-aldosterone ($66 \text{ ng kg}^{-1} \text{ min}^{-1}$), hydrocortisone ($33 \text{ ng kg}^{-1} \text{ min}^{-1}$), norepinephrine ($333 \text{ ng kg}^{-1} \text{ min}^{-1}$) and Arg⁸-vasopressin acetate ($0.17 \text{ ng kg}^{-1} \text{ min}^{-1}$). Drugs were obtained from Sigma Chemicals (St. Louis, MO, U.S.A.) and dissolved in 0.9% sodium chloride containing 1% bovine serum albumin (Fraction V) and 1.25% polyfructosan. At the end of the experiment, the left kidney was decapsulated, removed, cut in half, blotted dry and weighed.

Renal parameters

RPP (mmHg) was estimated as the mean femoral artery pressure when the suprarenal aortic cuff was inflated and as the mean carotid artery pressure when the infrarenal aortic cuff was inflated. Glomerular filtration rate ($\text{ml min}^{-1} \text{ g}^{-1}$) was measured by polyfructosan clearance. Urine flow ($\mu\text{L min}^{-1} \text{ g}^{-1}$) was determined by weighing, and sodium concentration by flame photometry (IL meter, model 243, Lexington, MA, U.S.A.) so as to calculate urinary sodium excretion ($\mu\text{moles min}^{-1} \text{ g}^{-1}$). Urinary concentration of cyclic GMP was determined by an enzyme immunoassay kit (Cayman Chemical Company, Ann Arbor, MI, U.S.A.) (Pradelles *et al.*, 1989) with which the minimum detectable concentration approached $0.04 \text{ pmol ml}^{-1}$ and the coefficient of variation was below 5%. All the parameters were normalized per gram of the left kidney weight.

Experimental protocol

The endogenous production of AII was blocked by an angiotensin converting enzyme (ACE) inhibitor, quinapril (10 mg kg^{-1} , i.v.) (Parke-Davis, Ann Arbor, MI, U.S.A.) so as to eliminate any interference due to changes in renal renin release. The efficiency of this inhibition during the whole of the experimental period is shown in Figure 1. The injection of angiotensin I (AI, 750 ng kg^{-1}) in five animals increased mean blood pressure by $37 \pm 5\%$ and lowered renal blood flow by $82 \pm 5\%$. These responses were fully abolished during the 2 h following quinapril injection.

Sixty minutes after the hormonal-cocktail infusion begun, quinapril (10 mg kg^{-1} , i.v.) was given 30 min prior to the start of the study. In a first protocol, four groups of animals were used. Controls received dimethyl sulphoxide (the solvent for TA) at the rate corresponding to that infused with the different doses of TA. Three doses of TA (5 , 10 and $30 \mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v.) were randomly allocated each to one group of rats 10 min prior to the start of the study. To confirm the specificity of the findings, TA ($10 \mu\text{g kg}^{-1} \text{ min}^{-1}$) was given to another group of animals in which PD 123319 ($50 \mu\text{g kg}^{-1} \text{ min}^{-1}$) was infused starting 15 min before. Finally, so as to check whether PD 123319 by itself could influence renal functions, an additional group of rats received PD 123319 alone at the same dose of $50 \mu\text{g kg}^{-1} \text{ min}^{-1}$. In both protocols, the renal functions were studied at a RPP of 90 mmHg, a level close to the basal mean blood pressure level, and, later on, at 110 and 130 mmHg. Urines were collected for 20, 10 and 10 min respectively. Arterial blood ($200 \mu\text{L}$) was sampled at the end of

each period. Urines collected at RPP of 130 mmHg were used for cyclic GMP assay, since at the lower levels of pressure the volume of urines did not allow all the measurements to be done.

Statistical analysis

Values are means $\pm$ s.e.mean. Two-way analysis of variance (ANOVA) was used to statistically evaluate the differences between groups over the whole range of RPP. The differences at a given level of RPP were assessed using the Student's *t*-test for unpaired data. A difference was considered statistically significant at $P < 0.05$.

Results

Effects of AT₂ receptor ligands on renal functions

The renal functions observed at a RPP of 90 mmHg, a level close to the basal mean blood pressure level in rats deprived of endogenous AII are shown in Table 1. Neither the infusion of TA nor that of PD 123319 changed significantly these baseline renal functions.

Figure 2 shows that in control animals, a stepwise elevation in RPP from 90 to 130 mmHg, did not change total renal blood flow and glomerular filtration rate, but induced 8 and 15 fold increases in urine flow and sodium excretion respectively. The lowest dose of TA ($5 \mu\text{g kg}^{-1} \text{min}^{-1}$) did not modify the effects of increasing renal perfusion pressure. When infused at the rate of $10 \mu\text{g kg}^{-1} \text{min}^{-1}$, TA did not affect, over the whole range of pressures, total renal blood flow and glomerular filtration rate, while it significantly decreased pressure-diuresis (ANOVA, $P < 0.001$) and natriuresis relationships (ANOVA, $P < 0.03$) by increasing the tubular sodium reabsorption (98.0 ± 0.2 and $99.0 \pm 0.3\%$ in control and TA-treated animals respectively at the RPP of 130 mmHg, $P < 0.05$). The $30 \mu\text{g kg}^{-1} \text{min}^{-1}$ dose of TA induced effects similar to those of $10 \mu\text{g kg}^{-1} \text{min}^{-1}$.

Figure 3 shows that, in animals deprived of endogenous AII, pressure-diuresis and natriuresis observed during the infusion of PD 123319 alone did not differ from those of controls although glomerular filtration rate was significantly increased (ANOVA, $P < 0.01$). The combined infusion of PD 123319 and TA did not change the relationships between RPP, renal blood flow and glomerular filtration rate. The decreases in pressure-diuresis and -natriuresis observed during infusion of TA ($10 \mu\text{g kg}^{-1} \text{min}^{-1}$) were fully abolished by PD 123319.

Figure 1 Averaged values ($n=5$) of mean blood pressure (MBP) and renal blood flow (RBF) in response to angiotensin I (AI) injections (750 ng kg^{-1}) before, 30, 60, 90 and 120 min after angiotensin converting enzyme inhibition by quinapril (10 mg kg^{-1}).

Table 1 Effects of AT₂ receptor ligands on baseline renal functions

	n	MBP (mmHg)	RBF ($\text{ml min}^{-1} \text{g}^{-1}$)	GFR ($\text{ml min}^{-1} \text{g}^{-1}$)	V ($\mu\text{l min}^{-1} \text{g}^{-1}$)	UNaV ($\mu\text{mol min}^{-1} \text{g}^{-1}$)
Controls	9	91 ± 1	6.5 ± 0.5	0.9 ± 0.1	4.1 ± 0.5	0.17 ± 0.04
TA-5	6	90 ± 1	6.5 ± 0.5	1.1 ± 0.3	3.7 ± 0.8	0.20 ± 0.04
TA-10	9	91 ± 1	6.4 ± 0.4	0.9 ± 0.1	3.5 ± 0.3	0.20 ± 0.05
TA-30	4	93 ± 2	7.4 ± 0.3	1.0 ± 0.1	4.2 ± 0.5	0.17 ± 0.05
PD	7	88 ± 2	5.9 ± 0.2	0.9 ± 0.1	3.0 ± 0.4	0.15 ± 0.02
PD + TA-10	7	91 ± 2	6.2 ± 0.4	1.2 ± 0.2	4.3 ± 0.5	0.25 ± 0.10

Mean blood pressure (MBP), renal blood flow (RBF), glomerular filtration rate (GFR), urine flow (V) and urinary sodium excretion (UNaV) observed at prevailing blood pressure in rats deprived of endogenous angiotensin II and infused with solvent (Controls) or TA at the dose of 5, 10 or $30 \mu\text{g kg}^{-1} \text{min}^{-1}$ (TA-5, TA-10 and TA-30) or PD 123319 ($50 \mu\text{g kg}^{-1} \text{min}^{-1}$, PD) or PD 123319 combined to TA (PD+TA-10).

Figure 2 Effects of TA on the relationships between renal perfusion pressure (RPP), renal blood flow (RBF), glomerular filtration rate (GFR), urine flow (V) and sodium excretion (UNaV) in rats deprived of endogenous angiotensin II and infused with solvent (Controls) or TA at the doses of 5, 10 or 30 $\mu\text{g kg}^{-1} \text{min}^{-1}$ (TA-5, TA-10 and TA-30).

Effect of AT₂ receptor stimulation on urinary excretion of cyclic GMP

As shown in Figure 4, urinary excretion of cyclic GMP, measured at the renal perfusion pressure of 130 mmHg, was significantly decreased during the infusion of TA alone (10 $\mu\text{g kg}^{-1} \text{min}^{-1}$). This decrease was transformed into an increase when TA was given in rats pretreated with PD 123319. Interestingly, PD 123319 alone also tended to increase the urinary excretion of cyclic GMP.

Discussion

The present work shows that the acute stimulation of AT₂ receptors with a new highly specific peptide agonist in adult rats, blunts pressure-natriuresis and -diuresis, and suggests that these effects of AT₂ receptors do not directly involve changes in cyclic GMP release.

The method developed by Roman & Cowley (1985) is one of the most widely used to study the acute pressure-natriuresis in anaesthetized rats. We applied this technique after ACE inhibition so as to block the endogenous production of AII and possibly enhance the renal sensitivity to AII (Richer *et al.*, 1983). The efficiency of ACE inhibition in this work was demonstrated by the lack of pressor response to AI injections during the whole experimental period. Among the compounds

having agonistic properties for AT₂ receptors, TA seems the most selective as it leaves intact AT₁ receptors at concentration above 10^{-5} M, while CGP 42112, starts having significant interactions with AT₁ receptors at the concentration of 10^{-6} M (Grouzmann *et al.*, 1995). Using this compound, we observed that at the doses of 5, 10 and 30 $\mu\text{g kg}^{-1} \text{min}^{-1}$, TA had no effect on the total renal blood flow. Since this latter is mainly controlled by AT₁ receptors (Keiser *et al.*, 1992; Lo *et al.*, 1995), this result suggests that even the highest dose of TA used, remained fully specific for AT₂ receptors. Contrasting with this lack of overall vascular effect, TA lowered pressure-natriuresis and -diuresis. These effects were dose-dependent partially only since, the 5 $\mu\text{g kg}^{-1} \text{min}^{-1}$ dose was devoid of action while the maximum effect was obtained with the 10 $\mu\text{g kg}^{-1} \text{min}^{-1}$ dose. Such a narrow dose-response relationship is difficult to explain. It might relate to the low number of AT₂ receptors available or to the high sensitivity of AT₂ receptors in our experimental conditions. The specificity of the observed effects of TA on natriuresis and diuresis was further confirmed by their disappearance after infusion of PD 123319 at a dose which does not affect AT₁ receptors (Macari *et al.*, 1993). Thus AT₂ receptor stimulation lowers pressure-natriuresis. According to this finding, Madrid *et al.*, (1997a) recently reported that in rats in which NO synthesis was blocked with N^ω-nitro-L-arginine methyl ester, AT₁ receptor blockade had no effect on the slope of the pressure-natriuresis curve, while PD 123319 normalized it thus indicating that the

Figure 3 Effects of renal perfusion pressure (RPP) on renal blood flow (RBF), glomerular filtration rate (GFR), urine flow (V) and sodium excretion (UNaV) in rats deprived of endogenous angiotensin II and infused with solvent (Controls) or TA (10 $\mu\text{g kg}^{-1} \text{min}^{-1}$, TA-10) or PD 123319 (50 $\mu\text{g kg}^{-1} \text{min}^{-1}$, PD) or PD 123319 combined to TA (PD+TA-10).

Figure 4 Urinary excretion of cyclic guanosine 3', 5' monophosphate (cyclic GMP) in rats deprived of endogenous angiotensin II and infused with solvent (Controls) or TA (10 $\mu\text{g kg}^{-1} \text{min}^{-1}$, TA-10) or PD 123319 (50 $\mu\text{g kg}^{-1} \text{min}^{-1}$, PD) or PD 123319 combined to TA (PD+TA-10). The renal perfusion pressure was set at 130 mmHg. * $P < 0.05$ vs controls; † $P < 0.05$, †† $P < 0.01$ vs TA-10.

pressure-natriuresis was under the control of AT₂ receptors. Such a function of AT₂ receptors is also in accordance with the fact that in acute conditions, AII lowers the pressure-natriuresis (Mattson *et al.*, 1991) while AT₁ receptor blockade

with losartan given acutely does not affect the pressure-natriuresis in rats (Kline & Liu, 1994; Lo *et al.*, 1995). In addition, recent studies showing that dietary sodium depletion increases the density of AT₂ receptors in adult rat kidneys (Ozono *et al.*, 1997) and decreases that of AT₁ receptors (Ruan *et al.*, 1997) reinforce the hypothesis that AT₂ receptors could be involved in sodium preservation. In that respect, since in the present experiment, as well as in our previous one (Lo *et al.*, 1995), the effects of AT₂ receptor stimulation or blockade were more marked when renal perfusion pressure was elevated, it can be speculated that AT₂ receptors contribute to the sodium losses which would follow acute increases in blood pressure.

The mechanisms of this action are difficult to approach, because little is known about not only the intracellular signaling pathways of AT₂ receptors but also about the mechanisms involved in pressure-natriuresis. Concerning the signaling pathways of AT₂ receptors *in vitro* studies conducted in tissues that selectively or mainly express these receptors, show that the activation of AT₂ receptors in R3T3 fibroblasts stimulates a phosphotyrosine phosphatase (Tsuzuki *et al.*, 1996) and decreases cyclic GMP formation in rat adrenal gland (Israel *et al.*, 1995). More recently, several kinases belonging to transduction cascades were shown to be altered by AT₂ receptor stimulation (Fischer *et al.*, 1998). Concerning the mechanisms involved in pressure-natriuresis, the most widely accepted hypothesis is that, in rats, they implicate increases in

the poorly regulated papillary blood perfusion (Roman *et al.*, 1988a) leading to rises in renal interstitial pressure (Granger, 1992) which will reduce the tubular sodium and fluid reabsorption. In that respect, the reported effects of AII on renal medullary circulation varied upon the experimental conditions. The intravenous infusion of AII at high doses did not modify (Nobes *et al.*, 1991) or increased (Parekh & Zou, 1996) medullary blood flow, an effect which was mediated by the release of vasodilator prostaglandins. The intrarenal infusion of AII at a physiological dose ($0.5 \text{ ng kg}^{-1} \text{ min}^{-1}$) decreased papillary flow without altering cortical renal blood flow and glomerular filtration rate (Faubert *et al.*, 1987). Consistently, AII receptor blockade with saralasin as well as ACE inhibition increased papillary blood flow in rats having received a bradykinin antagonist (Roman *et al.*, 1988b). Since AT₁ receptor blockade was found devoid of action on papillary blood flow (Madrid *et al.*, 1997b), it can be postulated that AII constricts medullary vessels through AT₂ receptors. Another possible mechanism is that AT₂ receptor stimulation might blunt NO release. This is suggested by the fact that, in the present work, as well as in our previous one (Lo *et al.*, 1995), AT₂ receptors were found more efficient at high than at normal pressure, a condition which, through elevated shear-stress, stimulates NO production, and might contribute to pressure-induced natriuresis by impairing the autoregulation of papillary flow (Fenoy *et al.*, 1995). To test this hypothesis and

although it is known that cyclic GMP is under the control of several circulating agents such as atrial natriuretic peptide, we used its urinary excretion as an index of renal NO synthesis (Siragy *et al.*, 1992). We observed that urinary excretion of cyclic GMP decreased during infusion of TA alone. However, when TA was given after PD 123319, urinary cyclic GMP excretion reached greater values than in controls. Finally, since PD 123319 given alone which, despite a surprising and unexplained increase in glomerular filtration rate, was devoid of effect on pressure-diuresis and -natriuresis, also increased urinary cyclic GMP, it became obvious that antinatriuretic properties of AT₂ receptor stimulation could not be explained by a decreased formation or release of cyclic GMP in the kidney.

In conclusion, using a new highly specific agonist, we confirm that AT₂ receptor stimulation blunts pressure-natriuresis, and can contribute to prevent the sodium losses induced by acute increases in blood pressure.

This work was supported by Centre National de la Recherche Scientifique. It has been presented as an oral communication at the Eighth European Meeting on Hypertension, Milan, Italy (June, 1997). We gratefully acknowledge J. Sacquet for her technical assistance. PD 123319 and quinapril were generous gifts of Parke-Davis Laboratories.

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(Received October 19, 1998)

Revised November 12, 1998

Accepted November 13, 1998)