Effect of noradrenaline, vasopressin and angiotensin II on renal prostaglandins in man

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Summary
1. To examine the response of renal prostaglandins (PG) to systemic and renal vasoconstriction noradrenaline (NA), arginine vasopressin (AVP) and angiotensin II (ANG II) were each infused into eight healthy female subjects for 3 h on different days. Urinary excretion of PGE₂, PGF₂α and 6-keto-PGF₁α was determined hourly.
2. NA and ANG II stimulated excretion of PGF₂α significantly, but not of PGE₂ or 6-keto-PGF₁α. AVP stimulated renal PGF₂α and 6-keto-PGF₁α significantly, but not PGE₂.
3. A weak correlation was found between urinary PGF₂α and diastolic blood pressure during NA and ANG II infusions, but not during AVP infusion.
4. The release of renal PG does not appear to constitute an obligatory and concomitant response to the blood pressure rise induced by the pressor agonists. The greater response of PGF₂α than of PGE₂ may result from a preferential direct effect on PGF₂α secretion or from an increased conversion of PGE₂ into F₂α.

Key words: angiotensin II, noradrenaline, renin, urinary prostaglandins E₂, F₂α and 6-keto-PGF₁α.

Abbreviations: ANG II, angiotensin II; AVP, arginine vasopressin; NA, noradrenaline; PG, prostaglandin; PRA, plasma renin activity; TX, thromboxane.

Introduction
The principal functions attributed to renal prostaglandins (PG) are modulation of renal blood flow and electrolyte excretion, activation of renin secretion, and antagonism to the antidiuretic action of vasopressin [1].

The vasodilatory properties of renal PG oppose the vasoconstrictor influences of endogenous pressor hormones such as noradrenaline (NA), angiotensin II (ANG II) and arginine vasopressin (AVP) [review in [2, 3]]. On the other hand these hormones participate actively in the process of PG biosynthesis in different renal compartments.

Several animal studies in vivo and in vitro have shown that NA, AVP and ANG II can stimulate the release of PG by the whole kidney [2, 3]. Other studies have demonstrated direct stimulation of PG by NA, AVP and ANG II on dispersed and/or cultured cells [4–8]. In vitro, production of renal PGE₂ and PGF₂α occurs mainly in the medulla whereas prostacyclin (PGI₂) is produced chiefly by the glomeruli and the cortical vessels [9]. Two clinical studies have shown that NA, AVP and ANG II all increase the urinary excretion of PGE₂ [10, 11]. Whether the mechanism of this stimulation is direct or indirect has not been elucidated.

The present study was designed to examine the acute effects of three vasoconstrictor hormones infused at three stepwise increasing dose rates on the urinary excretion of PGE₂, PGF₂α and 6-keto-PGF₁α, a stable metabolite of PGI₂, in human subjects.

Methods
Subjects
Eight healthy female medical students aged 22–27 years and weighing 49–63 kg participated in the study. They were taking no medication. They were fully informed and gave their written consent to the experimental protocol, which was approved by the Ethical Committee of the Depart-
ment of Medicine. During the 3 days preceding the study, the subjects observed a normocaloric diet containing 135 mmol of sodium/day and 70 mmol of potassium/day. The daily water intake ranged from 1.5 to 2 litres.

**Experimental protocol**

The three vasoactive hormones were tested on 3 consecutive days. The subjects remained supine overnight and during the whole morning. The tests comprised four periods of 1 h including one control period and three periods of infusion of each hormone at three stepwise increasing infusion rates: NA 20, 100, 200 ng min⁻¹ kg⁻¹; AVP (kindly provided by Ferring, Malmö, Sweden) 0.2, 1, 2 ng min⁻¹ kg⁻¹; ANG II (kindly provided by Ciba-Geigy, Basel, Switzerland) 1, 5, 10 ng min⁻¹ kg⁻¹.

The substances were dissolved at appropriate dilutions in 50 ml of 0.9% sodium chloride. The flow rate, maintained constant by an infusion pump with a plastic syringe, ranged from 0.03 to 0.3 ml/min for NA and AVP and from 0.025 to 0.25 ml/min for ANG II. The sequence of the tests was randomized for each subject. The infusions flowed in the cubital vein of one arm. Hourly blood samples were drawn from the other arm for the determination of electrolytes, creatinine, osmolality, plasma renin activity (PRA). NA and AVP. Blood pressure was recorded every 2 min by an automatic sphygmomanometer (Tonomed, SK, Germany). The mean hourly blood pressure was calculated from the last 10 measurements for each hour.

To ensure sufficient urinary output, fluid intake was 8.3 ml/kg the evening before each test and 3.3 ml h⁻¹ kg⁻¹ from 07.00 hours to 12.00 hours during the tests. Urines were collected for 24 h the day before the study and hourly during the tests by spontaneous voiding. Portions were immediately frozen at −20°C for the determination of prostaglandins. For technical reasons the infusion of ANG II was discontinued in one subject, who was withdrawn from this part of the study. Because of difficulties in collecting hourly urine during ANG II and AVP infusions, some urinary data are missing. Urinary 6-keto-PGF₁α was measured during the first and last hours only. For paired data analysis we have therefore considered only the subjects who completed the entire experimental protocol for each infusion.

**Analytical methods**

Sodium and potassium were measured by flame photometry, creatinine by Jaffé's colorimetric method, osmolality by depression of the freezing point (Advanced Digimatic Osmometer, Advanced Instruments Inc., MA, U.S.A.). PRA [12, 13] and plasma AVP [14] were measured by using specific radioimmunoassays. Plasma NA was determined by radioenzymatic assay [15]. Urinary excretion of PGE₂ and PGF₂α was estimated by radioimmunoassay as described before [16–18], with PGE₂ antiserum purchased from the Institut Pasteur (Paris, France) and PGF₂α antiserum kindly given by Dr M. Dunn (Division of Nephrology, Case Western Reserve University, Cleveland, OH, U.S.A.). With PGF₂α antiserum no significant cross-reaction with TXA₂, TXB₂, 15-keto-PGF₁α, PGI₂ or 6-keto-PGF₁α was observed. Tritiated PGE₂ and PGF₂α, with a stated specific radioactivity of 160 Ci/mmol were purchased from Radiochemicals Co., Amer- sham, U.K.

Urine (3 ml), acidified to pH 2.5–3.0 with citric acid, was extracted twice with 6 ml of ethyl acetate/cyclohexane (1:1, v/v). The extract was chromatographed on silicic acid columns by a stepwise elution with increasing concentrations of methanol in toluene/ethyl acetate (3:2, v/v), to separate PGE and PGF fractions. The recoveries measured by internal tritiated tracer were 69.1 ± 0.5% (n = 100) for PGE₂ and 77.4 ± 0.5% for PGF₂α. The inter-assay coefficients of variation were 7.8% (n = 33) and 12.5% (n = 33), respectively for PGE₂ and PGF₂α. No correction was made for blank values since they were not significantly different from zero.

The urinary excretion rate of prostacyclin was estimated by the radioimmunoassay of 6-keto-PGF₁α (method of T. Garcia, J. M. Dayer, M. B. Valloton, R. Loup & C. Wicht, unpublished work), a stable metabolite of prostacyclin. The 6-keto-PGF₁α antiserum was kindly given by Dr M. Dunn. Tritiated tracer with a stated specific radioactivity of 150 Ci/mmol was purchased from Radiochemicals Co. A portion (50 μl) of recovery tracer (3000 c.p.m.) was added to 27 ml of urine for the control hour or to 9 ml of urine for the infusion hour and for the 24 h collection. The extraction procedure used Sep-pak C18 cartridges (Waters Associates, Milford, MA, U.S.A.) [19]. Because of cross-reactivity of the antiserum with other PG, mainly PGE₂ and PGF₂α, the extract was chromatographed on HPLC (C18 radial compression cartridge, Wisp, Waters Associates) in reverse phase with water/acetonitrile/acetic acid (73.3/26.6/0.1, by vol.); 6-keto-PGF₁α was preincubated for 1 h with the antiserum before the addition of the tritiated tracer (100 μl = 2500 c.p.m.) for radioimmunoassay. The recovery of the tracer throughout the procedure was 46 ± 7% (so). Intra- and inter-assay coefficients of variation were 9 and 12% respectively.
Statistics

Results are expressed as means ± SEM. Statistical analysis was carried out with a two-tailed t-test for paired data, each subject being his own control for each parameter. P values < 0.05 were considered significant. No statistical comparison was made between the three substances infused.

Results

For the 24 h preceding infusion, urinary excretion of sodium, potassium and creatinine was 121 ± 13, 69 ± 7 and 11 ± 1 mmol/24 h respectively.

Effects of noradrenaline infusion

Each dose of NA induced a significant dose-related rise in systolic and diastolic blood pressure and a significant fall in pulse rate (Fig. 1). As shown in Table 1, there was no change in plasma sodium, potassium and osmolality. Plasma AVP remained stable. Plasma NA increased significantly at each successive dose. There was no change in urinary volume, sodium and potassium excretion, or PRA. Urinary osmolality increased. Creatinine clearance did not decrease significantly. Urinary PGE₂ increased by 148% and 6-keto-PGF₁α (227%) (Fig. 2).

Effects of arginine-vasopressin infusion

AVP induced a significant increase in diastolic blood pressure, without any change in systolic pressure or in pulse rate (Fig. 1). As shown in Table 1, plasma sodium and osmolality decreased significantly, whereas the changes in potassium concentration, PRA and NA were not significant. Plasma AVP concentration increased significantly. There was no change in urinary sodium or potassium excretion. Creatinine clearance did not decrease significantly. Urinary volume decreased significantly after the second dose of AVP. Urinary osmolality increased after the lowest dose of AVP. Urinary PGE₂ increased by 181% (n.s.), whereas PGE₂α increased significantly for the last 2 h (143% increase for the last hour) and 6-keto-PGF₁α for the last hour by 195% (Fig. 2). There was no significant relationship between urinary PGF₂α and diastolic blood pressure (r = 0.12, n.s.).

Effects of angiotensin II infusion

ANG II increased diastolic blood pressure after the first dose and systolic pressure after the second dose. Pulse rate fell significantly only during the second period of infusion (Fig. 1). As shown in

\[\text{Fig. 1. Effects of 3 h infusions of noradrenaline (NA), vasopressin (AVP) and angiotensin II (ANG II) on blood pressure (\(\nabla\), systolic; \(\Delta\), diastolic) and heart rate (\(\circ\)) in normal subjects. Mean values ± SEM are shown.}^*\text{P}<0.05;**\text{P}<0.01;***\text{P}<0.001.\]
**Table 1.** Effects of noradrenaline, vasopressin and angiotensin II on various plasma and urine variables.

Means ± SEM are shown. NS, Not significant; *P < 0.05, **P < 0.01, ***P < 0.001.

<table>
<thead>
<tr>
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<th>Noradrenaline</th>
<th>Vasopressin</th>
<th>Angiotensin</th>
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<td>C</td>
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<tr>
<td>Plasma sodium (mmol/l)</td>
<td>137.9 ± 0.6</td>
<td>137.8 ± 0.8</td>
<td>138.1 ± 0.5</td>
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<td>Plasma potassium (mmol/l)</td>
<td>4.0 ± 0.1</td>
<td>3.8 ± 0.1</td>
<td>3.9 ± 0.1</td>
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<td>Plasma osmolality (mosmol/kg)</td>
<td>294 ± 11.1</td>
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<tr>
<td>Urinary volume (ml/h)</td>
<td>99 ± 28</td>
<td>73 ± 23</td>
<td>99 ± 28</td>
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<tr>
<td>Urinary sodium (mmol/h)</td>
<td>2.1 ± 0.6</td>
<td>2.2 ± 0.8</td>
<td>2.7 ± 0.2</td>
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<tr>
<td>Urinary potassium (mmol/h)</td>
<td>1.5 ± 0.3</td>
<td>1.4 ± 0.3</td>
<td>1.7 ± 0.2</td>
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<tr>
<td>Urinary osmolality (mosmol/kg)</td>
<td>262 ± 24</td>
<td>225 ± 16</td>
<td>225 ± 16</td>
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<tr>
<td>Plasma renin activity (pmol h⁻¹ ml⁻¹)</td>
<td>1.5 ± 0.5</td>
<td>1.5 ± 0.5</td>
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<tr>
<td>Urinary 6-keto-PGF₁α (ng/h)</td>
<td>12 ± 2.4</td>
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**Fig. 2.** Effects of 3 h infusions of noradrenaline (NA), vasopressin (AVP) and angiotensin II (ANG II) on urinary excretion of prostaglandins E₂, F₂α and 6-keto-PGF₁α in normal subjects. Mean values ± SEM are shown. *P < 0.05.

Table 1, plasma sodium, osmolality and PRA decreased significantly at the end of the last hour. Plasma potassium concentration did not change. Plasma AVP increased significantly. NA did not change. Urinary sodium, potassium, volume and creatinine clearance decreased, and urinary osmolality increased, all significantly. Urinary PGE₂ excretion increased by 27% (n.s.). Urinary PGF₂α excretion increased significantly by 201% during the last hour. There was a significant weak relationship between urinary PGF₂α and diastolic blood pressure (r = 0.43, P < 0.05). Excretion of 6-keto-PGF₁α did not change (Fig 2).
Discussion

Three vasoactive hormones, NA, AVP and ANG II, were used in this study at pharmacological doses in order to compare their systemic and renal effects, and to establish dose–response curves in relation to different haemodynamic, renal and hormonal parameters. As might be expected from the dosages selected, the systemic vasoconstrictor effect of NA and ANG II was of comparable magnitude. As a result of its predominantly antidiuretic action, AVP was less potent and increased diastolic blood pressure only. Other investigators using comparable doses of AVP have reported a similar diastolic rise [20] or no pressor effect [21].

Renal PG, especially PGE₂ and PGI₂, may contribute to the defence against the intrarenal vasoconstriction caused by NA, AVP or ANG II [3, 22-24]. Conversely, PG inhibition induced by cyclo-oxygenase inhibitors has been shown to aggravate renal ischaemia and precipitate renal failure under certain circumstances [25, 26]. On the other hand, numerous studies in vitro have demonstrated that NA, AVP and ANG II have a PG stimulatory effect on different types of renal cells, suggesting direct action resulting from binding to specific receptors [8, 27, 28].

The aim of the present study was to determine whether a relation exists for each vasoconstrictor hormone between its systemic pressor effect and its ability to stimulate the release of renal PG. Other investigators pursuing a similar clinical approach have reported a selective stimulatory effect of pressor agonists which was absent in non-pressor analogues [10, 11]. This is borne out by our finding of a positive correlation between the rise in diastolic blood pressure and the change in PGF₂α during NA and ANG II infusions, but not during AVP infusion. Regarding the link between vasoconstriction and release of renal PG, a set of experiments in vitro and in vivo in control and diabetes insipidus rats have established that AVP and its non-pressor analogue dDAVP have a direct stimulatory effect on renal PG [28, 29]. We also observed that dDAVP increased PGE₂ and PGF₂α in man [30], as opposed to the PG decrease associated with antidiuresis in other studies [10, 31]. Moreover, like pressor agents, bradykinin can stimulate PG in vitro and in vivo [32].

It may well be that different cells, i.e. vascular, cortical, tubular, interstitial or medullary collecting tubular cells, respond differently to the pressor agents NA, ANG II and possibly AVP, or to the non-pressor agent dDAVP. Since we do not know the intrarenal cellular source of urinary PG in the present study, this remains a matter of speculation which can only be settled by experiments in vitro. It is generally accepted that urinary PG, at least PGE₂ and PGF₂α, have an intrarenal origin. In this clinical study, as in earlier ones [18, 33], the urinary excretion rate of PG was independent of urine flow. As far as prostacyclin (PGI₂) and its stable inactive metabolite 6-keto-PGF₁α are concerned, the issue is not yet definitively resolved, as a small fraction of systemic circulating PGI₂ may be filtered and excreted in urine, in addition to the local product of renal synthesis [34, 35]. However, the contribution of systemic PGI₂ to urinary PG appears to be minimal [36].

In interpreting our results, both the multiple sites of action of the pressor agonists and the compartmentalization of the sites of synthesis of renal PG sensitive to these hormones should be kept in mind [2]. One striking observation is the differences between the changes of excretion of the PG measured, namely PGE₂, PGF₂α, 6-keto-PGF₁α, NA, AVP and ANG II induced a much greater release of PGF₂α than of PGE₂, and the difference was not correlated with their respective basal levels. In other studies, the PGE₂ response to these agonists was slightly more marked than in this study and reached the level of significance, but no comparison with PGF₂α was made [10, 11]. The renomedullary interstitial cells and collecting duct cells, main sources of renal PG, secrete both PGE and PGF [1, 6], and renal production of PGE₂ and PGF₂α, may or may not be linked in vivo after acute stimulation [32, 37]. In the dog ANG II infusion increases the secretory PGE/PGE ratio [32]. Alternatively, PGE to PGF conversion may have occurred by activation of 9-keto-reductase [38]. Interestingly, it has been reported that dDAVP stimulated PGF more than PGE, as we observed in man, whereas AVP has an equipotent effect on PGE and PGF [28, 39]. The precise function of PGF₂α within the kidney has not yet been fully assessed. It does not modify the renal blood flow in the dog, but increases blood pressure, contracts non-vascular smooth muscle and mediates the vasoconstriction induced by bradykinin [1].

PGI₂, the most potent vasoactive PG originating from vascular structures, is the best candidate for countering vasoconstrictor agents. ANG II has been found to stimulate release of PGL₁ in various tissues [3], and NA to stimulate PGL₂ synthesis at least in dog kidney cells [5]. However, a net rise in urinary 6-keto-PGF₁α was only detected during AVP infusion. More recently, it has been shown that urinary 6-keto-PGF₁α is not influenced by AVP injected subcutaneously, whereas it increases slightly but significantly during NA infusion [11].

In conclusion, the rise in blood pressure induced by NA, AVP and ANG II is not associated with a concomitant increase in urinary excretion of PGE₂, PGF₂α and 6-keto-PGF₁α. The slight response of
PGE\(_2\) compared with the marked response of PGF\(_2\alpha\), may result from a preferential direct effect on PGF\(_2\alpha\) secretion or from increased conversion of PGE\(_2\) into PGF\(_2\alpha\). The respective intrarenal roles of prostaglandins E\(_2\), F\(_2\alpha\), and I\(_2\) in modulating or antagonizing the renal effects of vasopressor peptides in vivo require further elucidation.

Acknowledgments

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References


