The effect of medetomidine, an α_2 -adrenoceptor agonist, on plasma atrial natriuretic peptide levels, haemodynamics and renal excretory function in spontaneously hypertensive and Wistar-Kyoto rats

¹Heikki Ruskoaho & *Juhani Leppäluoto

Departments of Pharmacology and Toxicology and *Physiology, University of Oulu, Kajaanintie 52D, SF-90220 Oulu, Finland

1 The effects of the selective α_2 -adrenoceptor agonist, medetomidine, were assessed on plasma levels of immunoreactive atrial natriuretic peptide (IR-ANP), haemodynamics and on urine water and solute excretion in conscious, chronically cannulated, 7 month-old spontaneously hypertensive (SHR) and age-matched Wistar-Kyoto (WKY) rats, in order to examine the role of α_2 -adrenoceptors in the control of ANP secretion.

2 A 60 min i.v. infusion of medetomidine (0.2 or $0.6 \mu\text{g kg}^{-1} \text{min}^{-1}$) decreased heart rate dose-dependently in both strains. Medetomidine infusion ($0.6 \mu\text{g kg}^{-1} \text{min}^{-1}$) resulted in an increase in mean arterial pressure in WKY, whereas both doses decreased blood pressure in SHR. There was a slight increase in the right atrial pressure in both strains (WKY: $+1.18 \pm 0.26 \text{ mmHg}$; SHR: $+1.64 \pm 0.64 \text{ mmHg}$, NS) in response to infusion of $0.6 \mu\text{g kg}^{-1} \text{min}^{-1}$ of medetomidine.

3 No differences were found in resting plasma IR-ANP levels between WKY ($114 \pm 8 \text{ pg ml}^{-1}$, $n = 19$) and SHR ($117 \pm 10 \text{ pg ml}^{-1}$, $n = 21$). Infusion of equibradycardic doses of medetomidine increased dose-dependently plasma IR-ANP levels in WKY, but did not affect the plasma IR-ANP concentration in SHR rats.

4 Despite the different effect of medetomidine on ANP release in WKY and SHR rats, i.v. administration of medetomidine affected renal excretory functions similarly in both strains; urine flow and sodium excretion increased and urine osmolality decreased significantly, while there was no consistent change in urinary potassium excretion. Urine osmolality decreased to hypo-osmotic levels during the infusion of $0.6 \mu\text{g kg}^{-1} \text{min}^{-1}$ of medetomidine, suggesting a possible interaction between α_2 -adrenoceptor stimulation and the vasopressin system.

5 These results show that the α_2 -adrenoceptor agonist medetomidine increased plasma levels of ANP in WKY rats, probably through an increase in mean arterial and right atrial pressures, whereas the SHR had attenuated ANP release to α_2 -adrenoceptor stimulation. Our findings, that medetomidine caused marked natriuretic and diuretic effects in both strains and that these effects on the excretory functions of the kidneys were not related to changes in plasma levels of IR-ANP, demonstrate that changes in plasma ANP levels alone do not account for the diuretic and natriuretic effect of α_2 -agonists.

Introduction

Atrial natriuretic peptide (ANP) is a hormone released from cardiac atria which acts upon the kidneys, vasculature, adrenal glands and brain to regulate volume and blood pressure (Laragh, 1985;

Brenner & Ballerman, 1986; Needleman & Greenwald, 1986). The exact mechanism by which ANP is released is not known, although an increase in atrial pressure, presumably leading to augmented mechanical distension of the atrial wall, may be the primary physiological regulator (Lang *et al.*, 1985; Ruskoaho

¹ Author for correspondence.

et al., 1987). Little is known about other factors that may influence ANP release. Adrenaline, noradrenaline, vasopressin and other agents have been shown to increase the release of ANP into the circulation both in man and experimental animals, probably via their pressor effects (Manning *et al.*, 1985; Uehlinger *et al.*, 1986; Ruskoaho *et al.*, 1987; Sanfield *et al.*, 1987). In addition, clonidine, an α_2 -agonist, has been shown to increase plasma levels of ANP in conscious rats (Baranowska *et al.*, 1987a,b,c). However, because atrial pressure or other haemodynamic variables were not measured in these studies, the role of α_2 -adrenoceptors in the control of ANP release remains unclear.

The present study was thus undertaken to examine the effects of an α_2 -agonist, medetomidine, on plasma levels of immunoreactive ANP (IR-ANP), haemodynamics (mean arterial pressure, heart rate, right atrial pressure) and renal excretory function simultaneously in genetically hypertensive and age-matched normotensive control rats. Medetomidine has been shown to be a potent, selective and specific α_2 -adrenoceptor agonist both in experimental animals and in man (Savola *et al.*, 1986; Scheinin *et al.*, 1986; 1987; Virtanen *et al.*, 1988). Binding studies carried out in rat brain membranes showed a relative α_2/α_1 selectivity ratio of 1620 for medetomidine (compared to 220 for clonidine, 300 for UK 14304) and negligible binding to, or effects at, β -adrenoceptors and opiate, adenosine, dopamine, histamine or 5-hydroxytryptamine receptors (Virtanen *et al.*, 1988). Biochemical experiments in rats have indicated that the compound reduces the release and metabolism of noradrenaline in the central nervous system (Scheinin *et al.*, 1986). In anaesthetized rats, medetomidine reduced blood pressure and heart rate, and in the pithed rat it caused peripheral vasoconstriction and inhibition of tachycardia induced by preganglionic sympathetic nerve stimulation (Savola *et al.*, 1986). Idazoxan and other α_2 -adrenoceptor antagonists, but not prazosin, antagonized these effects (Savola *et al.*, 1986; Scheinin *et al.*, 1986).

Methods

Animals

Male 7 month-old spontaneously hypertensive rats (SHR) of the Okamoto-Aoki strain (F61) and age-matched Wistar-Kyoto (WKY) rats from the Department of Pharmacology colony at the University of Oulu, Oulu, Finland, were used. Both strains were originally obtained from Mollegaards Avslaboratorium, Skensved, Denmark. The rats were

housed in plastic cages in a room with controlled 40% humidity and a temperature of 22°C. A 12 h light/dark cycle (lights on 06 h 00 min) was maintained. The experimental protocols were approved by the Committee for Animal Experimentation of the University of Oulu.

Experimental procedures

The surgical preparation has been described previously (Ruskoaho & Leppäluoto, 1988). Briefly, rats were anaesthetized (for 30–40 min) with chloral hydrate (300 mg kg⁻¹ i.p.), the left femoral artery was cannulated for recording of blood pressure and heart rate and the left femoral vein for drug infusions. A cannula was inserted into the right atrium via the jugular vein for measurement of right atrial pressure. After surgery, rats were housed individually and had continuous access to food and water until the day of experiment.

The day after the operation, a rat was placed in the metabolic cage, and the arterial and right atrial catheters were attached to pressure transducers (model MP-15, Micron Instruments Inc., Los Angeles, CA, U.S.A.) and a Grass polygraph (model 7D, Grass Instruments, Quincy, Mass., U.S.A.) for mean arterial pressure, heart rate and right atrial pressure recording. The venous catheter was connected to an infusion pump (B. Braun PerfusorED, Braun Melsungen AG, FRG) for infusions of drug and replacement fluid.

After an equilibration period (30 min), haemodynamic variables were recorded in the conscious animals for 60 min (control period). Then, either vehicle (0.9% saline) or the α_2 -adrenoceptor agonist, medetomidine was administered i.v. as a continuous infusion at a rate of 66 μ l min⁻¹ for 60 min, followed by a recovery period of 60 min. The doses of medetomidine infused (0.2 or 0.6 μ g kg⁻¹ min⁻¹) were chosen based on preliminary experiments in conscious rats. These experiments revealed that infusion of medetomidine at these doses resulted in increases in plasma IR-ANP comparable to those observed earlier after acute volume expansion under identical experimental conditions (Ruskoaho & Leppäluoto, 1988). Arterial blood samples (0.8 ml) for plasma IR-ANP were taken 30 and 5 min before and at 10, 30, 60, and 90 min after the start of infusion. Each blood sample taken was replaced by an equal volume of blood from a donor rat. Urine was collected during control, infusion and recovery periods. To establish correlations between haemodynamic variables and renal excretory functions and plasma levels of IR-ANP, mean arterial pressure, heart rate and right atrial pressure were measured continuously throughout the experiments.

Urinary osmolality was measured with an Advanced wide-range osmometer 3W2 (Advanced Instruments, Inc., Needham Heights, Massachusetts, U.S.A.), and electrolytes were determined with FLM3 Flame Photometer (Radiometer, Copenhagen, Denmark). The volume of urine was measured gravimetrically.

Assay of immunoreactive atrial natriuretic peptide in plasma

All blood samples (0.8 ml) were taken into pre-cooled EDTA tubes on ice, immediately centrifuged (2000 *g*, 10 min, at +4°C) and the plasma stored at -20°C until assayed by radioimmunoassay (RIA). ANP was extracted by Sep-Pak C₁₈ cartridges from plasma as described previously (Ruskoaho & Leppäluoto, 1988). The recovery of added rat ANP₁₋₂₈ (50 pg) was 81.7 ± 3.3% (mean ± s.e.mean, *n* = 6). The ANP values were not corrected for recovery.

The plasma extracts were diluted with the radioimmunoassay buffer and incubated in duplicates of 100 µl with the same volume of the specific rabbit ANP antiserum at the final dilution of 1/25,000 (Vuolteenaho *et al.*, 1985; Ruskoaho & Leppäluoto, 1988). Synthetic rat ANP₁₋₂₈ (Sigma) ranging from 0 to 1250 pg per tube was used as standard. The ANP tracer was rat [¹²⁵I]-ANP₁₋₂₈ from Amersham, England. After incubation for 20–44 h at +4°C the bound and free fractions were separated by double antibody in the presence of 0.5 ml 1.2 M (NH₄)₂SO₄, pH 7. The sensitivity of the assay was 1 pg per tube. The intra-assay and inter-assay coefficients were 5% and 14%, respectively. The rat plasma extracts diluted serially displaced [¹²⁵I]-ANP in parallel with the synthetic ANP₁₋₂₈ standard and eluted (over 90%) in the reverse phase high performance liquid chromatography as rat ANP₁₋₂₈.

Materials

Medetomidine hydrochloride was a gift from Dr A. Karjalainen, Farnos Group Ltd, Farnos Research Center, Turku, Finland. Synthetic rat ANP₁₋₂₈ was obtained from Sigma Chemicals and heparin from Medica, Helsinki, Finland. Other chemicals were from Sigma Chemicals.

Data analysis

The results are expressed as mean ± s.e.mean. The data were analysed with two- or one-way analysis of variance (ANOVA). For the comparison of statistical significance between two independent groups, Student's *t* test for unpaired data was used. For the multiple comparison, one-way analysis of variance

Table 1 Body weight, baseline cardiovascular and urinary excretory variables and plasma levels of immunoreactive atrial natriuretic peptide (IR-ANP) in conscious 7 month-old spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats

	WKY (<i>n</i> = 19)	SHR (<i>n</i> = 21)
Body weight (g)	420 ± 6	388 ± 6***
Mean arterial pressure (mmHg)	125 ± 2	165 ± 3***
Heart rate (beats min ⁻¹)	336 ± 7	363 ± 8*
Right atrial pressure (mmHg)	3.0 ± 0.3	3.7 ± 0.5
Plasma IR-ANP (pg ml ⁻¹)	114 ± 8	117 ± 10
Urinary volume (ml h ⁻¹ 100 g ⁻¹)	0.47 ± 0.04	0.42 ± 0.05
U _{Na} V (mEq h ⁻¹ 100 g ⁻¹)	11.5 ± 2.4	19.7 ± 4.1
U _K V (mEq h ⁻¹ 100 g ⁻¹)	34.3 ± 3.0	25.3 ± 3.4
U _{osm} (mosm kg ⁻¹ H ₂ O)	521 ± 70	515 ± 45

Results are expressed as mean ± s.e.mean.
* *P* < 0.05 and *** *P* < 0.001 WKY vs SHR (Student's *t* test). U_{Na}V = urinary sodium excretion; U_KV = urinary potassium excretion; and U_{osm} = urine osmolality.

followed by the Bonferroni *t* test was used. Differences at the 95% level were considered significant.

Results

Effect of medetomidine infusion on haemodynamics in conscious SHR and WKY rats

The mean basal arterial pressure, as measured directly in chronically cannulated conscious rats, and heart rate were significantly higher in SHR than in WKY at the age of 7 months but their right atrial pressures did not differ significantly (Table 1). The body weight of SHR was lower than that of WKY rats (Table 1). Table 2 shows the effect of medetomidine infusions on haemodynamics in WKY and SHR. A sixty min infusion of 0.2 µg kg⁻¹ min⁻¹ of medetomidine decreased heart rate maximally by 23% (*F* = 3.85 for drug and time interaction, *P* < 0.01) in WKY and by 18% in SHR and an infusion of 0.6 µg kg⁻¹ min⁻¹ by 34% and by 35%, respectively.

The changes in mean arterial pressure evoked by medetomidine infusions were clearly different in WKY and SHR rats at both doses tested and showed an opposite pattern. Infusion of 0.6 µg kg⁻¹ min⁻¹ of medetomidine in WKY rats resulted in an increase in mean arterial pressure, whereas the lower dose of medetomidine had no significant effect (Table 2). In contrast, both doses of medetomidine decreased blood pressure in SHR

Table 2 Effects of a 60 min infusion of the α_2 -agonist medetomidine (Med) on cardiovascular variables in spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats

	Control	At 10 min	Infusion At 30 min	At 60 min	Recovery	F
<i>Heart rate (beats min⁻¹)</i>						
WKY						
Vehicle	346 ± 12	328 ± 13	313 ± 12	318 ± 14	325 ± 18	—
Med 0.2 µg kg ⁻¹ min ⁻¹	318 ± 8	277 ± 6	263 ± 14	245 ± 15	300 ± 9	F = 3.83; P < 0.01
Med 0.6 µg kg ⁻¹ min ⁻¹	343 ± 14	275 ± 23	226 ± 22	228 ± 22	318 ± 34	F = 12.51; P < 0.001
SHR						
Vehicle	361 ± 13	346 ± 7	334 ± 12	346 ± 12	337 ± 13	—
Med 0.2 µg kg ⁻¹ min ⁻¹	367 ± 12	337 ± 10	300 ± 13	300 ± 7	336 ± 12	F = 5.68; P < 0.001
Med 0.6 µg kg ⁻¹ min ⁻¹	361 ± 18	296 ± 13	243 ± 10	233 ± 11	321 ± 23	F = 15.06; P < 0.001
<i>Mean arterial pressure (mmHg)</i>						
WKY						
Vehicle	122 ± 3	120 ± 4	120 ± 3	116 ± 3	116 ± 2	—
Med 0.2 µg kg ⁻¹ min ⁻¹	123 ± 5	123 ± 5	130 ± 8	129 ± 9	118 ± 7	F < 2; NS
Med 0.6 µg kg ⁻¹ min ⁻¹	128 ± 3	138 ± 5	137 ± 6	138 ± 8	121 ± 4	F = 4.76; P < 0.003
SHR						
Vehicle	157 ± 6	155 ± 6	156 ± 7	156 ± 7	154 ± 9	—
Med 0.2 µg kg ⁻¹ min ⁻¹	171 ± 5	157 ± 6	141 ± 9	131 ± 7	134 ± 4	F = 9.11; P < 0.001
Med 0.6 µg kg ⁻¹ min ⁻¹	169 ± 4	148 ± 5	149 ± 6	152 ± 6	142 ± 4	F = 3.66; P < 0.01
<i>Right atrial pressure (mmHg)</i>						
WKY						
Vehicle	2.6 ± 0.5	2.5 ± 0.4	2.4 ± 0.4	2.3 ± 0.5	2.7 ± 0.5	—
Med 0.2 µg kg ⁻¹ min ⁻¹	3.4 ± 0.7	3.2 ± 0.6	3.3 ± 0.5	3.0 ± 0.5	2.9 ± 0.7	F = 2.46; NS
Med 0.6 µg kg ⁻¹ min ⁻¹	3.1 ± 0.6	3.6 ± 0.8	3.9 ± 0.8	3.3 ± 0.7	2.5 ± 0.6	F = 6.26; P < 0.001
SHR						
Vehicle	3.3 ± 0.7	3.0 ± 0.5	3.2 ± 0.6	3.4 ± 0.7	3.7 ± 0.6	—
Med 0.2 µg kg ⁻¹ min ⁻¹	3.3 ± 0.7	3.1 ± 0.7	2.9 ± 0.6	2.9 ± 0.6	2.4 ± 0.7	F = 2.00; NS
Med 0.6 µg kg ⁻¹ min ⁻¹	3.4 ± 0.5	4.0 ± 0.6	3.8 ± 0.4	3.2 ± 0.6	3.0 ± 0.7	F = 3.45; P < 0.02

Results are expressed as mean ± s.e.mean from 5–8 animals. Recovery = 30 min after the end of infusion. The data were analysed with two-way analysis of variance (ANOVA). F = drug and time interaction, medetomidine vs vehicle.

(0.2 µg kg⁻¹ min⁻¹: maximal decrease of 23%; and 0.6 µg kg⁻¹ min⁻¹: -10%).

The right atrial pressure increased in both strains in response to an infusion of 0.6 µg kg⁻¹ min⁻¹ of

medetomidine (Table 2). The maximal increases in right atrial pressure were noted between 5–30 min after the start of medetomidine infusion and were 1.18 ± 0.26 mmHg in WKY and 1.64 ± 0.64 mmHg

Table 3 Effects of a 60 min infusion of the α_2 -agonist medetomidine (Med) on plasma levels of immunoreactive atrial natriuretic peptide (IR-ANP in pg ml⁻¹) in spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats

	Control	At 10 min	Infusion At 30 min	At 60 min	Recovery	F
WKY						
Vehicle	93 ± 7	126 ± 20	133 ± 14	140 ± 21	131 ± 27	—
Med 0.2 µg kg ⁻¹ min ⁻¹	136 ± 18	179 ± 29	260 ± 68	216 ± 70	174 ± 35	F = 2.89; P < 0.09
Med 0.6 µg kg ⁻¹ min ⁻¹	112 ± 11	194 ± 31	261 ± 45	239 ± 35	148 ± 18	F = 10.57; P < 0.003
SHR						
Vehicle	123 ± 19	130 ± 20	128 ± 15	123 ± 17	125 ± 21	—
Med 0.2 µg kg ⁻¹ min ⁻¹	105 ± 18	114 ± 12	120 ± 18	108 ± 21	121 ± 12	F < 2; NS
Med 0.6 µg kg ⁻¹ min ⁻¹	120 ± 24	119 ± 22	149 ± 15	139 ± 19	120 ± 24	F < 2; NS

Results are expressed as mean ± s.e.mean from 6–8 animals. Recovery = 30 min after the end of infusion. The data were analysed with two-way analysis of variance (ANOVA). F = drug and time interaction, medetomidine vs vehicle.

in SHR ($t = 0.63$, NS, paired t test). The lower dose of medetomidine had no significant effect on right atrial pressure (Table 2).

Effect of medetomidine infusion on plasma ANP in conscious SHR and WKY rats

The resting values of plasma IR-ANP were similar in conscious 7 month-old WKY and SHR rats with indwelling catheters (Table 1). Basal IR-ANP levels in these rats were similar to those obtained earlier at this age in our strains (Ruskoaho & Leppäluoto, 1988). A marked difference was observed in the plasma IR-ANP response to medetomidine infusion between the two strains. In WKY rats, medetomidine increased dose-dependently the plasma concentration of IR-ANP: a maximal increase of $105 \pm 48\%$ after the lower dose and $196 \pm 62\%$ after the higher dose of medetomidine infusion was noted (Table 3). In contrast, the i.v. infusions of medetomidine did not affect plasma levels of IR-ANP in SHR rats (Table 3).

Urine volume and solute excretion in SHR and WKY rats

Table 1 shows the mean values of the renal excretory functions in conscious WKY and SHR rats measured before the infusion of medetomidine. No significant differences in urine flow, urinary sodium ($U_{Na}V$) and urinary potassium (U_KV) excretion or, in urine osmolality (U_{osm}) were found between these strains.

The mean values of the urinary excretory function in WKY and SHR during control (C), infusion (E), and recovery (R) periods are shown in Figure 1. Infusion of vehicle (0.9% saline) caused a slight increase in urine osmolality in WKY rats but had no significant effect on urine volume or $U_{Na}V$ and U_KV . A 60 min constant infusion of medetomidine dose-dependently increased urine flow and urinary sodium excretion in WKY and SHR rats, while urine osmolality decreased in both strains. Excretion of potassium was unaffected, except that it increased during the infusion of the lower dose of medetomidine in the WKY rats (Figure 1).

Discussion

The present results show that in the conscious normotensive and genetically hypertensive rats medetomidine caused marked bradycardia, an effect typical for clonidine and other α_2 -adrenoceptor agonists (Kobinger, 1978). Although decreasing similarly heart rate in both strains, the effect of medetomidine on blood pressure differed in WKY and SHR rats, causing a decrease in mean arterial pressure in SHR,

while in normotensive WKY it increased blood pressure. In anesthetized rats, medetomidine had a blood pressure action resembling that of clonidine, with an initial transient rise in blood pressure followed by a gradual decrease (Savola *et al.*, 1986). In pithed rats, its vasoconstrictor effect has been found to be antagonized by α_2 -adrenoceptor, but not α_1 -adrenoceptor blocking drugs (Savola *et al.*, 1986). Thus, it seems likely that the vasopressor response in WKY, as well as the smaller hypotensive response in SHR to the higher dose of medetomidine, are due to peripheral α_2 -adrenoceptor-mediated vasoconstriction. This lack of hypotensive response to medetomidine in WKY rats is also in agreement with our previous study in which an analogue of medetomidine had a similar effect (Savola *et al.*, 1985). Finally, the SHR strain shows a hyperactivity of the sympathetic nervous system of central origin (Folkow, 1982), which may explain the observed enhanced hypotensive response to medetomidine in SHR rats.

To examine the capacity of the heart to release ANP and the role of α_2 -adrenoceptors in the control of ANP secretion, we have compared here the effect of medetomidine infusion on plasma ANP levels in WKY and SHR rats. In normotensive WKY rats infusion of the selective α_2 -agonist medetomidine resulted in an increase in the plasma concentration of IR-ANP, while a similar infusion of medetomidine in hypertensive rats did not cause an ANP response. Previously Baranowska *et al.* (1987a,b,c) have demonstrated that clonidine (10 – $50 \mu\text{g}$ i.v.) elicits a marked increase of plasma IR-ANP in conscious rats (20 fold elevation after the highest dose). They also noted that yohimbine, an α_2 -adrenoceptor antagonist, decreased plasma IR-ANP levels and partially inhibited the increase of plasma IR-ANP induced by clonidine (Baranowska *et al.*, 1987b), suggesting that α_2 -adrenoceptors are important for ANP release. However, they did not measure haemodynamic parameters, though they noted that mean arterial pressure increased by more than 30 mmHg after a dose of $50 \mu\text{g}$ of clonidine, while a dose of $25 \mu\text{g}$ of clonidine (i.v.) had no pressor effect and caused a 10 fold increase in plasma IR-ANP (Baranowska *et al.*, 1987a). Our present results show that the haemodynamic effects seem to explain the increase of ANP secretion in response to α_2 -adrenoceptor stimulation in normotensive rats, because a significant increase in plasma IR-ANP levels during medetomidine infusion was accompanied by the pressor effect and increased right atrial pressure. In dogs, clonidine causes an elevation of left atrial pressure and left-ventricular end-diastolic pressure (Nayler *et al.*, 1968; Laubie & Schmitt, 1974).

Why medetomidine fails to cause ANP release in SHR is not clear. It is unlikely to be due to the impairment of right atrial stretch in SHR, because

Figure 1 Effects of a 60 min infusion of the α_2 -adrenoceptor agonist, medetomidine, on urinary excretory parameters in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. U_{vol} = urinary volume; $U_{Na V}$ = urinary sodium excretion; $U_K V$ = urinary potassium excretion; U_{osm} = urinary osmolality. After a control period (C) (60 min), medetomidine was administered by a constant infusion (0.2 or 0.6 $\mu\text{g kg}^{-1} \text{min}^{-1}$) (E) for 60 min. One recovery period (R) (60 min each) was performed after the medetomidine infusion was stopped. Results are mean of values in 5–8 animals; vertical lines indicate s.e.mean. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (one-way-ANOVA followed by the Bonferroni t test).

medetomidine produced similar increases in right atrial pressure in both strains. In contrast, a different pattern of blood pressure response to medetomidine was observed in SHR and WKY rats. The decreased mean arterial pressure, as clearly demonstrated in each experiment in SHR, may explain the absence of an increased release of ANP in response to medetomidine in these animals. Previous haemodynamic data obtained in dogs suggest that a fall in blood pressure may counteract the increase in left atrial pressure induced by α_2 -agonists (Nayler *et al.*, 1968). However, because hypotension induced by medetomidine did not decrease plasma IR-ANP levels in SHR, our results do not necessarily exclude the potential role of α_2 -adrenoceptors in the modulation of ANP release in SHR.

Clonidine and other α_2 -adrenoceptor agonists produce diuresis and increase the elimination of sodium in rats (Hoefke & Kobinger, 1966; Roman *et al.*, 1979; Bahr & Kauker, 1979; Miller, 1980; Gellai & Ruffolo, 1987; Gellai & Edwards, 1988). Their diuretic effect is not qualitatively influenced by the prior fluid and electrolyte status of the animal (Roman *et al.*, 1979), but the mechanism underlying the diuretic action of α_2 -agonists is not fully understood. In the present study, medetomidine increased urine flow rate and sodium excretion in both strains. Potassium excretion was also slightly increased with the suppressor dose of medetomidine in WKY consistent with the previous observation by Gellai & Ruffolo (1987) using another selective α_2 -agonist, BHT-933.

Based on *in vitro* studies, renal tubular α_2 -adrenoceptors may be involved in modulating the water flow and urinary sodium excretion by inhibition of the tubular action of vasopressin and other hormones (Pettinger *et al.*, 1987; Gellai & Edwards, 1988). Experimental evidence from *in vivo* studies indicates that α_2 -agonists decrease vasopressin secretion (Humpreys & Reid, 1975; Reid *et al.*, 1979; Roman *et al.*, 1979; Stranhoy *et al.*, 1982; Brooks *et al.*, 1986) as well as sympathetic nervous activity (DiBona, 1985); both of these actions probably contributing to the diuresis and urinary sodium excretion observed. In the present study, the diuretic action of medetomidine was associated with low levels of urine osmolality, maintained at 200 mosm kg⁻¹ of H₂O in WKY rats, substantially less than plasma osmolality (290 mosm kg⁻¹ of H₂O). The formation of urine hypotonic to plasma is indicative of a reduction in vasopressin-associated renal water reabsorption. However, further studies on the role of vasopressin are needed, since there is evidence that in conscious euvoletic animals administration of α_2 -agonists does not inhibit vasopressin secretion (Olsen, 1976; Gellai & Edwards, 1988).

Baranowska *et al.* (1987c), in their study of the effect of clonidine on ANP secretion, showed that the increment of ANP correlated with the diuretic effect of clonidine in normally-hydrated, conscious normotensive rats. Our results on the effect of medetomidine on plasma IR-ANP and renal excretory functions in WKY rats support their observations and demonstrate that enhanced ANP release may be involved in the mechanisms of α_2 -agonist-evoked

diuresis. The increased plasma levels of ANP in response to α_2 -adrenoceptor stimulation could also inhibit vasopressin release (Laragh, 1985; Brenner & Ballerman, 1986; Needleman & Greenwald, 1986) and thus enhance that diuretic effect. However, the present findings that medetomidine had a similar effect on the renal excretory functions in both strains, but did not affect plasma levels of IR-ANP in SHR rats suggest that changes in plasma ANP levels could not alone account for the diuretic and natriuretic effect of medetomidine in conscious SHR. Thus, the present study, together with previous results, suggest that the renal effects of α_2 -agonists may be explained by hormonal effects (vasopressin, ANP) in combination with renal tubular and autonomic effects, depending on the rat strain and experimental conditions.

In conclusion, our results clearly show that α_2 -adrenoceptor stimulation failed to increase plasma IR-ANP secretion in genetically hypertensive rats, but markedly increased ANP release in normotensive rats. This differing effect of medetomidine on ANP release in WKY and SHR rats was probably associated with differing mean arterial pressure responses. However, the lack of ANP response to α_2 -adrenoceptor stimulation in SHR may also represent a generalized attenuation of ANP release in these animals.

We thank Mrs Tuula Lumijärvi, Mrs Eila Pesonen and Ms Tuula Räisänen for expert technical assistance. This study was supported by the Academy of Finland, Sigrid Juselius Foundation and Paulo Foundation.

References

- BAHR, J. & KAUKER, M.L. (1979). Renal tubular site and mechanism of clonidine-induced diuresis in rats: clearance and micropuncture studies. *J. Pharmacol. Exp. Ther.*, **209**, 389–395.
- BARANOWSKA, B., GUTKOWSKA, J., CANTIN, M. & GENEST, J. (1987a). Effects of alpha-1 and alpha-2 adrenergic agonists on plasma immunoreactive atrial natriuretic factor (IR-ANF) in conscious rats. *Neuroendocrinol. Lett.*, **4**, 261–266.
- BARANOWSKA, B., GUTKOWSKA, J., TALBOT, P., GENEST, J. & CANTIN, M. (1987b). Plasma immunoreactive atrial natriuretic factor is inhibited by selective blockade of alpha-2 adrenergic receptors in conscious Sprague-Dawley rats. *Neurosci. Lett.*, **76**, 119–123.
- BARANOWSKA, B., GUTKOWSKA, J., CANTIN, M. & GENEST, J. (1987c). Plasma immunoreactive atrial natriuretic factor (IR-ANF) increases markedly after alpha-2 adrenergic stimulation with clonidine in normally-hydrated rats. *Biochem. Biophys. Res. Commun.*, **143**, 159–163.
- BRENNER, B.J. & BALLERMAN, B.M. (1986). Role of atrial peptides in body fluid homeostasis. *Circ. Res.*, **58**, 619–630.
- BROOKS, D.P., SHARE, L. & CROFTON, J.T. (1986). Central adrenergic control of vasopressin release. *Neuroendocrinol.*, **42**, 416–420.
- DiBONA, G.F. (1985). Neural regulation of renal function: role of renal alpha-adrenoceptors. *J. Cardiovasc. Pharmacol.*, **7**, S18–S23.
- FOLKOW, B. (1982). Physiological aspects of primary hypertension. *Physiol. Rev.*, **62**, 347–504.
- GELLAI, M. & EDWARDS, R.M. (1988). Mechanism of alpha-2 adrenoceptor agonist-induced diuresis. *Am. J. Physiol.*, **255**, F317–323.
- GELLAI, M. & RUFFOLO, R.R. (1987). Renal effects of selective alpha-1 and alpha-2 adrenoceptor agonists in conscious, normotensive rats. *J. Pharmacol. Exp. Ther.*, **240**, 723–728.
- HOEFKE, V.W. & KOBINGER, W. (1966). Pharmakologische Wirkungen des 2-(2,6-dichlor-phenylamino)-2-imidazolin-hydrochlorids, einer neuen, antihypertensiven substanz. *Arzneim. Forsch.*, **16**, 1038–1056.
- HUMPREYS, M.H. & REID, I.A. (1975). Suppression of anti-diuretic hormone secretion by clonidine in the anesthetized dog. *Kidney Int.*, **7**, 405–412.
- KOBINGER, W. (1978). Central alpha-adrenergic systems as

- targets for hypotensive drugs. *Rev. Physiol. Biochem. Pharmacol.*, **81**, 31–100.
- LAUBIE, M. & SCHMITT, H. (1974). Influence of autonomic blockade on the reduction in myocardial performance produced by clonidine. *Eur. J. Pharmacol.*, **25**, 56–65.
- LANG, R.E., THÖLKEN, H., GANTEN, D., LUFT, F.C., RUSKOAHO, H. & UNGER, Th. (1985). Atrial natriuretic factor – a circulating hormone stimulated by volume loading. *Nature*, **314**, 264–266.
- LARAGH, J.H. (1985). Atrial natriuretic hormone, the renin-aldosterone axis and blood pressure-electrolyte homeostasis. *N. Engl. J. Med.*, **313**, 1330–1340.
- MANNING, P.T., SCHWARTZ, D., KATSUBE, N.C., HOLMBERG, S.W. & NEEDLEMAN, P. (1985). Vasopressin-stimulated release of atriopeptin: endocrine antagonists in fluid homeostasis. *Science*, **229**, 395–397.
- MILLER, M. (1980). clonidine-induced diuresis in the rat: evidence for a renal site of action. *J. Pharmacol. Exp. Ther.*, **214**, 608–613.
- NAYLER, W.G., PRICE, J.M., SWANN, J.B., McINNES, I., RACE, D. & LOWE, T.E. (1968). Effect of the hypotensive drug St 155 (catapres) on the heart and peripheral circulation. *J. Pharmacol. Exp. Ther.*, **164**, 45–59.
- NEEDLEMAN, P. & GREENWALD, J.E. (1986). Atriopeptin: a cardiac hormone intimately involved in fluid, electrolyte and blood pressure homeostasis. *N. Engl. J. Med.*, **314**, 828–834.
- OLSEN, U.B. (1976). Clonidine-induced increase of renal prostaglandin activity and water-diuresis in conscious dogs. *Eur. J. Pharmacol.*, **36**, 95–101.
- PETTINGER, W.A., UMEMURA, S., SMYTH, D.D. & JEFFRIES, W.B. (1987). Renal alpha-2 adrenoceptors and the adenylate cyclase-cAMP system: biochemical and physiological interactions. *Am. J. Physiol.*, **252**, F199–F208.
- REID, I.A., NOLAN, P.L., WOLF, A. & NEIL, L.C. (1979). Suppression of vasopressin release by clonidine: effect of alpha-adrenoceptor antagonists. *Endocrinol.*, **104**, 1403–1406.
- ROMAN, R.J., COWLEY, A.W. & LECHERIE, C. (1979). Water diuretic and natriuretic effect of clonidine in the rat. *J. Pharmacol. Exp. Ther.*, **221**, 385–393.
- RUSKOAHO, H., LANG, R.E., TOTH, M., GANTEN, D. & UNGER, Th. (1987). Release and regulation of atrial natriuretic peptide. *Eur. Heart. J.* **8** (suppl B), 99–109.
- RUSKOAHO, H. & LEPPÄLUOTO, J. (1988). Immunoreactive atrial natriuretic peptide in ventricles, atria, hypothalamus, and plasma of genetically hypertensive rats. *Circ. Res.*, **62**, 384–394.
- SANFIELD, J.A., SHENKER, Y., GREKIN, R.J. & ROSEN, S.G. (1987). Epinephrine increases plasma immunoreactive atrial natriuretic hormone levels in humans. *Am. J. Physiol.*, **252**, E740–E745.
- SAVOLA, J.M., RUSKOAHO, H., PUURUNEN, J. & KÄRKI, N.T. (1985). Cardiovascular action of detomidine, a sedative and analgesic imidazole derivative with alpha-agonistic properties. *Eur. J. Pharmacol.*, **118**, 69–76.
- SAVOLA, J.M., RUSKOAHO, H., PUURUNEN, J., SALONEN, J. & KÄRKI, N.T. (1986). Evidence for medetomidine as a selective and potent agonist of alpha-2 adrenoceptors. *J. Autom. Pharmacol.*, **5**, 275–284.
- SCHEININ, H., VIRTANEN, R. & SCHEININ, M. (1986). Alpha-2 adrenoceptor agonists decrease free 3-methoxy-4-hydroxyphenylglycol in the rat cerebrospinal fluid. *Eur. J. Pharmacol.*, **123**, 115–121.
- SCHEININ, M., KALLIO, A., KOULU, M., VIIKARI, J. & SCHEININ, H. (1987). Sedative and cardiovascular effects of medetomidine, a novel selective alpha-2 agonist, in healthy volunteers. *Br. J. Clin. Pharmacol.*, **24**, 443–451.
- STRANHOY, J.W., MORRIS, M. & BUCKALEW, V.M. (1982). Renal effects of the antihypertensive, guanabenz, in the dog. *J. Pharmacol. Exp. Ther.*, **221**, 347–352.
- UEHLINGER, D.E., WEIDMANN, P., GNAEDINGER, M.P., SHAW, S. & LANG, R.E. (1986). Depressor effects and release of atrial natriuretic peptide during norepinephrine or angiotensin II infusion in man. *J. Clin. Endocrinol. Metab.*, **63**, 669–674.
- VIRTANEN, R., SAVOLA, J.M., SAANO, V. & NYMAN, L. (1988). Characterization of the selectivity, specificity and potency of medetomidine as an alpha-2 adrenoceptor agonist. *Eur. J. Pharmacol.*, **150**, 9–14.
- VUOLTEENAHO, O., ARJAMAA, O. & LING, N. (1985). Atrial natriuretic polypeptides (ANP): rat atria store high molecular weight precursor but secrete processed peptides of 25–35 amino acids. *Biochem. Biophys. Res. Commun.*, **129**, 82–88.

(Received October 7, 1988

Revised January 7, 1989

Accepted January 9, 1989)