British Journal of Pharmacology (1997) 121, 861 – 866

Renal $\alpha_{2a/d}$-adrenoceptor subtype function: Wistar as compared to spontaneously hypertensive rats

*H.D. Intengan & *,†,[1]D.D. Smyth

*Department of Pharmacology & Therapeutics and †Internal Medicine, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3

1 The $\alpha_{2a/d}$-adrenoceptor subtype in the rat kidney modulates solute excretion (osmolar clearance). Since the kidney plays a role in chronic regulation of blood pressure, altered renal function may be implicated in the development of hypertension. A second alteration - that of the $\alpha_{2a/d}$-adrenoceptor subtype gene - has also been correlated with hypertension in rats and man.

2 We hypothesized that as a consequence of the altered $\alpha_{2a/d}$-adrenoceptor subtype gene previously shown in spontaneously hypertensive (SH) rats, the increase in osmolar clearance following stimulation of the renal $\alpha_{2a/d}$-subtype would be attenuated in SH rats as compared to normotensive Wistar rats. In contrast, based on the theory that such functional unresponsiveness of the $\alpha_{2a/d}$-subtype would be genetically determined, we further hypothesized that in one kidney-one clip (1K-1C) rats, the response to stimulation of the renal $\alpha_{2a/d}$-subtype would be intact as compared to the normotensive Wistar 1K-sham rats.

3 Male rats were unilaterally nephrectomized under ether anaesthesia. In the 1K-1C rats, a silver clip (diameter 0.254 mm) was also placed around the left renal artery. On the experimental day, rats were administered pentobarbitone (50.0 mg kg^{-1}, i.p.). The carotid artery and jugular vein were cannulated for blood pressure monitoring and saline infusion. The ureter was catheterized for urine collection. A 31 gauge needle was advanced into the renal artery for infusion of the $\alpha_{2a/d}$-selective agonist, guanfacine (vehicle, 1.0, 3.0 and 10.0 nmol kg^{-1} min^{-1} in Wistar and SH rats; vehicle and 10.0 nmol kg^{-1} min^{-1} in Wistar 1K-sham and 1K-1C rats).

4 In Wistar rats, guanfacine dose-dependently increased urine flow and sodium excretion. An increase in osmolar clearance but not free water clearance was also observed. However, in SH rats guanfacine failed to alter urine flow, sodium excretion, osmolar and free water clearance. In contrast, in both Wistar 1K-sham and 1K-1C rats, guanfacine increased urine flow rate. Again, this response was due solely to an increase in osmolar clearance. At these doses, guanfacine did not alter blood pressure or creatinine clearance during the experiment.

5 In summary, the ability of the $\alpha_{2a/d}$-adrenoceptor subtype to mediate an increase in osmolar clearance was absent in a genetic model of hypertension, the SH rats. This effect was intact in an acquired model of hypertension (1K-1C rats). This suggested a defective modulation of solute excretion in SH rats which was probably due to alteration of the $\alpha_{2a/d}$-subtype gene and not secondary to the elevated blood pressure. The altered $\alpha_{2a/d}$-subtype gene and function may therefore play a causal role in the pathogenesis of hypertension.

Keywords: Kidney; hypertension; sodium excretion; $\alpha_{2a/d}$-adrenoceptor; guanfacine

Introduction

The kidney plays a major role in the chronic regulation of blood pressure via modulation of sodium and water excretion (Guyton *et al*., 1990; Hall *et al*., 1990; Guyton, 1991). Kidney cross-transplantation studies indicated that the post-transplant blood pressure in a recipient rat is determined by the donor kidney (Rettig *et al*., 1993; Raine, 1993; Churchill *et al*., 1995). However, the precise alteration(s) in renal function remain(s) unknown.

A potential link between the kidney and hypertension may be the $\alpha_{2a/d}$-adrenoceptor subtype. α_2-Adrenoceptor stimulation in the kidney has been documented to modulate renal function in the rat, although the function of the $\alpha_{2a/d}$-subtype in particular has not been determined. An increase in urine flow rate, osmolar clearance, free water clearance and sodium excretion have been demonstrated in response to various α_2-adrenoceptor agonists (Strandhoy *et al*., 1982; Gellai & Ruffolo, 1987; Stanton *et al*., 1987; Blandford & Smyth, 1988; 1991). The increase in free water clearance and osmolar clearance produced by α_2-adrenoceptor stimulation have been separated pharmacologically (Intengan & Smyth, 1996). The free water clearance was prazosin-sensitive whereas the osmolar clearance was naltrexone-sensitive. These findings suggested that two receptors were involved. Moreover, we have recently obtained the novel finding that stimulation of the $\alpha_{2a/d}$-adrenoceptor subtype with guanfacine, a selective $\alpha_{2a/d}$-subtype agonist, increased urine flow rate solely by increasing osmolar clearance (Intengan & Smyth, 1997).

A defect or alteration of the $\alpha_{2a/d}$-adrenoceptor subtype may therefore result in defective modulation of solute excretion, thereby predisposing the carrier to hypertension. An alteration of the $\alpha_{2a/d}$-subtype gene has been identified in man (Hoehe *et al*., 1988) and rats (Chun *et al*., 1991). These genetic alterations correlated with elevated blood pressure in both species (Pettinger *et al*., 1991; Lockette *et al*., 1995; Svetkey *et al*., 1996). We therefore hypothesized that as a consequence of the $\alpha_{2a/d}$-adrenoceptor gene alteration which correlated with hypertension, the previously found natriuretic function of the renal $\alpha_{2a/d}$-adrenoceptor subtype will be absent in spontaneously hypertensive (SH) rats. We further postulated that this lack of response to $\alpha_{2a/d}$-adrenoceptor stimulation was not secondary to the elevated blood pressure and therefore the response would be intact in an acquired model of hypertension, the one kidney-one clip (1K-1C) rats. In the present study, we used the selective $\alpha_{2a/d}$-adrenoceptor agonist, guanfacine, to assess the function of renal $\alpha_{2a/d}$-adrenoceptors in normotensive and hy-

[1] Author for correspondence.

pertensive rats. Guanfacine has an approximately 60 fold greater selectivity for the $\alpha_{2a/d}$-adrenoceptor over the α_{2b}-adrenoceptor subtype in radioligand binding studies (Uhlén & Wikberg, 1991). We determined the dose-related effects of guanfacine on renal function in Wistar (normotensive) and SH rats. We also determined the renal effects of guanfacine (10.0 nmol kg^{-1} min^{-1}) in normotensive Wistar one kidney-sham (1K-sham) and hypertensive Wistar 1K-1C rats.

Methods

Experimental preparation

The standard procedures have been previously described by Intengan & Smyth (1996). Briefly, male rats were obtained from the University of Manitoba Central Animal Care (Charles River Breeding Stock) and cared for according to regional animal care standards protocol. The rats were housed four per cage, fed a standard Purina rat chow diet with free access to tap water in cages at 22°C with a 12 h light/dark cycle.

Wistar and spontaneously hypertensive rats

Male Wistar and SH rats (8 weeks old) were used. Seven to ten days before the experiment, the right kidney was removed under ether anaesthesia via a flank incision. The animals were then administered a subcutaneous injection of the post-operative analgesic, buprenorphine (0.015 mg kg^{-1}).

Wistar sham and one kidney-one clip rats

The standard procedures have been previously described by Li *et al*. (1994). Male Wistar rats (100–125 g) were separated into two groups. In the first group (1K-1C), both kidneys were exposed under ether anaesthesia by an abdominal incision. A silver clip (diameter 0.254 mm) was placed around the left renal artery. The right kidney was then removed. In the second group (1K-sham), the surgery was identical to the 1K-1C rats except no clip was placed on the left renal artery. The animals were maintained for at least 28 days after surgery before experimentation.

Surgical preparation

On the day of the experiment, the rats (280–310 g) were anaesthetized with pentobarbitone (BDH Chemicals Ltd., Poole, U.K., 50.0 mg kg^{-1}, i.p.). Additional anaesthetic was administered as required in a bolus dose of 3.0 mg kg^{-1}, i.v. The rats were placed on a Harvard Animal Blanket Control unit with a rectal thermometer probe and the temperature was set for 37.5°C. A tracheotomy was performed, after which the animals were allowed to breathe spontaneously. The left carotid artery was cannulated with PE-60 tubing and connected to a Statham pressure transducer (Model P23Dc) and a Grass model 5 polygraph for the monitoring of blood pressure. The left jugular vein was cannulated with PE-160 tubing for the infusion of normal saline at 97 μl min^{-1} and additional anaesthetic as required. A left flank incision was performed and the remaining kidney exposed. The ureter was catheterized to facilitate the collection of urine into pre-weighed vials. Urine volume was determined gravimetrically. Finally, a 31 gauge stainless steel needle was advanced into the renal artery for the infusion of guanfacine or vehicle with a Harvard sage pump. The preparation was allowed to stabilize for 45 min. Immediately following the stabilization period, a 30 min control urine collection was obtained.

Wistar and SH rats

Dose-related response to guanfacine Following the control collection, the Wistar and SH rats received an intrarenal infusion (3.4 μl min^{-1}) of isotonic saline or guanfacine (1.0, 3.0, or 10.0 nmol kg^{-1} min^{-1}). The experiments were randomized, and each treatment group consisted of six animals. During the intrarenal infusion of saline or guanfacine, two consecutive 30 min urine collections were obtained

Renal response to furosemide To confirm that a difference in the renal response to guanfacine in the Wistar compared to SH rats was not common to all natriuretic drugs, an extra urine collection was obtained following the third collection in four Wistar control and four SH control rats. At the start of the fourth collection, furosemide, a natriuretic drug unrelated to the $\alpha_{2a/d}$-adrenoceptor, was administered as an intravenous bolus injection at a moderate dose (0.3 mg kg^{-1}).

Wistar 1K-sham and 1K-1C rats: renal response to guanfacine

Following the control collection, the 1K-sham and 1K-1C rats received an intrarenal infusion (3.4 μl min^{-1}) of isotonic saline or guanfacine (10.0 nmol kg^{-1} min^{-1}). The experiments were randomized and each treatment group consisted of six animals. During the intrarenal infusion of saline or guanfacine, two consecutive 30 min urine collections were obtained.

Sample analysis

At the end of each experiment, a blood sample was collected through the carotid artery catheter. Dye was injected through the renal artery line to confirm proper positioning of the needle. Creatinine levels in the urine and plasma were measured with a Beckman Creatinine 2 Analyzer. Urine and plasma osmolalities were determined with a Precision Systems Micro Osmometer. The sodium concentrations in urine and plasma were measured by a Nova Electrolyte Analyzer (model 13+). Free water clearance was calculated as the difference between urine flow rate and osmolar clearance.

Statistical analysis

Data are presented as the mean ± s.e. Statistical analysis was conducted by use of Systat software, version 5.0. The data were subjected to repeated measures of analysis of variance (ANOVA). Significant interactions were determined by Newman-Keul's multiple-comparison test. The furosemide data (i.e. only the absolute values determined from the extra collection at the end of control experiments) were analysed by Student's *t* test. Significance is denoted by asterisks, $*P<0.05$ and $**P<0.01$.

Drugs

Guanfacine (Wyeth-Ayerst) and furosemide were used in the present studies.

Data presentation

Guanfacine data The same levels of significance were found for absolute values as well as the calculated changes from the first to the third urine collection. Since there were only a few minor differences found between groups in the pretreatment baseline values, the data have been presented as changes from the first to the third urine collection to highlight the magnitude of responses between groups.

Furosemide data The above absolute values from the extra, fourth urine collection were analysed to avoid overlap in statistical analysis. The furosemide data have been presented as absolute values for the Wistar and SH rat groups.

Table 1 Wistar and SH rats: absolute baseline values before intrarenal infusion of vehicle or guanfacine (1.0, 3.0 and 10.0 nmol kg^{-1} min^{-1})

	Wistar				*SH*			
		Pre-guanfacine				*Pre-guanfacine*		
	Vehicle	*1.0*	*3.0*	*10.0*	*Vehicle*	*1.0*	*3.0*	*10.0*
Blood pressure (mmHg)	127±7	116±4	122±9	117±6	181±9	174±8	185±5	173±5
Creatinine clearance (ml min^{-1})	1.5±0.1	1.6±0.2	1.5±0.1	1.3±0.2	1.4±0.1	1.4±0.1	1.4±0.2	1.4±0.1
Urine flow rate (μl min^{-1})	24±3	20±3	17±3	16±2	9±1	8±2	8±1	11±2
Sodium excretion (μEq min^{-1})	3.0±0.6	2.4±0.5	1.6±0.3	2.0±0.3	1.0±0.1	0.9±0.3	1.1±0.3	1.4±0.3
Free water clearance (μl min^{-1})	–39±5	–54±4**	–36±4	–28±3*	–27±6	–28±4	–22±3	–33±5
Osmolar clearance (μl min^{-1})	63±7	74±5*	53±4*	44±3	36±6	33±11	31±3	44±5

Data shown are means±s.e.mean ($n=6$). *$P<0.05$ and **$P<0.01$ versus respective control.

Table 2 Wistar 1K-sham and 1K-1C rats: absolute baseline values before intrarenal infusion of vehicle or guanfacine (GF, 10.0 nmol kg^{-1} min^{-1})

	Pre-guanfacine			
	1K-sham		*1K-1C*	
	Vehicle	*GF*	*Vehicle*	*GF*
Blood pressure (mmHg)	124±4	109±5	178±10**	168±12**
Creatinine clearance (ml min^{-1})	1.6±0.3	1.4±0.2	1.5±0.2	1.3±0.1
Urine flow rate (μl min^{-1})	13±3	13±2	11±3	13±4
Sodium excretion (μEq min^{-1})	1.1±0.3	1.1±0.3	0.7±0.1	1.3±0.5
Free water clearance (μl min^{-1})	–39±5	–30±4	–33±3	–34±5
Osmolar clearance (μl min^{-1})	52±7	43±3	44±5	47±9

Data shown are means+s.e.mean ($n=6$). **$P<0.01$.

Results

Absolute baseline values

Baseline absolute values for groups before the administration of the experimental treatments (vehicle or guanfacine) were used as an indication of the effects for each group of the surgical preparation (Tables 1 and 2). Blood pressure was consistently elevated in the SH as compared to the Wistar rats and in the Wistar 1K-1C as compared to the Wistar 1K-sham rats (Tables 1 and 2). Within the different groups of Wistar rats, baseline values for all parameters were similar except for a minor variation in free water and osmolar clearance. Within the groups of SH rats, no significant differences were detected for baseline values of all parameters (Table 1). Within the groups of Wistar 1K-1C or 1K-sham rats, no significant differences were detected for baseline values of all parameters (Table 2).

Effect of intrarenal infusion of guanfacine in Wistar and SH rats

The effects of guanfacine have been shown as the change from the first to the third collection period (data not shown). In the groups of Wistar rats, the changes in blood pressure were similar and ranged from −9±3 to 2±2 mmHg. Likewise, in the groups of SH rats, blood pressure changes were similar between all treatment groups (ranging from −2±4 to 5±4 mmHg). The change in creatinine clearance between the final and the baseline values were also similar between experimental groups in Wistar (0.01±0.2 to −0.4±0.2 ml min^{-1}) and in the SH rats (−0.1±0.1 to −0.4±0.2 ml min^{-1}).

In the Wistar rats, guanfacine increased urine flow rate and sodium excretion significantly in a dose-related manner (Figure 1). This increase in urine flow rate was secondary to an increase in osmolar clearance and not free water clearance (Figure 2). However, in SH rats guanfacine failed to alter urine flow rate or sodium excretion (Figure 1). Accordingly, osmolar clearance as well as free water clearance were not affected by the administration of guanfacine (Figure 2). Variation in the baseline values of osmolar clearance in Wistar rats were minor compared to the magnitude of increase in osmolar clearance in rats treated with guanficine.

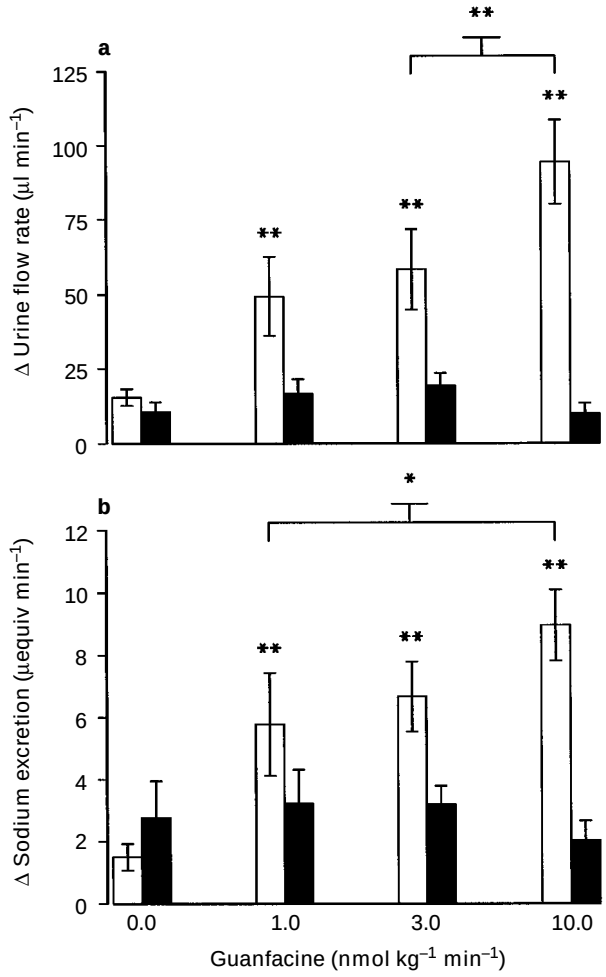

Figure 1 Effects of intrarenal infusion of 0.9% saline (vehicle) or guanfacine (1.0, 3.0 and 10.0 nmol kg^{-1} min^{-1}) on (a) urine flow rate and (b) sodium excretion in Wistar (open columns) and SH (solid columns) rats. Each group represents the mean±s.e. of the difference between final collection and baseline values of six experiments. *$P<0.05$ and **$P<0.01$ vs respective control or where indicated, between groups.

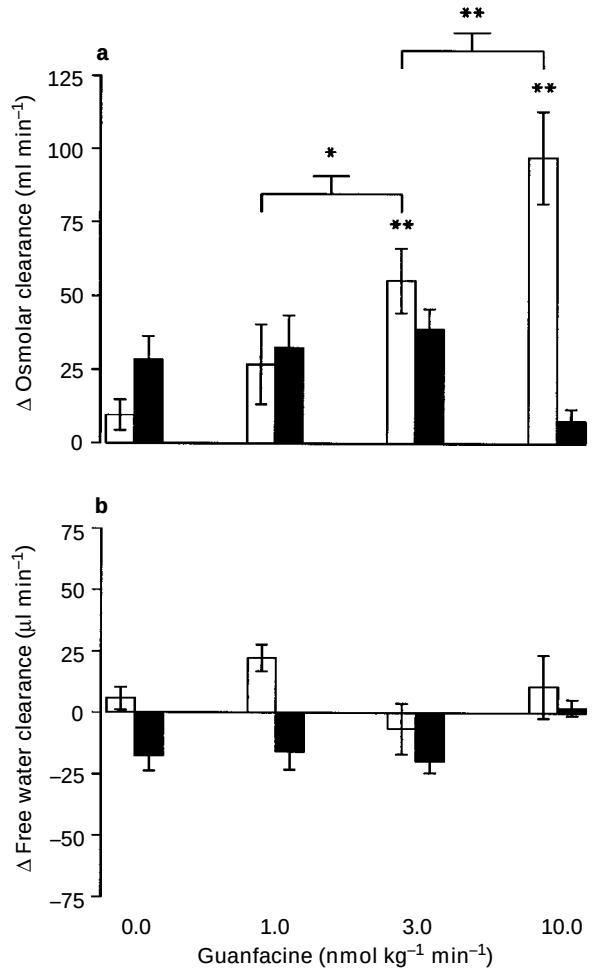

Figure 2 Effects of intrarenal infusion of 0.9% saline (vehicle) or guanfacine (1.0, 3.0 and 10.0 nmol kg^{-1} min^{-1}) on (a) osmolar clearance and (b) free water clearance in Wistar (open columns) and SH (solid columns) rats. Each group represents the mean ± s.e. of the difference between final collection and baseline values of six experiments. $*P<0.05$ and $**P<0.01$ vs respective control or where indicated, between groups.

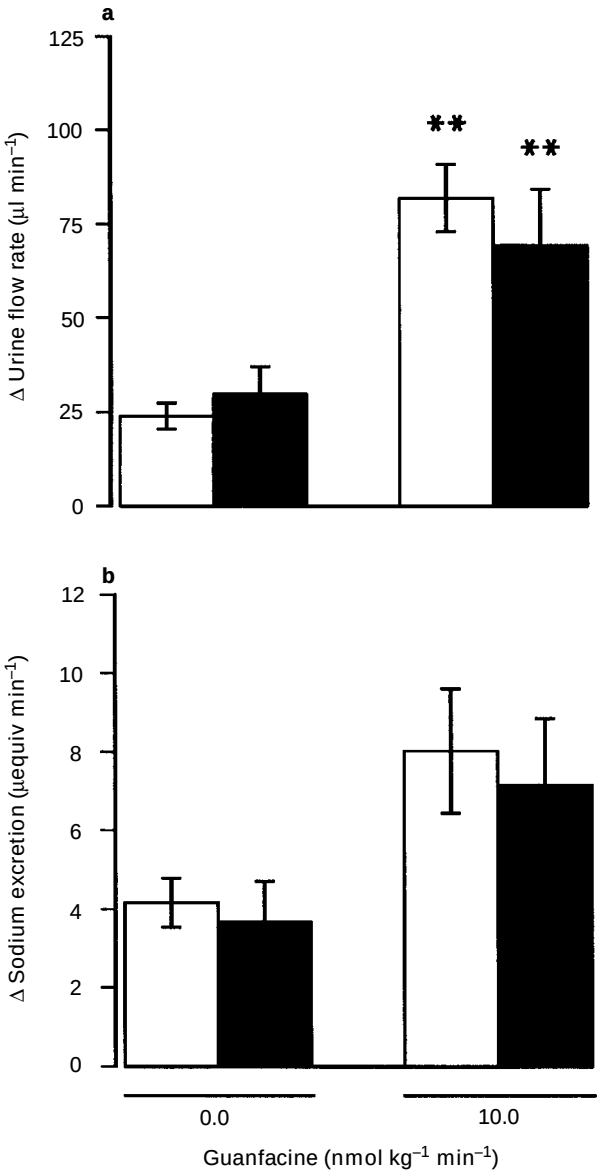

Figure 3 Effects of intrarenal infusion of 0.9% saline (vehicle) or guanfacine (10.0 nmol kg^{-1} min^{-1}) on (a) urine flow rate and (b) sodium excretion in Wistar 1K-sham (open columns) and 1K-1C (solid columns) rats. Each group represents the mean ± s.e. of the difference between final collection and baseline values of six experiments. $**P<0.01$ vs respective control.

Effects of intrarenal infusion of guanfacine in Wistar 1K-sham and 1K-1C rats

In Wistar 1K-sham rats, blood pressure was unaffected by pharmacological intervention. Blood pressure changes between the final and baseline collection ranged from 3 ± 2 mmHg (control) to 4 ± 3 mmHg (guanfacine). In Wistar 1K-1C rats, blood pressure changes were similar and ranged from −5 ± 8 mmHg (control) to 5 ± 2 mmHg (guanfacine). Creatinine clearance changes were similar between experimental groups in Wistar 1K-sham rats ranging from −0.04 ± 0.1 ml min^{-1} (control) to 0.2 ± 0.2 ml min^{-1} (guanfacine) and in Wistar 1K-1C rats ranging from −0.01 ± 0.2 ml min^{-1} (control) to 0.1 ± 0.1 ml min^{-1} (guanfacine).

Guanfacine increased urine flow rate in both the normotensive and hypertensive animals (Figure 3). This guanfacine-induced increase in urine flow rate was secondary to an increase in osmolar clearance. Free water clearance was not affected by guanfacine administration (Figure 4).

Effect of intravenous administration of furosemide in Wistar and SH rats

Absolute values have been presented for the fourth collection period which looked at the effects of furosemide. In the fourth collection period, blood pressure was elevated in the SH rats (173 ± 10 mmHg) as compared to the Wistar animals (108 ± 7 mmHg). Creatinine clearance was also similar between both groups (1.1 ± 0.4 and 1.1 ± 0.1 ml min^{-1}, respectively). Furosemide increased urine flow rate and sodium excretion in both strains to comparable levels. In the Wistar rats, urine flow rate increased to 123 ± 14 μl min^{-1} and sodium excretion increased to 17 ± 1.8 μEq min^{-1}. In SH rats, urine flow rate was increased to 86 ± 4 μl min^{-1} and sodium excretion increased to 13 ± 1.4 μEq min^{-1}. The furosemide-induced increase in urine flow rate was reflected solely by an increase in osmolar clearance in Wistar rats (157 ± 16 μl min^{-1}) and in SH rats (130 ± 11 μl min^{-1}). Free water clearance was not affected by furosemide administration in Wistar (−34 ± 6 μl min^{-1} · min^{-1}) and in SH (−44 ± 11 μl min^{-1}) rats.

Discussion

The present investigation has examined the effects of renal $\alpha_{2a/d}$-adrenoceptor stimulation in the SH rat, a genetic model

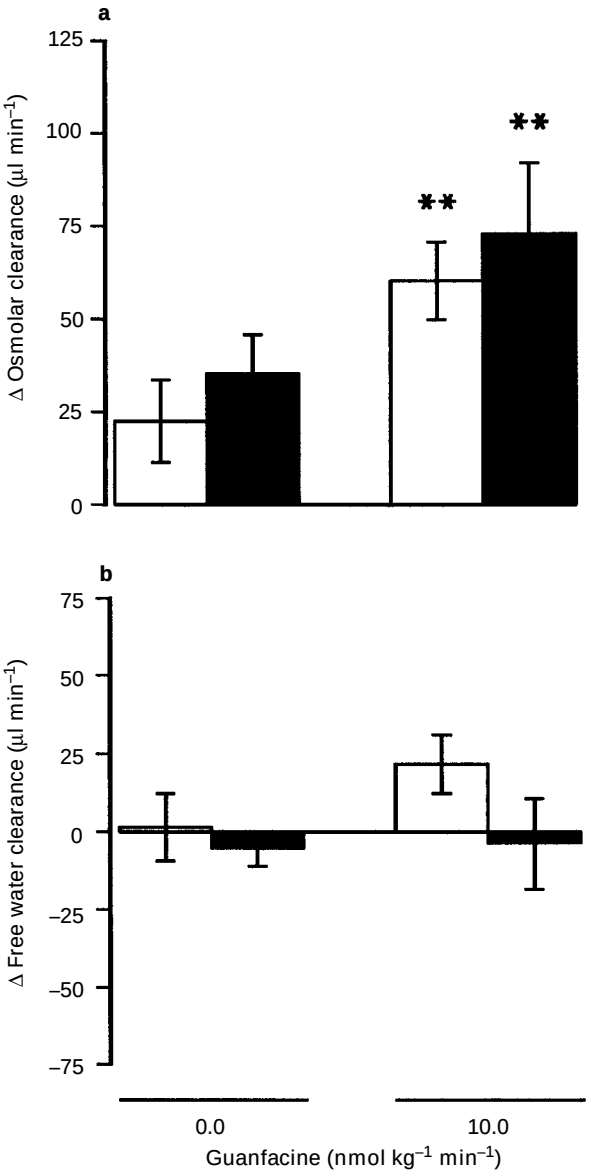

Figure 4 Effects of intrarenal infusion of 0.9% saline (vehicle) or guanfacine (10.0 nmol kg^{-1} min^{-1}) on (a) osmolar clearance and (b) free water clearance in Wistar 1K-sham (open columns) and 1K-1C (solid columns) rats. Each group represents the mean ± s.e. of the difference between final collection and baseline values of six experiments. $^{**}P<0.01$ vs respective control.

of hypertension. It was hypothesized that as in Sprague-Dawley rats, the $\alpha_{2a/d}$-subtype would mediate osmolar clearance in the normotensive Wistar control rats, whereas this function would be absent in the genetic hypertensive rat.

Following selective stimulation of the $\alpha_{2a/d}$-adrenoceptor subtype by infusion of guanfacine into the renal artery, an increase in urine flow rate, sodium excretion, and osmolar clearance were observed in Wistar but not in SH rats. This lack of response was not likely to be secondary to the elevated blood pressure. In the hypertensive 1K-1C rat, guanfacine increased urine flow rate and sodium excretion despite blood pressure levels comparable to those of the SH rats. Moreover, the unresponsiveness to guanfacine was not common to all natriuretic drugs. The unrelated natriuretic drug, furosemide, increased osmolar clearance in both Wistar and SH rats.

The decreased natriuretic activity in SH rats gives rise to the speculation that the alteration of the $\alpha_{2a/d}$-subtype gene found in these rats may contribute to the pathogenesis of hypertension. Chun *et al.* (1991) identified an allele of the $\alpha_{2a/d}$-adrenoceptor subtype gene in rats. This allele cosegregated with elevated blood pressure in the F2 generation of cross-bred WKY and SH rats (Pettinger *et al.*, 1991). Recent studies with Sabra salt-sensitive and salt-resistant rats are consistent with the conjecture that alteration of the $\alpha_{2a/d}$-subtype gene confers salt-sensitive hypertension (Le Jossec *et al.*, 1995). Radioligand binding studies showed that in Sabra salt-resistant rats, both $\alpha_{2a/d}$- and α_{2b}-adrenoceptors were detectable in renal cortical membranes. However, in the Sabra salt-sensitive rats, only the α_{2b}-adrenoceptor was detected whereas the $\alpha_{2a/d}$-adrenoceptor was absent. By use of reverse-transcription-cDNA amplification techniques, $\alpha_{2a/d}$- and α_{2b}-subtype mRNA were found in the renal cortex from both salt-sensitive and salt-resistant rats. The authors suggested that the absence of the $\alpha_{2a/d}$-adrenoceptor in the salt-sensitive rats may have been due to post-transcriptional or post-translational events occurring in these animals. Based on the natriuretic activity of the $\alpha_{2a/d}$-subtype which we have shown in Wistar and Sprague-Dawley rats, the absence of this receptor in the Sabra salt-sensitive rats may be contributing to the sensitivity to sodium which pre-disposes these animals to hypertension. The present data suggest the merit of investigating the function of renal $\alpha_{2a/d}$-adrenoceptors in Sabra salt-sensitive and salt-resistant rats.

The $\alpha_{2a/d}$-adrenoceptor subtype has also been detected in the human kidney. In the human kidney the $\alpha_{2a/d}$-subtype represents at least 80% of total renal α_2-adrenoceptor population (Motomura *et al.*, 1989). A restriction fragment length polymorphism (RFLP) for the $\alpha_{2a/d}$-adrenoceptor gene (located on chromosome 10) as well as abnormalities of the $\alpha_{2a/d}$-subtype in man have previously been found (Hoehe *et al.*, 1988). Normotensive children with one essential hypertensive parent have altered platelet $\alpha_{2a/d}$-adrenoceptor densities as compared to platelets from normotensive children with no family history of hypertension (Michel *et al.*, 1989). In the African-American population, a frequent coexistence of hypertension with salt-sensitivity has been documented. Furthermore, an association coexistence of hypertension with salt-sensitivity has been documented. Furthermore, an association exists between an RFLP of the $\alpha_{2a/d}$-subtype (6.3 kb and 6.7 kb alleles) and essential hypertension in African-American (Lockette *et al.*, 1995) and Caucasian subjects (Svetkey *et al.*, 1996). A four fold increase in the prevalence of 6.3 kb homozygotes was found in hypertensive versus normotensive subjects. A recent study also demonstrated that individuals carrying at least one copy of the 6.3 kb allele had a decreased natriuretic response following immersion in thermal neutral water (Freeman *et al.*, 1995). Again, the finding that the 6.3 kb allele correlated with hypertension and decreased sodium excretion was consistent with our findings that the natriuretic function of the $\alpha_{2a/d}$-subtype in SH rats was defective where an RFLP for this gene has also been correlated.

In the present study, we demonstrate the potential functional relevance between the $\alpha_{2a/d}$-adrenoceptor subtype RFLP and the elevated blood pressure in SH rats obtained by others. Stimulation of the renal $\alpha_{2a/d}$-adrenoceptor with guanfacine increased solute excretion in Wistar rats. However, in SH rats guanfacine failed to alter solute excretion. This decreased response is indicative of defective modulation of solute excretion in the SH rats which may be due to the alteration of the $\alpha_{2a/d}$-adrenoceptor gene. In an induced model of hypertension (1K-1C rats), the osmolar response to guanfacine was intact. Thus, the unresponsiveness of the SH rats to guanfacine was not secondary to the elevated blood pressure. These data suggest that the altered $\alpha_{2a/d}$-subtype gene and function may be involved in the onset of hypertension.

This work was supported by the Medical Research Council of Canada. H.D.I. is a recipient of the Canadian Hypertension Society/Pfizer/Medical Research Council of Canada Graduate Studentship. D.D.S is a recipient of the Scientist Award from the Medical Research Council of Canada. The authors wish to express their gratitude to Dr Gary Glavin for biostatistical consultation.

References

BLANDFORD, D.E. & SMYTH, D.D. (1988). Dose selective dissociation of water and solute excretion after renal α_2-adrenoceptor stimulation. *J. Pharmacol. Exp. Ther.*, **247,** 1181–1186.

BLANDFORD, D.E. & SMYTH, D.D. (1991). Potentiation of the natriuretic effect of clonidine following indomethacin in the rat. *Can. J. Physiol. Pharmacol.*, **69,** 1196–1203.

CHUN, M., SUN, L., SCHULTE, N., SCOFIELD, M. & PETTINGER, W.A. (1991). Restriction fragment length polymorphism (RFLP) mapped in spontaneously hypertensive rats (SHR) using human α_2-adrenoceptor probes. *FASEB J.*, **5,** A850. Abstract.

CHURCHILL, P.C., CHURCHILL, M.C., BIDANI, A.K. & RABITO, S.F. (1995). Kallikrein excretion in Dahl salt-sensitive and salt-resistant rats with native and transplanted kidneys. *Am.J. Physiol.*, **269,** F710–F717.

FREEMAN, K., FARROW, S., SCHMAIER, A., FREEDMAN, R., SCHORK, T. & LOCKETTE, W. (1995). Genetic polymorphism of the α_2-adrenergic receptor is associated with increased platelet aggregation, baroreceptor sensitivity, and salt excretion in normotensive humans. *Am. J. Hypertens.*, **8,** 863–869.

GELLAI, M. & RUFFOLO, R.R. (1987). Renal effects of selective α_1- and α_2-adrenoceptor agonists in conscious normotensive dogs. *J. Pharmacol. Exp. Ther.*, **240,** 723–728.

GUYTON, A.C. (1991). Blood pressure control - special role of the kidneys and body fluids. *Science*, **250,** 1813–1816.

GUYTON, A.C., HALL, J.E., COLEMAN, T.G. & MANNING, Jr, R.D. (1990). The dominant role of the kidneys in the long-term regulation of arterial pressure in normal and hypertensive states. In *Hypertension: Pathophysiology, Diagnosis, and Management*. ed. Laragh, J.H. & Brenner, B.M. New York, NY: Raven Press.

HALL, J.E., MIZELLE, H.L., HILDEBRANDT, D.A. & BRANDS, M.W. (1990). Abnormal pressure-natriuresis. A cause or consequence of hypertension? *Hypertension*, **15,** 547–559.

HOEHE, M.R., BERRETTINE, W.H. & LENTES, K.-U. (1988). DraI identifies a two allele DNA polymorphism in the human α_2-adrenergic receptor gene (ADRAR), using a 5.5 kb probe (p ADRAR). *Nucl. Acids. Res.*, **16,** 9070. Abstract.

INTENGAN, H.D. & SMYTH, D.D. (1996). Clonidine-induced increase in osmolar clearance and free water clearance via activation of two distinct α_2-adrenoceptor sites. *Br. J. Pharmacol.*, **119,** 663–670.

INTENGAN, H.D. & SMYTH, D.D. (1977). $\alpha_{2a/d}$-Adrenoceptor subtype stimulation by guanfacine increases osmolar clearance. *J. Pharmacol. Exp. Ther.*, (in press).

LE JOSSEC, M., TRIVALLE, C., CLOIX, J.-F., PECQUERY, R., GIUDICELLI, Y. & DAUSSE, J.-P. (1995). Differential distribution of α_2-adrenoceptor subtypes and messenger RNA expression between renal cortex of salt-sensitive and salt-resistant Sabra rats. *J. Hypertens.*, **13,** 781–790.

LI, P., PENNER, S.B. & SMYTH, D.D. (1994). Attenuated renal response to moxonidine and rilmenidine in one kidney-one clip hypertensive rats. *Br. J. Pharmacol.*, **112,** 200–206.

LOCKETTE, W., GHOSH, S., FARROW, S., MACKENZIE, S., BAKER, S., MILES, P., SCHORK, A. & CADARET, L. (1995). α_2-Adrenergic receptor gene polymorphism and hypertension in blacks. *Am. J. Hypertens.*, **8,** 390–394.

MICHEL, M.C., GALAL, O., STOERMER, J., BOCK, K.D. & BRODDE, O.-E. (1989). α- and β-Adrenoceptors in hypertension. II. Platelet α_2- and lymphocyte β_2-adrenoceptors in children of parents with essential hypertension. A model for the pathogenesis of the genetically determined hypertension. *J. Cardiovasc. Pharmacol.*, **13,** 432–439.

MOTOMURA, S., SHCNEPEL, B., SEHER, U., MICHEL, M.C. & BRODDE, O.-E. (1989). Properties of α_2-adrenoceptors in human myometrium and kidney: similarities with human platelets, but differences to rat kidney. *J. Hypertens.*, **7** (suppl 6), S50–S51.

PETTINGER, W.A., SUN, L., WOLFF, D.W., CHUN, M. & MCARDLE, S. (1991). Cosegregation of the α_2-adrenoceptor allele of hypertensive rats with an increase in blood pressure. *FASEB J.*, **5,** A850. Abstract.

RAINE, A.E.G. (1994). Hypertension and the kidney. *Br. Med. Bull.*, **50** (No.2), 332–341.

RETTIG, R., SCHMITT, B., PELZL, B. & SPECK, T. (1993). The kidney and primary hypertension: contributions from renal transplantation studies in animals and humans. *J. Hypertens.*, **11,** 883–891.

STANTON, B., PUGLISI, E. & GELLAI, M. (1987). Localization of α_2-adrenoceptor-mediated increase in renal Na^+, K^+, and water excretion. *Am. J. Physiol.*, **252,** F1016–F1021.

STRANDHOY, J.W., MORRIS, M. & BUCKALEW, V. (1982). Renal effects of the anti-hypertensive, guanabenz, in the dog. *J. Pharmacol. Exp. Ther.*, **256,** 606–616.

SVETKEY, L.P., TIMMONS, P.Z., EMOVON, O., ANDERSON, N.B., PREIS, L. & CHEN, Y.-T. (1996). Association of hypertension with β_2- and α_{2c10}-adrenergic receptor genotype. *Hypertension*, **27,** 1210–1215.

UHLÉN, S. & WIKBERG, J.E.S. (1991). Delineation of rat kidney α_{2a}- and α_{2b}-adrenoceptors with [3H]RX821002 radioligand binding: computer modeling reveals that guanfacine is an alpha2a-selective compound. *Eur. J. Pharmacol.*, **202,** 235–243.

(Received December 23, 1996
Revised March 12, 1997
Accepted March 19, 1997)