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Relationships between kallikrein secretion, kallikrein excretion and β-adrenoceptors in kidney cortical slices from neurogenic hypertensive dogs

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1 Sinoaortic denervation (SAD) in dogs is characterized by an increase in blood pressure and heart rate as well as the development of renal morphological lesions similar to those observed in essential hypertension in human subjects. To assess the effect of SAD on the secretion of kallikrein kinin systems (KKS), we studied the *in vitro* secretion of kallikrein by renal cortical slices of normal and neurogenic hypertensive dogs (1 and 18 months after SAD). The method using renal cortical slices allowed the study of secretion of kallikrein independently of renal perfusion pressure. The number of renal β-adrenoceptors was measured by [^{125}I]-cyanopindolol binding.

2 SAD was associated with a marked increase in urinary kallikrein excretion at one month and a significant decrease at 18 months when compared with controls. Both changes were statistically significant ($P<0.05$). Concurrently, a progressive increase in *in vitro* kallikrein secretion was observed ($+80\pm10\%$ and $+179\pm48\%$, 1 and 18 months after SAD, respectively). Moreover, the cortical slices obtained from sinoaortic denervated dogs contained more kallikrein than the control cortical slices ($+32\pm16\%$ and $+55\pm7\%$, 1 and 18 months after SAD, respectively).

3 Renal β-adrenoceptor number significantly ($P<0.05$) decreased 18 months after SAD from 18 ± 2 to 8 ± 3 fmol mg^{-1} protein without any change in affinity constant.

4 Although there was no test of association, because the number of renal β-adrenoceptors decreased whereas kallikrein secretion increased, the present data could suggest a β-adrenoceptor-mediated inhibition of kallikrein secretion. These results show that although the urinary kallikrein is decreased, the tissue secretory capacities are enhanced. This could suggest a renal compensatory mechanism possibly involved in tissue protection in dogs after SAD, although such a mechanism is not sufficient to reverse hypertension.

Keywords: Kallikrein; sinoaortic denervation; experimental hypertension; β-adrenoceptors

Introduction

Hypertensive diseases result from multiple factors, including disorders in a variety of humoral, hormonal and enzymatic systems; the kidney also plays a critical role in the development of hypertension. Some renal systems known to be either vasoconstrictor (renin-angiotensin system) or potentially vasodilator (prostaglandins or kallikrein-kinin systems (KKS)) are altered (Carretero & Scicli, 1989; Margolius, 1989). Decreased urinary kallikrein excretion appears to be a well-accepted feature of established hypertension in various human and experimental models (Carretero & Scicli, 1989). However, the causal relationships between reduced levels of urinary kallikrein excretion and the development of arterial hypertension have yet to be established. Until recently, urinary kallikrein has been regarded as a good index of the intrarenal KKS; however, inconsistencies between changes in urinary kallikrein excretion and tissue kallikrein content have been reported (Marin-Grez *et al.*, 1982; Girolami *et al.*, 1986). In fact, the excretion of kallikrein in the urine is only one aspect of the system. Not only is kallikrein released in urine (Corthorn *et al.*, 1979), but it is also found in lymph (De Bono & Mills, 1974) and in the venous effluent of an isolated perfused kidney (Misumi *et al.*, 1983; Van Leeuwen *et al.*, 1984). Urinary kallikrein excretion is the result of two influences: (1) direct factors acting at the site of kallikrein production and secretion (e.g. aldosterone) and (2) indirect factors such as perfusion pressure that can mask the direct effects. Thus, the study of kallikrein secretion by kidney slices, independent of perfusion pressure, is essential in studying KKS in arterial hypertension.

The aim of this study was (1) to investigate renal tissue kallikrein secretion in animals with arterial hypertension characterized by different levels of sympathetic tone, and (2) to compare tissue secretion from renal kidney slices and urinary excretion of kallikrein. To achieve this, we used a model of neurogenic arterial hypertension elicited by sino-aortic denervation (SAD) in dogs. This model is associated with a transient increase in sympathetic tone one month after surgical procedure (Valet *et al.*, 1989b). Functional (Valet *et al.*, 1989a) and morphological (Orfila *et al.*, 1993) renal alterations are similar between SAD dogs and human subjects with essential hypertension.

Methods

Eighteen male beagle dogs (mean age 2.0 ± 1.3 years) of 12-25 kg body weight (INSERM farm) were used in the experiments and were randomly divided into control ($n=8$) and sinoaortic denervated dogs ($n=10$). Kallikrein secretion was studied 1 ($n=6$) and 18 ($n=4$) months after SAD. Controls were submitted to sham operations. The release of kallikrein from kidney cortical slices was studied 1 ($n=4$) and 18 ($n=4$) months later.

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Sinoaortic denervation

Sinoaortic denervation (SAD) was performed as previously described (Damase-Michel *et al.*, 1987; Valet *et al.*, 1989b). Briefly, sinoaortic denervation involved two successive surgical procedures under α-chloralose (80 mg kg^{-1}, i.v.) anaesthesia. First, the carotid sinus nerve at the carotid bifurcation and the aortic depressor nerve in the cervical region were cut on the right side. Seven weeks later, the same surgical technique was used on the left side. The failure of noradrenaline (0.1, 1, 2 μg kg^{-1}, i.v.) and phenylephrine (0.1, 1 and 10 μg kg^{-1}, i.v.) to induce bradycardia after bilateral carotid and aortic sinus nerve section demonstrated the effectiveness of baroreceptor denervation. Nitroglycerine (1, 3, 10 and 30 μg kg^{-1}, i.v.) induced a dose-dependent decrease in blood pressure without any change in heart rate in sinoaortic denervated dogs. These tests were performed monthly, beginning immediately after SAD in order to exclude any possibility of reinnervation. Previous research (Valet *et al.*, 1989b) has shown that one month after surgey, SAD is associated with an increase in plasma catecholamine levels. Eighteen months later, sympathetic tone is normal (Valet *et al.*, 1989b). In our study, the dogs were maintained in individual cages under normal sodium diet. All investigations reported in this article were conducted in conformity with the guidelines for the care and use of animals.

Blood pressure and heart rate measurement in conscious dogs

Several days before any experiments, the animals were trained to stand still for 3–4 h on a Pavlov table and to become accustomed to blood sampling. Blood pressure was measured by means of a catheter introduced into the abdominal aorta under local anaesthesia (xylocaine 5%) and connected to a Honeywell recorded via a Gould P23ID transducer. The heart rate was measured with a heart period meter triggered by blood pressure. The cardiovascular parameters were always measured after a rest period of at least 30 min.

Urinary parameters

Before collecting urine, the animals were acclimatized for three days in individual cages, and the urine volume during the following two days was measured. Urinary samples were collected on two consecutive, but separate, 24 h periods to minimize kallikrein degeneration by urinary proteases. Urinary kallikrein activities were estimated by the kinogenase activity as described previously (Girolami *et al.*, 1987). Urinary protein was measured by the sulfosalicyl method (Davidson & Henry, 1969).

Biochemical plasma assays

Plasma catecholamines were measured before surgery, and 1 and 18 months later, by high performance liquid chromatography using electrochemical (amperometric) detection (Damase-Michel *et al.*, 1990). Briefly, fresh blood was collected (from a catheter introduced into the femoral artery 1 h earlier in order to prevent any stress) over lithium heparin with 10 mM sodium metabisulphite centrifuged at 2000 *g* for 10 min at 0°C. Plasma was stored at −80°C. Catecholamines were selectively isolated from the sample by adsorption on activated alumina, then eluted with 0.1 M acetic acid. Dihydroxybenzylamine was used as in internal standard to monitor recovery from this extraction step. The working electrode potential was set at 0.65 V against an Ag/AgCl reference electrode. The electrochemical detector response was linear for concentrations ranging from 10 pg ml^{-1} to 100 ng ml^{-1}; r = 0. 997 for noradrenaline, $r = 0.992$ for adrenaline. Under these conditions, the detection limit was 10 pg ml^{-1}. Intercoefficients of variance for plasma catecholamines performed over 5 days were 11% for noradrenaline and 12% for adrenaline. The intracoefficients of variance performed on three assays were 1% for noradrenaline and 7% for adrenaline.

Preparation and incubation of renal cortical slices

Renal cortical slices were prepared by a modification (Girolami *et al.*, 1987; 1990a,b) of the method currently used for renin secretion studies (Churchill *et al.*, 1983; Nakamura *et al.*, 1989).

At the end of the observation period (1 or 18 months), sinoaortic denervated and control dogs were killed by an overdose of intravenous pentobarbitone. The kidneys were quickly removed, decapsuled and placed in ice-cold saline solution. The renal cortex was cut with a razor blade and then sliced, approximately 0.3 mm thick, with the tissue slicer (TC-2 SORVAL). The slices were washed twice and equilibrated during 15 min at 37°C in an atmosphere of 95% O_2: 5% CO_2 in a modified Krebs Ringer bicarbonate solution (KRBS) which also served as an incubation buffer. The composition of the KRBS was as follows (in mM): NaCl 125, KCl 4, $CaCl_2$ 2.6, NaH_2PO_4 1.2, $MgSO_4$ 0.8, $NaHCO_3$ 25 and glucose 10. The pH of the solution was 7.4. All chemicals were reagent grade (Merck, Darmstadt, Germany). Cortical slices were incubated in a flask containing 2 ml of KRBS prewarmed to 37°C for 30 min under a continuous stream of 95% O_2: 5% CO_2.

For each of the 18 dogs, 12 flasks were prepared for incubation; thus a total of 216 flasks was used. Each flask contained 3.50 ± 1.25 mg of protein. The protein content was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Incubation was carried out in the dark to prevent photochemical alterations. At the end of the incubation period, the incubation medium was drawn up and centrifuged at 4,000 r.p.m. for 5 min at 4°C. The supernatant was stored at −20°C until kallikrein measurements. The tissue was also weighed and frozen until measurements after solubilization as previously reported (Girolami *et al.*, 1987).

Tissue homogenate

The kidneys were homogenized in cold 0.2 M tris(hydroxymethyl) aminomethane (Tris) buffer (pH 8.2 1 ml 100 mg^{-1} tissue) in a glass Teflon homogenizer using a Heidolph polytron device. After 30 s of homogenization in a refrigerated bath, the concentration of the medium was adjusted to 0.5% in deoxycholic acid sodium salt (Sigma) without any other change in the medium. The membrane-bound kallikrein was solubilized after 1 h of incubation at 4°C under gentle agitation (Girolami *et al.*, 1987). The solution was then centrifuged (45,000 *g* at 4°C for 30 min), and the pellet was homogenized and centrifuged to achieve further solubilization. The two supernatants were pooled to be used for kallikrein measurements. The concentration of deoxycholate or Triton X-100 was tested in a preliminary study and ranged from 0.5 to 5%. Dialysis of the samples to remove deoxycholic acid did no produce any significant difference in the results.

Kallikrein assays

Kallikrein activity was estimated by its kininogenase activity using a kinin radioimmunoassay as previously described (Girolami *et al.*, 1987). Heated citrated dog plasma was used as kininogen source. This crude source, after ammonium sulphate precipitation, was devoid of spontaneous kininogenase activity. Bradykinin (Bachgem, Switzerland) was used as a standard and [^{125}I]-iodobradykinin as a tracer. The bradykinin antibodies recognize only bradykinin and longer analogues, but not bradykinin fragments.

Kallikrein concentrations were measured as previously reported (Valet *et al.*, 1989a) by using a direct radioimmunoassay against purified dog urinary kallikrein. The antibody used recognizes the total form of kallikrein. Results are expressed in micrograms per min per milligram of creatinine (μg min^{-1} mg^{-1}) for urinary kallikrein excretion, in na-

nograms of immunoreactive kallikrein per 100 mg of tissue (ng 100 mg^{-1} tissue) when measured in tissue, or in nanograms of immunoreactive kallikrein released during 30 min of incubation per 100 mg of tissue when assessed in the incubation medium (ng 30 min^{-1} 100 mg^{-1} tissue). For each animal, the values of tissue kallikrein content or secretion were expressed by the mean value of the multiple determination (12 flasks). The recovery of purified kallikrein added to the incubation medium (both with and without tissue) was 84±11% indicating minor degradation in the absence of protease inhibitors. Previous studies (Girolami *et al.*, 1990a,b) have shown that kallikrein release in the incubation medium is linear in a time-dependent fashion for at least 40 min.

Identification of β-adrenoceptor sites

Renal cortex was dissected out, minced and homogenized in an ice-cold lysing buffer containing 5 mM Tris-HCl, 5 mM EDTA (pH=7.4) with an Ultraturrax device. The homogenate was then resuspended in a solution containing 250 mM sucrose, 50 mM Tris (pH=7.4) and centrifuged (400 *g* for 10 min, 4°C). The supernatant was washed in three volumes of buffer (50 mM Tris; $MgCl_2$ 0.5 mM; pH=7.4) and centrifuged (39,000 *g* for 10 min, 4°C). The resultant pellet was resuspended in a binding buffer (50 mM Tris; 0.5 mM $MgCl_2$; 120 mM NaCl, pH=7.4). Binding assays were extemporally performed. All experiments were performed in a binding buffer (see above) in a total volume of 200 μl. Membranes were incubated under constant shaking in the presence of increasing [^{125}I]-cyanopindolol concentrations ranging from 10 pM to 400 pM for 60 min at 37°C. For determination of total β-adrenoceptor number, non-specific binding was defined in the presence of (-)-adrenaline 10^{-4} M. Ritanserin (10^{-5} M) was added to all samples. The reaction was stopped by the addition of 4 ml of ice cold buffer. The separation of free and bound ligand was carried out by filtration through GF/C glass fibre filters under vacuum. The filters were washed twice with 10 ml buffer and then placed in vials. The radioactivity retained on the filters was counted in a Beckmann scintillation counter at an efficiency of 82%.

Computer-assisted analysis of binding data was performed with the EBDA-Ligand programme (McPherson, 1985). The protein concentration was determined by the method of Lowry *et al.* (1951) with serum albumin used as the standard.

Statistical analysis

All data are presented as mean values ± s.e.mean. Statistical analysis was performed by using analysis of variance followed by Student's *t* test for unpaired comparisons. The level of significance was $P<0.05$.

Results

Blood pressure and heart rate (Table 1).

No change in cardiovascular parameters occurred in controls (sham-operated animals). At 1 and 18 months after surgery, the systolic and diastolic blood pressures and heart rate of the SAD group were significantly increased ($P<0.05$) when compared with resting values or with sham-operated animals.

Plasma catecholamine assays (Table 1)

In the control group, the sham operation failed to modify plasma catecholamine concentrations. In the SAD group, plasma catecholamine levels increased one month after surgery, compared to control values ($P<0.05$). Eighteen months later, the levels of plasma catecholamines were not significantly different from the control values.

Urinary kallikrein excretion (Table 1).

Urinary kallikrein excretion (UKE) did not vary in the sham operated group. During the first month following surgery, the UKE of the SAD group exhibited a significant increase when compared to control values ($P<0.05$). UKE decreased ($P<0.05$) below control values 18 months after SAD.

Proteinuria

No significant proteinuria was detected in control animals. In contrast, a marked proteinuria appeared (1.0±0.1 g l^{-1}) in the SAD group one month after surgery and was significantly ($P<0.05$) higher after 18 months (3.4±0.5 g l^{-1}).

Kallikrein release from renal cortical slices (Figure 1)

In slices from control dogs, immunoreactive kallikrein was released at rates of 16.5±2.4 ng 30 min^{-1} 100 mg^{-1} tissue. After surgery, kallikrein release in the SAD group was significantly higher ($P<0.05$) than in controls (+80±10% 1 month after surgery, +179±48% after 18 months). The cortical slices obtained from the SAD group contained more kallikrein than the control cortical slices (respectively +32±16% and +55±7%, 1 and 18 months after SAD).

Renal β-adrenoceptors (Figure 2)

Renal β-adrenoceptors number (B_{max}) significantly ($P<0.05$) decreased 18 months after surgery in the SAD group. No significant change was observed in affinity constant (K_D).

Table 1 Cardiovascular, plasma and urinary parameters in sham-operated and sinoaortic denervated dogs before and after surgical procedure

	Sham-operated				*Sinoaortic denervated*			
	1 month ($n=4$)		18 months ($n=4$)		1 month (n-6)		18 months ($n=4$)	
	Before	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*
SBP (mmHg)	155±3	155±5	154±9	158±10	159±15	269±13*	157±17	204±10*
DBP (mmHg)	65±5	66±5	67±3	69±4	69±11	145±13*	68±13	116±5*
HR (b.p.m.)	76±6	79±8	77±5	77±6	78±5	135±5*	80±7	150±10*
Noradrenaline (nM)	1.34±0.09	1.36±0.15	1.40±0.15	1.44±0.17	1.35±0.10	6.11±1.17*	1.43±0.23	1.38±0.18
Adrenaline (nM)	0.86±0.04	0.85±0.06	0.91±0.09	0.90±0.10	0.89±0.03	3.52±1.1*	0.83±0.10	0.92±0.07
UKE (μg min^{-1} mg^{-1} creatinine)	0.65±0.09	0.63±0.11	0.63±0.14	0.66±0.13	0.70±0.18	6.36±2.38*	0.64±0.21	0.11±0.03*

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; UKE, urinary kallikrein excretion. Values are mean ± s.e.mean.

*$P<0.05$ compared with sham-operated dogs.

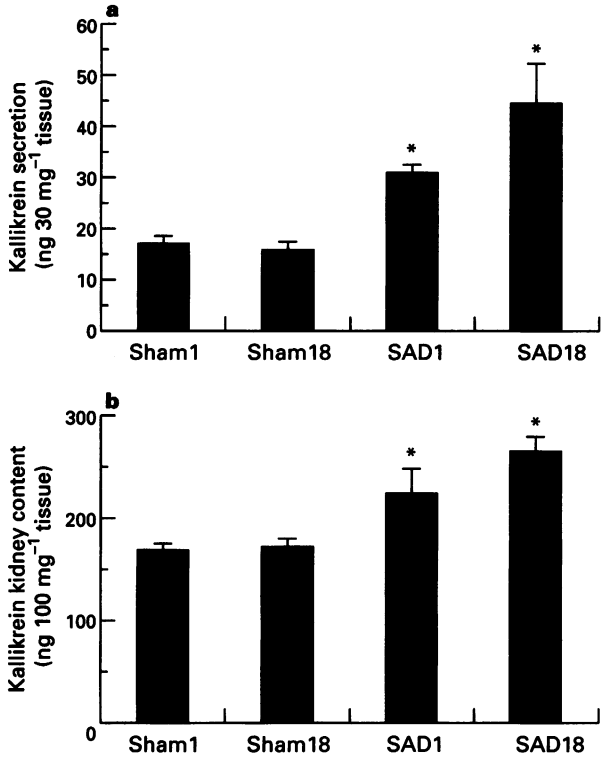

Figure 1 Kallikrein secretion (a) and content (b) of dog kidney cortical slices, 1 month (SAD1: $n=6$) or 18 months (SAD18: $n=4$) after sinoartic denervation and 1 month (Sham1: $n=4$) or 18 months (Sham18: $n=4$) after sham operation; 12 samples were measured for each animal. *$P<0.05$, compared with sham-operated animals.

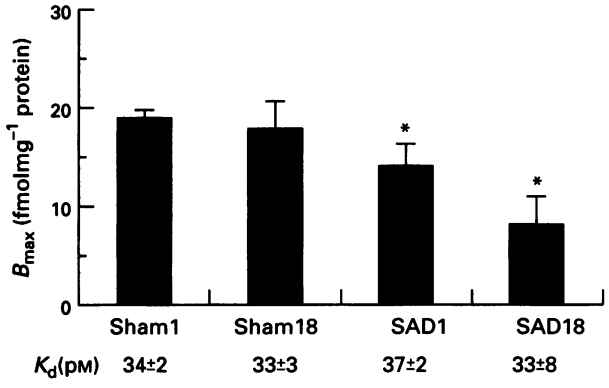

Figure 2 Maximum number of binding sites (B_{max}) and affinity constant measured with [^{125}I]-cyanopindolol of renal β-adrenoceptors 1 month (SAD1: $n=6$) or 18 months (SAD18: $n=4$) after sinoartic denervation and 1 month (Sham1: $n=4$) or 18 months (Sham18: $n=4$) after sham-operation. *$P<0.05$, compared with sham-operated animals.

Discussion

The aim of this study was to compare secretion of kallikrein in renal cortical slices and the urinary kallikrein excretion of normotensive dogs with neurogenic hypertensive animals. Both the model and the method used in this study are relevant in further understanding the role of renal kallikrein kinin systems (KKS) in the development of arterial hypertension. This animal model of hypertension is characterized by an increase in sympathetic tone one month after the section of the De Cyon and Hering's nerves, followed by a period of normalization of plasma catecholamine concentrations associated with functional (Valet *et al.*, 1989a) and morphological (Orfila *et al.*, 1993) renal alterations. Both an increase in sympathetic tone and normalization have been observed in human subjects who have essential hypertension (De Champlain, 1990; Goldstein & Kopin, 1990). Increased plasma catecholamine concentrations have been observed in some but not all patients with essential hypertension (Goldstein, 1983).

The method used in this study was also of interest. The use of kidney slices to measure the synthesis of kallikrein has been reported by Nustad & Vaaje (1975). The use of rat kidney slices to study the release of active kinogenase was first described by Nolly & Lama (1981). We modified that protocol in order to create a more sensitive method (Girolami *et al.*, 1990a,b). We were aware that indirect factors, such as renal perfusion pressure which is a well-known determinant of kallikrein secretion (Misumi *et al.*, 1983), can mask direct effects at the site of kallikrein production and secretion. The use of renal cortical slices permits observation of direct effects on the secretion of tissue kallikrein as well as potential tissue changes resulting from chronic changes in renal perfusion pressure.

The results at the end of each stage of the present study, (ie. in the first and 18th months), were significant and each of these stages merits a separate summary and discussion.

The first month of neurogenic hypertension was associated mainly with an increase in kallikrein content as measured by a release from renal cortical slices. Concurrently, plasma catecholamine levels and urinary kallikrein excretion (UKE) were increased. Such increases in tissue kallikrein secretion and urinary excretion are consistent and may be linked with sympathetic hyperactivity. There are a number of studies suggesting a relationship between the sympathetic system and kallikrein indicating that (1) bradykinin can stimulate catecholamine release from the adrenal medulla eliciting β_2-adrenoceptor activation and vasodilatation (Gardiner *et al.*, 1992) and (2) kallikrein release can be increased by β_1-adrenoceptor inhibition (Girolami *et al.*, 1990a). A direct relationship between adrenergic activity and UKE has been suggested in patients with phaeochromocytoma who exhibit high levels of UKE (Margolius *et al.*, 1972). In young spontaneously hypertensive rats, UKE has been found to be higher than in normotensive controls (Rasher *et al.*, 1980). However, other reports show evidence of the inhibitory effect of the adrenergic system on kallikrein secretion. For example, Mills & Obika (1977) reported that UKE increased after withdrawal of an arterial infusion of a non-vasoconstrictor dose of noradrenaline. Electrical stimulation of the renal nerve decreases kinogenase activity in cat urine and both intracerebroventricular injection of propranolol and peripheral chemical denervation stimulate urinary kallikrein release in rats (Albertini *et al.*, 1981). In a previous study (Girolami *et al.*, 1990a), we have shown that kallikrein release, and to a lesser degree biosynthesis, are inhibited by β_1-adrenoceptor activation, suggesting a negative feedback of catecholamine-induced kallikrein secretion due to the stimulation of β_1-adrenoceptors by catecholamines.

One can hypothesize that conflicting data from the literature can be explained by discrepancies in the experimental protocols and especially by the different durations of exposure to catecholamines. For example, electrical stimulation or injection of noradrenaline induces a short increase in catecholamine levels whereas, phaeochromocytoma and neurogenic hypertension can be considered as two situations when catecholamine levels are high for a long period. Such long term exposure to catecholamines can down regulate β-adrenoceptors, and a decrease in β-adrenoceptors has been reported previously in phaeochromocytoma (Valet *et al.*, 1988). Based on our hypothesis, parallel increases in urinary kallikrein activity, kallikrein release from cortical slices, and catecholamine secretion may be explained by a decrease in renal β-adrenoceptor density which is down regulated by the high plasma catecholamine levels. In a previous investigation of our model of hypertension using tissue β-adrenoceptor density, we found a decrease in lymphocyte (Valet *et al.*, 1989a) and cardiac (Galinier *et al.*, 1992) β-adrenoceptors. Thus, in hypertensive

dogs, an increase in kallikrein release associated with a decrease in the density of renal β-adrenoceptors suggests a diminished inhibitory effect of sympathetic tone.

One can argue that both the changes in kallikrein and the decrease in β-adrenoceptors are independent events occurring in response to the hypertension. The results of a previous study suggest that down regulation of the receptors and the presence of systemic hypertension may have independent roles in the increase in kallikrein excretion and content. In this previous study (Damase-Michel *et al.*, 1991), the same experimental model was used with an antihypertensive agent that did not directly interact with the autonomic nervous system. We used chronic treatment with cicletanine, an antihypertensive agent that stimulates arachidonic acid release (Deby *et al.*, 1988a) and increases the activity of two enzymes: (1) prostaglandin I_2 synthetase (Deby *et al.*, 1988b) and (2) cholesteryl ester hydrolase (Hajjar *et al.*, 1989). We found an enhancement in UKE (Damase-Michel *et al.*, 1991). This result is consistent with another study of SHR rats (Emond *et al.*, 1992) in which cicletanine increased UKE and KKS.

We can hypothesize that if the observed increase in KKS is only linked to the increase in systemic blood pressure, a normalization of blood pressure should induce a decrease in KKS and UKE: this was not the case in SAD dogs nor in SHR rats after cicletanine. Thus, the increase in KKS is not only due to the rise in blood pressure, but also to other phenomena such as changes in β-adrenoceptor number. The decrease in renal β-adrenoceptor number is an agreement with other data on phaeochromocytoma (Greenacre & Conolly, 1978; Valet *et al.*, 1988) and might be due to increased renal catecholamine content often seen in hypertensive states (Dawson & Oparil, 1987).

Several investigators have studied renal β-adrenoceptors in other models of genetic or acquired hypertension. Some authors have found an unaltered number of renal β-adrenoceptors (Yamada *et al.*, 1980; Freissmuth *et al.*, 1986). Others have reported an increase in renal adrenoceptors in rats with genetic (Yamada *et al.*, 1982; Struyker-Boudier *et al.*, 1986; Michel *et al.*, 1987) or acquired (Woodcock & Johnson, 1980; Michel *et al.*, 1989) hypertension. Such an increase has not been reported consistently in prehypertensive SHR rats (Michel *et al.*, 1987). The existence of an unknown factor that can overcome the down-regulation induced by catecholamines has been proposed to explain this surprising enhancement of renal β-adrenoceptors (Michel *et al.*, 1990).

The results of the present study at the end of 18 months were also of importance. In the 18 month hypertensive dogs, kallikrein release from cortical slices was significantly enhanced and cortical β-adrenoceptor number was reduced, whereas, plasma catecholamine levels were normalized. In contrast to values at the end of the first month, UKE was reduced during this second phase of hypertension. These findings are in agreement with previous results of hypertensive states, whether in man or in animal models, with the exception of mineralocorticoid hypertension in rats (Carretero & Scicli, 1989). These findings also confirm our hypothesis that the increase of kallikrein as measured by renal cortical slices is directly related to a decrease in the number of β-adrenoceptors. However, two facts must be noted: first, kallikrein release from slices and UKE are not necessarily linked. Previous research has demonstrated that kallikrein is not only released in urine but also in lymph and in the venous effluent of an isolated perfused kidney (Misumi *et al.*, 1983; Van Leeuwen *et al.*, 1984). Moreover, several studies have already suggested that tissue kallikrein content may follow a different pattern from urinary kallikrein, especially under pathological conditions involving changes in functional parameters such as perfusion pressure. For example, both kidneys of hypertensive rats with a two kidney-one clip showed a similar decrease in active tissue kallikrein, but exhibited different urinary kallikrein excretion rates (Carretero *et al.*, 1974). A reduced UKE may result in an increased storage of kallikrein in the kidney since kallikrein can be secreted from the cell through basolateral channels (Vio & Figueroa, 1985) to generate kinins in interstitial cells (Sigary *et al.*, 1993). The discrepancy between UKE and KKS may be explained by the renal damage rapidly associated with chronic changes in perfusion pressure. Morphological alterations appear as early as one month after SAD, and these lesions are dramatically more pronounced several months later. Changes occur at both the vascular and the glomerular levels. At the vascular level, there is a marked hyalinization and thickening of the media and small interlobular arteries. Changes at the glomerular level include mesangial lesions associated with focal glomerular sclerosis and thickening of Bowman's capsule (Orfila *et al.*, 1993). Secondly, renal β-adrenoceptors were diminished when catecholamines, as well as lymphocyte (Valet *et al.*, 1989b) and cardiac (Galinier *et al.*, 1992) β-adrenoceptors reached normal values.

Although, these results may be surprising, the findings are consistent with an increase in kallikrein secretion by renal slices and with the renal pressure diuresis volume regulation hypothesis for the long-term control of arterial pressure (Cowley, 1992). The inverse relationship between the density of β-adrenoceptors and kallikrein secretion may be important in considering the antihypertensive effect of β-blocking drugs. The results reported by Verbeuren *et al.* (1988) suggest that an increase in kallikrein activity may be involved in the vasodepressor action of β-blocking agents. In that study it was shown that the dilator response to the β-blocker, tertatolol, is dependent on the formation of cyclic GMP (Verbeuren *et al.*, 1988). In this respect, the recent availability of kinin antagonists may be useful in clarifying possible involvement of an increase in kallikrein activity following β-adrenoceptor blockade. Thus, the observed discordance between UKE and KKS might be explained by two mechanisms: (1) a decrease in β-adrenoceptors eliciting an enhancement in kallikrein secretion and (2) changes in perfusion pressure eliciting a decrease in UKE. Moreover, since a decrease in blood pressure by cicletanine induced a slight increase in UKE (Damase-Michel *et al.*, 1991), one can hypothesize that the antihypertensive agent prevents dramatic changes in perfusion pressure and in turn partially corrects the altered excretion of kallikrein. Probably, these changes are due to the stimulation of prostaglandin I_2 production and a subsequent relaxation of the glomerular smooth muscle.

In conclusion, the development of SAD-induced-hypertension exhibits functional and morphological renal lesions similar to those observed in human subjects with essential hypertension. In this animal model, there is a characteristic increase in renal kallikrein secretion and a decrease in renal β-adrenoceptor number. These results are consistent with our previous research using rats (Girolami *et al.*, 1990a). Both studies suggest β-adrenergic inhibition of kallikrein secretion. Our finding that tissue secretory capacities are enhanced when urinary kallikrein is decreased, suggests a compensatory renal mechanism. This mechanism may be involved in tissue protection although it may not be sufficient to reverse hypertension.

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References

ALBERTINI, R., DE GUEVARA, L.D., ASENJO, F. & BORIC, M. (1981). Effect of renal nerve stimulation on urine and tissue kininogenase activity in cats. *Hypertension Dallas*, **3**, suppl II, II 50–II 54.

CARRETERO, O.A. & SCICLI, A.G. (1989). Kinins, paracrine function and blood pressure. In *Endocrine Mechanisms in Hypertension*. ed. Laragh, J.H, Brenner, B.M. & Kaplan, N.M. pp. 219–239. New York: Raven Press.

CHURCHILL, P.C., CHURCHILL, M.C. & MCDONALD, F.D. (1983). Evidence that β1-adrenoceptor activation mediates isoproterenol-stimulated renin secretion in the rat. *Endocrinology*, **113**, 687–692.

CORTHORN, T., IMANARI, H., YOSHIDA, H., KAIZU, T., PIERCE, J.V. & PISANO, J.J. (1979). Isolation of prokallikrein from human urine. In *Advances in Experimental Medicine and Biology, Kinins-I*. ed. Moriya, M. & Suzuki, T. p. 120B. New York: Plenum Press.

COWLEY, A.W. (1992). Long term control of arterial blood pressure. *Physiol. Rev.*, **72**, 231–300.

DAMASE-MICHEL, C., GIROLAMI, J.P., BASCANDS, J.L., TRAN, M.A., MOATTI, J.P., PECHER, C., BERTHET, P., TARRADE, T. & MONTASTRUC, J.L. (1991). Effects of cicletanine on vasoactive systems in conscious sinoaortic denervated dogs. *Fundam. Clin. Pharmacol.*, **5**, 719–732.

DAMASE-MICHEL, C., MONTASTRUC, J.L., GHARIB, C., GEELEN, G., DE SAINT BLANQUAT, G. & TRAN, M.A. (1990). Effect of quinpirole, a specific dopamine DA2 agonist on the sympathoadrenal system in dogs. *J. Pharmacol. Exp. Ther.*, **252**, 770–777.

DAMASE-MICHEL, C., VALET, P. & MONTASTRUC, J.L. (1987). Nicardipine causes sympathetic activation not involving baroreflex tachycardia in conscious sinoaortic denervated dogs. *Eur. J. Pharmacol.*, **142**, 145–149.

DAVIDSON, I. & HENRY, J.B. (1969). *Clinical Diagnosis by Laboratory Methods*. 19th ed. p. 74. Philadelphia, Pa: WB Saunders Co.

DAWSON, R.J.R. & OPARIL, S. (1987). Renal catecholamines and α_2-adrenergic receptors in salt-related and genetic hypertension. *Pharmacology*, **34**, 131–142.

DE BONO, E. & MILLS, I.H. (1974). Simultaneous increases in kallikrein in renal lymph and urine during saline infusion. *J. Physiol.*, **241**, 127–128P.

DEBY, C., BOURGAIN, R.H., ANDRIES, R. & BRAQUET, P. (1988a). Modulation of the prostacyclin synthetase by cicletanine and drugs which affect ion transport. *Pharmacol. Res. Commun.*, **20**, 101–108.

DEBY, C., BOURGAIN, R.H., DEBY-DUPONT, G., PINCEMAIL, J., GARAIL, R. & BRAQUET, P. (1988b). Modulation of in vivo generation of prostanoïds by drugs affecting membrane ion transport. *Drugs. Exp. Clin. Res.*, **14**, 123–134.

DE CHAMPLAIN, J. (1990). Pre- and postsynaptic adrenergic dysfunctions in hypertension. *J. Hypertension*, **8**, S77–S85.

EMOND, C., BOMPART, G., RAKOTOARIVONY, J., BASCANDS, J.L., PECHER, C., ADAM, A., TARRADE, T., BERTHET, P. & GIROLAMI, J.P. (1992). Protective effect of cicletanine on hypertension-induced decreases in the renal kallikrein-kinin systems in stroke-prone spontaneously hypertensive rats. *J. Cardiovasc. Pharmacol.*, **20**, 601–608.

FREISSMUTH, M., KRAUPP, O., HAUSLEITHNER, V., TUISL, E. & SCHUTZ, W. (1986). Alterations of glomerular β-adrenoceptor-linked adenylate cyclase system in perinephritis hypertension. *J. Cardiovasc. Pharmacol.*, **8**, 60–66.

GALINIER, M., SENARD, J.M., VALET, P., DREAN, G., PLANAT, V., ARIAS, A., TRAN, M.A., & MONTASTRUC, J.L. (1992). Myocardial hypertrophy, cardiac β-adrenoceptors and adenylate cyclase activity during sinoaortic denervation in dogs. *Br. J. Pharmacol.*, **105**, 341–346.

GARDINER, S.M., KEMP, P.A., BENNET, T., BOSE, C., FOULKES, R. & HUGHES, B. (1992). Involvement of beta2-adrenoceptors in the regional hemodynamic responses to bradykinin in conscious rats. *Br. J. Pharmacol.*, **105**, 839–848

GIROLAMI, J.P., BASCANDS, J.L., PECHER, C. & SUC, J.M. (1987). Direct radioimmunoassay of rat urinary kallikrein: its application to the determination of active and inactive kallikrein after HPLC determination. *J. Immunoassay*, **8**, 115–130.

GIROLAMI, J.P., BASCANDS, J.L., PRADDAUDE, F. & SUC, J.M. (1986). Change in cortical active kallikrein during the onset of goldblatt hypertension in the rat. In *Kinins-IV*. ed. Greenbaum, L.M. & Margolius, H.S. pp. 279–283. New York: Plenum Press.

GIROLAMI, J.P., BASCANDS, J.L., VALET, P., PERCHER, C. & CABOS, G. (1990a). β1-adrenergic inhibition of kallikrein release from rat kidney cortical slices. *Am. J. Physiol.*, **258**, F1425–F1431.

GIROLAMI, J.P., ORFILA, C., CABOS-BOUTOT, G., PERCHER, C., BASCANDS, J.L. & COLLE, A. (1990b). Early acute effects of mercuric chloride on synthetis and release of kallikrein and on distal tubular morphology of rat renal cortical slices. *Renal Physiol. Biochem.*, **13**, 223–232.

GOLDSTEIN, D.S. (1983). Plasma catecholamines and essential hypertension. An analytical review. *Hypertension*, **5**, 86–99.

GOLDSTEIN, D.S. & KOPIN, I.J. (1990). The autonomic nervous system and catecholamines in normal blood pressure control and in hypertension. In *Hypertension: Pathophysiology, Diagnosis and Management*. ed. Laragh, J.H. & Brenner, B.M. pp. 711–747. New York: Raven Press.

GREENACRE, J.K. & CONOLLY, M.E. (1978). Desensitization of the beta adrenoceptor lymphocytes from normal subjects and patients with phaeochromocytoma: studies *in vivo*. *Br. J. Clin. Pharmacol.*, **5**, 191–197.

HAJJAR, D.P. & POMERANTZ, K.B. (1989). Cicletanine stimulates cholesteryl ester hydrolase activities and prostacyclin production in arterial smooth muscle cells. *Arch. Mal. Coeur*, **82**, 79–84.

LOWRY, O.H., ROSENBROUGH, H., FAN, M. & RANDALL, R.J. (1951). Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

MARGOLIUS, H.S. (1989). Tissue kallicreins and kinins regulation and roles in hypertensive and diabetic diseases. *Ann. Rev. Pharmacol. Toxicol.*, **29**, 343–364.

MARGOLIUS, H.S., GELLER, R.G., DE JONG, W., PISANO, J.J. & SJOERDSMA, A. (1972). Urinary kallikrein excretion in hypertension. *Circ. Res.*, **II**, 125–II.131.

MARIN-GREZ, M., SCHAECHTELIN, G., BONNER, G., SPECH, G. & GROSS, F. (1982). Renal kallikrein activity and urine kallikrein excretion in rats with experimental renal hypertension. *Clin. Sci.*, **63**, 349–354.

MCPHERSON, G.A. (1985). Analysis of radioligand binding experiments: a collection of computer programs for the IBM PC. *J. Pharmacol. Methods*, **14**, 213–218.

MICHEL, M.C., BRODDE, O.E. & INSEL, P.A. (1990). Peripheral adrenergic receptors in hypertension. *Hypertension*, **16**, 107–120.

MICHEL, M.C., KANCZIK, R., KHAMSSI, M., KNORR, A., SIEGL, H., BECKERINGH, J.J. & BRODDE, O.E. (1989). α- and β-adrenoceptors in hypertension: Cardiac and renal α1, β1 and β2-adrenoceptors in rat models of acquired hypertension. *J. Cardiovasc. Pharmacol.*, **13**, 421–431.

MICHEL, M.C., WANG, X.L., SCHLICKER, E., GOTHERT, M., BECKERINGH, J.J. & BRODDE, O.E. (1987). Increased beta2 adrenoceptor density in heart, lung, and kidney of spontaneously hypertensive rats. *J. Autonom. Pharmacol.*, **7**, 41–51.

MILLS, I.H. & OBIKA, L.F.O. (1977). A novel effect of intrarenal infusion of a non-vasoconstrictor dose of noradrenaline on renal function: relationship to renal kallikrein and prostaglandin. *J. Physiol.*, **267**, 21–22P.

MISUMI, J., ALHENC-GELAS, F., MARRE, M., MARCHETTI, J., CORVOL, P. & MENARD, J. (1983). Regulation of kallikrein and renin release by the isolated perfused rat. *Kidney Int.*, **24**, 58–65.

NAKAMURA, K.T., PAGE, W.V., SATO, T., KLINKEFUS, J.M. & ROBILLARD, J.E. (1989). Ontogeny of isoproterenol-stimulated renin secretion from renal cortical slices. *Am. J. Physiol.*, **256**, R1258–R1263.

NOLLY, H. & LAMA, C.M. (1981). Active and inactive kallikrein released by rat kidney cortical slices from normotensive and hypertensive rats. *Hypertension Dallas*, **3**, Suppl II, II.35–II.38.

NUSTAD, K. & VAAJE, K. (1975). Synthesis of kallikrein by rat kidney slices. *Br. J. Pharmacol.*, **53**, 229–234.

ORFILA, C., DAMASE-MICHEL, C., LEPERT, J.C., MONTASTRUC, J.L., SUC, J.M., MONTASTRUC, P. & GIROLAMI, J.P. (1993). Renal morphological changes after sinoaortic denervation in dogs. *Hypertension*, **21**, 758–766.

RASHER, W., BÖNNER, G., STOCKER, M. & GROSS, F. (1980). Increased kallikrein excretion in spontaneously hypertensive rats and its inhibition by 6-hydroxydopamine. *Naunyn-Schmied Arch. Pharmacol.*, **313**, 155–157.

SIGARY, H.M., JAFFA, A.A. & MARGOLIUS, H.S. (1993). Stimulation of renal interstitial bradykinin by sodium depletion. *Am. J. Hypertens.*, **6**, 863–866.

STRUYKER-BOUDIER, H.A.J., VERVOOT-PETERS, L.H.T.M., ROUSCH, M.J.M., SMITS, J.F.M. & THIJSSEN, H.H.W. (1986). Beta-adrenoceptors in kidney tubules of spontaneously hypertensive and normotensive rats. *Life Sci.*, **38**, 137–145.

VALET, P., BASCANDS, J.L., PECHER, C., CABOS, G., MOATTI, J.P., MONTASTRUC, J.L. & GIROLAMI, J.P. (1989a). Urinary kallikrein excretion following chronic sinoaortic denervation in conscious dogs. *Clin. Exp. Hypertens.*, **A11**, 215–231.

VALET, P., DAMASE-MICHEL, C., CHAMONTIN, B., DURAND, D., CHOLLET, F. & MONTASTRUC, J.L. (1988). Platelet α2- and leucocyte β2-adrenoceptors in phaeochromocytoma: effect of tumor removal. *Eur. J. Clin. Invest.*, **18**, 481–485.

VALET, P., DAMASE-MICHEL, C., MONTASTRUC, J.L. & MONTASTRUC, P. (1989b). Plasma catecholamines and adrenoceptors after chronic sinoaortic denervation in dogs. *Fund. Clin. Pharmacol.*, **3**, 307–320.

VAN LEEUWEN, B.H., GRINBLAT, S.M. & JOHNSTON, C.I. (1984). Release of active and inactive kallikrein from the isolated perfused kidney. *Clin. Sci.*, **66**, 207–215.

VERBEUREN, T.J., ZONNEKEYN, L.L., PROST, J.F., ROCHAT, C., THOMAS, J.R. & HERMANN, A.G. (1988). The beta adrenoceptor blocker tertatolol causes vasodilatation in the isolated perfused vasoconstricted rat kidney. *J. Pharmacol. Exp. Ther.*, **246**, 628–634.

VIO, C.P. & FIGUEROA, C.D. (1985). Subcellular localization of renal kallikrein by ultrastructural immunocytochemistry. *Kidney Int.*, **28**, 36–42.

WOODCOCK, E.A. & JOHNSON, C.I. (1980). Changes in tissue alpha- and beta-adrenergic receptors in renal hypertension in the rat. *Hypertension*, **2**, 156–161.

YAMADA, S., ISHIMA, T., TOMITA, T., HAYASHI, M. & HAYASHI, E. (1982). Increased renal beta adrenoceptors in stroke-prone spontaneously hypertensive rats. *Eur. J. Pharmacol.*, **83**, 149–150.

YAMADA, S., YAMAURA, H.I. & ROESKE, W.R. (1980). Alterations in central and peripheral adrenergic receptors in deoxycorticosterone/salt hypertensive rats. *Life Sci.*, **27**, 2405–2416.

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