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Effects of Hoe 140, a bradykinin B_2-receptor antagonist, on renal function in conscious normotensive rats

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1. The present study was designed to determine if endogenous kinins are involved in the regulation of arterial blood pressure and renal function in conscious rats given deoxycorticosterone enantate (DOC, 25 mg kg^{-1}, s.c., weekly) or vehicle for two weeks.

2. The bradykinin B_2-receptor antagonist, D-Arg[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (Hoe 140), at a dose of 300 μg kg^{-1}, s.c., blocked the hypotensive effect of 300 ng kg^{-1} bradykinin i.a., but it did not alter the blood pressure lowering action of 300 ng kg^{-1} acetylcholine or prostaglandin E_2. Inhibition of the response to bradykinin persisted up to 6 h after the administration of Hoe 140.

3. Administration of 300 μg kg^{-1} Hoe 140 s.c. four times a day did not alter mean blood pressure, renal blood flow, or renal function in rats given DOC-vehicle. However, it decreased urinary volume by 70% (from 48.2 ± 3.8 to 14.3 ± 3.7 ml 24 h^{-1}, $P<0.01$) and urinary secretion of sodium by 54% (from 1.02 ± 0.05 to 0.47 ± 0.16 mmol 24 h^{-1}, $P<0.01$) and potassium by 30% (from 2.93 ± 0.15 to 2.04 ± 0.15 mmol 24 h^{-1}, $P<0.05$) in DOC-treated rats. Mean blood pressure, glomerular filtration rate and total renal blood flow remained unchanged.

4. Our results suggest that endogenous kinins play a role in the regulation of renal excretion of water and sodium in the presence of elevated levels of DOC.

Keywords: Kinin antagonist; Hoe 140; bradykinin; kallikrein; renal blood flow; renal function; blood pressure

Introduction

Kinins are potent vasodilators, which acting as paracrine hormones, may be involved in the regulation of regional haemodynamics (Nolly *et al.*, 1990; Saed *et al.*, 1990; Wiemer *et al.*, 1991; Gardiner *et al.*, 1990; 1992) and renal excretion of water and sodium (Willis *et al.*, 1969; Carretero & Scicli, 1988). Alteration of the function of locally formed kinins, leading to predominance of vasoconstrictor tone and retention of water and salt, may contribute to the pathogenesis of arterial hypertension.

In 1985, five years after the proposal that the biological effects of kinins are mediated by two different receptor types, named B_1- and B_2-receptors (Regoli & Barabé, 1980), the first effective competitive antagonists of bradykinin were discovered (Vavrek & Stewart, 1985). B_2-receptor antagonists reportedly increase blood pressure (Carbonell *et al.*, 1988a), decrease renal blood flow (Seino *et al.*, 1988; Madeddu *et al.*, 1990a), and prevent the cardiovascular and renal effects of kininase inhibitors (Carbonell *et al.*, 1988b; Zimmerman *et al.*, 1990; Genden & Molineaux, 1991; Carretero & Scicli, 1991). Unfortunately, B_2-receptor antagonists of the first generation were characterized by low selectivity, residual agonist activity, and an ability to stimulate the release of renin, catecholamines and histamine (Mulinari *et al.*, 1987; Beierwaltes *et al.*, 1988; Rhaleb *et al.*, 1991). Thus, the effects caused by this class of antagonists might be unrelated to their kinin-blocking ability. In addition, their susceptibility to fast enzymatic degradation (Griesbacher *et al.*, 1989) limited their application to acute experiments.

Structure-activity studies, performed in order to eliminate the above described limitations, have resulted in a new series of potent, long-acting, selective, and specific B_2-receptor antagonists, devoid of agonist effect *in vitro* (Lembeck *et al.*, 1991; Hock *et al.*, 1991).

The present study was designed to evaluate the haemodynamic and renal effects caused by the administration of D-Arg[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin, a newly synthesized, B_2-receptor antagonist, which reportedly inhibits bradykinin-induced decreases in blood pressure in the rat for several hours (Wirth *et al.*, 1991). Experiments were conducted in conscious rats with or without pretreatment with deoxycorticosterone, which increases renal kallikrein activity and urinary kallikrein excretion (Geller *et al.*, 1972; Nakagawa *et al.*, 1991).

Methods

Male Wistar rats (Morini, Como, Italy) weighing between 270 and 330 g were housed at a constant room temperature with a 12-h light-dark cycle and had free access to tap water and rat chow. The following experimental protocols were performed in rats free to move in their own cages.

Experiment 1: Duration of action of Hoe 140 assessed by antagonism of the blood pressure response to bradykinin

With rats under light ether anaesthesia, a PE-10 tubing (Clay-Adams, Parsippany, NJ, U.S.A.) catheter was inserted into the left femoral artery and advanced into the abdominal aorta; a PE-50 tubing catheter was inserted into the left carotid artery and advanced into the descending thoracic aorta; both catheters were tunnelled under the skin and exteriorized at the back of the neck. Twenty-four hours later, mean arterial blood pressure (MBP) was measured by connecting the femoral catheter to a Statham transducer (Gould, Oxford, CA, U.S.A.) MBP was recorded on an S&W recorder (Medico Teknik, Albertslund, Denmark). The effects

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of intra-aortic bolus injections of bradykinin (300 ng kg^{-1} in 20 µl saline, via the carotid catheter) on MBP were tested before and at intervals after the administration of Hoe 140 (300 µg kg^{-1} in 0.1 ml saline, s.c.; $n = 7$) or vehicle ($n = 7$).

Experiment 2: Specificity of Hoe 140 assessed by inhibition of the blood pressure lowering effect of bradykinin, prostaglandin E_2 and acetylcholine

Rats underwent surgery as in experiment 1. Twenty-four hours after catheter implantation, Hoe 140 (300 µg kg^{-1} in 0.1 ml saline, $n = 7$) or vehicle ($n = 7$) was given subcutaneously. One hour later, the blood pressure lowering effect induced by an intra-aortic bolus injection of bradykinin (300 ng kg^{-1} in 20 µl saline) was evaluated. The same protocol was followed in rats given intra-aortic boluses of prostaglandin E_2 or acetylcholine (both at a dose of 300 ng kg^{-1} in 20 µl saline, $n = 7$ each group), instead of bradykinin.

Experiment 3: Effect of Hoe 140 on mean blood pressure in rats given either nonpressor or pressor doses of angiotensin II, phenylephrine or endothelin-1

Rats received weekly injections of DOC (25 mg kg^{-1} body weight, s.c.) or vehicle for two weeks. Then, with rats under light ether anaesthesia, two PE-10 tubing catheters (Clay-Adams, Parsippany, NJ, U.S.A.) were inserted into the left femoral artery and vein, advanced into the abdominal aorta and vena cava, respectively, tunnelled under the skin and exteriorized at the back of the neck. Experiments were conducted 24 h after catheter implantation.

First, the effect of Hoe 140 (300 µg kg^{-1} in 0.1 ml saline, s.c.) on MBP of rats with or without DOC pretreatment was tested during a 60 min infusion of saline (20 µl min^{-1}, i.v.), angiotensin II (3 ng kg^{-1} min^{-1}, i.v.), phenylephrine (7.5 ng kg^{-1} min^{-1}, i.v.) or endothelin-1 (7.5 ng kg^{-1} min^{-1}, i.v.); each group consisted of 6 rats.

In another set of experiments, rats received Hoe 140 (300 µg kg^{-1} in 0.1 ml saline, s.c.) or vehicle and 30 min later they were given bolus injections of either angiotensin II (30, 75 and 187 ng kg^{-1}, i.v.), or phenylephrine (0.6, 1.5 and 3.75 µg kg^{-1}, i.v.) or endothelin-1 (0.3, 0.75 and 3 µg kg^{-1}, i.v.). Each group ($n = 6$) received one vasoconstrictor. Each dose was given at 30 min intervals in random order, except for endothelin-1 which was injected at increasing doses. This procedure allowed return of MBP to control levels prior to subsequent injection.

Experiment 4: Effect of Hoe 140 on renal blood flow

Rats received weekly injections of DOC (25 mg kg^{-1} body weight, s.c.) or vehicle. After two weeks, they were anaesthetized with pentobarbitone sodium (50 mg kg^{-1}, i.p.) and, through a vertical mid-abdominal incision, miniaturized pulsed Doppler flowmeter probes (Crystal Biotech, Holliston, MA, U.S.A.) were placed and sutured around the left renal artery. The probe wires were tunnelled out of the back of the abdominal cavity, exteriorized and sutured on the back of the neck. One PE-10 catheter was inserted into the left femoral artery, advanced into the abdominal aorta and exteriorized at the back of the neck. Following closure of the incisions, rats were treated with ampicillin (7 mg kg^{-1}, s.c.) and allowed to recover for 5 days. After this time, the probe wires were connected to the alligator clips of Doppler cables whose terminations were plugged into a three-channel pulsed Doppler instrument (Pulse-Doppler, University of Iowa, Iowa City, IA, U.S.A.). Mean Doppler shift (electronically derived from the phasic signal) which corresponds to renal blood flow (Haywood *et al.*, 1981) was recorded on a Kipp and Zonen recorder (Delft, The Netherlands) before and at regular intervals after the injection of Hoe 140 (300 µg kg^{-1} four times a day, s.c.) or vehicle for 24 h. Zero flow was established by turning off the ultrasound signal; each group consisted of 6 rats. In our laboratory, comparison of instantaneous responses to graded doses of noradrenaline (from 0.2 to 2 nmol kg^{-1}) simultaneously detected by the pulsed Doppler system and an electromagnetic flowmeter indicates that changes in Doppler shift correlate very well with alterations in renal blood flow ($r = 0.988$, $P<0.01$) (Madeddu *et al.*, 1990b). In addition, comparison of measurements of renal blood flow by the clearance of *p*-[^{14}C]-aminohippuric acid and by the pulsed Doppler system over 6 h periods of time in conscious rats indicated that the two methods estimate changes in renal blood flow in a similar fashion ($r = 0.910$, $P<0.01$).

Experiment 5: Effect of Hoe 140 on renal function

Rats received weekly injections of DOC (25 mg kg^{-1} body weight, s.c.) or vehicle for two weeks. Then, urine collections over 24 h were obtained from rats in individual metabolic cages with free access to tap water but deprived of food. The same procedure was repeated two days later. Either vehicle- or DOC-treated rats were divided into two groups ($n = 9$ each); rats in group 1 received saline (100 µl four times a day, s.c.) on both occasions and rats in group 2 received saline on the day of the first urine collection and Hoe 140 (300 µg kg^{-1} four times a day, s.c.) on the day of the second urine collection. Blood (0.3 ml) was sampled by a small incision in the tail artery at the beginning and at the end of each urine collection to measure plasma creatinine.

Analytical procedures

MBP and Doppler renal blood flow (DRBF) are expressed in mmHg and kHz, respectively. Urinary volume (UV) was determined gravimetrically. Urinary sodium (U_{Na}) and potassium (U_K) were determine by flame photometry. Urinary osmolality was measured by determining the freezing point of urine with a Fisker Osmometer and expressed as mosmol excreted $24\ h^{-1}$. Urinary and plasma creatinine were measured by an automatic analyzer (Hitachi 704). Glomerular filtration rate was calculated as the clearance of endogenous creatinine.

All data are expressed as mean ± s.e.mean. Multivariate repeated measures analysis of variance was performed to test for interaction between time and the grouping factor. Then, univariate analysis of variance was used to test for differences among groups and over time. Differences between groups were determined by paired and unpaired *t* tests with the Bonferroni multiple comparison adjustment. Mathematical and statistical analyses were performed with a Statview II package, on a Macintosh IICX computer.

Substances

Bradykinin, angiotensin II, phenylephrine and endothelin-1 were purchasesd from Peninsula Laboratories (Belmont, CA, U.S.A.). The bradykinin antagonist, D-Arg[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin was synthesized by solid phase peptide synthesis by Hoechst AG (Frankfurt, Germany). Concentrated solutions (1–10 mg ml^{-1}) of these reagents were prepared in 0.9% NaCl and stored at −20°C until use. Deoxycorticosterone enantate (DOC) was donated by Shering Company (Milan, Italy).

Results

Experiment 1: Duration of action of Hoe 140 assessed by antagonism of the blood pressure response to bradykinin

Injection of bradykinin prior to subcutaneous administration of Hoe 140 or vehicle decreased MBP from 117 ± 2 and 116 ± 2 mmHg by 13 ± 1 and 12 ± 1 mmHg, respectively. Hoe 140 at 300 µg kg^{-1} exerted a long-lasting antagonistic

effect since the response to bradykinin was inhibited completely at 1 h and by 25% at 6 h (Figure 1). Inhibition by the antagonist was dose-related since in preliminary experiments we found that the blood pressure lowering effect of bradykinin was inhibited by 55% 1 h after 30 $\mu g\,kg^{-1}$ and was restored to normal at 6 h.

Experiment 2: Specificity of Hoe 140 assessed by inhibition of the blood pressure lowering effect of bradykinin, prostaglandin E_2 and acetylcholine

Bradykinin decreased MBP by 12 ± 1 mmHg (from 113 ± 2 to 101 ± 1 mmHg, $P<0.01$) in rats given vehicle; the blood

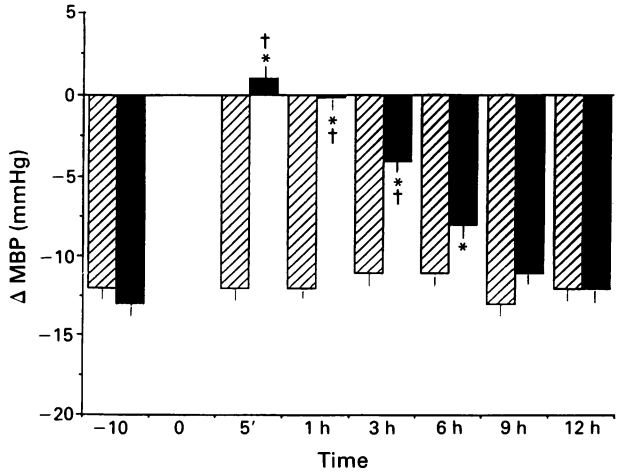

Figure 1 Inhibition of bradykinin-induced blood pressure lowering effect by 300 $\mu g\,kg^{-1}$ Hoe 140. Bradykinin (300 ng kg^{-1}) was given intra-arterially before and at intervals following the subcutaneous administration of vehicle or Hoe 140. Each column (Hoe 140 group, solid; vehicle group, hatched) represents the mean absolute change in mean blood pressure (MBP) of 7 rats; standard error of the mean is shown by vertical lines. *$P<0.05$ compared with time − 10'; † $P<0.05$ compared with vehicle group.

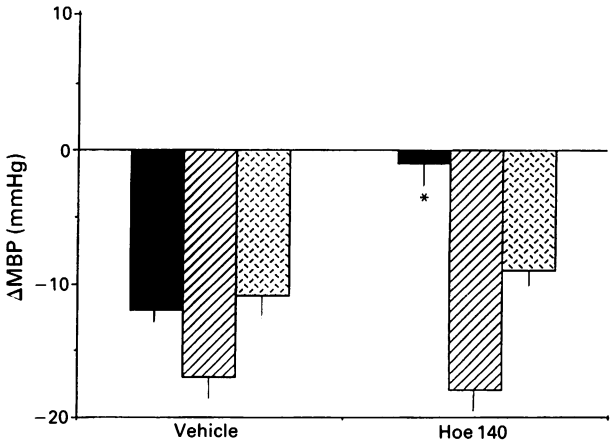

Figure 2 Specificity of Hoe 140 assessed by comparing the inhibition of the hypotensive response to bradykinin, prostaglandin E_2 or acetylcholine (300 ng kg^{-1}, i.a.). Rats received vehicle or Hoe 140 (300 $\mu g\,kg^{-1}$, s.c.) 1 h before the injection of vasodilators. Each column represents the mean absolute change in mean blood pressure (MBP) of 7 rats (solid columns, bradykinin; hatched columns, prostaglandin E_2; stippled columns, acetylcholine). Standard error of the mean is shown by vertical lines. *$P<0.05$ compared with vehicle group.

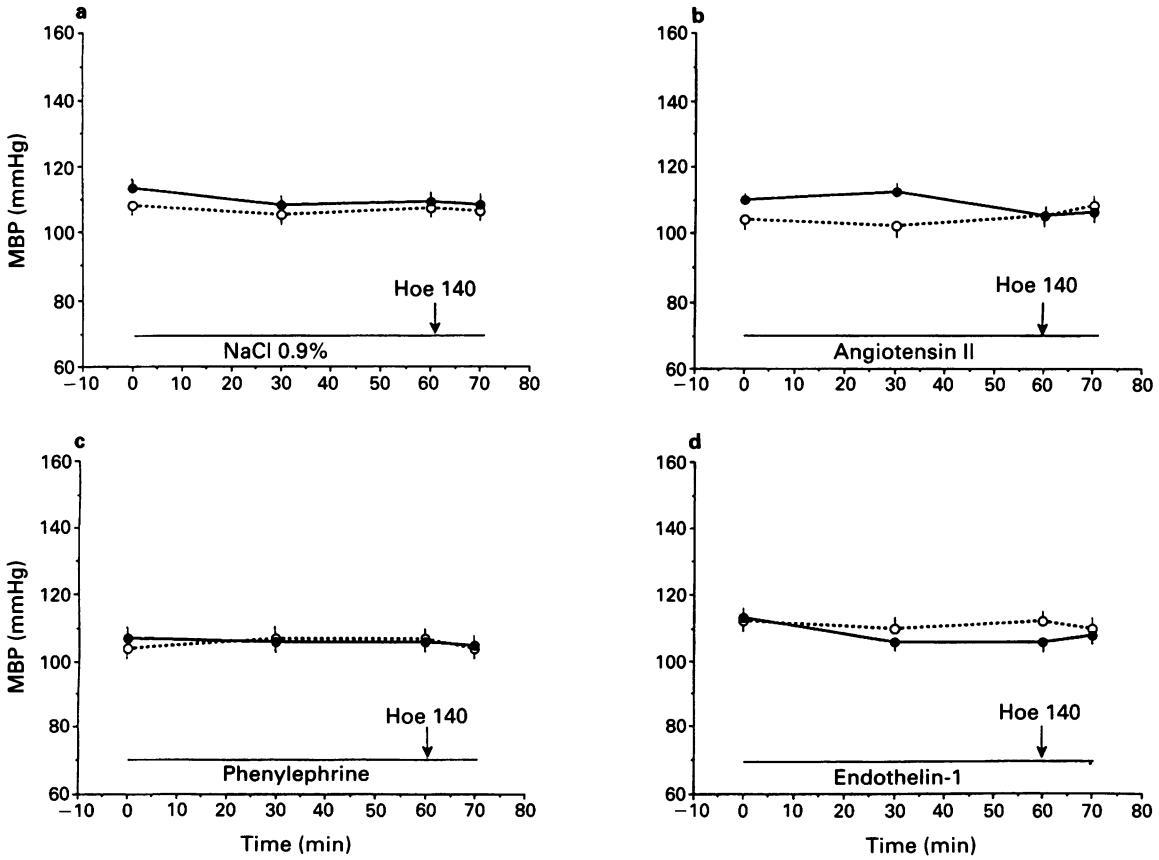

Figure 3 Effect of Hoe 140 (300 $\mu g\,kg^{-1}$, s.c.) on mean blood pressure (MBP) in rats with (solid lines) or without (broken lines) deoxycorticosterone (DOC) pretreatment during the infusion of (a) saline (20 $\mu l\,min^{-1}$, i.v.), (b) angiotensin II (3 ng $kg^{-1}\,min^{-1}$, i.v.), (c) phenylephrine (7.5 ng $kg^{-1}\,min^{-1}$, i.v.) or (d) endothelin-1 (7.5 ng $kg^{-1}\,min^{-1}$, i.v.). Each group consisted of 6 rats.

pressure lowering effect of bradykinin was abolished by Hoe 140 (before: 113 ± 1 mmHg, after bradykinin: 112 ± 1 mmHg, NS). The antagonist proved to be specific since it did not inhibit the effect of other unrelated vasodilators such as prostaglandin E_2 or acetylcholine (Figure 2).

Experiment 3: Effect of Hoe 140 on mean blood pressure in rats given either nonpressor or pressor doses of angiotensin II, phenylephrine or endothelin-1

As shown in Figure 3, Hoe 140 did not alter MBP in rats with or without deoxycorticosterone pretreatment during the infusion of vehicle or nonpressor doses of angiotensin II, phenylephrine, endothelin-1.

As shown in Figure 4, pretreatment with Hoe 140 did not alter the dose-pressor response curves to angiotensin II, phenylephrine, or endothelin-1.

Experiment 4: Effect of Hoe 140 on renal blood flow

As shown in Figure 5, administration of Hoe 140 over a period of 24 h did not alter MBP or renal blood flow in rats with or without DOC pretreatment.

Experiment 5: Effect of Hoe 140 on renal function

In rats given DOC-vehicle, no significant change in renal function was induced by Hoe 140 (Table 1).

In DOC-treated rats, Hoe 140 did not alter GFR [experimental group: vehicle (day 1), 3.16 ± 0.39 ml min^{-1}; Hoe 140 (day 2), 3.19 ± 0.13 ml min^{-1}, NS], [control group: vehicle (day 1), 2.91 ± 0.15 ml min^{-1}; vehicle (day 2), 2.87 ± 0.15 ml min^{-1}, NS]. As shown in Figure 6, Hoe 140 reduced urinary volume by 70% (from 48.2 ± 3.8 to 14.3 ± 3.7 ml $24\,h^{-1}$, $P<0.01$), Na^+ excretion by 54% (from 1.02 ± 0.05 to 0.47 ± 0.16 mmol $24\,h^{-1}$, $P<0.01$), K^+ excretion by 30% (from 2.93 ± 0.15 to 2.04 ± 0.15 mmol $24\,h^{-1}$, $P<0.05$) and osmolal excretion by 28% (from 18.32 ± 1.11 to 13.19 ± 1.18 mosmol $24\,h^{-1}$, $P<0.05$).

Discussion

Our results indicate that the compound Hoe 140 is a potent, specific and long-acting antagonist of bradykinin. When injected subcutaneously in rats that had received DOC-pretreatment, Hoe 140 caused a significant decrease in urinary volume and urinary sodium excretion without affecting MBP, total renal blood flow or glomerular filtration rate.

In the present study, we were able to demonstrate that Hoe 140 is specific *in vivo* as it did not alter the hypotensive effect caused by acetylcholine and prostaglandin E_2, thus confirming studies performed *in vitro* by Hock *et al.* (1991).

Hoe 140, at a dose which significantly inhibited the hypotensive effect of exogenous bradykinin for 24 h, did not

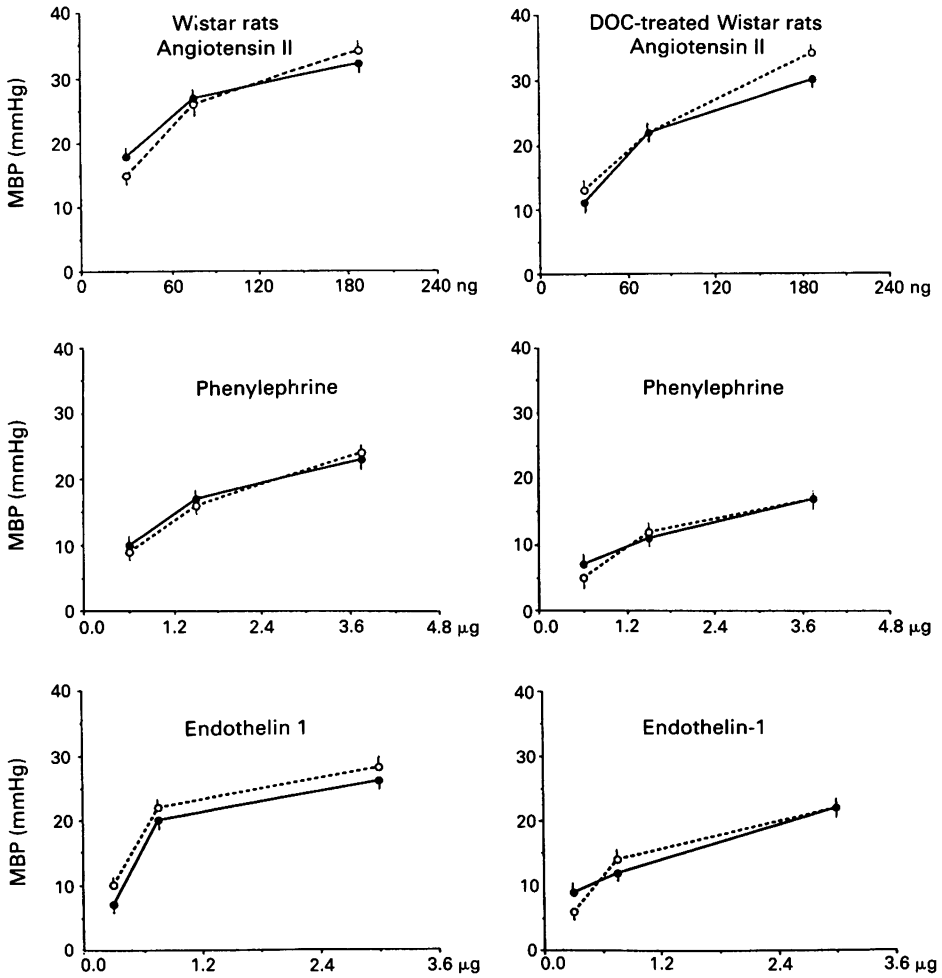

Figure 4 Dose-response curves to boluses of angiotensin II (30, 75 and 187 ng kg^{-1}, i.v.), phenylephrine (0.6, 1.5 and 3.75 µg kg^{-1}, i.v.) or endothelin-1 (0.3, 0.75 and 3 µg kg^{-1}, i.v.), in rats with (right panels) or without deoxycorticosterone (DOC) pretreatment (left panels), given 300 µg kg^{-1} Hoe 140 (solid lines) or vehicle (broken lines) subcutaneously. Each group ($n = 6$) received only one vasoconstrictor. Each dose of vasoconstrictor was given at 30 min intervals in random order except endothelin-1 which was injected at increasing doses. Mean blood pressure (MBP) returned to basal level prior to subsequent injection.

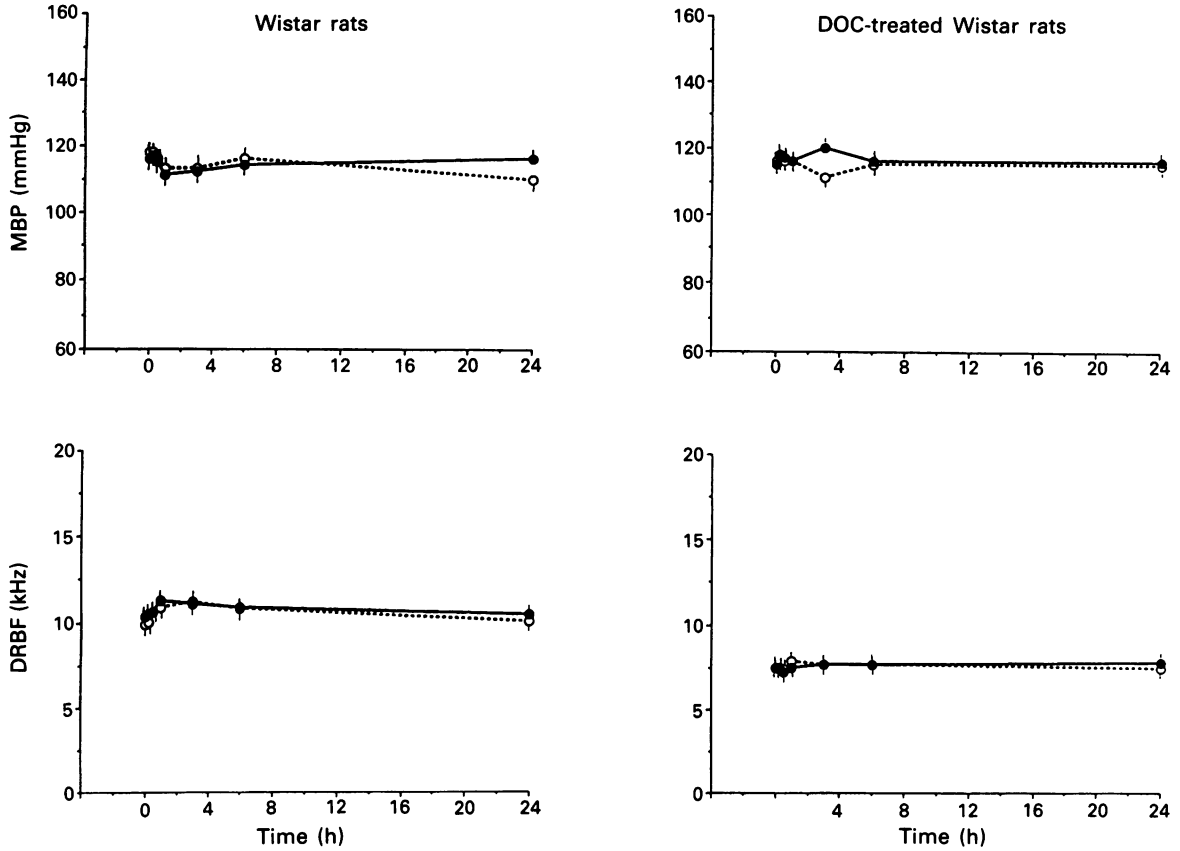

Figure 5 Effect of Hoe 140 (300 μg kg^{-1} four times a day, s.c., solid lines) or vehicle (broken lines) on mean blood pressure (MBP) and Doppler renal blood flow (DRBF) in rats with (right panels) and without (left panels) deoxycorticosterone (DOC)-pretreatment. Each group consisted of 6 rats.

Table 1 Effect of Hoe 140 on renal function in Wistar rats given deoxycorticosterone (DOC)-vehicle

	Control		*Experimental*	
Group treatment	*Vehicle*	*Vehicle*	*Vehicle*	*Hoe 140*
GFR (ml min^{-1})	2.9 ± 0.1	2.8 ± 0.1	2.8 ± 0.2	2.8 ± 0.1
UV (ml 24 h^{-1})	26.0 ± 2.1	25.2 ± 2.6	23.1 ± 1.1	22.5 ± 1.5
$U_{Na}V$ (mmol 24 h^{-1})	0.81 ± 0.13	0.75 ± 0.16	0.98 ± 0.17	1.08 ± 0.18
U_KV (mmol 24 h^{-1})	2.49 ± 0.24	2.65 ± 0.16	2.74 ± 0.16	3.17 ± 0.23
$U_{Osm}V$ (mosmol 24 h^{-1})	17.5 ± 1.6	16.8 ± 0.8	18.5 ± 0.5	18.7 ± 0.8

Two groups ($n = 9$ each) were studied: the control group received vehicle on both occasions, the experimental group received vehicle on the first occasion and Hoe 140 (300 μg kg^{-1} four times a day, s.c.) two days apart.

GFR, glomerular filtration rate; UV, urinary volume; $U_{Na}V$, urinary sodium excretion; U_kV, urinary potassium excretion; $U_{Osm}V$, urinary osmolal excretion.

alter MBP in normotensive rats. In addition, Hoe 140 did not affect MBP in rats given nonpressor or pressor doses of angiotensin II, phenylephrine or endothelin-1. Collectively, these results do not favour a major role for endogenous kinins in the regulation of arterial blood pressure in basal conditions in normotensive rats. However, kinins might modulate local and systemic vascular resistances in particular stimulated conditions (Weipert *et al.*, 1988; Berg *et al.*, 1989; Carbonell *et al.*, 1988b; Genden & Molineaux, 1991).

The present study was also undertaken to determine if endogenous kinins regulate renal haemodynamics and function in unanesthetized rats. Antagonists of the first generation reportedly decreased renal blood flow in dogs (Beierwaltes *et al.*, 1988) and rats (Madeddu *et al.*, 1990a). However, this effect was associated with stimulation of renin release (Beierwaltes *et al.*, 1988) and it was prevented by a cocktail of hormone receptor antagonists (Madeddu *et al.*, 1991), suggesting that it was caused by stimulation of neurohormonal reflexes rather than inhibition of endogenous kinins. The failure of Hoe 140 to affect renal blood flow in normotensive rats does not support a major role of kinins in the regulation of overall renal haemodynamics.

The finding that administration of Hoe 140 did not alter urinary volume, urinary sodium or potassium excretion over 24 h in normotensive, unanaesthetized rats is consistent with previous studies in which renal function was not affected by the administration of Fab fragments of kinin antibodies (Carretero & Scicli, 1991).

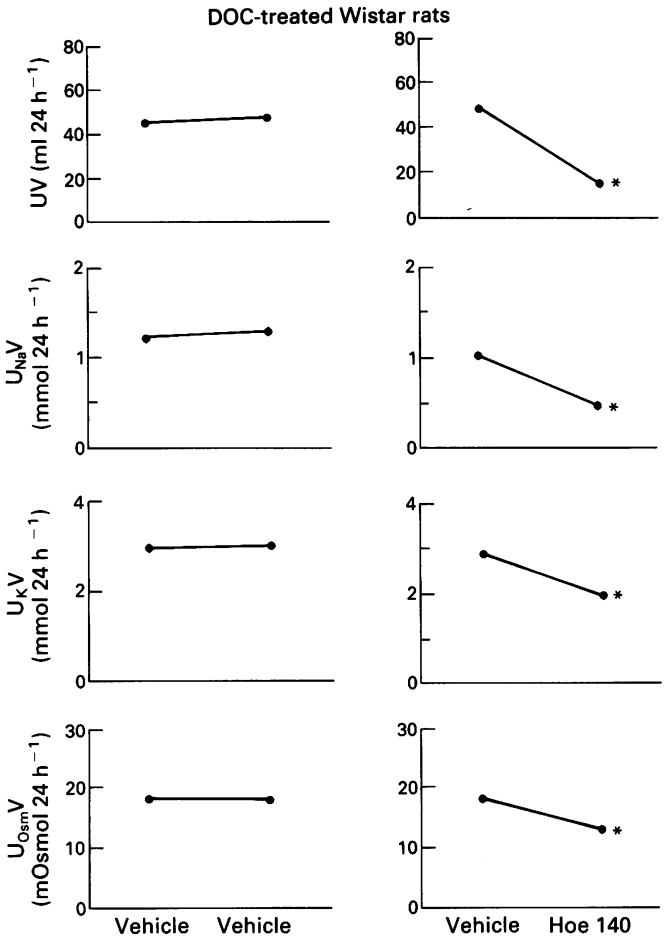

Figure 6 Effect of Hoe 140 (300 μg kg^{-1} four times a day, s.c.) or vehicle on urinary volume (UV), sodium ($U_{Na}V$), potassium (U_KV) and osmolal excretion ($U_{Osm}V$) in rats with deoxycorticosterone (DOC)-pretreatment. *$P<0.05$ *vs* vehicle.

By contrast, Hoe 140 reduced 24 h urinary volume by 70%, urinary sodium by 54%, and potassium excretion by 30% in DOC-treated rats. These results are consistent with a tubular effect of endogenous kinins since glomerular filtration rate was not modified and total renal blood flow remained unaltered. Inhibition of water and sodium reabsorption may have occurred at the level of the distal tubule where specific binding sites for bradykinin have been found (Tomita & Pisano, 1984).

Our study extends the observations of Tomiyama *et al.* (1991), who found that acute administration of the Fab fragments of kinin antibodies decreases urinary volume and sodium excretion without affecting blood pressure, renal blood flow, or glomerular filtration rate in rats given DOC acetate and salt. However, our study differs from that of Tomiyama regarding the longer duration of kinin inhibition and the fact that salt was not added to drinking water, a condition which could suppress renal kallikrein activity. By contrast, the antagonist D-Arg[Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin decreases urinary sodium excretion only when superimposed on the infusion of kininase inhibitors (Nakagawa *et al.*, 1990). Thus, failure of this antagonist to affect urinary sodium excretion in basal conditions may be attributable to its hydrolysis by kininases in the proximal tubule. Conversely, Hoe 140, which is excreted with urine in part as the intact compound in the rat (unpublished observations from Hoechst AG, Germany) possibly because of its resistance to kininases (Bond *et al.*, 1991), causes a decrease in urinary volume and sodium excretion. Collectively, these results suggest that kinins play a role in the regulation of electrolyte homeostasis by acting in the intratubular compartment of the kidney.

The finding that MBP of DOC-treated rats was similar to that of rats given DOC-vehicle is consistent with the observation that DOC alone does not induce sustained hypertension unless combined with chronic sodium loading and uninephrectomy. Activation of the renal kallikrein-kinin system by chronic administration of DOC (Geller *et al.*, 1972; Nakagawa *et al.*, 1991) may counteract the salt-retaining effect of DOC thus preventing the development of hypertension. Failure of Hoe 140, given over a period of 24 h, to alter MBP of DOC-treated rats does not rule out the possibility that the antidiuretic and antinatriuretic effect induced by inhibiting kinins causes fluid volume expansion leading over the long term to arterial hypertension.

In conclusion, our results indicate that endogenous kinins could play an important role in the regulation of water and sodium excretion by the kidney during chronic administration of DOC.

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