British Journal of Pharmacology (1998) 124, 197–205

http://www.stockton-press.co.uk/bjp

Effect of chronic bradykinin B_2 receptor blockade on blood pressure of conscious Dahl salt-resistant rats

[3]Hiroya Mukai, [1,4]Wayne R. Fitzgibbon, [1,2]David W. Ploth & Harry S. Margolius

Departments of Cell and Molecular Pharmacology and Experimental Therapeutics, and [1]Medicine, Medical University of South Carolina, and the [2]Ralph H. Johnson Veteran's Affairs Medical Center, Charleston, SC, U.S.A.

1 In this study 3 protocols were utilized to determine the role of endogenous kinins in the resistance of the inbred Dahl (Rapp) salt-resistant (SR/Jr) rats to high salt diet-induced blood pressure elevation.

2 The bradykinin B_2 receptor antagonist, Hoe 140 (D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8]-bradykinin) at doses of either 10–20 or 20–40 nmol day^{-1} (subcutaneously (s.c), via osmotic minipumps, for either 1 or 3 weeks during a high (8%) salt diet) effectively blocked or attenuated the hypotensive responses to 100–1000 ng of bradykinin.

3 In the first protocol, 5 week old SR/Jr rats treated with Hoe 140 (10–20 nmol day^{-1}, $n=9$, s.c., via osmotic minipumps) for 3 weeks and concomitantly fed high (8%) NaCl diet had significantly higher conscious tail cuff blood pressures (BPc) at 1 and 3 weeks when compared with rats treated with vehicle (0.9% NaCl, $n=6$). The differences in BPc between the 2 groups were 13 mmHg ($P<0.001$) after 1 week and 8 mmHg ($P<0.05$) after 3 weeks of treatment.

4 In the second protocol, 5 week old SR/Jr rats were treated with Hoe 140 (20–40 nmol day^{-1}, $n=8$, s.c., via osmotic minipumps) or vehicle ($n=8$) for 3 weeks. During the first week of treatment the rats were fed normal (0.8%) NaCl diet. The rats were then switched to 8% NaCl for 2 remaining weeks of the protocol. The mean BPc of Hoe 140-treated rats was not significantly different from that of the vehicle-treated rats when fed 0.8% NaCl diet. In contrast, rats treated with Hoe 140 and concomitantly fed high (8%) NaCl diet had significantly increased BPc (123 ± 2 vs 111 ± 1 mmHg, $P<0.001$ for the Hoe 140- and vehicle-treated rats, respectively).

5 In the third protocol, treatment with Hoe 140 (20–40 nmol day^{-1}, s.c., via osmotic minipumps) during high salt diet did not increase BPc in rats that were pre-exposed to the high salt diet for 2 weeks.

6 At the end of 3 weeks of study, blood pressure was measured via an arterial catheter during pentobarbitone-induced anaesthesia. Rats treated with Hoe 140 for 1 or 3 weeks had significantly lower mean arterial blood pressures than the vehicle-treated rats.

7 Our findings suggest that in SR/Jr rats, kinin activation of bradykinin B_2 receptors at least partially contributes to early regulatory mechanisms that resist an increase in blood pressure following exposure to a high salt diet. The mechanism underlying the decreased blood pressure during pentobarbitone anaesthesia of SR/Jr rats chronically treated with Hoe 140 has yet to be elucidated.

Keywords: Anaesthesia; blood pressure; bradykinin; Hoe 140; kinin antagonist; renal function; salt resistance

Introduction

The tissue kallikrein-kinin system participates in the regulation of cardiovascular function and blood pressure, and is probably involved in the pathogenesis of various forms of hypertension (Margolius, 1989; Carretero & Scicli, 1991; Bhoola *et al*., 1992). Kinins acting on local bradykinin B_2 receptors (Manning & Snyder, 1989; Fenoy *et al*., 1991; Lortie *et al*., 1992; Figueroa *et al*., 1995) could act as paracrine factors to regulate systemic blood pressure by increasing local vasodilatation or renal excretory function (Margolius, 1989; Carretero & Scicli, 1991; Vio *et al*., 1992). It appears that under most physiological conditions there is no or little tonic activity of the kallikrein-kinin systemic blood pressure (Bao *et al*., 1991; Madeddu *et al*., 1992; 1993) or renal excretory function (Roman *et al*., 1988; Fenoy *et al*., 1991; Mattson & Cowley, 1993). However, tissue kinins appear to contribute to the regulation of systemic blood pressure and/or renal function under conditions of altered salt intake (Majima *et al*., 1993; Siragy, 1993; Mukai *et al*., 1996; Alfie *et al*., 1996).

Studies utilizing potent, specific and long lasting (Hock *et al*., 1991; Wirth *et al*., 1991) bradykinin B_2 receptor antagonist, Hoe 140 (D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8]-bradykinin) or new 'knockout gene' techniques have partially clarified the role of the kallikrein-kinin system in the regulation of systemic blood pressure. Madeddu *et al*. (1993) showed that 6 weeks of treatment with Hoe 140 increased blood pressure in DOC-treated Wistar rats. Similarly, Hoe 140 treatment increased the blood pressure of Brown Norway Kitasato rats to hypertensive levels when they were fed a 2% salt diet – a level of dietary NaCl loading that did not increase the blood pressure of untreated Brown Norway Kitasato rats (Majima *et al*., 1993). Further, 1 or 2 weeks of 2% NaCl diet resulted in a marked increase in blood pressure in Brown Norway Katholiek rats of the Kitasato strain. This latter substrain of rats have barely detectable levels of circulating kininogens and of urinary kinin excretion. Thus, this substrain appears to be salt-sensitive because of the absence of, or attenuation of, kinin production (Majima *et al*., 1993). Recently, it was demonstrated that mice lacking the gene encoding the bradykinin B_2 receptor became hypertensive when placed on a high salt diet (Alfie *et al*., 1996). Taken together, the results of these studies suggest that the kallikrein-kinin system contributes to the defence of normoten-

[3] Present address: Cardiovascular and Renal Division, Teine Keijinkai Hospital, 1-2 Maeda, Teine-ku, Sapporo 006, Japan.
[4] Author for correspondence.

sion under conditions of hypertensive insult, especially high salt intake.

Inbred Dahl (Rapp) salt-resistant (SR/Jr) rats are known to resist the hypertensive stimulus of a high (8%) salt intake (Rapp, 1984; Sterzel *et al*., 1988). The mechanism that imparts this salt resistance is not known. In previous studies from this laboratory, we have shown that renal kallikrein synthesis rates, renal kallikrein levels and urinary excretion rates of kallikrein and kinins were significantly greater in 5–6 week old SR/Jr rats than in comparable inbred Dahl (Rapp) salt-sensitive (SS/Jr) rats (Rapp *et al*., 1978; Yamaji *et al*., 1988; Nishimura & Margolius, 1992). Further, renal kallikrein synthesis rates and renal kallikrein levels in SR/Jr rats were significantly greater than those observed for Sprague Dawley rats (Jaffa *et al*., 1987). These findings have led us to propose that kinins participate in the salt resistance of the SR/Jr rats. Thus, the purpose of the present study was to examine the hypothesis that endogenous kinins contribute at least in part to the salt resistance of SR/Jr rats. We tested this premise by measuring blood pressure and renal excretory function in response to a high salt diet in the absence or presence of chronic bradykinin B_2 receptor inhibition.

Methods

Animals

The experiments described in this manuscript were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with approval of our institutional Animal Research Committee. Four week old female Dahl SR/Jr rats ($n=74$) were obtained from Harlan Sprague Dawley (Indianapolis, IN) between January and November, 1993. The animals were housed in a dedicated room under the conditions of constant temperature (25°C) with a 12 h light/dark cycle and had free access to tap water and rat chow. Rats were housed in our animal facilities and fed regular rat chow (0.8% NaCl, Teklad, Madison, WI) for one week before the study.

Experimental protocols and procedures

Protocol 1: effects of Hoe 140 (10–20 nmol d^{-1}) on systolic blood pressure (BPc), heart rate (HR) and renal function in SR/Jr rats fed high (8%) NaCl diet for 3 weeks Five week old female SR/Jr ($n=15$) were implanted with an osmotic minipump (model 2001, Alza Corp., Palo Alto, CA) for the initial delivery of Hoe 140 or vehicle. Rats were weighed and then anaesthetized by intraperitoneal injection of 40 mg kg^{-1} pentobarbitone (Abbott Laboratories, North Chicago, IL). The back between the scapulae was shaved and a subcutaneous pocket was made through a skin incision. A minipump containing either the bradykinin B_2 receptor antagonist, Hoe 140 (10–14 nmol d^{-1} dissolved in 0.9% NaCl; gift from Hoechst AG, Frankfurt, Germany) ($n=9$) or vehicle ($n=6$) was placed in the pocket. The skin incision was then closed with wound clips. One week later, the rats were reweighed and the minipumps were replaced with new pumps (model 2002) as above to deliver 14–20 nmol d^{-1} for the next 2 weeks. All the procedures were performed under sterile conditions. The rats were fed an 8% NaCl diet (Teklad, Madison, WI) for the 3 weeks of treatment.

Protocol 2: effects of Hoe 140 (20–40 nmol d^{-1}) on BPc, HR and renal function in SR/Jr rats fed normal (0.8%) and then high (8%) NaCl diet for 2 weeks This protocol differs from the first in 2 aspects; we examined the effect of a higher dose of Hoe 140; and the rats were fed normal (0.8%) NaCl for the first week of treatment and then fed 8% NaCl for the following 2 weeks. Five week old female SR/Jr rats were implanted with minipumps as described above to deliver either Hoe 140 (20–40 nmol d^{-1}, $n=8$) or vehicle (0.9% Nacl, $n=8$).

Protocol 3: effects of Hoe 140 (20–40 nmol d^{-1}) on BPc, HR and renal function in SR/Jr rats prefed high (8%) NaCl diet for 2 weeks The effect of prior exposure to 8% NaCl diet before chronic blockade of bradykinin B_2 receptors was examined by use of the following protocol. Five week old female SR/Jr rats were fed 8% NaCl diet for 2 weeks. Then osmotic minipumps (model 2001) were inserted as described above to deliver Hoe 140 (20–40 nmol d^{-1}, $n=7$) or vehicle (0.9% NaCl, $n=14$). Both groups of rats received 0.2 ml of 5% dextrose in water, s.c., b.i.d. After recovery from the implantation surgery the rats were fed a 8% NaCl diet for one further week.

In each protocol, awake tail cuff blood pressure (BPc), heart rate and body weight measurements and renal clearance studies were performed as described below.

Blood pressure, heart rate and body weight On each of the last 3 days of each week (a baseline week and then the 3 experimental weeks after the commencement of either treatment with Hoe 140 or the high salt diet), the rats were gently placed in holders and warmed for about 15 min in a thermostatically controlled heating cabinet that suppled a continuous air flow at 25°C. Tail cuff blood pressure and HR were measured with tail plethysmography (KN-210, Natsume Corp., Tokyo, Japan). The first day of this measurement regime was used to acclimatize the rats and thus no data were recorded. On each of the other 2 days, BPc and HR were measured at least 15–20 times over a 20–30 min interval always keeping the last 10 measurements for analysis. The data from these 2 days were then averaged and the values taken as BPc and HR for the end of that week.

The rats were also weighed before the commencement of the experimental protocols and at the end of each experimental week.

Renal function studies At the end of each protocol, the rats were anaesthetized with pentobarbitone (50 mg kg^{-1}, i.p.), placed on a thermostatically-controlled heated table and maintained at 37°C. After tracheotomy, 3 polyethylene catheters were inserted into a jugular vein. Polyfructosan 10% (Inutest; Laevosan-Gesellschaft, Linz, Austria) and 2% *para*-aminohippurate (PAH; Merck, Sharp and Dohme, West Point, PA) in 0.9% NaCl were infused through one of the catheters and 0.9% NaCl through the second. The other catheter was used to deliver supplemental anaesthetic. The total infusion rate was 1.2 ml h^{-1} during the surgical preparation. The left femoral artery was cannulated for the measurement of blood pressure by use of a Statham pressure transducer and a polygraph (Grass Instrument Co., Quincy, MA). The urinary bladder was cannulated through an abdominal incision for collection of urine samples from the right kidney. The left kidney was exposed through a flank incision, carefully freed from the perirenal fat and supported in a lucite cup. The left ureter was then cannulated. Following completion of surgery, the polyfructosan and PAH solution and the 0.9% NaCl were each administered in priming volumes of 0.6 ml, followed by infusion rates of 0.6 ml h^{-1} (to give a total infusion rate of 1.2 ml h^{-1}). One hour was allowed to achieve steady state and then two, timed (30 min) urine collections were obtained.

Blood samples for measurement of plasma polyfructosan, PAH, Na^+ and haematocrit were taken between the 2 urine collection periods. At the end of the experiment, both kidneys were removed, blotted gently and weighed.

Protocol 4: effect of chronically infused Hoe 140 on the acute depressor response to exogenous bradykinin Five week old female SR/Jr rats ($n=22$) were implanted with osmotic minipumps (models 2001 and 2002, Alza Corp., Palo Alto, CA) as described above and treated with: (1) Hoe 140 (10–20 nmol d^{-1}, $n=7$), (2) Hoe 140 (20–40 nmol d^{-1}, $n=8$) and (3) vehicle (0.9% NaCl, $n=7$).

After implantation of the initial minipump, the rats were placed on the 8% NaCl diet. Rats from each treatment regime were divided into 2 groups and at either 1 and 3 weeks of treatment, decreases in mean arterial pressure (MAP) in response to a number of bolus injections of bradykinin (Sigma, St Louis, MO) were determined. On the day of study the rats were anaesthetized with pentobarbitone (50 mg kg^{-1}, i.p.) and a tracheotomy performed as described above. Either a jugular or a femoral vein was cannulated with PE-50 (Clay-Adams, Parsippany, NJ) for the infusion of 0.9% NaCl at 1.2 ml h^{-1} and PE-10 (Clay-Adams, Parsippany, NJ) for the infusion of supplemental administration of anaesthetic. Another PE-50 catheter was inserted into the left femoral artery and advanced into the abdominal aorta for direct measurement of blood pressure. A special catheter for the injection of bradykinin (PE-10 at the tip and PE-50 at the base) was inserted into the left carotid artery and advanced into the descending thoracic aorta. After an hour equilibration, the vasodepressor effects of bradykinin (100, 300, 500 and 1000 ng kg^{-1} in 50 μl of 0.9% NaCl) on the decrease in MAP were measured. Each dose of bradykinin was randomly injected twice, with 15 min between each of the eight injections.

Analytical procedures

Mean arterial pressure during anaesthesia (MAPa) was derived from direct arterial determination of systolic (SBP) and diastolic (DBP) blood pressures by use of the standard formula. Urine samples were collected under oil in preweighted containers and urine volumes were determined gravimetrically. Polyfructosan and PAH concentrations in plasma and urine were measured by modified colorimetric techniques (Smith *et al*., 1945; Fuhr *et al*., 1995). Glomerular filtration rate (GFR) and estimated renal plasma flow (ERPF) were determined from the clearance of polyfructosan and PAH, respectively. Plasma and urinary Na^+ concentrations were measured by flame photometry. Filtration fraction and urinary Na^+ excretion ($U_{Na}V$) were calculated from standard formulae.

Statistical analysis

The data are expressed as mean ± s.e. of mean. Tail-cuff BPc measurements for each treatment group were analysed by analysis of variance (ANOVA) for repeated measures. Differences in mean BPc, HR and clearance parameters between control and treatment groups were analysed by Student's unpaired *t*-test. Differences in the changes in MAPa following bradykinin injection were analysed by one-way ANOVA. Multiple comparisons of the means were subsequently tested by use of the Bonferroni/Dunn or Student-Newman-Keuls methods where appropriate. Analysis was performed with either Statview 512+ or SuperAnova (Abacus Concepts, Berkeley, CA). Differences were considered significant at $P<0.05$.

Results

Effect of chronic infusion of Hoe 140 (10–20 nmol d^{-1}) on the vasodepressor response to bradykinin

Chronic treatment with Hoe 140 for one week at a dose of 10–20 nmol d^{-1} markedly attenuated the vasodepressor action of bradykinin in SR/Jr rats fed 8% NaCl diet (Figure 1a). A similar or possibly enhanced degree of attenuation of the vasodepressor effect bradykinin was observed after 3 weeks of treatment (Figure 1b). The maximum attenuation was 80% at the lowest dose of bradykinin. Thus, chronic Hoe 140 treatment at 10–20 nmol d^{-1} markedly blocked vascular bradykinin B_2 receptors.

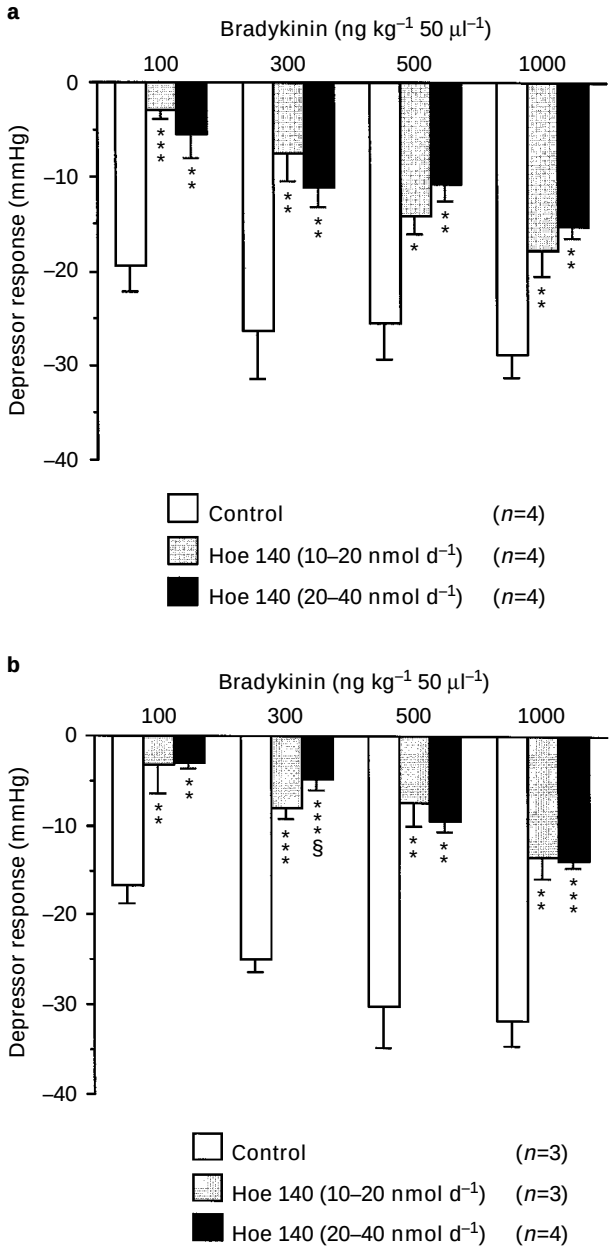

Figure 1 The depressor response to intra-arterial bradykinin in Dahl salt-resistant (SR/Jr) rats fed high (8%) NaCl diet with concomitant treatment with Hoe 140 or vehicle ($n=3-4$). Depressor responses were reduced after 1 (a) or 3 (b) weeks in rats treated with either 10–20 nmol d^{-1} or 20–40 nmol d^{-1} Hoe 140. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs vehicle-treated rats, §$P<0.05$ vs 10–20 nmol d^{-1} Hoe 140.

Protocol 1: effects of Hoe 140 (10–20 nmol d^{-1}) on BPc, HR and renal function in SR/Jr rats fed 8% NaCl for 3 weeks

Pretreatment BPc did not differ between the 2 groups (Figure 2). The BPc of vehicle-treated rats was not affected by 1 week of 8% NaCl (109±1 vs 107±2 mmHg), but was significantly elevated after 2 (118±1 mmHg, $P<0.001$) and 3 weeks (117±2 mmHg, $P<0.001$) of the 8% NaCl (Figure 2). In contrast, BPc of Hoe 140-treated rats increased significantly ($P<0.001$) after 1 week compared with pretreatment BPc (122±2 vs 104±2 mmHg), and remained elevated after 2 (122±2 mmHg, $P<0.001$) and 3 weeks (125±2 mmHg, $P<0.001$). BPc of Hoe 140-treated rats was significantly higher than that of vehicle-treated rats at the end of the first (122±2 vs 109±1 mmHg, $P<0.001$) and third weeks (125±2 vs 117±2 mmHg, $P<0.05$) of treatment. Thus, Hoe 140 promptly increased BPc of SR/Jr rats fed 8% NaCl by 13 mmHg.

For both groups, HR did not change over the 3 weeks of the study and did not differ between the groups at any time during the study. The mean HRs after 3 weeks of treatment were 429±31 and 411±8 beats min^{-1} for the Hoe 140 and vehicle-treated rats, respectively.

Chronic treatment with Hoe 140 did not alter the growth rate of the rats over the 3 weeks of study. The absolute increases in body weight were 70.4±2.4 g and 69.3±2.8 g for the Hoe 140 and vehicle-treated rats, respectively. The percentage increases in body weight were: Hoe 140-treated, 83.9±5.4%; vehicle-treated group, 87.5±4.8%.

At the end of 3 weeks of treatment, renal clearance data were obtained from five of the Hoe-treated and five of the vehicle-treated rats. Haemodynamic and excretory function were similar between the left and right kidneys for each animal. Consequently, in this and the subsequent protocols, only data from the left kidney are presented. SR/Jr rats treated with Hoe 140 had lower GFR ($P<0.001$) than vehicle-treated rats (Table 1). There were no significant differences in the other functional parameters between the 2 groups.

We also examined whether a higher dose of Hoe 140 would provide a complete blockade of vascular bradykinin B_2 receptors and whether chronic blockade of these receptors by the higher dose of Hoe 140 would induce a larger increase in blood pressure.

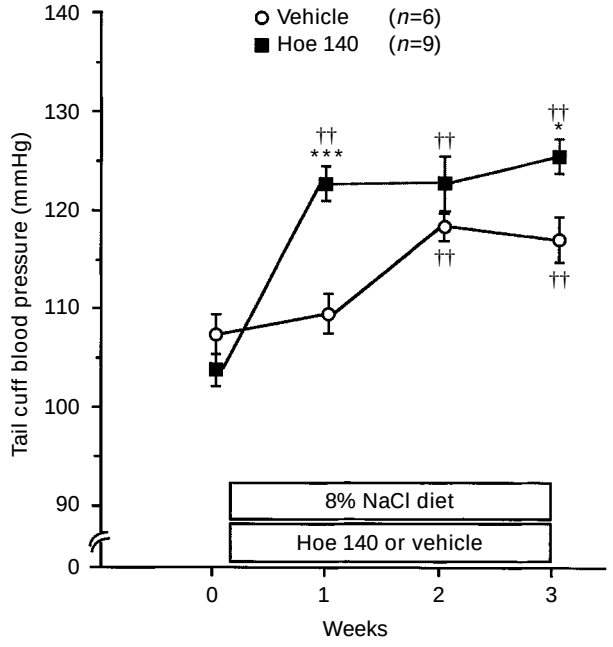

Figure 2 Systolic blood pressure of awake Dahl salt-resistant (SR/Jr) rats before and during high (8%) NaCl diet with concomitant treatment (via osmotic minipumps) with 10–20 nmol d^{-1} Hoe 140 or vehicle (Protocol 1). The kinin B_2 receptor antagonist increased blood pressure after one week of 8% NaCl diet. ††$P<0.001$ vs baseline BPc, *$P<0.05$, ***$P<0.001$ vs vehicle-treated rats.

Effect of chronic infusion of Hoe 140 (20–40 nmol d^{-1}) on the vasodepressor response to bradykinin

Although chronic treatment with the higher dose of Hoe 140 also markedly attenuated the vasodepressor action of bradykinin (Figure 1), the degree of blockade was similar to that for the lower dose of the antagonist. Nevertheless, we decided to explore the possibility that this high dose of the antagonist might produce more substantial changes in blood pressure and renal function of SR/Jr rats fed a high salt diet.

Protocol 2: effects of Hoe 140 (20–40 nmol d^{-1}) on BPc, HR and renal function in SR/Jr rats fed 0.8%/8% NaCl

Pretreatment BPc did not differ between the 2 groups (Figure 3). Treatment with the higher dose of Hoe 140 did not induce a significant increase in BPc when the rats were fed normal (0.8%) NaCl for one week (Figure 3). However, after the Hoe 140-treated rats were fed 8% NaCl diet for one week, BPc was significantly increased above the pretreatment level (123±2 vs 103±3 mmHg, $P<0.001$, for 2 and 0 weeks, respectively) and remained elevated above the pretreatment value following a further week of 8% NaCl diet (123±3 mmHg, $P<0.001$). In contrast to the first protocol, the BPc of the vehicle-treated rats did not change significantly during the 3 weeks of study. The BPc of Hoe 140-treated rats was significantly higher than that of the vehicle-treated rats after one (123±2 vs 111±1 mmHg, $P<0.001$) and 2 (123±3 vs 111±1 mmHg, $P<0.001$) weeks of the 8% NaCl diet.

As also observed in the first protocol, neither Hoe 140 treatment or diet significantly altered HR. Chronic treatment with Hoe 140 did not alter the growth rate of the rats over the 3 weeks of study. The absolute increases in body weight above the pretreatment value at the end of the first week (0.8% NaCl) were 21.8±2.9 g and 20.3±2.7 g for the vehicle- and Hoe 140-treated rats, respectively. The absolute (and percentage) increases in body weight above the pretreatment value at the end of the third week (following 2 weeks of the high salt diet)

Table 1 Renal function in SR/Jr rats treated with Hoe 140 (10–20 nmol day^{-1}) or vehicle while concomitantly fed 8% NaCl for 3 weeks (Protocol 1)

Parameter	*Vehicle* (*n*=5)	*Hoe 140* (*n*=5)
Urine flow rate (μl min^{-1} g^{-1} kwt)	11.7±2.3	14.0±1.9
Sodium excretion (nmol min^{-1} g^{-1} kwt)	2401±507	2025±256
Glomerular filtration rate (μl min^{-1} g^{-1} kwt)	883±18	647±31***
Estimated renal plasma flow (ml min^{-1} g^{-1} kwt)	2.84±0.40	2.29±0.11
Filtration fraction	0.33±0.04	0.28±0.01
Kidney wt (kwt) (g)	0.88±0.04	0.93±0.01

*** $P<0.001$.

were 53.9±2.6 g (51.6±4.4%) and 54.5±2.9 g (52.6±4.5%) for the vehicle- and Hoe 140-treated rats, respectively.

The renal haemodynamic and excretory function data obtained at the end of the 3 weeks of treatment are presented in Table 2. SR/Jr rats treated with Hoe 140 had a lower mean urine flow rate ($P<0.05$) than vehicle-treated rats (Table 2). There were no significant differences in the other functional parameters between the 2 groups.

Protocols 1-2: effect of anaesthesia on blood pressure of rats chronically treated with Hoe 140

We examined the effect of pentobarbitone anaesthesia on blood pressure of the Hoe 140-treated rats used in the renal clearance experiments, by comparing tail cuff blood pressure data obtained at the end of the third study week and mean arterial pressure during anaesthesia (MAPa). Since similar responses to concomitant Hoe 140 treatment during the high salt diet were observed in both protocols 1 and 2, the BPc data obtained on the last day of the 2 protocols were combined and are presented in Figure 4a. Similarly, MAPa data from the 2 protocols were combined and are presented in Figure 4b. As noted above for the individual protocols, rats treated with Hoe 140 for 3 weeks had significantly higher BP measured by tail plethysmography than those that received vehicle (124±2 vs 113±1 mmHg, $P<0.01$, $n=13$ and 12 for the Hoe 140 and vehicle-treated groups, respectively). In contrast, during pentobarbitone anaesthesia, MAP of the Hoe 140-treated rats was significantly lower than that determined for the control rats (102±2 vs 108±1 mmHg, $P<0.05$).

Figure 3 Systolic blood pressure of awake Dahl salt-resistant (SR/Jr) rats fed normal (0.8%) NaCl diet for one week and high (8%) NaCl diet for 2 weeks. The rats were concomitantly treated with 20–40 nmol d^{-1} Hoe 140 or vehicle during the 3 weeks of study (Protocol 2). The kinin B_2 receptor antagonist increased blood pressure only after the rats were fed the high NaCl diet. ††$P<0.001$ vs baseline BPc, **$P<0.01$ vs control rats.

Table 2 Renal function in SR/Jr rats treated with Hoe 140 (20–40 nmol day^{-1}) or vehicle while concomitantly fed 0.8% NaCl for 1 week and then 8% NaCl for 2 weeks (Protocol 2)

Parameter	*Vehicle* (*n*=8)	*Hoe 140* (*n*=8)
Urine flow rate ($\mu l\ min^{-1}\ g^{-1}$ kwt)	8.7±1.7	5.2±0.6*
Sodium excretion (nmol $min^{-1}\ g^{-1}$ kwt)	1660±269	1064±235
Glomerular filtration rate ($\mu l\ min^{-1}\ g^{-1}$ kwt)	857±72	853±54
Estimated renal plasma flow (ml $min^{-1}\ g^{-1}$ kwt)	3.59±0.55	3.65±0.53
Filtration fraction	0.30±0.04	0.26±0.03
Kidney wt (kwt) (g)	0.82±0.02	0.79±0.02

* $P<0.05$

Figure 4 Effect of anaesthesia on the blood pressure of rats chronically treated with Hoe 140 before or comcomitantly with being fed a high salt diet. Data from animals used in Protocols 1 and 2 were combined and are presented in this figure. In (a) mean tail cuff blood pressures (BPc) are presented for rats treated with Hoe 140 or vehicle; MAP were also obtained by direct measurement during anaesthesia with pentobarbitone (b). Despite a significantly higher blood pressure when conscious, during anaesthesia, the blood pressure of rats chronically treated with Hoe 140 was significantly lower than that obtained for control rats. $n=13$ and 12 for the Hoe 140 and vehicle-treated groups, respectively.

Protocol 3: effects of Hoe 140 (20–40 nmol d^{-1}) on BPc, HR and renal function in SR/Jr rats prefed high (8%) NaCl diet for 2 weeks

In the first 2 protocols, we observed that Hoe 140 treatment induced a significant increase in BPc after the rats had been switched from a 0.8% to a 8% NaCl for one week. In this protocol we sought to examine the effect of pre-exposure to the high salt diet on the blood pressure response to chronic bradykinin B_2 receptor blockade. There was no difference in BPc between the 2 groups after 2 weeks of the high salt intake (113 ± 1 vs 111 ± 4 mmHg for the vehicle and Hoe groups, respectively, Figure 5). In contrast to the first 2 protocols, in SR/Jr rats pre-exposed to 8% NaCl diet, treatment with Hoe 140 for one week did not increase BPc of rats concomitantly fed the high salt diet (120 ± 2 vs 111 ± 4 mmHg, week 3 and week 2, respectively, $P > 0.05$, Figure 5).

At the end of the 3 week study, renal clearance data were obtained from five of the Hoe-treated and eight of the vehicle-treated rats. The data are presented in Table 3. There were no significant differences in renal haemodynamic and excretory function between the 2 groups.

Table 3 Renal function in SR/Jr rats fed 8% NaCl for 2 weeks and then treated with Hoe 140 (20–40 nmol day^{-1}) or vehicle while concomitantly fed 8% NaCl for 1 further week (Protocol 3)

Parameter	*Vehicle* ($n=8$)	*Hoe 140* ($n=5$)
Urine flow rate (μl min^{-1} g^{-1} kwt)	19.4 ± 3.7	10.6 ± 1.8
Sodium excretion (nmol min^{-1} g^{-1} kwt)	2904 ± 676	1431 ± 445
Glomerular filtration rate (μl min^{-1} g^{-1} kwt)	622 ± 87	550 ± 98
Estimated renal plasma flow (ml min^{-1} g^{-1} kwt)	2.64 ± 0.20	2.38 ± 0.29
Filtration fraction	0.24 ± 0.03	0.23 ± 0.02
Kidney wt (kwt) (g)	0.90 ± 0.03	0.98 ± 0.04

Protocol 3: effect of anaesthesia on blood pressure of rats chronically treated with Hoe 140

We again examined the effect of pentobarbitone anaesthesia on blood pressure of rats used in the renal clearance experiments for protocol 3, by comparing mean tail cuff blood pressures obtained at the end of the third study week, and mean arterial pressures obtained during anaesthesia (MAPa). BPc data are presented in Figure 6a and the MAPa data are presented in Figure 6b. As noted above (as with the complete tail cuff data for this protocol), rats treated with Hoe 140 after pre-exposure to the high salt diet had similar mean tail cuff BP as vehicle-

Figure 5 Systolic blood pressure of awake Dahl salt-resistant (SR/Jr) rats fed the high salt diet. The rats were concomitantly treated with 20–40 nmol d^{-1} Hoe 140 or vehicle during the last week of the study (Protocol 3). The kinin B_2 receptor antagonist did not increase blood pressure when given subsequent to the high NaCl diet. †$P < 0.01$ vs baseline BPc.

Figure 6 Effect of anaesthesia on the blood pressure of rats chronically treated with Hoe 140 during a high salt diet. In (a), mean tail cuff blood pressures (BPc) are presented for rats treated with Hoe 140 or vehicle; MAP pressures were also obtained by direct measurement during anaesthesia with pentobarbitone (b). There was no significant difference in blood pressure between awake animals from the 2 groups. In contrast, during anaesthesia, the blood pressure of rats chronically treated with Hoe 140 was significantly lower than that obtained for control rats. $n=5$ and 8 for the Hoe 140 and vehicle-treated groups, respectively.

treated rats (119 ± 2 vs 115 ± 2 mmHg, $P > 0.05$, for the Hoe 140 ($n = 5$) and control ($n = 8$) groups, respectively). Interestingly, during pentobarbitone anaesthesia, MAPa of the Hoe 140-treated rats was significantly lower than that determined for the control rats (92 ± 2 vs 101 ± 2 mmHg, $P < 0.05$).

Discussion

The results of the present study show that the blood pressure of young Dahl/Rapp salt resistant rats (SR/Jr) is significantly increased by a high salt diet during concomitant chronic blockade of bradykinin B_2 receptors. This observation supports the hypothesis that endogenous kinins contribute at least in part to salt resistance in this strain. However, the finding that blockade of bradykinin B_2 receptors does not elevate blood pressure in SR/Jr rats pre-exposed to the high salt indicates that bradykinin B_2 receptor activation may contribute only to the early physiological response to high dietary salt intake. Further, pentobarbitone anaesthesia decreased the blood pressure of SR/Jr rats fed high salt diet and concomitantly treated with the bradykinin B_2 receptor antagonist, Hoe 140. To our knowledge this is the first time an effect of pentobarbitone anaesthesia on the blood pressure of rats undergoing chronic blockade of bradykinin B_2 receptors has been described. This finding suggests that there is a complex interaction between the kallikrein-kinin system and the central nervous system in the regulation of blood pressure under conditions of high dietary salt intake.

We investigated the effect of concomitant bradykinin B_2 receptor antagonism and high dietary salt intake in young (5–8 week old) SR/Jr rats. A possible confounding problem with examining the effect of treatment on blood pressure in young rats is an age and/or growth-related increase in BP independent of treatment or salt intake. Blood pressure increased from 7 to 9 weeks of age in Wistar rats fed high salt (Madeddu *et al*., 1995), from 6 to 8 weeks of age in outbred Dahl salt-resistant rats fed either a normal or high salt diet (Sterzel *et al*., 1988) and from 4 to 6 weeks of age in SR/Jr rats fed either a low or high salt diet (Rapp, 1984). In the present study, we observed an age and/or growth-related effect on BP. The tail-cuff blood pressures of the young SR/Jr rats in the vehicle-treated groups were slightly but significantly increased after 2 to 3 weeks of high salt intake. To minimize the confounding effects of age and/or growth in the present study, the vehicle-, and Hoe 140-treated rats were age-, and weight-matched at the start of each protocol. Further, for each appropriate variable, comparisons were not only made between the post-treatment and pretreatment means but also between the Hoe 140-treated and control groups at each weekly time point. Also, the animals were weighed at each time point and the growth rates for the experimental and the control groups determined. Since Hoe 140 treatment during high salt intake did not alter growth rate, it is unlikely that the effect of Hoe 140 on BP was due to a difference in growth rates between the experimental and control groups.

Recently, a number of investigators have compared differences in blood pressure between control and experimental groups obtained using tail plethysmography with those obtained in the same conscious rats by direct measurement of MAP (Majima *et al*., 1993; Madeddu *et al*., 1994; 1995). These investigators showed that similar effects of Hoe 140 on BP were observed with either technique. Experience in our laboratory suggests that, using our procedures, there was almost no difference in tail cuff blood pressure measurement between observers (Imamura & Fitzgibbon, unpublished observations), and there was a high degree of correlation between tail cuff and direct blood pressure measurements in conscious rats (Imamura, unpublished results). However, from these latter measurements we concluded that blood pressure measured by tail plethysmography did not equal systolic blood pressure determined directly. Further, similar differences in blood pressure between the same treatment groups were obtained from both tail plethysmography, when the rats were conscious, and direct measurement of mean arterial blood pressure during anaesthesia (Fitzgibbon *et al*., 1993; Imamura *et al*., 1995). Thus, we decided that during pentobarbitone anaesthesia, mean arterial blood pressure was the best parameter to use when testing the effect of treatment on blood pressure. We have no evidence to suggest that the differences in tail cuff blood pressure observed between the vehicle- and Hoe-treated rats in the present study represent anything other than actual differences in blood pressure.

We examined the effect of long-term treatment with Hoe 140 at doses lower than those used in previous studies ($10-40$ nmol day^{-1} compared to 75 nmol kg^{-1} day^{-1} or 5 mmol kg^{-1} day^{-1}). To characterize fully the effectiveness of bradykinin receptor blockade at these lower doses of Hoe 140, the hypotensive response to a range of bradykinin doses was measured under anaesthesia, following 1 or 3 weeks of Hoe 140 treatment with concomitant 8% salt diet. The effectiveness of bradykinin blockade did not differ between treatment regimes of either $10-20$ or $20-40$ nmol day^{-1} Hoe 140. We observed almost complete blockade of the hypotensive response to 100 ng kg^{-1} bradykinin and marked attenuation of the hypotensive response to bradykinin doses of $500-1000$ ng kg^{-1}. The findings suggest that at the doses used in the present study, Hoe 140 substantially blocked systemic bradykinin B_2 receptors. In contrast to our findings, in a series of studies in conscious rats, Madeddu and coworkers have shown that under control and experimental conditions long-term treatment with Hoe 140, at a dose of 75 nmol day^{-1}, i.p., completely abolished the hypotensive response to a bolus intra-arterial injection of 0.85 nmol ($\sim$1000 ng) kg^{-1} bradykinin (Madeddu *et al*., 1993; 1994; 1995). Taken together, these findings suggest that Hoe 140 may have only partially blocked bradykinin B_2 receptors in the present study.

Despite the possibility that only partial blockade of bradykinin receptors was achieved, SR/Jr rats treated with low dose Hoe 140 and concomitantly fed high salt (or treated with the receptor antagonist before being fed high salt) had significantly elevated tail cuff blood pressures compared to vehicle-treated rats. This finding suggests that kinins contribute to the maintenance of blood pressure of young SR/Jr rats during high salt intake. A role for endogenous kinins in the maintenance of blood pressure during high salt intake has been supported by a number of previous findings. Mice lacking the gene encoding the bradykinin B_2 receptor became hypertensive when placed on a high salt diet (Alfie *et al*., 1996). Brown Norway Katholiek rats (which have either no, or attenuated kinin production) have a marked increase in blood pressure with a 2% NaCl diet (Majima *et al*., 1993). Further, treatment with a very high dose of Hoe 140 (5 mmol kg^{-1} day^{-1}) increased BP of Brown Norway Kitasato rats fed a 2% salt diet to hypertensive levels (Majima *et al*., 1993). In contrast to the observations of Majima *et al*. (1993) and of the present study, chronic administration of Hoe 140 (at a dose lower than that used by Majima *et al*. (1993) but higher than that used in the present study) did not alter the BP of Wistar rats fed a high salt intake (Madeddu *et al*., 1995). Despite the inconsistency between the studies which used Hoe 140, taken

together, the findings from the present and previous studies support the proposal that the kallikrein-kinin system contributes to the defence of normotension during high salt intake.

High salt intake suppresses the renal kallikrein-kinin system (Yamaji *et al*., 1988; Nishimura & Margolius, 1992). This suppression is rapid, with interstitial levels of kinins markedly reduced within 24 h of presentation of a high salt diet (Siragy *et al*., 1994). Expression of mRNA for kallikrein has been shown to be downregulated by high salt intake in kidney, liver and salivary glands (Wang *et al*., 1996). This finding suggests that tissue kallikrein-kinin levels are suppressed by high salt intake. Thus, there is an apparent paradox between suppression of kallikrein-kinin levels by high salt intake and the contribution of the kallikrein-kinin system to the defence of normotension during high salt intake. Blood pressure homeostasis involves a complex interaction of humoral and neuronal vasoactive mechanisms. Some of these regulatory mechanisms play minor roles in blood pressure homeostasis under basal conditions but became important under conditions involving an insult to BP. Under both basal and stimulated conditions the loss of one vasoactive mechanism appears to be compensated by increased activity of at least one of the other regulatory mechanisms. Further, these humoral and neuronal vasoactive mechanisms have different time courses in response to an insult. Some of the regulatory systems act immediately after a challenge to maintain BP at normotensive levels, some act in the intermediate term, while others are slower to intitiate and act to maintain BP over the long term (Guyton *et al*., 1990). Some of the early responses may also act to initiate the later responses. In the present study, although SR/Jr rats treated with low dose Hoe 140 and concomitantly fed high salt (or treated with the receptor antagonist before being fed high salt) had significantly elevated BP, blockade of bradykinin B_2 receptors did not increase the BP of SR/Jr rats pre-exposed to the high salt diet. From this finding and from the previous findings of suppression of the kallikrein-kinin system by high salt intake, we conclude that kinins appear to contribute to an early physiological response to maintain BP at normotensive levels during high dietary salt intake. Kininogen expression (Wang *et al*., 1996) and urinary kallikrein activity (Emond *et al*., 1989) are upregulated by high salt intake while bradykinin B_2 receptor density is unchanged by high salt intake (Emond *et al*., 1989). Thus, it is possible that although kallikrein and kinin levels are downregulated, activity of the kallikrein-kinin system would be maintained after chronic exposure to high salt intake. However, the finding that treatment with Hoe 140 following pre-exposure to high salt does not increase BP argues against a role for kinins in the later responses in the defence of normotension.

We observed that during pentobarbitone anaesthesia, MAP of Hoe 140-treated rats was significantly lower than that of vehicle-treated rats. This effect of pentobarbitone on MAP occurred in groups of Hoe 140-treated rats that had conscious tail cuff BPs significantly higher than vehicle-treated rats or that had conscious tail cuff BPs not significantly different from vehicle-treated rats. This effect appears to be unique to Hoe 140-treated SR/Jr rats fed high salt as we know of no other study that has observed a similar interaction between chronic Hoe 140 treatment and anaesthesia in other strains of rats. The decreases in sympathetic outflow and blood pressure induced by pentobarbitone (Matsukawa *et al*., 1993) are most probably due to a decrease in the tonic activity of the central vasomotor center (Matsukawa & Ninomiya, 1989). Our finding that during pentobarbitone anaesthesia, MAP of Hoe 140-treated rats was significantly lower than that of vehicle-treated rats supports the possibility that there may be an increase in the effect of pentobarbitone on the role of the central vasomotor centre in the tonic control of blood pressure in SR/Jr rats treated with Hoe 140 and concomitantly fed high salt. Thus, blockade of bradykinin B_2 receptors appears to be associated with an increased role of the central nervous system in regulation of BP. It is unlikely that Hoe 140 penetrates the blood-brain barrier, so this association between blockade of bradykinin B_2 receptors and increased central vasomotor centre activity does not appear due to be a direct action of Hoe 140.

Kinins are vasodilatory, diuretic and natriuretic (Carretero & Scili, 1991). When fed a high salt diet, mice lacking the gene coding for the bradykinin B_2 receptor have higher BP and lower renal blood flow than control mice (Alfie *et al*., 1996). This finding is consistent with the proposal that kinin-mediated regulation of renal blood flow and renal excretory function contribute to the maintenance of BP following high salt intake. At the end of the present study, we measured renal plasma flow and excretory function during anaesthesia. In contrast to the study with bradykinin B_2 receptor knockout mice, we did not observe decreased renal haemodynamics in SR/Jr rats chronically treated with Hoe 140 and concomitantly fed a high salt diet. Further, we did not observe consistent decreases in renal excretory function for the Hoe 140-treated rats. In Protocol 1, only GFR and in Protocol 2, only urine flow rate were significantly lower, while in Protocol 3 urinary sodium excretion tended to be but was not significantly lower in Hoe 140- compared to vehicle-treated rats. Since MAP was lower in Hoe 140- compared to vehicle-treated rats during anaesthesia it is difficult to interpret these inconsistent differences in excretory function. Therefore, the present findings do not support the proposal that chronic blockade of kinin receptors results in decreased renal haemodynamic and excretory function of SR/Jr rats fed a high salt diet. Further, decreased renal haemodynamic and excretory function does not appear to contribute to the maintenance of a higher conscious BP in these Hoe 140-treated rats. However, the present findings do not rule out the possibility that decreased renal haemodynamic and excretory function contributed to the initial elevation of BP in the Hoe 140-treated rats fed a high salt diet.

In conclusion, during kinin receptor blockade, rats bred for resistance to salt induced-hypertension have an elevation of blood pressure in response to high salt diet. This finding supports the hypothesis that endogenous kinins contribute, at least in part, to the resistance to salt induced-hypertension by SR/Jr rats.

These studies were supported by grants from the National Institute of Health (HL-17705, HL-44671), Bethesda, MD and research funds provided by Dialysis Clinics Inc. We express our gratitude to Emma S. Murner and Phillip W. Yates for their expert technical assistance.

References

ALFIE, M.E., SIGMON, D.H. & CARRETERO, O.A. (1996). Bradykinin B_2 receptor knockout mouse: effect of high sodium intake. *J. Am. Soc. Neph.,* **7,** 1530 (Abst).

BAO, G., QUADRI, F., STAUSS, B., STAUSS, H., GHOLKE, P. & UNGER, T. (1991). Hoe 140, a new highly potent and long-acting bradykinin antagonist in conscious rats. *Eur. J. Pharmacol.,* **200,** 179–182.

BHOOLA, K.D., FIGUEROA, C.D. & WORTHY, K. (1992). Bioregulation of kinins: kallikreins, kininogens, and kininases. *Pharmacol. Rev.,* **44,** 1–80.

CARRETERO, O.A. & SCILI, A.G. (1991). Local hormonal factors (intracrine, autocrine, and paracrine) in hypertension. *Hypertension,* **18** (suppl I), I-58–I-69.

EMOND, C., BASCANDS, J.P., CABOS-BOUTOT, G., PECHER, C. & GIROLAMI, J.P. (1989). Effect of changes in sodium or water intake on glomerular B_2-kinin-binding sites. *Am. J. Physiol.,* **257,** F353–F358.

FENOY, F.J., SCILI, A.G., CARRETERO, O.A. & ROMAN, R.J. (1991). Effect of an angiotensin II and a kinin receptor antagonist on the renal hemodynamic response to captopril. *Hypertension,* **17,** 1038–1044.

FIGUEROA, C.D., GONZALEZ, C.B., GRIGORIEV, S., ALLA, S.A., HAASEMANN, M., JARNAGIN, K. & MÜLLER-ESTERL, W. (1995). Probing for the bradykinin B_2 receptor in rat kidney by anti-peptide and anti-ligand antibodies. *J. Histochem. Cytochem.,* **43,** 137–148.

FITZGIBBON, W.R., MARGOLIUS, H.S. & PLOTH, D.W. (1993). Effect of acute saline expansion on renal vasodilation capacity in Dahl salt resistant (SR/Jr) rats resistant to 2-kidney, 1 clip hypertension (2-K, 1C HT). *J. Am. Soc. Nephrol.,* **4,** 510 (Abst.).

FUHR, J., KACZMARCZYK, J. & KRUTTGEN, C.D. (1995). Eine einfache colorimetrische methode zur inulin-bestimmung fur nieren-clearance-untersuchungen bei stoffwecksel-gesunden und diabetiken. *Klin. Wochenschr.,* **33,** 729–730.

GUYTON, A.C., HALL, J.E., COLEMAN, T.G. & MANNING, R.D. JR. (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. pp. 1029–1052. New York: Raven Press Ltd.

HOCK, F.J., WIRTH, K., ALBUS, U., LINZ, W., GERHARDS, H.J., WIEMER, G., HENKE, ST., BREIPOHL, G., KÖNIG, W., KNOLLE, J. & SCHÖLKENS, B.A. (1991). HOE 140 a new potent and long acting bradykinin-antagonist: *in vitro* studies. *Br. J. Pharmacol.,* **102,** 769–773.

IMAMURA, A.K., MACKENZIE, H.S., LACY, E.R., HUTCHISON, F.N., FITZGIBBON, W.R. & PLOTH, D.W. (1995). Effects of chronic treatment with angiotensin converting enzyme inhibitor or an angiotensin receptor antagonist in two-kidney, one-clip hypertensive rats. *Kidney Int.,* **47,** 1394–1402.

JAFFA, A.A., MILLER, D.H., BAILEY, G.S., CHAO, J., MARGOLIUS, H.S. & MAYFIELD, R.K. (1987). Abnormal regulation of renal kallikrein in experimental diabetes and the effects of insulin on prokallikrein synthesis, activation and excretion. *J. Clin. Invest.,* **80,** 1651–1659.

LORTIE, M., REGOLI, D., RHALEB, N.E. & PLANTE, G.E. (1992). The role of B_1- and B_2- kinin receptors in the renal tubular and hemodynamic response to bradykinin. *Am. J. Physiol.,* **262,** R72–R76.

MADEDDU, P., ANANIA, V., PARPAGLIA, P.P., DEMONTIS, M.P., VARONI, M.V., PISANU, G., TROFFA, C., TONOLO, G. & GLORIOSO, N. (1992). Effects of HOE 140, a bradykinin B_2-receptor antagonist, on renal function in conscious normotensive rats. *Br. J. Pharmacol.,* **106,** 380–386.

MADEDDU, P., ANANIA, V., PARPAGLIA, P.P., DEMONTIS, M.P., VARONI, M.V., FATTACCIO, M.C. & GLORIOSO, N. (1993). Chronic kinin receptor blockade induces hypertension in deoxycorticosterone-treated rats. *Br. J. Pharmacol.,* **108,** 651–657.

MADEDDU, P., PARPAGLIA, P.P., DEMONTIS, M.P., VARONI, M.V., FATTACCIO, M.C. & GLORIOSO, N. (1994). Chronic inhibition of bradykinin B_2-receptors enhances the slow vasopressor response to angiotensin II. *Hypertension,* **23,** 646–652.

MADEDDU, P., PARPAGLIA, P.P., DEMONTIS, M.P., VARONI, M.V., FATTACCIO, M.C., ANANIA, V. & GLORIOSO, N. (1995). Early blockade of bradykinin B_2-receptors alters the adult cardiovascular phenotype in rats. *Hypertension,* **25,** 453–459.

MAJIMA, M., YOSHIDA, O., MIHARA, H., MUTO, T., MIZOGAMI, S., KURIBAYASHI, Y., KATORI, M. & OH-ISHI, S. (1993). High sensitivity to salt in kininogen-deficient Brown Norway Katholiek rats. *Hypertension,* **22,** 705–714.

MANNING, D.C. & SNYDER, S.H. (1989). Bradykinin receptors localized by quantitative autoradiography in kidney, ureter, and bladder. *Am. J. Physiol.,* **256,** F909–F915.

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

MATSUKAWA, K. & NINOMUYA, I. (1989). Anesthetic effects on tonic and reflex renal sympathetic nerve activity in awake cats. *Am. J. Physiol.,* **256,** R371–R378.

MATSUKAWA, K., NINOMUYA, I. & NISHIMURA, N. (1993). Effects of anesthesia on cardiac and renal sympathetic nerve activities and plasma catecholamines. *Am. J. Physiol.,* **265,** R792–R797.

MATTSON, D.L. & COWLEY, JR, A.W. (1993). Kinin actions on renal papillary blood flow and sodium excretion. *Hypertension,* **21,** 961–965.

MUKAI, H., FITZGIBBON, W.R., BOZEMAN, G., MARGOLIUS, H.S. & PLOTH, D.W. (1996). Bradykinin B_2 receptor antagonist increases chloride and water absorption in rat medullary collecting duct. *Am. J. Physiol.,* **271,** R352–R360.

NISHIMURA, M. & MARGOLIUS, H.S. (1992). A kinin receptor antagonist affects renal function of Dahl salt-resistant (SR/Jr) rats. *Hypertension,* **20,** 402 (Abstr.).

RAPP, J.P. (1984). Characteristics of dahl salt-susceptible and salt-resistant rats. In *Handbook of Hypertension, Vol 4, Experimental and Genetic Models of Hypertension.* ed. de Jong, W. pp. 286–295. Amsterdam: Elsevier.

RAPP, J.P., TAN, S.Y. & MARGOLIUS, H.S. (1978). Plasma mineralocorticoids, plasma renin and urinary kallikrein in salt-sensitive and salt-resistant rats. *Endocrine Res. Commun.,* **5,** 35–41.

ROMAN, R.J., KALDUNSKI, M.L., SCICLI, A.G. & CARRETERO, O.A. (1988). Influences of kinins and angiotensin II on the regulation of papillary blood flow. *Am. J. Physiol.,* **255,** F690–698.

SIRAGY, H.M. (1993). Evidence that intrarenal bradykinin plays a role in regulation of renal function. *Am. J. Physiol.,* **265,** E648–E654.

SIRAGY, H.M., IBRAHIM, M., JAFFA, A.A., MAYFIELD, R. & MARGOLIUS, H.S. (1994). Rat renal interstitial bradykinin, prostaglandin E2, and cyclic guanosine 3′,5′-monophosphate. effects of altered sodium intake. *Hypertension,* **23,** 1068–1070.

SMITH, H.W., FINKELSTEIN, N., ALIMINOSA, L., CRAWFORD, B. & GRABOR, M. (1945). Renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. *J. Clin. Invest.,* **24,** 388–404.

STERZEL, R.B., LUFT, F.C., GAO, Y., SCHNERMANN, J., BRIGGS, J.P., GANTEN, D., WALDHERR, R., SCHNABEL, E. & KRIZ, W. (1988). Renal disease and the development of hypertension in salt-sensitive rats. *Kidney Int.,* **33,** 1119–1129.

VIO, C.P., LOYOLA, S. & VELARDE, D.V. (1992). Localization of components of the kallikrein-kinin system in the kidney: relation to renal function. *Hypertension, Suppl 2,* **19,** II-10–II-16.

WANG, C., CHAO, C., CHEN, L., CHAO, L. & CHAO, J. (1996). High-salt diet upregulates kininogen and downregulates tissue kallikrein expression in Dahl-SS and SHR rats. *Am. J. Physiol.,* **271,** F824–F830.

WIRTH, K., HOCK, F.J., ALBUS, U., LINZ, W., ALPERMANN, H.G., ANAGNOSTOPOULOS, H., HENKE, ST., BREIPOHL, G., KÖNIG, W., KNOLLE, J. & SCHÖLKENS, B.A. (1991). HOE 140 a new potent and long acting bradykinin-antagonist: *in vivo* studies. *Br. J. Pharmacol.,* **102,** 774–777.

YAMAJI, I., MILLER, D.H., RAPP, J.P. & MARGOLIUS, H.S. (1988). Tissue kallikrein levels and synthesis in Dahl salt-sensitive and salt-resistant rats on low or high salt diets. *Am. J. Hypertens.,* **1,** 76A (Abstr.).

(Received December 12, 1997
Accepted January 26, 1998)