ELSEVIER

European Journal of Pharmacology 296 (1996) 267–276

ejp

Effects of angiotensin-converting enzyme and neutral endopeptidase inhibitors: influence of bradykinin

Isabelle Pham [a], Walter Gonzalez [a], Jean Doucet [a], Marie-Claude Fournié-Zaluski [b], Bernard P. Roques [b], Jean-Baptiste Michel [a,*]

[a] *INSERM U 367, 17 Rue du Fer-à-Moulin, 75005 Paris, France*

[b] *Laboratoire de pharmacochimie structurale et moléculaire, INSERM U 266-CNRS DURA 1500, Faculté de Pharmacie, 4 Avenue de l'Observatoire, 75006 Paris, France*

Received 15 May 1995; revised 17 October 1995; accepted 20 October 1995

Abstract

These experiments compare the effects of a neutral endopeptidase inhibitor, retrothiorphan, 1-[(1-mercaptomethyl-2-phenyl)ethyl]amino-1-oxopropanoic acid, a converting enzyme inhibitor, enalaprilat, and the combination of the two inhibitors on changes in blood pressure and renal function induced by exogenous and endogenous bradykinin in deoxycorticosterone acetate (DOCA)-salt rats. Enalaprilat potentiated the exogenous bradykinin-induced hypotensive responses while retrothiorphan potentiated the effects on urinary cyclic-GMP (cGMP) and bradykinin. The combination potentiated the exogenous bradykinin-induced hypotensive effects and the bradykinin-induced urinary excretion of cGMP, bradykinin and prostaglandin. The bradykinin B_2 receptor antagonist, Hoe 140, had no effect on the enalaprilat- and retrothiorphan-induced changes in blood pressure and renal function. In conclusion, while angiotensin-converting enzyme and neutral endopeptidase are involved in the vascular and renal catabolism of exogenous bradykinin, the effects of the peptidase inhibitors do not appear to depend on the protection of endogenous bradykinin under acute conditions in DOCA-salt rats.

Keywords: Bradykinin; Angiotensin-converting enzyme inhibitor; Neutral endopeptidase inhibitor; DOCA-salt rat

1. Introduction

Bradykinin appears to play a role in the control of blood pressure and sodium homeostasis by promoting vasodilation, diuresis and natriuresis (Scicli and Carretero 1986; Seymour et al., 1994). These vascular and renal effects may well involve the activation of endothelial nitric oxide synthase and phospholipase A_2 both in the circulation and in the kidney (Wiemer and Wirth, 1992, Cachofeiro and Nasjletti, 1991; D'Orléans-Juste et al., 1989).

Bradykinin catabolism occurs via several enzymatic pathways in vivo (Scicli and Carretero, 1986). Carboxypeptidase N cleaves the peptide at the Phe^8-Arg^9 bond, but angiotensin-converting enzyme and neutral endopeptidase are the two main enzymes involved in bradykinin degradation in vivo. Both enzymes cleave the peptide at the Pro^7-Phe^8 bond (Gafford et al., 1983) and are present within the vascular wall (Llorens-Cortes et al., 1992; Soleilhac et al., 1992) and in the kidney (Schulz et al., 1988). A combination of a neutral endopeptidase inhibitor and a converting enzyme inhibitor provides maximal protection against enzymatic degradation in endothelial cells in culture (Graf et al., 1991). Both enzymes are very abundant in the proximal tubule (Schulz et al., 1988). Their location in the brush border suggests that they may degrade the filtered bradykinin and other vasoactive peptides (Carone et al., 1976). However, their respective importance in this catabolic pathway for vasoactive peptides is not yet clear. Smits et al. (1990) reported that neutral endopeptidase is the major enzyme for bradykinin degradation in the kidney. Similarly, we found that neutral endopeptidase inhibition induces an increase in urinary bradykinin while converting enzyme inhibition has no significant effect on urinary

* Corresponding author. Tel.: 33-1-43364735; fax: 33-1-45356629.

SSDI 0014-2999(95)00706-7

bradykinin excretion (Pham et al., 1993). Neutral endopeptidase has several other substrates such as atrial natriuretic peptide, angiotensin I and II, and substance P (Roques et al., 1993) and potentiation of bradykinin levels in the urine by inhibition of neutral endopeptidase has raised the question about which substrate, atrial natriuretic peptide or bradykinin or both, is involved in the natriuretic effect of the neutral endopeptidase inhibitor. The effects of a bradykinin antibody or antagonist on the renal effects of neutral endopeptidase remain controversial (Bralet et al., 1991; Ura et al., 1987; Hirata et al., 1990) and we have also reported that neutral endopeptidase inhibition has similar effects on renal function and blood pressure in kininogen-kinin-deficient rats (Brown-Norway Kat) and in Wistar deoxycorticosterone acetate (DOCA)-salt rats (Pham et al., 1992).

The development and the pharmacology of dual angiotensin-converting enzyme and neutral endopeptidase inhibitors requires knowledge of the role of these enzymes in bradykinin catabolism and the involvement of bradykinin in the effects of converting enzyme inhibitor and/or neutral endopeptidase inhibitor (Fournié-Zaluski et al., 1994; Gonzalez Vera et al., 1995). The present study therefore investigates the actions of a neutral endopeptidase inhibitor, a converting enzyme inhibitor and the combination of the two inhibitors on the effects of bradykinin in the circulation and in the kidney of DOCA-salt rats. The first set of experiment compares the effects of a neutral endopeptidase inhibitor, a converting enzyme inhibitor and the combination of the two inhibitors on exogenous bradykinin-induced vascular and renal effects in vivo. The second set of experiments examines the effect of giving the specific bradykinin B_2 receptor antagonist, Hoe 140, before neutral endopeptidase or converting enzyme inhibitor, or the combination of the two inhibitors, to test the participation of endogenous bradykinin in the neutral endopeptidase and converting enzyme inhibitor-induced vascular and renal effects.

2. Materials and methods

2.1. Animals

Male Wistar rats (200–220 g) (Iffa-Credo, St Germain sur Abresles, France) were made DOCA-salt hypertensive. Unilateral nephrectomy was performed under anesthesia with ether and a pellet of deoxycorticosterone acetate (DOCA) (200 mg/kg body weight) was implanted subcutaneously. After surgery, the rats were fed on standard rat chow and the drinking water was supplemented with 1% NaCl and 0.2% KCl. Hypertension developed 4 weeks later.

2.2. Experimental design

Rats were anesthetized with ether and the right carotid artery was cannulated with a polyethylene catheter (Biotrol, Paris, France). The catheter was connected to a pressure transducer (Harvard, South Natick, MA, USA) and recorder (Ankersmit, Villeneuve d'Asq, France) for continuous blood pressure monitoring. The right jugular vein was cannulated with an elastomer catheter for injections, infusions and blood sampling. The two catheters were directed to the back of the neck and exteriorized. Urine samples were collected via a polyethylene catheter implanted in the bladder. Each rat was placed in a plastic box, saline infusion (1.2 ml/h) was begun immediately and the rat was allowed to recover for 180 min to stabilize blood pressure and diuresis. The experimental protocol was then carried out in unanesthetized conscious animals.

2.3. Effect of the treatments on exogenous bradykinin

After a baseline period of 30 min (period B), the rats were given the neutral endopeptidase inhibitor, retrothiorphan (25 mg/kg bolus injection + 25 mg/kg per h infusion), the converting enzyme inhibitor, enalaprilat (5 mg/kg bolus injection) or vehicle (control group) (period T). We have previously shown that this dose of retrothiorphan induces a 25% decrease in blood pressure in DOCA-salt hypertensive rats and that enalaprilat (5 mg/kg) normalizes blood pressure in renovascular hypertensive rats (Pham et al., 1993). 30 min later, bradykinin was injected at 0.5 (P 1), 1 (P 2) and 2 μg/kg (P 3) at 10-min intervals in the control group and at 20-min intervals in the treated groups because of prolonged decreases in blood pressure. The effects of retrothiorphan, enalaprilat and the combination of these two compounds on bradykinin-induced diuresis, natriuresis and decrease in blood pressure were examined versus control ($n = 10$ for each group). Blood pressure was monitored continuously and urine samples were collected for determination of diuresis, natriuresis, kaliuresis, urinary cyclic-GMP (cGMP), urinary bradykinin and urinary 6-keto-prostaglandin-$F_{1\alpha}$.

2.4. Effects of Hoe 140 on the peptidase inhibitors responses

The responses to the different peptidase inhibitors were compared with and without the bradykinin B_2 receptor antagonist Hoe 140. A 1-h baseline response was recorded and Hoe 140 (1 mg/kg i.v.) or its vehicle was injected. This dose completely abolished the decrease in blood pressure induced by bradykinin 2 μg/kg i.v. for 2 h. Retrothiorphan or enalaprilat or vehicle was given 15 min later. Blood pressure was monitored

continuously and urine samples were collected every 30 min as above. Eight groups of rats were compared: Hoe 140 + vehicle or neutral endopeptidase inhibitor or converting enzyme inhibitor or the combination of the two inhibitors ($n = 7$) and Hoe 140 vehicle + vehicle or neutral endopeptidase inhibitor or converting enzyme inhibitor or the combination of the two inhibitors ($n = 7$).

2.5. Compounds

Retrothiorphan, 1-[(1-mercaptomethyl-2-phenyl)ethyl]amino-1-oxopropanoic acid, is a potent neutral endopeptidase inhibitor ($K_i = 6$ nM) without an effect on ACE ($IC_{50} > 10\,000$ nM) (Roques et al., 1983). Retrothiorphan was synthesized in the Laboratoire de Pharmacochimie Moléculaire et structurale, INSERM U 266-CNRS D 1500 (M.-C. F-Z. and B.P.R.). Enalaprilat (MK 422) was provided by Merck-Sharp-Dohme and Hoe 140 was a generous gift from Dr Schoelkens (Hoescht, Frankfurt, Germany).

2.6. Biochemical assays

Urinary volume was determined gravimetrically and urinary flow (UV) was expressed in μl/min. Electrolyte concentrations were measured in fresh urine samples with an ion-selective electrode (Beckman, Brea, CA, USA). The excretion rates for sodium ($U_{Na}V$) and potassium were calculated and expressed in μmol/min. Urine samples were then frozen at −30°C.

Urinary cGMP ($U_{cGMP}V$) was measured in diluted urine (1/50) with a [^{3}H]cGMP radioimmunoassay (assay range from 0.25 to 8 pmol) (Amersham, Amersham, UK). Immunoreactive bradykinin was measured in diluted urine (1/100) with [^{125}I][Tyr^8]BK (assay range from 1.6 to 200 pg). The antibodies used did not crossreact with the bradykinin fragments produced by the neutral endopeptidase action of neutral endopeptidase (Alhenc-Gélas et al., 1981). The product of prostacyclin, 6-keto-prostaglandin-$F_{1\alpha}$, was determined in the urine by radioimmunoassay with [^{3}H]6-keto-prostaglandin-$F_{1\alpha}$ (assay range from 14 to 500 pg) (Amersham, Amersham, UK) after extraction with an Amprep column.

2.7. Statistical analysis

Data are expressed as means ± S.E.M. In the first study, a paired Student's t-test was performed to test the effect of exogenous bradykinin in the control group. A factorial two-way analysis of variance (ANOVA) was performed to test the interaction between retrothiorphan and enalaprilat on the peptidase inhibitor-induced changes at period T and then an ANOVA for repeated measures, restricted to control and each treatment group, was performed to test the effect of each treatment on the bradykinin-induced changes in blood pressure, diuresis and natriuresis and in the biochemical parameters. The effect of the treatment at each time was then tested by a factorial one-way ANOVA with Fisher PLSD.

In the second study, the interaction between the effect of the bradykinin antagonist Hoe 140 and the various inhibitors was evaluated by a factorial two-way ANOVA on mean arterial pressure, diuresis, natriuresis and the urinary parameters. If there was no effect of Hoe 140, an ANOVA for repeated measures, restricted to each treatment, was performed to test the interaction between the effect of Hoe 140 and time. The Hoe 140-treated groups and the vehicle group within each treatment were compared at each time by a factorial one-way ANOVA and Fisher PLSD test. Statistical significance was reached with $P < 0.05$.

3. Results

3.1. Effects of exogenous bradykinin in control rats

Bradykinin injections induced a transient decrease in mean arterial pressure (Table 1 and Fig. 1) and transient increases in diuresis and natriuresis in control DOCA-salt rats (Table 1). The effect of exogenous bradykinin on blood pressure was dose-dependent (Fig. 1) but the changes in diuresis and natriuresis were not.

Table 1
Effects of exogenous bradykinin (BK) on mean arterial pressure (MAP), diuresis (UV), natriuresis ($U_{Na}V$), kaliuresis (U_KV), and the urinary excretion of cGMP ($U_{cGMP}V$), bradykinin ($U_{BK}V$) and 6-keto-prostaglandin-$F_{1\alpha}$ ($U_{PG}V$) in control rats.

BK doses (μg/kg)	0	0.5	1	2
MAP (mmHg)	190 ± 8	181 ± 8.3 [a]	176.5 ± 7.5	177 ± 6.5 [a]
UV (μl/min)	43.4 ± 1.8	88.7 ± 17.5 [b]	84.5 ± 13.7 [b]	92.4 ± 17.6 [b]
U_KV (μmol/min)	2.2 ± 0.3	2.5 ± 0.4	2.5 ± 0.3	3.3 ± 0.4 [a]
$U_{Na}V$ (μmol/min)	5.9 ± 1.2	12.5 ± 2.4 [b]	11.2 ± 1.9 [a]	12.2 ± 2.2 [b]
$U_{cGMP}V$ (pmol/min)	20.2 ± 1.9	44.9 ± 11.3 [a]	33.8 ± 8.9	29.2 ± 5 [a]
$U_{BK}V$ (pg/min)	284.2 ± 69.4	498.2 ± 128	626.1 ± 162	478.2 ± 95 [a]
$U_{PG}V$ (pg/min)	32.3 ± 8.1	81.6 ± 36.8	80.3 ± 24.4	65.9 ± 19.4 [a]

[a] $P < 0.05$, [b] $P < 0.01$ vs. baseline.

Kaliuresis, urinary bradykinin, cGMP and 6-keto-prostaglandin-$F_{1\alpha}$ increased also with the bradykinin injections (Table 1).

3.2. Effects of the peptidase inhibitors on baseline parameters

The blood pressure of the four groups was similar at baseline (placebo 187 ± 8; retrothiorphan 178.5 ± 5; enalaprilat 180 ± 6; combination 188 ± 8 mmHg) and did not significantly change 30 min after injection of enalaprilat ($F = 1.6$; ns) or retrothiorphan ($F = 0.8$; ns) or their combination (interaction $F = 0.7$; ns) in DOCA-salt hypertensive rats (Fig. 1).

The effects of the peptidase inhibitors on renal parameters were evaluated by examining the differences between the effects obtained just before bradykinin injections (period T) and the baseline values. Enalaprilat had no significant effect on diuresis ($F = 1.5$; ns) (Fig. 2), natriuresis ($F = 1$; ns) (Fig. 3), kaliuresis ($F = 1.3$; ns) or the urinary excretion of cGMP ($F = 0.2$; ns) (Fig. 4a), bradykinin ($F = 1$; ns) (Fig. 4b) and prostaglandin ($F = 0.1$; ns) (Fig. 4c) but retrothiorphan significantly increased diuresis ($F = 7.9$; $P < 0.01$) (Fig. 2), natriuresis ($F = 7.8$; iP < 0.01) (Fig. 3), kaliuresis ($F = 11.2$; $P < 0.01$) and the urinary excretion of cGMP ($F = 7.4$; $P < 0.01$) (Fig. 4a), bradykinin ($F = 29$; $P < 0.001$) (Fig. 4b) and 6-keto-prostaglandin-$F_{1\alpha}$ ($F = 3.8$; $P < 0.05$) (Fig. 4c) 30 min after its administration. There was no significant interaction between enalaprilat and retrothiorphan for any parameter (Figs. 2, 3, 4a,b,c).

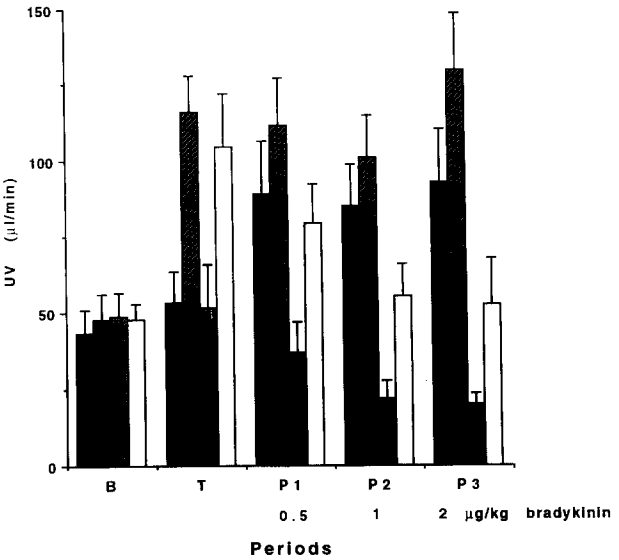

Fig. 2. Effect of retrothiorphan, enalaprilat and their combination on diuresis. B = baseline, T = peptidase inhibitors, P1 = bradykinin 0.5 µg/kg, P2 = bradykinin 1 µg/kg, P3 = bradykinin 2 µg/kg. Control: black bars; retrothiorphan: hatched bars; enalaprilat: grey bars; combination: open bars.

3.3. Interaction between peptidase inhibitors and exogenous bradykinin

The hypotensive responses were evaluated by examining the difference between the maximal decrease in mean arterial pressure obtained after the bradykinin injections and the mean arterial pressure just before

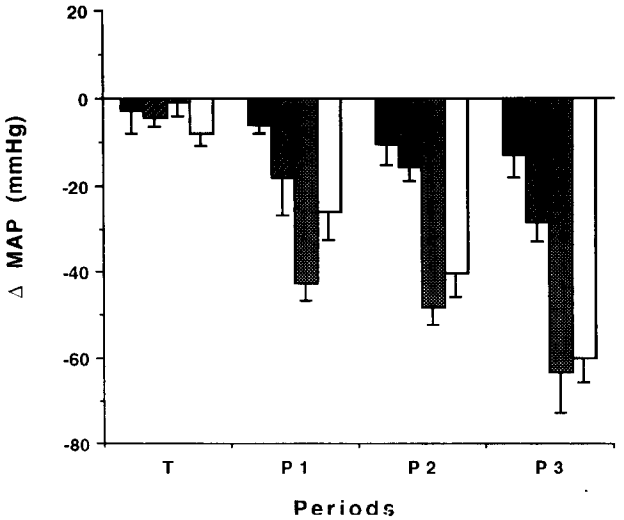

Fig. 1. Effect of retrothiorphan, enalaprilat and their combination on changes in blood pressure. T = peptidase inhibitors, P1 = bradykinin 0.5 µg/kg, P2 = bradykinin 1 µg/kg, P3 = bradykinin 2 µg/kg. Control: black bars; retrothiorphan: hatched bars; enalaprilat: grey bars; combination: open bars.

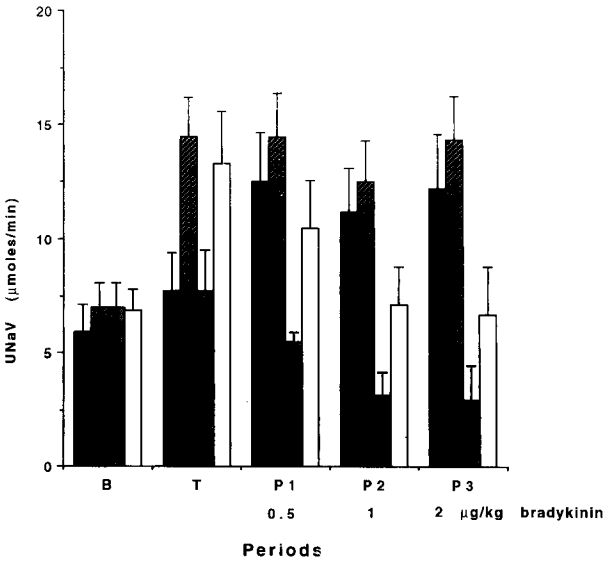

Fig. 3. Effect of retrothiorphan, enalaprilat and their combination on natriuresis. B = baseline, T = peptidase inhibitors, P1 = bradykinin 0.5 µ /kg, P2 = bradykinin 1 µg/kg, P3 = bradykinin 2 µg/kg. Control: black bars; retrothiorphan: hatched bars; enalaprilat: grey bars; combination: open bars.

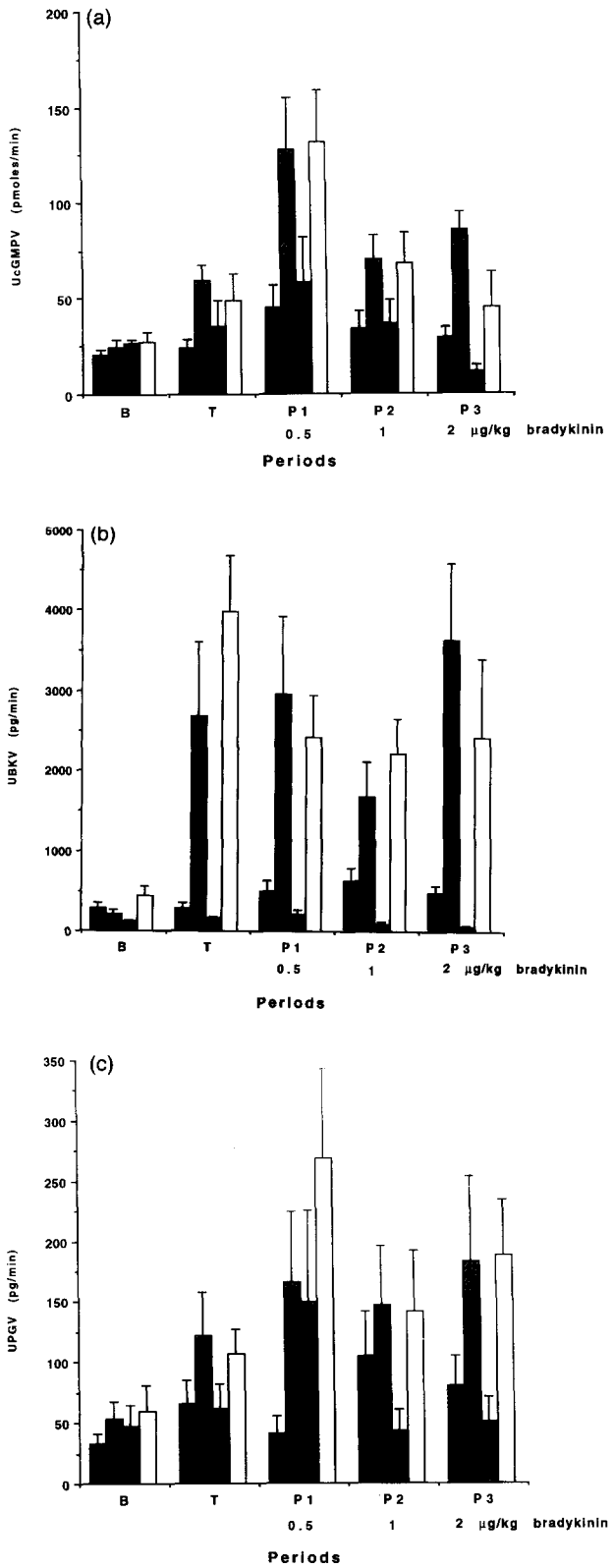

Fig. 4. Effect of retrothiorphan, enalaprilat and their combination on (a) urinary cGMP; (b) urinary bradykinin; (c) urinary 6-keto-prostagalandin $F_{1\alpha}$. B = baseline, T = peptidase inhibitors, P1 = bradykinin 0.5 µg/kg, P2 = bradykinin 1 µg/kg, P3 = bradykinin 2 µg/kg. Control: black bars; retrothiorphan: hatched bars; enalaprilat: grey bars; combination: open bars.

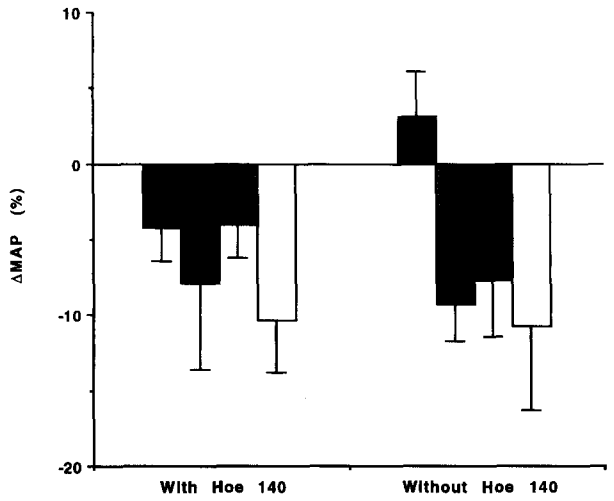

Fig. 5. Effect of Hoe 140 1 mg/kg on the maximal retrothiorphan (hatched bars)-, enalaprilat (grey bars)- and their combination (open bars)-induced changes in blood pressure vs. control (black bars).

the injections. Enalaprilat significantly potentiated the bradykinin-induced decrease in blood pressure (for all doses $P < 0.001$) and prolonged its duration (Fig. 1). The effects of retrothiorphan were significant on bradykinin-induced hypotension only with the highest dose of bradykinin ($F = 8.7$ $P < 0.01$) (Fig. 1). However this potentiation was smaller than that with enalaprilat. The combination also induced a decrease in blood pressure which was similar to the hypotensive effect of enalaprilat ($F = 1.2$; ns) (Fig. 1).

Enalaprilat significantly decreased bradykinin-induced diuresis ($F = 7.1$, $P < 0.001$) (Fig. 2) and natriuresis ($F = 4.9$, $P < 0.001$) (Fig. 3). It also decreased urinary cGMP at the 2 µg/kg bradykinin dose ($F = 8.8$, $P < 0.05$) (Fig. 4a) and urinary bradykinin excretion at the 1 ($F = 10.7$, $P < 0.01$) and 2 µg/kg bradykinin doses ($F = 18.9$, $P < 0.001$) (Fig. 4b). However, enalaprilat had a significant and positive effect on urinary 6-keto-prostaglandin-$F_{1\alpha}$ at the 0.5 µg/kg bradykinin dose ($F = 4.3$, $P < 0.05$) (Fig. 4c). Retrothiorphan had no significant effect on the bradykinin-induced diuresis ($F = 2.3$, ns) (Fig. 2) and natriuresis ($F = 1.5$, ns) (Fig. 3) while it potentiated urinary cGMP ($F = 2.6$, $P < 0.05$) (Fig. 4a) and bradykinin ($F = 4$, $P < 0.01$) (Fig. 4b) excretion. The effect on 6-keto-prostaglandin-$F_{1\alpha}$ was significant only at the 0.5 µg/kg bradykinin dose ($F = 3.9$, $P < 0.05$) (Fig. 4c).

The combination enalaprilat + retrothiorphan had no significant effect on bradykinin-induced diuresis ($F = 2.9$, ns) (Fig. 2) and natriuresis ($F = 2.6$, ns) (Fig. 3) while it had a significant and positive effect on urinary cGMP ($F = 2.5$, $P < 0.05$) (Fig. 4a) and bradykinin ($F = 4.7$, $P < 0.001$) (Fig. 4b). The effect on urinary prostaglandin was significant only at the 0.5 µg/kg ($F = 9.2$, $P < 0.001$) and the 2 µg/kg bradykinin doses ($F = 4.4$, $P < 0.05$) (Fig. 4c). The effects on di-

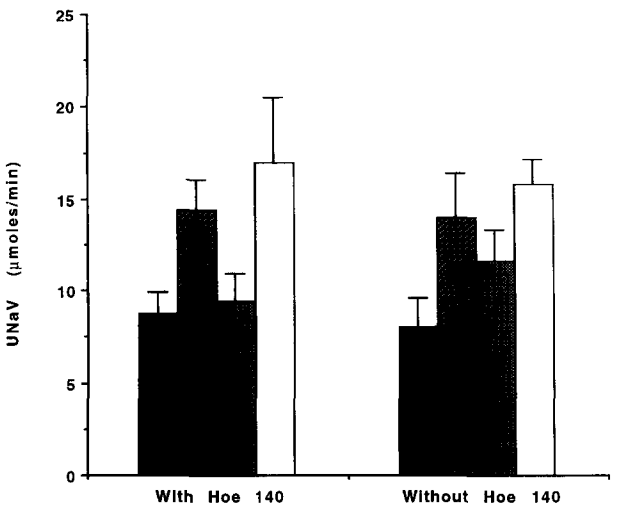

Fig. 6. Effect of Hoe 140 1 mg/kg on the maximal retrothiorphan (hatched bars)-, enalaprilat (grey bars)- and their combination (open bars)-induced changes in natriuresis vs. control (black bars).

uresis ($F = 4$; $P < 00.1$) and natriuresis ($F = 2.6$; $P < 0.05$) were significantly smaller than those obtained with retrothiorphan alone but the effects on urinary cGMP ($F = 0.9$; ns), bradykinin ($F = 1.3$; ns) and prostaglandin ($F = 0.6$; ns) were not different.

3.4. Effects of Hoe 140 on the different peptidases inhibitors

Hoe 140 tended to decrease blood pressure ($F = 3.9$; $P = 0.07$) (Fig. 5) but had no significant effect on diuresis ($F = 0.2$; ns) and natriuresis ($F = 1.9$; ns) (Fig. 6) in the control group. Factorial two-way ANOVA did not find an interaction between Hoe 140 and the peptidase inhibitors. ANOVA for repeated measures restricted to each peptidase inhibitor revealed no significant interaction between Hoe 140 and time on the retrothiorphan, enalaprilat and combination-induced changes in diuresis ($F = 0.6$; $F = 0.9$; $F = 0.7$; ns), natriuresis ($F = 1$; $F = 0.6$; $F = 0.7$; ns) (Fig. 6), urinary excretion of cGMP, bradykinin and prostaglandin (Table 2) and decrease in blood pressure ($F = 0.3$; $F = 0.5$; $F = 0.2$; ns) (Fig. 6).

4. Discussion

Margolius et al. reported that urinary kallikrein activity is increased in mineralocorticoïd-induced hypertension as occurs in DOCA-salt rats (Margolius et al., 1972) and primitive hyperaldosteronism (Margolius et al., 1971). We previously found that the urinary excretion of bradykinin is higher in DOCA-salt hypertensive rats than in spontaneously or in renovascular hypertensive rats (Pham et al., 1993). Some investigators reported recently that administration of bradykinin, Hoe 140, or the atrial natriuretic peptide antagonist, HS 142-1, induces a rise in blood pressure and a decrease in renal sodium excretion in DOCA-salt rats, indicating that both the bradykinin-kallikrein system and the natriuretic peptide may be involved in the regulation of blood pressure in this experimental model of hypertension (Madeddu et al., 1992; Hirata et al., 1993). Thus, the DOCA-salt hypertensive rat appears to be an accurate model in which to compare the role of neutral endopeptidase and angiotensin-converting enzyme in bradykinin metabolism.

Bolus injections of bradykinin induced a decrease in blood pressure and an increase in diuresis and natriuresis. The decrease in blood pressure was transient (< 1 min) and dose dependent. The renal effects also appeared to be rather transient (< 10 min). However, the lack of dose dependence suggests that there may be a remnant effect of repeated bradykinin injections on renal function. Several investigators have reported the effects of bradykinin on renal hemodynamics: exogenous bradykinin induces renal vasodilation (Willis et al., 1969) and a bradykinin antagonist reverses the captopril-induced increase in glomerular filtration rate and papillary blood flow (Fenoy et al., 1991; Mattson and Cowley, Jr., 1993). However, others have reported that kinins act rather by a tubular effect (Siragy, 1993;

Table 2
Effects of Hoe 140 (1 mg/kg) on retrothiorphan-, enalaprilat- and their combination-induced changes in kaliuresis (U_KV, μmol/min) and the urinary excretion of cGMP ($U_{cGMP}V$, pmol/min), bradykinin ($U_{BK}V$, pg/min) and 6-keto-prostaglandin-$F_{1\alpha}$ ($U_{PG}V$, pg/min).

	Control		Retrothiorphan		Enalaprilat		Combination	
	− [a]	+ [b]	− [a]	+ [b]	−	+	−	+
U_KV	2.3 ± 0.2	2.1 ± 0.3	2.8 ± 0.2	3.3 ± 0.2	2.6 ± 0.3	2 ± 0.4	3.2 ± 0.3	2.8 ± 0.4
F; P	0.9; ns		2.2; ns		1.4; ns		0.8; ns	
$U_{cGMP}V$	28.9 ± 3.9	33.3 ± 4	74.4 ± 11	80.8 ± 22.6	44.1 ± 2.7	35.1 ± 6.2	153.6 ± 52	113 ± 19
F; P	0.9; ns		0.2; ns		1; ns		1.5; ns	
$U_{BK}V$	392 ± 229	488 ± 155	1498 ± 443	1443 ± 495	716 ± 213	586 ± 197	1360 ± 424	1144 ± 338
F; P	0.04; ns		0.6; ns		0.9; ns		0.2; ns	
$U_{PG}V$	45.1 ± 14.5	22.3 ± 6.3	69 ± 37.4	47.1 ± 20.9	48.1 ± 25.8	62.7 ± 38.6	22.2 ± 5.7	27.2 ± 7.4
F; P	0.9; ns		0.3; ns		1.3; ns		0.05; ns	

[a] − Hoe 140; [b] + Hoe 140.

Lortie et al., 1992). In agreement, bradykinin receptors appear to be localized mostly in the collecting tubule (Tomita and Pisano, 1984). However, the effects of bradykinin in the distal tubule remain controversial: some have reported that bradykinin increases sodium excretion by inhibiting its tubular reabsorption by the amiloride-sensitive channel (Kauker, 1980; Zeidel et al., 1990) while others have found no effect of bradykinin (Rouch et al., 1991). The effects of bradykinin may be synergistic with those of atrial natriuretic peptide (Stoos et al., 1992). In vitro experiments show that the renal effects of bradykinin are mediated by nitric oxide and prostaglandin (Cachofeiro and Nasjletti, 1991; Garcia-Perez and Smith, 1984), as in endothelial cells (D'Orléans-Juste et al., 1989). Administration of NO-synthase inhibitors abolishes the effects of kinins and the kinin induced-increase in cGMP in isolated perfused kidney, in agreement with a mechanism depending on nitric oxide (Cachofeiro and Nasjletti, 1991), and bradykinin causes the production of prostaglandin by collecting duct cells in culture (Garcia-Perez and Smith, 1984). This latter effect involves the apical but not the basolateral side of the cells, in agreement with an intra-luminal effect of bradykinin (Garcia-Perez and Smith, 1984). Our results show that the renal effects of exogenous bradykinin are accompanied by an increase in urinary bradykinin, cGMP and the prostacyclin metabolite, 6-keto-prostaglandin-$F_{1\alpha}$. But it is difficult to determine whether these second messengers are produced by the renal cells, after an intraluminal action of bradykinin, or come from plasma.

The renal effects of the different peptidase inhibitors can be compared before the bradykinin injections. Enalaprilat had no significant effect on the renal parameters, diuresis, natriuresis and the renal excretion of cGMP, a prostacyclin metabolite, and bradykinin in DOCA-salt rats. Angiotensin-converting enzyme inhibition potentiates the effect of bradykinin on endothelial cells in culture (Wiemer and Wirth, 1992; Gräfe et al., 1993) and urinary prostaglandin is increased by a converting enzyme inhibitor (Miyamoto et al., 1981). Potentiation of the effects of bradykinin also appears to be involved in the effects of converting enzyme inhibitor via production of nitric oxide (Cachofeiro et al., 1992; Carbonell et al., 1988). However, we have previously reported that acute angiotensin-converting enzyme inhibition induces no increase in urinary bradykinin excretion under similar conditions although urinary bradykinin increases after neutral endopeptidase blockade (Pham et al. 1993). In contrast, neutral endopeptidase inhibitor enhances diuresis with an increase in the urinary excretion of cGMP, bradykinin and prostaglandin. Urinary cGMP is an indicator of the action of atrial natriuretic peptide (Wong et al., 1988) and we and other investigators have shown that neutral endopeptidase inhibition is marked by an increase in urinary cGMP (Pham et al., 1992; Sybertz et al., 1990). The neutral endopeptidase inhibitor candoxatril also causes an increase in urinary prostaglandins in human heart failure (Münzel et al., 1992). The increase in urinary cGMP and 6-keto-prostaglandin-$F_{1\alpha}$ probably indicates that neutral endopeptidase has been blocked by the inhibitor in the kidney and that the vasoactive peptides, bradykinin and atrial natriuretic peptide, have been protected from degradation. Thus, the renal effects of neutral endopeptidase inhibitor appear to be due to potentiation of both atrial natriuretic peptide and bradykinin. However the effect of retrothiorphan was significant on bradykinin-induced urinary cGMP, bradykinin excretion while it had no significant effect on bradykinin-induced diuresis and natriuresis. Although the hypotensive responses were rather small, they could have blunted the potentiation of diuresis, natriuresis induced by neutral endopeptidase inhibitor. Converting enzyme inhibitor has a negative effect on bradykinin-induced renal responses because of the large decreases in blood pressure, but it potentiated the urinary excretion of prostaglandin induced by a low dose (0.5 μg/kg) of bradykinin. The combination induced similar renal effects on the bradykinin-induced urinary excretion of cGMP and bradykinin as seen with retrothiorphan alone. Neutral endopeptidase and angiotensin-converting enzyme are present in the renal brush border in the same amount and could cleave the filtered bradykinin (Schulz et al., 1988; Carone et al., 1976). The neutral endopeptidase inhibitor-induced increase in urinary cGMP and bradykinin excretion and the potentiation of exogenous bradykinin-induced urinary excretion of cGMP, bradykinin and prostaglandin production by the neutral endopeptidase inhibitor may suggest that renal neutral endopeptidase is more important than renal angiotensin-converting enzyme in renal bradykinin catabolism. However, the significant protection of 0.5 μg/kg bradykinin-induced prostaglandin production by converting enzyme inhibitor also indicates that a role for angiotensin-converting enzyme in the renal catabolism of bradykinin cannot be excluded.

The effects of the peptidase inhibitors on blood pressure were not significant at 30 min. We have previously shown that the effect of retrothiorphan on blood pressure develops rather slowly in rats (Pham et al., 1992) and converting enzyme inhibitor usually has a weak hypotensive effect in DOCA-salt rats. Converting enzyme inhibitor potentiated and prolonged the exogenous bradykinin-induced decrease in blood pressure with reactive negative effects on diuresis and natriuresis. In contrast, neutral endopeptidase inhibitor only potentiated the hypotensive effect of a high dose of bradykinin (2 μg/kg) with no significant effect on bradykinin-induced changes in renal function. These

results confirm the major role of angiotensin-converting enzyme in bradykinin catabolism in the circulation. Angiotensin-converting enzyme is mainly a constitutive endothelial ectoenzyme. Neutral endopeptidase is also present in endothelial cells but in smaller amounts than angiotensin-converting enzyme (Llorens-Cortes et al., 1992; Soleilhac et al., 1992). It has been suggested that endothelial neutral endopeptidase is involved in 30–50% of bradykinin degradation in vitro (Llorens-Cortes et al., 1992; Graf et al., 1991). The high K_m for bradykinin and the turnover rate of neutral endopeptidase could also explain why angiotensin-converting enzyme is the main enzyme at low, physiological, concentrations of bradykinin and that neutral endopeptidase could be more efficient than angiotensin converting enzyme at high concentrations of bradykinin (Gafford et al., 1983). Thus, neutral endopeptidase could play a main role in bradykinin catabolism when angiotensin-converting enzyme is blocked. The combination of a neutral endopeptidase inhibitor and an angiotensin-converting enzyme inhibitor provides the best protection against degradation in endothelial cells in culture (Graf et al., 1991). Seymour et al. (1994) also reported that combination of a converting enzyme inhibitor and a neutral endopeptidase inhibitor potentiates intra-renal bradykinin-induced renal blood flow and natriuresis. But the combination of the two inhibitors did not potentiate the bradykinin-induced decrease in blood pressure more than converting enzyme alone under our experimental conditions. The major decreases in blood pressure obtained with the combination probably blunted any potentiation of diuresis and natriuresis. However, the effect of the combination was positive for the urinary biological markers.

Potentiation of endogenous bradykinin does not appear to be required for the effects of inhibition of neutral endopeptidase or angiotensin-converting enzyme under acute conditions. Hoe 140 at 1 mg/kg i.v. inhibits the effects of exogenous bradykinin in a model of inflammation in rat (Wirth et al., 1991) and blocked the hypotensive effect of bradykinin at a lower dose, when injected subcutaneously (Madeddu et al., 1992; Wirth et al., 1991). In preliminary experiments, Hoe 140 at 1 mg/kg i.v. completely blocked the exogenous bradykinin-induced hypotensive response for 2 h, although the 0.1 mg/kg dose appeared to block this response only for 1 h. Hoe 140 (1 mg/kg i.v.) never reversed even partially, the hypotensive and renal effects of neutral endopeptidase inhibitor and converting enzyme inhibitor. Some investigators have reported that a bradykinin antibody completely reverses the diuresis and increase in urinary cGMP induced by neutral endopeptidase inhibitor (Bralet et al., 1991; Ura et al., 1987), while others have reported that a bradykinin antagonist has no effect on the renal effects of thiorphan (Hirata et al., 1990). The discrepancy between these results may be explained by differences in the pharmacokinetics of the antagonists or antibodies. If bradykinin has an intraluminal tubular effect, these antagonists need to be filtered by the glomerulus and excreted by the kidney to reach their target. Only 4% of Hoe 140 appears to be found in an intact form in the urine (Dr K. Wirth, personal communication). This could explain the lack of effect of Hoe 140 on the renal response to neutral endopeptidase inhibitor, although the reabsorption of Hoe 140 cannot be excluded, in which case the intrarenal concentration would be higher. Moreover, the positive response of a high-weight kininogen deficient strain (Brown-Norway Kat) to retrothiorphan is not in agreement with an important role for bradykinin in the effect of the neutral endopeptidase inhibitor (Pham et al., 1992). In experimental heart failure, potentiation of bradykinin may be involved in the vascular effect of a combination of converting enzyme inhibitor and neutral endopeptidase inhibitor, although it does not appear to be involved in the vascular effect of neutral endopeptidase inhibitor alone (Trippodo et al., 1995). However, the blockade of atrial natriuretic peptide receptors by an antibody or an antagonist (HS 142-1) abolishes the hypotensive and renal effects of neutral endopeptidase inhibition (Hirata et al., 1990; Hirata et al., 1994). Thus, there is a consensus on the involvement of atrial natriuretic peptide in the effects of neutral endopeptidase inhibitor while the involvement of endogenous bradykinin under acute conditions remains controversial.

However, a role for bradykinin cannot be excluded in chronic inhibition and in particular at a high level of bradykinin since it rather acts in a paracrine and long-term way than in an endocrine and humoral way. Chronic blockade of bradykinin receptors with Hoe 140 partially reverses the antihypertensive effect of converting enzyme inhibitor in renovascular hypertension, although it has no effect in acute conditions (Bao et al., 1992) and enhances blood pressure in DOCA-salt rats when given as a chronic treatment (Madeddu et al., 1992).

In conclusion, our results show that bolus injections of bradykinin produce hypotensive and natriuretic responses which are probably mediated by nitric oxide-induced cGMP and prostacyclin in DOCA-salt hypertensive rat. The exogenous bradykinin-induced hypotensive and vascular responses are potentiated by angiotensin-converting enzyme inhibition and in part by neutral endopeptidase inhibition, indicating that angiotensin-converting enzyme and neutral endopeptidase are involved in the vascular catabolism of bradykinin. In contrast, diuretic and natriuretic responses are not potentiated by peptidase inhibition. However, the potentiation of the bradykinin-induced urinary excretion of cGMP, bradykinin and prostaglan-

din by peptidase inhibitors also indicates that neutral endopeptidase and angiotensin-converting enzyme in part are involved in renal catabolism of bradykinin. There is no evidence from experiments with the bradykinin receptor antagonist Hoe 140 that endogenous bradykinin is involved in the effects of inhibition of neutral endopeptidase and angiotensin-converting enzyme under acute conditions in DOCA-salt hypertensive rats. Nevertheless, these results do not exclude the possibility that endogenous bradykinin may play a role in the effects of chronic inhibition of neutral endopeptidase and/or angiotensin-converting enzyme in DOCA-salt hypertensive rats.

Acknowledgement

This work was supported by a grant from Institut de Recherche International Servier (IRIS), Courbevoie, France.

References

Alhenc-Gélas, F., J. Marchetti, J. Allegrini, P. Corvol and J. Menard, 1981, Measurement of kallikrein activity by kinin radioimmunoassay. Species differences in kinin production, Biochim. Biophys. Acta 677, 477.

Bao, G.,P. Gohlke, F. Qadri and T. Unger, 1992, Chronic kinin receptor blockade attenuates the antihypertensive effects of ramipril, Hypertension 20, 74.

Bralet, J., C. Mossiat, C. Gros and J. Schwartz, 1991, Thiorphan-induced natriuresis in volume-expanded rats: roles of endogenous atrial natriuretic factor and kinins, J. Pharmacol. Exp. Ther. 258, 807.

Cachofeiro, V. and A. Nasjletti, 1991, Increased vascular responsiveness to bradykinin in kidneys spontaneously hypertensive rats. Effects of *N*-nitro-L-arginine, Hypertension 18, 683.

Cachofeiro, V., T. Sakakibara and A. Nasjletti, 1992, Kinins, nitric oxide, and the hypotensive effect of captopril and ramiprilat in hypertension, Hypertension 19, 138.

Carbonell, L.F., O.A. Carretero, J.M. Stewart and A.G. Scicli, 1988, Effect of a kinin antagonist on the acute antihypertensive activity of enalaprilat in severe hypertension, Hypertension 11, 239.

Carone, F.A., T.N. Pullman, S. Oparil and S. Nakamura, 1976, Micropuncture evidence of rapid hydrolysis of bradykinin by rat proximal tubule, Am. J. Physiol. 230, 1420.

D'Orléans-Juste, P., G. de Nucci and J. Vane, 1989, Kinins act on B1 or B2 receptors to release conjointly endothelium-derived relaxing factor and prostacyclin from bovine aortic endothelial cells, Br. J. Pharmacol. 96, 920.

Fenoy, F.J., G. Scicli, O. Carretero and R.J. Roman, 1991, Effect of an angiotensin II and a kinin receptor antagonist on the renal hemodynamic response to captopril, Hypertension 17, 1038.

Fournié-Zaluski, M.-C., W. Gonzales, S. Turcaud, I. Pham, B. Roques and J.-B. Michel, 1994, Dual inhibition of angiotensin converting enzyme and neutral endopeptidase by the orally active inhibitor mixanpril I; a new therapeutic approach in hypertension, Proc. Natl. Acad. Sci. USA 91, 4072.

Gafford, J.T., R.A. Skidgel, E.G. Erdös and L.B. Hersh, 1983, Human kidney "enkephalinase", a neutral metalloendopeptidase that cleaves active peptides, Biochemistry 22, 3265.

Garcia-Perez, A. and W. Smith, 1984, Apical-basolateral membrane asymetry in canine cortical collecting tubule cells, J. Clin. Invest. 74, 63.

Gonzalez Vera, W., M.-C. Fournié-Zaluski, I. Pham, I. Laboulandine, B. Roques and J.-B. Michel, 1995, Hypotensive and natriuretic effects of RB 105, a new dual inhibitor of angiotensin converting enzyme and neutral endopeptidase in hypertensive rats, J. Pharmacol. Exp. Ther. 272, 343.

Graf, K., C. Bossaler, M. Gräfe, W. Auch-Schwelk, C.R. Baumgarten, J. Niehus and E. Fleck, 1991, Neutral endopeptidase is involved in endothelial degradation of kinins, Circulation 84 (Suppl. II), II-52.

Gräfe, M., C. Bossaler, K. Graf, W. Auch-Schwelk, C.R. Baumgarten, A. Hidebrandt and E. Fleck, 1993, Effect of angiotensin-converting enzyme inhibition on bradykinin metabolism by vascular endothelial cells, Am. J. Physiol. 264, H1493.

Hirata, Y., H. Matsuoka, H. Hayakawa, T. Sugimoto, E. Suzuki, K. Kangawa and H. Matsuo, 1990, Role of endogenous atrial natriuretic peptide in regulating sodium excretion in spontaneously hypertensive rats, Hypertension 17, 1025.

Hirata, Y., H. Matsuoka, E. Suzuki, H. Hayakawa, T. Sugimoto, Y. Matsuda, Y. Morishita, K. Kangawa, N. Minamino, H. Matsuo and T. Sugimoto, 1993, Role of endogenous atrial natriuretic peptide in DOCA-salt hypertensive rats. Effects of a novel nonpeptide antagonist for atrial natriuretic peptide receptor, Circulation 87, 554.

Hirata, Y., Y. Suzuki, E. Suzuky, H. Hayakawa, K. Kimura, A. Goto, Y. Matsuda and M. Omata, 1994, Mechanisms underlying the augmented responses of DOCA-salt hypertensive rats to neutral endopeptidase inhibition, J. Hypertens. 12 (Suppl. 3), S102.

Kauker, M.L., 1980, Bradykinin action on the efflux of luminal ^{22}Na in the rat nephron, J. Pharmacol. Exp. Ther. 214, 119.

Llorens-Cortes, C., H. Huang, P. Vicart, J.-M. Gasc, D. Paulin and P. Corvol, 1992, Identification and characterization of neutral endopeptidase in endothelial cells from venous or arterial origins, J. Biol. Chem. 267, 14012.

Lortie, M.,D. Regoli, N.-E. Rhaleb and G. Plante, 1992, The role of B1- and B2-kinin receptors in the renal tubular and hemodynamic response to bradykinin, Am. J. Physiol. 262, R72.

Madeddu, P., V. Anania, P.P. Parpaglia, M.P. Demontis, V.M. Varoni, G. Pisanu, C. Troffa, G. Tonolo and N. Glorioso, 1992, Effects of Hoe 140, a bradykinin B2-receptor antagonist, on renal function in conscious normotensive rats, Br. J. Pharmacol. 106, 380.

Margolius, H.S., R. Geller, J.J. Pisano and A. Sjoerdsma, 1971, Altered urinary kallikrein excretion in human hypertension, Lancet 2, 1063.

Margolius, H.S., R. Geller, W. De Jong, J.J. Pisano and A. Sjoerdsma, 1972, Altered urinary kallikrein excretion in rats with hypertension, Circ. Res. 30, 358.

Mattson, D. and A. Cowley Jr., 1993, Kinin actions on renal papillary blood flow and sodium excretion, Hypertension 21, 961.

Miyamoto, M., H. Koike, K. Ito and M. Yamazaki, 1981, Effects of captopril on urinary excretion of prostaglandins and electrolytes in spontaneously hypertensive rats, Eur. J. Pharmacol. 76, 187.

Münzel, T., S. Kurz, J. Holtz, R. Busse, H. Steinhauer, H. Just and H. Drexler, 1992, Neurohormonal inhibition and hemodynamic unloading during prolonged inhibition of ANF degradation in patients with severe chronic heart failure, Circulation 86, 1089.

Pham, I., A.-I. El Amrani, M.-C. Fournié-Zaluski, P. Corvol, B. Roques and J.-B. Michel, 1992, Effects of the selective neutral endopeptidase inhibitor, retrothiorphan, on renal function and blood pressure in normotensive and in DOCA-salt hypertensive rats, J. Cardiovasc. Pharmacol. 20, 847.

Pham, I., W. Gonzalez, A.-I. El Amrani, M.-C. Fournié-Zaluski, M. Philippe, I. Laboulandine, B. Roques and J.-B. Michel, 1993, Effects of converting enzyme inhibitor and neutral endopeptidase

inhibitor on blood pressure and renal function in experimental hypertension, J. Pharmacol. Exp. Ther. 265, 1339.

Roques, B., E. Lucas-Soroca, P. Chaillet, J. Costentin and M.-C. Fournié-Zaluski, 1983, Complete differenciation between enkephalinase and angiotensin converting-enzyme inhibition by retro-thiorphan, Proc. Natl. Acad. Sci. USA 80, 3178.

Roques, B., F. Noble, V. Dauge, M.-C. Fournié-Zaluski and A. Beaumont, 1993, Neutral endopeptidase 24.11. Structure, inhibition, experimental and clinical pharmacology, Pharmacol. Rev. 45, 87.

Rouch, A., L. Chen, S. Troutman and J. Schafer, 1991, Na^+ transport in isolated rat CCD: effects of bradykinin, ANP, clonidine, and hydrochlorothiazide, Am. J. Physiol. 260, F86.

Schulz, W.W., H.K. Hagler, L.M. Buja and E.G. Erdös, 1988, Ultrastructural localization of angiotensin I-converting enzyme (EC 3.4.15.1) and the neutral metalloendopeptidase (EC 3.4.24.11) in the proximal tubule of the human kidney, Lab. Invest. 59, 789.

Scicli, A.G. and O.A. Carretero, 1986, Renal kallikrein-kinin system, Kidney Int. 29, 120.

Seymour, A., J. Sheldon, P. Smith, M. Asaad and W. Rogers, 1994, Potentiation of the renal responses to bradykinin by inhibition of neutral endopeptidase 3.4.24.11 and angiotensin-converting enzyme in anesthetized dogs, J. Pharmacol. Exp. Ther. 269, 263.

Siragy, H., 1993, Evidence that intrarenal bradykinin plays a role in regulation of renal function, Am. J. Physiol. 265, E648.

Smits, G.J., D.E. Mc Graw and A.J. Trapani, 1990, Interaction of ANP and bradykinin during endopeptidase 24.11. inhibition: renal effects, Am. J. Physiol. 258, F1417.

Soleilhac, J.-M., E. Lucas, A. Beaumont, S. Turcaud, J.-B. Michel, P. Crine, M.-C. Fournié-Zaluski and B.P. Roques, 1992, A 94 kdalton protein, identified as neutral endopeptidase-24.11, can inactivate atrial natriuretic peptide in vascular tissue, Mol. Pharmacol. 41, 609.

Stoos, B.A., O.A. Carretero and J.L. Garvin, 1992, ANF and bradykinin synergistically inhibits transport in M-1 cortical collecting duct cell line, Am. J. Physiol. 263, F1.

Sybertz, E.J., P.J.S. Chiu, S. Vemupalalli, R. Watkins and M. Haslanger, 1990, Atrial natriuretic factor-potentiating and antihypertensive inhibitor, Hypertension 15, 152.

Tomita, K. and J.J. Pisano, 1984, Binding of (^{3}H)bradykinin isolated nephron segments of the rabbit, Am. J. Physiol. 246, F732.

Trippodo, N., B. Panchal and M. Fox, 1995, Repression of angiotensin II and potentiation of bradykinin contribute to the synergistic effects of dual metalloprotease inhibition in heart failure, J. Pharmacol. Exp. Ther. 272, 619.

Ura, N., O.A. Carretero and E.G. Erdös, 1987, Role of renal endopeptidase 24. 11 in kinins metabolism in vitro and in vivo, Kidney Int. 32, 507.

Wiemer, G. and K. Wirth, 1992, Production of cyclic GMP via activation of B1 and B2 kinin receptors in cultured bovine aortic endothelial cells, J. Pharmacol. Exp. Therap. 262, 729.

Willis, L.R., J.H. Ludens, J.B. Hook and H.E. Williamson, 1969, Mechanism of natriuretic action of bradykinin, Am. J. Physiol. 217, 1.

Wirth, K., F. Hock, U. Albus, W. Linz, H. Alpermann, H. Anagnostopoulos, S. Henke, G. Breipohl, W. König, J. Knolle and B. Schölkens, 1991, Hoe 140 a new potent and long acting bradykinin antagonist: in vivo studies, Br. J. Pharmacol. 102, 774.

Wong, K.R., M.H. Xie, L.B. Shi, F.Y. Liu, C.L. Huang, D.G. Gardner and M.G. Cogan, 1988, Urinary cGMP as a biological marker of the renal activity of atrial natriuretic factor, Am. J. Physiol. 255, F1220.

Zeidel, M., K. Jabs, D. Kikeri and P. Silva, 1990, Kinins inhibit conductive Na^+ uptake by rabbit inner medullary collecting duct cells, Am. J. Physiol. 258, F1584.