ELSEVIER

European Journal of Pharmacology 407 (2000) 317–325

ejp

www.elsevier.nl/locate/ejphar

Vascular catabolism of bradykinin in the isolated perfused rat kidney

Karim Bagaté [a,b], Leyla Develioglu [a], Michèle Grima [a], Wybren De Jong [a], William H. Simmons [c], Jean-Louis Imbs [a,d], Mariette Barthelmebs [a,b,*]

[a] *Institut de Pharmacologie, Faculté de Médecine, 11 Rue Humann, 67085 Strasbourg Cedex, France*
[b] *Laboratoire de Pharmacologie et de Physiologie Rénovasculaire (EMI-0015 INSERM), Faculté de Médecine, Strasbourg, France*
[c] *Department of Molecular and Cellular Biochemistry, Loyola University Chicago, Strich School of Medicine, Maywood, USA*
[d] *Service d'Hypertension Artérielle, Maladies Vasculaires et Pharmacologie Clinique, Hôpital Civil, Strasbourg, France*

Received 12 September 2000; accepted 22 September 2000

Abstract

Kinins in the circulation are rapidly metabolized by several different peptidases. The purpose of this study was to evaluate the contribution of membrane-bound peptidases to kinin metabolism in the renal circulation. Experiments were performed in vitro, in isolated rat kidneys perfused at a constant flow rate (8 ml/min) with Tyrode's solution. The effects of peptidase inhibitors were evaluated on the functional vasodilator response caused by bradykinin (30 nM) or [Tyr(Me)^8]bradykinin (10 nM) via activation of bradykinin B_2 receptors in kidneys precontracted with prostaglandin $F_{2\alpha}$. Angiotensin converting enzyme inhibitors, enalaprilat (3 μM), ramiprilat (1 μM) or lisinopril (1 μM), increased the bradykinin-induced renal vasodilation by 40% or more. Inhibitors of neutral endopeptidase (thiorphan or phosphoramidon, 10 μM), basic carboxypeptidase (DL-2-mercaptomethyl-3-guanidino-ethylthiopropanoic acid or MGTPA, 10 μM) and aminopeptidase P (apstatin, 20 μM) however did not enhance the renal vasodilator response elicited by kinins, whatever tested alone or in the presence of lisinopril. These findings indicate that angiotensin converting enzyme is the major peptidase whose inhibition potentiates the renal bradykinin B_2 receptor mediated vasodilator response of kinins. The relative contribution in this potentiation of inhibition of kinin inactivation and of cross-talk of angiotensin converting enzyme with bradykinin B_2 receptor remains however to be clarified.

Keywords: Bradykinin; Bradykinin B_2 receptor; Carboxypeptidase; Neutral endopeptidase; Angiotensin I converting enzyme; Aminopeptidase P; Renal vasculature, rat

1. Introduction

The kinin family consists of several peptides that contribute to the regulation of blood pressure and regional hemodynamics (Bhoola et al., 1992). Vasorelaxation via the activation of endothelial bradykinin B_2 receptors as well as vasoconstriction via the activation of bradykinin B_1 receptors on vascular smooth muscle cells have been described (Regoli and Barabé, 1980). This complex response pattern is also present in the renal circulation. We previously reported that the main response to bradykinin in the isolated perfused rat kidney consisted of bradykinin B_2 receptor-linked vasodilation while bradykinin B_1 receptor-linked vasoconstriction was time-dependently sensitized (Bagaté et al., 1999a). As in other blood vessels, the bradykinin B_2 receptor-related endothelium-dependent vasodilation was explained by the combined release of nitric oxide, cyclooxygenase-derived prostanoids and a cytochrome P450-derived hyperpolarizing factor (Bagaté et al., 1999c).

Since circulating levels of kinins are low and at least 10 times lower than tissue levels (Campbell et al., 1994), it is believed that bradykinin mainly exerts its physiological effects in an autocrine or paracrine fashion. Tissue peptide concentrations may depend on both local kinin-synthesizing pathways and tissue kininases activity. Endothelial cells are rich in kininogen binding sites and can release

* Corresponding author. Institut de Pharmacologie, Faculté de Médecine, 11 Rue Humann, 67085 Strasbourg Cedex, France. Tel.: +33-3-88-35-87-59; fax: +33-3-88-36-07-15.
E-mail address: mariette.barthelmebs@pharmaco-ulp.u-strasbg.fr (M. Barthelmebs).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00744-5

active kallikrein (Hasan et al., 1994; Nolly and Nolly, 1998). Kallikrein activity has been documented in the renal circulation (Gardes et al., 1990). Kininogen and kallikrein are also abundant in the distal nephron and kinins may diffuse from the renal interstitial fluid to the vascular compartment (Navar et al., 1996).

Kinins are rapidly metabolized by a variety of peptidases which are widely distributed in plasma and most cell types (Skidgel, 1992). Indeed, bradykinin half life is only 15 s in vivo and almost complete inactivation of bradykinin occurs in vitro during a single passage through the lung, the liver and the kidney (Prechel et al., 1995; Griswold et al., 1996, 1999). Membrane-bound peptidases, particularly on the surface of endothelial cells, seem remarkably efficient in the local vascular catabolism of bradykinin. The contribution of angiotensin converting enzyme (kininase II, EC 3.4.15.1) has been extensively documented by the measurement of bradykinin metabolites as well as by the effects of converting enzyme inhibitors on plasma and tissue kinin levels and bradykinin-induced vasodilator responses (Mombouli and Vanhoutte, 1991; Campbell et al., 1994). Recent evidence showed a contribution of aminopeptidase P (EC 3.4.11.9) to kinin metabolism (Orawski and Simmons, 1995). This peptidase has been localized by immunohistochemistry on capillary endothelial cells (Revann et al., 1991). Apstatin, a selective inhibitor of aminopeptidase P, decreased bradykinin catabolism and potentiated kinin-mediated vasodepressor response (Prechel et al., 1995; Kitamura et al., 1999). Less is known about the contribution of basic carboxypeptidases (kininases I) which are present in a plasma soluble form (carboxypeptidase N) and a membrane-bound form (carboxypeptidase M, EC 3.4.17.12) (Skidgel and Erdös, 1998). These enzymes can cleave the C-terminal basic arginine of bradykinin and generate desArg^{9}bradykinin, a selective bradykinin B_1 receptor agonist. Finally, neutral endopeptidase (EC 3.4.24.11) was detected in vascular endothelium and in smooth muscle cells (Graf et al., 1993; Gonzalez et al., 1998).

The aim of the present study was to evaluate the role of the different above mentioned peptidases in the local renal vascular catabolism of kinins. The kidney is rich in kininases and has often been used as a source to purify these enzymes (Gafford et al., 1983; Hooper et al., 1990). Their contribution to the vascular catabolism of kinins has however not clearly been established. Based on measurements of bradykinin metabolites in the venous effluent of the isolated perfused rat kidney, Griswold et al. (1999) recently reported that bradykinin catabolism in the rat kidney in vitro occurs with a major contribution of the aminopeptidase P pathway. Using a similar salt solution perfused kidney, we now investigate the contribution of the various membrane-bound peptidases in the local vascular catabolism of kinins on the basis of their main functional response, the bradykinin B_2 receptor-mediated vasorelaxation. Specific inhibitors of the well-known membrane-bound peptidases involved in kinin catabolism were used for this purpose. Preliminary results have been published as an abstract (Bagaté et al., 1999b).

2. Methods

2.1. Animals

Male Wistar rats (7–8 weeks old, 200–250 g, Janvier breeding, Le Genest St Isle, France) were used. Animals were housed in a room at 20°C with a 12 h light/dark cycle (light on at 6:00 a.m.) and allowed free access to tap water and standard food (AO4 pellets, UAR, Villemoisson/Orge, France). The rats stayed in our animal facility for at least 1 week before experiments. Experiments were performed in accordance with guidelines of the European Community and the French Government concerning the use of animals.

2.2. Preparation of the isolated perfused rat kidney

After sodium pentobarbital anesthesia (45 mg/kg i.p.), the right kidney was prepared with special care to avoid ischemia, and perfused via the mesenteric artery, in an open circuit, as described previously (Barthelmebs et al., 1996; Bagaté et al., 1999a). Briefly, the perfusion medium was a prewarmed (37°C), oxygenated (95% O_2/5% CO_2), colloid free Tyrode's solution of the following composition (mM): NaCl 137; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 1.1; NaH_2PO_4 0.42; $NaHCO_3$ 12; glucose 10; pH was adjusted at 7.4. Following a 45-min equilibration period, perfusion flow was adjusted at 8 ml/min and kept constant thereafter (Gilson Minipuls 3, Bioblock, Illkirch, France). Perfusion pressure was continuously monitored (Statham P23 Db tranducer, Statham Intruments, Hato Rey, Porto Rico) and recorded (Philips PM 8222, Philips, Bobigny, France) throughout the experiment.

2.3. Evaluation of vasodilator responses

The renal vascular tone of the isolated kidney was increased by continuous perfusion of prostaglandin $F_{2\alpha}$ at a concentration (0.5–3 μM) sufficient to induce a steady increase in perfusion pressure of about 20 mm Hg. Bradykinin or [Tyr(Me)^8]bradykinin, a preferential bradykinin B_2 receptor agonist, were subsequently tested at 30 min intervals (two or three experimental periods) on preconstricted kidneys. Such a protocol was previously shown to avoid occurrence of desensitization to the bradykinin B_2 receptor-mediated renal vasodilation (Bagaté et al., 1999a). Acetylcholine and sodium nitroprusside were included as respective markers for endothelium-dependent and -independent vasodilator reactivity (Barthelmebs et al., 1996). Thus, a supramaximal concentration of acetylcholine (30

nM) was tested after each concentration of a bradykinin B_2 receptor agonist and a single supramaximal concentration of sodium nitroprusside (10 μM) was tested at the end of the experiments. The vasodilator responses were evaluated at the maximum of the response and in some cases, every minute during the perfusion of bradykinin. Relaxations are expressed as percentage reversal of the prostaglandin $F_{2\alpha}$-induced preconstriction.

2.4. Evaluation of vasodilator responses in the presence of inhibitors of kinin catabolism

Three protocols were used to test the effects of the inhibitors of kinin metabolism. In the first protocol, a single concentration of bradykinin (30 nM) that caused maximum apparent renal vasorelaxation in the isolated rat kidney under basal conditions, in the absence of ACE inhibitors (Bagaté et al., 1999a) was tested, in the absence (first period) and subsequently in the presence of the inhibitors (second period, perfusion with the inhibitors starting 5 to 15 min before that of the agonist). This protocol was used to evaluate the effects of inhibition of basic carboxypeptidase by DL-2-mercaptomethyl-3-guanidino-ethylthiopropanoic acid (MGTPA, 10 μM), or inhibition of neutral endopeptidase by thiorphan (10 μM) or phosphoramidon (10 μM), or inhibition of aminopeptidase P by apstatin (20 μM), or inhibition of angiotensin converting enzyme by enalaprilat (3 μM) or ramiprilat (1 μM), the active metabolites of enalapril and ramipril. The concentrations of inhibitors used in our study have been shown previously to be efficient in blocking the various peptidases (Regoli et al., 1986; Hooper et al., 1992; Prechel et al., 1995; Zhang et al., 1998).

In the second protocol, bradykinin or [Tyr(Me)^8]-bradykinin were tested at two concentrations (1 and 30 nM, 1 and 10 nM, respectively) at a 30-min interval. Responses obtained under control conditions were compared with responses obtained in other kidneys perfused with the converting enzyme inhibitor, lisinopril (1 μM).

In the third protocol, the effects of sequential inhibition of kininases were analysed on the renal vasodilator response elicited by [Tyr(Me)^8]bradykinin (10 nM). After the responses had been obtained in the presence of lisinopril (1 μM) during a first experimental period, thiorphan, MGTPA or apstatin were added to the perfusate together with lisinopril during the second or third experimental periods.

Acetylcholine and sodium nitroprusside were included in the three protocols as described above.

2.5. Drugs

The following drugs were used: acetylcholine hydrochloride, apstatin trifluoroacetate salt, bradykinin, lisino-

Table 1
Effect of inhibitors of basic carboxypeptidase (MGTPA, 10 μM), neutral endopeptidase (thiorphan or phosphoramidon, 10 μM), angiotensin converting enzyme (enalaprilat, 3 μM; ramiprilat, 1 μM) or aminopeptidase P (apstatin, 20 μM) on the renal vasodilator responses to bradykinin or [Tyr(Me)^8]bradykinin, acetylcholine and sodium nitroprusside

First period		Second period		Final period
Bradykinin (30 nM) (% relaxation)	Acetylcholine (30 nM) (% relaxation)	Bradykinin (30 nM) (% relaxation)	Acetylcholine (30 nM) (% relaxation)	Sodium nitroprusside (10 μM) (% relaxation)
Control (5)		MGTPA		
34.0 ± 2.1	77.1 ± 6.3	25.5 ± 3.3[a]	72.7 ± 8.9	98.2 ± 2.3
Control (6)		Thiorphan		
32.7 ± 2.8	78.3 ± 6.3	34.2 ± 4.4	69.7 ± 7.5	108.9 ± 7.1
Control (6)		Phosphoramidon		
60.5 ± 6.1	77.1 ± 4.2	51.5 ± 5.5[b]	69.4 ± 5.4	103.6 ± 1.2
Control (6)		Enalaprilat		
37.3 ± 3.3	80.4 ± 7.7	52.8 ± 6.6[b]	79.2 ± 5.7	97.3 ± 5.9
Control (7)		Ramiprilat		
32.8 ± 4.2	87.4 ± 4.2	45.9 ± 4.5[a]	85.7 ± 4.3	106.7 ± 4.9
[Tyr(Me)^8]bradykinin (10 nM) (% relaxation)	Acetylcholine (30 nM) (% relaxation)	[Tyr(Me)^8]bradykinin (10 nM) (% relaxation)	Acetylcholine (30 nM) (% relaxation)	Sodium nitroprusside (10 μM) (% relaxation)
Control (4)		Apstatin		
29.1 ± 0.8	81.7 ± 3.7	33.6 ± 1.6	86.8 ± 3.1	95.7 ± 1.8

Bradykinin, [Tyr(Me)^8]bradykinin and acetylcholine were administered in the absence (first period) and in the presence of the inhibitors (second period). Sodium nitroprusside was tested at the end of the second period. Vasorelaxation is expressed as percentage reversal of preconstriction induced by prostaglandin $F_{2\alpha}$. Results are given as means ± S.E.M. with number of experiments given in brackets. Results were statistically analysed by two-way variance analysis on repeated measurements or paired *t*-test.

[a] $P < 0.05$ as compared to responses in the absence of inhibitors (first experimental period).

[b] $P < 0.01$ as compared to responses in the absence of inhibitors (first experimental period).

pril, phosphoramidon, sodium nitroprusside (all from Sigma, St. Quentin Fallavier, France); enalaprilat (Merck Sharp & Dohme Research, Rahway, USA); MGTPA (Calbiochem-Novabiochem, La Jolla, France); prostaglandin $F_{2\alpha}$ thromethamine salt (Dinolytic®, Pharmacia & Upjohn, Guyancourt, France); ramiprilat (Aventis, Frankfurt, Germany); sodium pentobarbital (Nembutal®, Sanofi Santé Animale, Libourne, France); sodium heparinate (Léo, St. Quentin Yvelines, France); thiorphan (Bâle Biochimie, Voisins-les-Bretonneaux, France), [Tyr(Me)^8]bradykinin (Dr. Regoli, Sherbrooke, Canada). All other chemicals were of pro-analysis quality from Merck (Darmstadt, Germany).

Kinins were prepared as stock solutions (1 mg/ml in distilled water), stored in aliquots at −20°C and diluted to the required final concentration with 0.9% saline just prior to use. To avoid adsorption of peptides, perfusion material was coated with a 1% silicone solution (Aquasil, Interchim, Monluçon, France). Inhibitors of kinin metabolism were dissolved in distilled water and freshly prepared before each experiment.

2.6. Statistical analysis

Results are expressed as means ± S.E.M. Differences were tested for statistical significance by the unpaired or paired Student's *t*-test or the two-way variance analysis on repeated measurements when appropriate. Multiple comparisons between groups were performed by Tukey's test or Newman–Keuls test. A *P*-value less than 0.05 was considered significant. All statistics were run with GraphPad Prism (GraphPad, San Diego, USA) or SigmaStat (SPSS, Chicago, USA).

3. Results

3.1. Characteristics of isolated perfused rat kidneys

After pentobarbital anesthesia, overall mean arterial blood pressure of the rats before starting kidney perfusion amounted 89 ± 1.6 mm Hg ($n = 67$). Kidneys were perfused at a fixed flow rate of 8 ml/min, allowing an approximate physiological perfusion pressure of about 77 ± 2.5 mm Hg. Accordingly, calculated renal vascular resistance averaged 10.1 ± 0.2 mm Hg min/ml. The renal vascular tone was increased by 22 ± 0.4 mm Hg at a mean prostaglandin $F_{2\alpha}$ concentration of 1.1 ± 0.1 μM. Bradykinin (30 μM) under basal conditions elicited a mean renal vasorelaxation of 38.7 ± 2.4% (decrease in prostaglandin $F_{2\alpha}$-induced tone), a value consistent with our previous results (Bagaté et al., 1999a). Sodium nitroprusside (10 μM) elicited an overall complete relaxation (100.4 ± 1.3% reversion of prostaglandin $F_{2\alpha}$-induced precontraction), while the response of acetylcholine (30 nM) averaged 81.7 ± 3.1% relaxation.

3.2. Effects of MGTPA on the renal vascular response elicited by bradykinin

The inhibition of basic carboxypeptidase by MGTPA (10 μM) did not potentiate the renal vasodilator response elicited by 30 nM bradykinin (Table 1). Bradykinin-induced vasodilation was even 16% lower during the second experimental period when compared with the first one in the absence of the inhibitor. The vasodilator responses elicited by acetylcholine and sodium nitroprusside in the same kidneys remained unchanged in the presence of MGTPA. MGTPA was devoid of any effect per se on the preconstricted renal vasculature.

3.3. Effects of thiorphan or phosphoramidon on the renal vascular response elicited by bradykinin

Whatever the inhibitor of neutral endopeptidase used (thiorphan or phosphoramidon, 10 μM), no increase in

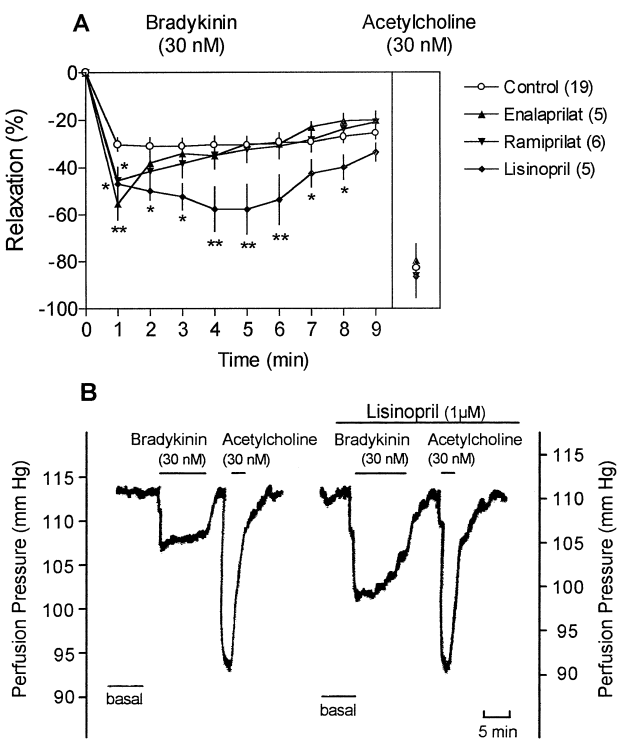

Fig. 1. Effects of angiotensin converting enzyme inhibitors on the renal vasodilator response caused by bradykinin. Time-course of the effects of enalaprilat (3 μM), ramiprilat (1 μM) and lisinopril (1 μM) on the bradykinin-induced vasodilator response are given in the upper part (A). Typical recordings of the vasorelaxation induced by bradykinin (30 nM) in the absence and in the presence of lisinopril (1 μM) are shown in the lower part (B). Basal tone of the isolated perfused kidney was restored by a continuous perfusion of prostaglandin $F_{2\alpha}$. The kinin was perfused during 9 min at a dose achieving a 30 nM concentration in the perfusate. Acetylcholine was used as a marker for endothelium-dependent vascular reactivity. Vasorelaxation is expressed as percentage reversal of preconstriction induced by prostaglandin $F_{2\alpha}$. Results are given by means ± S.E.M. with number of experiments given in brackets. Results were statistically analysed by two-way variance analysis on repeated measurements. $^{*}P < 0.05$, $^{**}P < 0.01$ as compared to responses in the absence of converting enzyme inhibitors (control).

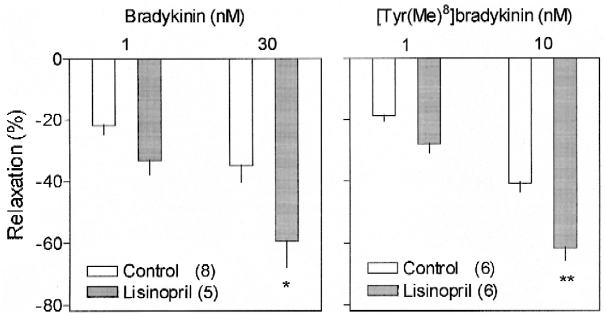

Fig. 2. Effects of lisinopril (1 μM) on the vasodilator responses elicited by bradykinin and [Tyr(Me)^8]bradykinin, a selective bradykinin B_2 receptor agonist. The two peptides were tested in separate experiments. Both of them were administrated in the absence (control) and in the presence of lisinopril in different kidneys. Vasorelaxation is expressed as percentage reversal of preconstriction induced by prostaglandin $F_{2\alpha}$. Results are given by means ± S.E.M. with number of experiments given in brackets. Results were statistically analysed by two-way variance analysis on repeated measurements. $^{*}P < 0.05$, $^{**}P < 0.01$ as compared to responses in the absence of lisinopril.

bradykinin-elicited renal vasodilation was observed (Table 1). Moreover, as also reported above with MGTPA, the vasodilator response to bradykinin was decreased by about 15% in the presence of phosphoramidon although the responses to acetylcholine and sodium nitroprusside were unchanged. The neutral endopeptidase inhibitors were devoid of any effect per se on the preconstricted renal vasculature.

3.4. Effects of enalaprilat and ramiprilat on the renal vascular response elicited by bradykinin

Enalaprilat (3 μM) as well as ramiprilat (1 μM) potentiated the renal vasodilator response elicited by 30 nM bradykinin (Table 1). The kinin-induced vasodilation was enhanced by about 40% in the presence of each converting enzyme inhibitor. This effect particularly occurred at the beginning of the kinin perfusion and faded rapidly thereafter (Fig. 1A). The potentiating effect of enalaprilat and ramiprilat was selective for bradykinin since the vasodilator responses to acetylcholine and sodium nitroprussiate remained unchanged. The angiotensin converting enzyme inhibitors were devoid of any effect per se on the preconstricted renal vasculature.

3.5. Effects of lisinopril on the renal vascular responses elicited by bradykinin and [Tyr(Me)^8]bradykinin

The renal vasodilator response caused by bradykinin was potentiated in the presence of lisinopril (1 μM). A typical recording is shown in Fig. 1B. This potentiation was more marked than observed with enalaprilat or ramiprilat and persisted during the period of kinin perfusion (Fig. 1A). A similar degree of potentiation was observed with lisinopril for the vasodilator response elicited by [Tyr(Me)^8]bradykinin (Fig. 2). The effect of lisinopril was particularly obvious at the highest concentration of bradykinin and [Tyr(Me)^8]bradykinin used where the enhancement of relaxation averaged 65% and 50%, respectively. This effect of lisinopril was selective for kinins since lisinopril failed to modify the renal vasorelaxation induced by acetylcholine (Fig. 1) or sodium nitroprusside (100.2 ± 5.0 vs. 98.9 ± 4.3% in control rats). Lisinopril also had no effect per se on the preconstricted renal vasculature.

3.6. Effects of apstatin on the renal vascular response elicited by [Tyr(Me)^8]bradykinin

The inhibition of aminopeptidase P by apstatin (20 μM) did not potentiate the renal vasodilator response elicited by

Table 2
Effect of combined inhibition of angiotensin converting enzyme (lisinopril, 1 μM) with the inhibition of basic carboxypeptidase (MGTPA, 10 μM), neutral endopeptidase (thiorphan, 10 μM) or aminopeptidase P (apstatin, 20 μM) on the renal vasodilator responses elicited by [Tyr(Me)^8]bradykinin, acetylcholine and sodium nitroprusside

First period		Second or third period		Final period
[Tyr(Me)^8]bradykinin (10 nM) (% relaxation)	Acetylcholine (30 nM) (% relaxation)	[Tyr(Me)^8]bradykinin (10 nM) (% relaxation)	Acetylcholine (30 nM) (% relaxation)	Sodium nitroprusside (10 μM) (% relaxation)
Lisinopril (5)		*Lisinopril + MGTPA*		
35.7 ± 2.7	86.6 ± 1.1	30.8 ± 1.9	79.4 ± 3.2[a]	91.8 ± 2.5
Lisinopril (5)		*Lisinopril + thiorphan*		
38.0 ± 4.0	90.2 ± 1.2	25.7 ± 2.3[b]	85.5 ± 1.5	94.4 ± 1.7
Lisinopril (4)		*Lisinopril + apstatin*		
39.6 ± 2.2	88.7 ± 2.2	37.7 ± 1.5	83.5 ± 2.7	93.5 ± 1.6

[Tyr(Me)^8]bradykinin and acetylcholine were administered in the presence of lisinopril alone (first period) and after combined inhibition of peptidases (second or third period). Sodium nitroprusside was tested at the end of the protocol. Vasorelaxation is expressed as percentage reversal of preconstriction induced by prostaglandin $F_{2\alpha}$. Results are given as means ± S.E.M. with number of experiments given in brackets. Results were statistically analysed by two-way variance analysis on repeated measurements.

[a] $P < 0.05$ as compared to response in the presence of lisinopril alone (first experimental period).

[b] $P < 0.01$ as compared to response in the presence of lisinopril alone (first experimental period).

10 nM [Tyr(Me)^8]bradykinin (Table 1). The vasodilator responses to acetylcholine and sodium nitroprusside in the same kidneys also remained unchanged in the presence of apstatin. Apstatin per se caused a slight concentration-dependent renal vasoconstriction, increasing renal perfusion pressure by 3.7 ± 0.9 and 7.8 ± 1.6 mm Hg, respectively at 10 and 20 μM ($n = 8$).

3.7. Combined effects of lisinopril with MGTPA, thiorphan or apstatin on the renal vascular responses elicited by [Tyr(Me)^8]bradykinin

In the presence of lisinopril, any subsequent inhibition of another peptidase, basic carboxypeptidase by MGTPA (10 μM), neutral endopeptidase by thiorphan (10 μM) or aminopeptidase P by apstatin (20 μM), did not further potentiate the renal vasorelaxation induced by [Tyr(Me)^8]-bradykinin (Table 2). Thiorphan even seemed to decrease the potentiation caused by lisinopril. Also, in the presence of lisinopril, none of the inhibitors caused any effect per se on the preconstricted renal vasculature.

4. Discussion

Using a blood-free Tyrode's solution perfused rat kidney, we evaluated the contribution of various kininases to the renal vascular catabolism of bradykinin. Only membrane-bound peptidases readily accessible to kinins in the circulation were addressed. The contribution of the peptidases was evaluated by changes induced by specific inhibitors in both the preconstricted renal vasculature (considered as basal) and in kinin elicited bradykinin B_2 receptor-mediated renal vasodilator response. Present results show that none of the inhibitors, MGTPA for basic carboxypeptidase, thiorphan and phosphoramidon for neutral endopeptidase, apstatin for aminopeptidase P but also enalaprilat, ramiprilat and lisinopril for angiotensin converting enzyme, was able to induce renal vasorelaxation per se in the isolated kidney whose tone had been previously restored by prostaglandin $F_{2\alpha}$. Since converting enzyme inhibitors have repeatedly been shown in vivo to induce acute renal vasodilation linked to enhanced levels of endogenous bradykinin (Navar et al., 1996), our in vitro kidney model may differ by having a lower rate of endogenous kinin synthesis. This defect may relate to a deficit in kininogen since the addition of kininogen to the perfusate of the isolated rat kidney was able to restore kinin synthesis (Gardes et al., 1990). Species differences may also exist since captopril was able to produce a bradykinin mediated relaxation in isolated dog renal arteries (Malomvölgyl et al., 1995). Unexpectedly, apstatin caused some degree of renal vasoconstriction. Although the underlying mechanism has not been explored, it is noteworthy that neuropeptide Y, a potent renal vasoconstrictor, is a fairly good substrate for aminopeptidase P (Bischoff et al., 1997; Orawski and Simmons, 1995). However, we cannot exclude that under different experimental conditions maintaining the endogenous bradykinin synthesis, apstatin may have a vasodilator effect on the renal blood flow since aminopeptidase P seems particularly efficient in degrading endogenous kinins (Kitamura et al., 1999).

The peptidase inhibitors differently affected the vascular response to exogenous bradykinin. Only inhibitors of angiotensin converting enzyme did potentiate the kinin-induced renal vasorelaxation when used alone. The potentiating effect of lisinopril was similar for [Tyr(Me)^8]-bradykinin, the selective bradykinin B_2 receptor agonist. Substitution of Phe^8 by Tyr(Me)^8 in bradykinin does not modify its inactivation by angiotensin converting enzyme (Prof. D. Regoli, personal communication). The contribution of angiotensin converting enzyme to the vascular catabolism of kinins has been documented. It accounted for nearly 70% of bradykinin metabolism in the rat lung, heart and coronary circulations as shown by the measurements of [^{3}H]bradykinin and its metabolites in the absence and presence of converting enzyme inhibitors (Prechel et al., 1995; Dendorfer et al., 1997; Ersahin and Simmons, 1997). The contribution of angiotensin converting enzyme (28%) may however have been underestimated in the study of Griswold et al. (1999) using an isolated perfused kidney since kinin metabolites generated by converting enzyme can be further degraded by aminopeptidase P at the N-terminal site (Orawski and Simmons, 1995). Bradykinin is a good substrate for angiotensin converting enzyme, even better than angiotensin I itself with $kcat/K_m$ of 1250 and 125 min^{-1} μM^{-1}, respectively (Erdös and Skidgel, 1985). Bradykinin-(1–7) was detected in the kidney at a concentration of about 50% of the parent peptide (Campbell et al., 1994). Moreover, angiotensin converting enzyme inhibitors increased renal levels of kinins in vivo (Campbell et al., 1994; Matsuda et al., 1999) and potentiated the renal vasodilation to intrarenal bradykinin (Seymour et al., 1994). Furthermore, the renal vasodilator response elicited by converting enzyme inhibitors was partially blocked by icatibant, a bradykinin B_2 receptor antagonist, also suggesting a role of kinins in this response (Fitzgibbon et al., 1996; Heller et al., 1997).

The three angiotensin converting enzyme inhibitors tested in our study caused a similar degree of potentiation of the bradykinin elicited vasodilation but differed in the duration of their effect although they were all used at concentrations completely inhibiting kidney angiotensin converting enzyme activity (Hooper et al., 1992). Some inhibition of aminopeptidase P has been reported with enalaprilat and ramiprilat but not with lisinopril (Hooper et al., 1992) and also not when bradykinin was used as substrate (Orawski and Simmons, 1995; Lloyd et al., 1996).

Besides the protection of kinins from degradation, converting enzyme inhibitors may also potentiate bradykinin mediated vasodilation by a cross-talk of angiotensin con-

verting enzyme with endothelial bradykinin B_2 receptor. Enalaprilat was shown to enhance bradykinin binding, to maintain bradykinin B_2 receptors in a high affinity state and to block receptor desensitization and internalization (Minshall et al., 1997; Benzing et al., 1999). Experiments on cultured cells transfected with cDNA of the human bradykinin B_2 receptor provided evidence that angiotensin converting enzyme inhibitors did not directly interact with the bradykinin B_2 receptor but potentiated bradykinin effects only when cells coexpressed the angiotensin converting enzyme, possibly forming heterodimers (Minshall et al., 1997; Marcic et al., 2000). The potentiation of bradykinin was observed with agents interacting with a single active site of the peptidase, on the N-domain or on the C-domain (Marcic et al., 1999) but did not require the cytosolic and transmembrane domains of angiotensin converting enzyme (Marcic et al., 2000). This property of converting enzyme inhibitors to stabilize bradykinin B_2 receptor probably contributes to the enhanced vascular response to bradykinin occurring in our experiments. Testing RMP-7, a bradykinin B_2 receptor agonist resistant to peptidases (Shimuta et al., 1999), would be useful in the future to elucidate this point.

Present results also show that the inhibition of aminopeptidase P by apstatin does not modify renal vasodilation to exogenous [Tyr(Me)^8]bradykinin. This kinin which does not differ from bradykinin at the N-terminal side may also be a substrate of aminopeptidase P (Orawski and Simmons, 1995). Apstatin (20 μM) has been shown to cause maximal survival of kinins in the isolated rat lung in the presence of a converting enzyme inhibitor (Prechel et al., 1995). Bradykinin is one of the best endogenous substrates for aminopeptidase P although kinetic parameters show that hydrolysis occurs at a lower efficiency than with angiotensin converting enzyme ($k\text{cat}/K_m = 34$ min^{-1} μM^{-1}; Orawski and Simmons, 1995). The identification of the kinin metabolites in the venous effluent of isolated perfused organs combined with the use of enzyme inhibitors allowed to evaluate its contribution to 30% in kinin degradation in the rat pulmonary and coronary circulations (Prechel et al., 1995; Ersahin and Simmons, 1997). In the isolated rat kidney however, a majority of bradykinin metabolites was recovered as the final fragments of the inactivation steps involving aminopeptidase P (Griswold et al., 1999). The conclusion of the authors that aminopeptidase P is the major operational bradykinin degrading pathway in the isolated kidney, seems right from a biochemical point of view but includes cleavage by aminopeptidase P of metabolites, bradykinin-(1–7) and bradykinin-(1–5), generated by angiotensin converting enzyme. Since these metabolites are already functionally inactive, this may explain discrepancies between our results and those of Griswold et al. (1999). Since apstatin has been shown to become functionally effective only after converting enzyme inhibition on various responses to exogenous bradykinin (Kitamura et al., 1999; Kim et al., 2000), we also tested its effects in the presence of lisinopril but without any change in the kinin elicited renal vasodilation.

Neutral endopeptidase is the third enzyme of which we analysed the possible contribution in the renal vascular catabolism of kinins. However, neither phosphoramidon nor thiorphan, relative selective inhibitors of neutral endopeptidase, enhanced the renal vasodilator response to bradykinin when tested alone or after inhibition of converting enzyme by lisinopril. Neutral endopeptidase activity has been characterized in the three layers of aorta, adventitia, smooth muscle and endothelial cells (Gonzalez et al., 1998). However, although it seems to participate to some extent in bradykinin degradation in cultured endothelial cells (Graf et al., 1993; Gonzalez et al., 1998), its inhibition did not affect kinin metabolism in the perfused rat coronary circulation (Dumoulin et al., 1998), or in the isolated perfused heart (Dendorfer et al., 1997). The kidney is particularly rich in neutral endopeptidase (Gafford et al., 1983). Inhibition of the enzyme has been shown to increase the urinary excretion of intact bradykinin but did not affect renal tissue level of kinins (Ura et al., 1987; Nomura et al., 1995; Campbell et al., 1998). Neutral endopeptidase has mainly been localized along the proximal and distal nephron from where it is secreted into tubular fluid (Roques et al., 1993; Casarini et al., 1999) and contributes to 68% of kinin inactivation in urine (Ura et al., 1987). In agreement with the lack of a significant vascular effect of thiorphan and phosphoramidon in our study, neutral endopeptidase inhibitors did not affect renal hemodynamics in anesthetized dogs or rats (Ura et al., 1987; Seymour et al., 1994; Takagawa et al., 1999). They however enhanced urinary bradykinin concentration and salidiuresis (Nomura et al., 1995). The high K_m of neutral endopeptidase for bradykinin (120 μM; Gafford et al., 1983) also means that the enzyme may be active at rather high concentrations of kinin which probably occur only in urine.

Finally, we investigated the contribution of basic carboxypeptidase M in renal vascular kinin degradation. Selective inhibition of the enzyme by MGTPA was however without effect on bradykinin induced vasorelaxation, independent of the fact that angiotensin converting enzyme had been inhibited or not. MGTPA was also inactive in bradykinin catabolism in cultured human umbilical endothelial cells (Graf et al., 1993) suggesting that carboxypeptidase M may not contribute significantly to vascular kinin metabolism. The enzyme, also present in the distal tubule (Casarini et al., 1999), seems however to contribute to about 9% of kinin degradation in urine (Ura et al., 1987).

Taken together, our results based on a functional response in the isolated perfused rat kidney, indicate that only inhibitors of angiotensin converting enzyme were able to potentiate kinin-mediated vasodilation. The relative contribution in this potentiation of inhibition of kinin inactivation and of cross-talk of angiotensin converting

enzyme with bradykinin B_2 receptor remains however to be clarified. Our results also demonstrate that the other degradation pathways of kinins via aminopeptidase P, neutral endopeptidase or basic carboxypeptidase M seem silent for the renal vascular response to bradykinin. These differences probably are related to differences in amounts of enzyme present in the renal vasculature in addition to differences in affinity for kinin substrates.

Acknowledgements

The authors are indebted to Prof. D. Regoli and Dr. F. Gobeil (Medical School, Sherbrooke, Canada) for the gift of [Tyr(Me)^8]bradykinin, to Dr. B. Schölkens (Aventis, Frankfurt, Germany) for the gift of ramiprilat and to Mr. Arfi (Pharmacia & Upjohn, Guyancourt, France) for the gift of Dinolytic®. Mariette Barthelmebs is a Research Director in the C.N.R.S.

References

Bagaté, K., Develioglu, L., Imbs, J.L., Michel, B., Helwig, J.J., Barthelmebs, M., 1999a. Vascular kinin B1 and B2 receptor-mediated effects in the rat isolated perfused kidney. Br. J. Pharmacol. 128, 1643–1650.

Bagaté, K., Grima, M., De Jong, W., Imbs, J.L., Barthelmebs, M., 1999b. Bradykinin (BK) vascular catabolism in the isolated rat kidney. Fundam. Clin. Pharmacol. 13 (Suppl. 1), 155s.

Bagaté, K., Grima, M., De Jong, W., Helwig, J.J., Imbs, J.L., Barthelmebs, M., 1999c. Signal transduction pathways involved in kinin B2 receptor stimulation in the isolated rat kidney. Fundam. Clin. Pharmacol. 13 (Suppl. 1), 254s.

Barthelmebs, M., Krieger, J.P., Grima, M., Nisato, D., Imbs, J.L., 1996. Vascular effects of [Arg^8]vasopressin in the isolated perfused rat kidney. Eur. J. Pharmacol. 314, 325–332.

Benzing, T., Fleming, I., Blaukat, A., Müller-Esterl, W., Busse, R., 1999. Angiotensin-converting enzyme inhibitor ramiprilat interferes with the sequestration of the B2 kinin receptor within the plasma membrane of native endothelial cells. Circ. 99, 2034–2040.

Bhoola, K.D., Figueroa, D., Worthy, K., 1992. Bioregulation of kinins: kallicreins, kininogens, and kininases. Pharmacol. Rev. 44, 1–80.

Bischoff, A., Avramidis, P., Erdbrügger, W., Münter, K., Michel, M.C., 1997. Receptor subtypes Y_1 and Y_5 are involved in the renal effects of neuropeptide Y. Br. J. Pharmacol. 120, 1335–1343.

Campbell, D.J., Kladis, A., Duncan, A.M., 1994. Effects of converting enzyme inhibitors on angiotensin and bradykinin peptides. Hypertension 23, 439–449.

Campbell, D.J., Anastasopoulos, F., Duncan, A.M., James, G.M., Kladis, A., Briscoe, T.A., 1998. Effects of neutral endopeptidase inhibition and combined angiotensin converting enzyme and neutral endopeptidase inhibition on angiotensin and bradykinin peptides in rats. J. Pharmacol. Exp. Ther. 287, 567–577.

Casarini, D.E., Boim, M.A., Stella, R.C.R., Schor, N., 1999. Endopeptidases (kininases) are able to hydrolyze kinins in tubular fluid along the rat nephron. Am. J. Physiol. 277, F66–F74.

Dendorfer, A., Wolfrum, S., Wellhoner, P., Korsman, K., Dominiak, P., 1997. Intravascular and interstitial degradation of bradykinin in isolated perfused rat heart. Br. J. Pharmacol. 122, 1179–1187.

Dumoulin, M.J., Adam, A., Blais, C., Lamontagne, D., 1998. Metabolism of bradykinin by the rat coronary vascular bed. Cardiovasc. Res. 38, 229–236.

Erdös, E.G., Skidgel, R.A., 1985. Structure and functions of human angiotensin I converting enzyme (kininase II). Biochem. Soc. Trans. 13, 42–44.

Ersahin, C., Simmons, W.H., 1997. Inhibition of both aminopeptidase P and angiotensin-converting enzyme prevents bradykinin degradation in the rat coronary circulation. J. Cardiovasc. Pharmacol. 30, 96–101.

Fitzgibbon, W.R., Jaffa, A.A., Mayfield, R.K., Ploth, D.W., 1996. Role of kinins in the renal response to enalaprilat in normotensive and hypertensive rats. Hypertension 27, 235–244.

Gafford, J.T., Skidgel, R.A., Erdös, E.G., Hersh, L.B., 1983. Human kidney «enkephalinase», a neutral metalloendopeptidase that cleaves active peptides. Biochemistry 22, 3265–3271.

Gardes, J., Baussant, P., Corvol, P., Menard, J., Alhenc-Gelas, F., 1990. Effect of bradykinin and kininogens in isolated rat kidney vasoconstricted by angiotensin II. Am. J. Physiol. 258, F1273–F1281.

Gonzalez, W., Soleilhac, J.M., Fournie-Zaluski, M.C., Roques, B.P., Michel, J.B., 1998. Characterization of neutral endopeptidase in vascular cells, modulation of vasoactive peptide levels. Eur. J. Pharmacol. 345, 323–331.

Graf, K., Grafe, M., Bossaller, C., Niehus, J., Schulz, K.D., Auch-Schwelk, W., Fleck, E., 1993. Degradation of bradykinin by neutral endopeptidase (EC 3.4.24.11) in cultured human endothelial cells. Eur. J. Clin. Chem. Clin. Biochem. 31, 267–272.

Griswold, J.A., Beall, C.V., Baker, C.R.F., Little, D.T., Little, G.H., Behal, F.J., 1996. Bradykinin metabolism in the liver and lung of the rat. J. Surg. Res. 66, 12–20.

Griswold, J.A., Baker, C.R.F., Little, D.T., Little, G.H., Behal, F.J., 1999. Bradykinin metabolism in perfused rat kidney. Shock 11, 372–379.

Hasan, A.A.K., Cines, D.B., Zhang, J., Schmaier, A.H., 1994. The carboxyl terminus of bradykinin and amino terminus of the light chain of kininogens comprise an endothelial cell binding domain. J. Biol. Chem. 269, 31822–31830.

Heller, J., Kramer, H.J., Horacek, V., 1997. Comparative effects of the angiotensin II receptor blocker EXP 3174 and of the angiotensin-converting enzyme inhibitor captopril on renal glomerular hemodynamics in the dog. Kidney Blood Pressure Res. 20, 391–397.

Hooper, N.M., Hryszko, J., Turner, A.J., 1990. Purification and characterization of pig kidney aminopeptidase P: a glycosyl-phosphatidyl-inositol-anchored ectoenzyme. Biochem. J. 267, 509–515.

Hooper, N.M., Hryszko, J., Oppong, S.Y., Turner, A.J., 1992. Inhibition by converting enzyme inhibitors of pig kidney aminopeptidase P. Hypertension 19, 281–285.

Kim, K.S., Kumar, S., Simmons, W.H., Brown, N.J., 2000. Inhibition of aminopeptidase P potentiates wheal response to bradykinin in angiotensin-converting enzyme inhibitor-treated humans. J. Pharmacol. Exp. Pharmacol. 292, 295–298.

Kitamura, S.I., Carbini, L.A., Simmons, W.H., Scicli, A.G., 1999. Effects of aminopeptidase P inhibition on kinin-mediated vasodepressor responses. Am. J. Physiol. 276, H1664–H1671.

Lloyd, G.S., Hryszko, J., Hooper, N.M., Turner, A.J., 1996. Inhibition and metal ion activation of pig kidney aminopeptidase P. Dependence on nature of substrate. Biochem. Pharmacol. 52, 229–236.

Malomvölgyl, B., Koltai, M.Z., Hadhazy, P., Pogatsa, G., Magyar, K., 1995. Captopril produces endothelium-dependent relaxation of dog isolated renal arteries. Potential role of bradykinin. Arch. Int. Pharmacodyn. 330, 39–52.

Marcic, B., Deddish, P.A., Jackman, H.L., Erdös, E.G., 1999. Enhancement of bradykinin and resensitization of its B_2 receptor. Hypertension 33, 835–843.

Marcic, B., Deddish, P.A., Skidgel, R.A., Erdös, E.G., Minshall, R.D., Tan, F., 2000. Replacement of the transmembrane anchor in angiotensin I-converting enzyme (ACE) with a glycosylphosphatidylinositol tail affects activation of the B_2 bradykinin receptor by ACE inhibitors. J. Biol. Chem. 275, 16110–16118.

Matsuda, H., Hayashi, K., Arakawa, K., Naitoh, M., Kubota, E., Honda, M., Matsumoto, A., Suzuki, H., Yamamoto, T., Kajiya, F., Saruta, T., 1999. Zonal heterogeneity in action of angiotensin-converting enzyme

inhibitors on renal microcirculation: role of intrarenal bradykinin. J. Am. Soc. Nephrol. 10, 2272–2282.

Minshall, R.D., Tan, F., Nakamura, F., Rabito, S.F., Becker, R.P., Marcic, B., Erdös, E.G., 1997. Potentiation of the actions of bradykinin by angiotensin-converting enzyme inhibitors. The role of expressed human bradykinin B_2 receptors and angiotensin-converting enzyme in CHO cells. Circ. Res. 81, 848–856.

Mombouli, J.V., Vanhoutte, P.M., 1991. Kinins and endothelium-dependent relaxations to converting enzyme inhibitors in perfused canine arteries. J. Cardiovasc. Pharmacol. 18, 926–927.

Navar, L.G., Inscho, E.W., Majid, D.S.A., Imig, J.D., Harrison-Bernard, L.M., Mitchell, K.D., 1996. Paracrine regulation of renal microcirculation. Physiol. Rev. 76, 425–536.

Nolly, H., Nolly, A., 1998. Release of endothelial-derived kallikrein, kininogen and kinins. Biol. Res. 31, 169–174.

Nomura, N., Shimamoto, K., Ura, N., Iwata, M., Aoyama, T., Takagawa, Y., Iimura, O., 1995. The role of kinins and atrial natriuretic peptide on the renal effects of neutral endopeptidase inhibitor in normotensive and hypertensive rats. Clin. Exp. Hypertens. 17, 1219–1231.

Orawski, A.T., Simmons, W.H., 1995. Purification and properties of membrane-bound aminopeptidase P from rat lung. Biochemistry 34, 11227–11236.

Prechel, M.M., Orawski, A.T., Maggiora, L.L., Simmons, W.H., 1995. Effect of a new aminopeptidase P inhibitor, apstatin, on bradykinin degradation in the rat lung. J. Pharmacol. Exp. Ther. 275, 1136–1142.

Revann, T.R., Ryan, J.W., Berryer, P., Valido, F., 1991. Aminopeptidase P is disposed on guinea-pig vascular endothelium and some epithelia. Fed. Am. Soc. Exp. Biol. J. 5, A1579.

Regoli, D., Barabé, J., 1980. Pharmacology of bradykinin and related kinins. Pharmacol. Rev. 32, 1–38.

Regoli, D., Drapeau, G., Rovero, P., Dion, S., Rhaleb, N.E., Barabé, J., D'Orléans-Juste, P., Ward, P., 1986. Conversion of kinins and their antagonists into B_1 receptor activators and blockers in isolated vessels. Eur. J. Pharmacol. 127, 219–224.

Roques, B.P., Noble, P., Dauge, V., Fournié-Zaluski, M.C., Beaumont, A., 1993. Neutral endopeptidase 24-11: structure, inhibition, and experimental and clinical pharmacology. Pharmacol. Rev. 45, 87–146.

Seymour, A.A., Sheldon, J.H., Smith, P.L., Asaad, M., Rogers, W.L., 1994. Potentiation of 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, 263–270.

Shimuta, S.I., Barbosa, A.M.R.B., Borges, A.C.R., Paiva, T.B., 1999. Pharmacological characterization of RMP-7, a novel bradykinin agonist in smooth muscle. Immunopharmacology 45, 63–67.

Skidgel, R.A., 1992. Bradykinin-degrading enzymes: structure, function, distribution, and potential roles in cardiovascular pharmacology. J. Cardiovasc. Pharmacol. 20 (Suppl. 9), S4–S9.

Skidgel, R.A., Erdös, E.G., 1998. Cellular carboxypeptidases. Immunol. Rev. 161, 129–141.

Takagawa, Y., Ura, N., Nanba, M., Agata, J., Higashiura, K., Torii, T., Takada, M., Shimamoto, K., 1999. The contribution of nitric oxide to diuretic and natriuretic effects of renal kinins in normotensive rats. Hypertens. Res. 22, 129–134.

Ura, N., Carretero, O.A., Erdös, E.G., 1987. Role of renal endopeptidase 24.11 in kinin metabolism in vitro and in vivo. Kidney Int. 32, 507–513.

Zhang, X., Nasjletti, A., Xu, X., Hintze, T.H., 1998. Neutral endopeptidase and angiotensin-converting enzyme inhibitors increase nitric oxide production in isolated canine coronary microvessels by a kinin-dependent mechanism. J. Cardiovasc. Pharmacol. 31, 623–629.