Cardiovascular effects of intrathecally administered bradykinin in the rat: characterization of receptors with antagonists

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- 1 The effects of intrathecal (i.t.) pretreatment with selective B₁ or B₂ kinin receptor antagonists were studied on the cardiovascular response to i.t. injection of bradykinin (BK) in conscious freely moving rats.
- 2 BK (81 pmol) produced an increase in mean arterial pressure (MAP: 9–13 mmHg) and decrease in heart rate (HR: 20–30 beats min⁻¹) that reached a maximum 2 min after injection.
- 3 The BK-induced cardiovascular responses were dose-dependently and reversibly reduced by four antagonists with the following rank order of potency: Tyr, D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK = D-Arg[Tyr³,D-Phe⁷,Leu⁸]-BK = D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK > D-Arg[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK (Hoe 140). These compounds failed to alter the cardiovascular response to i.t. injection of 8.1 nmol of substance P.
- 4 Other compounds acting on the B₂ receptor, namely D-Arg[Hyp³,Gly⁶,Leu⁸]-BK, D-Arg[Hyp³,D-Phe⁷]-BK, D-Arg[Hyp³,Thi⁵,D-Phe⁷]-BK and D-Arg[Hyp³,Gly⁶,D-Phe⁷,Leu⁸]-BK or on the B₁ receptor, [Leu⁸]-desArg⁹-BK, did not influence the cardiovascular responses to BK at doses devoid of intrinsic activity on MAP and HR.
- 5 None of the kinin receptor antagonists caused motor impairment, respiratory arrest or persisting cardiovascular changes.
- 6 These results confirm that the cardiovascular effects induced by i.t. BK are mediated by the activation of a B₂ receptor in the rat spinal cord. However, the rank order of potency of antagonists does not conform to the classical B₂ functional site characterized in peripheral tissues.

Keywords: Bradykinin; kinin antagonists; kinin receptor subtypes; spinal cord; cardiovascular responses

Introduction

Compelling evidence suggests that bradykinin (BK) is a mediator of pain and inflammation in the periphery (Regoli & Barabé, 1980; Marceau *et al.*, 1983) and a neuromodulator in the central nervous system (Corréa *et al.*, 1979; Diz, 1985; Lopes & Couture, 1992; Lopes *et al.*, 1992; 1993). The biological activities of BK and of other naturally-occurring kinins (kallidin and T-kinin) are considered to be mediated primarily by two BK receptor types denoted as B₁ and B₂, of which B₁ is induced *de novo* following tissue injury or inflammation (Marceau *et al.*, 1983; deBlois *et al.*, 1989) whereas the B₂ receptor is found to subserve the majority of physiological functions under normal conditions (Regoli, 1984; Taylor *et al.*, 1989; Bathon & Proud, 1991). The B₂ receptor was recently cloned from a rat uterus cDNA library; the deduced amino acid sequence (366 residues) contains seven putative membrane-spanning domains and shows a sequence similarity to members of the G protein-coupled receptor family (McEachern *et al.*, 1991).

Autoradiographic studies (Steranka *et al.*, 1988; Lopes *et al.*, 1993) and binding assays (Fujiwara *et al.*, 1989; Sharif & Whiting, 1991) have revealed the presence of BK binding sites (identified as B₂ receptors) in the guinea-pig spinal cord. Immunoreactive BK has been found to be present in the central nervous system including the spinal cord and the cerebrospinal fluid of different species such as rats, dogs and man (Scicli *et al.*, 1984; Perry & Snyder, 1984; Thomas *et al.*, 1984; Kariya *et al.*, 1985; Hermann *et al.*, 1986). Moreover, the concentrations of BK, T-kinin and kininogen were found to be increased in the rat spinal cord after mechanical injury

(Xu *et al.*, 1991). Recently, we have shown that application of BK intrathecally in conscious rats leads to an increase in arterial blood pressure by activating the sympathoadrenal system. The accompanying bradycardia was ascribed to a vagal reflex involving sensory C-fibres (Lopes & Couture, 1992). It was suggested that the cardiovascular effect of BK is mediated by the activation of a B₂ receptor in the spinal cord as des-Arg⁹-BK, a highly selective agonist at B₁ receptors, was much less potent than BK, kallidin and T-kinin which were equipotent (Lopes & Couture, 1992). However, the B₂ receptor hypothesis needs to be confirmed by the use of selective antagonists. [Leu⁸]-desArg⁹-BK is a potent and competitive antagonist of the B₁ receptor but inactive on the B₂ receptor (Regoli & Barabé, 1980). Among the B₂ receptor antagonists described so far, Hoe 140 (D-Arg[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK) is considered to be the most potent in several *in vitro* and *in vivo* assays (Hock *et al.*, 1991; Wirth *et al.*, 1991; Lembeck *et al.*, 1991), and is selective for B₂ sites since it is inactive at B₁ receptors (Rhaleb *et al.*, 1991). Nevertheless, several studies using new selective antagonists have highlighted the possible existence of subclasses of B₂ receptors in the periphery (Llona *et al.*, 1987; Rifo *et al.*, 1987; Braas *et al.*, 1988; Steranka *et al.*, 1988; Plevin & Owen, 1988; Taylor *et al.*, 1989; Stewart & Vavrek, 1990; Rhaleb *et al.*, 1991) as well as in the central nervous system (Sharif & Whiting, 1991; Seguin *et al.*, 1992). More recently, two new kinin functional sites denoted as B_{2A} (in the rabbit jugular vein) and B_{2B} (in the guinea-pig ileum) have been described (Regoli *et al.*, 1992).

In the present study, selective antagonists for the B₁ and B₂ receptors were used in an attempt to characterize the kinin receptor subtype which mediates the spinal action of BK on the cardiovascular system of conscious freely moving rats.

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Methods

Animal preparation

Male Wistar rats (Charles River, St-Constant, Qué., Canada) weighing 200–250 g were anaesthetized with an intraperitoneal (i.p.) injection of 65 mg kg⁻¹ sodium pentobarbitone (Nembutal, M.T.C. Pharmaceuticals, Mississauga, Ont., Canada) and implanted with two polyethylene tubes (Intramedic, Clay Adams, NJ, U.S.A.) as described previously (Hasséssian *et al.*, 1988). Briefly, the first catheter (PE-50) filled with physiological saline (0.9% w/v NaCl) containing sodium heparin (100 iu ml⁻¹; Sigma, St. Louis, MO, U.S.A.) was inserted into the medial tail artery. The second catheter (PE-10) was inserted into the spinal subarachnoid space via an incision made in the dura at the atlanto-occipital junction and projected to the 9th thoracic segment (T-9).

Following surgery, the animals were housed individually in a plastic cage for a 24 h recuperation period. The catheters were drawn through the grid cage top and supported upright so that the animals were capable of moving freely in the cage and had free access to food pellets and tap water. Only rats which presented normal behaviour and no apparent motor dysfunction were used in the study. The correct positioning of the i.t. catheter was verified by post-mortem examination at the end of experiments.

Monitoring of cardiovascular parameters

Experiments were performed, 24 h after surgery, in conscious freely moving rats. Water bottles were removed from resident cages at the start of experiments and the cage was covered with an opaque cloth to eliminate visual stimuli during the course of the experiment. Arterial blood pressure was recorded from the intra-arterial catheter connected to a Statham pressure transducer (P231D). Heart rate (HR) was measured with a cardiac tachograph (model 7P4) triggered by the arterial pressure pulses. Both signals were monitored on a Grass polygraph model 79D (Quincy, Mass., U.S.A.).

Experiments started when animals were in a resting state with a stable basal blood pressure and HR. The rats were then challenged with an i.t. injection of 30 µl of artificial cerebrospinal fluid (CSF, aqueous solution of (mM): NaCl 128.6, KCl 2.6, MgCl₂ 2.0 and CaCl₂ 1.4; pH adjusted to 7.2–7.4). The catheter void volume was 20 µl. Only rats that did not exhibit changes of blood pressure and HR to i.t. injection of CSF for 30 min were used further in the study.

Experimental protocol

After testing CSF, the animals were injected with 81 pmol BK in a volume of 10 µl. The peptide and the CSF flush (20 µl) were given within a total period of 60 s. This dose of BK was selected from a dose-response curve determined in a previous study (Lopes & Couture, 1992). The kinin receptor antagonists were initially administered i.t. in a single dose of 0.7–0.8 nmol (1.0 µg), 2–3 min prior to the second i.t. injection of 81 pmol BK. This period corresponds to the time required for BK to reach its maximal cardiovascular response. BK injections were given 30 min apart and no tachyphylaxis was observed under these experimental conditions (Lopes & Couture, 1992). The response to BK in the presence of an antagonist was compared with that obtained in the absence of antagonist as measured in the same rat. If no blockade was observed, the rats were challenged a second time with the same antagonist at a dose 10 fold higher. On the other hand, if the cardiovascular response to BK was significantly reduced, BK was injected alone 1 h later to determine whether the antagonist blocks in a reversible manner. If the response to BK was entirely reversible, the same antagonist was tested either at higher or lower doses in random order against the same dose of BK (81 pmol). In other rats, the blocking antagonists were tested the same way

against 8.1 nmol substance P (SP) to determine their specificity with respect to BK receptors. The potential residual agonistic activities of increasing doses of kinin antagonists were measured for 30 min in separate experiments. Care was taken to detect behavioural or toxic effects by the antagonists.

Maximal changes in MAP and HR have been calculated from the differences between the recordings made 1 min before peptide injection and the maximal values obtained within 3 min after injection.

Peptides

[Leu⁸]-desArg⁹-BK, D-Arg[Hyp³,Gly⁶,D-Phe⁷,Leu⁸]-BK, D-Arg[Hyp²,Thi^{5,8},D-Phe⁷]-BK, D-Arg[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK (Hoe 140), D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK, D-Arg[Tyr³,D-Phe⁷,

Figure 1 Effects of various bradykinin (BK) antagonists on the maximal changes in mean arterial pressure (MAP; a) and heart rate (HR; b) induced by the i.t. injection of 81 pmol BK. BK was injected before (□) and 2–3 min after (▨) the intrathecal injection of D-Arg[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK (Ana₄), D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK (Ana₅), D-Arg[Tyr³,D-Phe⁷,Leu⁸]-BK (Ana₆) or Tyr,D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK (Ana₇) at the indicated doses. Each point represents the mean ± s.e. mean results of 8–10 rats in each group. The statistical significance of the differences between CSF (□) and BK was calculated with the ANOVA and the Dunnett test as indicated by **P* < 0.05; ***P* < 0.01. The statistical significance of the differences between values before and after treatment with the antagonist was calculated with Student's *t* test for paired samples: †*P* < 0.05; ††*P* < 0.01. Respective baseline MAP (mmHg) and HR (beats min⁻¹) were 86.1 ± 2.8 and 340 ± 22 for Ana₄, 91.4 ± 2.9 and 345 ± 12 for Ana₅, 90.5 ± 2.3 and 349 ± 16 for Ana₆, 89.5 ± 4.0 and 373 ± 25 for Ana₇.

Leu⁸]-BK, Tyr,D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK, D-Arg[Hyp³,D-Phe⁷]-BK, and D-Arg[Hyp³,Gly⁶,Leu⁸]-BK were used in the present study. The selectivity and antagonist activity of these compounds have been published elsewhere (Rhaleb *et al.*, 1991; Regoli *et al.*, 1992). SP, BK and [Leu⁸]-desArg⁹-BK were purchased from Hükabel Scientific Ltd. (Montréal, Québec, Canada). Hoe 140 was made available from Hoechst AG (Frankfurt, Germany). All other peptides were synthesized by the solid-phase method and purified by high-performance liquid chromatography (Drapeau & Regoli, 1988). Their structure was assessed by fast atomic bombardment (FAB) mass spectrometry. All peptides were dissolved directly in CSF and stock solutions (1 mg ml⁻¹) were divided into 100 µl aliquots and stored at -20°C until used. Daily dilutions were made in CSF.

Statistical analysis of data

The results are expressed as means ± s.e.mean. Statistical significance of differences were evaluated with Student's *t* test for paired samples. A one-way analysis of variance (ANOVA) with a *post-hoc* Dunnett test was used for multiple comparisons with one control group. Only probability values (*P*) less than 0.05 were considered to be statistically significant.

Results

Cardiovascular response to bradykinin administered intrathecally

The i.t. administration of 81 pmol BK, at the T-9 spinal cord level to freely moving rats, caused transient increases in mean arterial blood pressure (MAP) of 9–13 mmHg and decreases in heart rate (HR) of 20–30 beats min⁻¹ that reached a maximum 2 min after injection (Figure 1). At this dose of BK, the pressor effect returned to baseline values within 10 min while the bradycardia lasted for about 15–20 min.

Furthermore, no tachyphylaxis was observed when 3–4 successive injections of 81 pmol BK were given 30 min apart. The i.t. injection of a similar volume of CSF was without appreciable effect on MAP and HR (Figure 1). Baseline MAP and HR were not significantly different among groups (Figure 1 and Table 1).

Effects of BK receptor antagonists on the cardiovascular response to BK

The receptor responsible for the cardiovascular response induced by the i.t. injection of BK was characterized with a series of antagonists selective either for the B₂ or the B₁ kinin receptor (Table 1 and Figure 1). When administered intrathecally 2–3 min prior to 81 pmol BK, four analogues out of nine blocked in a dose-dependent manner the cardiovascular response to BK with the following rank order of potency: Tyr,D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK = D-Arg[Tyr³,D-Phe⁷,Leu⁸]-BK = D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK > D-Arg[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK (Hoe 140). The first three compounds blocked significantly the MAP and HR responses to BK at a molar ratio antagonist/agonist of 10:1, whereas the last compound blocked only at a dose 100 fold (7.6 nmol) higher than BK (Figure 1). The antagonism was no longer present when BK was re-injected alone 1 h later (data not shown). Hoe 140 (767 pmol) was also tested for longer pretreatment periods (10, 20 and 30 min) to determine whether its apparent weakness was dependent on the equilibration time with the B₂ receptor before BK was injected. Such a possibility is however unlikely as this dose of Hoe 140 was inactive against BK whatever the time-exposure (*n* = 8, data not shown). D-Arg[Hyp³,Gly⁶,D-Phe⁷,Leu⁸]-BK and D-Arg[Hyp³,Thi^{5,8},D-Phe⁷]-BK had no significant effect on the cardiovascular response elicited by 81 pmol BK when administered at doses 10 and 100 times higher than the agonist (Table 1). The antagonistic activities of these compounds could not be measured at doses higher than 40 nmol as they produced direct effects on MAP (Table 1). D-Arg[Hyp³,D-Phe⁷]-BK and D-Arg[Hyp³,Gly⁶,Leu⁸]-BK were unable to block BK at doses

Table 1 Effects of kinin receptor antagonists on mean arterial pressure (MAP) and on the MAP response to 81 pmol bradykinin

Antagonist	n	Dose (nmol)	Basal MAP (mmHg)	Direct effect on MAP (mmHg)	Changes in MAP to BK (mmHg)	
					Before antagonist	After antagonist
[Leu ⁸]-desArg ⁹ -BK	8	CSF	91.8 ± 5.6	1.7 ± 0.8	7.1 ± 1.3	8.3 ± 0.8
		1.0		2.4 ± 0.9		
		10.3		1.9 ± 1.1		
	4	51.3	96.1 ± 8.0	10.0 ± 2.0**	12.9 ± 2.6	16.3 ± 2.3
		102.7		9.2 ± 2.6**		
D-Arg[Hyp ³ ,Gly ⁶ ,D-Phe ⁷ ,Leu ⁸]-BK	8	CSF	96.1 ± 8.0	0.6 ± 0.6	11.9 ± 2.5	11.1 ± 4.6
		0.8		3.3 ± 1.5		
		8.2		3.3 ± 1.7		
	4	41.0	93.8 ± 5.1	39.5 ± 6.4**	15.0 ± 3.9	12.5 ± 2.3
		82.1		38.5 ± 9.7**		
D-Arg[Hyp ² ,Thi ^{5,8} ,D-Phe ⁷]-BK	10	CSF	93.8 ± 5.1	0.0 ± 1.2	11.3 ± 3.3	8.6 ± 1.7
		0.8		3.2 ± 1.5		
		8.4		1.5 ± 0.8		
	4	41.9	88.0 ± 4.4	6.7 ± 1.0**	10.4 ± 1.2	10.4 ± 1.2
		83.7		11.0 ± 2.2**		
D-Arg[Hyp ³ ,D-Phe ⁷]-BK	8	CSF	91.5 ± 3.7	0.4 ± 1.2	11.3 ± 3.3	8.6 ± 1.7
		0.8		4.1 ± 1.3		
		8.3		4.5 ± 1.3		
		41.7		10.2 ± 1.2**		
D-Arg[Hyp ³ ,Gly ⁶ ,Leu ⁸]-BK	8	CSF	91.5 ± 3.7	0.6 ± 0.9	11.3 ± 3.3	8.6 ± 1.7
		0.9		2.4 ± 0.7		
		8.6		5.6 ± 1.1		
		42.8		10.3 ± 1.9**		

Values represent the mean ± s.e.mean of (*n*) rats. Statistical differences between values before and after the i.t. injection of the antagonist is indicated by †*P* < 0.05. Statistical comparison to CSF values is indicated by **P* < 0.05 and ***P* < 0.01.

Figure 2 Typical tracings showing the arterial blood pressure (BP) response to 8.1 nmol substance P (SP) before (a) and after (b) 0.8 nmol D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK (Ana₅) injected intrathecally in one rat. ΔHR represents the maximal changes in heart rate measured 5 min after SP administration in 6 rats.

up to 100 times that of the agonist. At a fairly high dose (42 nmol), only D-Arg[Hyp³,Gly⁶,Leu⁸]-BK reduced significantly the BK-induced increase in MAP although the analogue displayed an intrinsic effect on MAP (Table 1). The B₁ antagonist, [Leu⁸]-desArg⁹-BK, at doses ranging from 1 nmol to 10.3 nmol, failed to antagonize the cardiovascular response to 81 pmol BK. At higher doses, this analogue also displayed a direct pressor effect.

Intrathecal effect of B₂ receptor antagonists against substance P

The i.t. injection of 8.1 nmol substance P produced an increase of MAP and HR as reported earlier (Hassessian *et al.*, 1988). This cardiovascular response to SP was not significantly different from that obtained in animals which received a prior i.t. injection of Tyr,D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK, D-Arg[Tyr³,D-Phe⁷,Leu⁸]-BK, D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK or D-Arg[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK at doses of 0.7–0.8 nmol. An example is given in Figure 2 with the analogue D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK.

Intrinsic activity of BK antagonists

The intrinsic activity of each antagonist administered at increasing doses was assessed on MAP in separate groups of rats. All analogues failed to produce any significant effect on MAP when administered alone at doses lower than 8–10 nmol. However, with doses 4–5 times higher, most of the antagonists showed an increase in MAP similar to that observed with 81 pmol BK. D-Arg[Hyp³,Gly⁶,D-Phe⁷,Leu⁸]-BK (41.0 nmol) and Tyr,D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK (35.8 nmol) produced the greatest effect on MAP with maximal increases of 39.5 ± 6.4 mmHg (Table 1) and 41.2 ± 12.0 mmHg, respectively (data not shown). Note that the pressor effects evoked by these two analogues were 2–3 times greater than that evoked by 81 pmol BK and could not be reached with a supramaximal dose of BK (81 nmol) (Lopes & Couture, 1992). All other antagonists, at doses ranging from 40–100 nmol, caused a small (about 10 mmHg) but significant change in MAP (Table 1). At the doses tested, neither BK nor BK analogues produced motor impairment, respiratory arrest or persisting cardiovascular changes.

Discussion

In a previous study, we have shown that BK, kallidin and T-kinin are equipotent in eliciting increases in MAP and decreases in HR when injected intrathecally at the ninth

thoracic segment of the conscious rat (Lopes & Couture, 1992). In contrast, des-Arg⁹-BK, a selective agonist at the B₁ receptor, was 1000 fold less potent. On the basis of this finding, it was concluded that the cardiovascular effect induced by BK applied to the spinal cord is mediated by B₂ receptors. Likewise, the pressor response to BK administered intracerebroventricularly in rats was attributed to activation of B₂ receptors (Lindsey *et al.*, 1989). In the present study, the hypothesis of the existence of B₂ receptors in the spinal cord of the rat has been confirmed by the use of antagonists. While the B₁ receptor antagonist [Leu⁸]-desArg⁹-BK had no effect, four B₂ receptor antagonists blocked in a dose-dependent and reversible manner the cardiovascular response to BK: D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK = D-Arg[Tyr³,D-Phe⁷,Leu⁸]-BK = Tyr,D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK > D-Arg[Hyp³,Thi⁵,D-Tic⁷,Oic⁸]-BK (Hoe 140) when pre-injected intrathecally at molar antagonist/agonist ratio equal or superior to 10. However, this rank order of potency for B₂ receptor antagonists does not conform to that found in peripheral tissues, where Hoe 140 has been shown to be the most active compound (Hock *et al.*, 1991; Rhaleb *et al.*, 1991; Regoli *et al.*, 1992). Moreover, several compounds which have been shown to be antagonists in peripheral tissues, namely D-Arg[Hyp³,Gly⁶,Leu⁸]-BK, D-Arg[Hyp³,D-Phe⁷]-BK, D-Arg[Hyp²,Thi⁵,D-Phe⁷]-BK and D-Arg[Hyp³,Gly⁶,D-Phe⁷,Leu⁸]-BK were unable to reduce the effects of BK, while other compounds (D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK, D-Arg[Tyr³,D-Phe⁷,Leu⁸]-BK and Tyr,D-Arg[Hyp³,D-Phe⁷,Leu⁸]-BK) pre-injected at the same molar doses (generally 810 pmol) were found to reduce significantly the cardiovascular effects of BK. The first group of compounds which were found to be inactive have also been shown to act as partial agonists in peripheral organs (Regoli *et al.*, 1992). The major discrepancy between the central and peripheral effects of kinin receptor antagonists is the weakness of Hoe 140 which cannot be attributed to differences in metabolic degradation since Hoe 140 has been shown to be more resistant to peptidases than the other antagonists (Wirth *et al.*, 1991; Lembeck *et al.*, 1991). Although other factors such as the rate of diffusion into nervous structures cannot be completely ruled out, it is possible that the functional site involved in the spinal cardiovascular effect of BK is somewhat different from that found in the vascular endothelium or in various smooth muscles. The BK receptor of the rat spinal cord may be similar to the high-affinity site (denoted B_{2C}) described in the guinea-pig brain and lung membranes by Seguin *et al.* (1992) at which Hoe 140 displays low affinity.

Most analogues of BK (B₁ or B₂ receptor antagonists) exhibited intrinsic activities at doses 50 fold greater than that of BK (0.81 nmol) required to elicit the maximal effect (Lopes & Couture, 1992). It is unknown, whether these cardiovascular changes (increase of BP and decrease of HR) are attributable to direct actions on B₂ receptors (agonist effect) or to non specific effects in the spinal cord. Nevertheless, none of the analogues tested caused distressed behaviour, hindlimb paralysis or death of the animals. Moreover, the four analogues which blocked the BK-induced cardiovascular effects were inactive against the blood pressure and heart rate increases induced by the i.t. injection of SP, supporting the interpretation that they act specifically on B₂ receptors in the spinal cord. On the other hand, the i.t. injection of an antagonist of SP (± CP-96,345) did not modify the effects of BK (Pham & Couture, 1993), while blocking those of SP. This indicates that BK is not acting via the release of SP. This possibility had to be excluded since *in vitro* quantitative receptor autoradiographic studies have shown that [¹²⁵I-Tyr⁸]-BK receptor binding sites are localized predominantly in superficial layers of the dorsal horn in the rat and guinea-pig spinal cord, where numerous endings of thin sensory myelinated (Aδ) and non-myelinated (C) fibres containing SP are found (Lopes *et al.*, 1992; 1993). Although the selective destruction of these fibres with the neurotoxin, capsaicin, reduced significantly the BK radiolabelled sites in

the rat dorsal horn (Lopes *et al.*, 1992), this treatment had no effect on the pressor response to BK (Lopes & Couture, 1992) thus ruling out the possible involvement of other sensory neuropeptides such as calcitonin gene-related peptide, galanin, somatostatin and cholecystokinin as mediators of BK-induced cardiovascular responses. In fact, these peptides are depleted from sensory C-fibres together with SP by capsaicin (Holzer, 1988; Maggi, 1991).

A rather low, but significant density of specific [125 I-Tyr 8]-BK binding sites was also detected in the intermediolateral horn where cell bodies and dendrites of preganglionic sympathetic fibres are located (Lopes *et al.*, 1992; 1993). These specific BK sites may be involved in mediating the spinal action of kinins on the cardiovascular system. Moreover, the possibility that BK activates preganglionic sympathetic fibres through interneurons and other transmitters present in this tissue cannot be excluded. However, compelling evidence rules out peripheral and supraspinal sites of action for i.t. BK and related analogues (Lopes & Couture, 1992).

In the rat and guinea-pig spinal cord, we found that Hoe 140 was the most potent displacer of [125 I-Tyr 8]-BK specific binding followed by D-Arg[Hyp 3 ,D-Phe 7 ,Leu 8]-BK, Tyr,D-Arg[Hyp 3 ,D-Phe 7 ,Leu 8]-BK, D-Arg[Tyr 3 ,D-Phe 7 ,Leu 8]-BK (Lopes *et al.*, 1992; 1993). This rank order of potency, and in particular the high activity of Hoe 140, contrasts with the results obtained in the present study. These findings suggest that the functional B $_2$ site involved in the cardiovascular effects of BK is different from the major (abundant) site

labelled by [125 I-Tyr 8]-BK and occupied by Hoe 140. It thus appears that the sites located in superficial layers of the dorsal horn as revealed by autoradiographic studies, are not involved in the spinal cardiovascular action of BK.

In summary, the i.t. injection of BK in the conscious rat produces a transient increase in MAP and decrease in HR which appear to be mediated by the activation of a B $_2$ receptor in the spinal cord. This hypothesis is supported by the finding that the cardiovascular response to BK is blocked in a dose-dependent, reversible and specific manner by the intrathecal administration of antagonists selective for the B $_2$ but not for the B $_1$ receptor. The rank order of potency of the antagonists suggests, however, that the B $_2$ receptor involved in the cardiovascular action of BK belongs to a functional site which, pharmacologically, does not conform to the B $_2$ receptors that are found in a large variety of peripheral tissues.

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