Central nervous system kinin receptors and the hypertensive response mediated by bradykinin

¹C.J. Lindsey, C.R. Nakaie & ²D.T.O. Martins

Department of Biophysics, Escola Paulista de Medicina, 04034 São Paulo, SP, Brazil

- 1 Bradykinin (Bk) administered intracerebroventricularly to the rat causes an increase in arterial pressure.
- 2 Analogues of Bk with agonist and antagonist activity were injected, over a wide dose-range, into the posterior region of the fourth ventricle of unanaesthetized rats implanted with permanent ventricular cannulae, and blood pressure was measured directly from the abdominal aorta.
- 3 The analogues Ile-Ser-Bk (T-kinin) and Lys-Lys-Bk, which interact with both B₁ and B₂ Bk receptors, produced pressor effects similar to those of Bk, although of greater duration, whereas des-Arg⁹-Bk, a B₁-receptor agonist, had no effect.
- 4 The B₁-antagonist des-Arg⁹-[Leu⁸]-Bk did not alter the Bk pressor response, but D-Arg-[Hyp³, Thi^{5,8}, D-Phe⁷]-Bk, which interacts both with B₁- and B₂-receptors blocked the responses to Bk, T-kinin and Lys-Lys-Bk and caused parallel shifts to the right of the Bk dose-response curves. Neither antagonist, by itself, had any effect on blood pressure.
- 5 It is concluded that the central pressor response to Bk is mediated by receptors of the B₂ subtype.

Introduction

Bradykinin (Bk), when injected into the systemic circulation, usually causes vasorelaxation and consequently, a temporary decrease in blood pressure (Rocha e Silva *et al.*, 1949). However, the same peptide administered to the cerebral ventricles (Graeff *et al.*, 1969; Pearson *et al.*, 1969) or into the cerebral circulation (Lang & Pearson, 1968), causes a marked and sustained increase in the mean arterial pressure. This hypertensive response to Bk has been documented in rabbits (Graeff *et al.*, 1969), rats (Pearson *et al.*, 1969), cats (Lang & Pearson, 1968) and dogs (Pearson & Lang, 1969). In rats, the pressor response to intraventricularly injected Bk has been ascribed to the stimulation of Bk receptors in the lateral septal area (Correa & Graeff, 1975), or alternatively, in areas adjacent to the ventral portion of the third ventricle (Lewis & Phillips, 1984). However, there are data which suggest that the pressor response to the peptide results from the stimulation of sites on the dorsal surface of the medulla (Lindsey *et al.*, 1988 and unpublished observations), probably in the area of the fourth ven-

tricle. In fact, Bk injected into the posterior region of the fourth ventricle is about twenty times as potent, has a considerably shorter latency and a larger maximal effect than when injected into the lateral ventricle (Lindsey *et al.*, 1988).

Only recently have specific antagonists been developed for the Bk receptors. With the advent of antagonists, however, two subpopulations of Bk receptors have been described. The B₁-receptor which is present in the rabbit aorta (Regoli & Barabé, 1980) and the rat renal artery (Guimarães *et al.*, 1986), is stimulated selectively by des-Arg⁹-Bk (DABk) and is blocked by des-Arg⁹-[Leu⁸]-Bk (DALBk). The B₂-receptors, present in arteries and other tissues are stimulated by Bk and many analogues and are blocked by a series of Bk derivatives with a D-phenylalanine substitution in position 7 (Vavrek & Stewart, 1985), such as D-Arg-[Hyp³, Thi^{5,8}, D-Phe⁷]-Bk (DAHTDBk). In contrast to the B₁-agonists and antagonists, the B₂-agonists or antagonists are not selective and in general also interact with the B₁-receptors (Regoli *et al.*, 1986).

Other kinins of physiological or practical interest have also been developed. For example, Lys-Lys-Bk (LLBk), a kinin agonist which is exceptionally resistant to degradation by kininase activity (Roblero *et*

¹ Author for correspondence.

² Permanent address: Universidade Federal de Mato Grosso, 78000 Cuiabá, MT, Brazil.

Table 1 Kinin receptor agonists and antagonists employed in the present study

Peptides	Agonist activity	Antagonist activity	Amino acid sequence
Bradykinin (Bk)	B ₁ B ₂		Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
Lys-Lys-Bk (LLBk)	B ₁ ? B ₂		Lys-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
des-Arg ⁹ -Bk (DABk)	B ₁		Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe
T-kinin (TK)	B ₁ ? B ₂		Ile-Ser-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
des-Arg ⁹ -[Leu ⁸]-Bk (DALBk)		B ₁	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu
D-Arg-[Hyp ³ ,Thi ^{5,8} ,D-Phe ⁷]-Bk (DAHTDBk)		B ₁ B ₂	D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Phe-Thi-Arg

al., 1973; Lindsey *et al.*, 1987), is a potential tool to detect *in vivo* kininase activity in experimental situations where the use of kininase inhibitors is precluded. Another agonist of interest, Ile-Ser-Bk or T-kinin (TK) was first obtained by the incubation of rat plasma with trypsin (Okamoto & Greenbaum, 1983a), the classical method for obtaining Bk from human plasma. T-kinin is thus a candidate for the role of endogenous kinin in the rat.

Central Bk receptors have still to be classified. The objective of this study was to characterize the receptors which mediate the central pressor response to Bk, by using analogues which interact with different subpopulations of receptors.

Methods

The peptides used in this study (Table 1) were synthetic products made in this laboratory, with the exception of D-Arg-[Hyp³,Thi^{5,8},D-Phe⁷]-Bk which was kindly supplied by Dr J.M. Stewart from the University of Colorado (Denver, CO, U.S.A.).

Four month old female normotensive Wistar rats (NR), weighing approximately 200 g, with a mean arterial pressure of 111 ± 3 mmHg, were used. The animals were anaesthetized with a mixture of pentobarbitone and chloral hydrate and permanent cannulae were placed in the posterior portion of the fourth ventricle (-11.4 mm antero-posterior, 2.4 mm vertical and 0.0 mm lateral in stereotaxic coordinates, Paxinos & Watson, 1986). The cannulae were

anchored to the skull by jeweller's screws embedded in dental acrylic cement. Following the intracerebro-ventricular surgery a polyethylene catheter (PE10 connected to PE50), filled with heparinized saline, was placed in the abdominal aorta, through the left femoral artery. The other end of the cannula was slipped beneath the skin and exteriorized on the back of the animal. After surgery, the rats were individually housed in plastic cages ($30 \times 20 \times 10$ cm) which also served as recording chambers. Two days after implantation surgery the effect of centrally administered Bk and analogues on the mean arterial blood pressure was recorded, in the unanaesthetized and unrestrained animals, with a Narco P-1000B pressure transducer and a DMP-4B physiograph (Narco Biosystems, Houston, TX, U.S.A.). Dose-response curves for Bk, TK and LLBk were obtained by injecting $1 \mu\text{l}$ saline containing various concentrations of the peptides at 30 min intervals, and no more than three doses were administered to each animal. Proper cannula placement was verified histologically in all subjects by an injection of dye at the end of the experiment. The rat brains were excised and placed in 10% formaldehyde for two weeks before histology. The lack of diffusion of the dye into the expected ventricular space was the criterion for excluding an animal.

Regression lines obtained for the linear parts of the dose-response curves were compared for critical differences following covariance analyses and tests for regression, linearity and parallelism (Snedecor & Cochran, 1967). For differences between independent means, Student's *t* test was used.

Table 2 Hypertensive effect of kinins injected into the posterior region of the fourth ventricle

	ED ₅₀ (pmol)	Latency (s)	Duration (s)	Maximal effect (mmHg)
Bk	32	16 ± 10	91 ± 36	31 ± 15
TK	51	24 ± 12	$155 \pm 40^*$	30 ± 11
LLBk	6*	18 ± 11	$175 \pm 26^*$	31 ± 7
DABk (>11,000)	—	—	—	No effect

Values are the mean $\pm$ s.d. of at least 10 animals.

* Significantly different from Bk ($P < 0.05$). For key to abbreviations used see Table 1.

Results

Effect of kinin agonists on arterial pressure

The kinin agonists, Bk, DABk, TK and LLBk were injected into the posterior region of the fourth ventricle of normotensive Wistar rats. Bradykinin, TK or LLBk, in the pmol range, produced increases in the mean arterial pressure which in some animals reached 60 mmHg above baseline. The response to Bk occurred with a mean latency of 16 s and lasted an average of 90 s. T-kinin and LLBk exhibited similar latencies but the effects of both peptides were of longer duration (Table 2). No effect was observed with DABk, an agonist of the smooth muscle B_1 -receptor, although it was injected in doses from 8 to 11,000 pmol.

The dose-response curves for the three peptides which presented pressor activity are shown in Figure 1. The kinins had the same maximal effect with an average value of 30 mmHg. From Figure 1, it can be seen that LLBk ($ED_{50} = 6$ pmol) is approximately 5 times more potent than Bk. The regression lines corresponding to the linear portion of the dose-response curve to Bk and TK did not differ significantly from each other.

Effect of antagonists on the central kinin pressor response

For the antagonism studies, the analogues were mixed with Bk in the appropriate concentrations so that 1 μ l contained the desired amount of agonist and antagonist.

Preliminary experiments showed that the inhibitor DAHTDBk rapidly blocks the Bk receptors. The intracerebroventricular injection of the antagonists five or ten min before administration of Bk did not enhance inhibition. Thirty min after DAHTDBk no inhibition of the pressor effect could be observed.

The Bk antagonists DALBk (400–11,500 pmol) and DAHTDBk (200–1800 pmol), when injected into the fourth ventricle alone, did not cause alterations in the mean arterial pressure ($n = 10$).

The B_1 -receptor antagonist DALBk (10–6000 pmol) did not alter the hypertensive response to Bk (Figure 2). However, DAHTDBk (600 pmol) blocked the responses to Bk, TK and LLBk (Figure 3). The pressor effect of Bk was inhibited in a dose-dependent manner by 200–1200 pmol of DAHTDBk, which caused parallel displacements of the Bk dose-response curves (Figure 4). With a higher dose of DAHTDBk (1800 pmol) it was impossible to obtain a full dose-response curve because of the appearance of a hypotensive response, in approximately 60% of the animals. In these animals the decrease in arterial pressure appeared alone or following a hypertensive

Figure 1 Log dose-response curves for the systemic pressor effects, measured as changes in the mean arterial pressure (Δ MAP), of bradykinin (O), Lys-Lys-bradykinin (●) and T-kinin (■) injected into the fourth ventricle of normotensive Wistar rats. The injection volume was 1 μ l in all cases. The regression lines corresponding to the linear portion of the curves were homogeneous regarding parallelism. The curve for Lys-Lys-bradykinin differed significantly from the others ($P < 0.05$, analyses of covariance). Each point represents the mean obtained from 8 to 10 rats. Vertical lines show s.e.mean.

Figure 2 Effect of bradykinin (Bk 30 and 120 pmol) on the mean arterial pressure (Δ MAP) in the absence (open columns) and presence (hatched columns) of 6 nmol of the bradykinin B_1 -antagonist, des-Arg⁹-[Leu⁸]-bradykinin (DALBk). The solid columns represent the effect of DALBk alone. Each column represents the mean obtained from 8 rats; vertical lines show s.e.mean.

Figure 3 Inhibitory effect of 0.6 nmol D-Arg-[Hyp³, Thi^{5,8}, D-Phe⁷]-Bk on the pressor effect of bradykinin (BK), T-kinin (TK) or Lys-Lys-Bk (LLBk). The responses to the agonists (open columns) were significantly ($P < 0.05$) reduced in the presence of the antagonist (hatched columns). Each column represents the mean obtained from 9 rats; vertical lines show s.e.mean.

Figure 4 Log dose-response curves obtained for bradykinin (Bk) on the mean arterial pressure (Δ MAP) alone (○) and in the presence of different doses (●, 0.2 nmol; ■, 0.6 nmol; ▲, 0.8 nmol; △, 1.2 nmol) of D-Arg-[Hyp³, Thi^{5,8}, D-Phe⁷]-Bk. The regression lines were homogeneous as regards parallelism and differed significantly from each other ($P < 0.05$, analyses of covariance). Each point is the mean of determinations carried out in 15 animals. Vertical lines show s.e.mean.

response of small amplitude. The remaining animals presented blunted pressor responses to 7600 pmol of Bk. Neither the agonist alone nor the antagonist, at the highest doses employed, caused a hypotensive response.

Discussion

Bradykinin, TK or LLBk, when injected intracerebroventricularly into the posterior region of the fourth ventricle, consistently produced an increase in the mean arterial pressure. The response to TK was similar in pattern to that of Bk or LLBk. The query as to which kinin is the mediator of this effect is an open question since no kinin has been identified as a mediator of any physiological function in the central nervous system. Bradykinin, its higher homologues or novel kinins such as TK are possible candidates for this role in the rat. Since TK has been obtained from the incubation of rat plasma with trypsin (Okamoto & Greenbaum, 1983a), it has been postulated that the Bk analogue Ile-Ser-Bk or TK is one of the endogenous kinins in the rat. Apparently TK is a mediator of the inflammatory response in the rat (Barlas *et al.*, 1985; Sakamoto *et al.*, 1987).

The differences in the duration of the response to the three kinins, as well as the greater potency of LLBk, may be ascribed to pharmacokinetic properties of the Bk analogues. TK (Okamoto & Greenbaum, 1983b) and LLBk (Roblero *et al.*, 1973), respectively, are more resistant to kininase activity than Bk. TK has been shown to have a lower specific activity than Bk (Okamoto & Greenbaum, 1983b), thus the final biological activity of TK (or other peptides) results from an interplay of affinity, efficacy and resistance to degradation.

In contrast to the results obtained with Bk, TK and LLBk, no noteworthy cardiovascular effects were observed following intraventricular administration of the specific B₁-agonist DABk. This result is in agreement with a previous experiment, in which DABk was injected into the lateral ventricle of rats (Correa & Graeff, 1974), and indicates that the Bk receptors involved in the central pressor response are not of the B₁ subtype. This is also supported by the finding that the response to Bk was not antagonized by the B₁-antagonist DALBk. On the other hand, the responses to Bk, TK and LLBk were antagonized by the B₂-antagonist DAHTDBk, indicating that the receptors which mediate the central hypertensive response in normotensive rats are, in all probability, of the B₂ type. The parallel displacement to the right of the Bk dose-response curves in the presence of DAHTDBk indicates that this compound acts as a competitive antagonist in this system. Another antagonist [Thi^{5,8}, D-Phe⁷]-Bk with

similar properties to those of DAHTDBk has been shown to inhibit the pressor action of Bk in spontaneously hypertensive rats (Lindsey *et al.*, 1988).

The hypotensive response that was observed following a high dose of Bk in conjunction with antagonist suggests the existence of a site where kinins exert a centrally mediated vasodilator response. This effect could occur in response to stimulation of systems with low sensitivity to Bk which are not blocked by the antagonist. Alternatively, the hypotensive effect could be exerted at a site which is relatively less accessible to the antagonist than to Bk. The increase in the net charge of DAHTDBk, in relation to that of Bk given by the extra D-Arg, could hinder diffusion of inhibitor in tissue.

Another aspect which was examined was whether the antagonists portray biological activity on the central nervous system receptors, as is the case in

some other preparations (Llona *et al.*, 1987; Braas *et al.*, 1988). The B₁- and B₂-antagonists DALBk or DAHTDBk, administered over a wide dose range, were devoid of any effect on blood pressure.

The central nervous system receptors which mediate the pressor response to Bk are stimulated by analogues which interact with B₂ Bk receptors and are inhibited by B₂ competitive antagonists. No evidence was found that B₁-receptors are involved in this response, since B₁-agonists or antagonists did not have any effect on blood pressure or inhibitory action on the Bk pressor response.

This work was done with the technical assistance of Cleber Cicero de Souza Silva and was supported by grants from FINEP, FIEC and FAPESP. D.T.O.M. is a Coordenacao de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) Postgraduate Fellow.

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(Received October 24, 1988

Revised February 28, 1989

Accepted March 14, 1989)