*Br. J. Pharmacol.* (1991), **103**, 1851–1856 

# Kinin receptors of the central nervous system of spontaneously hypertensive rats related to the pressor response to bradykinin

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

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

1 Kinin analogues bradykinin (BK), T-kinin, Met-Lys-BK, Lys-Lys-BK, Des-Arg$^9$-BK with agonist activity and D-Arg$^0$-Hyp$^3$-Thi$^{5,8}$-D-Phe$^7$-BK (DAHTDBK) and Arg$^9$-Leu$^8$-BK with antagonist activity were injected into the posterior portion of the fourth cerebral ventricle of unanaesthetized rats implanted with permanent cannulae and arterial pressure was measured directly from the abdominal aorta.

2 The spontaneously hypertensive rats (SHR) were more sensitive than normotensive Wistar rats (NWR) to the pressor effect of BK and other kinin analogues. The SHR did not differ in sensitivity of the pressor response to centrally administered angiotensin II or endothelin-1.

3 Experiments with selective kinin agonists and antagonists revealed that in the SHR, as in the NWR, the receptors which mediated the central pressor response are of the $BK_2$ subtype.

4 Measurements of the pressor activity of kinins with different degrees of susceptibility to degradation, as well as experiments with kininase inhibitors, enalaprilat and CPP-Ala-Ala-Phe-pAB, suggest that the kininase activity in the central nervous system of SHR is reduced in comparison to that of NWR.

5 The SHR also showed increased sensitivity to BK and Lys-Lys-BK, compared with the NWR, when the kinins were injected following the administration of a mixture of the kininase inhibitors, suggesting that mechanisms other than kininase activity may play a role in the increased sensitivity of the SHR to the central pressor action of kinins.

6 An *in vivo* characterization of the kinin receptors which mediate the central pressor response showed that the interaction with DAHTDBK was reversible and of competitive nature. The $pA_2$ *in vivo* estimated for the kinin receptors of the SHR was 0.7 logarithm units larger than that obtained in the NWR.

7 The kinin receptors which mediate the central BK pressor effect in the SHR are of the $BK_2$ subtype and are similar to receptors in the NWR. The increased sensitivity to kinins in the SHR may be explained, at least in part, by their decreased kininase activity. At present it is impossible to conclude whether the difference observed in the $pA_2$ represents an increased affinity of the kinin receptors or can be attributed to differences amongst strains in the enzymatic degradation of the antagonist.

**Keywords:** Bradykinin; central nervous system; kinin receptors; pressor effect; spontaneously hypertensive rats

## Introduction

The intracerebroventricular (i.c.v.) administration of bradykinin (BK) causes an increase in the mean arterial pressure (MAP) of rats (Pearson & Lang, 1969) and other species (Pearson & Lang, 1967; Lang & Pearson, 1968; Graeff *et al.*, 1969). Apparently this pressor response, which depends on a functionally intact adrenergic efference (Correa & Graeff, 1974), is mediated by kinin receptors in the medulla oblongata in or adjacent to the fourth cerebral ventricle (Lindsey *et al.*, 1988). The kinin receptors which mediate the central pressor response in the normotensive Wistar rat (NWR) are of the $BK_2$ subtype (Lindsey *et al.*, 1989) according to the classification of BK receptors in peripheral tissues established by Regoli & Barabé (1980).

The spontaneously hypertensive rat (SHR) showed increased sensitivity to the pressor action of i.c.v.-administered kinins in comparison to normotensive Wistar rats (NWR) (Lindsey *et al.*, 1988). The increased responsiveness observed in the SHR could be, in principle, related to alterations at any of the different levels of the neuroeffector system which determine short term changes in arterial pressure. The mechanisms which modulate the haemodynamic effect of kinins applied to the central nervous system include alterations in kininase activity, receptor sensitivity or population subtype, central sympathetic reactivity and vascular smooth muscle responsiveness. The aim of this study was to examine pharmacological aspects of the central kinin receptors which mediate the pressor response in the SHR. The classification of these receptors was obtained with the use of agonists and antagonists which interact with $BK_1$ and $BK_2$ kinin receptors (Regoli & Barabé, 1980; Vavrek & Stewart, 1985). An *in vivo* pharmacological characterization of receptors in SHR and NWR was attempted. These experiments were complemented with an investigation of the central pressor effect of angiotensin II (AII) and of kinin analogues with distinct pharmacodynamic or pharmacokinetic properties.

## Methods

Four-month old female spontaneously hypertensive and normotensive Wistar rats, weighing approximately 200 *g*, with a mean arterial pressure of $148 \pm 16$ and $111 \pm 12$ mmHg, respectively, 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 (11.0 mm antero-posterior, 7.9 mm vertical and 0.0 mm lateral from bregma in SHR and 11.7 mm antero-posterior, 7.5 mm vertical and 0.0 mm lateral in NWR) (Paxinos & Watson, 1986). The cannulae were anchored to the skull by jeweller's screws embedded in dental acrylic cement. Following the i.c.v. surgery a polyethelene 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 the surgery, the rats were individually housed in plastic cages (30 × 20 × 10 cm) which also served as recording chambers. Two days after implantation surgery the effect of centrally

[1] Permanent address: Universidade Federal de Mato Grosso, 78000 Cuiabá, MT, Brazil.
[2] Author for correspondence.

administered BK and analogues on the mean arterial blood pressure (MAP) was recorded, in the unanaesthetized and unrestrained animals, with a Narco P-1000B pressure transducer and a DMP-4B physiograph (Narco Biosystem, Houston, TX, U.S.A.). Dose-response curves for BK, TK, MLBK and LLBK were obtained by injecting 1 $\mu$l saline containing various concentrations of peptides at 30 min intervals; no more than three doses were administered to each animal. Delivery of the injected volume took slightly less than 1 s. Latency for the manifestation of the pressor response to peptides was defined as the time elapsed between the moment of the injection and the onset of the pressor effect of the $ED_{50}$ dose of each peptide. Proper cannula placement was verified histologically in all rats 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 the 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 dose-response curves were compared for critical differences following covariance analyses and tests for regression, linearity and parallelism (Snedecor & Cochran, 1980). The $ED_{50}$ values and respective confidence intervals were estimated by a linear calibration method (Snedecor & Cochran, 1980). For differences between independent means, Student's $t$ test was used, preceded by analysis of variance in the case of multiple comparisons.

The peptides bradykinin (BK), Met-Lys-BK (MLBK), T-kinin (Ile-Ser-BK, TK) Lys-Lys-BK (LLBK), des-Arg$^9$-BK (DABK), des-Arg$^9$-Leu$^8$-BK (DALBK), angiotensin II (AII) are synthetic products made in this laboratory. D-Arg-[Hyp$^3$, Thi$^{5,8}$-D-Phe$^7$]-BK (DAHTDBK) and Thi$^{5,8}$-D-Phe$^7$-BK (TDBK) were kindly supplied by Dr J.M. Stewart from the University of Colorado (Denver, CO, U.S.A.) and endothelin-1 was purchased from Sigma Chemical Co. The peptidase inhibitor, N-[1(RS)-carboxy-3-phenyl-propyl]-Ala-Ala-Phe-pAB (CPP-Ala-Ala-Phe-pAB) was a kind gift from Dr M. Orlowski, Mount Sinai School of Medicine, University, New York, NY, U.S.A., and enalaprilat was kindly provided by Merck Sharp & Dohme.

## Results

### *Effect of kinin agonists on arterial pressure of SHR and NWR*

Bradykinin produced a pressor response in SHR, typically lasting several minutes following the injection of the peptide into the fourth cerebral ventricle. The effect, which led to an increase of the MAP of up to 60 mmHg in some animals, was similar to the response observed in the NWR. However, the SHR responded to doses of 1 pmol or less whereas the normotensive animals responded only to doses 4 to 8 times larger. Both NWR and SHR presented pressor responses to the kinin analogues TK, MLBK or LLBK. Full dose-response curves were obtained for each analogue (Figure 1). The potency ratios, estimated by comparison of the $ED_{50}$ values obtained in NWR and SHR (Table 1), show that BK was seven times more potent in the SHR, TK eight times as potent, LLBK four times and MLBK three times as potent as in NWR. The parameters regarding the pressor effect of the kinins injected into the fourth ventricle are shown in Table 1.

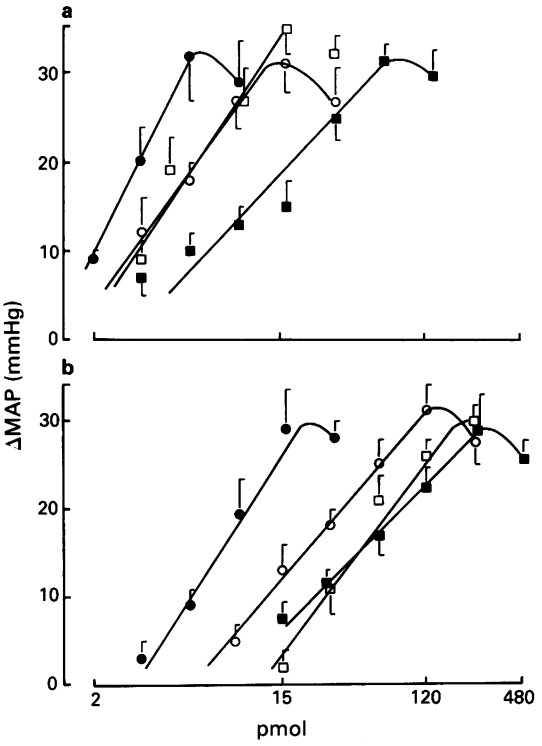


**Figure 1** Log dose-response curves for the effect of kinins injected into the fourth cerebral ventricle upon the mean arterial pressure (MAP) of SHR (a) and NWR (b). The curves for bradykinin (BK) (○), Lys-Lys-BK (LLBK) (●), Met-Lys-BK (MLBK) (■) and T-kinin (□) in the SHR differed significantly ($P \leq 0.001$) from those in the NWR. The linear portion of the curves were parallel with the exception of the curve for MLBK which was not parallel to that for LLBK. Each point represents the mean of 10–15 determinations and the s.e.mean are indicated by vertical bars.

**Table 1** Pressor effect of kinins injected into the fourth ventricle of SHR and NWR

| | $ED_{50}$ (pmol) | | Latency (s) | | Maximal effect (mmHg) | | Duration (min) | |
|---|---|---|---|---|---|---|---|---|
| *Peptide* | *SHR* | *NWR* | *SHR* | *NWR* | *SHR* | *NWR* | *SHR* | *NWR* |
| LLBK | 1, 5*† (0.2–8) | 6† (2–19) | 11 ± 8*§ | 16 ± 9§ | 32 ± 16 | 29 ± 11 | 5 ± 4 | 5 ± 4 |
| BK | 3 (0.7–14) | 22 (1–238) | 9 ± 8 | 16 ± 10 | 31 ± 14 | 31 ± 15 | 4 ± 4 | 4 ± 3 |
| TK | 4, 5 (0.7–25) | 43 (7–240) | 13 ± 8 | 20 ± 9 | 35 ± 14 | 30 ± 9 | 2 ± 2† | 3 ± 3 |
| MLBK | 14† (1–87) | 45 (8–240) | 14 ± 8 | 18 ± 8 | 31 ± 9 | 29 ± 11 | 6 ± 5 | 8 ± 8 |

BK, bradykinin; LLBK, Lys-Lys-BK; TK, T-kinin; MLBK, Met-Lys-BK.
The $ED_{50}$ values and respective 95% confidence limits (shown in parentheses) were calculated from linear portions of the dose-response curves (Figure 1).
The values for latency, maximal effect and duration represent the mean ± s.d. of at least twenty determinations.
* All values of the column differ ($P < 0.05$) from the respective values obtained in the NWR.
† Differs significantly ($P < 0.05$) from all values of the same column.
§ The latency values for BK and LLBK differ ($P < 0.05$) from those for TK and MLBK.

Among the kinins studied, LLBK was the most potent in the SHR, BK and TK ranked after LLBK and MLBK was the least potent analogue. Almost the same order was followed in the NWR, with the difference that BK was more potent than TK and MLBK, which were equipotent in normotensive animals. The mean time elapsed for the onset of the pressor effect in the hypertensive animals ranged from 9 to 14 seconds, depending on the kinin. The shortest latencies were observed for BK and LLBK whereas longer latencies were observed for TK and MLBK (Table 1). The latency for manifestation of the pressor effect in the NWR showed a significant correlation ($r = 0.98$) to the values obtained in the SHR. However, in the normotensive rats the latencies observed for all the kinins studied were approximately 50% longer than in their hypertensive counterparts. No difference was observed between NWR and SHR in the duration of the pressor response to kinins. The effects of BK and TK had shorter durations while MLBK had the longest lasting effect in both strains of rats. An inverse correlation ($r = 0.97$) was observed between the potency ratios of kinins in NWR and SHR and the duration of the pressor response. The mean arterial maximal effect of approximately 30 mmHg was the same for all kinins in both SHR and NWR (Figure 1).

### *Effects of angiotensin II and endothelin-1 on arterial pressure*

Angiotensin II injected into the fourth ventricle of SHR or NWR produced an increase in the MAP with the same maximal effect (33 ± 11 mmHg for SHR and 31 ± 17 mmHg for NWR) as BK. However, AII was 50 and 250 times less potent than BK in the NWR and in the SHR, respectively (Figure 2). The latency for the onset of the pressor response was significantly shorter for AII than for any of the kinins (Table 2). The pressor effect of AII lasted from 8 to 10 min and was significantly longer than that observed for the kinins with the exception of MLBK in the NWR. Angiotensin II was equipotent in the SHR and NWR and there was no significant difference in the duration of the pressor effect, although the SHR showed a significantly shorter latency for the manifestation of that effect. Endothelin-1 like AII and BK, produced a pressor response in the NWR and SHR. Endothelin-1 was equipotent in SHR and NWR ($ED_{50} = 5.0$ and 5.2 pmol respectively) and the maximal effect of 31 ± 8 and 29 ± 11 mmHg was achieved with the dose of 10 pmol in SHR and NWR respectively.

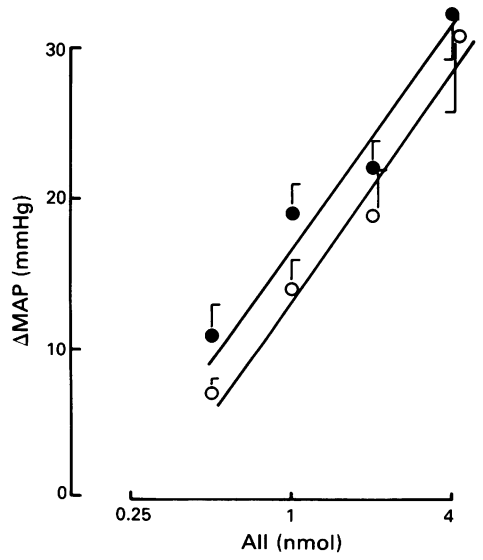


**Figure 2** Linear portions of the log dose-response curves for the systemic pressor effect of angiotensin II (AII) injected into the fourth cerebral ventricle of NWR (○) and SHR (●) rats. The linear portion of the curves were parallel and did not differ significantly from each other ($P > 0.2$). Each point represents the mean of at least 8 observations and the s.e.mean are indicated by vertical bars.

**Table 2** Effect of angiotensin II injected into the fourth cerebral ventricle of SHR and NWR

| | $ED_{50}$ (pmol) | *Latency* (s) | *Maximal effect* (mmHg) | *Duration of response* (min) |
|---|---|---|---|---|
| SHR | 830 (150–4283) | 1 ± 1* | 33 ± 11 | 7 ± 5 |
| NWR | 1137 (196–6383) | 4 ± 3 | 31 ± 17 | 8 ± 6 |

The $ED_{50}$ values and 95% confidence limits (in parentheses) were calculated from the log-dose response curves of Figure 2. The values for latency, maximal effect and duration of the pressor response are represented by the mean ± s.d. of at least 20 animals.
* Significantly different ($P < 0.01$) from NWR.

### *Effect of kininase inhibitors on the response to kinins*

The enzyme inhibitors were injected into the fourth cerebral ventricle dissolved in 2 μl of saline 10 min before the administration of kinins. Preliminary experiments showed that maximal potentiation of the BK pressor effect was observed from 5 to 15 min following the administration of the inhibitors. Pretreatment with a mixture of the kininase inhibitors enalaprilat (0.6 μmol) and CPP-Ala-Ala-Phe-pAB (2.4 μmol), injected into the fourth ventricle 10 min before the administration of the kinins, potentiated the effect of BK in the NWR and the SHR (Table 3). The pressor effect of LLBK was not altered by pretreatment with the enzyme inhibitors. Interpolation of the effect in the presence or absence of kininase inhibitors on the dose-response curves from Figure 1, revealed that BK was potentiated approximately fivefold in the NWR and threefold in the SHR. In the presence of the kininase inhibitors the SHR continued to show increased sensitivity to the pressor effects of BK or LLBK.

### *Effect of agonists and antagonists on the central pressor response to bradykinin in the SHR*

For the antagonism studies, the analogues were mixed with BK in the appropriate concentrations so that 1 μl 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 5 or 10 min before administration of BK did

**Table 3** Effect of the kininase inhibitors enalaprilat (0.6 μmol) and CPP-Ala-Ala-Phe-pAB (2.4 μmol) on the pressor effect (MAP) of bradykinin (BK) or Lys-Lys-BK (LLBK) injected into the fourth cerebral ventricle of NWR and SHR

| | *Control MAP* (mmHg) | *Kininase inhibitors MAP* (mmHg) |
|---|---|---|
| NWR (BK 8 pmol) | 4 ± 8 | 20 ± 13* |
| SHR (BK 2 pmol) | 12 ± 9 | 29 ± 9* |
| NWR (LLBK 2 pmol) | 6 ± 5 | 9 ± 7 |
| SHR (LLBK 1 pmol) | 7 ± 7 | 7 ± 7 |

The values represent the mean ± s.d. of 20 animals.
The kininase inhibitors, dissolved in saline, were injected into the fourth cerebral ventricle 10 min before injection of BK or LLBK.
Differs significantly from controls ($P < 0.001$, paired $t$ test).

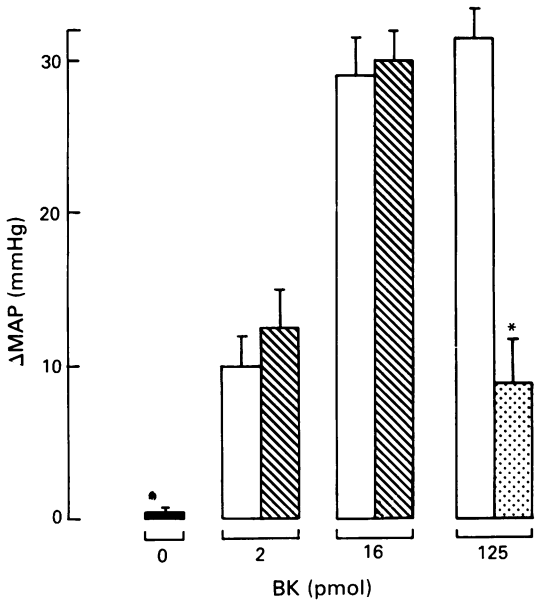


**Figure 3** Effect of 12 nmol of the $BK_1$ receptor antagonist des-Arg$^9$-Leu$^8$-bradykinin (DALBK, hatched columns) or 12 nmol of the $BK_2$ antagonist Thi$^{5,8}$-D-Phe$^7$-BK (TDBK, stippled column) on the response to 60 or 125 pmol of bradykinin (BK, open columns). The solid column shows the effect of DALBK alone. Each column is the mean of 5 to 10 determinations and the s.e.mean are indicated by vertical bars.

not enhance inhibition. Thirty minutes after DAHTDBK, no inhibition of the pressor effect could be observed. No change in MAP was observed after the administration of the $BK_1$ bradykinin agonist DABK in the dose range of 8–11,500 pmol

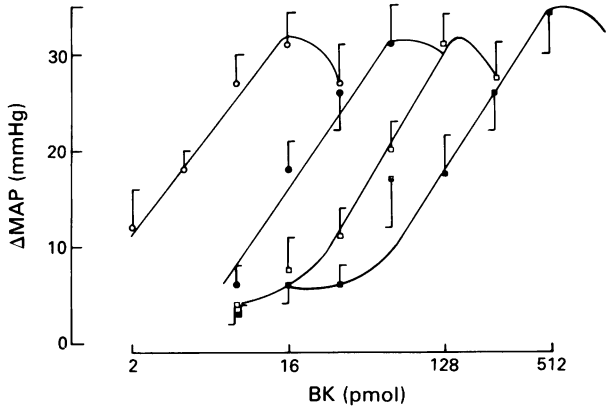


**Figure 4** Log dose-response curves obtained for bradykinin (BK) on the mean arterial pressure (ΔMAP) alone (○) and in the presence of different doses (●, 0.08; □, 0.2; ■, 0.8 nmol) of D-Arg-[Hyp$^3$, Thi$^{5,8}$-D-Phe$^7$]-BK. The regression lines were parallel and differed significantly from each other ($P < 0.01$). Each point is the mean of determinations carried out in 10 to 15 animals and the s.e.mean are indicated by vertical bars.

**Table 4** Inhibition of the pressor effect of Lys-Lys-bradykinin (LLBK) by the antagonist D-Arg$^0$-Hyp$^3$-Thi$^{5,8}$-D-Phe$^7$-BK (DAHTDBK) in SHR and NWR

| | *LLBK* (pmol) | *DAHTDBK* (pmol) | *Antagonist/agonist ratio* (DAHTDBK/LLBK) |
|---|---|---|---|
| SHR | 8 | 30 | 3, 7 |
| NWR | 16 | 100 | 6, 3 |

Dose of LLBK used to cause a mean pressor effect of 27 to 29 mmHg in SHR and NWR.
Dose of antagonist which caused a mean reduction of 50% in the maximal pressor effect of LLBK.

($n = 8$ per dose). The $BK_1$ antagonist DALBK, in doses up to 11,500 pmol, did not alter blood pressure in the SHR nor did DALBK, administered i.c.v. simultaneously with the agonist, interfere with the pressor response to BK (Figure 3). On the other hand, the $BK_2$ antagonist TDBK, at a molar ratio of 10:1 with respect to the agonist, produced a 70% inhibition of the effect of 125 pmol BK (Figure 3). The pressor effect of BK was also inhibited by another $BK_2$ antagonist. Different amounts of DAHTDBK produced parallel shifts to the right of the dose-response curves for BK (Figure 4). Schild plots (Arunlakshana & Schild, 1959) of the data from Figure 4 and similar data previously obtained in normotensive animals (Lindsey *et al.*, 1989) yielded linear plots ($r = 0.98$ for SHR and $r = 0.99$ for NWR) with similar slopes (−1.01 for SHR and −1.16 for NWR). The $pA_2$ *in vivo* was estimated to be 10.66 for SHR and 9.91 for NWR. Another experiment, designed to estimate the potency of the antagonist in the SHR was carried out with LLBK as an agonist instead of BK. The dose of antagonist DAHTDBK sufficient to reduce by 50% the response of an equipotent dose of LLBK was estimated in both strains of rats. Table 4 shows that the agonist: antagonist ratio was 6 for the NWR and approximately 4 for the SHR.

## Discussion

The spontaneously hypertensive rats are more sensitive than normotensive rats to the systemic pressor action of kinins injected into the fourth cerebral ventricle. Bradykinin, TK, MLBK and LLBK are from three to seven times as potent in the SHR than in the NWR. Another significant difference between the two strains is the shorter latency for the manifestation of the pressor response to the kinins in the hypertensive animals. At present it is difficult to ascertain whether the increased sensitivity observed in the SHR is a selective trait related to the central action of kinins on blood pressure or whether it is due to widespread alterations in the SHR including aspects of neuronal reactivity. The finding that the SHR is not more sensitive than the NWR to the pressor effect of AII or endothelin injected into the fourth cerebral ventricle indicates that the increased sensitivity would be specific for BK. There is convincing evidence that the SHR display a hyperreactivity of the sympathetic nervous system (Okamoto *et al.*, 1967; Takahashi & Buñag, 1980), and this might explain the increased pressor responses to centrally administered BK in anaesthetized and pithed SHR (Takahashi & Buñag, 1980). However the anaesthesia and deafferentation affect the sympathetic tone and sympathetic/parasympathetic balance in an unpredictable manner, rendering impossible any comparison with the present experiments.

The present data, obtained in conscious rats, suggest that pharmacodynamic and pharmacokinetic aspects of the handling of kinins in the central nervous system may play a relevant role in the increased responsiveness of the SHR to BK and analogues. The biological activity of kinins in a given system results from the interplay of affinity, efficacy and susceptibility to degradation. Molecular degradation carried out by kininases represents the most important mechanism known to reduce the biological activity of kinins, and the differences in $ED_{50}$ ratios for each kinin in the NWR and SHR probably reflect a decreased kininase activity in the hypertensive animals. The two kinins LLBK (Roblero *et al.*, 1973; Lindsey *et al.*, 1987) and MLBK (Roblero *et al.*, 1973) which are more resistant to kininase activity showed lower potency ratios when the respective $ED_{50}$ values for the pressor effect were compared in the NWR and SHR. The relationship between susceptibility to degradation and potency ratio between strains is bolstered by the inverse correlation which is observed between these ratios and the duration of the pressor response, a parameter which itself may represent an index of kininase activity. A number of peptidases are believed to be active in metabolizing kinins in the central nervous system. Angiotensin converting enzyme (ACE), or kininase II has been

detected in neural tissue (Yang & Neff, 1972) and cerebrospinal fluid (Wigger & Stalcup, 1978) probably as an ACE isoenzyme (Strittmatter *et al.*, 1985). Enalaprilat, in the nanomolar range, inhibits ACE activity (Shapiro & Riordan, 1984). Other enzymes, such as EC 3.4.24.15, (Orlowski *et al.*, 1983), EC 3.4.24.19 (Oliveira *et al.*, 1990) or EC 3.4.21.26 (Wilk, 1983) are also found in neuronal tissue and inactivate several neuropeptides. CPP-Ala-Ala-Phe-pAB inhibits EC 3.4.24.15 with an affinity constant ($K_i$) of 30 nM (Orlowski, personal communication) and the same compound was shown to inhibit 80% of the kininase activity detected in hypothalamic slices of the rat brain (McDermott *et al.*, 1987). Preliminary experiments on the inhibition of the kininase activity in the fourth cerebral ventricle carried out in our laboratory support a similar conclusion. In order to assess the contribution of kininase activity to the response to centrally administered kinins, the pressor effect of BK and LLBK was examined in rats pretreated with an association of CPP-Ala-Ala-Phe-pAB and enalaprilat. Inhibitors were injected intracerebroventricularly in doses which could reach concentrations 5 and 6 orders of magnitude greater than the respective inhibition constants, provided that no losses occurred and that the inhibitors were homogeneously distributed in cerebrospinal fluid. Pretreatment with kininase inhibitors potentiated the response to BK in the NWR approximately five times and in the SHR three times. The response to LLBK, on the other hand, was not significantly altered in either strain by the inhibitors, supporting the assumption that LLBK is relatively resistant to degradation. Notwithstanding pretreatment with kininase inhibitors, the SHR still showed increased sensitivity to the pressor action of kinins in comparison to the NWR. This result suggests that mechanisms other than reduced kininase activity may contribute to the increased sensitivity to centrally administered kinins observed in the hypertensive rats.

The $BK_1$ receptor agonist DABK did not cause any alteration in the mean arterial pressure following its administration to the fourth cerebral ventricle of SHR and neither did the simultaneous administration of a selective antagonist of $BK_1$ receptors alter the central pressor response to BK. The pressor action of BK, however, was antagonized by the $BK_2$ antagonists TDBK and DAHTDBK, suggesting that the receptors which mediate the central effect of BK on blood pressure are of the $BK_2$ subtype. Apparently the receptors which mediate the central pressor response in the SHR are of the same subtype as those which mediate the same response in the NWR (Lindsey *et al.*, 1989). The antagonist DAHTDBK interacts with the central $BK_2$ receptors in competitive and reversible fashion as is shown by the parallel displacements to the right of the BK dose-response curves when the agonist was injected simultaneously with different concentrations of the antagonist. The $pA_2$ *in vivo* (Tallarida *et al.*, 1979) estimated for the kinin receptors of the SHR was 10.66, 0.7 log units larger than the $pA_2$ obtained for the kinin receptors in the NWR. It has been advanced that a difference in 0.5 or more in the $pA_2$ values for the same antagonist can be considered preliminary evidence for different types of receptors (Furchgott, 1972). However, since the $pA_2$ were determined *in vivo*, without control of any of the drug removal mechanisms, it is impossible to ascertain whether the difference in $pA_2$ values represents an increased affinity of the SHR receptors for the antagonist or is a consequence of differences in peptidase activity in SHR and NWR. Nevertheless, differential metabolism of the antagonist amongst strains, which could lead to a difference in the estimated $pA_2$ is apparently not an important factor. The DAHTDBK molecule is probably very resistant to kininase activity as may be deduced by modifications introduced into the antagonist's chemical structure. Data on the duration of the antagonist's action in the fourth cerebral ventricle support the assumption that DAHTDBK is relatively resistant to degradation (Lindsey *et al.*, 1989).

BK, LLBK, MLBK and TK probably have the same intrinsic activity since the maximal responses obtained were the same for the four kinins. Significant differences were observed in the potency of BK analogues. BK, which has the greatest affinity for the $BK_2$ receptors in different preparations (Regoli & Barabé, 1980; Manning *et al.*, 1986), was supplanted by LLBK in potency, probably because of the latter analogues exceptional resistance to enzymatic degradation. Differences among the kinins in the latency for the manifestation of the pressor response are difficult to interpret. BK and LLBK, which have the greatest affinity for the receptor, also showed significantly shorter latencies than TK or MLBK and, curiously, the latencies observed for BK, LLBK and MLBK are in the inverse order of the relative affinity for the kinin receptor in the guinea-pig ileum (Manning *et al.*, 1986). A causal relation between the greatest affinity of the kinin receptors and the shorter latency for manifestation of the pressor response in the SHR is improbable as many other mechanisms underlie the chain of events leading to centrally mediated pressor response.

The kinin receptors which mediate the central pressor response to BK in the SHR are of the $BK_2$ subtype, similar to the same receptors in the NWR. Distinct mechanisms comprising a decreased kininase activity and possibly a greater affinity of the receptors for the agonists, contribute to the increased sensitivity of the SHR to the pressor effect of centrally administered kinins.

The authors are indebted to Dr Aron Jurkiewicz from the Department of Pharmacology of the Escola Paulista de Medicina for valuable discussions of this manuscript throughout its preparation. This work was done with the technical assistance of Cléber Cícero de Souza Silva and was supported by grants from FINEP, FAPESP and CNPQ. D.R.F. is a FAPESP Undergraduate Trainee Fellow.

## References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48–58.

CORREA, F.M.A. & GRAEFF, F.G. (1974). Central mechanism of the hypertensive action of intraventricular bradykinin in the unanesthetized rat. *Neuropharmacology*, **13**, 65–75.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Catecholamines*. ed. Blaschko, H. & Muscholl, E. p. 283. New York: Springer-Verlag.

GRAEFF, F.G., PELA, I.R. & ROCHA E SILVA, M. (1969). Behavioural and somatic effects of bradykinin injected into cerebral ventricles of unanesthetized rabbits. *Br. J. Pharmacol.*, **37**, 723–732.

LANG, W.J. & PEARSON, L. (1968). Studies on the pressor responses produced by bradykinin and kallidin. *Br. J. Pharmacol. Chemother.*, **32**, 330–338.

LINDSEY, C.J., BENDHACK, L.M. & PAIVA, A.C.M. (1987). Effect of pH on the inhibition of angiotensin converting activity by enalaprilat in the rat perfused mesenteric vascular bed. *J. Pharmacol. Exp. Ther.*, **243**, 292–296.

LINDSEY, C.J., FUJITA, K. & MARTINS, D.T.O. (1988). The central pressor effect of bradykinin in normotensive and hypertensive rats. *Hypertension*, **2**, 126–129.

LINDSEY, C.J., NAKAIE, C.R. & MARTINS, D.T.O. (1989). Central nervous system kinin receptors and the hypertensive response mediated by bradykinin. *Br. J. Pharmacol.*, **97**, 763–768.

MANNING, D.C., VAVREK, R., STEWART, J.M. & SNYDER, S.H. (1986). Two bradykinin binding sites with picomolar affinities. *J. Pharmacol. Exp. Ther.*, **237**, 504–512.

McDERMOTT, J.R., GIBSON, A.M. & TURNER, J.D. (1987). Involvement of endopeptidase 24.15 in the inactivation of bradykinin by rat brain slices. *Biochem. Biophys. Res. Commun.*, **146**, 154–158.

OKAMOTO, K., NOSAKA, S., YAMORI, Y. & MATSUMO, M. (1967). Participation of neural factor in the pathogenesis of hypertension in the spontaneously hypertensive rat. *Jpn. Heart J.*, **8**, 168.

OLIVEIRA, E.S., LEITE, P.E.P., SPILLANTINI, M.G., CAMARGO, A.C.M. & HUNT, S.P. (1990). Localization of endo-oligopeptidase (EC3.4.22.19) in the rat nervous tissues. *J. Neurochem.*, **55**, 1114–1121.

ORLOWSKI, M., MICHAUD, C. & CHU, T.G. (1983). A soluble metalloendopeptidase from rat brain Purification of the enzyme and determination of specificity with synthetic and natural peptides. *Eur. J. Biochem.*, **135,** 81.

PAXINOS, G. & WATSON, C. (1986). The Rat Brain in Stereotaxic Coordinates. Centrecourt, Australia: Academic Press.

PEARSON, L. & LANG, W.J. (1967). The nature of the pressor responses produced by bradykinin and kallidin. *Aust. J. Exp. Biol. Med. Sci.*, **45,** 35.

PEARSON, L. & LANG, W.J. (1969). A comparison in conscious and anaesthetized dogs of the effect on blood pressure of bradykinin, kallidin, eledoisin and kallikrein. *Eur. J. Pharmacol.*, **2,** 83–87.

REGOLI, D. & BARABE, J. (1980). Pharmacology of bradykinin and related kinins. *Pharmacol. Rev.*, **32,** 1–46.

ROBLERO, J., RYAN, J.W. & STEWART, J.M. (1973). Assay of kinins by their effect on blood pressure. *Res. Commun. Chem. Pathol.*, **6,** 207–212.

SCHACHTER, M., UCHIDA, Y., LONGRIDGE, D.J., LABEDZ, T., WHALLEY, E.T., VAVREK, R.J. & STEWART, J.M. (1987). New synthetic antagonists of bradykinin. *Br. J. Pharmacol.*, **92,** 851–855.

SHAPIRO, R. & RIORDAN, J.F. (1984). Inhibition of angiotensin converting enzyme: dependence on chloride. *Biochemistry*, **23,** 5234–5240.

SNEDECOR, G.W. & COCHRAN, W.G. (1980). In *Statistical Methods.* pp. 149–392. Iowa City: The Iowa State University Press.

STRITTMATTER, S.M., THIELE, E.A., KAPILOFF, M.S. & SNYDER, S.H. (1985). A rat brain isozyme of angiotensin-converting enzyme. *J. Biol. Chem.*, **260,** 9825–9832.

TAKAHASHI, H. & BUÑAG, R.D. (1981). Centrally induced cardiovascular and sympathetic nerve responses to bradykinin in rats. *J. Pharmacol. Exp. Ther.*, **216,** 192–197.

TALLARIDA, R.J., COWAN, A. & ADLER, M.W. (1979). $PA_2$ and receptor differentiation: a statistical analysis of competitive antagonism. *Life Sci.*, **25,** 637–654.

VAVREK, R.J. & STEWART, J.M. (1985). Competitive antagonists of bradykinin. *Peptides*, **6,** 161.

WIGGER, H.J. & STALCUP, S.A. (1978). Distribution and development of angiotensin converting enzyme in the fetal and newborn rabbit. *Lab. Invest.*, **38,** 581–585.

WILK, S. (1983). Prolyl endopeptidase. *Life Sci.*, **33,** 2149–2157.

YANG, H.Y.T. & NEFF, N.H. (1972). Distribution and properties of angiotensin-converting enzyme of rat brain. *J. Neurochem.*, **21,** 1035–1036.

*(Received January 21, 1991*
*Revised March 5, 1991*
*Accepted March 26, 1991)*