British Journal of Pharmacology (2004) 142, 285–296
www.nature.com/bjp

Correlation between brain bradykinin receptor binding sites and cardiovascular function in young and adult spontaneously hypertensive rats

[1]Frank Cloutier, [1]Brice Ongali, [1]Maria M. Campos, [2]Gaétan Thibault, [3]Witold Neugebauer & *,[1]Réjean Couture

[1]Department of Physiology, Faculty of Medicine, Université de Montréal, C.P. 6128, Succursale Centre-ville, Montréal, Québec, Canada H3C 3J7; [2]Clinical Research Institute, Université de Montréal, Montréal, Québec, Canada H2W 1R7 and [3]Institute of Pharmacology, Faculty of Medicine, Université de Sherbrooke, Sherbrooke, Québec, Canada JIH 5N4

1 Intracerebroventricular (i.c.v.) effects of bradykinin (BK) B_1 and B_2 receptor agonists and antagonists were assessed on mean arterial blood pressure (MAP) and heart rate (HR) in awake unrestrained spontaneously hypertensive rats (SHR, aged of 8 and 16 weeks) and age-matched Wistar Kyoto rats (WKY). Quantitative *in vitro* autoradiographic studies were also performed on the brain of both strains with specific radioligands for B_2 receptors [^{125}I]HPP-Hoe 140 and B_1 receptors [^{125}I]HPP-des-Arg^{10} and Hoe140.

2 MAP increased linearly with doses of BK (81–8100 pmol) and the amplitudes were significantly greater in SHR, particularly at 16 weeks. While BK evoked a negative linear trend on HR (bradycardia) in WKY, a positive one (tachycardia) was observed in adult SHR. In both strains, BK-induced pressor response was blocked by equimolar doses of B_2 receptor antagonist, D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK (Hoe 140), but not by B_1 receptor antagonist, AcLys[D-βNal^7, Ile^8]des-Arg^9-BK (R-715).

3 B_1 receptor agonists (Sar-[D-Phe^8]-des-Arg^9-BK, des-Arg^9-BK, des-Arg^{10}-Kallidin) and antagonist (R-715 alone or with Hoe 140) had no or marginal effect on MAP and HR at doses up to 8100 pmol in SHR and WKY.

4 Higher densities of specific [^{125}I]HPP-Hoe 140 labelling were found in discrete brain areas of SHR, especially in regions associated with cardiovascular function. Low levels of [^{125}I]HPP-[des-Arg^{10}]-Hoe140 binding sites were seen in WKY and SHR, yet densities were significantly greater in midbrain and cortical regions of SHR aged of 16 weeks. Contrary to SHR, ageing caused a downregulation of B_2 and B_1 receptor binding sites in specific brain nuclei in WKY.

5 It is concluded that the hypersensitivity of the pressor response to i.c.v. BK in SHR occurs during both the early and established phases of hypertension in parallel with the enhancement of B_2 receptor binding sites in various cardiovascular brain centres. In contrast, brain B_1 receptors do not seem to participate in the central pressor effects of kinins nor in the maintenance of hypertension in SHR.
British Journal of Pharmacology (2004) **142,** 285–296. doi:10.1038/sj.bjp.0705759

Keywords: Kinins; bradykinin; B_1 and B_2 receptors; brain; blood pressure; hypertension; autoradiography

Abbreviations: Amb, ambiguus nucleus; aCSF, artificial cerebrospinal fluid; BSA, bovine serum albumin; CGA, central gray (alpha part); DA, dorsal hypothalamic area; DTT, dithiothreitol; HR, heart rate; i.c.v., intracerebroventricular; LDDM, laterodorsal thalamic nucleus (dorsomedial); LDVL, laterodorsal thalamic nucleus (ventrolateral); MAP, mean arterial blood pressure; NTS, nucleus tractus solitarius; PA5, paratrigeminal nucleus; PDTg, posterodorsal tegmental nucleus; PIPES, piperazine-*N,N′-bis*[2-ethanesulphonic-acid]; Pn, pontine nucleus; Pyt, pyramidal tract; SHR, spontaneously hypertensive rat; SCP, superior cerebellar peduncle; SP5, spinal trigeminal tract; VMH, ventromedial hypothalamic nucleus; VPL, ventral posterolateral thalamic nucleus; VPM, ventral posteromedial thalamic nucleus; WKY, Wistar Kyoto rat

Introduction

Kinins are known as vasoactive peptides released by kallikrein from kininogens during tissue injury and noxious stimulation. This family includes bradykinin (BK), kallidin (KD) and their active kininase I metabolites (des-Arg^9-BK and des-Arg^{10}-KD) whose biological effects are mediated by two transmembrane G-protein-coupled receptors named B_1 and B_2 (Regoli & Barabé, 1980; Marceau *et al*., 1998; Couture *et al*., 2001). Unlike B_2 receptors, which are constitutively expressed in most tissues, B_1 receptors are generally underexpressed in healthy animals and humans but induced and functionally expressed by cytokines, bacterial endotoxins and following tissue injury (Marceau *et al*., 1998).

All components of the kallikrein–kinin system have been identified within the central nervous system, and activation of

*Author for correspondence; E-mail: couturer@physio.umontreal.ca
Advance online publication: 5 April 2004

B_2 receptors exerts modulatory effects on arterial blood pressure through neuronal autonomic pathways (Couture & Lindsey, 2000). In comparison to normotensive Wistar Kyoto rat or WKY, spontaneously hypertensive rat (SHR) showed increased sensitivity to the pressor action of BK when injected into the lateral brain ventricle (Buñag & Takahashi, 1981; Lindsey *et al*., 1988), fourth ventricle (Lindsey *et al*., 1988; Martins *et al*., 1991), rostral ventrolateral medulla (Privitera *et al*., 1994) and thoracic spinal cord (Cloutier *et al*., 2002). Although acute and chronic B_2 receptor blockade with antagonists injected centrally failed to alter systemic arterial blood pressure in SHR (Lindsey *et al*., 1988; Madeddu *et al*., 1990; 1994; Martins *et al*., 1991), evidence for a central tonic influence of kinins on blood pressure may be found in this model. Inhibition of kininase II, the major metabolic pathway for kinins, by intracerebroventricular (i.c.v.) injection of captopril caused a rise in blood pressure that was blocked by a B_2 receptor antagonist, suggesting a prominent role for kininase II and endogenous kinins in SHR (Madeddu *et al*., 1990).

Data with regard to the functional relevance of B_1 receptors in the brain of SHR are conflicting. Martins *et al*. (1991) reported that neither the B_1 receptor agonist des-Arg^9-BK nor the B_1 receptor antagonist des-Arg^9-Leu^8-BK injected into the fourth ventricle altered blood pressure in SHR or WKY. In contrast, Alvarez *et al*. (1992) reported that the same antagonist injected into the lateral ventricle caused a long-lasting reduction in blood pressure and heart rate (HR) in SHR but not in WKY. Using a more stable B_1 agonist (Sar-[D-Phe^8]-des-Arg^9-BK), Emanueli *et al*. (1999) reported that the i.c.v. activation of B_1 receptors in SHR and WKY evokes increases in blood pressure, and that the peptidase-resistant B_1 antagonist AcLys[D-βNal^7, Ile^8]des-Arg^9-BK (R-715) lowers blood pressure in SHR. Moreover, i.c.v. injection of antisense oligodeoxynucleotides targeted to B_1 receptor mRNA produced a profound blood pressure reduction that persisted more than 48 h in SHR (Emanueli *et al*., 1999).

A recent pharmacological and autoradiographic study conducted in 16-week-old SHR and WKY showed that the cardiovascular changes induced by the spinal injection of kinins are mediated exclusively by B_2 receptor activation (Cloutier *et al*., 2002). B_2 receptor binding sites were found to increase from the age of 8–16 weeks in the thoracic spinal cord of SHR. Conversely, B_1 receptor binding sites were present in young (8 weeks) but not in old (16 and 24 weeks) SHR and WKY (Ongali *et al*., 2003a). Other differences between young and old SHR have been described such as the concentration of kinins (Khan *et al*., 1995) and kininase II activity (Israel & Saavedra, 1987) in the cerebrospinal fluid, which are, respectively, reduced and increased in adult SHR. Thus, ageing has a profound influence on the kinin system and may account for some discrepancies in the literature. Housing conditions in a pathogen-free environment is also a prerequisite if one intends to study the inducible B_1 receptor as it is expressed in animals diagnosed with an established infection (Siebeck *et al*., 1998).

The present study was undertaken to re-evaluate the relative contribution of B_1 and B_2 receptors in the cardiovascular responses to brain kinins in SHR and age-matched WKY. This was achieved with: (i) a pharmacological approach following the i.c.v. injection of stable selective B_1 and B_2 receptor agonists and antagonists in 8-week-old SHR (early phase of hypertension) and 16-week-old SHR (established hypertension) and age-matched WKY; and (ii) an extensive quantitative analysis of the anatomical distribution of binding sites for both kinin receptors in the brain of SHR and WKY using *in vitro* autoradiography.

Methods

Animal source and care

Male SHR (8 and 16 weeks old) ($n=40$) and age-matched WKY ($n=31$) were purchased pathogen free at least 1 week prior to the experiments from Charles River, St Constant, Québec, Canada and Harlan, Indianapolis, IN, U.S.A. Equal number of matched WKY and SHR were purchased from the same source at each occasion. They were housed individually in plastic cages under a 12 h light–dark cycle in a room with controlled temperature (23°C) and humidity (50%) with food (Charles River Rodent) and tap water available *ad libitum*. The care of animals and research protocols were in compliance with the guiding principles for animal experimentation as enunciated by the Canadian Council on Animal Care and approved by the Animal Care Committee of our University.

Surgery

Rats were anaesthetised with an intraperitoneal (i.p.) injection of 65 mg kg^{-1} sodium pentobarbitone (Somnotol; MTC Pharmaceuticals, Cambridge, Ontario, Canada). The head of the rat was fixed to a stereotaxic apparatus (David Kopf Instrumentation, Tujunga, CA, U.S.A.), and one midline incision was made on the scalp. The angle of the head was adjusted according to the horizontal plan with respect to both *bregma* and *lambda* reference points. A hole was drilled in the skull according to stereotaxic coordinates: 8–week-old rats – 1.0 mm caudal to the *bregma*, 1.5 mm lateral to the midline, 4.5 mm vertical from the skull surface; 16-week-old rats – 0.6 mm caudal to the *bregma*, 1.3 mm lateral to the midline, 5 mm vertical from the skull surface. An i.c.v. cannula (PE-20; Fisher Scientific, Ontario, Canada) was inserted with a guide into the right lateral ventricle (i.c.v.) and fixed to the skull with dental cement (Reliance Dental MFG Co., Worth, IL, U.S.A.). Thereafter, the rats were allowed to recover in individual plastic cages (40 cm × 23 cm × 20 cm) and housed in the same controlled conditions. The correct position of the i.c.v. catheter was verified by post-mortem examination at the end of experiment.

After 5 days, rats were reanaesthetised with sodium pentobarbitone (65 mg kg^{-1}, i.p.) and an intravascular siliconised (Sigmacote) PE-50 catheter, filled with physiological saline containing 100 IU ml^{-1} heparin sodium salt, was inserted into the abdominal aorta through the femoral artery for direct blood pressure recording and exteriorised at the back of the neck. Before both surgeries, the animals received the antibiotics trimethoprime and sulphadiazine (Tribissen 24%, 30 mg kg^{-1}, s.c., Schering Canada Inc., Pointe Claire, Québec, Canada). Ketoprophen, an anti-inflammatory and analgesic drug, was given during the first surgery only (Anafen, 5 mg kg^{-1}, s.c., Merial Canada Inc., Baie d'Urfé, Québec, Canada). Recovery from anaesthesia was monitored closely under a warming lamp to maintain the body temperature of

animals. Thereafter, rats were housed individually in polyethylene cages with a top grid and returned to their resident room. Experimental protocols were initiated 24 h later, in awake and unrestrained rats.

Measurement of cardiovascular parameters

Blood pressure and HR were measured, respectively, with a Statham pressure Transducer (P23ID) and a cardiac tachometer (model 7P4) (triggered by the arterial blood pressure pulse) coupled to a Grass polygraph (model 79; Grass Instruments Co., Quincy, MA, U.S.A.). The cardiovascular response was measured 1 h after the rats were transported to the testing room. They remained in their resident cage, but the top grid was removed and they had no more access to the food and water during experiments (5–6 h). When resting blood pressure and HR were stable, rats received an i.c.v. injection of 1 μl artificial cerebrospinal fluid (aCSF).

Experimental protocols

Dose–response curves to i.c.v. agonists SHR (8 and 16 weeks old) ($n=7$–9) and age-matched WKY ($n=7$–10) were used on 3 consecutive days. On days 1 and 2, rats initially received an i.c.v. injection of aCSF (1 μl) followed 15 min later by increasing doses of either BK (81, 202, 405, 810 and 8100 pmol) or the metabolically stable B_1 receptor agonist Sar-[D-Phe^8[-des-Arg^9-BK (81, 810 and 8100 pmol) (Regoli *et al*., 1998) to construct a complete dose–response curve. Rats received only one of the two agonists at random on the same day. The two prototypic B_1 receptor agonists des-Arg^9-BK (8100 pmol) and des-Arg^{10}-KD (8100 pmol) (Regoli *et al*., 1998) were injected following the highest dose of Sar-[D-Phe^8]-des-Arg^9-BK. On the third day, the highly selective and metabolically stable B_1 receptor antagonist AcLys[D-βNal^7, Ile^8]des-Arg^9-BK (R-715, 81, 810 and 8100 pmol) (Regoli *et al*., 1998) was administered. Increasing doses of B_1 agonists and R-715 were given at 40–60 min intervals, while 60–90 min intervals were left between increasing doses of BK. No desensitisation of the cardiovascular response to BK was seen under these conditions in pilot experiments. Peptides were administered in a volume of 1 μl of vehicle followed by 4 μl volume of aCSF, which corresponds to the void volume of the catheter. Each dose was calculated per rat in 1 μl solution.

Effects of i.c.v. kinin receptor antagonists

SHR (8 and 16 weeks old) ($n=6$–7) and age-matched WKY ($n=6$–7) initially received 405 or 810 pmol BK, and 1 h after were given i.c.v. an equimolar dose of B_2 receptor antagonist, D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK (Hoe 140), and then 3 min later was given the same dose of BK. The agonist was reinjected alone 24 h later to assess the reversibility of any blockade observed with the antagonist on the preceding day.

In another group of 16-week-old SHR ($n=6$), the B_1 antagonist R-715 (8100 pmol) was tested i.c.v. 3 min prior to BK (405 pmol). The same group received 24 h later, R-715 (8100 pmol) and Hoe 140 (8100 pmol) together, and the effect on mean arterial blood pressure (MAP) and HR was continuously recorded for up to 24 h postinjection.

Tissue preparation for autoradiography

Autoradiographic studies were performed according to the procedures described previously (Cloutier *et al*., 2002; Ongali *et al*., 2003b). For this purpose, two groups of four WKY and two groups of four SHR (8 and 16 weeks old) not submitted to any surgery were used. After killing under carbon dioxide inhalation and decapitation, whole brains were immediately removed, frozen in 2-methyl butane cooled at -45 to -55°C with liquid nitrogen and then stored at -80°C until use. Matched whole brains of the same strain were mounted together (two brains per gelatine bloc) and serially cut into 20 μm thick coronal sections with a cryostat at temperature varying between -10 and -12°C. Adjacent sections were taken for experiments using B_1 and B_2 receptor ligands and alternatively thaw-mounted on 0.2% gelatine/0.033% chromium potassium sulphate-coated slides, and stored at -80°C. Sets of three slides were used for total binding and two sets for nonspecific binding. About 400 slides $\times$ three sections per slide were obtained per bloc $\times$ two blocs giving a total of 2400 sections per group of four WKY or SHR.

In vitro *receptor autoradiography*

On the day of experiments, sections were thawed at room temperature and preincubated for 30 s in 25 mM piperazine-*N*,*N*′-*bis*[2-ethanesulphonic-acid] (PIPES) buffer (pH 7.4; 4°C). Thereafter, slides were incubated for 90 min at room temperature in 25 mM PIPES buffer containing: 1 mM 1,10-phenanthroline, 1 mM dithiothreitol (DTT), 0.014% bacitracin, 0.1 mM captopril, 0.2% bovine serum albumin (BSA) (protease free) and 7.5 mM magnesium chloride in the presence of 150 pM [^{125}I]HPP-desArg^{10}-Hoe 140 (B_1 receptor ligand) or 200 pM [^{125}I]HPP-Hoe 140 (B_2 receptor ligand). Peptides were iodinated by means of the chloramine T method (Hunter & Greenwood, 1962), and the specific activity of both ligands was calculated to be approximately 2000 c.p.m. fmol^{-1} or 1212 Ci mmol^{-1}. The nonspecific binding was determined in the presence of 1 μM of unlabelled ligands (HPP-desArg^{10}-Hoe 140 for B_1 receptor and HPP-Hoe 140 for B_2 receptor). To ascertain the specificity of the labelled B_2 radioligand, the same concentration of unlabelled B_1 ligand was added to the solution. Likewise, the same concentration of the unlabelled B_2 ligand was added to the labelled B_1 ligand solution. At the end of the incubation period, slides were transferred sequentially through four rinses of 4 min each in 25 mM PIPES (pH 7.4; 4°C), dipped for 15 s in distilled water (4°C) to remove the excess of salts and air-dried. Kodak Scientific Imaging Films BIOMAX™ MS® were juxtaposed onto the slides in the presence of [^{125}I]-microscales and exposed at room temperature for 3 days (B_1 ligand) or 2 days (B_2 ligand). The films were developed in D-19 (Kodak developer) and fixed in Kodak Ektaflo. Densitometric readings were performed with an image analysis system (MCID™, Imaging Research Inc., Ontario, Canada). A standard curve from [^{125}I]-microscales was used to convert density levels into fentomoles per milligram of tissue (fmol mg^{-1} tissue).

Concentrations of radioligands were chosen on the basis of previous studies (Murone *et al*., 1997; Cloutier *et al*., 2002; Ongali *et al*., 2003b) and correspond to maximal specific binding on the saturation curves (B_{max}). The dissociation constant (K_d) was similar in SHR and WKY for B_1 receptor

(30 pM) and B_2 receptor (25 pM) radioligands as shown in the spinal trigeminal nucleus (Figure 1). Hence, increases in the density of kinin receptor binding sites in SHR are likely due to increases in the number of receptors and not due to changes in receptor affinity. Moreover, the absolute nonspecific B_1 and B_2 receptor binding in SHR and WKY was the same, suggesting that the increase in specific binding (determined by subtracting nonspecific labelling from total binding) measured in SHR was due to a true increase of kinin receptor protein (Figure 1). Similar results were obtained in the other selected regions of the brain. Nonspecific binding defined in the presence of 1 μM unlabelled ligands accounted for about 50 and 40% of total binding for B_1 and B_2 receptors, respectively, and no differences in nonspecific binding were found between-rat groups in both strains and ages. The number of replicates analysed for each brain region was a minimum of three per animal. The anatomical structures and their nomenclature were taken from the atlas of Paxinos & Watson (1998).

Drugs and solutions

The composition of aCSF was (in mM): NaCl 128.6, KCl 2.6, $MgCl_2$ 2.0 and $CaCl_2$ 1.4; pH adjusted to 7.2. BK (MW: 1060.3) and des-Arg^9-BK (MW: 904.1) were purchased from Peninsula laboratories (San Carlos, CA, U.S.A.). Des-Arg^{10}-KD (MW: 1032.2) was purchased from Bachem Bioscience Inc. (King of Prussia, PA, U.S.A.). Hoe 140 (1305.7), R-715 (1140.5) and Sar-[D-Phe^8]-des-Arg^9-BK (975.2) were obtained from Dr D. Regoli of Sherbrooke University (Sherbrooke, Québec, Canada). HPP-desArg^{10}-Hoe 140 (3-4 hydroxyphenyl-propionyl-desArg^9-D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK) and HPP-Hoe 140 (3-4 hydroxyphenyl-propionyl-D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK) were developed from the selective B_1 receptor antagonist desArg^{10}-Hoe 140 (Wirth *et al*., 1991) and the B_2 receptor antagonist Hoe 140 or Icatibant (Hock *et al*., 1991), respectively. Bacitracin, BSA (protease free), captopril, DTT, heparin sodium salt (porcine, grade 1-A), magnesium chloride, PIPES, 1,10-phenanthroline and Sigmacote were purchased from Sigma-Aldrich Canada Ltd (Oakville, Ontario, Canada). Autoradiographic [^{125}I]-microscales (20 μm) and Kodak Scientific imaging film BIOMAX™ MS (double coated, 24 cm × 30 cm) were purchased from Amersham Pharmacia Biotech Canada. Antagonists and agonists were dissolved directly in aCSF. The stock solutions (8100 pmol μl^{-1}) of agonists and antagonists were stored in aliquots of 10 μl at −20°C until use.

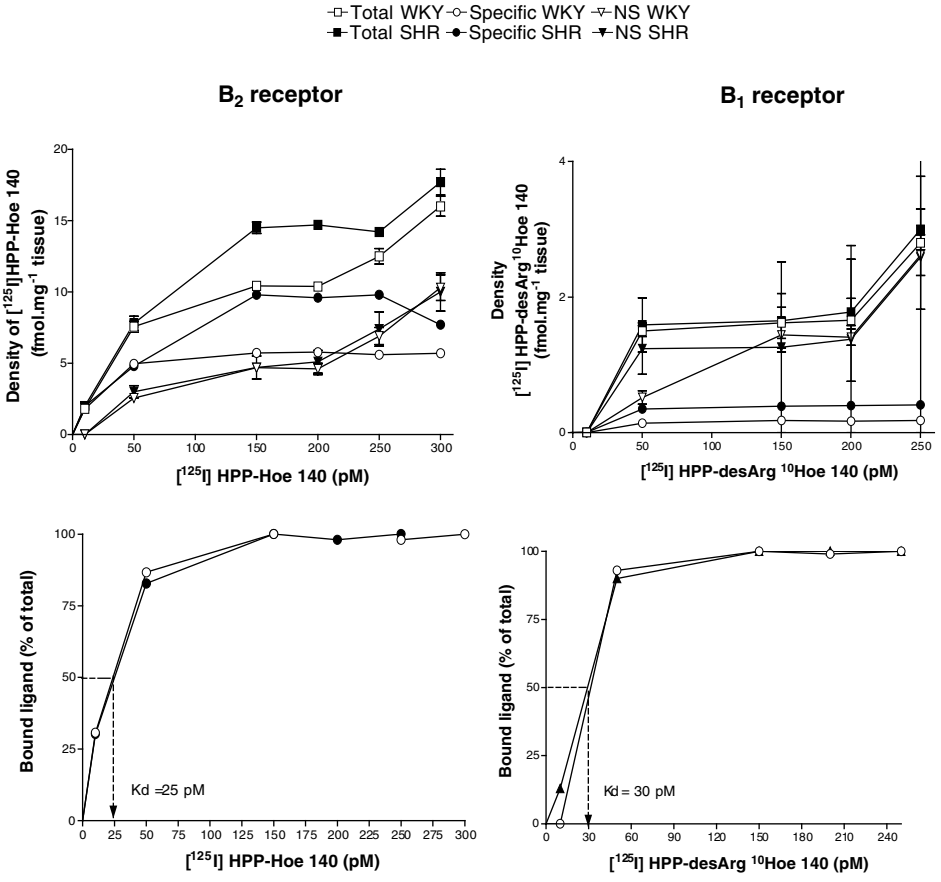

Figure 1 Amount of the B_2 receptor radioligand [^{125}I]HPP-Hoe 140 (left panels) and B_1 receptor radioligand [^{125}I]HPP-[des-Arg^{10}]-Hoe140 (right panels) bound to the spinal trigeminal nucleus (SP5) of 8-week-old SHR ($n=4$) and age-matched WKY ($n=4$) as a function of their concentrations. Specific binding is calculated as the mathematical difference between total and nonspecific (NS) binding, which persists in the presence of 1 μM of unlabelled ligand (upper panels). The affinity of the binding, which is expressed as a dissociation constant (K_d), was calculated as the concentration of B_2 or B1 radioligand that results in 50% of maximal specific binding (lower panels).

Statistical analysis of data

Results are expressed as means ± s.e.m. Statistical significance of differences were evaluated with Student's *t*-test on unpaired (between groups) or paired (within the same group) samples. Multiple comparisons were analysed with the analysis of variance (ANOVA). Data from MAP and HR were transformed in two variables: maximal values and area under the curve (AUC). These two variables were analysed for significance using a $2 \times 2 \times 4$ factorial ANOVA with strains at two levels, age at two levels and doses at four levels. This analysis allows the between-rats evaluation of strain, age and their interaction, as well as the within-rats evaluation of BK kinetic effects and the mixed interactions of strain and age with BK kinetics. Data from autoradiographic receptor binding were analysed for significance using a 2×2 factorial ANOVA with strains at two levels and age at two levels. Differences in the number of rats per group were weighted using orthogonalised coefficients (Draper & Smith, 1981). For all variables, the critical level of significance was set at 5%.

Table 1 Baseline values for MAP, HR and body weight in 8- and 16-week-old WKY and SHR

	Basal MAP (mm Hg)	*Basal HR* (beats min^{-1})	*Body weight* (g)	n
8 week-old				
WKY	103 ± 4	335 ± 10	211 ± 5	10
SHR	154 ± 5***	354 ± 14	190 ± 7	14
16 week-old				
WKY	116 ± 5	298 ± 17	321 ± 5	13
SHR	166 ± 4***,†	342 ± 8	315 ± 4	18

Data are means ± s.e.m. of *n* rats. Statistical comparison between SHR and age-matched WKY (*) or between 8- and 16-week-old SHR (†) is indicated by $^{\dagger}P<0.05$; $^{***}P<0.001$.

Results

In vivo *studies*

Baseline MAP values in SHR were significantly higher at the age of 8 and 16 weeks, and particularly at 16 weeks old, when compared to age-matched WKY. However, baseline HR values did not differ significantly between strains or age. Moreover, the body weight was not significantly different between WKY and SHR at either age (Table 1).

Effects of BK on MAP and HR in SHR and WKY

The effects of five increasing doses of BK on MAP in 8- and 16-week-old SHR and WKY are shown in Figure 2. BK (81–8100 pmol) evidenced dose- and time-dependent increases in MAP that peaked at 2–3 min postinjection. The ANOVA

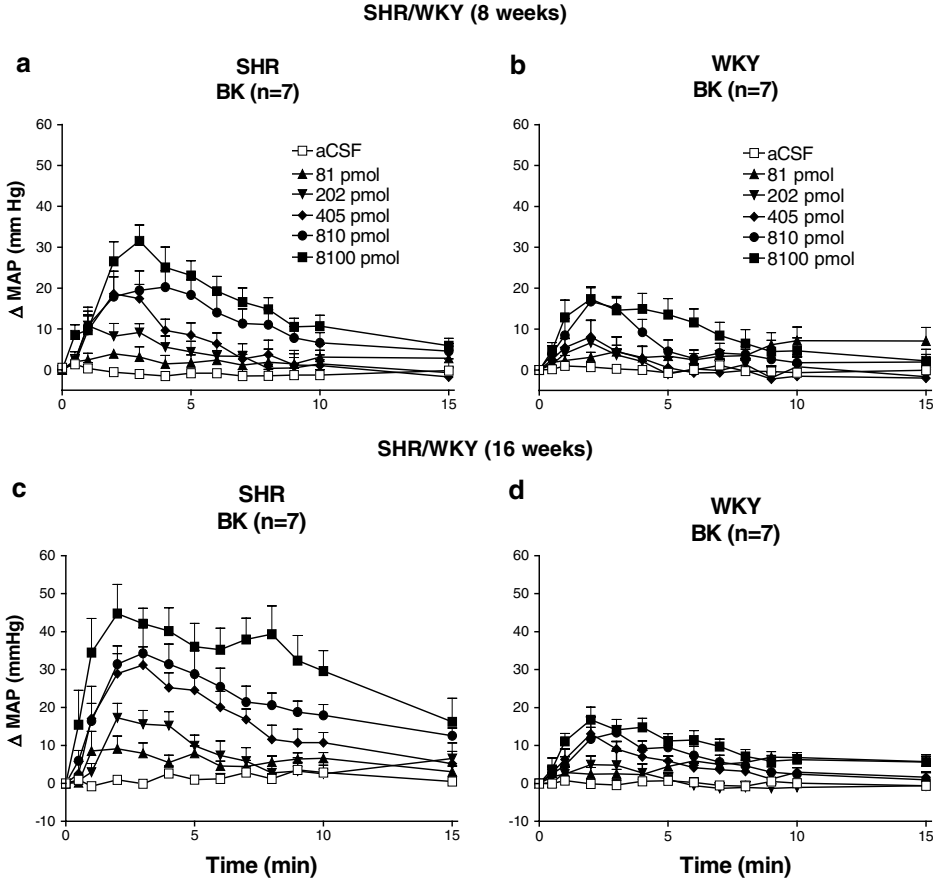

Figure 2 Time-course effects on changes in mean arterial blood pressure (ΔMAP) evoked by five increasing doses of BK injected i.c.v. in 8- and 16-week-old SHR (a and c) and age-matched WKY (b and d). Each point represents means ± s.e.m. of seven rats.

shows a significant effect of BK on maximal changes in MAP when compared to aCSF values for both WKY ($F_{1,54}=25.06$) and SHR ($F_{1,54}=144.76$) (Figure 3a). Maximal changes in MAP differed significantly between strains ($F_{1,18}=25.94$) and ages ($F_{1,18}=8.51$) and revealed strain by age interaction ($F_{1,18}=5.46$). Whereas no age effect was found in WKY ($F_{1,18}=0.004$), an age effect was found in SHR ($F_{1,18}=13.95$). In WKY, maximal changes in MAP increased linearly with doses ($F_{1,54}=8.54$) but much less than in SHR ($F_{1,54}=143.07$). The 16-week-old SHR were more sensitive to BK than the 8-week-old SHR ($F_{1,18}=8.51$). In WKY, no quadratic trend was observed ($F_{1,54}=0.09$) but a weak quadratic trend was seen in SHR ($F_{1,54}=5.73$). These qualitative differences between SHR and WKY in BK pressor responses led to a significant strain by dose interaction ($F_{3,72}=13.52$).

When MAP responses were expressed as AUC (Figure 3c), differences in strains ($F_{1,18}=22.16$) and ages ($F_{1,18}=8.84$) were observed with a significant strain by age interaction ($F_{1,18}=5.43$).This interaction is explained by an absence of age effect in WKY and a significant one in SHR ($F_{1,18}=0.011$; $F_{1,18}=14.25$, respectively). Both a strain by dose ($F_{3,72}=9.78$) and age per dose ($F_{3,72}=4.56$) interactions were found. Pressor effects to BK were significant in both WKY ($F_{1,54}=6.66$) and SHR ($F_{1,54}=68.77$). However, the BK effect did not vary significantly from 81 to 8100 pmol in WKY ($F_{linear,54}=2.86$ and $F_{quad,54}=0.92$), while it increased dose dependently in SHR ($F_{linear,54}=78.30$ and $F_{quad,54}=1.38$).

As illustrated in Figure 3b, the maximal changes in HR induced by BK (81–8100 pmol) show a strain by age interaction ($F_{1,24}=6.09$) since no changes were found between 8 and 16 weeks old in WKY ($F_{1,24}=0.13$) but in SHR ($F_{1,24}=9.82$), and because the differences between the two strains were observed only in the 16-week-old rat ($F_{1,24}=24.57$). The maximal changes in HR induced by BK differed between the two strains leading to significant interactions with the comparison of BK to aCSF values ($F_{1,96}=5.45$) and with the linear trend of BK ($F_{1,96}=18.25$). Indeed, the pharmacological effect of BK was observed only in SHR ($F_{1,72}=5.27$). A negative linear trend was observed in WKY ($F_{1,72}=9.93$), while a positive linear trend was observed in SHR ($F_{1,72}=8.35$).

When HR responses to BK were expressed as AUC (Figure 3d), SHR evidenced a significant increase in comparison to WKY ($F_{1,24}=7.77$). BK induced in both strains a significant augmentation of the AUC ($F_{1,72}=12.73$). However, this augmentation was dose linear dependent only in SHR ($F_{1,72}=18.98$) leading to a strain by linear trend interaction ($F_{1,96}=5.10$).

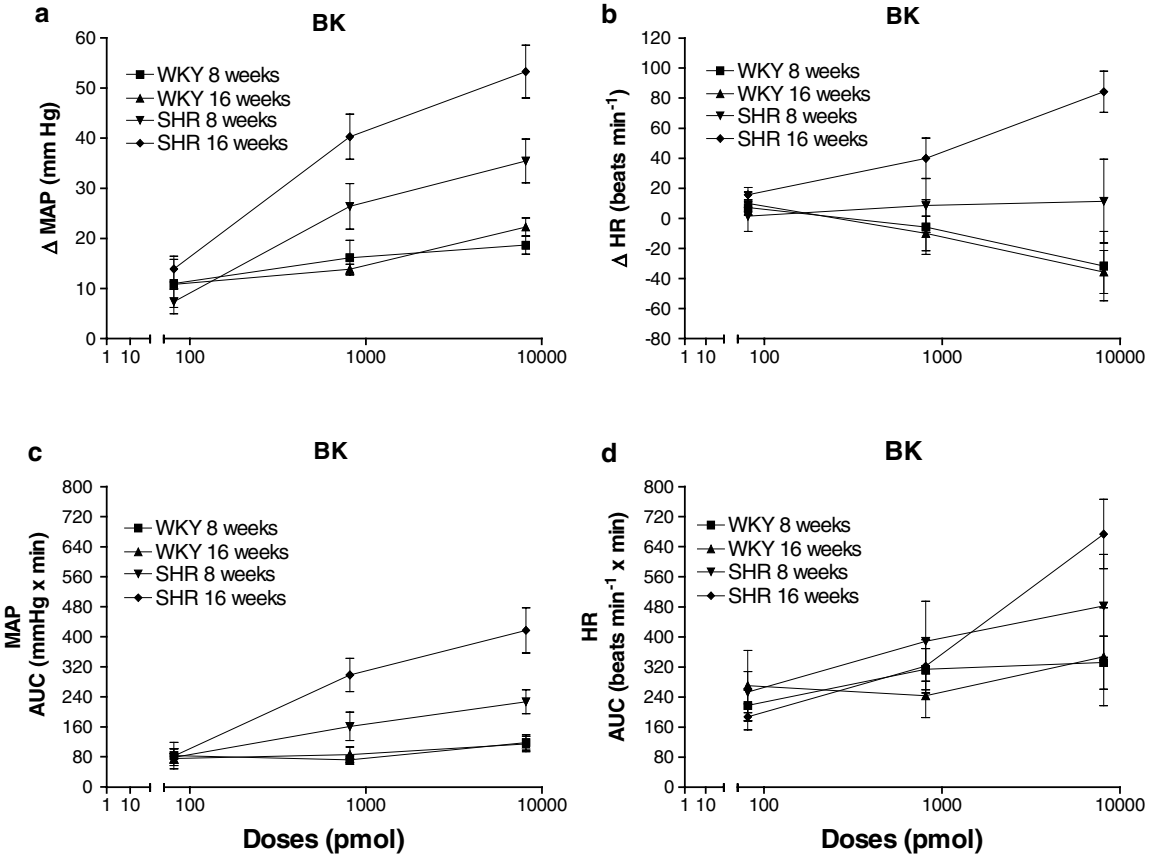

Figure 3 Dose–response curves to BK on MAP (a and c) and HR (b and d). Shown are maximal changes (a and b) and AUC for a period of 15 min (c and d). Values represent means ± s.e.m. of seven rats. aCSF values are: ΔMAP (mmHg): 2.0 ± 1.2 (WKY, 8 weeks), 1.7 ± 1.2 (WKY, 16 weeks), 1.8 ± 0.9 (SHR, 8 weeks), 1.4 ± 0.7 (SHR, 16 weeks); AUC MAP (mmHg × min): 42.1 ± 18.7 (WKY, 8 weeks), 20.7 ± 8.5 (WKY, 16 weeks), 25.5 ± 12.5 (SHR, 8 weeks), 29.6 ± 13.1 (SHR, 16 weeks); ΔHR (beats min^{-1}): 4.3 ± 5.3 (WKY, 8 weeks), −4.3 ± 5.2 (WKY, 16 weeks), 4.3 ± 9.7 (SHR, 8 weeks), −1.4 ± 8.8 (SHR, 16 weeks); AUC HR (beats min^{-1} × min): 152.0 ± 42.3 (WKY, 8 weeks), 121.2 ± 46.3 (WKY, 16 weeks), 203.0 ± 34.8 (SHR, 8 weeks), 190.6 ± 55.0 (SHR, 16 weeks).

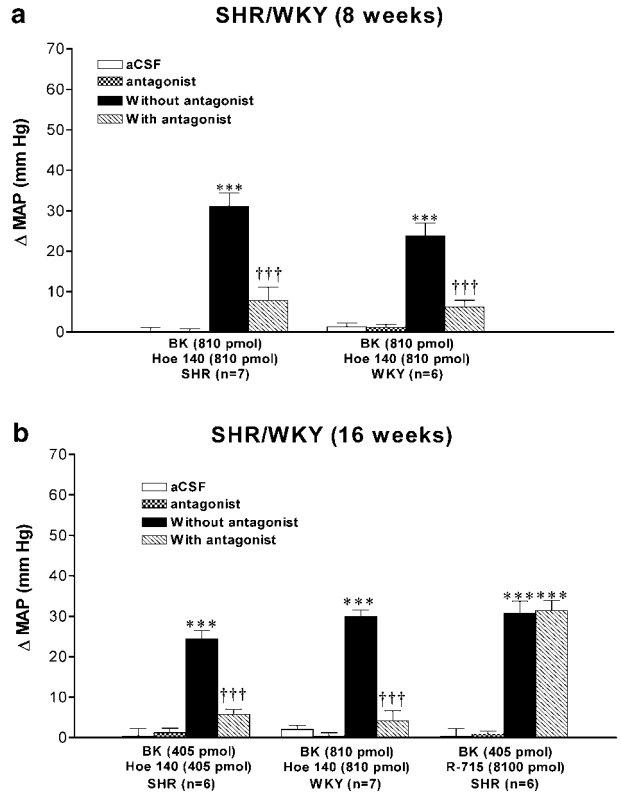

Figure 4 Maximal changes in mean arterial blood pressure (ΔMAP) induced by 405 or 810 pmol BK injected i.c.v. in 8-week-old (a) and 16-week-old (b) SHR and age-matched WKY without or with kinin antagonists (Hoe 140 or R-715). Data are means ± s.e.m. of (*n*) rats. Statistical comparison to aCSF values (*) or to the agonist without Hoe 140 (†) is indicated by ***,††† $P<0.001$.

Effects of BK under B_2 receptor blockade in SHR and WKY

The B_2 receptor antagonist Hoe 140 was tested against the maximal MAP increase induced by 405 or 810 pmol BK in SHR and WKY. The MAP response evoked by BK was blocked by the prior i.c.v. injection of Hoe 140 (at equimolar dose with BK, 3 min earlier) in both strains at the age of 8 weeks (Figure 4a) and 16 weeks (Figure 4b). In all groups, the pressor response to BK was completely back to preantagonist values when the agonist was reinjected alone 24 h later (data not shown). Hoe 140 was devoid of any direct effect on MAP (Figure 4) and HR (data not shown).

Lack of effect of B_1 receptor antagonist in SHR and WKY

The pressor response to BK (405 pmol) was not affected by the prior i.c.v. injection of R-715 (8100 pmol) in 16-week-old SHR (Figure 4b). Moreover, i.c.v. injection of R-715 (81–8100 pmol) failed to alter MAP (Figure 5c) and HR (Figure 5f) for a period up to 24 h postinjection when compared to aCSF values in both strains and ages. Coadministration of Hoe 140 and R-715 (8100 pmol each) did not alter blood pressure for a period up to 24 h postinjection in SHR (data not shown).

Lack of effect of B_1 receptor agonists on MAP and HR in SHR and WKY

The cardiovascular effects of three B_1 receptor agonists in 8- and 16-week-old SHR and WKY are shown in Figure 5. I.c.v. injection of des-Arg^9-BK (8100 pmol) or des-Arg^{10}-KD (8100 pmol) did not significantly alter MAP and HR when compared to aCSF values in SHR and WKY at both ages (Figure 5a and d). Whereas Sar-[D-Phe^8]-des-Arg^9-BK (81–8100 pmol) was inactive on MAP and HR in young and adult WKY, it evoked a small but significant effect on MAP at the two lowest doses in 8-week-old SHR. However, this agonist had no significant pressor effect in 16-week-old SHR (Figure 5b). Moreover, Sar-[D-Phe^8]-des-Arg^9-BK also failed to alter significantly HR in SHR at 8 and 16 weeks (Figure 5e).

Autoradiographic studies

Autoradiographic distribution and density values of B_1 and B_2 receptors in WKY and SHR aged of 8 and 16 weeks are depicted in Figure 6 and in Tables 2 and 3, and are described herein.

B_2 receptor distribution

Data show moderate levels of B_2 receptor binding sites in several distinct brain regions of WKY. At the midbrain level, B_2 receptor labelling (ranging between 1.0 ± 0.0 and 4.0 ± 0.5 fmol mg^{-1} tissue) was found in thalamic- and hypothalamic-related areas, such as laterodorsal thalamic nuclei (LDDM and LDVL), ventral posteromedial thalamic nuclei (VPM and VPL), whole hypothalamus, dorsal hypothalamic area (DA), ventromedial hypothalamic nucleus (VMH) as well as in amygdala and hippocampus. These values were significantly decreased in the thalamus, VPM and amygdala of 16-week-old WKY (Table 2). Greater density values (5.3 ± 1.0 to 5.9 ± 1.0 fmol mg^{-1} tissue) were seen in the hindbrain, including the posterodorsal tegmental nucleus (PDTg) and the spinal trigeminal tract (SP5) of 8- and 16-week-old WKY (Table 2). In some hindbrain areas such as the pontine nucleus (Pn), paratrigeminal nucleus (PA5), nucleus tractus solitarius (NTS), pyramidal tract (Pyt) and ambiguus nucleus (Amb), density values measured in WKY (8 weeks) were also significantly reduced in 16-week-old WKY (Table 2).

In SHR, the pattern of B_2 receptor brain distribution was quite similar than that observed in WKY, although values in several structures were significantly higher in SHR. B_2 receptor-specific labelling in SHR was markedly increased in several regions, including the amygdala, Pn, PDTg, central gray (alpha part) (CGA), SP5 and PA5 indistinctly of the age (Table 2). When compared to age-matched WKY, the density of B_2 receptor binding sites was significantly augmented in the hippocampus of SHR at 8 weeks, and in thalamus, hypothalamus, DA, VMH, superior and inferior colliculus, superior cerebellar peduncle (SCP), NTS, Pyt and Amb of SHR at 16 weeks (Table 2). Moreover, adult SHR exhibited significant increase of B_2 receptor density values in DA, VMH and SCP when compared to young SHR. In contrast, B_2 receptor-specific labelling measured in hippocampus was decreased in older SHR (Table 2).

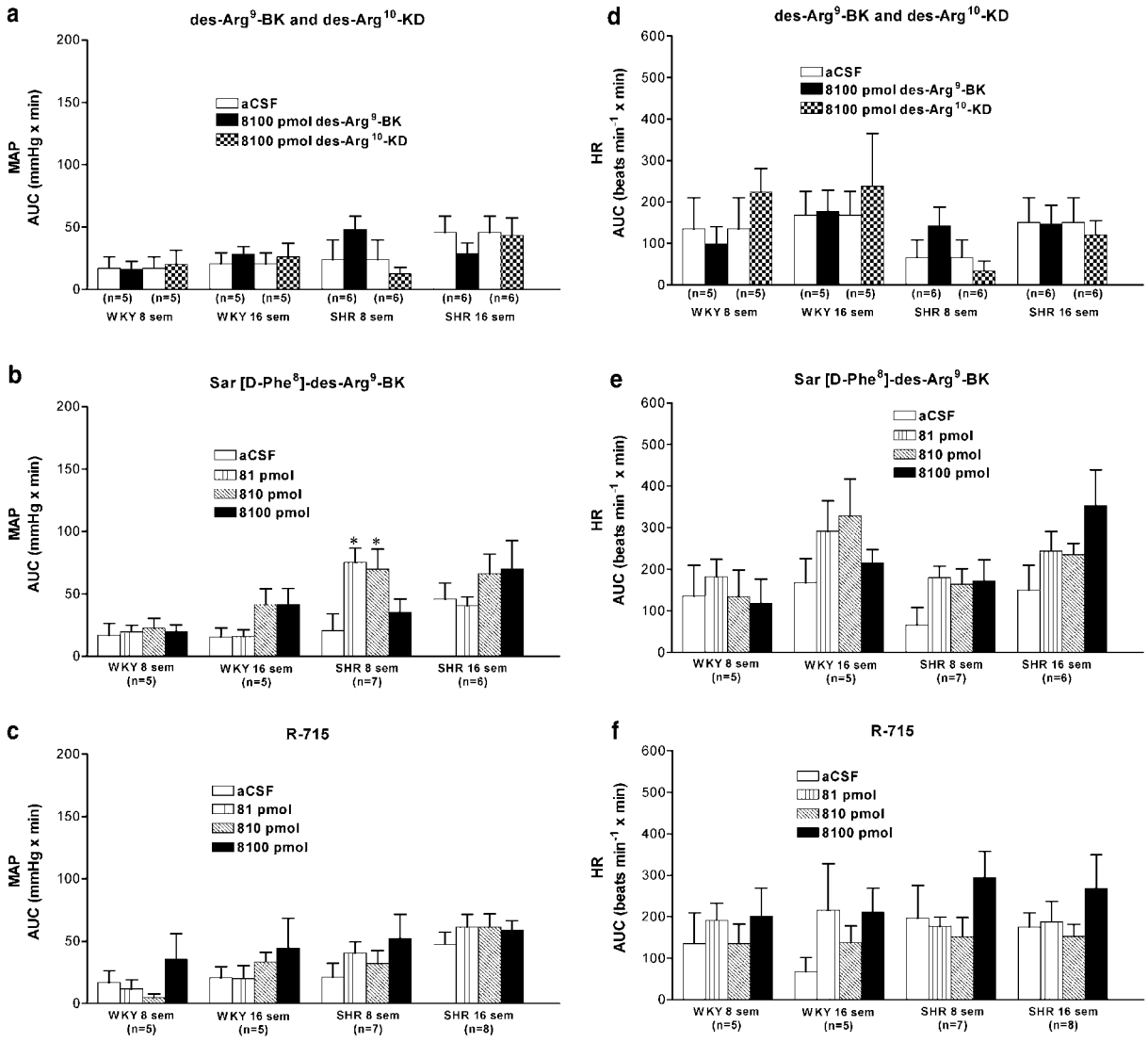

Figure 5 I.c.v. effects of kinin B_1 receptor agonists (des-Arg^9-BK, des-Arg^{10}-KD, Sar-[D-Phe^8]-des-Arg^9-BK) and antagonist (R-715) on MAP (a–c) and HR (d–f) in 8- and 16-week-old SHR and age-matched WKY. Data represent means$\pm$s.e.m. of the AUC for a period of 15 min in (*n*) rats. Statistical comparison to aCSF (*) values is indicated by $*P<0.05$.

B_1 receptor distribution

With respect to B_1 receptors, low levels of specific binding sites were seen in both WKY and SHR of 8 and 16 weeks of age (ranging between 0.02 ± 0.01 and 1.90 ± 0.30 fmol mg^{-1} tissue). In 8-week-old WKY, B_1 receptor densities were notably higher than those measured in 16-week-old rats; significant differences were seen in structures including occipital cortex, colliculus, subiculum, Pn and PDTg (Figure 6, Table 3). B_1 receptor densities differed significantly between WKY and SHR indistinctly of the age, and were higher in VPM, hypothalamus, DA, VMH and PDTg of SHR. When compared to age-matched WKY, B_1 receptor densities were significantly enhanced (6–19-fold) in 16 weeks SHR in all cortical regions, thalamus, LDDM, LDVL, VPL, amygdala, hippocampus, colliculus and subiculum (Table 3). B_1 receptor densities were also significantly greater in all cortical regions, LDDM, LDVL, VPL, amygdala and hippocampus of 16-week-old SHR when compared to 8 weeks SHR.

Discussion

In vivo *studies*

Our results are consistent with a number of studies which have reported increased pressor responses to BK administered into the lateral or fourth cerebral ventricles or in specific cardiovascular centres of the brain stem and thoracic spinal cord in SHR (Buñag & Takahashi, 1981; Lindsey *et al*., 1988; Martins *et al*., 1991; Privitera *et al*., 1994; Couture & Lindsey, 2000; Cloutier *et al*., 2002). However, the present study shows for the first time that the hypersensitivity to BK occurs during the early phase of hypertension and continues to develop thereafter. Our data with selective antagonists do not support, however, a contribution of endogenous kinins on cerebral B_2 and B_1 receptors in the maintenance of arterial hypertension in SHR nor in the tonic control of blood pressure in WKY.

Sympathetic hyperactivity has been consistently demonstrated in SHR (Takeda & Buñag, 1978; Buñag & Takeda,

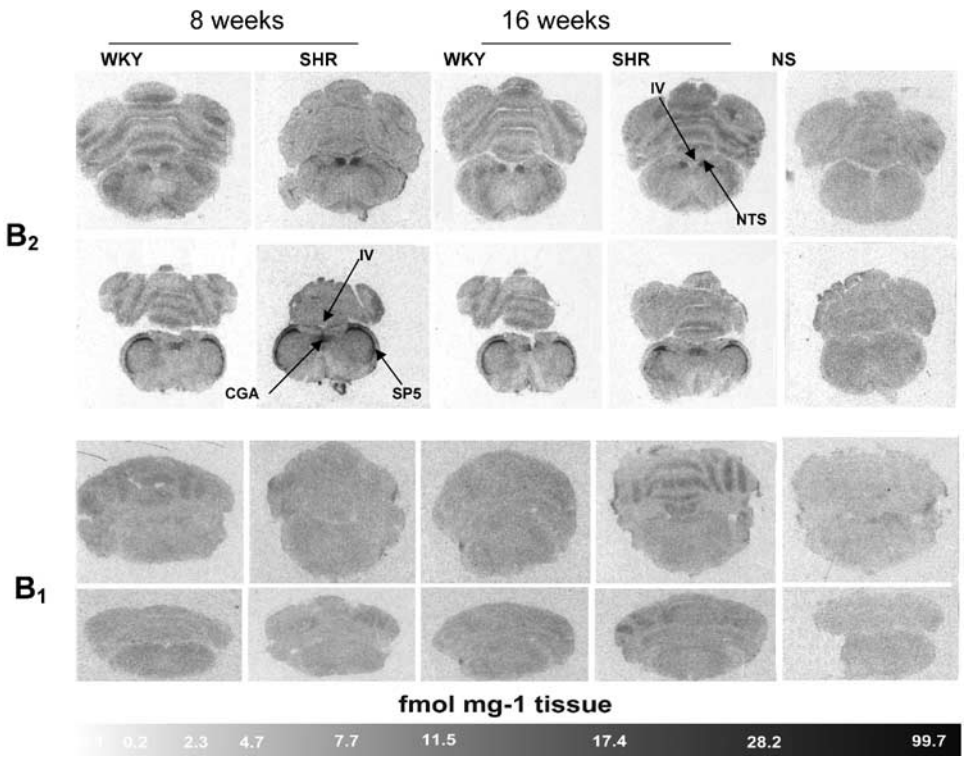

Figure 6 Autoradiographic distribution of kinin B_1 and B_2 receptors in the brain of 8- and 16-week-old WKY and SHR. Shown are autoradiograms representing total binding of [^{125}I]HPP-desArg^{10}-Hoe 140 (B_1 receptor ligand) or [^{125}I]HPP-Hoe 140 (B_2 receptor ligand). Nonspecific (NS) binding in the presence of 1 μM of HPP-Hoe 140 (B_2 receptors) and HPP-[des-Arg^{10}]-Hoe 140 (B_1 receptors) are also shown. Pictures are presented as obtained with the image analysis system. CGA, central gray (alpha part); SP5, spinal trigeminal tract; NTS, nucleus tractus solitarius; IV, fourth ventricle.

Table 2 Densities of specific B_2 receptor binding sites measured in the rat brain of 8- and 16-week-old WKY and SHR

	8 week-old		*16 week-old*		
	WKY	*SHR*	*WKY*	*SHR*	
Brain area					$F_{1,12}$
Midbrain					
Thalamus	2.95±0.29	2.06±0.25	1.48±0.39†	2.97±0.64*	7.92[c]
LDDM	4.01±0.46	3.03±0.11	2.29±0.74	2.99±0.56	2.61
LDVL	3.94±0.64	3.09±0.22	2.20±0.76	3.15±0.79	1.93
VPM	3.31±0.39	2.43±0.22	1.71±0.38†	3.05±0.75	5.45[c]
VPL	3.05±0.46	2.58±0.11	1.94±0.34	3.04±0.78	2.58
Hypothalamus	3.01±0.25	2.78±0.27	1.95±0.61	3.63±0.40 *	5.56[c]
DA	3.07±0.34	2.26±0.11	1.86±0.58	4.60±0.62***,††	14.72[c]
VMH	3.29±0.21	2.91±0.27	2.09±0.68	4.55±0.62**,†	8.45[c]
Amygdala[a,b]	1.14±0.02	1.24±0.06	0.87±0.06	0.98±0.42	0.02
Hippocampus	1.01±0.03	1.65±0.12***	0.94±0.10	0.89±0.15†††	10.14[c]
Hindbrain					
Colliculus	2.09±0.12	2.43±0.21	0.67±0.38	3.29±0.98**	4.48[c]
Subiculum	2.53±0.14	2.61±0.26	1.29±0.49	2.62±0.91	1.37
Pn[a,b]	1.91±0.13	2.98±0.34	0.45±0.26	1.04±0.46	0.56
PDTg[a]	5.23±0.97	11.30±1.08	5.95±1.07	10.78±0.85	0.39
CGA[a]	3.33±0.41	4.88±0.58	3.88±1.10	7.71±0.93	2.01
SCP	4.67±1.07	3.74±0.94	2.24±0.88	6.56±0.43**,†	9.26[c]
SP5[a]	5.72±0.42	12.94±0.53	5.69±0.36	10.68±1.11	2.71
PA5[a,b]	4.23±0.47	9.21±0.64	1.78±0.54	5.23±0.99	1.22
NTS	3.24±0.30	3.60±0.72	0.79±0.24†	4.17±1.14**	4.66[c]
Pyt	1.75±0.30	2.01±0.15	0.26±0.16†	2.14±0.39***	9.26[c]
Amb	2.09±0.39	1.72±0.91	0.22±0.19†	2.94±0.52**	7.45[c]

Data are means±s.e.m. of four rats. Values represent densities measured in fmol mg^{-1} tissue.
[a]Significant difference between WKY and SHR but not dependent on age.
[b]Significant difference between 8 weeks and 16 weeks but not dependent on strains.
[a,b]Significant differences between both strains and ages.
[c]F-values showing strain and age interaction. In that case, significant differences are between SHR and age-matched WKY (*$P<0.05$; **$P<0.01$; ***$P<0.001$) or between 8 and 16 weeks in each strain ($^{\dagger}P<0.05$; $^{\dagger\dagger}P<0.01$; $^{\dagger\dagger\dagger}P<0.001$).

Table 3 Densities of specific B_1 receptor binding sites measured in the rat brain of 8 and 16 week-old WKY and SHR

	8 week-old		*16 week-old*		
	WKY	*SHR*	*WKY*	*SHR*	
Brain area					$F_{1,12}$
Cortical regions					
Parietal cortex	0.41±0.21	0.52±0.19	0.07±0.02	1.35±0.33**,†	7.29[c]
Occipital	0.81±0.16	0.88±0.26	0.22±0.02†	1.88±0.15**,†††	21.25[c]
Perihinal/entorhinal	0.33±0.19	0.64±0.22	0.16±0.01	1.57±0.22***,††	8.89[c]
Midbrain					
Thalamus	0.37±0.16	0.44±0.17	0.08±0.02	0.87±0.17**	5.98[c]
LDDM	0.45±0.06	0.45±0.16	0.08±0.03	1.10±0.21***,††	14.02[c]
LDVL	0.46±0.10	0.43±0.23	0.09±0.02	1.04±0.25**,†	7.69[c]
VPM[a]	0.44±0.19	0.56±0.21	0.08±0.02	0.76±0.17	2.89
VPL	0.44±0.19	0.41±0.07	0.14±0.07	0.82±0.17**,†	7.41[c]
Hypothalamus[a]	0.39±0.15	0.54±0.18	0.10±0.01	0.80±0.20	3.33
DA[a]	0.44±0.18	0.50±0.26	0.09±0.01	0.86±0.17	3.96
VMH[a]	0.30±0.08	0.44±0.18	0.13±0.02	0.83±0.20	4.08
Amygdala	0.52±0.20	0.45±0.16	0.14±0.01	1.90±0.30***,†††	21.41[c]
Hippocampus	0.34±0.10	0.54±0.16	0.17±0.04	1.35±0.29***,††	7.87[c]
Hindbrain					
Colliculus	0.72±0.13	0.66±0.13	0.12±0.03††	0.65±0.15**	5.93[c]
Subiculum	0.73±0.07	0.76±0.12	0.15±0.01†††	0.85±0.13***	12.31[c]
Pn	0.65±0.15	0.26±0.11*	0.02±0.01†††	0.07±0.07	5.07[c]
PDTg[a,b]	0.29±0.02	0.41±0.09	0.13±0.01†††	0.26±0.08	0.02
CGA	0.13±0.09	0.30±0.06	0.12±0.02	0.10±0.03	3.12
SCP	0.14±0.10	0.38±0.12	0.10±0.02	0.18±0.06	0.85
SP5	0.17±0.11	0.39±0.16	0.18±0.03	0.22±0.12	0.57
PA5	0.28±0.15	0.25±0.15	0.16±0.03	0.13±0.06	0.00
NTS	0.20±0.13	0.42±0.20	0.17±0.04	0.19±0.12	0.53
Pyt	0.18±0.16	0.12±0.09	0.08±0.02	0.10±0.02	0.17
Amb	0.08±0.06	0.30±0.18	0.17±0.04	0.18±0.14	0.74

Data are means±s.e.m. of four rats. Values represent densities measured in fmol mg^{-1} tissue.
[a]Significant difference between WKY and SHR but not dependent on age.
[b]Significant difference between 8 and 16 weeks but not dependent on strains.
[a,b]Significant differences between both strains and ages.
[c]F-values showing strain and age interaction. In that case, significant differences are between SHR and age-matched WKY (*$P<0.05$; **$P<0.01$; ***$P<0.001$) or between 8 and 16 weeks in each strain (†$P<0.05$; ††$P<0.01$; †††$P<0.001$).

1979; de Champlain, 1990) and seems to increase in older animals (Judy *et al*., 1979). As pressor effects induced by i.c.v. BK are partly mediated by the activation of the sympathetic nervous system (Buñag & Takahashi, 1981; Takahashi & Buñag, 1981; Qadri *et al*., 1999), it is likely that the greater BK-induced pressor responses derive at least partly from an exaggerated sympathetic tone in SHR. Changes in kininase II activity cannot provide an explanation for the hypersensitivity to BK since it was found higher in the CSF of adult SHR, suggesting a higher metabolic activity for kinins (Israel & Saavedra, 1987).

The present pharmacological study confirms earlier reports suggesting that central administration of BK and related peptides causes cardiovascular changes through the activation of B_2 receptors in normotensive and hypertensive rats (for a review see Couture & Lindsey, 2000), although other studies suggested that B_1 receptors may also play a role (Alvarez *et al*., 1992; Emanueli *et al*., 1999). Using the same pharmacological B_1 receptor agonists and antagonists (and from the same supplier), we failed to reproduce the results of Emanueli *et al*. (1999), which showed pressor effects with B_1 receptor agonists (including the stable agonist Sar-[D-Phe^8]-des-Arg^9-BK) and antihypertensive effect with the B_1 receptor antagonists (R-715). This discrepancy cannot be explained by the age or the gender of rats as in both studies male were used and we took into consideration the early and established phases of hypertension in this model. However, the inducible behaviour of B_1 receptors may explain these conflicting results. Indeed, this receptor is highly inducible by a wide range of externally applied stressors, including inflammatory cytokines (TNFα, IL-1β) and bacterial products (Marceau *et al*., 1998). It is worth mentioning that instrumentation with i.c.v. cannula induces localised and robust expression of TNFα and IL-1β mRNA in the tissue surrounding the lesion (Zhang & Rivest, 2001). Also, brain injury is known to activate the transcriptional nuclear factor-κB (NF-κB) (Nonaka *et al*., 1999; Pennypacker *et al*., 2000), which is involved in the induction of B_1 receptors (Marceau *et al*., 1998; Couture *et al*., 2001). One should also pay attention to the housing condition of animals in a pathogen-controlled environment, as B_1 receptors are associated with pre-existing infection (Siebeck *et al*., 1998). In our study, antibiotics and an anti-inflammatory analgesic (ketoprophen) were used before surgery to reduce infections and inflammation following trauma caused by surgery. Thus, utilisation of antibiotics and ketoprophen may have prevented the induction of B_1 receptors by cytokines and bacterial agents in our colony of SHR and WKY, which in addition were housed in our animal facilities under controlled and well-identified viral contaminants.

Autoradiographic studies

Autoradiographic results demonstrate that B_2 receptors are widely distributed throughout the brain of WKY. This widespread distribution of B_2 receptors is consistent with other studies performed in the rat brain (Chen *et al*., 2000; Couture & Lindsey, 2000; Ongali *et al*., 2003b) and other animal species, including guinea-pig (Fujiwara *et al*., 1988; 1989; Privitera *et al*., 1991), sheep (Murone *et al*., 1997) and human (Buck *et al*., 2002). Taken together, these data reinforce the idea suggesting a physiological role for B_2 receptors in the CNS. Interestingly, our results also indicate that brain B_2 receptors are subjected to downregulation in specific brain nuclei of 16 weeks old WKY in comparison to 8 weeks old rats. This suggests an age- and tissue-dependent regulation of central B_2 receptors in WKY.

Densities of B_2 receptor binding sites were seen markedly enhanced in some brain areas of both 8- and 16-week-old SHR, in comparison with their normotensive controls. Thus, the increased number of B_2 receptors may represent an important contributory factor to the enhanced responsiveness to BK in SHR. B_2 receptors were upregulated mostly in the hindbrain nuclei, which are thought to be the most relevant sites of action of i.c.v. kinins (Couture & Lindsey, 2000). Likewise, the hypersensitivity of the pressor response to intrathecal BK in SHR was correlated with increased level of B_2 receptor binding sites in the thoracic spinal cord (Cloutier *et al*., 2002). This is in keeping with the age-related increase of B_2 receptor binding sites in the spinal cord of SHR (Ongali *et al*., 2003a) and of B_2 receptor mRNA levels in the hypothalamus of SHR (Qadri *et al*., 2002). Evidence was also provided that B_2 receptors are upregulated in several medullary cardiovascular centres of *post-mortem* human donors afflicted by arterial hypertension (Buck *et al*., 2002). Whether or not the observed changes of central kinin receptors reflect a secondary effect of hypertension rather that an underlying mechanism has been addressed in a recent study (Ongali *et al*., 2003a). In the latter study, it was concluded that the greater density of B_2 receptor binding sites occurring in the thoracic spinal cord of SHR is unlikely secondary to arterial hypertension because it was further increased in adult SHR, which had undergone a chronic antihypertensive therapy from the age of 4 weeks with angiotensin-1-converting enzyme inhibitors or with an angiotensin AT_1 receptor antagonist. Thus, alterations of central kinin receptors may reflect a genetic trait associated to hypertension. It is however unknown at the present time whether or not these alterations contribute to the development of arterial hypertension. Nevertheless, all these latter studies including ours contrast with a recent autoradiographic study, which failed to detect significant difference in B_2 receptor binding sites in the nucleus of the solitary tract, area postrema, dorsal motor nucleus of the vagus and caudal subnucleus of the spinal trigeminal nucleus of adult WKY and SHR using [^{125}I-Tyr^0]-BK as radioligand (Privitera *et al*., 2003). Discrepancies may result from differences in methodology or the radioligand used; for instance, an agonist (e.g. [Tyr^0]-BK) may cause internalisation of the receptor that does not occur with an antagonist such as HPP-Hoe 140 (Marceau *et al*., 1998).

In addition to the pharmacological study performed with i.c.v. injections of selective B_1 receptor agonists and antagonist, our autoradiographic study does not support a role for B_1 receptors in the central action of kinins nor in the maintenance of hypertension in SHR. A similar conclusion for the B_1 receptor was reached at the level of the spinal cord in SHR (Cloutier *et al*., 2002; Ongali *et al*., 2003a). Densities of B_1 receptor binding sites were very low in all examined brain structures in young SHR, and only small differences were found between SHR and WKY at the age of 8 weeks despite the occurrence of hypersensitivity to i.c.v. BK. B_1 receptor binding sites increased in some brain regions of 16-week-old SHR, while B_1 receptor density values declined with ageing in WKY. The exact meaning of this opposite regulation of B_1 receptors in mature SHR and WKY is still unknown. Most elevated densities of B_1 receptors in SHR were seen in cortical areas, thalamic regions, amygdala and hippocampus. B_1 receptors were also upregulated in some pressor-related hypothalamic areas of 16-week-old SHR, such as DA and VMH (Wardener, 2001); however, it is uncertain whether or not these areas can be stimulated by i.c.v. kinins. The latter data are congruent with the increased B_1 receptor mRNA expression in the hypothalamus of 12- to 13-week-old SHR (Qadri *et al*., 2002). Thus one cannot exclude a putative role for B_1 receptors in hypothalamic functions unrelated to the cardiovascular control in SHR.

Conclusion

Our study shows that the hypersensitivity of the pressor response to i.c.v. BK in SHR occurs not only during the established phase of hypertension but also during its early phase in parallel with the enhancement of B_2 receptor binding sites in various cardiovascular areas of the brain. On the other hand, pharmacological and autoradiographic results do not support a primary role for brain B_1 receptors neither in the central pressor effect of kinins nor in the maintenance of hypertension in SHR.

We acknowledge Dr R. Elie (Université de Montréal) for his invaluable help and expertise in the statistical analysis of the data and Dr D. Regoli (Sherbrooke Université, Sherbrooke, Canada) for the donation of kinin B_1 and B_2 receptor agonists and antagonists. This work was supported by a grant-in-aid to R.C. from the Canadian Institutes of Health Research (MT-14379). F. Cloutier and B. Ongali were the holders of PhD studentships from the Fonds de la Recherche en Santé du Québec and the Republic of Gabon, respectively. M.M. Campos was awarded a postdoctoral Fellowship from The Research Group on Autonomic Nervous System at Université de Montréal.

References

ALVAREZ, A.L., DELORENZI, A., SANTAJULIANA, D., FINKIELMAN, S., NAHMOD, V.E. & PIROLA, C.J. (1992). Central bradykininergic system in normotensive and hypertensive rats. *Clin. Sci.,* **82,** 513–519.

BUCK, H.S., ONGALI, B., THIBAULT, G., LINDSEY, C.J. & COUTURE, R. (2002). Autoradiographic detection of kinin receptors in the human medulla of control, hypertensive, and diabetic donors. *Can. J. Physiol. Pharmacol.,* **80,** 249–257.

BUÑAG, R.D. & TAKAHASHI, H. (1981). Exaggerated sympathetic responses to bradykinin in spontaneously hypertensive rats. *Hypertension,* **3,** 433–440.

BUÑAG, R.D. & TAKEDA, K. (1979). Sympathetic hyperresponsiveness to hypothalamic stimulation in young hypertensive rats. *Am. J. Physiol.,* **237,** R39–R44.

CHEN, E.Y., EMERICH, D.F., BARTUS, R.T. & KORDOWER, J.H. (2000). B_2 bradykinin receptor immunoreactivity in rat brain. *J. Comp. Neurol.,* **427,** 1–18.

CLOUTIER, F., BUCK, H.S., ONGALI, B. & COUTURE, R. (2002). Pharmacologic and autoradiographic evidence for an up-regulation of kinin B_2 receptors in the spinal cord of spontaneously hypertensive rats. *Br. J. Pharmacol.,* **135,** 1641–1654.

COUTURE, R., HARRISSON, M., VIANNA, R.M. & CLOUTIER, F. (2001). Kinin receptors in pain and inflammation. *Eur. J. Pharmacol.,* **429,** 161–176.

COUTURE, R. & LINDSEY, C.J. (2000). Brain kallikrein–kinin system: from receptors to neuronal pathways and physiological functions. In: *Handbook of Chemical Neuroanatomy,* ed. Quirion, R., Björklund, A. & Hökfelt, T. Vol. 16: Peptide Receptors, Part I, pp. 241–300.

DE CHAMPLAIN, J. (1990). Pre- and post-synaptic adrenergic dysfunctions in hypertension. *J. Hypertens.,* **8** (Suppl 7), S77–S85.

DRAPER, N.R. & SMITH, H. (1981). Applied regression analysis. *Wiley Series in Probability and Mathematical Statistics,* 2nd edn. New York: John Wiley & Sons Inc. 275–278.

EMANUELI, C., CHAO, J., REGOLI, D., CHAO, L., NI, A. & MADEDDU, P. (1999). The bradykinin B_1 receptor and the central regulation of blood pressure in spontaneously hypertensive rats. *Br. J. Pharmacol.,* **126,** 1769–1776.

FUJIWARA, Y., MANTIONE, C.R., VAVREK, R.J., STEWART, J.M. & YAMAMURA, H.I. (1989). Characterization of [^{3}H]bradykinin binding sites in guinea-pig central nervous system: possible existence of B_2 subtypes. *Life Sci.,* **44,** 1645–1653.

FUJIWARA, Y., MANTIONE, C.R. & YAMAMURA, H.I. (1988). Identification of B_2 bradykinin binding sites in guinea-pig brain. *Eur. J. Pharmacol.,* **147,** 487–488.

HOCK, F.J., WIRTH, K., ALBUS, U., LINZ, W., GERHARDS, H.J., WIEMER, G., HENKE, ST., BREIPOHL, G., KÖNIG, W., KNOLLE, J. & SCHÖLKENS, B.A. (1991). Hoe 140 a new potent and long acting bradykinin-antagonist: *in vitro* studies. *Br. J. Pharmacol.,* **102,** 769–773.

HUNTER, W.M. & GREENWOOD, F.C. (1962). Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature,* **194,** 495–496.

ISRAEL, A. & SAAVEDRA, J.M. (1987). High angiotensin converting enzyme (kininase II) activity in the cerebrospinal fluid of spontaneously hypertensive adult rats. *J. Hypertens.,* **5,** 355–357.

JUDY, W.V., WATANABE, A.M., MURPHYW, R., APRISON, B.S. & YU, P.L. (1979). Sympathetic nerve activity and blood pressure in normotensive backcross rats genetically related to the spontaneously hypertensive rat. *Hypertension,* **6,** 598–604.

KHAN, I.M., MILLER, D.H., STRICKLAND, J., MARGOLIUS, H.S. & PRIVITERA, P.J. (1995). Brain kallikrein–kinin system abnormalities in spontaneously hypertensive rats. *Hypertension,* **25**, (Part 1) 524–530.

LINDSEY, C.J., FUJITA, K. & MARTINS, T.O. (1988). The central pressor effect of bradykinin in normotensive and hypertensive rats. *Hypertension,* **11** (Suppl 1), 126–129.

MADEDDU, P., GLORIOSO, N., SORO, A., TONOLO, G., MANUNTA, P., TROFFA, C., DEMONTIS, M.P., VARONI, M.V. & ANANIA, V. (1990). Brain kinins are responsible for the pressor effect of intracerebroventricular captopril in spontaneously hypertensive rats. *Hypertension,* **15,** 407–412.

MADEDDU, P., GLORIOSO, N., VARONI, M.V., DEMONTIS, M.P., FATTACCIO, M.C. & ANANIA, V. (1994). Cardiovascular effects of brain kinin receptor blockade in spontaneously hypertensive rats. *Hypertension,* **23** (Suppl 1), I189–I292.

MARCEAU, F., HESS, J.F. & BACHVAROV, D.R. (1998). The B_1 receptors for kinins. *Pharmacol. Rev.,* **50,** 357–386.

MARTINS, D.T.O., FIOR, D.R., NAKAIE, C.R. & LINDSEY, C.J. (1991). Kinin receptors of the central nervous system of spontaneously hypertensive rats related to the pressor response to bradykinin. *Br. J. Pharmacol.,* **103,** 1851–1856.

MURONE, C., PAXINOS, G., McKINLEY, M.J., OLDFIELD, B.J., MÜLLER-ESTERL, W., MENDELSOHN, F.A. & CHAI, S.Y. (1997). Distribution of bradykinin B_2 receptors in sheep brain and spinal cord visualized by *in vitro* autoradiography. *J. Comp. Neurol.,* **381,** 203–218.

NONAKA, M., CHEN, X.H., PIERCE, J.E., LEONI, M.J., MCINTOSH, T.K., WOLF, J.A. & SMITH, D.H. (1999). Prolonged activation of NF-kappaB following traumatic brain injury in rats. *J. Neurotrauma.,* **16,** 1023–1034.

ONGALI, B., BUCK, H.S., CLOUTIER, F., LEGAULT, F., REGOLI, D., LAMBERT, C., THIBAULT, G. & COUTURE, R. (2003a). Chronic effects of angiotensin-converting enzyme inhibition on kinin receptor binding sites in the rat spinal cord. *Am. J. Physiol. (Heart Circ. Physiol.),* **284,** H1949–H1958.

ONGALI, B., CAMPOS, M.M., BREGOLA, G., RODI, D., REGOLI, D., THIBAULT, G., SIMONATO, M. & COUTURE, R. (2003b). Autoradiographic analysis of rat brain kinin B_1 and B_2 receptors: normal distribution and alterations induced by epilepsy. *J. Comp. Neurol.,* **461,** 506–519.

PAXINOS, G. & WATSON, C. (1998). *The Rat Brain in Stereotaxic Coordinates,* 4th edn. San Diego, CA: Academic Press.

PENNYPACKER, K.R., KASSED, C.A., EIDIZADEH, S. & O'CALLAGHAN, J.P. (2000). Brain injury: prolonged induction of transcription factors. *Acta Neurobiol. Exp.,* **60,** 515–530.

PRIVITERA, P.J., BECKSTEAD, R.M., YATES, P. & WALGREN, R. (2003). Autoradiographic localization of [^{125}I-Tyr^0]bradykinin binding sites in brains of Wistar–Kyoto and spontaneously hypertensive rats. *Cell. Mol. Neurobiol.,* **23,** 805–815.

PRIVITERA, P.J., THIBODEAUX, H. & YATES, P. (1994). Rostral ventrolateral medulla as a site for the central hypertensive action of kinins. *Hypertension,* **23,** 52–58.

PRIVITERA, P.J., DAUM, P.R., HILL, D.R. & HILEY, C.R. (1991). Autoradiographic visualization and characteristics of [^{125}I]bradykinin binding sites in guinea-pig brain. *Brain Res.,* **577,** 73–79.

QADRI, F., HAUSER, W., JOHREN, O. & DOMINIAK, P. (2002). Kinin B_1 and B_2 receptor mRNA expression in the hypothalamus of spontaneously hypertensive rats. *Can. J. Physiol. Pharmacol.,* **80,** 258–263.

QADRI, F., BÄURLE, L., HÄUSER, W., RASCHER, W. & DOMINIAK, P. (1999). Centrally bradykinin B_2-receptor-induced hypertensive and positive chronotropic effects are mediated *via* activation of the sympathetic nervous system. *J. Hypertens.,* **17,** 1265–1271.

REGOLI, D., NSA ALLOGHO, S., RIZZI, A. & GOBEIL, F. (1998). Bradykinin receptors and their antagonists. *Eur. J. Pharmacol.,* **348,** 1–10.

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

SIEBECK, M., SCHORR, M., SPANNAGL, E., LEHNER, M., FRITZ, H., CHERONIS, J.C. & WHALLEY, E.T. (1998). B_1 kinin receptor activity in pigs is associated with pre-existing infection. *Immunopharmacology,* **40,** 49–55.

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

TAKEDA, K. & BUÑAG, R.D. (1978). Sympathetic hyperactivity during hypothalamic stimulation in spontaneously hypertensive rats. *J. Clin. Invest.,* **62,** 642–648.

WARDENER, H.E. (2001). The hypothalamus and hypertension. *Physiol. Rev.,* **81,** 1599–1658.

WIRTH, K., BREIPOHL, G., STECHL, J., KNOLLE, J., HENKE, S. & SCHÖLKENS, B.A. (1991). Des-Arg^9-D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (des-Arg^{10}-[Hoe140]) is a potent bradykinin B_1 receptor antagonist. *Eur. J. Pharmacol.,* **205,** 217–218.

ZHANG, J. & RIVEST, S. (2001). Anti-inflammatory effects of prostaglandin E_2 in the central nervous system in response to brain injury and circulating lipopolysaccharide. *J. Neurochem.,* **76,** 855–864.

(Received December 17, 2003
Revised February 2, 2004
Accepted February 24, 2004)