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The bradykinin B_1 receptor and the central regulation of blood pressure in spontaneously hypertensive rats

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1 We evaluated if the brain bradykinin (BK) B_1 receptor is involved in the regulation of blood pressure (BP) in conscious rats.

2 Basal mean BP and HR were 115 ± 2 and 165 ± 3 mmHg and 345 ± 10 and 410 ± 14 beats min^{-1} in Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR), respectively. Intracerebroventricular (i.c.v.) injection of 1 nmol B_1 receptor agonist Lys-desArg^9-BK significantly increased the BP of WKY and SHR by 7 ± 1 and 19 ± 2 mmHg, respectively. One nmol Sar[D-Phe^8]-desArg^9-BK, a kininase-resistant B_1 agonist, increased the BP of WKY and SHR by 19 ± 2 and 17 ± 2 mmHg, respectively and reduced HR in both strains.

3 I.c.v. injection of 0.01 nmol B_1 antagonists, LysLeu^8-desArg^9-BK or AcLys[D-βNal^7,Ile^8]-desArg^9-BK (R715), significantly decreased mean BP in SHR (by 9 ± 2 mmHg the former and 14 ± 3 mmHg the latter compound), but not in WKY. In SHR, the BP response to R715 was associated to tachycardia.

4 I.c.v. Captopril, a kininase inhibitor, increased the BP of SHR, this response being partially prevented by i.c.v. R715 and reversed into a vasodepressor effect by R715 in combination with the B_2 antagonist Icatibant.

5 I.c.v. antisense oligodeoxynucleotides (ODNs) targeted to the B_1 receptor mRNA decreased BP in SHR, but not in WKY. HR was not altered in either strain. Distribution of fluorescein-conjugated ODNs was detected in brain areas surrounding cerebral ventricles.

6 Our results indicate that the brain B_1 receptor participates in the regulation of BP. Activation of the B_1 receptor by kinin metabolites could participate in the pathogenesis of hypertension in SHR.

Keywords: Brain; hypertension; genetic; kinins; DNA; fluorescence

Abbreviations: Ang II, angiotensin II; BP, blood pressure; BK, bradykinin; DABK, des-Arg^9-bradykinin; DALBK, des-Arg^9-Leu^8-bradykinin; HR, heart rate; i.c.v., intracerebroventricular; Lys-BK, Lys-bradykinin or kallidin; Lys-DABK, Lys-desArg^9-bradykinin; LysLeu^8-DABK, LysLeu^{8}desArg^9-bradykinin; ODNs, oligodeoxynucleotides; R715, AcLys[D-βNal^7,Ile^8]desArg^9-bradykinin; Sar[D-Phe^8]DABK, Sar[D-Phe^8] desArg^9-bradykinin; SHR, spontaneously hypertensive rats; WKY, Wistar Kyoto rats

Introduction

The kallikrein-kinin system is present in exocrine glands, kidney, cardiovascular system, brain, and pituitary and it has been implicated in a plethora of physiological functions such as the regulation of local and systemic haemodynamics, vascular permeability, neuronal activation, and passage of water and electrolytes across epithelia (see Clements, 1989; Margolius, 1995). Kinins are local hormones released by kallikrein from the substrate kininogen. They act on two receptor subtypes, B_1 and B_2, classified according to the relative potency of natural agonists (see Regoli & Barabé, 1980). While the B_2 receptor is typically activated by bradykinin (BK) and Lys-bradykinin (Lys-BK), the B_1 receptor is selectively sensitive to kinin metabolites without the C-terminal arginine residue, e.g. des-Arg^9-BK (DABK) and Lys-des-Arg^9-BK (Lys-DABK). Although most of the central and peripheral effects of kinins are known to be mediated by the B_2 receptor, which is constitutively expressed in different cell types, recent reports suggest a potential role of the B_1 receptor in pathophysiology. For instance, it might be implicated in inflammation, hyperalgesia, hyperthermia, and experimental diabetes (Dray & Perkins, 1993; Davis *et al*., 1994; Coelho *et al*., 1997; Zuccolo *et al*., 1996). In these conditions, the B_1 receptor is apparently rapidly upregulated.

Evidence exists that all the components of the kallikrein-kinin system are present in the rat brain (Correa *et al*., 1979; Chao *et al*., 1983; Perry & Snyder, 1984; Scicli *et al*., 1984; Simson *et al*., 1985; Lopes & Couture, 1997) and that activation of the brain B_2 receptor by endogenous kinins exerts modulatory effects on systemic blood pressure (BP) (Lindsley *et al*., 1988; Yang *et al*., 1989; Madeddu *et al*., 1990; 1996; Alvarez *et al*., 1992; Priviteira *et al*., 1994; Khan *et al*., 1995; Caligiorne *et al*., 1996). In particular, inhibition of brain kininase II, one of the enzymes responsible for kinin degradation, increases the already elevated BP of spontaneously hypertensive rats (SHR) through the B_2 receptor (Madeddu *et al*., 1990). Possible participation of the B_1 receptor in this hypertensive effect has yet to be determined. Recently, Raidoo & Bhoola (1997) have demonstrated that the B_1 receptor is localized on neurones of the human brain at the level of the thalamus, spinal cord and hypothalamus, providing an anatomical basis for a possible role of this

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subtype receptor in the central regulation of BP. However, functional studies performed so far did not reach consistent conclusions. Martins *et al*. (1991) reported that neither the B_1 receptor agonist DABK nor the antagonist des-Arg^9-Leu^8-BK (DALBK) injected into the fourth cerebral ventricle altered the BP of Wistar Kyoto (WKY) or SHR, nor did the antagonist interfere with the BP response to i.c.v. BK. In contrast, Caligiorne *et al*. (1996) showed that DALBK, microinjected into the nucleus of tractus solitarii of WKY rats prevented the cardiovascular effects of exogenous BK, and Alvarez *et al*. (1992) reported that brain B_1 receptor blockade lowers BP in SHR but not in normotensive rats. In some of the above reported studies, full understanding of the potential role of the B_1 receptor might have been precluded by the pharmacokinetic properties and short half-life of first-generation analogue-antagonists. These limitations were overcome by the recent introduciton of second-generation B_1 antagonists (Drapeau *et al*., 1993; Gobeil *et al*., 1996), characterized by enhanced selectivity and resistance to kininases, and by the availability of antisense oligodeoxynucleotides (ODNs), targeted to B_1 receptor mRNA.

In the present study, we applied these newly developed strategies to evaluate if kinins participate in the central regulation of BP through the activation of the B_1 receptor. In addition, we determined whether this receptor plays a role in the acute hypertensive response to i.c.v. Captopril, a kininase inhibitor, in SHR.

Methods

Male WKY and SHR (Charles River, Milan, Italy) weighing between 220 and 260 g were housed at a constant temperature ($24 \pm 1^{\circ}C$) and humidity ($60 \pm 3\%$) with a 12 h light/dark cycle and had free access to tap water and rat chow. All procedures complied with the standards for care and use of animal subjects as stated in *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Academy of Sciences, Bethesda, MD, U.S.A.). In addition, in compliance with the guidelines established by the Institutional Animal Care and Research Advisory Committee of Sassari University, animals were only used once and not reused in any other group. Experiments were performed in conscious, unrestrained rats (unless specified), 5 days after cerebroventricular cannula implantation and 24 h after insertion of intra-arterial catheter if haemodynamic measurements were required.

Surgical procedures

To implant cerebroventricular cannulas, rats were anaesthetized with ketamine chloridrate (45 mg kg^{-1} body weight, Parke-Davis, Milan, Italy) and diazepam (5 mg kg^{-1} body weight, Roche, Milan, Italy). A 22 gauge stainless steel cannula fitted into a 3×4 mm membrane-valve plastic block (Umberto Danuso, Milan, Italy) was placed stereotaxically into the left lateral cerebral ventricle (1.5 mm lateral and 1.0 mm posterior to the bregma, and 4.5 mm deep from the skull surface), as described previously (Madeddu *et al*., 1990).

For haemodynamic measurements, a polyethylene catheter (PE-10 connected to a PE-50, Clay Adams, Parsippany, NJ, U.S.A.) was filled with heparin-treated saline, inserted into the left femoral artery of rats under light ether anaesthesia, and advanced into the abdominal aorta. The catheter was then tunnelled under the skin and brought out of the back of the neck. Mean BP and HR were measured with a Statham transducer (Gould), connected to the arterial catheter, and recorded on a Quartet polygraph (Basile).

I.c.v. administration of agonists and antagonists of BK receptors

After a 15 min stabilization period, unrestrained WKY and SHR (at least $n = 6$ per group) received one of the following compounds by i.c.v. route: the B_1 receptor agonists Sar[D-Phe^8]desArg^9-BK (Sar[D-Phe^8]DABK, 0.1, 1, or 10 nmol) or LysDABK (1 nmol); the B_1 receptor antagonists, LysLeu^{8}des-Arg^9-BK (LysLeu^8-DABK, 0.01 nmol) or AcLys[D-βNal^7, Ile^8]desArg^9-BK (R715, 0.01 nmol); the B_2 receptor antagonist, D-Arg,[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (Icatibant, 1 nmol); vehicle (phosphate buffered saline, PBS, pH 7.4). Volume injection was 5 μl followed by additional 5 μl PBS to flush the cannula. Injections were made with a 25 μl syringe (Hamilton, Reno, NV, U.S.A.). Mean BP and HR were continuously recorded prior to and at least for 10 min following the injection of agonists, antagonists, or vehicle.

An additional set of experiments was performed to determine the selectivity of BK antagonists. To this aim, we evaluated the BP responses to i.c.v. Sar[D-Phe^8]DABK (1 nmol) or Ang II (0.5, 1, or 10 nmol) in SHR ($n = 6$ per group) pre-treated 15 min in advance with i.c.v. R715 (0.01 nmol), Icatibant (1 nmol), or PBS (vehicle).

I.c.v. Captopril administration to rats pre-treated with i.c.v. B_1 or B_2 receptor antagonists

After a 15 min stabilization period, 0.01 nmol R715 ($n = 6$), 1 nmol Icatibant ($n = 6$), R715 + Icatibant in combination ($n = 5$), or vehicle (PBS, $n = 6$) were injected by i.c.v. route. Captopril (1 mg in 10 μl PBS) was injected by i.c.v. route 10 min later. The mean BP of conscious, unrestrained SHR was continuously recorded prior to and for 30 min following Captopril administration. HR was not determined in these experiments.

Administration of ODNs

After 15 min stabilization period, a single 50 μg dose of sense, antisense, or scrambled ODNs (in 5 μl of PBS) was injected in conscious, unrestrained WKY or SHR by i.c.v. route (at least $n = 6$ per group). ODN injection was immediately followed by 5 μl PBS to flush the cannula. Mean BP and HR were recorded under basal conditions and for 15 min at 1, 4, 6, 8, 24, 36, 48, 72 and 96 h after ODN injection.

In a separate set of experiments, the haemodynamic effects of the systemic administration of ODNs were tested in SHR. Mean BP and HR were recorded for 15 min under basal conditions and for 15 min at 1, 4, 6, 8, and 24 h after the intra-arterial injection of a single dose (50 μg in 200 μl PBS) of antisense ($n = 5$) or sense ($n = 4$) ODNs.

I.c.v. Sar[D-Phe^8]DABK in SHR pre-treated with B_1 receptor ODNs

To test the efficacy of antisense ODNs in preventing the pressor effect induced by the activation of brain B_1 receptors, conscious, unrestrained SHR were injected with a single i.c.v. dose of the sense ($n = 7$) or antisense ($n = 6$) ODNs (50 μg in 5 μL PBS). Twenty-four hours later, mean BP was recorded prior to and for 10 min following the i.c.v. injection of 1 nmol of Sar[D-Phe^8]DABK in 5 μl PBS. HR was not determined in these experiments.

Analysis of the distribution of i.c.v. antisense B_1 ODNs

For analysis of brain distribution, antisense ODNs were conjugated with fluorescein isothiocyanate at both the 5′ and 3′ ends. SHR were injected by i.c.v. route with fluorescein-conjugated antisense B_1 ODN (50 μg in 5 μl PBS) followed by 5 μl PBS to flush the cannula. One, 3, or 6 h later, the rats ($n=2$ for each time point) were anaesthetized with ether, and transcardially perfused with cold PBS (30 ml), followed by buffered neutral formaldehyde (30 ml). The brain was then removed and incubated in buffered neutral formaldehyde for 2 h. After fixation, brains were washed briefly in distilled water and then placed in 70% ethanol until sectioning. Sections (5 μm) were mounted and observed by laser scanning confocal microscopy.

Substances

Lys-DABK, Sar[D-Phe^8]DABK, LysLeu^8-DABK and R715 were synthesized at the Department of Pharmacology of Sherbrooke Medical University, Canada. Icatibant was a gift from Hoechst AG, Germany. Captopril was purchased from Sigma-Aldrich (Milan, Italy). ODNs for the rat B_1 receptor were synthesized at the Department of Biochemistry and Molecular Biology, Medical University of South Carolina (Ni *et al*., 1998). Sense B_1-ODN$_a$, 5′-AGG TCA CTG TGG ATG CGC-3′, and antisense B_1-ODN$_a$, 5′-CGC CAT CCA CAG TGA CCT-3′, encompass the translation start codon, and the scrambled B_1-ODN$_a$ is 5′-TAA GCC TCC CTC CAG CAG-3′. Sense B_1-ODN$_b$, 5′-TGG CAG CAA CGA CAG AGG-3′, and antisense B_1-ODN$_b$, 5′-CCT CTG TCG TTG CTG CCA-3′, encompass the sequence 210 bp downstream of the translation start codon, and the scrambled B_1-ODN$_b$ is 5′-GCA CCT TGT TGC CCT GCT-3′. ODNs were made resistant to nucleases by DNA backbone phosphorothioation.

Statistical analysis

All data are expressed as mean$\pm$s.e.mean. Multivariate repeated measures analysis of variance was performed to test interactions between time and grouping factors. If an interaction was detected due to treatment factor, then ANOVA was performed for each treatment against time-matched controls. Comparison of absolute changes from baseline between each experimental group and time-matched controls was performed for each time point by unpaired Student's *t*-test. Within each group, changes from baseline were analysed by paired Student's *t*-test. A probability (P) value less than 0.05 was considered significant.

Results

Effects of i.c.v. B_1 receptor agonists on mean BP and HR

Absolute baseline values and peak responses are reported in Table 1. As shown in Figure 1A, i.c.v. injection of 1 nmol Sar[D-Phe^8]DABK or LysDABK increased the mean BP of WKY rats (19 ± 2 and 7 ± 1 vs 2 ± 1 mmHg in vehicle-treated rats at 2 min, $P<0.05$ for both comparisons). The pressor effect induced by Sar[D-Phe^8]DABK was significantly greater than that of LysDABK.

At 0.1 nmol Sar[D-Phe^8]DABK increased the mean BP of WKY rats by 9 ± 2 mmHg, while 10 nmol was equipotent to 1 nmol (17 ± 2 vs 19 ± 2 mmHg, respectively, at 2 min).

As shown in Figure 1B, i.c.v. injection of 1 nmol Sar[D-Phe^8]DABK or LysDABK caused similar pressor effects in SHR (18 ± 3 and 20 ± 2 vs 6 ± 3 mmHg in vehicle-treated rats at 2 min, $P<0.05$ for both comparisons).

At 0.1 nmol, Sar[D-Phe^8]DABK did not affect the mean BP of SHR, while the highest dose tested (10 nmol) was equipotent to 1 nmol. No difference was detected between WKY and SHR regarding the dose-BP response curve to Sar[D-Phe^8]DABK.

As shown in Figure 1C and D, HR was transiently reduced in both strains in response to i.c.v. Sar[D-Phe^8]DABK.

Effects of i.c.v. B_1 or B_2 receptor antagonists on mean BP and HR

In WKY rats, i.c.v. injection of 0.01 nmol R715, LysLeu^8-DABK, or PBS did not affect mean BP or HR. By contrast, as shown in Figure 2 both antagonists decreased the mean BP of SHR (-9 ± 2 and -14 ± 3 vs 2 ± 3 mmHg in vehicle-treated rats at 15 min, $P<0.05$ for both comparisons). A transient tachycardia was observed in correspondence with the hypotensive response induced by R715 in SHR. A typical trace of the haemodynamic responses to R715 in SHR is reproduced in Figure 3.

The selectivity of R715 was evaluated in SHR by testing the ability of this compound to prevent the central pressor effects of Sar[D-Phe^8]DABK or Ang II. At 0.01 nmol, R715 antagonized the pressor response to 1 nmol Sar[D-Phe^8]DABK

Table 1 Baseline and experimental mean blood pressure and heart rate values

Drug	*Strain*	*Baseline values (Mean BP and HR)*	*Experimental values (Mean BP and HR)*	*Time after i.c.v.*	P
LysDABK (1 nmol)	WKY	117 ± 3 mmHg HR N.D.	124 ± 3 mmHg HR N.D.	2 min	$P<0.01$
Sar[D-Phe^8]DABK (1 nmol)	WKY	111 ± 2 mmHg 345 ± 10 beats min^{-1}	130 ± 2 mmHg 328 ± 11 beats min^{-1}	2 min	$P<0.01$ $P<0.05$
LysDABK (1 nmol)	SHR	170 ± 8 mmHg HR N.D.	190 ± 9 mmHg HR N.D.	2 min	$P<0.01$
Sar[D-Phe^8]DABK (1 nmol)	SHR	159 ± 3 mmHg 410 ± 9 beats min^{-1}	177 ± 4 mmHg 392 ± 8 beats min^{-1}	2 min	$P<0.01$ $P<0.05$
LysLeu^8-DABK (0.01 nmol)	SHR	170 ± 5 mmHg HR N.D.	155 ± 3 mmHg HR N.D.	15 min	$P<0.05$
R175 (0.01 nmol)	SHR	170 ± 3 mmHg 415 ± 5 beats min^{-1}	158 ± 4 mmHg 435 ± 4 beats min^{-1}	15 min	$P<0.05$ $P<0.05$
Antisense B_1-ODN$_a$	SHR	173 ± 7 mmMg 413 ± 9 beats min^{-1}	137 ± 6 mmHg 415 ± 9 beats min^{-1}	36 h	$P<0.01$ $P=$N.S.
Antisense B_1-ODN$_b$	SHR	161 ± 8 mmHg 410 ± 7 beats min^{-1}	145 ± 8 mmHg 409 ± 9 beats min^{-1}	36 h	$P<0.05$ $P=$N.S.

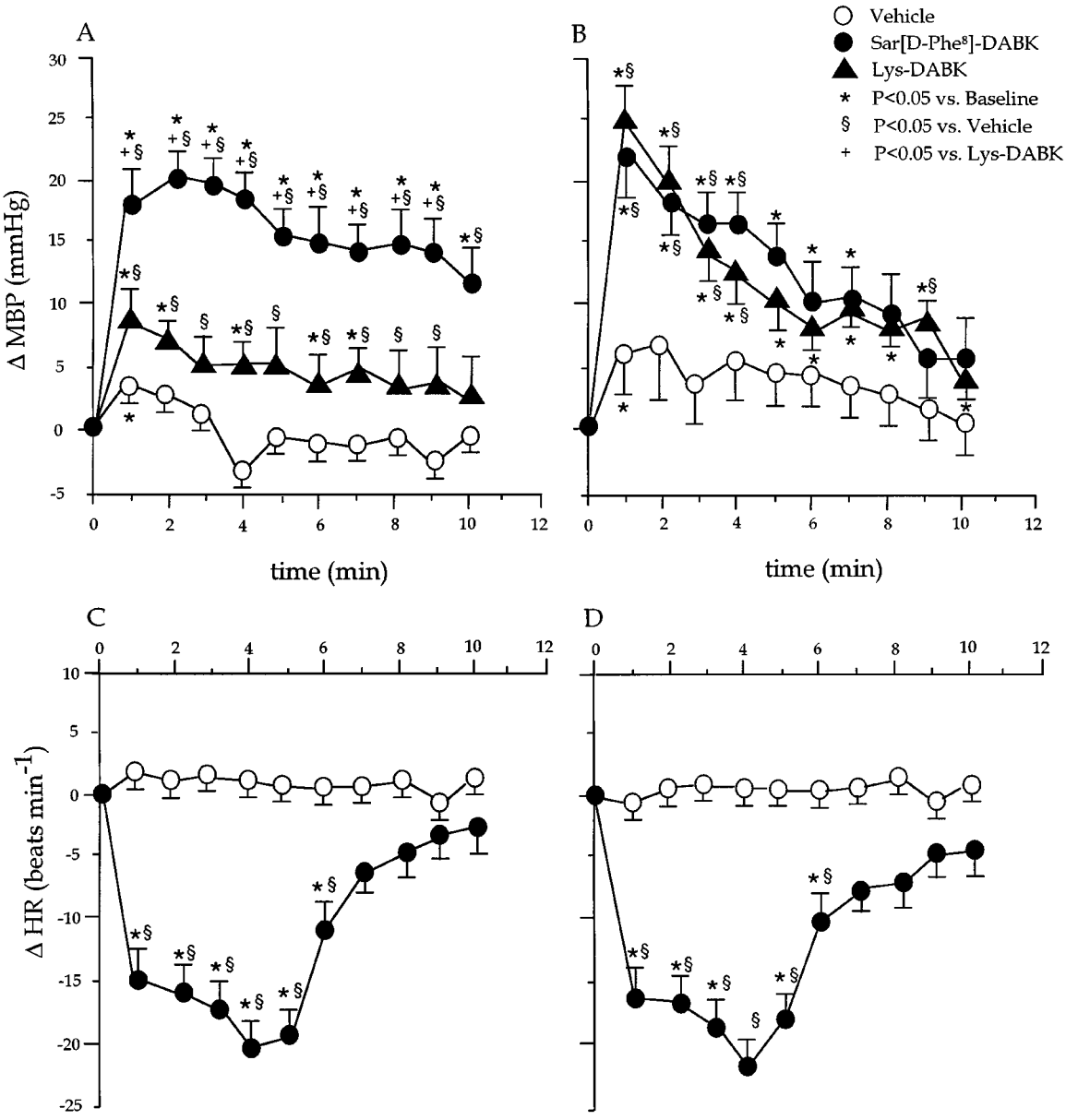

Figure 1 Line graphs show the absolute mean blood pressure (ΔMBP) and heart rate (ΔHR) changes from baseline induced in conscious WKY (panels A and C) and SHR (panels B and D) by the i.c.v. administration of 1 nmol Lys-DABK (WKY: $n=6$ and SHR: $n=10$), Sar[D-Phe^8]-DABK (WKY: $n=6$ and SHR: $n=9$), or vehicle (WKY: $n=10$ and SHR: $n=9$). Each point represents the mean ± s.e.mean.

by 80% (3±2 vs 14±2 mmHg in vehicle-treated rats, $P<0.05$), while the response to i.c.v. Ang II did not differ in SHR pre-treated with the B_1 antagonist or vehicle (5±2 vs 4±2 mmHg at 0.5 nmol Ang II, 10±2 vs 11±3 mmHg at 1 nmol Ang II, and 35±4 vs 36±5 mmHg at 10 nmol Ang II).

I.c.v. injection of Icatibant neither altered the basal mean BP or HR of WKY rats and SHR nor the BP responses to Sar[D-Phe^8]DABK or Ang II in SHR (data not shown).

Effects of B_1 or B_2 receptor antagonists on i.c.v. Captopril-induced mean BP changes

In SHR, i.c.v. Captopril induced a pronounced increase in mean BP, which peaked at 1 min (39±5 mmHg) and lasted for 15 min (Figure 4). The pressor effect induced by Captopril was partially prevented by i.c.v. R715 and completely abolished by i.c.v. Icatibant. Pre-treatment with R715 and Icatibant in combination reversed the pressor response to Captopril into a vasodepressor effect (−22±8 mmHg at 1 min).

Effects of B_1 receptor ODNs on mean BP and HR

In WKY rats, mean BP and HR were not affected by i.c.v. sense, scrambled, or antisense ODNs. By contrast, as shown in Figure 5, the mean BP of SHR was reduced by the i.c.v. injection of antisense B_1-ODN$_a$ (−31±5, −36±8, −28±11 mmHg vs −9±4, −4±3 and 4±2 mmHg in controls given sense B_1-ODN$_a$ at 24, 36, and 48 h, respectively, $P<0.05$ for each comparison). No BP effect was detected in rats given i.c.v. scrambled B_1-ODN$_a$. HR was not altered by antisense, sense, or scrambled B_1-ODN$_a$ (Figure 5). A typical trace of the haemodynamic responses to B_1-ODN$_a$ in SHR is reproduced in Figure 6.

Consistent with the above results was the finding of a hypotensive response to i.c.v. antisense B_1-ODN$_6$ in SHR

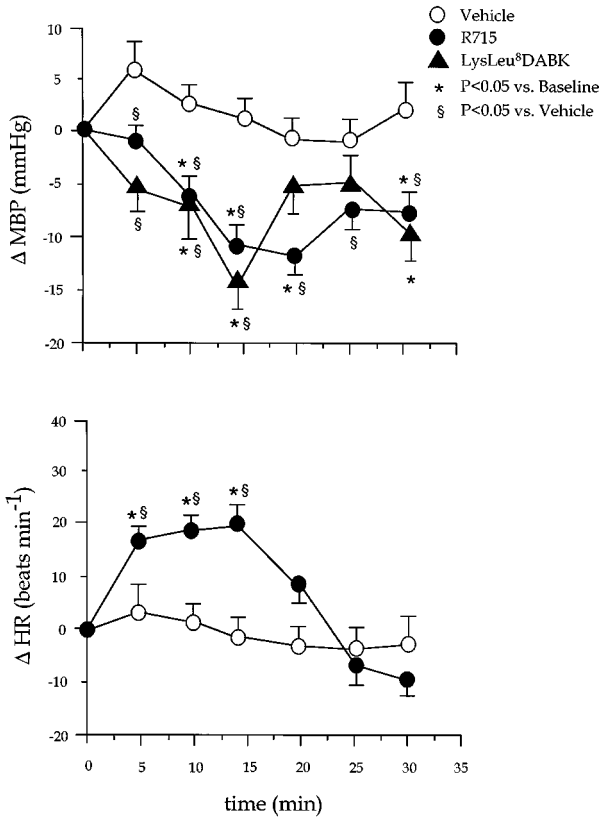

Figure 2 Line graphs show the absolute mean blood pressure (ΔMBP) and heart rate (ΔHR) changes from baseline induced by i.c.v. administration of 0.01 nmol LysLeu^{8}DABK ($n=6$), AcLys[D-βNal^7,Ile^8]DABK (R715, $n=7$), or vehicle ($n=10$) in conscious SHR. Each point represents the mean$\pm$s.e.mean.

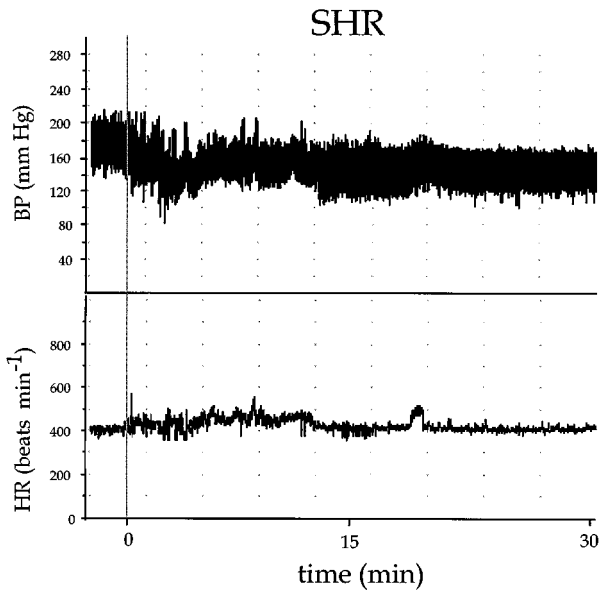

Figure 3 Typical trace of the blood pressure and heart rate responses to 0.01 nmol AcLys[D-βNal^7,Ile^8]DABK (R715) in a conscious SHR. The antagonist was injected by i.c.v. route at time 0.

(from 161 ± 8 to 145 ± 8 mmHg at 36 h, $P<0.05$). No change in mean BP was observed after i.c.v. injection of the corresponding sense or scrambled B_1-ODN$_b$ (data not shown). HR was not altered by antisense, sense, or scrambled B_1-ODN$_b$.

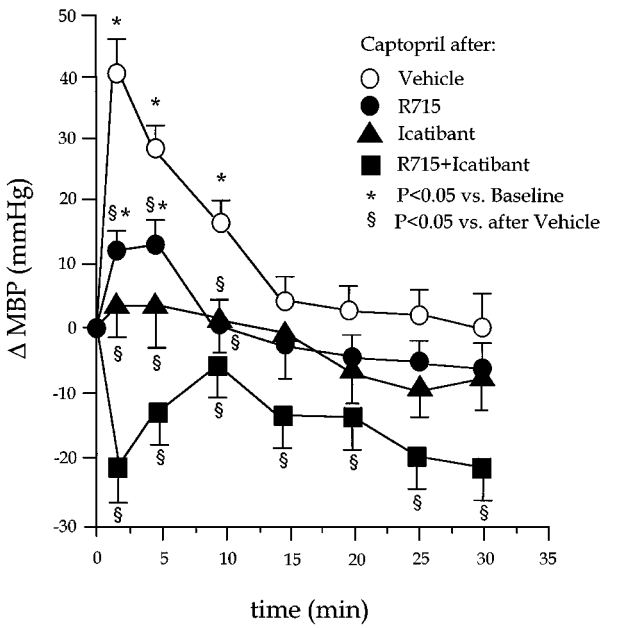

Figure 4 Line graphs show the absolute mean blood pressure changes (ΔMBP) from baseline induced by i.c.v. administration of 1 mg Captopril in conscious SHR pre-treated with i.c.v. PBS (vehicle, $n=6$), 0.01 nmol AcLys[D-βNal^7,Ile^8]DABK (R715, $n=6$), 1 nmol D-Arg,[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (Icatibant, $n=6$), or R715 and Icatibant in combination ($n=5$). Each point represents the mean$\pm$s.e.mean.

No change in mean BP or HR was observed in SHR following the intra-arterial injection of sense or antisense B_1-ODN$_a$ (data not shown).

Effects of i.c.v. ODN-pre-treatment on Sar[D-Phe^8]DABK-induced changes in mean BP

In SHR pre-treated with i.c.v. sense B_1-ODN$_a$, i.c.v. administration of 1 nmol Sar[D-Phe^8]DABK increased mean BP by 15 ± 5 mmHg at 2 min. The pressor response to i.c.v. Sar[D-Phe^8]DABK was completely blocked by pre-treatment with i.c.v. antisense B_1-ODN$_a$.

Distribution of i.c.v. antisense B_1 receptor ODNs

A strong fluorescent signal was detected in the brain of SHR 1 h after the i.c.v. injection of fluorescein isothiocyanate-conjugated antisense B_1-ODN$_a$. The maximal intensity was recognized around the lateral ventricles, at the level of the hippocampus, and around the third ventricle, at the hypothalamus periventricularis. Intranuclear uptake of antisense ODN$_a$ by individual cells was detected at greater magnifications. Fluorescent signal was still evident 6 h after the injection of antisense B_1-ODN$_a$. Similar results were obtained after i.c.v. administration of antisense B_1-ODN$_b$.

Discussion

In the present study, we have shown that activation of the B_1 receptor in the brain by selective agonists increases BP in either WKY or SHR. Blocking or knocking-down of the B_1 receptor exerts a BP lowering effect in SHR, but not in WKY rats.

The pressor response to i.c.v. BK has been known for a long time and this effect was referred to the activation of the B_2

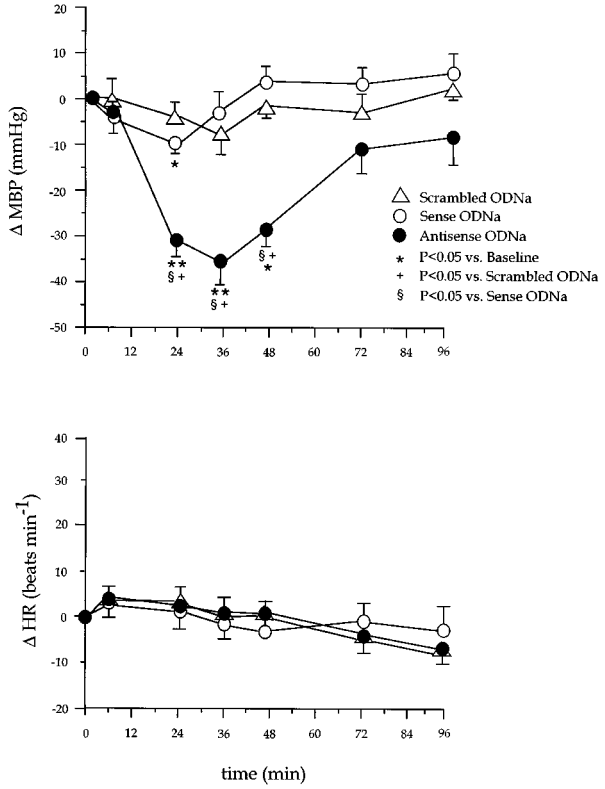

Figure 5 Line graphs show the absolute mean blood pressure (ΔMBP) and heart rate (ΔHR) changes from baseline induced by i.c.v. administration of 50 μg antisense ($n=9$), sense ($n=10$), or scrambled ($n=6$) oligodeoxynucleotides (ODN_a) targeted to the translation initiation codon of B_1 receptor mRNA in conscious SHR. Each point represents the mean ± s.e.mean.

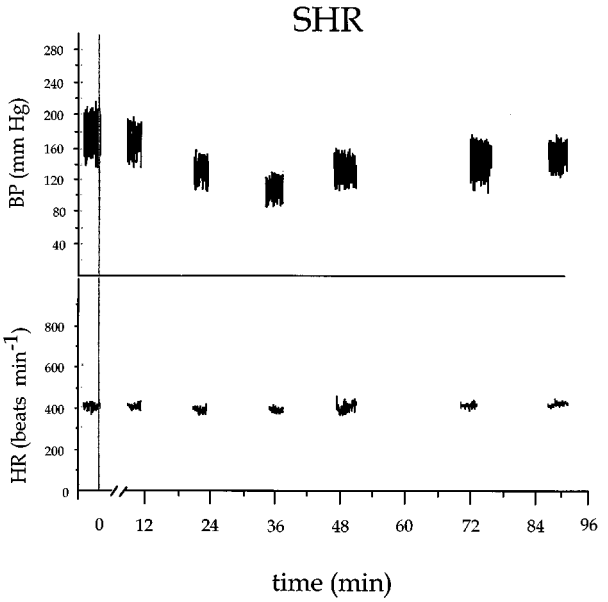

Figure 6 Typical trace of the blood pressure and heart rate responses to 50 μg antisense oligodeoxynucleotides (ODN_a) in a conscious SHR. ODN_a was injected by i.c.v. route at time 0.

receptor in the brain. Instead, data regarding the functional relevance of the B_1 receptor in the brain are scarce and contradictory. This mainly reflects the limitations of first-generation receptor agonists and antagonists, with particular regard to short half-life (precluding diffusion from the ventricular system to deep areas of the brain), and low affinity for the receptor. In the present study, we exploited the availability of the newly synthesized analogues Sar[D-Phe^8]DABK and R715 (Drapeau *et al*., 1993; Gobeil *et al*., 1997). Substitution of the aromatic residue Phe with its D-enantiomer in position 8 of the former compound has been shown to increase the affinity for the B_1 receptor. Moreover, Sar[D-Phe^8]DABK is resistant to ACE, aminopeptidase M, and neutral endopeptidase 24.11, while the corresponding unmodified sequence, Lys-DABK, the most potent natural kinin known to stimulate the B_1 receptor, is very sensitive to all these peptidases (see Marceau, 1995). On the other hand, the use of D-βNal in position 7, combined with Ile in position 8 and AcLys at the N-terminal gave the most active B_1 receptor antagonist, R715, which is also partially resistant to enzymatic degradation (Gobeil *et al*., 1996).

We found that activation of B_1 receptors in the brain increases BP in both WKY rats and SHR. However, in normotensive rats the natural ligand Lys-DABK caused milder pressor effects compared with the synthetic kinin analogue Sar[D-Phe^8]DABK which is resistant to enzymatic degradation. By contrast, the two compounds were equipotent in SHR. These findings are compatible with the possibility that kininase activity is reduced in the brain of SHR compared with WKY rats (Martins *et al*., 1991). Consequently, metabolic inactivation of endogenous ligands for the B_1 receptor could play an important role in autoregulatory processes preventing activation of the receptors in normotensive rats, while alteration of this protective mechanism may contribute to increase BP in SHR. Relevant to the pathways implicated in the BP response to B_1 receptor activation is the observation that prostaglandins are involved in the analgesic effects of B_1 antagonism in chronic inflammation models (Davis *et al*., 1996).

To explore the relevance of endogenous kinins in the central regulation of BP we used the distinct approaches of blocking or knocking down B_1 receptors in the brain. In agreement with Alvarez *et al*. (1992), participation of the B_1 receptor in the pathogenesis of genetic hypertension is supported by the finding that B_1 receptor blockade by two different selective antagonists induced a vasodepressor response in SHR, whereas the same compounds were ineffective in normotensive WKY rats. As suggested above, these differences might be related to elevation in des-Arg^9-kinins in brain of the hypertensive strain, due either to enhanced formation or reduced catabolism or both. Alternatively, receptor expression, density and/or distribution may be different between WKY and SHR. Consistent with this possibility are the results obtained with semiquantitative RT–PCR Southern blot analysis showing increased expression levels of the B_1 receptor gene in the brain of SHR (Julie Chao and Aiguo Ni, unpublished observations 1999).

Antisense ODNs represent a newly developed strategy particularly attractive for its application to the living brain. Affinity for the targeted gene and ability to enter intra-cellular compartments limit the length of the ODN sequence to 15–25 nucleotides. ODNs of 18 nucleotides have been successfully used in neuropharmacology (see Wahlestedt, 1994) as well as in studies aimed to evaluate the role of the brain renin-angiotensin system in the pathogenesis of genetic hypertension (Wielbo *et al*., 1995).

Using this approach, we were able to confirm the information provided by B_1 receptor antagonists. In fact, two

distinct antisense ODNs targeted to non-overlapping regions of the B_1 receptor mRNA were able to lower the BP of SHR, but they were ineffective in WKY rats. Antisense ODNs exerted delayed, but more prolonged BP effects compared with receptor antagonists, a result compatible with a kinetic model in which target protein is slowly degraded.

Although preliminary experiments have demonstrated the ability of antisense ODNs to significantly reduce B_1 receptor protein in cultured neuroblastoma cells transfected with B_1 receptor cDNA (Julie Chao, unpublished observations 1999), evaluation of the effects of antisense ODNs on protein levels was precluded by the failure to demonstrate specific B_1 binding sites in the whole brain even under basal conditions (data not shown). These results are attributable to two reasons. First, the cell culture system is very clean as compared to the crude membrane preparation from whole brain. Second, the expression level of the B_1 receptor in transfected cells is very high compared to that of the rat brain (Simonato *et al*., 1997). Unfortunately, RT–PCR Southern blot analysis, a method extremely sensitive to detect the low levels of B_1 receptor mRNA with many orders of amplification, is not suitable for documenting the translational arrest by ODNs. However, even in the absence of a direct demonstration, antisense-induced reduction in B_1 receptor protein is suggested by the finding that the pressor response to i.c.v. Sar[D-Phe^8]DABK was blocked by pre-treatment with i.c.v. antisense B_1-ODN_a.

It should be noted that one of the controls used for antisense ODNs, the scrambled ODN_a, showed 90% identity to a portion of the rat PLA_2 sequence according a search on the Genbank database. This should not affect the interpretation of our results since the scrambled ODN was devoid of haemodynamic effects. A modest transient drop in BP of SHR was observed in the presence of the sense ODN_a. However, this response was significantly less in magnitude and duration than that found in SHR given the corresponding antisense ODN_a or antisense ODN_b that was designed to target another non-overlapping region of B_1 receptor mRNA sequence. Confirmation by a second antisense ODN is generally considered an important proof of specificity (see Wahlestedt, 1994).

It is possible that resting BP has affected the response to manipulation of the B_1 receptor, a common problem when comparing hypertensive rats with normotensive controls. However, this appears to be unlikely in consideration of the fact that B_1 antagonists or antisense ODNs are devoid of any haemodynamic effect in WKY rats not only under basal conditions but also following elevation of BP by peripheral administration of a nitric oxide synthase inhibitor (Paolo Madeddu, unpublished observations 1999). Finally, failure of intra-arterial administration to alter the BP of SHR discounts the possibility that the effects observed after i.c.v. injection are due to leakage of antisense ODNs into the peripheral circulation.

The effects of antisense ODNs on BP were not associated with any change in HR. The long-lasting duration of antisense-induced hypotension might have allowed an adaptive response to occur thus resetting baroreceptor sensitivity. Indeed, the acute changes in BP induced by agonism or antagonism of the B_1 receptor were accompanied by reflex HR responses. The absence of tachycardia following activation of B_1 receptors is consistent with previous studies showing that B_1 agonists are devoid of effects on sympathetic outflow to the heart (Baurle *et al*., 1998).

As indicated by laser confocal microscopy 1 h was sufficient for antisense ODNs to be taken-up by brain regions adjacent to the lateral and third ventricles. In particular, they were detected at the level of structures (hypothalamus and thalamus) that are implicated in the regulation of cardiovascular homeostasis. Interestingly, this distribution corresponds to the localization of B_1 receptors recognized by studies on human neurones using polyclonal antibodies and standard immunolabelling techniques (Raidoo & Bhoola, 1997). A limitation of the present study is that fluorescent signal was not evaluated throughout the medulla. Thus, we cannot say whether ODNs have reached brainstem areas relevant for the regulation of BP and HR. In addition, non-neural elements which indirectly influence neurons (e.g. the vasculature and the associated connective tissue, the leukocytes recruited following the application of foreign instrumentation) might have been targeted by antisense ODNs.

Instrumentation with i.c.v. cannulas might have enhanced the functional relevance of the B_1 receptor. Inducibility of the B_1 receptor has been previously reported in various preparations, in which the expression is augmented by pharmacological or surgical manoeuvres (Davis *et al*., 1994; Davies & Hagen, 1994; Coelho *et al*., 1997; Zuccolo *et al*., 1996; Simonato *et al*., 1997). However, this possibility does not account for the difference between strains regarding the BP responses to receptor antagonists or antisense ODNs, since both WKY and SHR underwent a similar surgical procedure.

We also evaluated if the functional relevance of des-Arg^9-kinins acting on the B_1 receptor may be augmented by pharmacological manipulation of kinin degradation. Inhibition of brain kininase II (the predominant metabolizing enzyme of BK and Lys-BK) by Captopril causes an exaggerated pressor response in SHR attributable to activation of the B_2 receptor (Madeddu *et al*., 1990), as confirmed here with the use of Icatibant. In the same study, we have demonstrated that a large dose of Captopril is necessary to achieve complete inhibition of kininase II, thus enhancing endogenous kinins to levels high enough to exert pressor effects. The dose of Icatibant used here is able to block the brain B_2 receptor completely (Madeddu *et al*., 1994), while not interfering with other receptors. Our present results also favour participation of the B_1 receptor in the pressor effect of i.c.v. Captopril. Not only does R715 blunt this pressor response but, in combination with Icatibant, it reverts Captopril effect into a vasodepressor response. Thus, we speculate that following kininase II inhibition the functional importance of the B_1 receptor is enhanced because of a shift of kinin degradation through kininase I in the direction of des-Arg^9-BK (Israel & Saavedra, 1987; Lamontagne *et al*., 1995).

In conclusion, our results demonstrate that endogenous desArg^9-kinin metabolites exert a systemic vasopressor action in SHR by activating brain B_1 receptors. Furthermore, the enhanced BP response to kininase II inhibition together with the hypotensive effects exerted by B_1 antagonists and antisense ODNs strongly support the hypothesis that SHR are characterized by an abnormal sensitivity of central regulatory systems to kinins and related metabolites. This dysfunction might be relevant in the pathogenesis of essential hypertension.

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