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SPECIAL REPORT

In vivo evidence for B_1-receptor synthesis induction by heat stress in the rat

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A hypotensive effect of intravenously injected [des-Arg^9]-bradykinin was found in Wistar rats following acute heat stress. This effect was similar to that of intravenously injected bradykinin and was observed 6, 18 and 24 h following an increase in rectal temperature at 42°C for 20 min (H6, H18 and H24 groups, respectively). In contrast, [des-Arg^9]-bradykinin had no effect on blood pressure in control or sham conditions, early on (3 h) or later on (72 h) after heat stress (Ctl, H3 and H72 groups, respectively), while the response to bradykinin was maintained. The hypotension induced by [des-Arg^9]-bradykinin in groups H6, H18 and H24 was comparable to that induced in rats pretreated with a small amount of endotoxin 24 h earlier (LPS group). The hypotensive response in group H24 was totally blunted by [des-Arg^{10}]-Hoe 140 (a potent B_1 receptor antagonist) infused at a rate of 10 μg min^{-1}. These results suggest that heat stress induces the synthesis of vascular B_1 receptors in the rat.

Keywords: Hypotension; heat stress; endotoxin; [des-Arg^9]-bradykinin; bradykinin

Introduction Kinins exert their vascular effects through the activation of two different types of receptors, responsive to [des-Arg^9]-bradykinin and bradykinin named B_1 and B_2 receptors, respectively (Regoli & Barabé, 1980). While the hypotensive effect of bradykinin is present in most animals, [des-Arg^9]-bradykinin has little or no *in vivo* effect on the blood pressure in rats or rabbits. However, a hypotensive response to [des-Arg^9]-bradykinin has been seen in animals pretreated with a small amount of bacterial endotoxin (Regoli *et al*., 1981). Induction of B_1-receptors has also been observed in the heart following a sequence of ischaemia reperfusion (Chahine *et al*., 1993). The present study was thus undertaken in order to determine whether [des-Arg^9]-bradykinin could induce a hypotensive response *in vivo* following heat stress and to compare this response to that obtained with endotoxin pretreatment. The effects of bradykinin and the B_1- and B_2-receptor antagonists, [des-Arg^{10}]-Hoe 140 and Hoe 140 (D-Arg [Hyp^3 Thi^5, D-Tic^7, Oic^8]-bradykinin; Icatibant), respectively, were also investigated.

Methods This investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1985). The experiments were performed on male Wistar rats, weighing 250–350 g. The systemic arterial blood pressure was measured and only the animals with a mean arterial pressure >70 mmHg at the end of the stabilization period were studied. Eight groups were studied: a control group (Ctl; $n=8$) free of any intervention, a sham group (Sham; $n=8$) which was only anaesthetized with pentobarbitone sodium (25 mg kg^{-1}, pentobarbital, Sanofi Santé, France), 24 h before the experimental protocol, a lipopolysaccharide (LPS) group ($n=8$) which was administered 10 μg kg^{-1} LPS (*Escherichia Coli*, serotype 0111: B4, Sigma, France) via the penian vein under light ether anaesthesia, 24 h before the experimental protocol. Five groups H3, H6, H18, H24, H72 ($n=6$ for each group) were submitted to heat stress 3, 6, 18, 24 and 72 h respectively, before the experimental protocol. Before the heat stress the rats anaesthetized with pentobarbitone sodium (25 mg kg^{-1}, i.p.) were placed in an environmental chamber, under an infrared light. The temperature was recorded by means of a rectal probe. Heat exposure was performed in order to increase the internal temperature to 42±0.5°C for 20 min. For the experimental protocol, the animals were anaesthetized with pentobarbitone sodium (50 mg kg^{-1}, i.p.), polyethylene tubes were inserted in the penian vein and in the left carotid artery for the monitoring of the systemic arterial blood pressure through a transducer connected to a Mac Lab recording system (Mac Lab 4S, AD Instruments). The B_2- and B_1-receptor agonists, bradykinin and [des-Arg^9]-bradykinin (Bachem, France) were injected in the penian vein randomly at

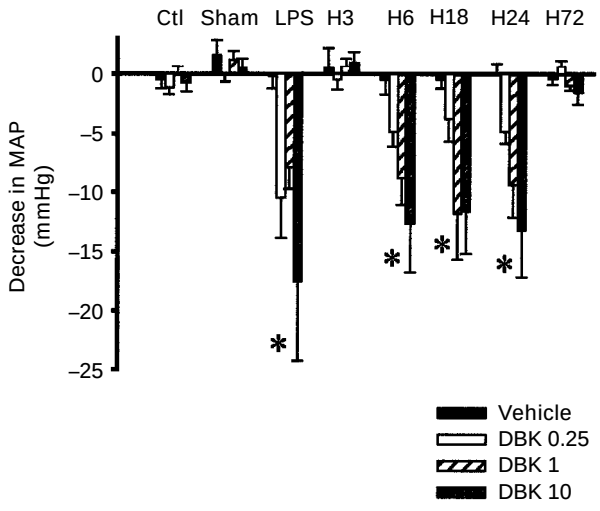

Figure 1 Hypotensive effects of intravenously injected [des-Arg^9]-bradykinin on mean arterial pressure (MAP) in control group (Ctl), 24 h after anaesthesia with pentobarbitone sodium (Sham), 24 h following LPS pretreatment (LPS) and 3 (H3), 6 (H6), 18 (H18), 24 (H24) or 72 (H72) h following heat stress. Vehicle was sodium chloride (0.9%). [des-Arg^9]-bradykinin (DBK) was given at doses of 0.25, 1 and 10 μg ml^{-1}. Columns show means±s.e.mean ($n=8$). *$P<0.05$ compared to control group (ANOVA).

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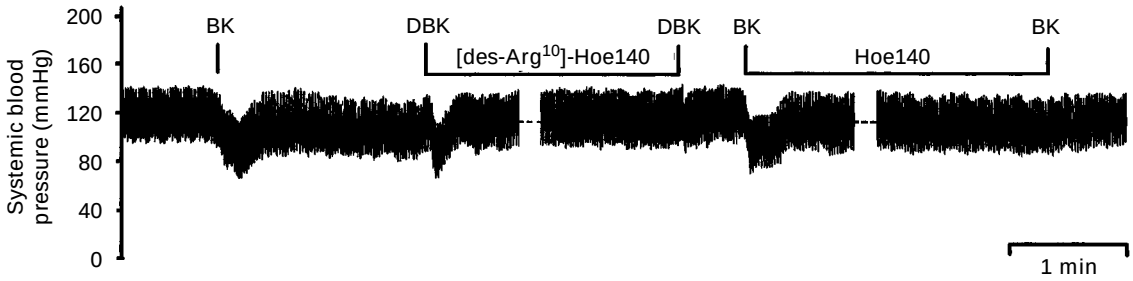

Figure 2 Typical patterns of the changes in systemic arterial blood pressure induced by bradykinin (BK) and [des-Arg^9]-bradykinin (DBK) in the rat 24 h after heat stress before and after B_1-receptor antagonist ([des-Arg^{10}]-Hoe 140) and B_2-receptor antagonist (Hoe 140). Agonists were injected intravenously ($10\,\mu g\,ml^{-1}$). Antagonists were infused intra-arterially at a dose of $10\,\mu g\,min^{-1}$.

doses of 0.25, 1 and 10 μg kg^{-1}. A vehicle (NaCl 0.9%) injection was also performed. Finally, a separate H24 group was injected intra-arterially with a B_2-receptor antagonist (Hoe 140; RBI, U.S.A.) and a B_1-receptor antagonist ([des-Arg^{10}]Hoe 140; RBI, U.S.A.) at a dose of 10 μg min^{-1} before intravenous bradykinin or [des-Arg^9]-bradykinin injection at 10 μg kg^{-1}.

Results The baseline blood pressure was not statistically different between the groups (119 ± 11 mmHg for Sham, 119 ± 6 mmHg for H24; 121 ± 10 mmHg for LPS; $n = 8$ each group. In all the groups studied, bradykinin induced a similar and dose-dependant hypotensive response (data not shown).

[des-Arg^9]-bradykinin was without effect in Ctl, Sham, H3 and H72 groups but induced a dose-dependant hypotensive effect in LPS, H6, H18 and H24 groups (ANOVA, Figure 1). Pharmacological blockade of B_1 receptors, by [des-Arg^{10}]-Hoe 140 infusion, in H24 rats abolished the hypotensive response to [des-Arg^9]-bradykinin (from -25 ± 8 to -1 ± 2 mmHg, $P < 0.05$), and decreased significantly that of bradykinin (from -32 ± 5 to -22 ± 1 mmHg, $P < 0.05$) (Figure 2). The hypotensive effect of bradykinin was totally abolished by prior B_2-receptor blockade with Hoe 140 infusion (from -32 ± 5 to 0 ± 1 mmHg, $P < 0.05$, Figure 2).

Discussion This study provides the first demonstration of a hypotensive effect of [des-Arg^9]-bradykinin, a B_1 receptor agonist, following acute heat stress in the rat.

In physiological conditions, this peptide has no effect on systemic blood pressure in the rat (Tokumasu *et al*., 1995) or on vascular reactivity (Marceau *et al*., 1980). However, in rats pretreated with lipopolysaccharide, a hypotensive response to [des-Arg^9]-bradykinin occurs which has been attributed to the *de novo* synthesis of B_1 receptors (Tokumasu *et al*., 1995). Interleukin 1β and epidermal growth factor have been shown to accelerate this up-regulation process in rabbit isolated aorta (Deblois *et al*., 1988), an observation in favour of the implication of the cytokines network. Plasma levels of interleukin 1β, interleukin 6, interferon γ and transforming growth factor β are also increased following whole body hyperthermia (Kekow *et al*., 1995).

One of the characteristics of smooth muscle preparations sensitive to [des-Arg^9]-bradykinin is an increase in response from an initial null level, as a function of incubation time *in vitro* or of delay after endotoxin injection *in vivo* (Bouthillier *et al*., 1987). This was also seen in our study since there was no effect of [des-Arg^9]-bradykinin 3 h after heat stress.

Finally, the hypotensive effect of [des-Arg^9]-bradykinin can be attributed to activation of B_1 receptors since it was totally blunted by [des-Arg^{10}]-Hoe 140. Although this antagonist is not absolutely selective for B_1 receptors (Rhaleb *et al*., 1992) and, indeed, partially inhibited the hypotensive effect of bradykinin, a B_2-receptor mediated effect, the agonist [des-Arg^9]-bradykinin shows high selectivity for B_1-receptors.

Finally, to support the hypothesis of induction of B_1 receptors, measurements of mRNA are currently under investigation.

In conclusion, acute heat stress induces a pharmacological response to [des-Arg^9]-bradykinin *in vivo* in the rat. This hypotensive response may be attributed to a *de novo* synthesis of B_1-receptors.

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