ELSEVIER

European Journal of Pharmacology 274 (1995) 225–228

ejp

Short communication

A hypotensive response induced by des-Arg^9-bradykinin in young Brown/Norway rats pretreated with endotoxin

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Received 13 October 1994; revised 7 December 1994; accepted 16 December 1994

Abstract

A hypotensive effect of intravenously injected des-Arg^9-bradykinin was found in Brown/Norway strain young male rats which were pretreated with a small amount of endotoxin 24 h before the experiment, whereas the hypotensive effect of bradykinin was unaffected by the endotoxin. The potency of des-Arg^9-bradykinin for the hypotensive effect was comparable to that of bradykinin. On the other hand, in endotoxin-pretreated aged rats, this effect of des-Arg^9-bradykinin was not observed. Only during the intravenous infusion of des-Arg^9[Leu^8]bradykinin, a bradykinin B_1 receptor antagonist, was the hypotensive effect of des-Arg^9-bradykinin inhibited, whereas that of bradykinin was potentiated. After the end of infusion of des-Arg^9[Leu^8]bradykinin, the response to des-Arg^9-bradykinin rapidly recovered. The results suggest the possibility that des-Arg^9-bradykinin might play a role in inflammatory diseases.

Keywords: Hypotension; Bradykinin B_1 receptor antagonist; Bradykinin; Des-Arg^9-bradykinin; Endotoxin

1. Introduction

Bradykinin and related kinins have a variety of potent pharmacological actions, such as increasing vascular permeability, eliciting a systemic hypotensive response, and so on. These actions are attributable to the activation of two different types of receptors, designated B_1 and B_2, which are responsive to des-Arg^9-bradykinin and bradykinin, respectively (Regoli and Barabé, 1980).

Des-Arg^9-bradykinin, which is a degradation product of bradykinin generated by kininase I, has little effect in vivo on the blood pressure of normal rats, whereas bradykinin has a potent hypotensive effect in most animals (Schroder, 1970). However, it has been reported that a hypotensive response to des-Arg^9-bradykinin could be induced in rabbits several hours after pretreatment with a small amount of bacterial endotoxin (lipopolysaccharide) (Regoli et al., 1981). Vasorelaxant effects of des-Arg^9-bradykinin mediated by bradykinin B_1 receptors on rabbit isolated mesentric artery (Churchill and Ward, 1986; Deblois and Marceau, 1987) and dog renal artery (Rhaleb et al., 1989) have been reported. The bradykinin B_1 receptor has also been implicated in the protective effects of bradykinin on the ischaemic heart (Chahine et al., 1993).

In the present study, we compared the des-Arg^9-bradykinin-induced hypotensive response in young and old rats pretreated with a small dose of endotoxin, and also studied the effect of a bradykinin B_1 receptor antagonist on this response in vivo.

2. Materials and methods

2.1. Measurement of systemic blood pressure

The experiments were performed on Brown Norway (B/N) strain, specific pathogen-free, male rats bred at Kitasato University (Oh-ishi et al., 1984). The rats were divided into two groups, the young group, consisting of 6- to 8-week-old animals and the old group, consisting of 10- to 12-month-old rats. Both groups of rats were administered 30 μg/kg of lipopolysaccharide via a tail vein under light ether anaesthesia 24 h before measurement of systemic arterial blood pressure. After

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SSDI 0014-2999(94)00765-9

anaesthesia with pentobarbitone sodium (50 mg/kg i.p., Nembutal, Abott, North-Chicago, IL, USA), two polyethylene tubes were inserted, one into the right femoral vein for agent injection and one into the right femoral artery for monitoring of the systemic pressure by a transducer (200TP, Nihon Koden Co., Tokyo, Japan) connected to a polygraph (6000M, Nihon Koden Co., Tokyo, Japan). Another cannula was also inserted into the left femoral vein for infusion of a bradykinin B_1 receptor antagonist. The maximal decrease in systemic mean blood pressure after injection of bradykinin or des-Arg^9-bradykinin was compared with that just before injection of an agent.

2.2. Drugs

Bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, Peptide Institute Co., Osaka, Japan) and des-Arg^9-bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe, Peptide Institute Co.) were dissolved at a concentration of 10^{-4} M in sterile physiological saline containing 0.1% gelatin at −35°C and diluted to an appropriate concentration just before use. Des-Arg^9-[Leu^8]bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu, Peptide Institute Co.), a bradykinin B_1 receptor antagonist (Regoli et al., 1977), was dissolved in sterile saline. Lipopolysaccharide (*Escherichia coli* 0111:B4, Sigma Chemical Co., St. Louis, MO, USA) was dissolved at 30 μg/ml in sterile saline.

2.3. Statistics

All data are expressed as means ± S.E.M. The data were analyzed by two-way analysis of variance, and individual groups were compared by means of the Student's *t*-test. A *P* value less than 0.05 was considered as significant.

3. Results

3.1. Dose-dependent des-Arg^9-bradykinin-induced decrease in the systemic blood pressure of lipopolysaccharide-pretreated young rats

When the rats received no pretreatment with lipopolysaccharide, bradykinin (3–30 nmol/kg i.v.) caused dose-dependent hypotension in blood pressure of both old and young rats (Fig. 1A and B). The drop in blood pressure of old rats was not significantly different from that of young rats. On the other hand, des-Arg^9-bradykinin, even at a dose of 30 nmol/kg, did not cause any hypotension in rats of either age not pretreated with lipopolysaccharide (Fig. 1C and D).

When the old rats were pretreated with a small amount of lipopolysaccharide injected intravenously 24 h before anaesthesia for the experiment, the hypotension induced by bradykinin was almost the same as that obtained when the animals were not pretreated with lipopolysaccharide (Fig. 1A). Also des-Arg^9-bradykinin did not cause any significant fall in blood pressure in such pretreated animals (Fig. 1C). In the young rats sensitized with lipopolysaccharide, the hypotensive response induced by bradykinin tended to be greater than that of the non-sensitized young rats, but there was no significant difference (Fig. 1B). However, a dose-dependent hypotension was induced by des-Arg^9-bradykinin (−17.2 ± 3.2 mm Hg, −26.9 ± 3.9 mm Hg, and −50.7 ± 7.8 mm Hg at 3, 10, and 30 nmol/kg, respectively) in the sensitized young rats (Fig. 1D) 2–3 h after the beginning of the experiment. The values of this des-Arg^9-bradykinin-induced hypotension obtained from the sensitized young rats were significantly different from those of the non-sensitized young rats at all doses used and were comparable to those induced by bradykinin on a molar basis. In the experiments ($n = 3$) in which the time of lipopolysaccharide sensitization was 6 h, the dose-dependent hypotensive responses (−7.8 ± 2.4 mm Hg, −12.8 ± 4.2 mm Hg, and −20.7 ± 3.7 mm Hg at 3, 10, and 30 nmol/kg, respectively) to

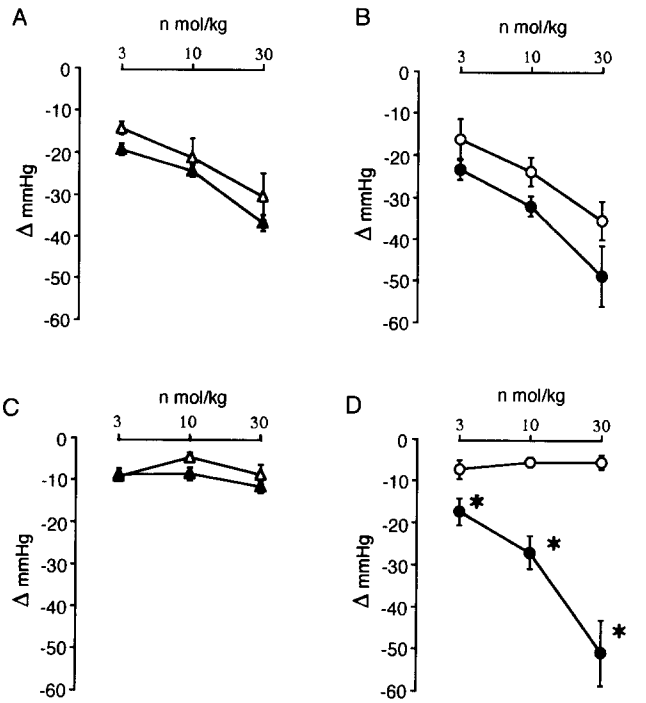

Fig. 1. Hypotensive responses to bradykinin (A and B) and des-Arg^9-bradykinin (C and D) in young (B and D, circles) and old rats (A and C, triangles) pretreated (closed symbols) or not (open symbols) with endotoxin. The ordinates indicate the maximal changes in mean systemic blood pressure after injections of bradykinin or des-Arg^9-bradykinin, with doses shown on the abscissae. Each point represents the mean of 4–7 experiments, and vertical bars show S.E.M. $^*P < 0.05$, Student's *t*-test for the comparison with the data from the rats receiving no pretreatment with lipopolysaccharide.

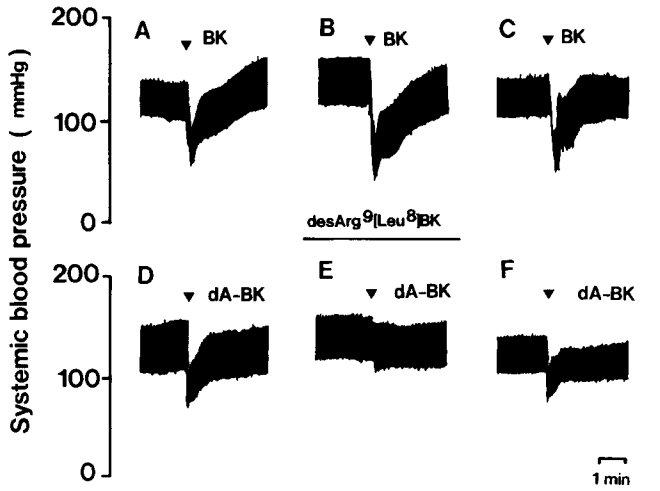

Fig. 2. Typical patterns of the changes in systemic blood pressure induced by bradykinin (BK, upper panel, A, B, and C) and des-Arg^9-bradykinin (dA-BK, lower panel, D, E, and F) in young rats pretreated with endotoxin and the effect of the B_1 receptor antagonist des-Arg^9[Leu^8]bradykinin on these hypotensive responses. Bradykinin and des-Arg^9-bradykinin (30 nmol kg^{-1}, each) were injected as boluses into the right femoral vein and the receptor antagonist was infused into the left femoral vein at a constant rate (20 μg/min) indicated by the horizonal bar. A and D are the results before the infusion of the antagonist, B and E are those during the infusion, and C and F are those 20 min after termination of the infusion. These results were obtained from one rat. Closed triangles indicate the injection of bradykinin or des-Arg^9-bradykinin.

des-Arg^9-bradykinin of the young rats were significantly smaller than those after the 24-h sensitization.

3.2. *Inhibition of des-Arg^9-bradykinin-induced hypotension by infusion of a bradykinin B_1 receptor antagonist*

Fig. 2 indicates typical patterns of the effect of intravenous infusion of the bradykinin B_1 receptor antagonist, des-Arg^9[Leu^8]bradykinin (20 μg/min), on the hypotension induced by bradykinin and des-Arg^9-bradykinin in young rats sensitized with lipopolysaccharide 24 h before ($n = 3$). The infusion of des-Arg^9[Leu^8]bradykinin caused a slight rise in the mean arterial blood pressure. The hypotensive response to 30 nmol/kg of bradykinin changed from -39.0 ± 3.1 mm Hg to -69.5 ± 8.6 mm Hg after the intravenous infusion of des-Arg^9[Leu^8]bradykinin; it was significantly ($P < 0.05$) potentiated. The response to 30 nmol/kg of des-Arg^9-bradykinin was significantly ($P < 0.05$) diminished by the treatment with des-Arg^9[Leu^8]bradykinin; -41.0 ± 2.1 mm Hg for the control and -13.8 ± 4.5 mm Hg for the treatment. After the end of the infusion of des-Arg^9[Leu^8]bradykinin, the response to bradykinin and des-Arg^9-bradykinin recovered to the level seen before infusion; -41.0 ± 10.1 mm Hg for bradykinin and -28.9 ± 4.2 mm Hg for des-Arg^9-bradykinin, respectively.

4. Discussion

The hypotensive response to des-Arg^9-bradykinin in the rabbit (Regoli et al., 1981) has become a standard model for induction of a bradykinin B_1 receptor in vivo. In rats, only in vitro experiments using arterial tissues had so far been reported (Marceau et al., 1980; Boschov et al., 1984). We now showed that, when young rats were pretreated with lipopolysaccharide, the hypotensive responses to des-Arg^9-bradykinin occurred, whereas the response induced by bradykinin, which is elicited via the bradykinin B_2 receptor, did not change. In these young rats pretreated with lipopolysaccharide, the potency of des-Arg^9-bradykinin on the hypotensive effect was comparable to that of bradykinin on a molar basis, whereas des-Arg^9-bradykinin did not cause the hypotensive response in the rats that had not received the lipopolysaccharide pretreatment. Thus, we can consider that the hypotensive response induced by des-Arg^9-bradykinin occurred via the bradykinin B_1 receptor, which is probably newly generated (Deblois et al., 1988), because infusion of a bradykinin B_1 receptor antagonist inhibited this response and also because of the specificity of des-Arg^9-bradykinin for the B_1 receptor, as it was also reported that des-Arg^9-bradykinin did not affect the hypotensive responses induced by substance P and [Tyr(Me)^8]bradykinin, a potent bradykinin B_2 agonist (Regoli et al., 1981). The des-Arg^9-bradykinin-induced response was not observed in the young rats without lipopolysaccharide pretreatment. The response 24 h after lipopolysaccharide pretreatment was greater than that 6 h after the pretreatment. Thus, lipopolysaccharide might be involved in the induction of the bradykinin B_1 receptor. However, this response had not developed within 2 h after the start of the kinin injection. When the repeated injection of bradykinin or des-Arg^9-bradykinin every 20–30 min was performed, the hypotensive response to des-Arg^9-bradykinin first occurred 3–4 h after the beginning of the kinin treatment. This result would suggest that there might be another factor involved in the induction of the bradykinin B_1 receptor, aside from the pretreatment with lipopolysaccharide. These results agree with those of an experiment in which rabbits were used (Regoli et al., 1981). Furthermore, we found that the induction could arise only in the young rats, but not in the old rats. Although the reason for the lack of induction in the old rats is not yet known, aging might be also a potential factor in the induction of the bradykinin B_1 receptor.

The effect of a bradykinin B_1 receptor antagonist (des-Arg^9[Leu^8]bradykinin) infused into the femoral vein on the hypotensive response induced by des-Arg^9-bradykinin was rapidly reversible, because the response induced by des-Arg^9-bradykinin had recovered after the end of the infusion of the antagonist. A

similar result was reported when des-Arg^{10}[Leu^{9}]kallidin was used as an antagonist (Regoli et al., 1981). This result suggests that this antagonist may be proteolytically degraded in the blood where many proteolytic enzymes are present as kininases. It is well known that bradykinin and related kinins are rapidly broken down in the blood. Thus, it can be thought that in vivo des-Arg^{9}[Leu^{8}]bradykinin can act not only as an antagonist of the bradykinin B_1 receptor, but also as a competing substrate for the several proteolytic enzymes instead of bradykinin and des-Arg^{9}-bradykinin in blood (Marceau and Regoli, 1991). In this study, we found that the infusion of des-Arg^{9}[Leu^{8}]bradykinin caused the potentiation of the hypotensive response to bradykinin. This phenomenon might be ascribed to a protective effect of des-Arg^{9}[Leu^{8}]bradykinin on the degradation of bradykinin as a substrate for kininase.

In conclusion, the induction of a hypotensive response to des-Arg^{9}-bradykinin after sensitization with endotoxin in rats was confirmed. However, this induction occurred only in young rats, not in old ones. The potency of des-Arg^{9}-bradykinin for this hypotensive response was the same as that of bradykinin on a molar basis. Thus, this potent action of des-Arg^{9} on systemic blood pressure suggests the possibility that des-Arg^{9}-bradykinin might play a role in some diseases, such as inflammation and septic shock.

Acknowledgement

This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (No. 04454534).

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