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Plasma extravasation mediated by lipopolysaccharide-induction of kinin B1 receptors in rat tissues

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The present study was performed to: (a) evaluate the effects of kinin B_1 (Sar{D-Phe^8}-des-Arg^9-BK; 10 nmol/kg) and B_2 (bradykinin (BK); 10 nmol/kg) receptor agonists on plasma extravasation in selected rat tissues; (b) determine the contribution of a lipopolysaccharide (LPS) (100 μg/kg) to the effects triggered by B_1 and B_2 agonists; and (c) characterize the selectivity of B_1 ({Leu^8}desArg^9-BK; 10 nmol/kg) and B_2 (HOE 140; 10 nmol/kg) antagonists as inhibitors of this kinin-induced phenomenon. B_1 and B_2 agonists were shown to increase plasma extravasation in the duodenum, ileum and also in the urinary bladder of the rat. LPS pretreatment enhanced the plasma extravasation mediated only by the B_1 agonist in the duodenum, ileum, trachea, main and segmentar bronchi. These effects were prevented by the B_1. but not the B_2 antagonist. In normal rats, the B_2 antagonist inhibited the effect of B_2 agonist in all the tissues analyzed. However, in LPS-treated rats, the B_2 antagonist was ineffective in the urinary bladder.

These results indicate that kinins induce plasma extravasation in selected rat tissues through activation of B_1 and B_2 receptors, and that LPS selectively enhances the kinin effect on the B_1 receptor in the duodenum, ileum, trachea and main and segmentar bronchi, and may increase B_1 receptor expression in these tissues.

Keywords: Kinin receptors, Plasma extravasation, Lipopolysaccharide, Bradykinin, B_1 expression

Introduction

Kinins are powerful pro-inflammatory peptides that are released from their precursors, the kininogens, by proteolytic cleavage of specific and non-specific kininogenases.[1] Pharmacological actions of kinins are mediated by B_1 and B_2 receptors, the distribution of which has been studied through the use of specific and selective antagonists,[2–4] and the analysis of protein expression levels.[5]

The kinin B_2 receptor is constitutively expressed in several different cell types and tissues, and most of the actions of kinins are mediated by this receptor.[6–8] In contrast, B_1 receptors are rarely expressed constitutively but, rather, their expression is induced by experimental interventions such as exposure of tissue *in vivo* or *in vitro* to bacterial lipopolysaccharides (LPS), interleukin-1β or to ultraviolet irradiation.[6]

Previously, we exploited the effect of LPS on kinin B_1 receptors to demonstrate the role of these receptors in mediating the vasodilatation in vascular beds of pretreated rats.[4] These results were observed only when we used the B_1 agonist, Sar[D-Phe^8]-des-Arg^9-BK, which is resistant to metabolism by angiotensin-converting enzyme, neutral endopeptidase and aminopeptidases.[9]

In the present study, we investigated the kinin B_1 receptor-mediated plasma extravasation in two sections of the gastrointestinal tract, in the airways and the urinary bladder, in normal and LPS-treated rats.

Materials and methods

Evans blue, HOE 140 (icatibant) and formamide were obtained from Sigma (St. Louis, MO, USA). Bradykinin (BK), Sar[D-Phe^8]-des-Arg^9-BK, and [Leu^8]desArg^9-BK were gifts from D. Regoli (Department of Pharmacology at the Université de Sherbrooke, Canada). The LPS used in this study was from *Escherichia coli* (serotype 0127:B8) from Difco (Detroit, MI, USA).

Conscious male Wistar rats (200–300 g) were used in these experiments. Protein extravasation was evaluated by measuring tissue content of Evans blue dye, as previously described.[10] In brief, EB (20 mg/kg of a solution containing 25 mg/ml in 0.9% NaCl) was injected alone or concomitantly with either kinin B_2 receptor agonist (BK; 10 nmol/kg) or the kinin B_1 receptor agonist (Sar[D-Phe^8]-des-Arg^9-BK; 10 nmol/kg), in control animals and animals pretreated 24 h

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earlier with LPS (100 μg/kg). All reagents were administered through the caudal vein. In other experiments, normal and LPS-treated animals were injected with a selective kinin B_2 receptor antagonist (HOE 140; 10 nmol/kg), or a selective kinin B_1 receptor antagonist ([Leu^8]desArg^9-BK; 10 nmol/kg), 5 min before the application of B_1 or B_2 agonists.

Evans blue was administered and, after 10 min, the animals were decapitated and exsanguinated. The thorax was cut open and the lungs were perfused with 20 ml of 0.9% NaCl (10 ml/min) via a cannula inserted into the pulmonary artery through the right ventricle, to remove the intravascular pulmonary dye. The trachea, main and segmentar bronchi, duodenum, ileum and urinary bladder were dissected and weighed. One-half of each organ was put in formamide while the other half was dried at 50°C for 24 h. The concentration of Evans blue dye in the tissues was determined at 620 nm, using an enzyme-linked immunosorbent assay plate reader, and was expressed in micrograms per gram of dry weight tissue to avoid error due to edema.[10]

Results are expressed as means (SEM, and data obtained in various groups of animals were compared by analysis of variance followed by a *post-hoc* Neuman–Keuls' test, when necessary. $p < 0.05$ was considered significant.

Results

In the first series of experiments, we studied the effects of a kinin B_2 receptor agonist (bradykinin) on plasma extravasation in control and LPS-treated rats (Fig. 1). Bradykinin (10 nmol/kg) increased the plasma extravasation in the duodenum, ileum, urinary bladder, trachea, and main and segmentar bronchi by 43, 30, 78, 58, 27, and 29%, respectively. The injection of the B_2 receptor antagonist HOE 140 (10 nmol/kg), before BK, reduced the plasma extravasation to control values in all tissues analyzed in this work. Pretreatment with a LPS (100 μg/kg), for 24 h, resulted in increased plasma extravasation only in the urinary bladder, by 59%. In LPS-treated rats, the injection of B_2 agonist produced results similar to those observed with BK in control animals. Thus, LPS did not exert any significant influence on plasma extravasation induced by B_2 agonist. Previous injection with the B_2 antagonist in LPS-treated rats significantly reduced the plasma extravasation induced by BK in the duodenum (31%), ileum (22%), trachea (32%), and main (33%) and segmentar bronchi (29%), but not in the urinary bladder. As expected, the B_1 antagonist ([Leu^8]desArg^9-BK; 10 nmol/kg) had no effect on plasma extravasation induced by BK in LPS-treated rats.

In the second series of experiments, we studied the effects of a selective B_1 agonist (Sar[D-Phe^8]-des-Arg^9-BK; 10 nmol/kg) on plasma extravasation in control and LPS-treated rats (Fig. 2). This B_1 agonist increased the plasma extravasation in the duodenum (33%), ileum (35%), and trachea (38%). The injection of a selective B_1 antagonist ([Leu^8]desArg^9-BK), before the administration of B_1 agonist, did not prevent the plasma extravasation in the duodenum, ileum and trachea induced by B_1 agonist, suggesting a partial agonist activity on the B_2 receptor. The injection of B_1 agonist in LPS-treated rats increased plasma extravasation in the urinary bladder (54%), and main (29%) and segmentar (48%) bronchi. An increase in plasma extravasation was also observed in the duodenum (56%), ileum (38%) and trachea (37%), similar to that observed in response to B_1 agonist, in non-LPS treated rats. In LPS-pretreated rats, the B_1 antagonist injected before the B_1 agonist significantly reduced the plasma extravasation in all tissues studied, with the exception of the urinary bladder. The B_2 antagonist did not prevent the plasma extravasation mediated by the B_1 agonist in LPS-pretreated rats.

Discussion

The results presented indicate that kinins are able to induce plasma extravasation in all the tissues of rats analyzed in this study, namely the duodenum, ileum, urinary bladder, trachea, and main bronchi and segmentar bronchi. The effects appear to be mediated by B_1 and/or B_2 receptors. B_1 receptor-mediated plasma extravasation responses were enhanced after LPS treatment in the duodenum, ileum, trachea, main and segmentar bronchi, in which the B_1 agonist (Sar[D-Phe^8]-des-Arg^9-BK) induced a significant increase in the dye content, suggesting that this toxin promotes the formation of B_1 receptor in the rat, as it does in the rabbit and other animal species.[11,12]

It is generally accepted that, while the expression of B_2 receptors is constitutive, expression of B_1 receptors is induced by tissue injury/inflammation.[11] In this study, the presence and the *de novo* expression of B_1 receptors following LPS treatment was found in main and segmentar bronchi, the duodenum and the ileum, and was demonstrated by the fact that the specific and selective B_1 antagonist ([Leu^8]desArg^9-BK) completely prevented the combined effect of LPS and B_1 agonist. In addition, the B_2 antagonist was found to be inactive against B_1 agonist. In the urinary bladder, LPS alone induced a significant increase in plasma extravasation, but did not promote *de novo* expression of B_1 receptors because the B_1 antagonist had no effect against LPS treatment. Recently, studies have provided evidence for the expression of kinin B_1 receptors mediating bladder smooth muscle contraction after cyclophosphamide-induced inflammation in rats.[8] Also, other authors showed evidence for the time-dependent induction of B_1 receptor expression in mice mediating the contraction of urinary bladder.[13] However, our results suggest that the LPS-induced increase in plasma extravasation

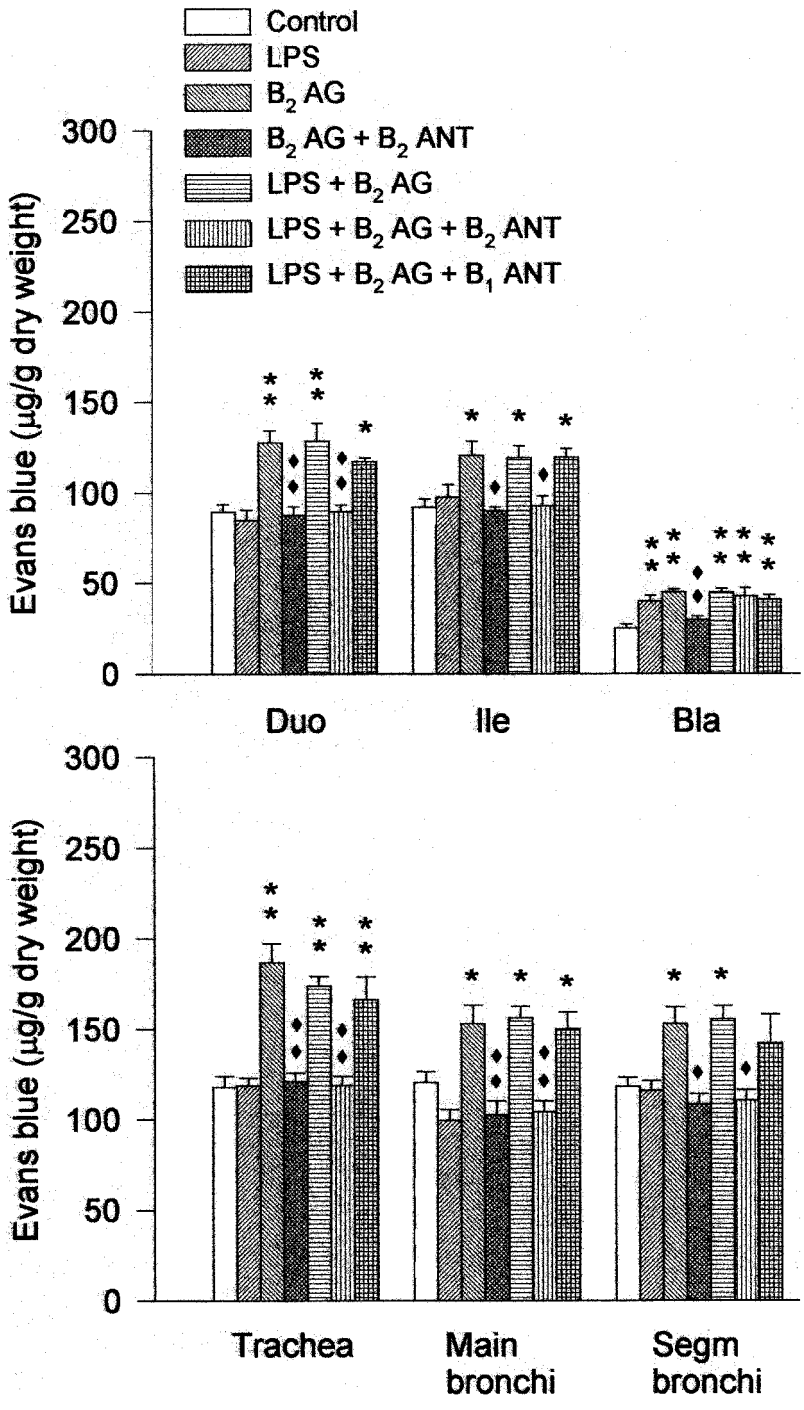

FIG. 1. Effects of kinin B_2 agonist, B_2 antagonist and lipopolysaccharide (LPS), alone and in combination, on plasma extravasation in various organs of the rat. Duo, Duodenum; Ile, ileum; Bla, urinary bladder; Segm bronchi, segmentar bronchi. Columns indicate the means, and vertical bars the standard error of six rats. LPS, LPS-treated rats; B_2 AG, B_2 agonist; B_2 ANT, B_2 antagonist; B_1 ANT, B_1 antagonist. * $p < 0.05$, ** $p < 0.01$, significance with respect to controls, and ♦$p < 0.05$, ♦♦$p < 0.01$ significance as compared with B_2 agonist and LPS + B_2 agonist.

observed in the urinary bladder is caused by mechanisms that may be independent of kinin receptor activation. This finding requires further investigation.

The B_1 receptor is known to be constitutively expressed *in vivo* both in the dog coronary system and in the cat pulmonary vascular bed.[11] In this study, we used the same B_1 agonist (Sar[D-Phe^8]-des-Arg^9-BK) as that used previously[8] because this drug is more resistant to enzymatic metabolism than the alternative B_1 agonist desArg-BK.[6,9] Our results showed that B_1 receptor-mediated plasma extravasation occurred in the duodenum, ileum and trachea of normal rats. Moreover, the effect of this drug was not inhibited by

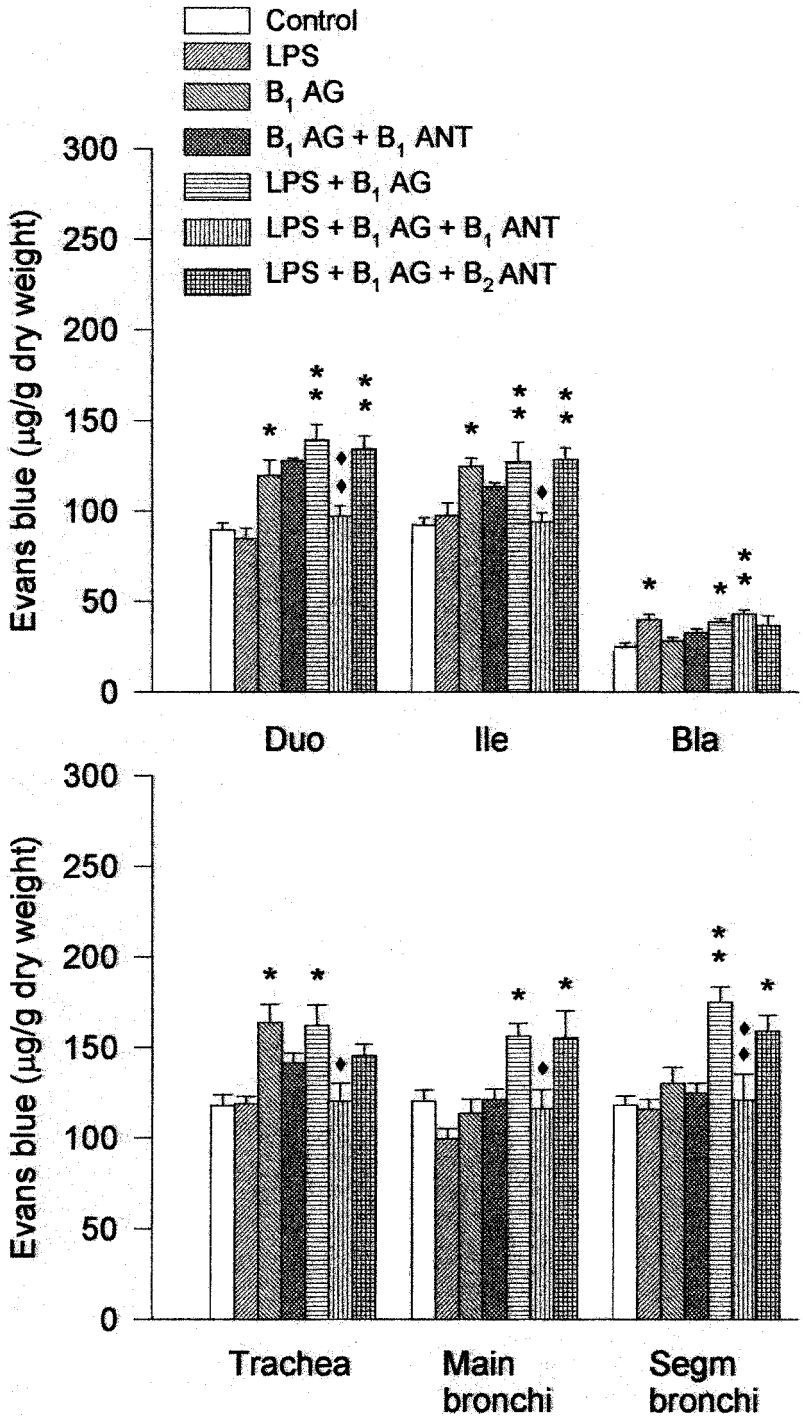

FIG. 2. Effects of kinin B_1 agonist, B_1 antagonists and lipopolysaccharide (LPS), alone and in combination, on plasma extravasation in various organs of the rat. Duo, Duodenum; Ile, ileum; Bla, urinary bladder; Segm bronchi, segmentar bronchi. Columns indicate the means, and vertical bars the standard error of six rats. LPS, LPS-treated rats; B_1 AG, B_1 agonist; B_1 ANT, B_1 antagonist; B_2 ANT, B_2 antagonist. * $p < 0.05$, ** $p < 0.01$, significance with respect to controls, ♦$p < 0.05$, ♦♦$p < 0.01$ significance as compared with B_1 agonist and LPS + B_1 agonist.

treatment with the B_1 antagonist, suggesting a partial agonist activity of the B_1 agonist at the B_2 receptor. On the contrary, the treatment with LPS, which significantly increased the effect of the B_1 agonist in the duodenum, ileum, trachea, main and segmentar bronchi, was blocked by the B_1 antagonist but not by the B_2 antagonist. Increased plasma extravasation has been attributed to B_2 receptors.[6] Our results are consistent with other vascular responses mediated by B_1 receptors observed in LPS-pretreated rabbits[14] and rats.[4,5,15]

Bradykinin increased plasma extravasation in all tissues analyzed in this report. This effect appears to be due to activation of B_2 receptors as it was not

enhanced by the pretreatment of the animal with LPS and this effect was blocked by the B_2 antagonist. This conclusion is further supported by the finding that the B_1 antagonist was found to be inactive against bradykinin-induced plasma extravasation.

It is therefore concluded that bradykinin and the enzyme-resistant B_1 agonist (Sar[D-Phe^8]-des-Arg^9-BK) promote plasma extravasation by acting on two receptor types, B_1 and B_2. Furthermore, we showed that B_2 receptors are present in rat duodenum, ileum, urinary bladder, trachea and main and segmentar bronchi, mediating plasma extravasation. Most importantly, we demonstrated that LPS is able to promote the *de novo* formation of kinin B_1 receptors, which subsequently mediate plasma extravasation in these tissues.

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