The present study was performed to: (a) evaluate the effects of kinin B₁ (Sar[D-Phe⁸]-des-Arg⁹-BK; 10 nmol/kg) and B₂ (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 B₁ and B₂ agonists; and (c) characterize the selectivity of B₁ ([Leu⁸]desArg⁹-BK; 10 nmol/kg) and B₂ (HOE 140; 10 nmol/kg) antagonists as inhibitors of this kinin-induced phenomenon. B₁ and B₂ 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 B₁ agonist in the duodenum, ileum, trachea, main and segmentar bronchi. These effects were prevented by the B₁, but not the B₂ antagonist. In normal rats, the B₂ antagonist inhibited the effect of B₁ agonist in all the tissues analyzed. However, in LPS-treated rats, the B₂ antagonist was ineffective in the urinary bladder.

These results indicate that kinins induce plasma extravasation in selected rat tissues through activation of B₁ and B₂ receptors, and that LPS selectively enhances the kinin effect on the B₁ receptor in the duodenum, ileum, trachea and main and segmentar bronchi, and may increase B₁ receptor expression in these tissues.

Keywords: Kinin receptors, Plasma extravasation, Lipopolysaccharide, Bradykinin, B₁ 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. Pharmacological actions of kinins are mediated by B₁ and B₂ receptors, the distribution of which has been studied through the use of specific and selective antagonists, and the analysis of protein expression levels.

The kinin B₂ receptor is constitutively expressed in several different cell types and tissues, and most of the actions of kinins are mediated by B₁ and B₂ receptors, the distribution of which has been studied through the use of specific and selective antagonists, and the analysis of protein expression levels.

The kinin B₂ receptor is constitutively expressed in several different cell types and tissues, and most of the actions of kinins are mediated by this receptor. In contrast, B₁ 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.

Previously, we exploited the effect of LPS on kinin B₁ receptors to demonstrate the role of these receptors in mediating the vasodilatation in vascular beds of pretreated rats. These results were observed only when we used the B₁ agonist, Sar[D-Phe⁸]-des-Arg⁹-BK, which is resistant to metabolism by angiotensin-converting enzyme, neutral endopeptidase and aminopeptidases.

In the present study, we investigated the kinin B₁ 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-Phe⁸]-des-Arg⁹-BK, and [Leu⁸]desArg⁹-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. 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 B₂ receptor agonist (BK; 10 nmol/kg) or the kinin B₁ receptor agonist (Sar[D-Phe⁸]-des-Arg⁹-BK; 10 nmol/kg), in control animals and animals pretreated 24 h
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<sub>2</sub> receptor antagonist (HOE 140; 10 nmol/kg), or a selective kinin B<sub>1</sub> receptor antagonist ([Leu<sup>8</sup>]desArg<sup>9</sup>-BK; 10 nmol/kg), 5 min before the application of B<sub>1</sub> or B<sub>2</sub> 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.<sup>10</sup>

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. <i>p</i> < 0.05 was considered significant.

**Results**

In the first series of experiments, we studied the effects of a kinin B<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> agonist. Previous injection with the B<sub>2</sub> 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<sub>1</sub> antagonist ([Leu<sup>8</sup>]desArg<sup>9</sup>-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<sub>1</sub> agonist (Sar[D-Phe<sup>11</sup>]-desArg<sup>9</sup>-BK; 10 nmol/kg) on plasma extravasation in control and LPS-treated rats (Fig. 2). This B<sub>1</sub> agonist increased the plasma extravasation in the duodenum (33%), ileum (35%), and trachea (38%). The injection of a selective B<sub>1</sub> antagonist ([Leu<sup>8</sup>]desArg<sup>9</sup>-BK), before the administration of B<sub>1</sub> agonist, did not prevent the plasma extravasation in the duodenum, ileum and trachea induced by B<sub>1</sub> agonist, suggesting a partial agonist activity on the B<sub>2</sub> receptor. The injection of B<sub>2</sub> 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<sub>1</sub> agonist, in non-LPS treated rats. In LPS-pretreated rats, the B<sub>2</sub> antagonist injected before the B<sub>1</sub> agonist significantly reduced the plasma extravasation in all tissues studied, with the exception of the urinary bladder. The B<sub>2</sub> antagonist did not prevent the plasma extravasation mediated by the B<sub>1</sub> 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<sub>1</sub> and/or B<sub>2</sub> receptors. B<sub>1</sub> receptor-mediated plasma extravasation responses were enhanced after LPS treatment in the duodenum, ileum, trachea, main and segmentar bronchi, in which the B<sub>1</sub> agonist (Sar[D-Phe<sup>11</sup>]-desArg<sup>9</sup>-BK) induced a significant increase in the dye content, suggesting that this toxin promotes the formation of B<sub>1</sub> receptor in the rat, as it does in the rabbit and other animal species.<sup>11,12</sup>

It is generally accepted that, while the expression of B<sub>2</sub> receptors is constitutive, expression of B<sub>1</sub> receptors is induced by tissue injury/inflammation.<sup>11</sup> In this study, the presence and the <i>de novo</i> expression of B<sub>1</sub> 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<sub>1</sub> antagonist ([Leu<sup>8</sup>]desArg<sup>9</sup>-BK) completely prevented the combined effect of LPS and B<sub>1</sub> agonist. In addition, the B<sub>2</sub> antagonist was found to be inactive against B<sub>1</sub> agonist. In the urinary bladder, LPS alone induced a significant increase in plasma extravasation, but did not promote <i>de novo</i> expression of B<sub>1</sub> receptors because the B<sub>1</sub> antagonist had no effect against LPS treatment. Recently, studies have provided evidence for the expression of kinin B<sub>2</sub> receptors mediating bladder smooth muscle contraction after cyclophosphamide-induced inflammation in rats.<sup>8</sup> Also, other authors showed evidence for the time-dependent induction of B<sub>2</sub> receptor expression in mice mediating the contraction of urinary bladder.<sup>13</sup> However, our results suggest that the LPS-induced increase in plasma extravasation...
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. In this study, we used the same B$_1$ agonist (Sar[D-Phed]-des-Arg$^9$-BK) as that used previously because this drug is more resistant to enzymatic metabolism than the alternative B$_1$ agonist desArg-BK. 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...
treatment with the B₁ antagonist, suggesting a partial agonist activity of the B₁ agonist at the B₂ receptor. On the contrary, the treatment with LPS, which significantly increased the effect of the B₁ agonist in the duodenum, ileum, trachea, main and segmental bronchi, was blocked by the B₁ antagonist but not by the B₂ antagonist. Increased plasma extravasation has been attributed to B₂ receptors. Our results are consistent with other vascular responses mediated by B₁ receptors observed in LPS-pretreated rabbits and rats.

Bradykinin increased plasma extravasation in all tissues analyzed in this report. This effect appears to be due to activation of B₂ receptors as it was not
enhanced by the pretreatment of the animal with LPS and this effect was blocked by the B₂ antagonist. This conclusion is further supported by the finding that the B₁ antagonist was found to be inactive against bradykinin-induced plasma extravasation.

It is therefore concluded that bradykinin and the enzyme-resistant B₁ agonist (Sar[D-Phe⁸]-des-Arg⁹-BK) promote plasma extravasation by acting on two receptor types, B₁ and B₂. Furthermore, we showed that B₂ receptors are present in rat duodenum, ileum, urinary bladder, trachea and main and segmental bronchi, mediating plasma extravasation. Most importantly, we demonstrated that LPS is able to promote the de novo formation of kinin B₁ receptors, which subsequently mediate plasma extravasation in these tissues.

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