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Contribution of B_2 receptors for bradykinin in Arthus reaction-induced plasma extravasation in wild-type or B_2 transgenic knockout mice

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1 The aim of the present study was to investigate the contribution of bradykinin (BK) B_1 and B_2 receptors in a model of type III hypersensitivity, the reverse passive Arthus reaction (RPA), in wild-type mice and transgenic B_2 knockout littermates.

2 BK (10 μg mouse^{-1}) or bovine serum albumin (0.5 mg mouse^{-1}) induced a sustained Evans blue extravasation for more than 80 min in naive or rabbit anti-bovine serum albumin-treated mice (RPA model), respectively. The response to the two stimuli was prevented by the B_2 receptor antagonist, HOE-140, but not by [Leu^8]desArg^9-BK (B_1 receptor antagonist).

3 In contrast to the wild-type littermates, RPA and bradykinin were unable to trigger an increase in plasma extravasation in B_2 knockout mice.

4 Furthermore, endothelin-1 (5 μg mouse^{-1}) and a selective NK-1 receptor agonist [Sar^9,Met(O$_2$)^{11}]-SP (20 μg mouse^{-1}), triggered a significant increase in peritoneal plasma extravasation in both wild-type and B_2 knockout animals.

5 A pretreatment with indomethacin (200 μg mouse^{-1}) significantly reduced the RPA-induced but not the BK-induced increase in Evans blue extravasation. Furthermore, RPA, but not BK, triggered a significant indomethacin-sensitive increase in peritoneal prostaglandin E_2 content.

6 Our results suggest a pivotal role for B_2 receptors in the mechanism of plasma extravasation which occurs during the reverse passive Arthus reaction in the mouse. Moreover, our results suggest an important contribution of prostanoids in the plasma leakage mechanisms triggered by RPA but not by bradykinin.

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Keywords: B_2 receptors; B_2 knockout mice; Arthus reaction; HOE-140; prostaglandin E_2

Abbreviations: B_1, B_1 receptor; B_2, B_2 receptor: BK, bradykinin; IL-8, interleukin-8; i.p., intraperitoneal; [Leu^8]desArg^9-BK, Leucine^8, desArginine^9 Bradykinin; NK-1, neurokinin-1 Receptor; PAF, platelet-activating factor; PGE_2, prostaglandin E_2; RPA, reverse passive Arthus reaction; [Sar^9,Met(O$_2$)^{11}]-SP, Sarcosine^9, Methionine^{11} Sulphoxide, Substance P

Introduction

The reverse passive Arthus reaction (RPA), a model of type III hypersensitivity, involves inflammatory and haemorrhagic lesions which can be elicited by the formation of immune complexes in the peritoneal cavity (Steil *et al*., 1995). The induction of RPA leads to a sequence of events, including activation of the complement system, polymorphonuclear cell infiltration and the release of an array of inflammatory mediators, such as platelet-activating factor (PAF) and prostaglandins (Steil *et al*., 1995; Moreno, 1993). The generation and release of inflammatory mediators following RPA leads to increased endothelial permeability in the small venules triggering plasma extravasation (Rossi *et al*., 1992; Teixeira *et al*., 1994). This plasma extravasation may be triggered by at least two different mechanisms, first by the direct action of mediators, like bradykinin (BK) on endothelial cells, and second by neutrophil-dependent mediators, such as PAF, IL-8 and the complement component 5a (Proud & Kaplan, 1988; Collins *et al*., 1991; Wedmore & William, 1981).

Bradykinin (BK), a potent proinflammatory nonapeptide synthesized *de novo* at the sites of tissue damage, reproduces many of the cardinal signs of inflammation, such as plasma extravasation and vasodilation in both chronic (Cruwys *et al*., 1994) and acute inflammatory models (Campos & Calixto, 1995; Ahluwalia & Perretti, 1996). Its actions can be mediated directly by the activation of BK receptors located on targeted tissues and indirectly by the release of nitric oxide or other inflammatory agents, such as neuropeptides and prostaglandins (Regoli *et al*., 1996; Steil *et al*., 1995; Figini *et al*., 1995;). Two distinct bradykinin receptor subtypes, B_1 and B_2, have been identified by Regoli & Barabé (1980). The two mammalian genes encoding the bradykinin receptors have been identified by molecular gene cloning; these receptors are G-protein coupled (Menke *et al*., 1994; Eggerickx *et al*., 1992; Hess *et al*., 1992; McEachern *et al*., 1991; McIntyre *et al*., 1993; Powell *et al*., 1993).

In 1995, Borkowski and his colleagues generated the transgenic B_2 knockout mice model. These mice are fertile

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and visually indistinguishable from their wild-type littermates (Borkowski, *et al.,* 1995). Total repression of the B_2 receptors abolishes the BK-induced hypotension as well as the endothelium-dependent vasodilation *in vitro* in the murine model (Alfie *et al*., 1996; Berthiaume *et al*., 1997).

The aim of the present study was to first investigate the contribution of BK, B_1 and B_2 receptors in RPA by the use of selective B_1 and B_2 agonists and antagonists in wild-type mice as well as transgenic B_2 knockout littermates. The putative role of prostaglandins in the induction of plasma extravasation in the RPA reaction was then evaluated in the same above-mentioned models.

Methods

Mice

The C57b1/6 B_2 receptor knockout mice (22–23 grams) were initially supplied by Dr Howard Chen (Merk, Rahway, U.S.A.) and are now routinely bred in our institution (Université de Sherbrooke). These mice are kept in the same normal conditions as their wild-type littermates of the same genetic background (C57b1/6).

Peritoneal plasma extravasation induced by BK

After anaesthetizing the animals with ketamine and xylazine (2.25 and 0.28 mg mouse^{-1}, respectively; i.p.), Evans blue (0.1 mg mouse^{-1}) was injected 5 min later into the penile vein with a sterile $30_G1/2$ needle. BK or the B_1 agonist, desArg^9-BK (DBK) (2–20 μg mouse^{-1}), was subsequently injected intraperitoneally. After the injection of BK, the mice were sacrificed (at fixed timed points 10, 30, 40 and 80 min) by decapitation and exsanguination. The peritoneal cavity of each animal was then washed with a fixed volume (1 ml) of thermostated (37°C) phosphate-buffered saline (PBS; pH 7.4) and the concentration of Evans blue dye (from 200 μl of peritoneal fluids) as an index of vascular permeability was determined by spectrophotometry at a wavelength of 620 nm (Jancar *et al*., 1988). In some other experiments, indomethacin (200 μg mouse^{-1}; i.p.) was injected 1 h prior to the BK injection. In another series of experiments, ET-1 (5 μg mouse^{-1}; i.p.) or [Sar^9, Met $(O_2)^{11}$]-SP (20 μg mouse^{-1} i.p.) was injected by the same route as BK and considered as positive controls of plasma extravasation in wild-type and B_2 knockout mice.

The effect of HOE-140 or [Leu^8]desArg^9-BK (10 μg mouse^{-1}; i.p., 30 min prior to Evans blue and BK injection) was tested against the BK-induced plasma extravasation in wild-type animals. Finally, HOE-140 (10 μg mouse^{-1}; i.p., 30 min prior) was tested against the ET-1 or the [Sar^9,Met$(O_2)^{11}$]-SP-induced plasma extravasation.

Peritoneal plasma extravasation induced by RPA

The reverse passive Arthus reaction was initiated in the peritoneal cavity by the i.p. injection of rabbit anti-bovine albumin (0.5 mg mouse^{-1}). Four hours later, the animals were injected with Evans blue (0.1 mg mouse^{-1}) and then challenged with bovine serum albumin (0.5 mg mouse^{-1}; i.p.). The animals were then sacrificed at fixed time points (10, 30, 40 and 80 min) and the concentration of Evans blue in the peritoneal cavity subsequently determined. In selected experiments, the antagonists HOE-140 and [Leu^8]desArg^9-BK (10 μg mouse^{-1}; i.p.) or indomethacin (200 μg mouse^{-1} i.p.) were injected 30–60 min before the administration of albumin. In some other control experiments, the albumin antiserum used for the RPA reaction was tested *per se* and was confirmed to have no effect on plasma extravasation and prostaglandin E_2 (PGE_2) release.

For both BK and RPA-induced peritoneal plasma extravasation experiments, volume-dependent effects were routinely controlled through the administration of fixed volumes of PBS administered by the same route as BK or bovine serum albumin, respectively. Although these volume controls did not significantly alter basal extravasation, each series of experiments was corrected for these volumetric effects.

Determination of PGE_2 concentration in the peritoneal cavity

Prostaglandin E_2 concentration in the washing fluid of the peritoneal cavity was determined by radioimmunoassay (Salmon, 1978). The antiserum against PGE_2 has a 100% cross-reactivity with PGE_2, 28% with PGA_1, 7% with PGA_2, less than 1.5% with $PGF_{2\alpha}$, 5% with $PGF_{1\alpha}$, 13% with PGB_1 and less than 6% with PGB_2. The limit of detection of the assay was established at 0.15 ng ml^{-1}.

Drugs

BK, desArg^9-BK, [Leu^8]desArg^9-BK and [Sar^9,Met$(O_2)^{11}$]-SP were synthesized by solid-phase synthesis and purified by high-pressure chromatography in our institution by Dr Witold Neugebauer, according to a previously reported method (Drapeau & Regoli, 1988). HOE-140 was supplied by Hoechst (Frankfurt, Germany). ET-1 was purchased from American Peptide Company (California, U.S.A.). Indomethacin, phosphate-buffered saline (PBS; pH 7.4), albumin antiserum, anti PGE_2 and albumin were obtained from Sigma (St. Louis, U.S.A.). Ketamine and xylazine were purchased from MTC Pharmaceuticals Company (Cambridge, Ontario, Canada). Tritiated (^{3}H) PGE_2 was purchased from Amersham (Oakville, Ontario, Canada).

Statistics

Results are expressed in terms of means ± s.e.means. Statistical significance was evaluated with the Student's *t*-test. *P* values of 0.05 and lower were considered significant.

Ethics

The care of the animals and all the research protocols conformed to the guiding principles for animal experimentation, as enunciated by the Canadian Council on Animal Care and approved by the Ethical Committee on Animal Research of the Université de Sherbrooke.

Results

BK or RPA induce an increase in plasma extravasation in the mouse

When injected into the peritoneal cavity of wild-type animals, BK (10 μg mouse^{-1}) induced plasma extravasation in a dose-dependent manner. The threshold for this response was 2 μg and the ED_{50} value was approximately 5.7 μg. No significant plasma extravasation occured in BK-injected B_2 knockout

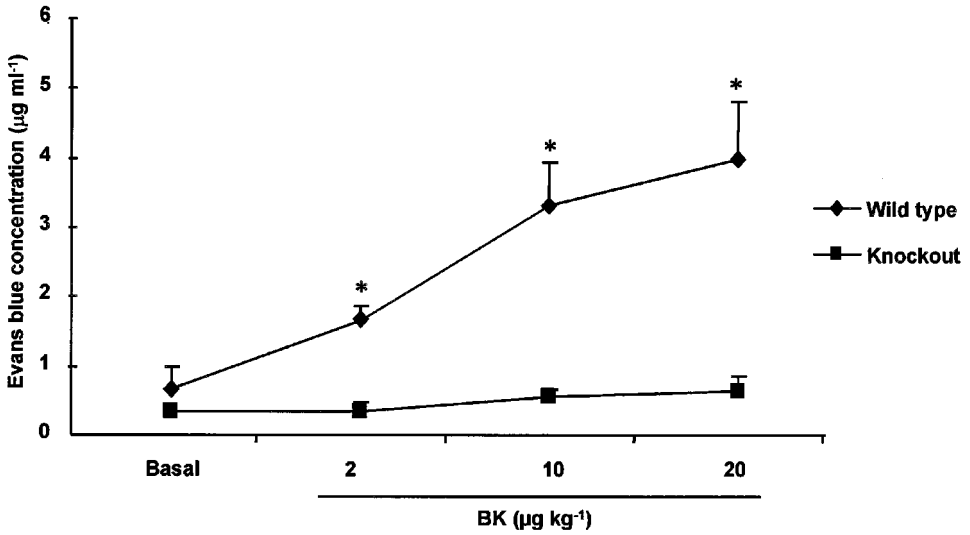

Figure 1 BK-induced plasma extravasation in wild-type and B_2 knockout mice. Each point represents the concentration (μg ml^{-1}) of Evans blue dye in the peritoneal cavity of mice. Data are shown as mean$\pm$s.e.mean ($n=6-15$ per group). *$P<0.05$ when compared to basal.

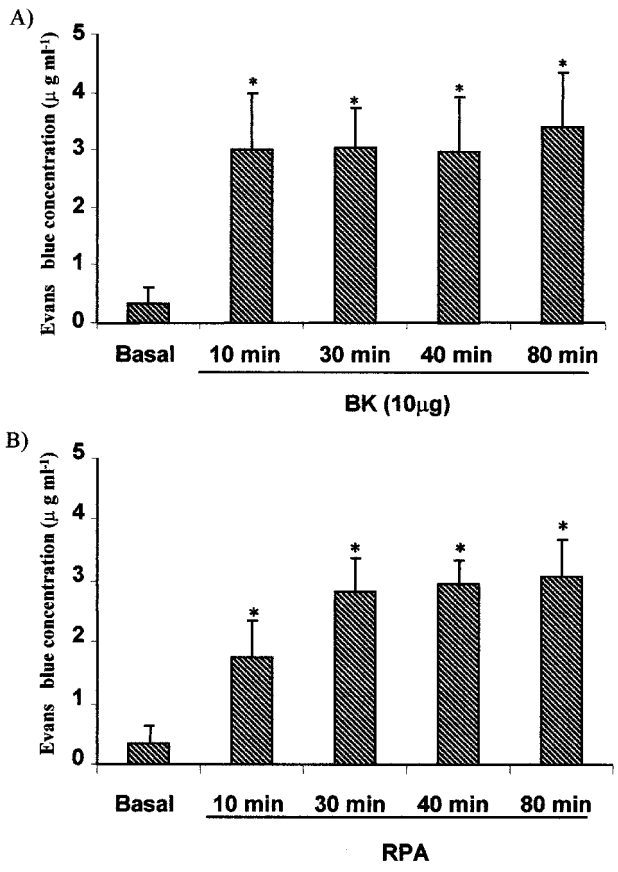

Figure 2 Plasma extravasation induced by BK (A) or RPA (B) in wild-type mice at different time points. Data are shown as mean$\pm$s.e.mean ($n=5-15$ per group). *$P<0.05$ when compared to basal at 10 min. RPA: Reverse Passive Arthus Reaction.

mice at any dosage (Figure 1). Plasma extravasation induced by BK or albumin (in sensitized animals) reached maximal levels at 10 or 30 min, respectively, after induction and accumulated for up to 80 min in wild-type littermates (Figure 2A,B).

Lack of B_1 receptor contribution in the BK-induced increase in plasma extravasation

When injected into the peritoneal cavity of wild-type mice, BK (10 μg mouse^{-1}), but not desArg^9-BK (10 μg mouse^{-1}), induced plasma extravasation in control animals, whereas both the B_1 or B_2 receptor agonists were without effect in B_2 knockout mice (Figure 3). The effects of HOE-140 (5 and 10 μg mouse^{-1}) and [Leu^8]desArg^9-BK (10 μg mouse^{-1}) were tested against the BK-induced increase in plasma extravasation in wild-type animals; the former completely inhibited the increase of extravasation triggered by BK whereas the later antagonist failed to reduce the same response (Figure 3). As positive controls for plasma extravasation in B_2 knockout mice, both ET-1 and [Sar^9,Met(O$_2$)^{11}]-SP induced a significant plasma extravasation in wild-type and B_2 knockout mice (Figure 4). In addition, HOE-140 (10 μg mouse^{-1}) did not affect the response to ET-1 or [Sar^9,Met(O$_2$)^{11}]-SP in wild-type mice (Figure 4).

Contribution of B_2 receptors in RPA-induced plasma extravasation

As previously mentioned, induction of RPA produced a marked increase of plasma extravasation in the peritoneal cavity of wild-type mice (Figure 2B). HOE-140 (10 μg mouse^{-1}), but not [Leu^8]desArg^9-BK (10 μg mouse^{-1}), abolished the RPA-induced plasma extravasation in wild-type animals (Figure 5). Furthermore, the RPA-induced extravasation observed in wild-type mice was abolished in B_2 knockout mice (Figure 5).

PGE_2 release into the peritoneal cavity

To investigate the possible mechanism of plasma extravasation after induction of RPA or following BK injection in mice, we measured the PGE_2 concentration in the peritoneal cavity of these animals. BK-injected wild-type mice did not show a significant enhancement of peritoneal PGE_2 concentration (Table 1). In contrast, RPA induced a marked increase in peritoneal PGE_2 release in both wild-type and B_2 knockout

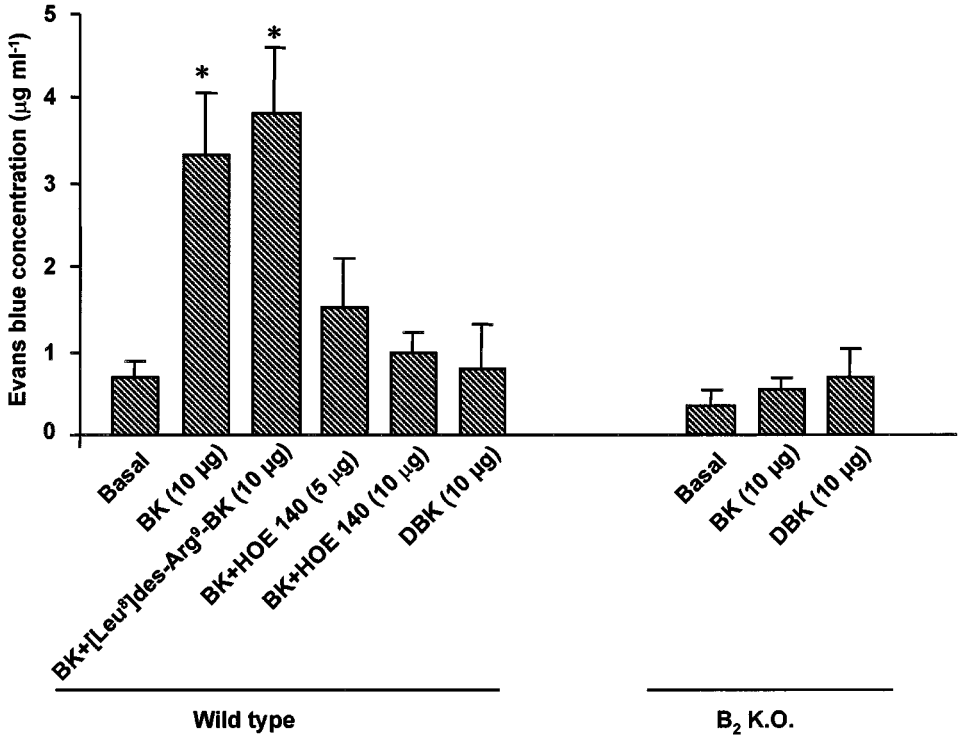

Figure 3 Contribution of B_2 but not B_1 receptors in BK-induced extravasation (10 μg mouse^{-1}). Selective B_1 ([Leu^8]desArg^9-BK (DBK); 10 μg mouse^{-1}) and B_2 (HOE-140; 5 and 10 μg mouse^{-1}) antagonists were tested against the BK-stimulated plasma extravasation. Each column represents the concentration (μg ml^{-1}) of Evans blue dye in the peritoneal cavity of mice. Data are shown as mean$\pm$s.e.mean ($n=6-15$ per group). *$P<0.05$ when compared to basal.

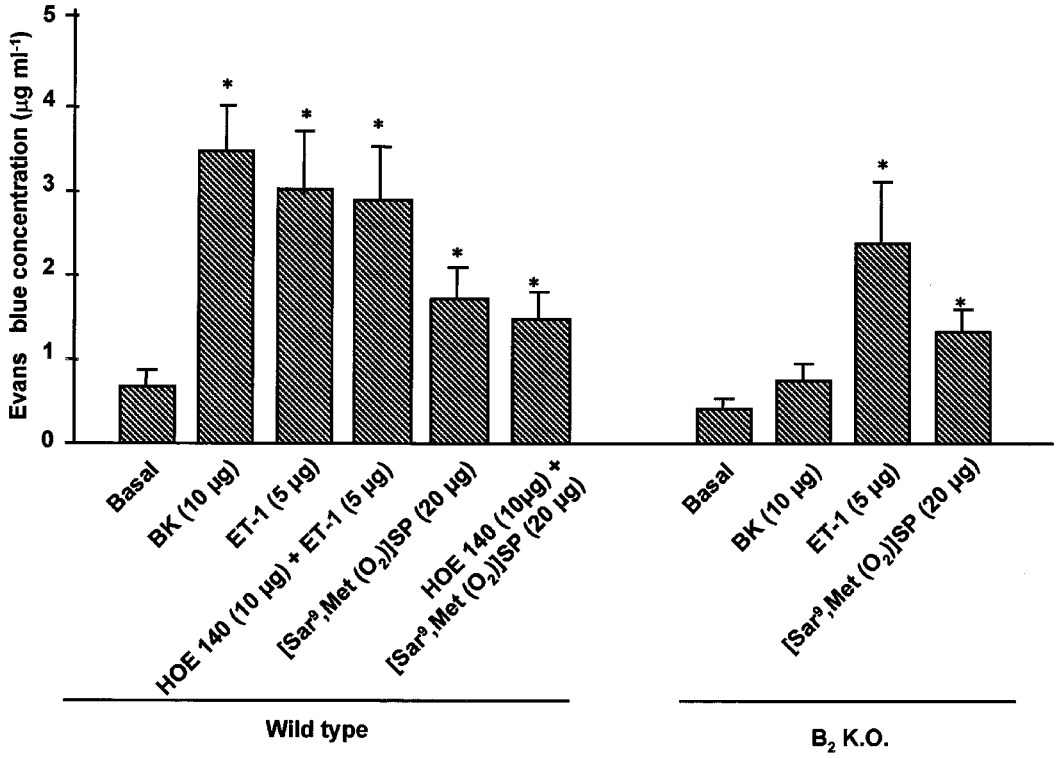

Figure 4 Plasma extravasation induced by BK (10 μg mouse^{-1}), ET-1 (5 μg mouse^{-1}) and selective NK_1 agonist ([Sar^9,Met(O$_2$)^{11}]-SP; 20 μg mouse^{-1}) in wild-type and B_2 knockout mice. HOE-140 (10 μg mouse^{-1}) was tested against ET-1 and [Sar^9,Met(O$_2$)^{11}]-SP. Data are shown as mean$\pm$s.e.mean ($n=6-15$ per group). *$P<0.05$ when compared to basal.

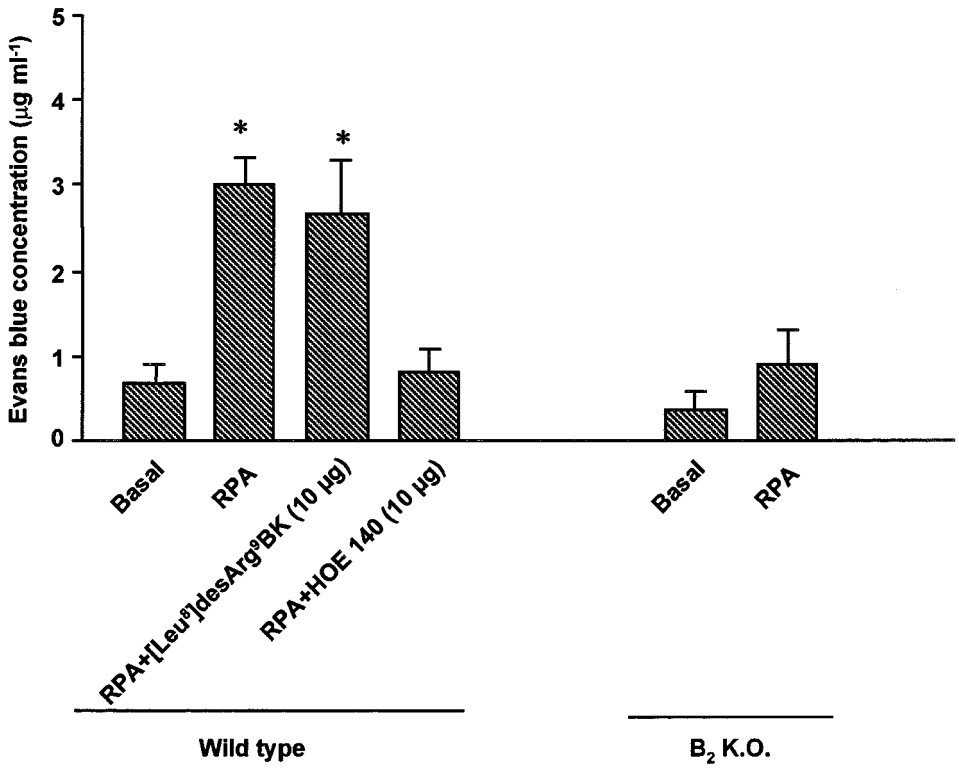

Figure 5 Plasma extravasation induced by RPA in wild-type and B_2 knockout mice. Selective B_1 ([Leu^8]desArg^9-BK; 10 μg mouse^{-1}) and B_2 (HOE-140; 10 μg mouse^{-1}) antagonists were tested against the RPA-induced plasma extravasation. Each column represents the concentration (μg ml^{-1}) of Evans blue dye in the peritoneal cavity of mice. Data are shown as mean$\pm$s.e.mean ($n=6-15$ per group). *$P<0.05$ when compared to basal.

Table 1 RPA-induced increases in PGE_2 levels in the mouse peritoneal fluid

	PGE_2 (ng ml^{-1})
Basal	0.15$\pm$0.08
BK	0.26$\pm$0.24
RPA	0.64$\pm$0.4*
RPA + HOE-140	0.37$\pm$0.18*
RPA + INDO	<0.15
RPA K.O.	0.8$\pm$0.5*

Data represent mean$\pm$s.e.mean ($n=6-9$ per group). *$P<0.05$ when compared to basal. <0.15: undetectable levels.

mice. HOE-140 was ineffective against the RPA-induced release of peritoneal PGE_2.

Effect of indomethacin on plasma extravasation induced by bradykinin and RPA

Finally, we investigated the effect of indomethacin (200 μg mouse^{-1}) on the RPA or BK-induced plasma extravasation in the peritoneal cavity of wild-type mice. Indomethacin did not alter the plasma extravasation response to BK (10 μg mouse^{-1}) (BK: 3.3$\pm$0.78, INDO + BK: 3.15$\pm$0.7 ng ml^{-1}), yet significantly reduced the same response triggered by RPA (RPA: 3.1$\pm$0.69, INDO + RPA: 1.48$\pm$0.6 ng ml^{-1}; $P<0.05$) ($n=6-9$ per group).

Discussion

In our study, RPA induced a significant plasma extravasation in wild-type C57bl/6 mice; this extravasation was absent in transgenic B_2 receptor knockout mice. In addition, a selective B_2 antagonist, HOE-140, completely inhibited the plasma extravasation induced by RPA in wild-type mice. Our results therefore suggest a pivotal contribution of kinins and B_2 receptors in the chain of events leading to plasma extravasation in this immune complex-triggered inflammation model. There are at least two possible mechanisms for kinin synthesis during the early stage of RPA: immune complex-mediated and neutrophil-mediated. It is well known that formation of immune complexes could lead to cell activation and lysis, resulting in the release of kininogenase which could then act on low molecular weight kininogen and generate kinins. The identification of tissue kallikrein, low and high molecular weight kininogen and plasma kallikrein in neutrophils (Figueroa & Bhoola, 1989; Figueroa *et al*., 1990; 1992 Henderson *et al*., 1992) suggests a contribution of kinins in diapedesis and infiltration of inflammatory cells into inflamed sites (Bhoola *et al*., 1992). Besides kallikreins, neutrophils contain a number of other enzymes which are stored intracellulary in cytoplasmic granules. These enzymes, similarly to tissue kallikreins, have the capacity to generate kinins from kininogens (Movat *et al*., 1973; Greenbaum, 1979; Lupke *et al*., 1982) and may be implicated in the inflammatory responses.

The importance of B_2 receptors in the RPA-induced plasma leakage properties, illustrated here by the use of HOE-140 and in B_2 knockout mice, is consistent with the pivotal observations of Boyce *et al*. (1996). In that earlier study, it was shown that knocking-out of the B_2 receptors abolished the oedema response to carrageenin in those transgenic animals.

Our results also show that bradykinin-induced plasma extravasation was abolished by HOE-140 (a selective B_2 antagonist) and was absent in transgenic B_2 knockout mice. Furthermore, indomethacin failed to abolish the increase in

plasma extravasation induced by bradykinin. In addition, we did not detect any significant differences in peritoneal concentration of PGE_2 in BK-injected wild-type mice. This data illustrates the contribution of B_2 receptors in plasma extravasation induced by BK and also indicates that plasma extravasation is not due to the release of PGE_2. On the other hand, the fact that ET-1 and [Sar^9,$Met(O_2)^{11}$]-SP induced extravasation in the knockout mice as well as in wild-type littermates suggests that the kinin pathway is not involved in the plasma leakage properties of other vasoactive peptides, such as endothelin-1 or neurokinins.

Induction of RPA resulted in a significant increase in peritoneal PGE_2 concentration in wild-type or B_2 receptor knockout mice. Furthermore, HOE-140 failed to inhibit the increase of PGE_2 concentration in the peritoneal cavity. Our results also indicated that indomethacin significantly reduced the plasma extravasation induced by RPA. Thus, we show that cyclo-oxygenase products are implicated in a RPA-induced extravasation process (also shown by Moreno, 1993). On the other hand, the fact that RPA triggered a similar peritoneal release of PGE_2 in wild-type or B_2 knockout mice supports the notion that prostaglandins and B_2 receptor activation may be involved in plasma extravasation through distinct mechanisms. One possibility would be that RPA-induced PGE_2 release would be upstream of B_2 receptor activation. A more plausible explanation however would be that indomethacin would effectively interfere with the RPA-induced activity of exudation-potentiating mediators (such as arachidonic acid metabolites) but not of permeability-increasing mediators (such as bradykinin), as suggested initially by Williams & Peck (1977).

It had been previously demonstrated that factors other than kinins, such as platelet-activating factor, mast cell degranulation, nitric oxide and prostaglandins (Rossi *et al*., 1992; Ramos *et al*., 1994; Steil *et al*., 1995; Moreno 1993), are also involved in the inflammatory reaction and plasma extravasation induced by RPA. Most of these studies have been performed in the later stage of RPA and it is possible that kinins may also be involved in more chronical situations, even though this aspect was not investigated in the present study. The activated complement components generated from the formation of the immune complexes lead to the mast cell degranulation. Interestingly bradykinin may also induce mast cell degranulation without binding to a specific bradykinin receptor (Johnson & Erdös, 1973; Lee & Pearce, 1990). It is therefore suggested that BK synthesis may potentiate markedly several of the cardinal factors involved in the RPA-induced inflammatory responses.

It also seems that, in the early stage of RPA, there is no implication of B_1 receptors as illustrated by the lack of effect of the B_1 antagonist [Leu^8]desArg^9-BK. Interestingly, the peptidic B_1 antagonist used in the present study had no agonistic effect in naive nor sensitized mice. Although these results differ from the observation of Allogho *et al*. (1998), the lack of agonistic properties of the B_1 antagonist had already been demonstrated in the mouse perfused mesenteric bed (Berthiaume *et al*., 1997).

We also found useful to use transgenic B_2 knockout mice in the present study. Indeed, these animals allowed us to confirm the importance of B_2 receptors in the RPA reaction without administering yet another drug (i.e. antagonist) in an already complex experimental protocol.

The pathology of autoimmune diseases, such as rheumatoid arthritis, is also associated with the activation of immune complexes which seems to be instrumental in the pathogenesis of these diseases (Bailey & Sturm, 1983). Therefore, the study of inflammatory responses and the exploration of mechanisms involved in RPA have some relevance to that which occurs in immune diseases and may be useful in designing new drugs and treatments for these conditions.

In summary, our study illustrated the implication of B_2 but not B_1 receptors in the early stage of RPA. However, the results of the present study do not preclude a significant role for B_1 receptors at later stages of the RPA reaction. Furthermore, we suggest a significant role for a cyclo-oxygenase product in the RPA but not BK-induced peritoneal plasma extravasation in the mouse.

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