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Up-regulation of [^{3}H]-des-Arg^{10}-kallidin binding to the bradykinin B_1 receptor by interleukin-1β in isolated smooth muscle cells: correlation with B_1 agonist-induced PGI_2 production

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1 Binding of the specific bradykinin B_1 receptor agonist, [^{3}H]-des-Arg^{10}-kallidin (-KD) was investigated in smooth muscle cells (SMC) isolated from rabbit mesenteric arteries (RMA).

2 [^{3}H]-des-Arg^{10}-KD specifically bound to interleukin-1 (IL-1)-treated RMA-SMC in a saturable fashion with an equilibrium dissociation constant (K_D) of 0.3–0.5 nM. The number of binding sites per cell was 20,000–35,000. Kinins inhibited [^{3}H]-des-Arg^{10}-KD binding to RMA-SMC with an order of potency very similar to that observed in typical B_1 specific bioassays: des-Arg^9-bradykinin (BK)$\approx$KD$\gg$BK. Furthermore, the B_1 receptor antagonist [Leu^8]des-Arg^9-BK inhibited [^{3}H]-des-Arg^{10}-KD binding with an IC_{50} of 43 nM as expected for its effect at B_1 receptors. The B_2 receptor antagonists, NPC 567 and Hoe 140 only affected [^{3}H]-des-Arg^{10}-KD binding at very high concentrations ($IC_{50} = 0.8\ \mu M$ and $IC_{50} > 10\ \mu M$, respectively).

3 Des-Arg^9-BK (B_1 agonist) and [Hyp^3]Tyr(Me)^8-BK (B_2 agonist) did not induce prostacyclin (PGI_2) production by RMA-SMC. Lipopolysaccharide (LPS) treatment of the cells did not affect the B_1 agonist response whereas IL-1β treatment produced a 7 fold increase in des-Arg^9-BK-stimulated PGI_2 production. IL-1β also stimulated the response to B_2 agonists.

4 Des-Arg^9-BK-induced PGI_2 secretion in IL-1-primed RMA-SMC was mediated by B_1 receptors since it was inhibited by [Leu^8]des-Arg^9-BK (IC_{50} = 56–73 nM) but not by Hoe 140. High concentrations of NPC 567 ($IC_{50} = 2.4\ \mu M$) were required to inhibit PGI_2 production induced by B_1 agonists.

5 IL-1-treated RMA-SMC displayed a 5 fold increase in the number of B_1 receptors without modification of the affinity constant, thus establishing a possible relationship between the receptor density and the IL-1-primed B_1 response.

6 LPS treatment of the cells induced a 4 fold increase in B_1 receptor number without modifying PGI_2 secretion. This observation suggests that IL-1 but not LPS, in addition to increase in the number of receptors, signals the cell to permit the coupling of B_1 receptors to the PLA_2/cyclo-oxygenase pathway.

Keywords: [^{3}H]-des-Arg^{10}-kallidin; B_1 receptor; binding; signal transduction

Introduction

Pathophysiological stimuli such as tissue trauma, inflammation, allergy or anoxia, activate tissue or plasma kallikrein which cleaves low and high-molecular weight kininogen into bradykinin (BK) or kallidin (lysBK/KD). BK preferentially acts on B_2 receptors whereas the biological active carboxipeptidase fragments des-Arg^9-BK/des-Arg^{10}-KD activate B_1 receptors. These two receptor types have been distinguished on the basis of their selectivity for agonists and antagonists (Regoli & Barabé, 1980). Stimulation of the B_2 receptor by BK induces a large number of effects which have been extensively reviewed (see for reviews, Regoli *et al.*, 1990a,b; Farmer, 1992; Dray & Perkins, 1993). Under non pathological conditions, a great number of kinin-sensitive tissues display a B_2 kinin specificity whereas B_1 responsiveness is acquired following trauma (Regoli & Barabé, 1980). In a number of smooth muscle preparations, obtained from rabbit vascular tissues (Marceau *et al.*, 1980; Regoli *et al.*, 1981; Bouthillier *et al.*, 1987; Deblois & Marceau, 1987; Pruneau & Bélichard, 1993), human colon (Boschov *et al.*, 1984) or rat duodenum (Couture *et al.*, 1981), the B_1 response increases with the incubation time or after treatment with lipopolysaccharides (LPS). In addition, *in vivo* treatment of rabbits with bacterial extract induces a state of responsiveness in the cardiovascular system (Regoli *et al.*, 1981; Marceau *et al.*, 1984; Deblois *et al.*, 1989). Such a feature has led to the hypothesis that the B_2 receptor might play a more significant role in the earlier stages of inflammatory process whereas B_1 receptor would become more important for the maintenance of the response.

Binding experiments performed on whole pieces of rabbit anterior mesenteric vein (Barabé *et al.*, 1982) have shown that LPS-mediated B_1 sensitization was due to an increase in B_1 receptor expression. IL-1β seems to be a key mediator in LPS-induced B_1 agonist sensitization since it was found to stimulate a number of B_1 responses (Deblois *et al.*, 1988; 1991) and was released *in vitro* by vascular and immune cells upon stimulation by LPS (Libby *et al.*, 1986a,b) or by B_1 agonist (Tiffany & Burch, 1989) and *in vivo* by LPS treatment (Cannon *et al.*, 1989).

Finally, a part of the hypotensive effect of B_1 agonists in LPS-treated rabbit is due to prostaglandin release (Drapeau *et al.*, 1991). The relaxation of dog renal (Rhaleb *et al.*, 1989) and rabbit mesenteric arteries (Churchill & Ward, 1986; Deblois & Marceau, 1987) was inhibited by the cyclo-oxygenase inhibitor, indomethacin. The direct effects of B_1 agonists on prostacyclin (PGI_2) production by IL-1-treated smooth muscle cells has also been described (Levesque *et al.*, 1993).

In order to investigate the interaction of B_1 agonists with the cellular receptors as well as the effects of LPS and IL-1β, the binding of [^{3}H]-des-Arg^{10}-KD was investigated in smooth muscle cells isolated from rabbit superior mesenteric arteries. The treatment of RMA-SMC by LPS or IL-1β strongly increased the number of [^{3}H]-des-Arg^{10}-KD binding sites.

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However, IL-1β but not LPS stimulated B_1-induced PGI_2 production. These results suggest that the IL-1-stimulated B_1 agonist response is not solely due to an increase in B_1 receptor expression but depends on IL-1 specific signalling events.

Methods

SMC isolation and cell culture

Rabbit mesenteric artery smooth muscle cells (RMA-SMC) were obtained as described by Ross (1971). Briefly, the superior mesenteric artery was aseptically excised and placed in Dulbecco's modified Eagle's medium (DMEM), 10% foetal calf serum (FCS) and 2% penicillin (100 u ml^{-1})/ streptomycin (100 $\mu g\ ml^{-1}$). The artery was dissected free of outer adventitial layers and cut longitudinally. Endothelial lining was removed by gently rubbing the intimal surface with filter paper. Small pieces of tissue were incubated in DMEM, 10% FCS and 1% penicillin/streptomycin. After 6 days, the tissue was removed and adherent cells were cultured until confluence. Then, cells were obtained for the next passage with a brief trypsin (0.05%)-EDTA (0.5 mM) treatment. Their identity as smooth muscle cells was checked by indirect immunofluorescence for α-actin using a monoclonal mouse anti α-actin antibody (1A4). Binding experiments were conducted with the same cells as those used to evaluate PGI_2 production. After the 6th passage, the cells were unable to reach confluence and lost B_1 specific binding sites.

Equilibrium binding studies

In standard equilibrium binding experiments, the cells (3×10^5) were incubated in 500 μl of NaCl 137 mM, KCl 2.7 mM, BSA 0.1%, bacitracin 140 $\mu g\ ml^{-1}$, captopril 20 μM, DTT 0.1 mM and HEPES-Tris 20 mM pH 7.4 (binding buffer), at 4°C in the presence of indicated concentrations of [^{3}H]-des-Arg^{10}-KD. After 4 h incubation, the cells were filtered through polyethyleneimine (0.3%)-treated Whatman GF/C filters, under reduced pressure. The filters were immediately washed twice with 5 ml of cold binding buffer solution without protease inhibitors. For measurement of nonspecific binding, unlabelled des-Arg^9[Leu^8]-BK was included in the medium at a final concentration of 10^{-5} M. Inhibition of [^{3}H]-des-Arg^{10}-KD by different kinins was measured at equilibrium in the presence of 0.35 nM [^{3}H]-des-Arg^{10}-KD.

Measurement of PGI_2 production

PGI_2 was measured as the stable 6-keto-$PGF_{1\alpha}$ with the Cayman assay kit and according to the manufacturer's instructions. The cells were seeded in 96-well plates at 6000 cells/well. Cells at confluence were cultured in the absence or in the presence of LPS (1 $\mu g\ ml^{-1}$) or IL-1β (10 u ml^{-1}) for 20 h in complete culture medium. At the end of the incubation time, the medium was removed, the cells washed once and incubated with 0.15 ml of phosphate buffered saline solution supplemented with 0.5 mg ml^{-1} gelatin and 10 μM of captopril containing the kinin agonist. After 30 min incubation period at 37°C, the supernatants were collected for 6-keto-$PGF_{1\alpha}$ measurement. When used, antagonists were added 30 min before stimulation with the specific agonist.

Materials

[^{3}H]-des-Arg^{10}-KD (60–120 Ci $mmol^{-1}$) was obtained from New England Nuclear (Les Ulis, France). New Zealand white rabbits (2–2.5 kg) were supplied from local sources. DMEM, PBS, penicillin, streptomycin and foetal calf serum (FCS) were obtained from Gibco BRL (Eragny, France). Monoclonal mouse anti α-smooth muscle actin antibodies were from Dako (Trappes, France). Bradykinin and kallidin were from Sigma (La Verpillière, France). des-Arg^9-BK, [Leu^8]des-Arg^9-BK, D-Arg [Hyp^3,D-Phe^7]-BK (NPC 567) and [Hyp^{3}Tyr(Me)^8]-BK were from Bachem (Bubendorf, Switzerland). Hoe 140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK) was custom-synthesized. IL-1β was obtained from Boehringer (Meylan, France). The Cayman assay kit (PGI_2) was purchased from Interchim (Montluçon, France).

Results

Binding of [^{3}H]-des-Arg^{10}-KD to IL-1-treated RMA-SMC

Preliminary kinetics studies have shown that the binding of [^{3}H]-des-Arg^{10}-KD (0.2–1 nM) to SMC at 4°C reached equilibrium within 4 h. Figure 1 illustrates a typical equilibrium binding experiment of [^{3}H]-des-Arg^{10}-KD to RMA-SMC treated with IL-1β. Specific binding, determined as the difference between total and nonspecific binding, was a saturable function of [^{3}H]-des-Arg^{10}-KD concentration. A Scatchard plot of the data (inset Figure 1) was consistent with the existence of a single class of sites with an average equilibrium dissociation constant (K_D) of 0.3–0.5 nM and an average number of 20,000–35,000 binding sites per cell. The K_D value was not modified when frozen cells were used but the number of binding sites decreased by approximately a factor of 2.5–3.

Effects of kinin agonists and antagonists on [^{3}H]-des-Arg^{10}-KD binding to IL-1-treated RMA-SMC

The inhibition curves of [^{3}H]-des-Arg^{10}-KD binding to IL-1-treated SMC obtained in the presence of increasing concentrations of various kinin agonists are shown in Figure 2a. Kinin agonists inhibited [^{3}H]-des-Arg^{10}-KD binding with a rank order of potency similar to that determined for their activity in B_1 specific biological assays: des-Arg^9-BK (IC_{50} = 7.7 nM) $\approx$ KD (IC_{50} = 8.2 nM) $\gg$ BK (IC_{50} = 3800 nM). The selective B_1 antagonist [Leu^8]des-Arg^9-BK also displaced [^{3}H]-des-Arg^{10}-KD binding with a half maximum concentration of

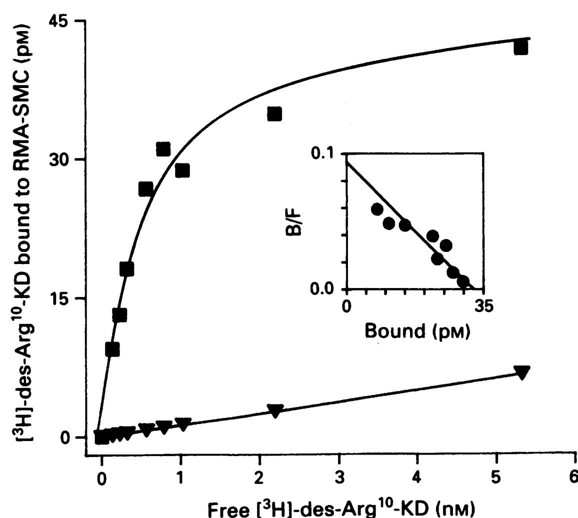

Figure 1 Binding analysis of [^{3}H]-des-Arg^{10}-KD to interleukin-1 (IL-1)-treated rabbit mesenteric artery smooth muscle cells (RMA SMC). Main panel: 3×10^5 cells (passage 5) were incubated at 4°C in 500 μl of binding buffer and in the presence of increasing concentrations of [^{3}H]-des-Arg^{10}-KD without (■, $n = 3$) or with (▼, $n = 3$) 10^{-5} M [Leu^8]des-Arg^9-BK. After 4 h, the cells were filtered under vacuum through GF/C filters and bound radioactivity was measured by a scintillation counter. Inset, Scatchard plot of the specific [^{3}H]-des-Arg^{10}-KD binding component: K_D = 0.35 mM, B_{max} = 35,520 sites/cell (32 pM).

43 nM which is close to the pA_2 measured in rabbit aorta contraction (Regoli *et al.*, 1990b). In addition, the typical specific B_2 antagonists Hoe 140 and NPC 567 inhibited [^{3}H]-des-Arg^{10}-KD binding only at very high concentrations: IC_{50} (Hoe 140) > 10 μM and IC_{50} (NPC 567) = 0.8 μM (Figure 2b). These values are far from those found in B_2-specific bioassays such as the inhibition of BK-mediated rabbit jugular vein contraction which is antagonized by NPC 567 and Hoe 140 with pA_2 values of 8 and 9.2, respectively (Regoli *et al.*, 1990a; Rhaleb *et al.*, 1992).

IL-1 but not LPS stimulates B_1 agonist-induced high level of PGI_2 production by RMA-SMC

Recently, it was shown that B_1 agonist response in rabbit aorta SMC was coupled to PGI_2 production (Levesque *et al.*, 1993). Figure 3a shows that increasing concentrations of the B_1 agonist, des-Arg^9-BK, had no significant effect on PGI_2 production by untreated SMC. LPS (1 μg ml^{-1}) alone did not modify the secretion of prostacyclin measured in the absence or presence of des-Arg^9-BK. Identical results were obtained with 10 μg ml^{-1} of LPS. In contrast, 24 h incubation of SMC with 10 u ml^{-1} of IL-1β markedly enhanced the basal level of PGI_2 from 7 to 19 pg/well. Addition of des-Arg^9-BK produced a 7 fold increase in PGI_2 secretion from 19 to an average of 130 pg/well with a half maximum effect at 20 nM (Exp.1) and 14 nM (Exp.2) (Figure 3a, Exp.2). Indeed, PGI_2 production was mediated by des-Arg^9-BK acting at the B_1 receptor since it was inhibited by the selective B_1 antagonist [Leu^8]Arg^9-BK with an IC_{50} of 73 nM (Figure 3b). The B_2 antagonist, Hoe 140, did not inhibit des-Arg^9-BK-induced PGI_2 production (Figure 3b). As in binding assays, the less specific B_2 antagonist, NPC 567, inhibited PGI_2 production only at the high concentrations: IC_{50} = 2.4 μM.

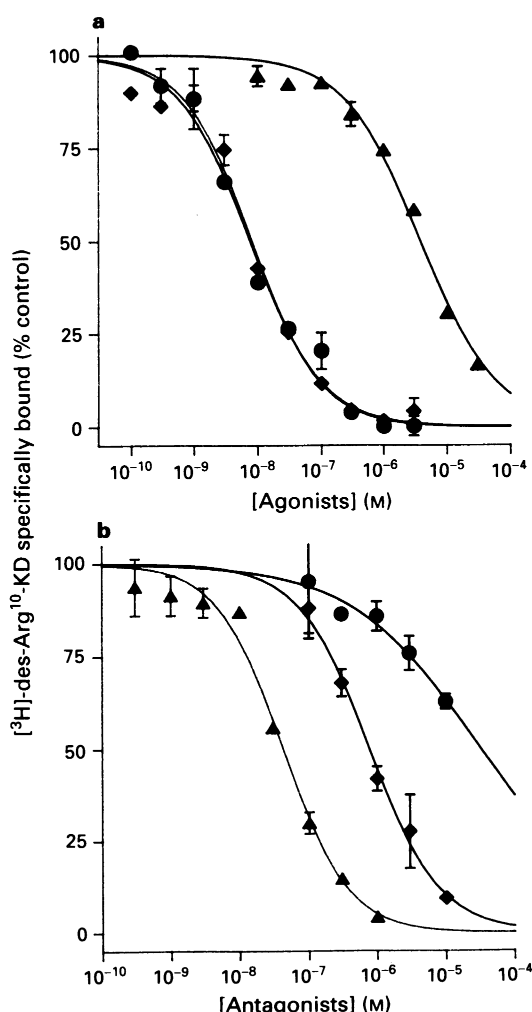

Figure 2 Effects of various kinin derivatives on [^{3}H]-des-Arg^{10}-KD binding to RMA-SMC. IL-1-treated cells (3 × 10^5, 20 h) were incubated at 4°C with 0.35 nM [^{3}H]-des-Arg^{10}-KD in the presence of the indicated concentrations of (a) des-Arg^9-BK (●), kallidin (◆), and bradykinin (▲); (b) [Leu^8]des-Arg^9-BK (▲), NPC 567 (◆) and Hoe 140 (●). After 4 h the cells were filtered as in Figure 1 and the radioactivity was determined; 100% corresponds to the specific binding of [^{3}H]-des-Arg^{10}-KD (1500–2000 d.p.m.) without drugs; values are means ± s.d. of triplicate determinations.

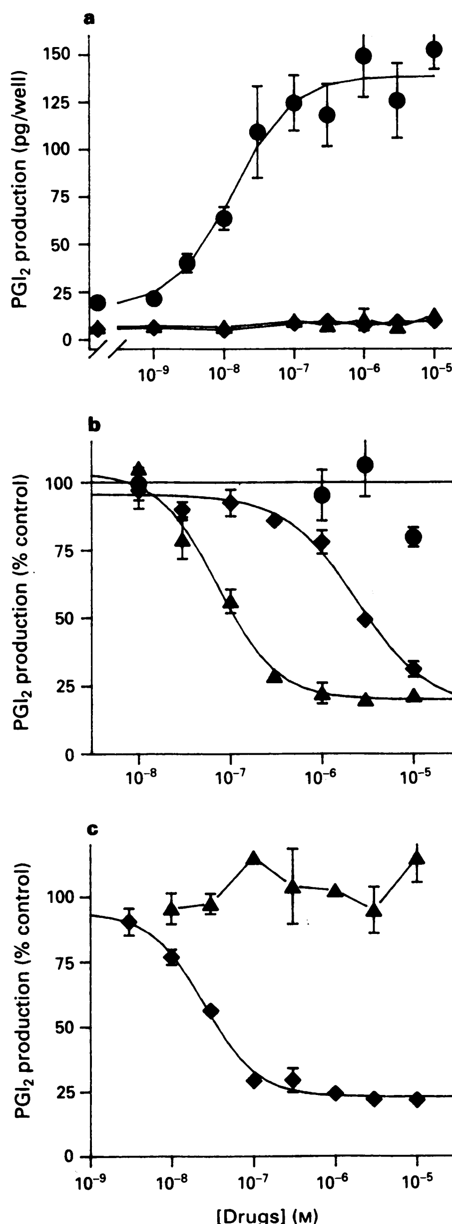

Figure 3 Interleukin 1β (IL-1β) treatment of RMA-SMC enhanced B_1- and B_2-mediated-prostacyclin (PGI_2) secretion. (a) Cells (6000/well) were cultured at confluence in 96 well plates and then incubated without (▲) or with 1 μg ml^{-1} lipopolysaccharide (LPS) (◆) or 10 u ml^{-1} IL-1β (●). After 20 h, the cells were stimulated for 30 min by increasing concentrations of des-Arg^9-BK and the amount of PGI_2 in the supernatant was determined. (b) and (c) IL-1-treated cells were incubated with the indicated concentrations of [Leu^8]des-Arg^9-BK (▲), NPC 567 (◆) or Hoe 140 (●) for 30 min. Then, cells were stimulated for an additional 30 min with (b) the B_1 agonist des-Arg^9-BK (10^{-7} M) or (c) the B_2 agonist [Hyp^3,Tyr(Me)^8]-BK (10^{-8} M). The amount of PGI_2 secreted was measured in the cell supernatant as the stable 6 keto-$PGF_{1\alpha}$ by assay kit as described in Methods. Values are the means ± s.d. of triplicate determinations.

Stimulation of PGI_2 production in IL-1-primed SMC was not restricted to B_1 agonists since it was also found when the B_2 agonist [Hyp^{3}Tyr(Me)^8]-BK was used as stimulating agent. In this case, PGI_2 secretion was blocked by low concentrations of the B_2 specific antagonist, NPC 567 (IC_{50} = 25 nM) but not by the B_1 antagonist, [Leu^8]des-Arg^9-BK up to 10^{-5} M (Figure 3c).

B_1 agonist-induced PGI_2 production in IL-1-treated RMA-SMC is not mediated by nitric oxide

It has recently been proposed that exogenous or endogenous nitric oxide acts on cyclo-oxygenase (COX) to increase the production of prostglandin E_2 (PGE_2) (Salvemini *et al.*, 1993). In this regard, B_1 agonist-induced PGI_2 production in IL-1-treated cells was investigated in the presence of NO inhibitors. Figure 4 shows that neither N^G-nitro-monomethyl-L-arginine (L-NMMA) nor aminoguanidine, the most selective inhibitor of inducible NO synthase, affected the B_1 agonist-induced PGI_2 production by Il-1-treated RMA-SMC. Hence, the difference in LPS and IL-1β-induced PGI_2 secretion was not due to a selective effect of IL-1β on endogenous NO production.

IL-1β and LPS up-regulate [^{3}H]-des-Arg^{10}-KD binding sites in RMA-SMC

After 20 h incubation of RMA-SMC with or without IL-1β, the cells were assessed for [^{3}H]-des-Arg^{10}-KD binding. Figure 5 shows that IL-1 pretreatment of SMC did not modify the equilibrium dissociation constant (K_D(control) = 0.39 nM – K_D (IL-1) = 0.38 nM) but strongly increased the number of binding sites per cell (B_{max}(control) = 6800; 4 pM to B_{max}(IL-1) = 34,300; 23 pM).

LPS, which is already known to induce *de novo* synthesis of specific proteins, increased the number of [^{3}H]-des-Arg^{10}-KD binding sites, (B_{max}(LPS) = 26,200 sites/cell; 17 pM) without significantly modifying the affinity (K_D(LPS) = 0.38 nM). These results suggest that the difference observed in B_1-stimulated PGI_2 production following IL-1 or LPS treatment was not exclusively due to an increase in B_1 receptor expression.

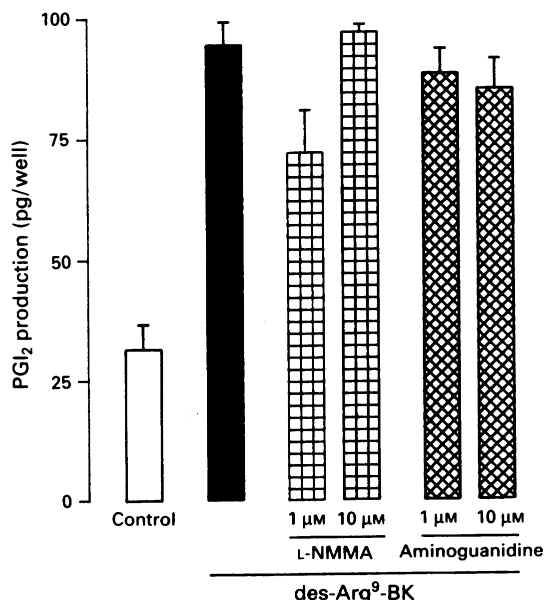

Figure 4 Effects of nitric oxide synthase inhibitors on prostacyclin (PGI_2) production. Cells were cultured at confluence in 96 well plates and then incubated in the presence of 10 u ml^{-1} interleukin-1β (IL-1β). After 20 h, the cells were first incubated without or with L-NMMA or aminoguanidine at the indicated concentrations and then stimulated for 30 min with 10^{-7} M des-Arg^9-BK. PGI_2 production was measured as in Figure 3. Columns are the means ± s.d. of triplicate determinations.

Discussion

In the present study, we describe the characterization of the B_1 receptor as well as its coupling to PGI_2 production in LPS- and IL-1-treated RMA-SMC.

The binding of kinins to the B_1 type receptor has already been described on whole pieces of rabbit mesenteric veins using [^{3}H]-des-Arg^9-BK as a ligand (Barabé *et al.*, 1982). However, as noticed by the authors, such binding experiments suffer from a number of difficulties including the variability from one vein to another and the achievement of binding saturability.

Preliminary experiments revealed that [^{3}H]-des-Arg^{10}-KD bound poorly to isolated RMA-SMC. Since IL-1β has been demonstrated *in vitro* to stimulate the effects of des-Arg^9-BK, [^{3}H]-des-Arg^{10}-KD binding was further investigated in RMA-SMC treated with IL-1β for 20 h. [^{3}H]-des-Arg^{10}-KD was

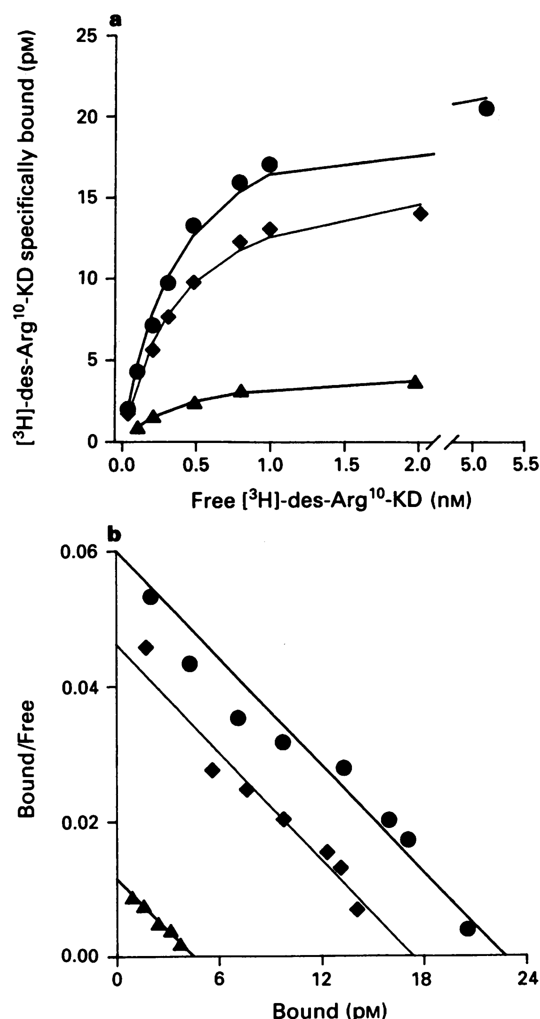

Figure 5 Up-regulation by interleukin-1β (IL-1β) and lipopolysaccharide (LPS) or [^{3}H]-des-Arg^{10}-KD binding sites in RMA-SMC. (a) RMA-SMC at confluence were cultured in the absence (▲, n = 3) or in the presence of 10 u ml^{-1} IL-1β (●, n = 3) or 1 μg ml^{-1} LPS (◆, n = 3). After 20 h, the cells were harvested for saturation binding experiments: 3 × 10^5 cells in 500 μl of binding buffer were incubated at 4°C in the presence of increasing concentrations of [^{3}H]-des-Arg^{10}-KD. After 4 h, the cell bound radioactivity was separated from free by rapid filtration and determined. Nonspecific binding was obtained with 10^{-5} M [Leu^8]des-Arg^9-BK. (b) Scatchard analysis of the specific [^{3}H]-des-Arg^{10}-KD binding component.

found to bind to IL-1-treated SMC in a specific and saturable manner. These binding sites appeared to be physiologically relevant since the K_D value of 0.30–0.5 nM (Figure 1) was comparable to the pD_2 of des-Arg[10]-KD (8.60) measured in rabbit aortic contraction (Regoli *et al.*, 1990b). Three important observations supported the fact that [^{3}H]-des-Arg[10]-KD bound to the B_1 receptor type: (i) the agonists des-Arg[9]-BK, kallidin and bradykinin inhibited binding with a rank order of potency identical to that determined in B_1 specific bioassays; (ii) the specific B_1 antagonist [Leu[8]]des-Arg[9]-BK inhibited [^{3}H]-des-Arg[10]-KD binding with an IC_{50} (43 nM) close to its pA_2 (7.27) measured in rabbit aorta (Regoli *et al.*, 1990b); (iii) the specific B_2 antagonist Hoe 140 had little effect on [^{3}H]-des-Arg[10]-KD binding.

The role of prostanoid secretion in the relaxation of the rabbit mesenteric arteries following B_1 agonist stimulation has been well established (Churchill & Ward, 1986; Deblois & Marceau, 1987). Isolated RMA-SMC in culture have been demonstrated to release arachidonic acid upon treatment with des-Arg[9]-BK (Tropea *et al.*, 1993). Accordingly, des-Arg[9]-BK stimulation of the IL-1-primed RMA-SMC produced a 7 fold increase in PGI_2 production as compared to untreated cells. Conversely, LPS treatment did not enhance the response to des-Arg[9]-BK. Furthermore des-Arg[9]-BK was demonstrated to induce PGI_2 production by interacting at B_1 receptor since B_1 but not B_2 specific antagonists were able to block the des-Arg[9]-BK response. The B_2 agonist [Hyp[3]]Tyr (Me)[8]-BK was also capable of inducing PGI_2 production in IL-1-treated cells, thus demonstrating the existence of functional B_2 receptors in RMA-SMC as already described (Tropea *et al.*, 1993).

In order to gain further insight into the molecular mechanism associated with IL-1 stimulation, the binding of [^{3}H]-des-Arg[10]-KD was investigated on cells treated with either LPS or IL-1β for 20 h. Saturation binding experiments clearly demonstrated that LPS and IL-1β increased the number of binding sites by a factor of 4 and 5 respectively, without significantly modifying the affinity constant. At first glance, these results suggest that the stimulation of B_1 agonist response by IL-1β might be due to an increase in the number of B_1 binding sites. However, they also point to the fact that an increase in receptor number might not be sufficient to promote B_1-induced PGI_2 release since LPS induced an increase in the number of B_2 receptors without affecting PGI_2 production. Obviously, it is possible that the difference in [^{3}H]-des-Arg[10]-KD binding sites observed between LPS and IL-1β treatment (26,200 to 34,300 sites/cell) accounts for IL-1-mediated PGI_2 production. Alternatively, IL-1β may act on a downstream event involved in B_1 receptor signalling. For instance, IL-1β has been described as inducing cyclooxygenase type 2 synthesis (COX-2) in endothelial cells (Habib *et al.*, 1993), fibroblasts and macrophages (O'Banion *et al.*, 1992). Although LPS also activates COX-2 synthesis in macrophages, it is possible that in RMA-SMC, COX-2 synthesis would be selectively induced by IL-1β but not by LPS.

Nitric oxide has recently been shown to activate cyclooxygenase activity (Salvemini *et al.*, 1993). Moreover, the induction of nitric oxide synthase in rat lung homogenates from LPS-treated rats was partially inhibited by the IL-1 receptor antagonist (Szabo *et al.*, 1993). However, NO synthase inhibitors did not modify IL-1-mediated PGI_2 production.

Further investigations are needed to confirm that the difference between LPS and IL-1β results from a preferential coupling of IL-1β to COX induction. LPS injection into rabbit induced a sensitization to des-Arg[9]-BK as well as an increase in IL-1β plasma concentration (Cannon *et al.*, 1989). LPS also renders vascular cells in culture able to produce IL-1 (Libby *et al.*, 1986a,b). Injection of IL-1β into rabbit enhanced vascular responses to the kinin metabolite, des-Arg[9]-BK (Deblois *et al.*, 1991). Thus, the finding that IL-1β greatly stimulates des-Arg[9]-BK to produce PGI_2 is in accordance with the notion of IL-1β as a key mediator responsible for the inducing effect.

At present, we do not know if LPS is able to induce IL-1β secretion by RMA-SMC which in turn would produce an increase in [^{3}H]-des-Arg[10]-KD binding sites and PGI_2 secretion. The observation that LPS is poor inducer of PGI_2 production does not favour this hypothesis. Although LPS does not prime RMA-SMC for B_1 agonist-induced PGI_2 production, the fact that it does increase B_1 receptor number suggests that LPS may be able to prime other B_1-mediated biological effects.

During the preparation of this manuscript a paper was published (Schneck *et al.*, 1994) describing the characterization of B_1 receptor in rabbit isolated aortic cells. The binding affinity for [^{3}H]-des-Arg[10]-KD was in the same range as reported here but the number of B_1 receptors per cell was very low (680 sites/cell) and did not exceed 4250 sites/cell following EGF treatment.

Thus, the presence of B_1 binding sites in RMA-SMC makes these cells a very attractive model to characterize the molecular features of the receptor and obtain further insight into the mechanisms of its regulation.

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