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Short communication

Rat peritoneal macrophages express endothelin ET_B but not endothelin ET_A receptors

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Abstract

The properties of endothelin receptors on rat peritoneal macrophages were examined in in vitro receptor autoradiographic binding experiments and in a reverse transcription-polymerase chain reaction (RT-PCR) study. Dense and specific [^{125}I]endothelin-1 binding sites were detected on the macrophages. [^{125}I]Tyr^{13}–Suc–[Glu^{9},$Ala^{11,15}$]–endothelin-1(8-21), IRL1620, a selective endothelin ET_B receptor ligand, but not [^{125}I](*N*-[(hexahydro-1-azepinyl)carbonyl])L-Leu(1-Me)D-Trp–D-Tyr, PD151242, a selective endothelin ET_A receptor ligand, specifically bound to rat macrophages ($K_d = 0.75 \pm 0.19$ nM, $B_{max} = 7.77 \pm 2.50$ fmol/mg). RT-PCR experiments also showed the expression of endothelin ET_B receptor mRNA, but not endothelin ET_A receptor mRNA, in these macrophages. These results indicate that rat peritoneal macrophages apparently express the endothelin ET_B receptor but not the endothelin ET_A receptor. © 1997 Elsevier Science B.V.

Keywords: Endothelin ET_B receptor; Macrophage; RT-PCR (reverse transcription-polymerase chain reaction); Receptor autoradiography; (Rat)

1. Introduction

The endothelins have a variety of biological activities both in cardiovascular and non-cardiovascular tissues, including monocytes/macrophages. Endothelin-1 and endothelin-3 have been found in cultured human macrophages and in the brain of patients with HIV encephalopathy (Ehrenreich et al., 1990, 1993). Endothelin-1 also increases $[Ca^{2+}]_i$, protein phosphorylation and O_2 production in human alveolar macrophages (Haller et al., 1991), and the production of prostaglandins, thromboxane A_2 and superoxide in alveolar macrophages (Ninomiya et al., 1992; Kojima et al., 1996). The actions of endothelins are mediated through at least two distinct subtypes of receptors, termed the endothelin ET_A and the endothelin ET_B receptors (Arai et al., 1990; Sakurai et al., 1990), and these receptors are widely distributed in a number of tissues and cell types (Hori et al., 1992). In atherosclerotic arteries, the endothelin ET_B receptor has been detected on macrophages within atherosclerotic plaques (Bacon et al., 1996), suggesting that the endothelin ET_B receptor on macrophages may have an important role in pathological conditions. Here, we used in vitro receptor autoradiography and reverse transcription-polymerase chain reaction (RT-PCR) to examine the properties of the endothelin receptors expressed on rat peritoneal macrophages, and found that these macrophages expressed only the endothelin ET_B receptor and not the endothelin ET_A receptor.

2. Materials and methods

2.1. Macrophage preparation

Anesthetized male Wistar rats weighing about 200 g were injected intraperitoneally with 25 ml of sterile phosphate-buffered saline (PBS, pH 7.2) and the ascitic liquid was removed and centrifuged ($200 \times g$, 10 min). The pellet was washed with PBS and resuspended in RPMI 1640 supplemented with 10% of heat-inactivated fetal calf serum, and incubated in humidified 5% CO_2 at 37°C for 2 h in a plastic dish to allow for macrophage adherence.

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After a wash step, more than 95% of the adherent cells were judged to be macrophages according to Giemsa staining.

2.2. Quantitative receptor autoradiography

The adherent cells, macrophages, were collected in a tube and centrifuged (15 000 × *g*, 10 min) at 4°C, and the pellet was frozen by immersion in isopentane at −30°C. 20-μm-thick sections were cut in a cryostat at −18°C, thaw-mounted onto gelatin-coated slides, and stored overnight under vacuum at 4°C. Sections were then labeled in vitro with [^{125}I]endothelin-1, [^{125}I]Tyr^{13}–Suc–[Glu^{9},Ala^{11,15}]–endothelin-1(8-21), IRL1620 (a selective radioligand for the endothelin ET_B receptor (Watakabe et al., 1992)), (New England Nuclear, USA) or [^{125}I](*N*-[(hexahydro-1-azepinyl)carbonyl])L-Leu(1-Me)D-Trp–D-Tyr, PD151242 (a selective radioligand for the endothelin ET_A receptor (Davenport et al., 1994)) (Amersham, UK) in 2.0 ml of incubation buffer, according to our method (Sakurai-Yamashita et al., 1997). [^{125}I]Endothelin-1 binding density in sections was quantified by using our method with the computerized radioluminographic imaging-plate system with [^{125}I]standards ([^{125}I]micro-scales, Amersham, UK) (Yamashita et al., 1994).

2.3. RNA preparation and RT-PCR

Purified macrophages were subjected to RNA extraction by the acid–guanidium–phenol–chloroform procedure. The primers for RT-PCR of the endothelin ET_A and the endothelin ET_B receptors are sense, GAAGTCGTCCGTGGGCATCA (538–557) and antisense, CTGTGCTGCTCGCCCTTGTA (753–734) for the endothelin ET_A receptor, and sense, TTACAAGACAGCCAAAGACT (1004–1023) and antisense, CACGATAGAGGACAATGAAGAT (1568–1549) for the endothelin ET_B receptor. Both receptors were amplified by 30 cycles (denaturation at 94°C for one min, annealing at 60°C for one min, and elongation at 72°C for one min) as previously reported (Shigematsu et al., 1996).

3. Results

Dense [^{125}I]endothelin-1 binding sites were detected on rat peritoneal macrophages (Fig. 1A–A). Co-incubation of [^{125}I]endothelin-1 with 1 μM of unlabeled endothelin-1 completely inhibited [^{125}I]endothelin-1 binding (Fig. 1A and B), indicating that specific [^{125}I]endothelin-1 binding

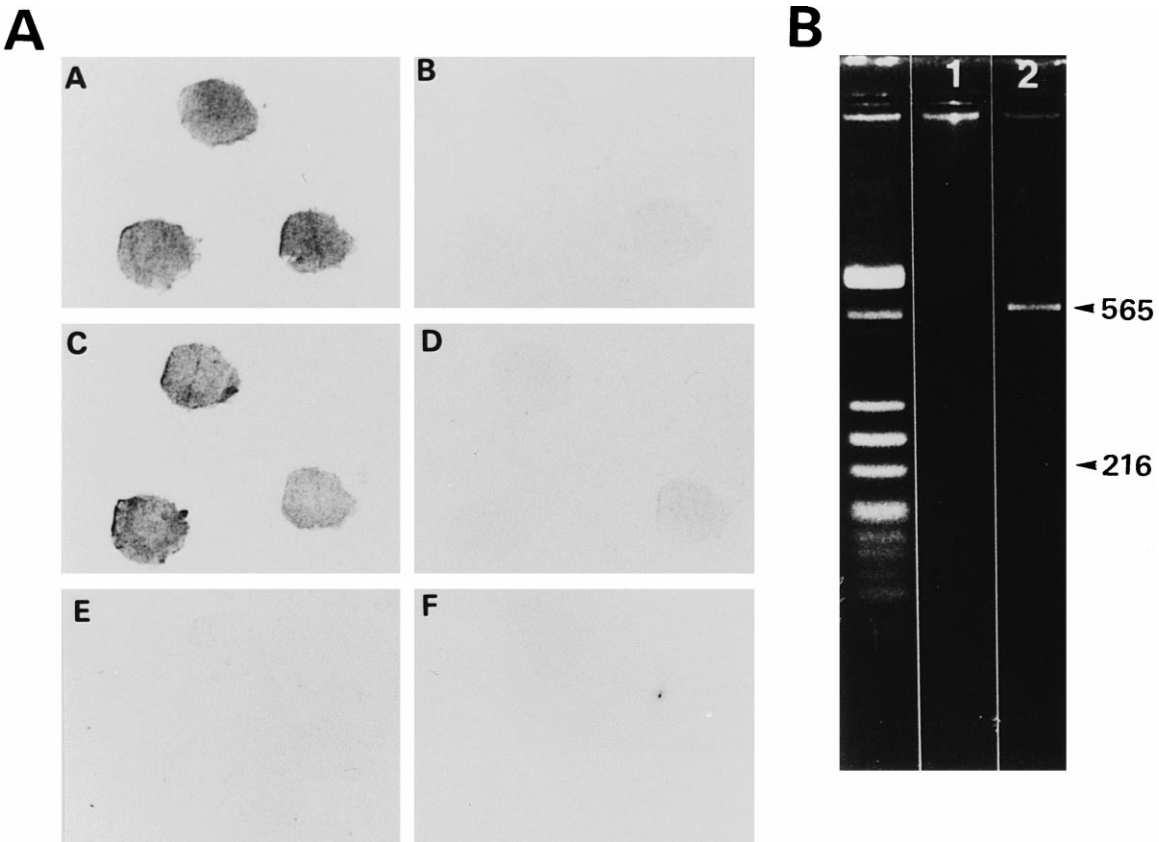

Fig. 1. RT-PCR and autoradiographic evidence of the endothelin ET_A and ET_B receptors on rat macrophages. (A) Consecutive sections were labeled with [^{125}I]endothelin-1 (A and B), [^{125}I]IRL1620 (C and D) and [^{125}I]PD151242 (E and F) in the absence (total binding, A, C and E) or presence (non-specific binding, B, D and F) of each non-labeled ligands (1 μM). Note clear and dense [^{125}I]endothelin-1 and [^{125}I]IRL1620 binding but no [^{125}I]PD151242 binding. (B) Lanes 1 and 2 show ethidium bromide-stained PCR products of the endothelin ET_A (216 bp) and ET_B (565 bp) receptors in a 2% agarose gel, respectively. Note the clear staining of the endothelin ET_B product and the absence of the endothelin ET_A product.

sites are present on these macrophages. To examine the subtype of these specific [^{125}I]endothelin-1 binding sites on macrophages, consecutive sections were incubated with [^{125}I]IRL1620 (a selective radioligand for the endothelin ET_B receptor) or [^{125}I]PD151242 (a selective radioligand for the endothelin ET_A receptor), respectively. Clear and dense [^{125}I]IRL1620 binding sites were also detected (Fig. 1A–C). Binding was abolished by co-incubation with 1 μM of unlabeled IRL1620 (Fig. 1A–D) or ET-1 (data not shown). No significant binding of [^{125}I]PD151242 was detected (Fig. 1A–E). Fig. 1B shows the ethidium bromide staining of the RT-PCR products on the 2% agarose gels. PCR of the rat peritoneal macrophages, using specific primer for the endothelin ET_B receptor, amplified the predicted product (565 bp, lane 2). However, the PCR product for the endothelin ET_A receptor was not detected (216 bp, lane 1). As clear bands of both the endothelin ET_A and ET_B receptors have been detected in rat placenta with the same primer and under the same conditions (Shigematsu et al., 1996), the absence of the endothelin ET_A receptor on rat macrophages in the present study was not due to technical problems.

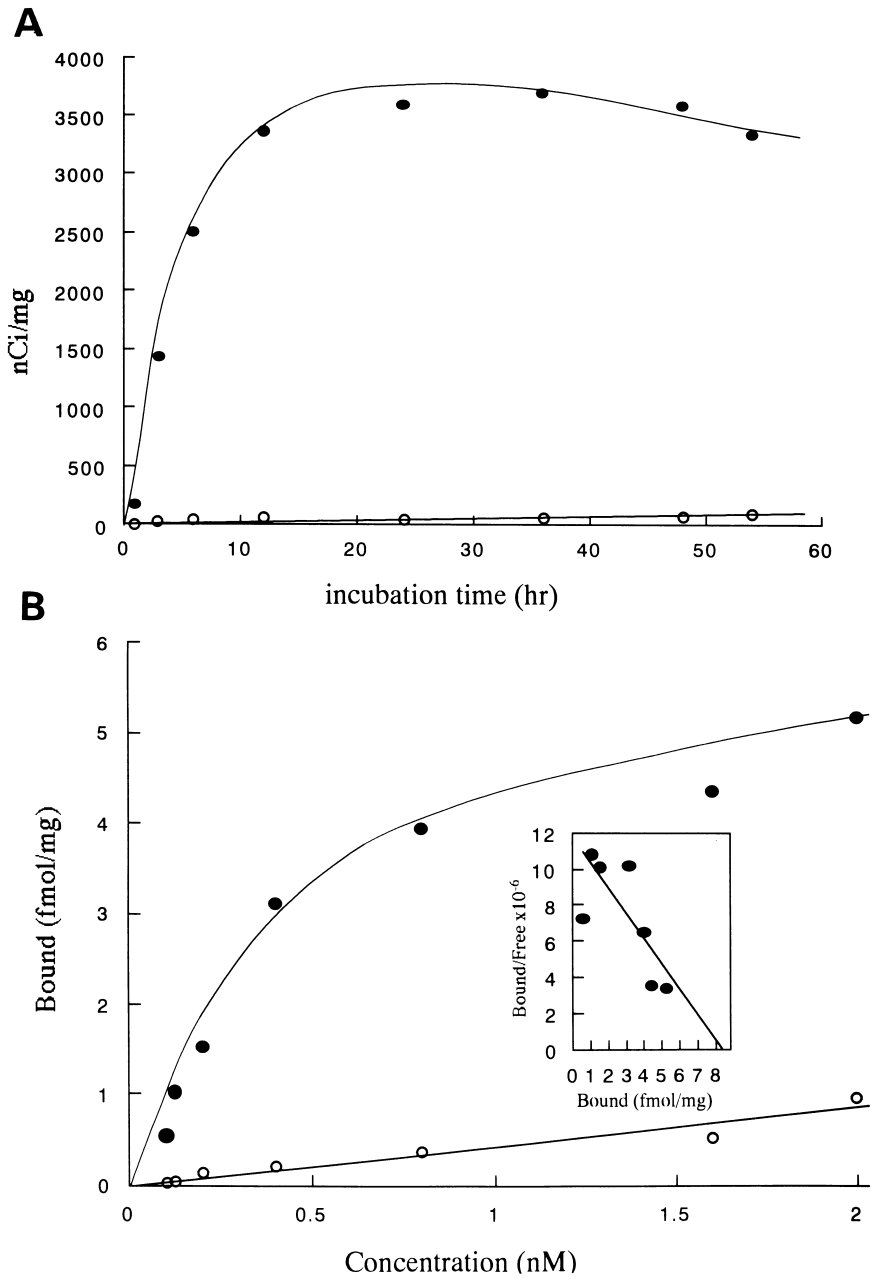

Fig. 2. Time-course and saturation binding study of [^{125}I]IRL1620 binding to rat macrophages. (A) Time-course: Consecutive sections were incubated with 1 nM of [^{125}I]IRL1620 for various times at 4°C. (B) Saturation study: Consecutive sections were incubated for 24 h with increasing concentrations of [^{125}I]IRL1620 (100 pM to 2000 pM). $K_d = 0.75 \pm 0.19$ nM, $B_{max} = 7.77 \pm 2.50$ fmol/mg. – · –: total binding, – ◦ –: non-specific binding.

The characteristics of the endothelin ET_B receptor on rat macrophages were examined by using a selective endothelin ET_B receptor ligand, [^{125}I]IRL1620. Binding of [^{125}I]IRL1620 (1 nM) to rat macrophages increased with time and was saturated after about 20–24 h (Fig. 2A). Therefore, the consecutive sections were incubated for 24 h with increasing concentrations of [^{125}I]IRL1620 (100 to 2000 pM), in the absence (total binding) or presence of 1 μM IRL1620 (non-specific binding). Specific [^{125}I]IRL1620 binding to rat peritoneal macrophages was saturable and a single site with a K_d of 0.75 ± 0.19 nM (mean ± S.E.) and a B_{max} of 7.77 ± 2.50 fmol/mg (Fig. 2B), calculated by using the program LIGAND (BIOSOFT, UK).

4. Discussion

Autoradiographic evidence showing that only [^{125}I]IRL1620 but not [^{125}I]PD151242 bound to rat peritoneal macrophages, and RT-PCR reactions detecting only the endothelin ET_B receptor products support strongly the notion that the endothelin ET_B receptor but not the endothelin ET_A receptor is expressed by and is present on rat peritoneal macrophages.

The present results correspond with those of previous studies suggesting the presence of the endothelin ET_B receptor on macrophages and monocytes. Mouse peritoneal macrophages have been reported to possess specific binding sites for [^{125}I]endothelin-1 and [^{125}I]endothelin-3 with similar affinity and to be increased by the intracellular Ca^{2+} concentration, possibly via the endothelin ET_B receptor (Kishino et al., 1991; Shimamoto et al., 1993). Addition of monocytes or macrophages to the medium increased the vascular contractile potency of endothelin-1, possibly through their own BQ-123 (an endothelin ET_A receptor antagonist)-insensitive receptor, the endothelin ET_B receptor (Magazine et al., 1994). Furthermore, endothelin ET_B receptors have been detected on macrophages within atherosclerotic plaques of patients (Bacon et al., 1996).

Endothelin-1 increases the production of cytokines in macrophages (Millul et al., 1991; Ninomiya et al., 1992; MacMillen et al., 1993). The microglia/macrophages that aggregate in the pyramidal cell layer of the rat hippocampus CA1 subfield after transient forebrain ischemia, possess the endothelin ET_B receptor (Yamashita et al., 1994). Thus, the endothelin ET_B receptor located on macrophages may be involved in inflammatory processes leading to cell injury in peripheral as well as central tissues. The K_d value of [^{125}I]IRL1620 binding to macrophages was 0.75 ± 0.19 nM, and we reported that the endothelin ET_B receptor on the microglia/macrophage aggregated in the CA1 pyramidal cell layer had a K_d of 0.78 ± 0.21 nM (Yamashita et al., 1994). Thus these endothelin ET_B receptors have a similar affinity.

Isoproterenol and salbutamol, β_2-adrenoceptor agonists, increase cAMP in human alveolar macrophages, a murine macrophage cell line (Chambaut-Guerin and Thomopoulos, 1987; Beusenberg et al., 1990), and inhibit lipopolysaccharide-induced tumor necrosis factor-α production in murine peritoneal macrophages (Ignatowski and Spengler, 1995). The endothelin ET_B receptors on rat peritoneal macrophages may be involved in functions via $[Ca^{2+}]_i$ signaling and cAMP signaling by decreasing the accumulation of cAMP stimulated by other stimulators such as β_2-adrenoceptor agonists, because endothelin-1 increases $[Ca^{2+}]_i$ in human alveolar macrophages and mouse peritoneal macrophages (Haller et al., 1991; Kishino et al., 1991), and endothelin ET_B receptors on cultured endothelial cells are linked to not only Gq protein, but also to Gi protein (Eguchi et al., 1993).

In summary, the present study demonstrated that rat peritoneal macrophages express the endothelin ET_B receptor but not the endothelin ET_A receptor. The pathophysiological significance of the endothelin ET_B receptor on macrophages in atherosclerosis and ischemic neuronal injury should be considered.

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