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Pro-inflammatory responses in human monocytes are β_1-adrenergic receptor subtype dependent☆,☆☆

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ABSTRACT

Stress induced circulating catecholamines are hypothesized to selectively activate adrenergic receptors (ARs) on immunocompetent cells modulating their inflammatory response to trauma or environmental toxins. We characterized changes in expression of a pro-inflammatory cytokine modulated by β-AR activation in human primary and immortalized monocytes that had been simultaneously stimulated with lipopolysaccharide (LPS). Results from cytokine antibody arrays demonstrated that half-maximal effective concentrations of the selective β-AR agonist isoproterenol (Iso) qualitatively increased LPS-mediated expression of the soluble cytokine, interleukin-1β (IL-1β). Semi-quantitative immunoblot techniques confirmed a synergistic increase of IL-1β production in both LPS stimulated THP-1 cells and primary human monocytes co-incubated with Iso. Immunoblot techniques as well as radioligand binding studies were also used to characterize the heterogeneous expression of β_1- and β_2-AR subtypes on THP-1 cells. β-AR activation is classically associated with generation of cAMP in many tissues and cell types. Therefore, using the method of Schild, we generated Iso concentration–response curves in the presence of fixed subtype-selective β-AR antagonist concentrations to demonstrate that β_1-AR activation was exclusively linked with the generation of cAMP in THP-1 cells. Furthermore, use of a selective kinase inhibitor demonstrated that Iso potentiated the expression of soluble IL-1β through activation of cAMP-dependent protein kinase A. Finally, discriminating concentrations of subtype-selective β-AR antagonists revealed that β_1-AR stimulation alone accounted for the synergistic production of IL-1β in LPS stimulated monocytes co-incubated with Iso. These results demonstrate a unique synergistic pro-inflammatory response mediated through a β_1-AR cAMP-dependent mechanism in LPS-challenged monocytic cells.

1. Introduction

During acute infections monocytes are the primary producers of cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α), which contribute to the microbicide activity of

Abbreviations: ARs, adrenergic receptors; B_{max}, maximal specific binding of radioligand; CGP 20712A, [1-(2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino)-3-(4-(1-methyl-4-trifluoromethyl-2-imidazoylyl)phenoxy)-2-propanol methanesulfonate, 2-hydroxy-5-(2-(hydroxy-3-(4-((1-methyl-4-trifluoromethyl)-1H-imidazol-2-yl)phenoxy)propyl)aminoethoxy)benzamide; EC_{50}, molar concentration of receptor agonist that produces 50% of its maximal effect; ELISA, Enzyme-Linked Immunosorbent Assay; FBS, fetal bovine serum; H-89, N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride; HBSS, Hank's balance salt solution; IBMX, 1-methyl-3-isobutylxanthine; IC_{50}, molar concentration of competitive ligand that inhibits radioligand binding by 50%; ICI 118,551, erythro-*dl*-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol; ^{125}I-CYP, (−)3-[^{125}I]iodocyanopindolol; IL-1β, interleukin-1β; Iso, isoproterenol; K_b, equilibrium dissociation constant of competitive receptor antagonist determined from functional assay; K_d, equilibrium dissociation constant of radioligand; K_i, equilibrium dissociation constant of competitive ligand determined from radioligand binding study; kDa, kilodalton; LPS, lipopolysaccharide; MNL, mononuclear leukocytes; PDE, phosphodiesterase; PKA, protein kinase A; PVDF, polyvinylidene difluoride; THP-1, human monocytic cell line; TIMP-2, tissue inhibitor of metalloproteinase-2; TNF-α, tumor necrosis factor α.

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the innate immune system (Cavaillon and Adib-Conquy, 2005). In chronic inflammatory conditions such as atherosclerosis, generation of IL-1β and TNF-α by these same immunocompetent cells induces damage to endothelial cells, which is a major factor in the development of vascular plaques (Loppnow et al., 2008). Understanding how to regulate monocyte production of IL-1β, TNF-α and other innate cytokines may prove useful in the treatment of sepsis or slowing the progression of cardiovascular diseases caused by atherosclerotic lesions.

Increased sympathetic responses associated with acute infection or tissue injury constitute an important regulatory mechanism that optimizes innate inflammatory responses (Calcagni and Elenkov, 2006). For instance, the sympathetic neurotransmitter norepinephrine has been shown to have a variety of effects on the innate immune response *in vivo*, including acting as a chemotactic agent for monocytes as well as inhibiting the bacterial endotoxin initiated production of TNF-α (Straub et al., 2000; van der Poll et al., 1994). In the same manner, prolonged or inappropriate stimulation of the sympathetic nervous system on innate immune responses can lead to excess inflammation or uncontrolled infection resulting in pathological effects, including toxic shock and tissue damage (Spengler et al., 1990; Murray et al., 2000). Therefore, understanding the mechanisms that modulate sympathetic regulation of monocyte function and thereby innate cytokine production is important in the development of therapeutic strategies to selectively regulate the inflammatory response to diseases in which immunocompetent cells of the innate immune system play an important pathological function.

In humans, responses to the endogenous catecholamines norepinephrine and epinephrine are mediated through activation of three independent families of adrenergic receptors (ARs), which include within each group three characterized receptor subtypes (α_{1a}, α_{1b}, α_{1d}, α_{2a}, α_{2b}, α_{2c}, β_1, β_2, β_3; see review by Guimarães and Moura, 2001) Early studies have characterized high β-AR expression levels from an *ex vivo* preparation of human mononuclear leukocytes (MNL), which comprise a mixed population of monocytes, lymphocytes (adaptive immunocompetent cells) and platelets (Motulsky et al., 1986). A subsequent investigation utilizing oral administration of subtype-selective β-AR antagonists to block isoproterenol (Iso) induced changes in β-AR density and generation of cAMP documented a β_2-AR population in this same MNL preparation (van Tits et al., 1990). Although an elegant analysis, this investigation did not quantify direct receptor interactions for subtype-selective β-AR competitive antagonists and thus heterogeneous expression of β-AR subtypes cannot be ruled out in this mixed population of immunocompetent cells. Nonetheless, *ex vivo* and *in vitro* preparations of human monocytes are considered to solely express the β_2-AR subtype, whose activation has further been shown to have "anti-inflammatory" effects resulting in dampening of the innate immune response to infection or injury (Farmer and Pugin, 2000; Mizuno et al., 2005; van der Poll et al., 1994). However, complicating the literature are reports of β_1-AR subtype expression in preparations of human monocytes or "pro-inflammatory" responses attributed to β_2-AR activation, suggesting a pluripotent β_2-AR effect in these same cells (Kavelaars et al., 1997; Szelenyi et al., 2006; Talmadge et al., 1993). In this study we tested the hypothesis that pro-inflammatory outcomes of β-AR activation in monocytes are not due to the bi-functional ability of a single receptor, but instead are related to the signaling capacity for a specific β-AR subtype expressed in a heterogeneous receptor population. We explored the dichotomous β-AR inflammatory response in human monocytes that had been simultaneously incubated with the bacterial endotoxin, lipopolysaccharide (LPS). We characterize the expression of both β_1- and β_2-AR subtypes on human monocytes, which when stimulated concomitantly with LPS and Iso generated a unique synergistic increase in IL-1β production. Using subtype-selective receptor antagonists, we observed that this novel pro-inflammatory response is associated with exclusive activation of the β_1-AR subtype and was functionally correlated to the generation of cAMP along with subsequent activation of protein kinase A (PKA). Our results are the first to demonstrate, using classical pharmacological techniques, a "pro-inflammatory" effect of β_1-AR activation in human monocytes that have been pathogenically challenged to initiate an inflammatory response.

2. Experimental

2.1. Materials and methods

2.1.1. Cell culture

A human monocytic cell line, THP-1 (ATCC, Manassas, VA) was propagated using standard cell culture conditions (37 °C/5% CO_2) in RPMI 1640 medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose and 10 mM HEPES (complete media), supplemented with 10% heat inactivated fetal bovine serum (FBS; Atlanta Biologicals, Lawrenceville, GA). Confluent THP-1 cells (10^6 cells/mL) were washed in serum-free complete medium, allowed to become quiescent for 30 min before preincubating with or without AR antagonists or inhibitors of PKA for 60 min prior to addition of the selective β-AR agonist, Iso (Sigma–Aldrich, St. Louis, MO) and/or an inflammatory receptor agonist, LPS (Sigma–Aldrich, St. Louis, MO).

2.1.2. Isolation of primary human monocytes

The lymphocyte layer was obtained from the peripheral blood of healthy adults. Monocytes were separated by centrifugation at 600 × *g* for 20 min at 22 °C using a 30–45–60% Percoll density gradient. The monocyte-enriched fraction was removed from the 30–45% gradient interface, washed and resuspended in complete media containing 10% heat inactivated FBS. 10^6 cells/mL were washed in serum-free medium and allowed to become quiescent for 30 min prior to the addition of Iso (Sigma–Aldrich, St. Louis, MO) and/or LPS (Sigma–Aldrich, St. Louis, MO).

2.1.3. Cytokine antibody arrays

A commercially available cytokine array system was used to simultaneously detect multiple inflammation-related factors generated by THP-1 cells according to the manufacturer's instructions (RayBiotech, Norcross, GA). Briefly, quiescent THP-1 cells were stimulated by LPS in the absence or presence of Iso for 2 h. Cells were gently pelleted and 1 mL of the supernatant used to cover the membrane containing the arrayed antibodies. After an overnight incubation at 4 °C, the membrane was washed and incubated at 22 °C for 2 h with biotin-conjugated antibodies. After washing, membranes were incubated at 22 °C for 60 min with diluted horseradish peroxidase-conjugated streptavidin. Bound antibody was visualized by digital photography (UVP, Upland, CA) using ECL imaging solutions from the manufacturer (RayBiotech, Norcross, GA).

2.1.4. Membrane preparation for receptor binding

Crude THP-1 cell membranes were prepared as previously described (Rojanathammanee et al., 2009). Briefly, suspended cells were transferred to a 50 mL conical tube and twice washed by centrifugation at 1000 × *g* using cold Hank's balance salt solution (HBSS). The intact cell pellet was resuspended in 10 mL of 0.25 M sucrose containing 10 μg/mL bacitracin, 10 μg/mL benzamidine, 10 μg/mL leupeptin, and 20 μg/mL phenylmethylsulfonylfluoride. The cells were disrupted by freezing followed by homogenization of the thawed suspension using 20 strokes from a loose fitting Dounce homogenizer (B) pestle. This mixture was then centrifuged at 1260 × *g* for 5 min at 4 °C. Buffer A (20 mM HEPES, pH 7.5, 1.4 mM