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HUMAN NEUTROPHILS AND HL-60 CELLS DO NOT POSSESS α_2-ADRENOCEPTORS

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Abstract—Human neutrophils have been reported to possess both α_2- and β_2-adrenoceptors. While activation of β_2-adrenoceptors is known to inhibit *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP)-induced superoxide anion (O_2^-) production, the functional role of α_2-adrenoceptors is not known. We studied the effects of a range of structurally unrelated α_2-adrenoceptor agonists on fMLP-induced O_2^- production and UTP-induced increases in cytosolic free calcium concentration ($[Ca^{2+}]_i$) in human neutrophils. No effect of α_2-adrenoceptor agonists was seen on either fMLP-induced O_2^- production or UTP-induced increases in $[Ca^{2+}]_i$. α_2-Adrenoceptor agonists by themselves had no effect on either O_2^- production or $[Ca^{2+}]_i$. We then studied a model for neutrophils, differentiated HL-60 cells and human erythroleukaemia (HEL) cells, a cell line known to possess α_2-adrenoceptors. While the α_2-adrenoceptor agonists 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline (UK 14304) and 5-allyl-2-amino-5,6,7,8-tetrahydro-4*H*-thiazolo-[4,5-*d*]azepin-dihydrochloride increased the $[Ca^{2+}]_i$ in HEL cells, they had no effect by themselves on either $[Ca^{2+}]_i$ or UTP-induced increases in $[Ca^{2+}]_i$ in differentiated HL-60 cells. Activation of high-affinity GTPase by UK 14304 was seen in membranes from HEL cells but not in membranes from differentiated HL-60 cells. Similarly, a selective α_2-adrenoceptor antagonist, [^{3}H]2-(2-methoxy-1,4-benzodioxan-2yl)-2 imidazoline, bound specifically and saturably to membranes from HEL cells, but not to membranes from HL-60 promyelocytes or differentiated HL-60 cells. Taken together, these data suggest that neither HL-60 promyelocytes nor differentiated HL-60 cells possess α_2-adrenoceptors, and that the lack of functional responses to α_2-adrenoceptor agonists in human neutrophils is due to the absence of α_2-adrenoceptors.

Human neutrophils and HL-60 leukaemic cells possess receptors for the chemotactic peptide *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP†) which activates a superoxide (O_2^-)-forming NADPH oxidase (EC 1.6.99.9) via G-proteins (for reviews see Refs 1–3). fMLP-stimulated O_2^- production is inhibited by activation of receptors linked to stimulation of adenylyl cyclase (EC 4.6.1.1; e.g. β_2-adrenoceptors, see Ref. 1). In contrast, the role of receptors linked to *inhibition* of adenylyl cyclase in modulating superoxide production is not known. Human neutrophils have been reported to have binding sites for α_2-adrenoceptors [4] and modulation of adenylyl cyclase activity by α_2-adrenergic drugs has also been reported in these cells [4, 5]. However, the effect of α_2-adrenergic agonists on fMLP-stimulated O_2^- production has not been reported. Therefore, we studied the effects of α_2-adrenoceptor agonists on fMLP-stimulated O_2^- production and its modulation by the α-adrenoceptor agonist isoprenaline. In addition, we studied a model of neutrophils, HL-60 promyelocytes and differentiated HL-60 cells (see Ref. 1), and a cell line known to have both α_2-adrenoceptors and functional responses to α_2-adrenoceptor activation, human erythroleukaemia (HEL) cells [6]. Furthermore, we studied the effects of α_2-adrenoceptor agonists on high affinity GTPase (EC 3.6.1.-) and binding of [^{3}H]RX821002, a specific α_2-adrenoceptor ligand, to membranes from HEL cells and HL-60 cells. We show here that, unlike in HEL cells, there is no evidence for the presence of α_2-adrenoceptors in human neutrophils and HL-60 cells.

MATERIALS AND METHODS

Materials. fMLP, UTP, ATP, adrenaline–HCl, isoprenaline–HCl, clonidine–HCl, oxymetazoline–HCl, phentolamine–HCl, yohimbine–HCl and thrombin were all obtained from Sigma Chemie (Deisenhofen, Germany). Fura-2 acetoxymethylester (Fura-2/AM) was purchased from Boehringer Mannheim (Mannheim, Germany). 5-Allyl-2-amino-5,6,7,8-tetrahydro-4*H*-thiazolo-[4,5-*d*]-azepin-dihydrochloride (BHT 920) was a kind gift of Boehringer Ingelheim (Ingelheim, Germany). 5-Bromo-6-(2-imidazolin-2-ylamino)-quinoxaline (UK 14304) was a kind gift of Pfizer (Sandwich, U.K.). [^{3}H]2-(2-Methoxy-1,4-benzodioxan-2yl)-2 imidazoline ([^{3}H]RX821002, 57 Ci/mmol) was obtained from Amersham (Braunschweig, Germany). All other reagents were of analytical grade or the best available commercial grade and obtained from standard commercial suppliers. Unless otherwise stated, substances were initially dissolved

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† Abbreviations: BHT 920, 5-allyl-2-amino-5,6,7,8-tetrahydro-4*H*-thiazolo-[4,5-*d*]azepin-dihydrochloride; $[Ca^{2+}]_i$, cytosolic free Ca^{2+} concentration; db-cAMP, dibutyryl cyclic AMP; DMSO, dimethyl sulphoxide; fMLP, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine; Fura-2/AM, Fura-2 acetoxymethylester; HEL cells, human erythroleukaemia cells; O_2^-, superoxide anion; RX821002, 2-(2-methoxy-1,4-benzodioxan-2yl)-2 imidazoline; UK 14304, 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline.

in deionized water and stock solutions were of 10 mM. Adrenaline was dissolved in 1 mM HCl, fMLP was dissolved in dimethyl sulphoxide (DMSO) and Fura-2/AM was dissolved in DMSO to give a 2 mM stock. Stock solutions were stored at −20°.

Preparation of human neutrophils. Heparinized blood was obtained by venipuncture from healthy volunteers of either sex who had taken no drugs for at least 3 weeks. Neutrophils were isolated by dextran sedimentation and centrifugation through Ficoll-hypaque [7]. Cell preparations contained more than 98% viable neutrophils as judged by Trypan blue dye exclusion and Pappenheim-stained smears.

Cell culture. HL-60 cells were grown in suspension culture in RPMI-1640 medium supplemented with 10% (v/v) horse serum, 1% (v/v) non-essential amino acids, 2 mM L-glutamine, 50 U of penicillin/mL and 50 μg of streptomycin/mL in a humidified atmosphere with 7% CO_2 at 37°. To induce differentiation, HL-60 cells were seeded at a density of 1×10^6/mL and were cultured for 48 hr in the presence of 0.2 mM dibutyryl cAMP (db-cAMP) or for 120 hr in the presence of 160 mM DMSO [8, 9]. HEL cells were grown identically, except that 10% (v/v) foetal calf serum was used instead of horse serum [10]. Cells were harvested by centrifugation for 10 min at 250 *g*. Cell preparations contained more than 90% viable cells as judged by Trypan blue exclusion.

O_2^- production. O_2^- production was monitored by continuous measurement of ferrycytochrome *c* reduction that was inhibitable by superoxide dismutase (EC 1.15.1.1), using an Uvikon 810 dual-beam spectrophotometer (Kontron, Eching, Germany). Reaction mixtures (0.5 mL) contained 1×10^6 neutrophils, 100 μM ferricytochrome *c* and a buffer consisting of 138 mM NaCl, 6 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM Na_2HPO_4, 5 mM $NaHCO_3$, 5.5 mM glucose and 20 mM HEPES (adjusted to pH 7.4 with NaOH). Reaction mixtures were preincubated at 37° for 3 min. O_2^- production was initiated by addition of fMLP. Test substances or their solvents were added 3 min before fMLP except for isoprenaline, which was added 15 sec before addition of fMLP, and in some experiments α_2-adrenoceptor agonists were substituted for fMLP. Maximal O_2^- production was calculated from the steady-state portions of the absorbance curves [7].

Measurement of $[Ca^{2+}]_i$. Cytosolic calcium concentration $[Ca^{2+}]_i$ was determined with the dye Fura-2/AM as described previously [10, 11]. Briefly, neutrophils, HL-60 or HEL cells were suspended at 1×10^7 cells/mL in a buffer consisting of 138 mM NaCl, 6 mM KCl, 1 mM $MgSO_4$, 1 mM Na_2HPO_4, 5 mM $NaHCO_3$, 5.5 mM glucose and 20 mM HEPES, pH 7.4, supplemented with 1% (w/v) bovine serum albumin. Fura-2/AM was added to give a final concentration of 4 μM and cells were incubated for 10 min at 37°. Thereafter, the cells were diluted to 5×10^6 cells/mL in the above buffer and incubated for a further 45 min. Following this incubation period the cells were diluted to 0.5×10^6 cells/mL and were centrifuged for 10 min at 250 *g* to remove extracellular dye. The cells were then resuspended in the above buffer at 1×10^6 cells/mL and kept at room temperature until measurement of $[Ca^{2+}]_i$. One millilitre of this suspension was added to acryl fluorescence cuvettes (Sarstedt, Nümbrecht, Germany), further diluted with 1 mL of the above buffer and $CaCl_2$ added to give a final concentration of 1 mM. Fluorescence was determined at 37° under constant stirring at 10^3 rpm, using a Ratio II spectrofluorometer (Aminco, Silver Spring, U.S.A.). Cells were incubated for 3 min at 37° prior to addition of stimuli. The excitation wavelength was 340 nm and the emission wavelength was 500 nm. Fluorescence signals were calibrated after lysis of the cells with 0.1% (w/v) Triton X-100 (maximal fluorescence) and subsequent addition of 20 mM EGTA (minimal fluorescence). Peak cytosolic Ca^{2+} was calculated according to equation 6 in Grynkiewcz *et al.* [12].

Preparation of membranes. HL-60 or HEL cells were homogenized by nitrogen cavitation and membranes were prepared as described in Ref. 13. Aliquots of cell membranes were thawed and centrifuged for 10 min at 30,000 *g* and 4° in 1.5 mL of 10 mM triethanolamine–HCl, pH 7.4. Membranes were suspended at either 0.2–0.4 (GTPase assay) or 1.5–2.0 (binding assay) mg/mL in the above buffer and immediately used for the respective experiments. Protein was determined by the method of Lowry *et al.* [14].

Measurement of high-affinity GTPase activity. [γ-^{32}P]GTP was synthesized according to the method of Johnson and Walseth [15]. High-affinity GTPase activity was measured according to the method of Seifert *et al.* [11] with minor modifications. The reaction mixtures (100 μL) contained membranes (5.0–7.0 μg protein/tube), 0.5 μM [γ-^{32}P]GTP (0.1 μCi/tube), 0.5 (HL-60 membranes) or 5 (HEL membranes) mM $MgCl_2$, 0.1 mM EGTA, 0.1 mM ATP, 1 mM adenosine 5′-[β,γ-imido]-triphosphate, 5 mM creatine phosphate, 40 μg of creatine kinase, 1 mM dithiothreitol and 0.2% (w/v) bovine serum albumin in 50 mM triethanolamine–HCl, pH 7.4. Preliminary experiments showed that the above $MgCl_2$ concentrations were optimal for studying stimulation of GTPase activity in HL-60 and HEL membranes. In addition, the reaction mixtures contained the various drugs and hormones at the final concentrations indicated in Results. The reaction mixtures without [γ-^{32}P]GTP were preincubated at 25° for 3 min, and the reaction was then started by addition of [γ-^{32}P]GTP. The reaction was terminated after 15 min by the addition of activated charcoal in 20 mM KH_2PO_4, pH 2.0. Low-affinity GTPase activity was determined in the presence of a high concentration of GTP (50 μM) and was subtracted from the total GTPase activity measured at 0.5 μM GTP. Low-affinity GTPase activity in HL-60 promyelocytes, db-cAMP- and DMSO-differentiated HL-60 cells was less than 5% of total GTPase activity and less than 20% of the total GTPase activity in HEL cells (data not shown).

Binding. Membranes were thawed and prepared as described above. The methoxy derivative of idazoxan, [^{3}H]RX821002, was used as the probe as it appears to bind to all described subtypes of α_2-adrenoceptors with high affinity and shows low non-specific binding [16–19], and appears to provide better resolution of low receptor numbers than [^{3}H]yohimbine [17, 18]. Total [^{3}H]RX821002 binding

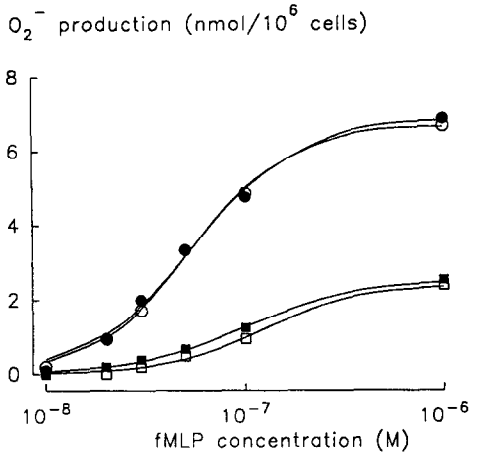

Fig. 1. Effects of BHT 920 and isoprenaline on fMLP-stimulated O_2^- production in human neutrophils. O_2^- production was initiated by the addition of fMLP at the concentrations indicated. BHT 920 was added 3 min before fMLP; isoprenaline was added 15 sec before fMLP. (○) fMLP alone; (□) fMLP in the presence of isoprenaline (0.01 μM); (●) fMLP in the presence of BHT 920 (10 μM); (■) fMLP in the presence of both BHT 920 and isoprenaline. The graph shows the results of a single experiment. The standard deviation for duplicates was generally less than 10% of the mean and has been omitted for clarity. Similar results were seen in at least four further experiments from different donors.

was determined by incubation of membranes (50–70 μg/tube) in a buffer containing 0.5 mM $MgCl_2$, 1 mM EDTA and 50 mM Tris–HCl, pH 7.4 (final volume 100 μL). Non-specific binding was determined in the presence of phentolamine (10 μM). The assay solution was preincubated at 25° for 3 min without [^{3}H]RX821002, and binding was initiated by addition of [^{3}H]RX821002 at the concentrations indicated in Fig. 3. After 30 min the assay mixtures were filtered through fiberglass filters (Whatman GF/B) presoaked with 1% (w/v) polyethylene imine (Fluka, Buchs, Switzerland). The filters were washed twice with 5 mL of ice-cold buffer consisting of 0.5 mM $MgCl_2$, 50 mM Tris–HCl, pH 7.4. The radioactivity trapped on the filters was determined by liquid scintillation spectrophotometry. The number of sites (B_{max}) and K_D were determined from the Scatchard plot and non-linear curve fitting of the saturation curve using the program Sigmaplot 4.0 (Jandel, U.S.A.).

RESULTS

Superoxide production

fMLP induced substantial and concentration-dependent production of O_2^- in human neutrophils (Fig. 1). The β-adrenoceptor agonist isoprenaline did not stimulate O_2^- production by itself, but significantly inhibited O_2^- production stimulated by 1 μM fMLP in a concentration-dependent manner with an IC_{50} of approximately 3 nM and a maximum of 30 nM (data not shown). The α_2-adrenoceptor agonist BHT 920 (10 μM) by itself did not stimulate O_2^- production (data not shown) and did not alter either fMLP-stimulated O_2^- production or the isoprenaline-induced inhibition of fMLP-stimulated O_2^- production (see Fig. 1). Similarly, BHT 920 (0.1–10 μM) did not reverse the inhibition of fMLP-stimulated O_2^- production induced by isoprenaline (3 and 10 nM, data not shown). Essentially similar results were obtained with the α_2-adrenoceptor agonists UK 14304, oxymetazoline and clonidine (all at 10 μM, data not shown).

$[Ca^{2+}]_i$

UTP (10 μM) induced consistent increases in $[Ca^{2+}]_i$ in HEL cells, human neutrophils. HL-60 promyelocytes and DMSO-differentiated HL-60 cells (Fig. 2). UK 14304 (10 μM) produced a substantial rise in $[Ca^{2+}]_i$ in HEL cells (see Fig. 2). The effect of UK 14304 was inhibited by the selective α_2-adrenoceptor antagonist yohimbine (10 μM, see Fig. 2). In contrast, in human neutrophils, HL-60 promyelocytes and DMSO-differentiated HL-60 cells, UK 14304 (10 μM) was ineffective (see Fig. 2). Similar results were seen with BHT 920 (10 μM) and adrenaline (1 μM) in the presence of propranolol (10 μM) (data not shown). BHT 920 (10 μM) and UK 14304 (10 μM) did not affect the UTP-induced increases in $[Ca^{2+}]_i$ in neutrophils and DMSO-differentiated HL-60 cells (data not shown).

GTPase activity

Table 1 shows the effects of various substances on high-affinity GTPase, the enzymic activity of α-subunits of G-proteins, in membranes from db-cAMP- and DMSO-differentiated HL-60 cells and HEL cells. High-affinity GTPase activity was significantly increased by fMLP in membranes from differentiated HL-60 cells and by thrombin in the membranes from HEL cells (Table 1). High-affinity GTPase activity in HEL membranes was also clearly activated by UK 14304 (see Table 1). This effect of UK 14304 was blocked by yohimbine (1 μM; data not shown). In contrast, UK 14304 had no effect on high-affinity GTPase activity in membranes from differentiated HL-60 cells (see Table 1).

Binding

Membranes from HEL cells showed saturable, specific high-affinity binding of [^{3}H]RX821002, with a K_D of 0.5 ± 0.1 nM and a B_{max} of 123 ± 4 fmol/mg protein (Fig. 3) which appear to correspond to the α_{2A}-adrenoceptor subtype [20]. Membranes from undifferentiated and differentiated HL-60 cells showed no saturable, specific high-affinity binding (see Fig. 3).

DISCUSSION

The aim of this study was to determine the functional role of α_2-adrenoceptors in human myeloid cells. Neutrophils have been reported to have α_2-adrenergic binding sites [4]. α_2-Adrenoceptor blockers have also been described as enhancing accumulation of cyclic AMP in response to noradrenaline [4]. Furthermore, α_2-adrenoceptor stimulation has been reported to inhibit adenylyl

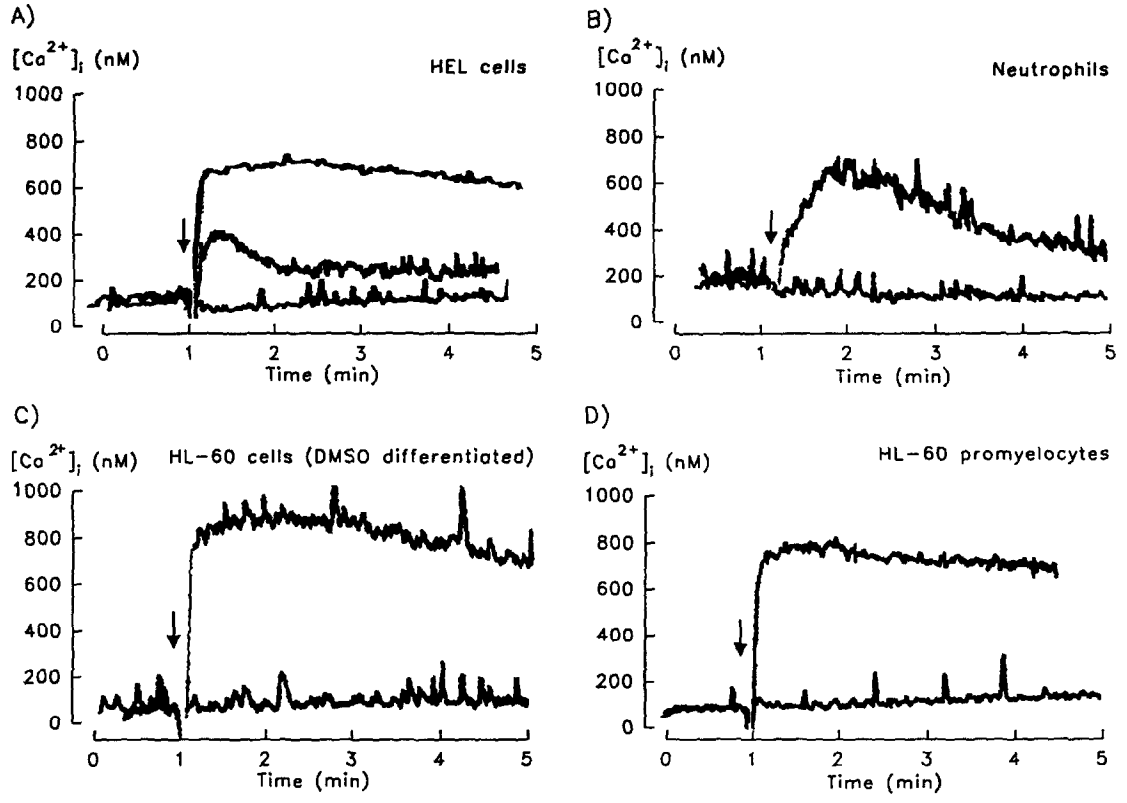

Fig. 2. The effect of the α_2-adrenoceptor agonist UK 14304 on $[Ca^{2+}]_i$ in HEL cells, neutrophils and HL-60 cells. In all panels the upper trace represents the effect of UTP (10 μM) on $[Ca^{2+}]_i$ in cells loaded with Fura-2/AM. In panel A the middle trace shows the effect of UK 14304 (10 μM) on $[Ca^{2+}]_i$ in HEL cells. The lower trace shows the effect of UK 14304 in the presence of yohimbine (10 μM). In panels B, C and D the lower trace shows the lack of effect of UK 14304 (10 μM) on $[Ca^{2+}]_i$ in human neutrophils, DMSO-differentiated HL-60 cells and HL-60 promyelocytes, respectively. Stimuli were added at the point indicated by an arrow. Experiments were performed in duplicate and similar results were seen in at least three further independent experiments.

Table 1. The effect of the α_2-adrenoceptor agonist UK 14304 on high affinity GTPase in membranes from HEL cells or HL-60 cells differentiated with DMSO or db-cAMP

	High affinity GTPase activity (pmol P_i/mg protein/min)		
		HL-60 cells differentiated with:	
	HEL cells	DMSO	db-cAMP
Control	19 ± 3	22 ± 4	15 ± 6
Thrombin	30 ± 7* (61%)	ND	ND
fMLP	ND	32 ± 3* (49%)	23 ± 9* (46%)
UK 14304	23 ± 3* (23%)	23 ± 5 (6%)	15 ± 6 (0%)

GTPase activity was determined by measurement of γ-$^{32}P_i$ released from [γ-^{32}P]-GTP as described in Materials and Methods. Thrombin (1 U/mL), fMLP (1 μM) and UK 14304 (10 μM) were present where indicated. Stimuli were added 3 min before the reaction was initiated by addition of [γ-^{32}P]GTP.

ND indicates that the experiment was not performed.

Values represent the means ± SD of two to three experiments on separate membrane preparations. Determinations in each experiment were performed in quadruplicate. Values in brackets represent the percentage increase over basal GTPase activity.

* Represents a significant difference from basal values ($P > 0.05$, Wilcoxon test).

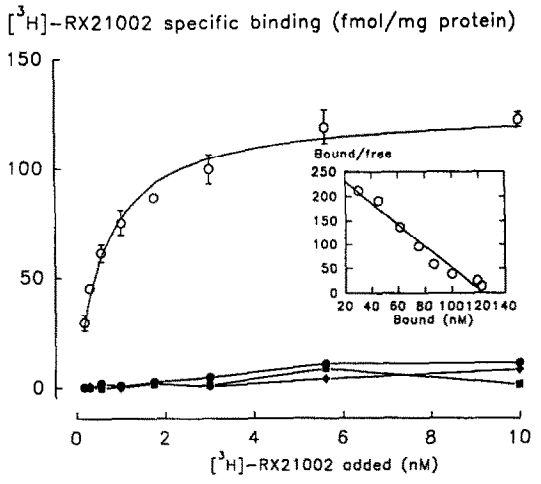

Fig. 3. Specific binding of [^{3}H]RX821002 to membranes of HEL and HL-60 cells. Binding of [^{3}H]RX821002 to membranes of HEL and HL-60 cells was performed as described in Materials and Methods with [^{3}H]RX821002 at the concentrations indicated. Non-specific binding was determined with phentolamine (10 μM). Each point represents the average of two to three experiments on separate membrane preparations. Each experiment was performed in triplicate. Membranes from HEL cells (○) showed specific, saturable, high-affinity binding (Scatchard plot inset); non-specific binding at saturation was 19 ± 5% of total. Membranes from HL-60 promyelocytes (●), or HL-60 cells differentiated with db-cAMP (■) or DMSO (◆) showed no saturable high affinity binding. SD bars are omitted from the HL-60 curves for clarity.

cyclase in neutrophil membranes and reduce cyclic AMP accumulation in intact neutrophils [5]. These reports suggest that human neutrophils contain functional α_2-adrenoceptors. Therefore, activation of α_2-adrenoceptors on human neutrophils would be expected to attenuate the effects of β-adrenoceptor agonists, which inhibit fMLP-stimulated O_2^- production, presumably through activation of adenylyl cyclase (see Ref 1). However, in the present study, the selective, potent and structurally unrelated α_2-adrenoceptor agonists BHT 920 and UK 14304 failed to attenuate the inhibitory effects of isoprenaline (see Fig. 1). Furthermore, both BHT 920 and UK 14304 had no effect on fMLP-stimulated O_2^- production alone. The partial agonists clonidine and oxymetazoline (α_{2A}-adrenoceptor selective) also had no effect on either fMLP-stimulated O_2^- production or the inhibition by isoprenaline of fMLP-stimulated O_2^- production. Thus, over a range of structural classes and potencies of α_2-adrenoceptor agonists, no effects on O_2^- production were seen. A possible interpretation of the results of the present study is that α_2-adrenoceptors are compartmentalized in intact cells, so that they cannot interact with G_i to inhibit adenylyl cyclase activated by β-adrenoceptors. Therefore, we sought a more direct functional correlate of α_2-adrenoceptor activation.

In many cell types α_2-adrenoceptors modulate $[Ca^{2+}]_i$. In some cell types, such as HEL cells and smooth muscle cells, α_2-adrenoceptor activation results in an increase in $[Ca^{2+}]_i$ [6, 21, 22]. In platelets α_2-adrenoceptor activation results in potentiation of $[Ca^{2+}]_i$ increases produced by other stimuli [23]. We studied neutrophils and a widely used model of neutrophils, differentiated HL-60 cells. Differentiated HL-60 cells express many of the receptors found in neutrophils, including β-adrenoceptors [24], express similar G-proteins (G_{i2}, G_{i3} and G_s [25]), and the receptors are functionally coupled to G-proteins and effectors [1]. Thus, if neutrophils express α_2-adrenoceptors, it might be reasonably assumed that HL-60 cells would also express α_2-adrenoceptors. We also studied HEL cells, a cell line known to possess α_2-adrenoceptors [21], activation of which increases $[Ca^{2+}]_i$ [6] as a positive control. As found for O_2^- production, a range of α_2-adrenoceptor agonists, belonging to structurally unrelated classes, was ineffective in either producing an increase in $[Ca^{2+}]_i$ or potentiating the effect of UTP in human neutrophils or differentiated HL-60 cells (Fig. 2 and unpublished data). However, UK 14304, adrenaline and BHT 920 produced clear effects in HEL cells with UK 14034 and adrenaline giving increases in $[Ca^{2+}]_i$ similar to those reported by Michel *et al.* [6] (Fig. 2 and unpublished data).

We then sought further evidence that α_2-adrenoceptors functionally coupled to G-proteins are present in neutrophils of HL-60 cells. Activation of receptors linked to G-proteins results in stimulation of the high-affinity GTPase associated with the α-subunits (see Refs 26, 27). Thus, if α_2-adrenoceptors were being activated, we should observe an increase in high-affinity GTPase activity. For these experiments and the binding experiments (see below) we used only membranes from the HL-60 myeloid cell line and HEL cells as there is no practical way to exclude completely the presence of platelets in neutrophil preparations. Platelets have a high number of α_2-adrenoceptors [16], and the presence of even a small proportion of their membranes could produce a false positive result.

UK 14304 produced a significant increase in GTPase activity in membranes from HEL cells (see Table 1). However, UK 14304 did not produce a significant increase in high-affinity GTPase activity in membranes from differentiated HL-60 cells (see Table 1). Receptor-mediated activation of high-affinity GTPase could be demonstrated in membranes from the differentiated HL-60 cells, as shown by the response to fMLP (see Table 1). Thus, we have no evidence for functional coupling of α_2-adrenoceptors to G-proteins in HL-60 cells.

The above results raised the question of whether HL-60 cells actually have α_2-adrenoceptor binding sites. Therefore, we studied the binding of [^{3}H]-RX821002, an α_2-adrenoceptor antagonist reported to have superior properties for detecting α_2-adrenoceptors [16, 17], to membranes from HEL and HL-60 cells. Membranes from HEL cells demonstrated specific, saturable, high-affinity binding of [^{3}H]RX821002, consistent with the increases in GTPase activity and increases in $[Ca^{2+}]_i$ (see Fig. 3). The B_{max} and K_D values for binding of [^{3}H]-RX821002 to HEL membranes in the present study are consistent with previously published data [6, 16].

In contrast, neither membranes from the HL-60 promyelocytes nor from the differentiated HL-60 cells demonstrated saturable high-affinity binding (see Fig. 3).

Taken together the results of the present study suggest that HL-60 promyelocytes and differentiated HL-60 cells do not possess α_2-adrenoceptors. This also suggests that the lack of effect of α_2-adrenoceptor agonists on O_2^- production in neutrophils in the present study is therefore due to a lack of α_2-adrenoceptors. While it might appear unusual for β-adrenoceptor stimulation to be unopposed by α_2-adrenoceptors, there are other examples of cells which show β-adrenoceptor-mediated stimulation of adenylyl cyclase without concomitant α_2-adrenoceptor-mediated inhibition (e.g. cardiac myocytes, see reviews in Refs 22, 28).

The question arises as to the reason for the discrepancy between the results in the present study on intact neutrophils and the results of Panosian and Marinetti [4] and Verghese *et al.* [5] in neutrophil membranes. Differences between the binding characteristics of [^{3}H]RX821002 and [^{3}H]yohimbine are unlikely to explain this, if anything [^{3}H]RX821002 should be a more sensitive ligand that [^{3}H]yohimbine [16–19]. A possibility is that in both membrane and whole cell preparations the presence of platelet membranes and platelets, respectively, could be responsible. Panosian and Marinetti [4] reported that their neutrophil preparations had typically less than two platelets per neutrophil. As platelets can have a B_{max} of 168 fmol/mg protein as determined by [^{3}H]yohimbine binding [16], this number of cells would be a significant source of binding sites in any membrane preparation. The B_{max} of 5 fmol/mg protein that Panosian and Marinetti [4] found with [^{3}H]yohimbine in neutrophil membranes could well be due to uncorrected platelet contamination.

A similar argument applies to the modulation of cyclic AMP levels reported in preparations of intact neutrophils [4] and adenylyl cyclase activity in membranes [5], as platelets have an adenylyl cyclase which is 10–20-fold more active than that of neutrophils [29], and which shows substantial inhibition by the platelet α_2-adrenoceptors [29]. Thus, a significant proportion of the observed adenylyl cyclase activity (and its modulation) could be due to platelets. Furthermore, Bokoch [29] using highly purified neutrophil membranes, found α_2-adrenoceptor agonists did not inhibit adenylyl cyclase. In contrast to measurements of adenylyl cyclase activity or cAMP content, measurement of O_2^- production is free from interference from residual platelets, as platelets do not produce O_2^- in response to fMLP.

In conclusion, using various experimental approaches to rule out the possible effect of platelet contamination, we could not find any evidence that neutrophils have functional α_2-adrenoceptors. In the light of our findings the reported presence and effects of these receptors are most likely due to the almost unavoidable contamination by platelets in neutrophil preparations.

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