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Short communication

G protein-coupled receptor for nicotinic acid in mouse macrophages

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Abstract

The use of the HDL-elevating drug nicotinic acid in the treatment and prevention of atherosclerotic disease is limited by the frequent induction of skin flushing. The therapeutic effects of nicotinic acid are attributed to inhibition of lipolysis in adipose tissue via a G protein-coupled receptor, whereas the mechanism of flush induction by release of prostaglandin D_2 from macrophages is not understood. In this study, we investigated if macrophages contain nicotinic acid receptors. Specific guanine nucleotide sensitive binding sites for [^{3}H]nicotinic acid were detected in membranes from mouse RAW 264.7 macrophages. Nicotinic acid and related heterocycles stimulated activation of pertussis toxin-sensitive G proteins. The rank orders of potency in macrophage membranes were identical for inhibition of [^{3}H]nicotinic acid binding and G protein activation, and were pharmacologically indistinguishable from that of the G protein-coupled nicotinic acid receptor in spleen membranes. These results indicate that the effects of nicotinic acid on macrophages, spleen and probably adipocytes are mediated via an identical, unique G protein-coupled receptor.

Keywords: Nicotinic acid; Macrophage; RAW 264.7; G protein-coupled receptor; GTPγS; Acipimox

1. Introduction

The ability of the hypolipidemic drug nicotinic acid to elevate high density lipoprotein cholesterol to a greater extent than other drugs is of particular interest in the treatment of atherosclerotic diseases [1]. Nicotinic acid and its metabolically stable derivative acipimox inhibit adenylate cyclase in adipocytes through a pertussis toxin-sensitive G protein [2,3], which leads to decreased phosphorylation of hormone-sensitive lipase and inhibition of its activity. Skin flushing is a common side effect of nicotinic acid. Flushing is prevented or attenuated by cyclooxygenase inhibitors, which, however, do not affect the lipid-lowering effect of nicotinic acid [4]. This vasodilation has been attributed to the release of prostaglandin D_2 in skin [5]. Since the prostaglandin D_2 release is not accompanied by a release of histamine, it is assumed that the cell type which releases the prostaglandin is not the mast cell [5]. The formation of prostaglandin D_2 has been attributed largely to a glutathione-requiring prostaglandin D synthase localized mainly in antigen-presenting cells [6]. However, there is no direct evidence for the presence of nicotinic acid receptors in these cells in skin. Since the activation of nicotinic acid receptors in skin macrophages might be the reason for the release of prostaglandin D_2, the present study was set up to investigate if macrophages express G protein-coupled receptors for nicotinic acid, which have been characterized previously in rat adipocyte and spleen membranes [7], or if a nicotinic acid binding site distinct from this receptor is detectable.

2. Materials and methods

RAW 264.7 mouse macrophages (European Collection of Cell Cultures) were cultured in Dulbecco's modified Eagle's medium (DMEM) with 2 mM glutamine, 100 U/mL penicillin, 200 μg/mL streptomycin and 10% fetal bovine serum (FBS) in 175 cm^2 flasks at 37° in the presence of 5% CO_2.

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Abbreviations: Acipimox, 5-methylpyrazine-2-carboxylic acid-1-oxide; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate; DPBS, Dulbecco's phosphate buffered saline; GDPβS, guanosine-5′-*O*-(2-thio)diphosphate; GTPγS, guanosine-5′-*O*-(3-thio)triphosphate.

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Confluent cultures were split 1:15 every 3–4 days. Incubations with pertussis toxin (50 ng/mL) were performed for 20 hr. Membranes from adherent and nonadherent cells were prepared separately after mechanical removal of adherent cells and removal of medium by centrifugation (1 min at 1,000 *g*) and washing in Dulbecco's phosphate buffered saline (DPBS) without Ca^{2+} and Mg^{2+} ions. DPBS was discarded. All further steps were performed at 0–4°. Cells were homogenized in 0.32 mol/L sucrose with a motor-driven glass-Teflon pestle. Further steps were performed as described for rat organs [7]. Protein concentration was determined according to Peterson [8], using bovine serum albumin (BSA) as standard.

[^{3}H]Nicotinic acid binding was performed as described [7] with 100 μg membrane protein in the presence of 1 mmol/L $MgCl_2$, 200 U/mL penicillin G and 200 μg/mL streptomycin. K_d, K_i and B_{max} values were calculated by nonlinear curve fitting using SCTFIT [9]. [^{35}S]GTPγS binding was determined using 3 μg membrane protein/tube. Incubations (100 μL) contained 50 mmol/L Tris–HCl pH 7.4, 1 mmol/L EDTA, 5 mmol/L $MgCl_2$, 1 mmol/L dithiothreitol, 10 μmol/L GDP, 0.5 U/mL adenosine deaminase, 0.0225% Triton-X114, 0.5% BSA, 50,000 cpm (0.2 nmol/L) [^{35}S]GTPγS and were performed for 90 min at 25°. Incubations were terminated by filtration through GF/B glass fiber filters, followed by two washes with 4 mL 50 mmol/L Tris–HCl pH 7.4, 5 mmol/L $MgCl_2$, 0.02% 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate (CHAPS). EC_{50} values for G protein activation were calculated by fitting the data to sigmoid dose–response curves with SigmaPlot. K_d, K_i and EC_{50} values are given as geometric means with 95% confidence limits. B_{max} values are arithmetic means ± SEM. All data are from at least three independent experiments.

Potassium pyrazole-3-carboxylate was synthesized by addition of 5 mL of KOH (2 mol/L) to the solution of pyrazole-3-carboxylic acid ethyl ester [10] (0.42 g, 3 mmol) in methanol (2 mL). The mixture was stirred for 6 hr at ambient temperature. Solvents were removed under reduced pressure and ethanol (4 mL) was added. The precipitate was filtered off and washed with ethanol. Impurities were less than 1%, as determined by HPLC (C_{18} column, 3.9 mm × 150 mm, mobile phase: 5% acetonitrile in H_2O with 0.1% H_3PO_4).

3. Results and discussion

Membranes from adherent and nonadherent RAW 264.7 macrophages displayed specific [^{3}H]nicotinic acid binding. The affinity of [^{3}H]nicotinic acid measured in saturation experiments was identical to that previously found for the nicotinic acid receptor in rat adipocyte and spleen membranes [7] (Table 1). The density of [^{3}H]nicotinic acid binding sites in mouse macrophage membranes was considerably lower than in membranes from adipocytes or spleen (Table 1). Competition experiments with pyridazine-4-carboxylic acid, acipimox and potassium pyrazole-3-carboxylate revealed an almost identical pharmacological profile of the [^{3}H]nicotinic acid binding site in mouse macrophage membranes and the nicotinic acid receptor in rat spleen membranes (Table 1). Binding of [^{3}H]nicotinic acid to macrophage membranes was inhibited by increasing concentrations of guanine nucleotides, GTPγS being more potent than guanosine-5′-*O*-(2-thio)diphosphate (GDPβS, Fig. 1). Pretreatment of the cells with pertussis toxin reduced specific [^{3}H]nicotinic acid binding to 20.8 ± 3.8% of control binding (not shown). Since the pharmacological profile, the inhibition of [^{3}H]nicotinic acid binding by guanine nucleotides and the pertussis toxin sensitivity suggested that the [^{3}H]nicotinic acid binding site in macrophage membranes was indeed the G protein-coupled nicotinic acid receptor, we investigated if nicotinic acid and related heterocycles induced activation of G proteins in macrophage membranes. Nonstimulated binding of [^{35}S]GTPγS to membranes from adherent RAW macrophages amounted to 2305 ± 435 cpm/3 μg protein and was increased by maximally stimulating concentrations

Table 1
Ligand binding to nicotinic acid receptors

Compound	RAW macrophages		Adipocytes[a]	Spleen[a]
	Adherent	Nonadherent		
[^{3}H]Nicotinic acid				
K_d (nmol/L)	21.4 (17.2–26.6)	22.9 (13.1–39.9)	43.5 (39.5–48.0)	22.8 (18.6–27.9)
B_{max} (fmol/mg)	258 ± 17	284 ± 63	1518 ± 115	1078 ± 145
Pyridazine-4-carboxylic acid				
K_i (nmol/L)	130 (54.8–310)	132 (71.2–244)	ND[b]	121 (81.7–179)
Acipimox				
K_i (nmol/L)	235 (160–345)	227 (91.4–564)	ND[b]	309 (263–362)
K^+ pyrazole-3-carboxylate				
K_i (nmol/L)	1230 (670–2170)	1010 (930–1100)	ND[b]	594 (522–675)

[a] Data from Ref. [7].
[b] ND, not determined.

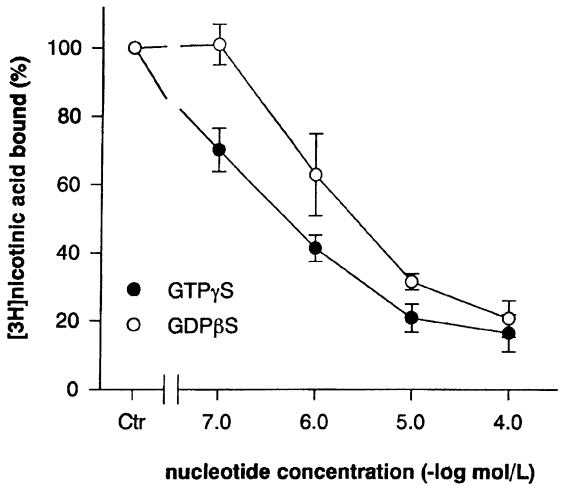

Fig. 1. Guanine nucleotide inhibition of [^{3}H]nicotinic acid binding to macrophage membranes. [^{3}H]Nicotinic acid (20 nmol/L) was incubated with membranes from adherent macrophages (100 μg) in the absence or presence of increasing concentrations of GTPγS or GDPβS. Data are means ± SEM from three independent experiments. Similar results were obtained with membranes from nonadherent cells (not shown).

of nicotinic acid (100 μM) to 206 ± 12% of the basal level. The rank order of potency of agonists in G protein activation was identical to the rank order in inhibition of [^{3}H]nicotinic acid binding (Fig. 2; nicotinic acid > pyridazine-4-carboxylic acid > acipimox > potassium pyrazole-3-carboxylate). Nicotinamide at 1 mM induced only 15% of the stimulation achieved with nicotinic acid. The potency and efficacy of these agonists were identical in membranes from adherent and nonadherent RAW macrophages (not shown). The stimulation of [^{35}S]GTPγS binding by nicotinic acid was completely prevented by pretreatment of the macrophages with pertussis toxin, indicating that G proteins of the G_i/G_o subtype are activated by the nicotinic acid receptor in these cells.

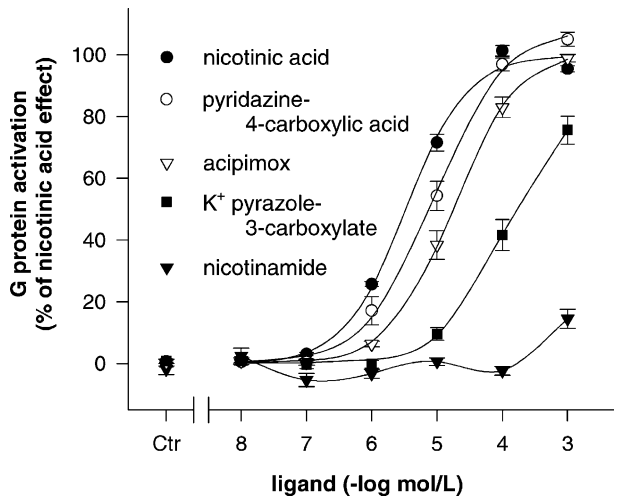

Fig. 2. Nicotinic acid receptor-mediated G protein activation in membranes from macrophages. [^{35}S]GTPγS binding to membranes from adherent cells was stimulated by nicotinic acid (EC_{50} 2.55 μmol/L (1.33–4.91 μmol/L)), pyridazine-4-carboxylic acid (7.23 μmol/L (3.66–14.3 μmol/L)), acipimox (14.9 μmol/L (9.16–24.4 μmol/L)), potassium pyrazole-3-carboxylate (91.5 μmol/L (66.9–125 μmol/L)) and nicotinamide (>1 mmol/L). Results are means ± SEM from three independent experiments. Similar results were found in [^{35}S]GTPγS binding experiments with membranes from nonadherent macrophages (not shown).

In summary, our results for the first time provide direct evidence for the presence of nicotinic acid receptors coupled to pertussis toxin-sensitive G proteins in macrophages. These receptors may be responsible for the release of prostaglandin D_2 from skin macrophages, which causes skin flushing, the most frequently observed adverse effect of nicotinic acid when used as an antihyperlipidemic drug. On the other hand, it is unknown whether these receptors in macrophages are possibly also involved in the beneficial effects of nicotinic acid. The relevant role of macrophage activation in the pathogenesis of atherosclerotic disease is well established. Hormone-sensitive lipase, which is regulated by cellular cAMP levels via phosphorylation by protein kinase A, is present not only in adipocytes, but also in macrophages. In macrophages and other cell types, it plays a key role in cholesterol metabolism, since it also acts as a neutral cholesterol ester hydrolase [11]. Therefore, nicotinic acid may act on macrophages to release prostaglandin D_2 as well as a local regulator of cholesterol ester hydrolysis. Based on the pharmacological profile obtained, we have no evidence for a heterogeneity of nicotinic acid receptors in macrophages, since only one binding site for nicotinic acid was detectable. There were also no differences between the pharmacological profiles of this receptor in spleen and macrophage membranes. Based on G protein activation studies, the nicotinic acid receptors in spleen and adipocyte membranes displayed identical pharmacological profiles [7]. The EC_{50} values obtained for the nicotinic acid receptor in macrophage membranes in the present study are in good agreement with the results obtained for this receptor in adipocyte membranes [7]. Therefore it seems presently improbable to pharmacologically target the antilipolytic effects with adipocyte-selective drugs, which would avoid the induction of skin flushing.

It has to be kept in mind, however, that the present results refer to a receptor expressed in a mouse cell line. In future studies, it will be necessary to investigate if nicotinic acid receptors are also present in primary human macrophages. In addition, direct evidence for the physiological role of these receptors in human macrophages is required, e.g. in the release of prostaglandin D_2 or, potentially, in the regulation of the activity of neutral cholesterol ester hydrolase.

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