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Enhancement of arachidonic acid signaling pathway by nicotinic acid receptor HM74A

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Abstract

HM74A is a G protein-coupled receptor for nicotinic acid (niacin), which has been used clinically to treat dyslipidemia for decades. The molecular mechanisms whereby niacin exerts its pleiotropic effects on lipid metabolism remain largely unknown. In addition, the most common side effect in niacin therapy is skin flushing that is caused by prostaglandin release, suggesting that the phospholipase A_2 (PLA_2)/arachidonic acid (AA) pathway is involved. Various eicosanoids have been shown to activate peroxisome-proliferator activated receptors (PPAR) that play a diverse array of roles in lipid metabolism. To further elucidate the potential roles of HM74A in mediating the therapeutic effects and/or side effects of niacin, we sought to explore the signaling events upon HM74A activation. Here we demonstrated that HM74A synergistically enhanced UTP- and bradykinin-mediated AA release in a pertussis toxin-sensitive manner in A431 cells. Activation of HM74A also led to Ca^{2+}-mobilization and enhanced bradykinin-promoted Ca^{2+}-mobilization through Gi protein. While HM74A increased ERK1/2 activation by the bradykinin receptor, it had no effects on UTP-promoted ERK1/2 activation.Furthermore, UTP- and bradykinin-mediated AA release was significantly decreased in the presence of both MAPK kinase inhibitor PD 098059 and PKC inhibitor GF 109203X. However, the synergistic effects of HM74A were not dramatically affected by co-treatment with both inhibitors, indicating the cross-talk occurred at the receptor level. Finally, stimulation of A431 cells transiently transfected with PPRE-luciferase with AA significantly induced luciferase activity, mimicking the effects of PPARγ agonist rosiglitazone, suggesting that alteration of AA signaling pathway can regulate gene expression via endogenous PPARs.

Keywords: G protein-coupled receptor (GPCR); Nicotinic acid (niacin); HM74A; Arachidonic acid (AA); Extracellular-signal-regulated kinases 1 and 2 (ERK1/2); Peroxisome-proliferator activated receptors (PPAR)

Nicotinic acid (niacin), a member of the water-soluble vitamin B complex, has been used as a lipid-lowering drug for several decades [1]. The pharmacological effects of nicotinic acid include increasing high density lipoprotein (HDL) and decreasing very low and low density lipoprotein (VLDL and LDL) and lipoprotein Lp(a) and triglycerides [2–4]. The primary action of nicotinic acid was shown to inhibit lipolysis in adipose tissue [5]. Recently, niacin was shown to bind G protein-coupled receptor human HM74A and its mouse homologue PUMA-G with high affinity ($K_d = 60$–90 nM) and act as an agonist for both receptors [6–10]. HM74A expression is restricted to adipose tissue and macrophages [6,8,9]. Upon niacin activation, HM74A couples to inhibitory G protein and decreases adenylate cyclase activity [8,11]. In adipocytes, decrease in cAMP level will lead to inhibition of hormone-sensitive lipase that in turn results in decreased liberation of free fatty acid [11]. However, the underlying molecular mechanisms for niacin/HM74A actions remain largely unknown. Moreover, the most common side effect of niacin treatment is skin

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flushing. The release of prostaglandin D_2 in skin was believed to be the cause [12,13]. Cyclooxygenase inhibitors such as acetylsalicylic acid can attenuate skin flushing caused by niacin and its analogue acipimox [14,15], indicating that the phospholipase A_2 (PLA_2)/arachidonic acid (AA) pathway is involved. During preparation of this manuscript, Offermanns and co-workers demonstrated that PUMA-G/HM74A knock-out mice were in lack of niacin-induced skin flushing, a phenomenon involving prostaglandin D_2 and E_2 receptors [16]. Moreover, various eicosanoids and related molecules including PGA1 and 2, PGD1 and 2, PGJ2, and hydroxyeicosatetraenoic acids (HETEs) have been shown to activate peroxisome-proliferator activated receptors (PPARα, γ, and δ) [17,18]. Moreover, 15-deoxy- $\Delta^{12,14}$-prostaglandin (15d-PGJ_2), the major metabolite of PGD_2, was identified as the most potent endogenous PPARγ ligand and promoted adipocyte differentiation [19]. Therefore, niacin, possibly through HM74A, may alter lipid and lipoprotein metabolism by activating nuclear receptors such as PPARs [20]. It has been well established that upon activation certain GPCRs stimulate cytosolic Ca^{2+}-dependent phospholipase A_2 ($cPLA_2$) that catalyzes liberation of arachidonic acid from membrane phospholipids. AA is the precursor for various eicosanoids including prostaglandins, thromboxanes, and leukotrienes that serve a broad spectrum of biological functions upon activation of their cognate receptors. Therefore, we aimed to investigate the possible effects of HM74A on arachidonic acid pathway. We previously characterized HM74A expression in a human epidermoid carcinoma cell line, A431 [21]. It was reported previously that PPARγ was expressed in A431 cells and mediated the profound effects of ultraviolet B radiation on epidermal cells [22]. Therefore we utilized A431 cells to examine the potential HM74A-mediated effects on arachidonic acid production. We also investigated another important signaling aspect of many GPCRs, ERK1/2 activation, upon HM74A activation in A431 cells. Finally, we studied AA-induced reporter gene expression under the control of PPAR-response element. These data provided new evidence for understanding the molecular mechanisms underlying the clinical effects of niacin.

Materials and methods

Cell culture and chemicals. A431 cells were obtained from ATCC (CRL-1555) at passage 28. They were grown in Dulbecco's modified Eagle's media (Invitrogen Inc., Carlsbad, CA) supplemented with 10% (v/v) fetal bovine serum (Invitrogen Inc., Carlsbad, CA). PD 098059, GF 109203X, UTP, ATP, bradykinin, nicotinic acid, PMA (Phorbol 12-myristate 13-acetate), arachidonic acid, and ionomycin (Ca^{2+} ionophore) were all obtained from Sigma (St. Louis, MO). [^{3}H]Arachidonic acid (NET-298Z, 200 Ci/mmol) was obtained from Perkin-Elmer (Shelton, CT). Human recombinant epidermal growth factor (EGF) was obtained from R&D systems (Minneapolis, MN).

Measurement of arachidonic acid release. A431 cells were seeded at 400 K/well in 24-well tissue culture plates. The next day, the cells were incubated with 0.25 μCi [^{3}H]arachidonic acid in 0.5 mL growth medium overnight. Before assay, the incubation medium was removed from cells and cells were extensively washed with assay medium (DMEM + 0.1% essential fatty acid free BSA). The cells were then stimulated with various stimuli in assay medium for 40 min and all cell culture medium was collected for counting [^{3}H] on Tri-carb scintillation counter (Perkin-Elmer, Shelton, CT). For MAPK kinase and PKC inhibitor assay, the cells were pretreated with 30 μM PD98059 or 10 μM GF 109203X or both for 30 min and then stimulated with various stimuli.

Measurement of intracellular calcium mobilization. Intracellular calcium mobilization was measured in A431 cells grown in black clear-bottom 96-well plates. The cells were grown to confluency and then loaded with the calcium-sensitive fluorescent dye fluo-3 AM (Molecular Probes, Inc., Eugene, OR, USA) in assay buffer (25 mmol/L Hepes, 125 mmol/L NaCl, 1 g/L glucose, 0.1% BSA, 5 mmol/L KCl, 0.5 mmol/L $CaCl_2$, and 0.5 mmol/L $MgCl_2$, pH 7.45). Changes in ligand-induced calcium-dependent intracellular fluorescence were measured with a fluorometric plate reader (FDSS; Hamamatsu Corp., Bridgewater, NJ, USA). In the FDSS protocol, fluorescence was measured continuously upon treatment with stimuli. For PTX treatment, cells were pretreated with 50 ng/mL PTX overnight prior to the calcium mobilization assay.

Measurement of ERK1/2 activation. A431 cells were seeded at 40 K/well in 96-well plates. The next day, the cells were treated with serum-free DMEM overnight. The cells were then treated with various reagents as stated in figure legends for 10 min prior to cell lysis. Activated ERK1/2 was measured with Surefire™ Cellular ERK kinase assay kit basically following the manufacturer's instructions (Perkin-Elmer, Shelton, CT). The alpha screen signal was measured on Envision (Perkin-Elmer, Shelton, CT).

Transient transfection and measurement of luciferase activity. A431 cells were seeded in T-75 flasks one day before transfection. On the day of transfection, cells usually reached 50–70% confluency and were transfected with 15 μg PPRE-Luciferase plasmid (the putative PPRE in the COX-2 promoter as well as a consensus PPRE for the rat acyl-CoA oxidase gene cloned in pGL3-basic plasmid, a gift from Dr. Jeffrey Travers, Indiana University School of Medicine) [22] and 90 μL FuGENE 6 reagent basically following the manufacturer's instructions (Roche Diagnostics, Indianapolis, IN). Twenty-four hours after transfection, cells from T-75 flask were collected and seeded into 24-well plate and incubation was continued for another 24 h. Cells were subsequently stimulated with various stimuli for 24 h in the presence of 0.5% charcoal-treated FBS and then lysed with 150 μL lysis buffer (Promega E153X). Luciferase activity was measured by adding 150 μL Steady-Glo® Luciferase substrate (Promega E253X). Luminescence was counted on Topcount (Perkin-Elmer, Shelton, CT).

Results

Enhancement of ATP-, UTP-, and bradykinin-stimulated arachidonic acid release upon HM74A activation

We have previously demonstrated the expression of endogenous HM74A receptors in A431 cells and their coupling to inhibitory G protein [21]. In order to understand the potential role of HM74A receptor in modulating the arachidonic acid pathway, we first examined whether or not HM74A activation by nicotinic acid may lead to arachidonic acid release. As shown in Fig. 1, 30 μM nicotinic acid did not induce significant amount of arachidonic acid release above the basal level in A431 cells. Since several subtypes of P2Y purinergic receptors (e.g., $P2Y_1$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$, Gi- and Gq-coupled), ionotropic P2X purinergic receptors, and