Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 345 (2006) 29–37
BBRC
www.elsevier.com/locate/ybbrc

Enhancement of arachidonic acid signaling pathway by nicotinic acid receptor HM74A

Yuting Tang *, Lubing Zhou, Joseph W. Gunnet, Pamela G. Wines, Ellen V. Cryan, Keith T. Demarest

Endocrine Therapeutics and Metabolic Disorders, The Johnson and Johnson Pharmaceutical Research and Development, L.L.C., 1000 Rt. 202, Raritan, NJ 08869, USA

Received 10 April 2006
Available online 25 April 2006

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

* Corresponding author.
E-mail addresses: ytang@prdus.jnj.com, ytang69@yahoo.com (Y. Tang).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.04.051

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

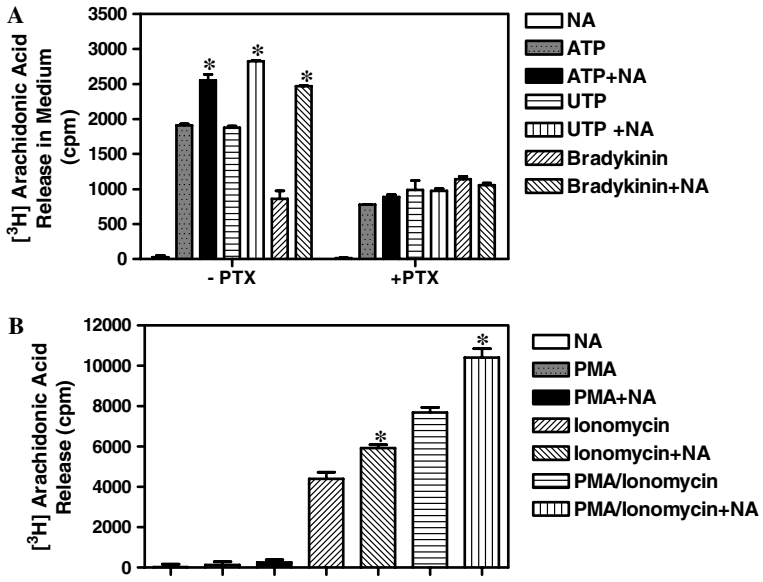

Fig. 1. Enhancement of purinergic receptors-, bradykinin receptor-, and PMA/ionomycin-induced AA release by HM74A in a PTX-sensitive way. (A) A431 cells in 24-well plates were loaded with [^{3}H]-AA overnight in the presence or absence of 50 μg/ml PTX and washed thoroughly with assay medium (DMEM + 0.1% fatty acid free BSA). Cells were then treated in assay medium with 100 μM ATP, 100 μM UTP or 30 μM bradykinin in the presence or absence of 30 μM nicotinic acid (NA) for 40 min and cell culture medium was then collected and counted for [^{3}H]. The assay was performed for at least three independent times. Data shown are from one representative experiment. Each data point is mean ± SD of duplicates. All data (cpm) were subtracted with cpm from vehicle-treated samples. *$p < 0.05$, indicating the difference when compared to the same treatment without NA from 3–5 experiments as stated in the text. (B) Cells were treated with PMA 100 ng/mL in the presence or absence of 0.5 μg/mL ionomycin with or without 30 μM nicotinic acid. Data shown are average from three independent experiments.

bradykinin receptors (Gq-coupled) were reported to be present in A431 cells [23,24], we went on to investigate whether HM74A may enhance arachidonic acid release mediated by those receptors. As depicted in Fig. 1A, nicotinic acid (30 μM) co-treatment synergistically enhanced AA release stimulated by 100 μM UTP (by 54 ± 5%, $n = 5$, compared to UTP alone), 100 μM ATP (by 33 ± 1%, $n = 3$, compared to ATP alone), or 30 μM bradykinin (by 148 ± 9%, $n = 5$, compared to bradykinin alone). When we pretreated A431 cells with 50 ng/mL pertussis toxin (PTX) overnight, the synergistic effects of nicotinic acid were completely abolished (Fig. 1A). While ATP- and UTP-induced AA release was inhibited by about 50% upon PTX pretreatment, bradykinin-induced AA release was not affected by PTX pretreatment. Therefore, AA release via activation of purinergic receptors was seemingly mediated by both Gi and Gq proteins and possibly Ca^{2+} influx, whereas bradykinin receptors are predominantly coupled to Gq protein in A431 cells.

Furthermore, we examined the possibility of a PKC activator, PMA, for inducing AA release in A431cells. PMA alone did not induce significant AA release up to 1 μg/mL (100 ng/mL in Fig. 1B and data not shown for 1 μg/mL treatment), while 500 ng/mL ionomycin alone led to significant AA release (Fig. 1B). Moreover, co-treatment with ionomycin and PMA further elevated AA release, confirming that activation of PLA_2 is a Ca^{2+}-dependent event and promoted by PKC activation [25,26]. More interestingly, activation of HM74A by nicotinic acid also enhanced ionomycin- (by 36 ± 8%, $n = 3$) and PMA/ionomycin-induced AA release (by 40 ± 6%, $n = 3$) (Fig. 1B) and the synergistic effects were also PTX-sensitive (data not shown).

HM74A stimulates calcium mobilization and synergistically enhances bradykinin-mediated calcium mobilization in a PTX-sensitive way

Above findings strongly suggested that activation of HM74A might stimulate phospholipase C to induce Ca^{2+} mobilization and activate PKC, although those effects might not be robust enough to cause significant AA release in medium alone and only became prominent when enhancing the effects of other stimuli. Therefore, we sought to examine Ca^{2+} mobilization upon HM74A activation. As shown in Fig. 2A, treatment of A431 cells with 0.1, 1, and 10 μM nicotinic acid induced increasing amount of intracellular Ca^{2+} release. The effect was abolished by overnight PTX pretreatment, indicating a Gi-dependent mechanism and most likely mediated by βγ subunit [27]. We went further to examine whether co-treatment of A431 cells with nicotinic acid will enhance Gq-coupled bradykinin receptor-promoted Ca^{2+} mobilization. As shown in Fig. 2B, bradykinin induced dose-dependent intracellular Ca^{2+} release. Co-treatment with nicotinic acid significantly increased bradykinin-promoted Ca^{2+} mobilization and shifted the

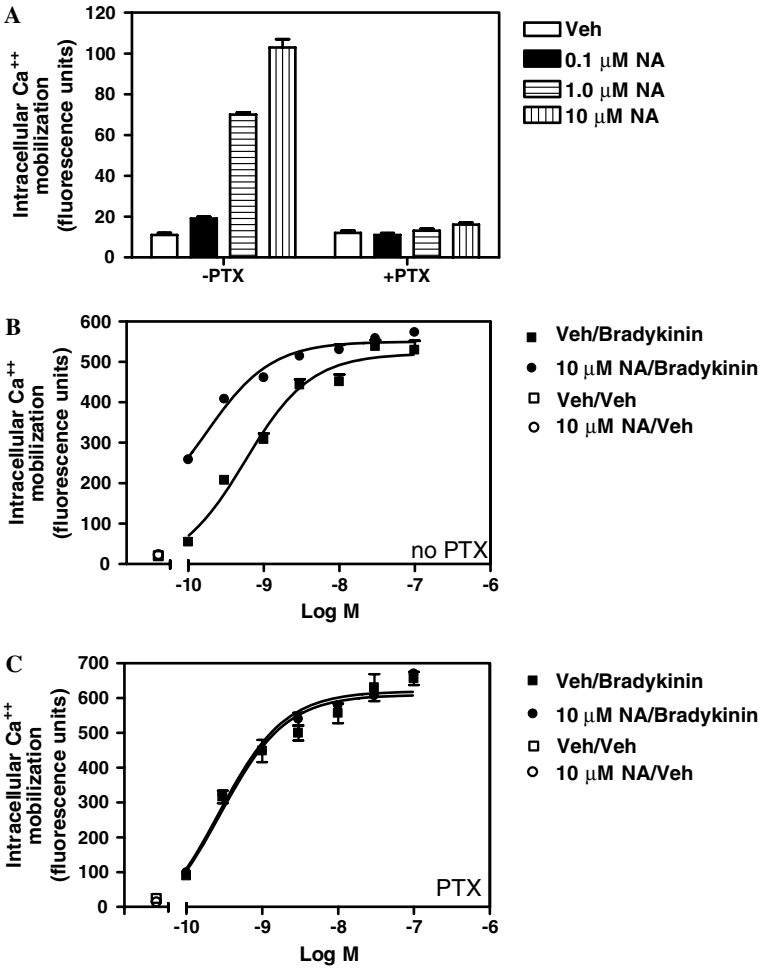

Fig. 2. HM74A stimulated Ca^{2+} mobilization and synergistically enhanced bradykinin-induced Ca^{2+} mobilization. A431 cells were seeded in 96-well plates and pretreated with or without 50 ng/mL PTX overnight. Cells were then washed extensively with assay buffer and loaded with Ca^{2+}-sensitive fluorescence dye fluo-3 for 1 h at RT followed by first addition with vehicle or nicotinic acid. Fluorescence was recorded continuously on FDSS as described in Materials and methods. Five minutes later, cells were stimulated with bradykinin and fluorescence recording continued for another five minutes. (A) Ca^{2+} mobilization was stimulated with nicotinic acid treatment and abolished by PTX pretreatment. (B,C) Five minutes after first addition, intracellular Ca^{2+} returned to basal level and then cells were stimulated by addition of bradykinin. Bradykinin induced significant dose-dependent intracellular Ca^{2+} mobilization that was enhanced by nicotinic acid treatment. The experiment was performed for three independent times. Data shown are from one representative assay performed in triplicate.

bradykinin dose-response curve to the left. The synergistic effect of nicotinic acid was also abolished by PTX pretreatment (Fig. 2C).

HM74A enhances ERK1/2 activation by bradykinin but not by UTP

It has been well established that AA release from membrane phospholipids in response to GPCR stimulation was promoted by activation of cytosolic Ca^{2+}-dependent phospholipase A_2 (cPLA$_2$) [28]. Activation of cPLA2 includes phosphorylation and translocation of the enzyme to the cell membranes [29,30]. Both PKC and ERK1/2 have been shown to contribute to the activation of cPLA2 [31–33]. Extracellular-signal-regulated kinases 1 and 2 (ERK1/2), members of mitogen-activated protein kinase (MAPK) family, have emerged as important effectors for cell surface GPCRs (for review, see [34,35]). Regulation of ERK1/2 by GPCRs is highly complex and often dependent on receptors and cellular context. Here we first examined ERK1/2 activation upon stimulation of HM74A with nicotinic acid. As shown in Fig. 3, 30 μM nicotinic acid treatment alone activated a small increase of ERK1/2 phosphorylation. Both UTP and bradykinin significantly stimulated ERK1/2 activation. Interestingly, ERK1/2 was synergistically activated by co-treatment with bradykinin and nicotinic acid. In contrast, HM74A activation exerted no effects on UTP-mediated ERK1/2 activation. As expected, PD 098059, a specific inhibitor for ERK1/2 activation by inhibiting MAPK kinase, abolished nicotinic acid-, UTP-, and bradykinin-promoted ERK1/2 activation and inhibited 100 ng/mL EGF-induced ERK1/2 activation by about 50%.

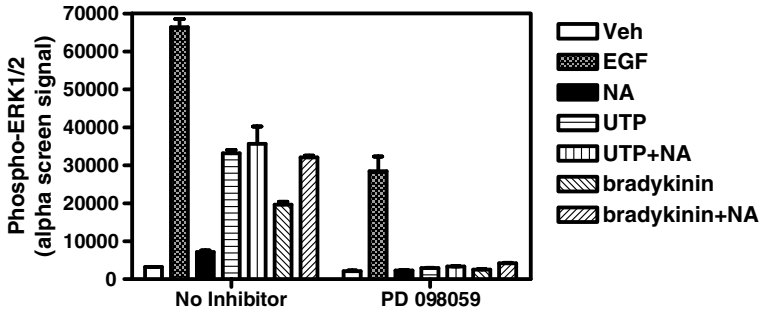

Fig. 3. HM74A synergistically activated ERK1/2 phosphorylation induced by bradykinin but not that of UTP in A431 cells. Cells were seeded in 96-well plates and starved with serum-free DMEM overnight. Cells were first treated with or without 30 μM PD 098059 for 30 min at 37 °C with 5% CO_2 and then stimulated with 100 μM UTP or 30 μM bradykinin in the presence or absence of 30 μM nicotinic acid (NA) for 10 min. EGF-treated cells (100 ng/ml) were used as positive control. Cells were then lysed and ERK1/2 phosphorylation was measured using Surefire™ ERK kinase assay method as described in Materials and methods. Data shown are from one representative experiment. Each data point is mean ± SD of duplicates.

AA release in A431 cells is dependent on both PKC and ERK1/2 activation and the synergistic effects of HM74A occur upstream of PKC and ERK1/2 at the receptor level

We next sought to determine the roles of PKC and ERK1/2 in mediating AA release and the synergistic effects of HM74A in A431 cells. A431 cells were pretreated with 10 μM GF 109203X (a specific PKC inhibitor) or 30 μM PD 098059 or both for 30 min before stimulation with UTP or bradykinin in the absence or presence of nicotinic acid. As shown in Fig. 4, both GF 109203X and PD 098059 decreased UTP-induced AA release ($47 \pm 2\%$, $n = 3$ and $35 \pm 4\%$, $n = 5$), while co-treatment with both inhibitors exhibited more pronounced effects ($66 \pm 3\%$ reduction, $n = 3$). Similarly, bradykinin-mediated AA release was decreased by $21 \pm 9\%$ ($n = 4$) and $29 \pm 4\%$ ($n = 3$) upon PD 098059 and GF 109203X treatment, respectively. Combination of both inhibitors lowered bradykinin-mediated AA release by $43 \pm 4\%$ ($n = 3$). However, the percentage increase of UTP- and bradykinin-induced AA release by

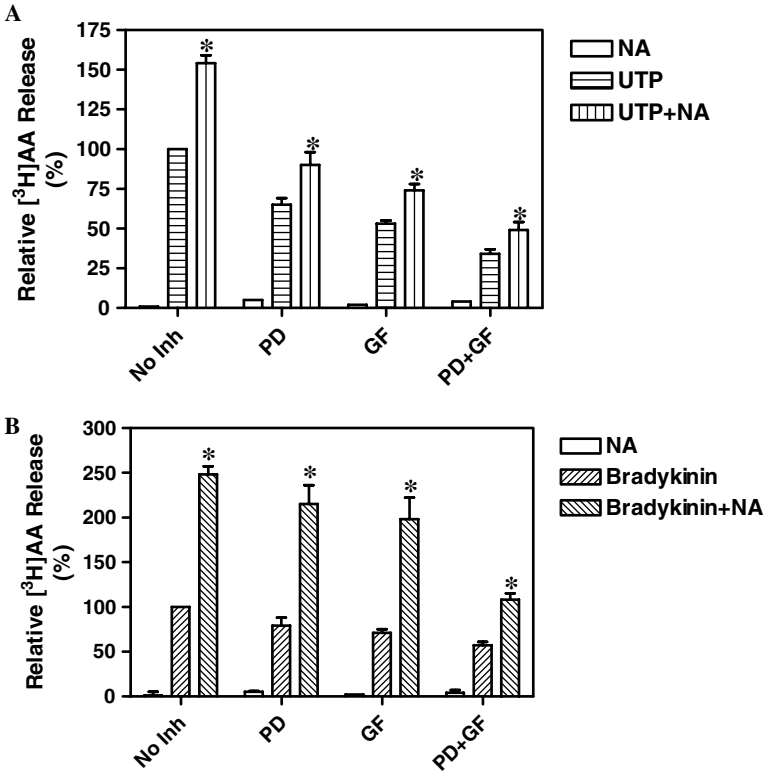

Fig. 4. Both PKC and ERK1/2 contributed to AA release in A431 cells and the synergistic effects of HM74A occurred at the receptor level. Cells were pretreated with 30 μM PD 098059, 10 μM GF 109203X or both for 30 min and then treated with 100 μM UTP (A) or 30 μM bradykinin (B) in the presence or absence of 30 μM nicotinic acid for 40 min. Net AA release from 100 μM UTP (A) or 30 μM bradykinin (B) treatment in the absence of any inhibitors was set at 100%. Data shown are means ± SE of 3–5 independent experiments. *$p < 0.05$, indicating the difference when compared to the same treatment without NA.

HM74A was basically maintained in the presence of both inhibitors, indicating that the cross-talk occurred at the receptor level. In summary, both PKC and ERK1/2 contributed to AA release in A431 cells. The synergistic effects of HM74A on AA release occurred upstream of PKC and ERK1/2.

Activation of nuclear receptor PPAR by AA pathway

The eicosanoids generated from AA pathway were demonstrated to activate PPARs [17,18]. We hypothesized that production of AA might provide a stage for cross-talk between cell surface receptor HM74A and nuclear receptor PPARs which play a broad spectrum of roles in lipid metabolism. Since A431 cells express endogenous PPARγ [22], we transiently transfected A431 cells with a luciferase reporter gene under the control of PPAR response elements [22]. As shown in Fig. 5, 10 μM rosiglitazone (PPARγ agonist) induced luciferase expression by about 4-fold. Treatment with 4 and 20 μM AA also led to significant induction of luciferase (2.3- and 3.5-fold, respectively). As reported previously, the PPRE promoter used here did not generally result in high level of reporter gene expression [22]. Therefore, we did not observe significant induction of luciferase activity upon UTP or bradykinin stimulation in the presence or absence of nicotinic acid (data not shown). Nonetheless, the results obtained from AA-treated cells strongly suggested that activation of AA pathway might lead to up-regulation of PPAR target genes and eventually the physiological effects of those genes.

Discussion

Here we reported that activation of HM74A receptors by nicotinic acid enhanced AA signaling pathway by potentiating AA release in A431 cells. Such effects of HM74A not only strongly implicate that activation of HM74A under in vivo physiological conditions may lead to activation of nuclear receptors such as PPARs and consequently regulation of genes involved in lipid metabolism, but also shed some light on the possible role of HM74A in mediating the skin-flushing symptom during niacin therapy.

AA release upon activation of various GPCRs has been well documented. Cytosolic Ca^{2+}-dependent phospholipase A_2 was shown to be responsible for catalyzing GPCR-mediated AA release. It has been generally established that cPLA2 undergoes phosphorylation by PKC and/or MAPK such as ERK1/2 or both and translocates in a Ca^{2+}-dependent way to cell membranes where it catalyzes AA liberation from phospholipids [29–33]. Both sustained elevation of intracellular Ca^{2+} level [26] and activities of protein kinases such as PKC and ERK1/2 are indispensable for AA release, although the roles of PKC and ERK1/2 vary depending on the receptors and cell types [32,36,37]. Consistent with this notion, only in the presence of Ca^{2+} ionophore did PMA, a PKC activator, promote significant AA release in A 431 cells (Fig. 1B). In addition, purinergic receptor-mediated AA release was inhibited more than that of bradykinin induced by PKC and MAPK kinase inhibitor co-treatment in A431 cells (66% and 43%, respectively, Fig. 4). Gq-coupled receptor can induce AA release since they activate phospholipase C to generate second messengers inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) which in turn mobilize intracellular Ca^{2+} and activate PKC, respectively. For instance, AA-release was reported for thrombin receptor, bradykinin receptor, α1 adrenergic receptor, endothelin1 receptor, and UTP-preferring P2Y receptors in various cell types [28,36–41]. Although Gi-coupled receptors have not been reported very often to stimulate significant AA release on their own, α2B adrenergic receptor can induce small AA release in LLC PK1 cells [42]. Here we showed that activation of HM74A alone in A431 cells did not lead to significant AA release. Nevertheless, HM74A significantly enhanced purinergic receptor- and bradykinin receptor-mediated AA release in a PTX-sensitive manner, indicating a Gi-dependent mechanism. Consistently, we showed that activation of HM74A receptor led to Ca^{2+} mobilization (an effect much smaller than that of Gq-coupled bradykinin receptor, Fig. 3A and B) and synergistic

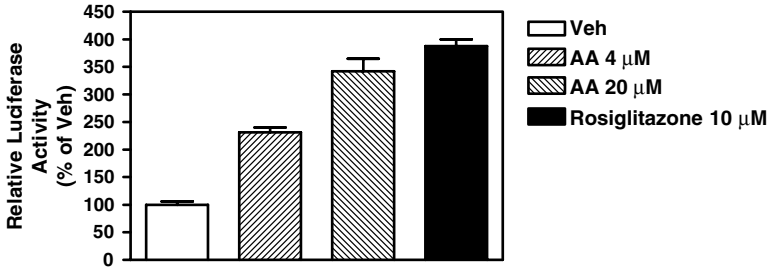

Fig. 5. Arachidonic acid induced PPRE-Luciferase expression through endogenous PPARγ in A431 cells. A431 cells were transiently transfected with PPRE-Luciferase in 75-cm^2 flasks, then seeded in 24-well plates, and treated with vehicle, 30 μM nicotinic acid, 4 μM AA, 20 μM AA, or 10 μM rosiglitazone for 24 h in DMEM in the presence of 0.5% charcoal-treated FBS. Cells were then lysed and luciferase activity was measured. Luciferase activity of vehicle-treated sample was set at 100%. Data shown are means ± SD of three independent experiments.

enhancement of bradykinin-induced Ca^{2+} mobilization in a PTX-sensitive way. Coupling of Gi-coupled receptors to phospholipase C by Gβγ subunit was previously reported for adenosine A1 receptor [27] (for review in the aspect of GPCR signaling, see [43,44]). Offermanns and co-workers demonstrated Ca^{2+} elevation in mouse macrophages that expressed PUMA-G upon nicotinic acid stimulation [16]. The synergistic effects between Gi and Gq receptors on regulating AA release have been reported previously [37,39,40]. For instance, adenosine A1 receptor enhanced AA release by thrombin receptor and cholecystokinin receptor [37,40]. Similar phenomenon was also observed for rhodopsin and purinergic receptor in CHO cells [39]. Although the precise underlying mechanism whereby Gi- and Gq-receptors cross-talk with each other remains to be further elucidated, G protein βγ subunit exchange between receptors has been demonstrated to be responsible by several groups using Gβγ sequester i.e., the C-terminus of beta-adrenoceptor kinase 1 (residues 495–689) or transient expression of Gβγ subunits [27,45].

In recent years, ERK1/2 activation by GPCR has been emerging as a new paradigm of GPCR signaling (for review, see [34,35]) and highly complex and cell type-dependent. As shown by Tunaru and co-authors, HM74A did stimulate ERK1/2 activation when over-expressed in CHO-K1 cells [10]. Here endogenous HM74A only moderately promoted activation of ERK1/2 in A431 cells. However, HM74A enhanced bradykinin-promoted ERK1/2 activation, but not that of UTP, once again underscoring the complexity of ERK1/2 regulation by GPCRs.

A431 cells were developed from epidermal origin. The physiological function of a high level of HM74A receptor in these cells is not clear at the present time. In niacin therapy, patients must take a high dose (typically 1.5–2 g/day) that often results in a common side effect of skin flushing [1]. The release of prostaglandin D_2 in skin was believed to be the cause [13,15]. COX (cyclooxygenase) inhibitors such as acetylsalicylic acid can attenuate skin flushing [13], indicating that the phospholipase A_2 (PLA_2)/arachidonic acid pathway is involved. Moreover, niacin-induced skin flushing was substantially attenuated in schizophrenic patients and this abnormality was correlated to increased PLA_2 activity in those patients [46–48]. Most recently, Offermanns and co-workers demonstrated that PUMA-G knock-out mice were in lack of niacin-induced skin flushing [16]. They further showed that mice lacking cyclooxygenase, prostaglandin D2 receptor or E2 receptor exhibited decreased skin flushing upon niacin administration, indicating that AA pathway is involved in niacin/HM74A-mediated skin flushing in mice [16]. Ross and co-workers also previously established that niacin-induced skin flushing in rat was abrogated by acetylsalicylic acid pretreatment [49]. Here in this report, we demonstrated for the first time that HM74A did enhance AA signaling pathway in an epidermal cell line. Our finding was also enlightening in terms of addressing the question about what types of cells are responsible for prostaglandin production and consequent vasodilation in skin [16,50]. Activation of AA pathway by HM74A receptors also provided us some evidence to further understand the diverse effects of niacin, possibly through transcription factors such as PPARs, on lipid metabolism. Although it is well accepted that HM74A inhibits hormone-sensitive lipase by coupling to Gi protein and inhibiting adenylate cyclase in adipocytes, most of the therapeutic effects of niacin on lipid profiles remain to be elucidated on the molecular level. Various eicosanoids including prostaglandins generated from AA have been demonstrated to be activators of PPARs (peroxisome-proliferator activated receptor, α, γ, and δ) [17,18], a family of nuclear receptors playing critical roles in regulating lipid and lipoprotein metabolism (for review, see [51]). In particular, 15-deoxy-$\Delta^{12,14}$-prostaglandin (15d-PGJ_2, the major metabolite of prostaglandin D_2) was identified as the most potent endogenous PPARγ ligand [19] and modulated adipocyte differentiation. Here we showed that treatment of A431 cells transiently transfected with PPRE-luciferase with AA mimicked the effects of PPARγ agonist rosiglitazone as to luciferase induction. Consistent with our findings, Rubic and her colleagues found that nicotinic acid could stimulate the transcription of several genes involved in lipid metabolism including PPARγ, CD36 (an important scavenger receptor for uptake of oxidized lipoproteins), and ABCA1 (a key transporter for efflux of cellular cholesterol to apolipoprotein A-I containing particles) in monocytoid cells [52]. However, they did not illustrate the possible role of HM74A in niacin-induced gene transcription. HM74A was reported to express in mouse macrophages [6]. We also found that HM74A receptors were expressed in human macrophages/monocytes (Tang and Zhou, unpublished data). It is unclear why Rubic et al. observed increased cAMP production in their monocytoid cells upon 1 mM nicotinic acid stimulation since HM74A is known to couple to Gi protein in HEK293, CHO, and adipocytes [8,11]. Nonetheless, the potential link between HM74A and PPAR may also occur through adenylate cyclase pathway in addition to AA pathway. Indeed, cAMP-responsive transcription factor CREB negatively regulates PPARγ in the liver and hence controls hepatic lipid metabolism [53]. Moreover, intracellular cAMP was also found to define a negative feedback regulation for AA release mediated by P2Y receptor in MDCK-D1 cells [54]. On the other hand, prostaglandin E2 derived from AA was also demonstrated to be secreted from cells and bind to cell surface EP2/4 receptor which in turn activates adenylate cyclase in an autocrine/paracrine way [55,56]. Taken together, AA and/or adenylate cyclase signaling pathway may serve as platform for cross-talk between cell surface G protein-coupled HM74A and transcription factors such as PPARs, although further investigation needs to be undertaken to delineate the signaling events.

Acknowledgments

We greatly appreciate the generous gift of PPRE-Luciferase plasmid from Dr. Jeffrey Travers, Indiana University School of Medicine. The authors thank Dr. Patricia Pelton for critical reading of the manuscript.

References

[1] R.W. Mahley, T.P. Bersot, Drug therapy for hypercholesterolemia and dyslipidemia, in: Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th ed., McGraw-Hill, New York, 2002, pp. 971–1002.
[2] L.A. Carlson, A. Hamsten, A. Asplund, Pronounced lowering of serum levels of lipoprotein Lp(a) in hyperlipidaemic subjects treated with nicotinic acid, J. Int. Med. 226 (1989) 271–276.
[3] A. Gurakar, J.M. Hoeg, G. Kostner, N.M. Papadopoulos, H.B. Brewer Jr., Levels of lipoprotein Lp(a) decline with neomycin and niacin treatment, Atherosclerosis 57 (1985) 293–301.
[4] C.J. Packard, J.M. Stewart, J.L. Third, H.G. Morgan, T.D. Lawrie, J. Shepherd, Effects of nicotinic acid therapy on high-density lipoprotein metabolism in type II and type IV hyperlipoproteinaemia, Biochim. Biophys. Acta 618 (1980) 53–62.
[5] L.A. Carlson, Studies on the effect of nicotinic acid on catecholamine stimulated lipolysis in adipose tissue in vitro, Acta Med. Scand. 173 (1963) 719–722.
[6] A. Lorenzen, C. Stannek, A. Burmeister, I. Kalvinsh, U. Schwabe, G protein-coupled receptor for nicotinic acid in mouse macrophages, Biochem. Pharmacol. 64 (2002) 645–648.
[7] A. Lorenzen, C. Stannek, H. Lang, V. Andrianov, I. Kalvinsh, U. Schwabe, Characterization of a G protein-coupled receptor for nicotinic acid, Mol. Pharmacol. 59 (2001) 349–357.
[8] T. Soga, M. Kamohara, J. Takasaki, S.-i. Matsumoto, T. Saito, T. Ohishi, H. Hiyama, A. Matsuo, H. Matsushime, K. Furuichi, Molecular identification of nicotinic acid receptor, Biochem. Biophys. Res. Commun. 303 (2003) 364–369.
[9] A. Wise, M. Foord Steven, J. Fraser Neil, A. Barnes Ashley, N. Elshourbagy, M. Eilert, M. Ignar Diane, R. Murdock Paul, K. Steplewski, A. Green, J. Brown Andrew, J. Dowell Simon, G. Szekeres Philip, G. Hassall David, H. Marshall Fiona, S. Wilson, B. Pike Nicholas, Molecular identification of high and low affinity receptors for nicotinic acid, J. Biol. Chem. 278 (2003) 9869–9874.
[10] S. Tunaru, J. Kero, A. Schaub, C. Wufka, A. Blaukat, K. Pfeffer, S. Offermanns, PUMA-G and HM74 are receptors for nicotinic acid and mediate its anti-lipolytic effect, Nat. Med. 9 (2003) 352–355.
[11] Y. Zhang, J. Schmidt Robert, P. Foxworthy, R. Emkey, K. Oler Jennifer, H. Large Thomas, H. Wang, W. Su Eric, K. Mosior Marion, I. Eacho Patrick, G. Cao, Niacin mediates lipolysis in adipose tissue through its G-protein coupled receptor HM74A, Biochem. Biophys. Res. Commun. 334 (2005) 729–732.
[12] J.D. Morrow, W.G. Parsons 3rd, L.J. Roberts 2nd, Release of markedly increased quantities of prostaglandin D2 in vivo in humans following the administration of nicotinic acid, Prostaglandins 38 (1989) 263–274.
[13] J.D. Morrow, J.A. Awad, J.A. Oates, L.J. Roberts 2nd, Identification of skin as a major site of prostaglandin D2 release following oral administration of niacin in humans, J. Invest. Dermatol. 98 (1992) 812–815.
[14] A. Edlund, L. Musatti, A. Wennmalm, Acipimox stimulates skin blood flow by a cyclo-oxygenase-dependent mechanism, Eur. J. Clin. Pharmacol. 39 (1990) 37–41.
[15] R.H. Stern, J.D. Spence, D.J. Freeman, A. Parbtani, Tolerance to nicotinic acid flushing, Clin. Pharmacol. Ther. 50 (1991) 66–70.
[16] Z. Benyo, A. Gille, J. Kero, M. Csiky, M.C. Suchankova, R.M. Nuesing, A. Moers, K. Pfeffer, S. Offermanns, GPR109A (PUMA-G/HM74A) mediates nicotinic acid-induced flushing, J. Clin. Invest. 115 (2005) 3634–3640.
[17] K. Yu, W. Bayona, C.B. Kallen, H.P. Harding, C.P. Ravera, G. McMahon, M. Brown, M.A. Lazar, Differential activation of peroxisome proliferator-activated receptors by eicosanoids, J. Biol. Chem. 270 (1995) 23975–23983.
[18] R. Hertz, I. Berman, D. Keppler, J. Bar-Tana, Activation of gene transcription by prostacyclin analogues is mediated by the peroxisome-proliferators-activated receptor (PPAR), Eur. J. Biochem./FEBS 235 (1996) 242–247.
[19] S.A. Kliewer, J.M. Lenhard, T.M. Willson, I. Patel, D.C. Morris, J.M. Lehmann, A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation, Cell 83 (1995) 813–819.
[20] C.D. Meyers, V.S. Kamanna, M.L. Kashyap, Niacin therapy in atherosclerosis, Curr. Opin. Lipidol. 15 (2004) 659–665.
[21] L. Zhou, Y. Tang, E.V. Cryan, K.T. Demarest, Human Epidermoid A431 Cells Express Functional Nicotinic Acid Receptor HM74A, Mol. Cell. Biochem. (in press).
[22] Q. Zhang, D. Southall Michael, M. Mezsick Steven, C. Johnson, C. Murphy Robert, L. Konger Raymond, B. Travers Jeffrey, Epidermal peroxisome proliferator-activated receptor gamma as a target for ultraviolet B radiation, J. Biol. Chem. 280 (2005) 73–79.
[23] N.N. Huang, D.J. Wang, F. Gonzalez, L.A. Heppel, Multiple signal transduction pathways lead to extracellular ATP-stimulated mitogenesis in mammalian cells: II. A pathway involving arachidonic acid release, prostaglandin synthesis, and cyclic AMP accumulation, J. Cell. Physiol. 146 (1991) 483–494.
[24] J. van Baal, J. de Widt, N. Divecha, J. van Blitterswijk Wim, Translocation of diacylglycerol kinase theta from cytosol to plasma membrane in response to activation of G protein-coupled receptors and protein kinase C, J. Biol. Chem. 280 (2005) 9870–9878.
[25] N.G. Kudryavtseva, K.M. Popov, T.V. Bulargina, Mobilization of arachidonic acid in human A431 cells in the presence of epidermal growth factor, calcium ions, and cyclic AMP, Biomed. Sci. (London) 1 (1990) 295–299.
[26] T. Hirabayashi, K. Kume, K. Hirose, T. Yokomizo, M. Iino, H. Itoh, T. Shimizu, Critical duration of intracellular Ca2+ response required for continuous translocation and activation of cytosolic phospholipase A2, J. Biol. Chem. 274 (1999) 5163–5169.
[27] J.M. Dickenson, S.J. Hill, Involvement of G-protein betagamma subunits in coupling the adenosine A1 receptor to phospholipase C in transfected CHO cells, Eur. J. Pharmacol. 355 (1998) 85–93.
[28] L.L. Lin, A.Y. Lin, J.L. Knopf, Cytosolic phospholipase A2 is coupled to hormonally regulated release of arachidonic acid, Proc. Natl. Acad. Sci. USA 89 (1992) 6147–6151.
[29] E.A. Nalefski, L.A. Sultzman, D.M. Martin, R.W. Kriz, P.S. Towler, J.L. Knopf, J.D. Clark, Delineation of two functionally distinct domains of cytosolic phospholipase A2, a regulatory Ca(2+)-dependent lipid-binding domain and a Ca(2+)-independent catalytic domain, J. Biol. Chem. 269 (1994) 18239–18249.
[30] M.A. Gijon, D.M. Spencer, A.L. Kaiser, C.C. Leslie, Role of phosphorylation sites and the C2 domain in regulation of cytosolic phospholipase A2, J. Cell Biol. 145 (1999) 1219–1232.
[31] L.L. Lin, M. Wartmann, A.Y. Lin, J.L. Knopf, A. Seth, R.J. Davis, cPLA2 is phosphorylated and activated by MAP kinase, Cell 72 (1993) 269–278.
[32] M.A. Lal, P.R. Proulx, R.L. Hebert, A role for PKC epsilon and MAP kinase in bradykinin-induced arachidonic acid release in rabbit CCD cells, Am. J. Physiol. 274 (1998) F728–F735.
[33] R.A. Nemenoff, S. Winitz, N.X. Qian, V. Van Putten, G.L. Johnson, L.E. Heasley, Phosphorylation and activation of a high molecular weight form of phospholipase A2 by p42 microtubule-associated protein 2 kinase and protein kinase C, J. Biol. Chem. 268 (1993) 1960–1964.
[34] K.L. Pierce, L.M. Luttrell, R.J. Lefkowitz, New mechanisms in heptahelical receptor signaling to mitogen activated protein kinase cascades, Oncogene 20 (2001) 1532–1539.

[35] T.D. Werry, P.M. Sexton, A. Christopoulos, Ins and outs of seven-transmembrane receptor signalling to ERK, Trends Endocrinol. Metab. 16 (2005) 26–33.
[36] G.Y. Sun, J. Xu, M.D. Jensen, S. Yu, W.G. Wood, F.A. Gonzalez, A. Simonyi, A.Y. Sun, G.A. Weisman, Phospholipase A2 in astrocytes. Responses to oxidative stress, inflammation, and G protein-coupled receptor agonists, Mol. Neurobiol. 31 (2005) 27–41.
[37] J.M. Dickenson, S.J. Hill, Transfected adenosine A1 receptor-mediated modulation of thrombin-stimulated phospholipase C and phospholipase A2 activity in CHO cells, Eur. J. Pharmacol. 321 (1997) 77–86.
[38] M. Xing, P.A. Insel, Protein kinase C-dependent activation of cytosolic phospholipase A2 and mitogen-activated protein kinase by alpha 1-adrenergic receptors in Madin–Darby canine kidney cells, J. Clin. Invest. 97 (1996) 1302–1310.
[39] C.D. Dickerson, E.R. Weiss, The coupling of pertussis toxin-sensitive G proteins to phospholipase A2 and adenylyl cyclase in CHO cells expressing bovine rhodopsin, Exp. Cell Res. 216 (1995) 46–50.
[40] J.M. Dickenson, S.J. Hill, Synergistic interactions between human transfected adenosine A1 receptors and endogenous cholecystokinin receptors in CHO cells, Eur. J. Pharmacol. 302 (1996) 141–151.
[41] L. Trevisi, S. Bova, G. Cargnelli, G. Ceolotto, S. Luciani, Endothelin-1-induced arachidonic acid release by cytosolic phospholipase A2 activation in rat vascular smooth muscle via extracellular signal-regulated kinases pathway, Biochem. Pharmacol. 64 (2002) 425–431.
[42] D. Cussac, S. Schaak, C. Denis, H. Paris, Alpha 2B-adrenergic receptor activates MAPK via a pathway involving arachidonic acid metabolism, matrix metalloproteinases, and epidermal growth factor receptor transactivation, J. Biol. Chem. 277 (2002) 19882–19888.
[43] J.H. Exton, Regulation of phosphoinositide phospholipases by hormones, neurotransmitters, and other agonists linked to G proteins, Annu. Rev. Pharmacol. Toxicol. 36 (1996) 481–509.
[44] S.G. Rhee, Regulation of phosphoinositide-specific phospholipase C, Annu. Rev. Biochem. 70 (2001) 281–312.
[45] U. Quitterer, M.J. Lohse, Crosstalk between Gai- and Gaq-coupled receptors is mediated by Gbg exchange, Proc. Natl. Acad. Sci. USA 96 (1999) 10626–10631.
[46] S. Smesny, G. Berger, T. Rosburg, S. Riemann, S. Riehemann, P. McGorry, H. Sauer, Potential use of the topical niacin skin test in early psychosis—a combined approach using optical reflection spectroscopy and a descriptive rating scale, J. Psychiatr. Res. 37 (2003) 237–247.
[47] H. Tavares, J. Yacubian, L. Talib Leda, R. Barbosa Nadia, F. Gattaz Wagner, Increased phospholipase A2 activity in schizophrenia with absent response to niacin, Schizophr. Res. 61 (2003) 1–6.
[48] E. Messamore, F. Hoffman William, A. Janowsky, The niacin skin flush abnormality in schizophrenia: a quantitative dose-response study, Schizophr. Res. 62 (2003) 251–258.
[49] S.D. Turenne, M. Seeman, B.M. Ross, An animal model of nicotinic-acid-induced vasodilation: effect of haloperidol, caffeine and nicotine upon nicotinic acid response, Schizophr. Res. 50 (2001) 191–197.
[50] N.B. Pike, Flushing out the role of GPR109A (HM74A) in the clinical efficacy of nicotinic acid, J. Clin. Invest. 115 (2005) 3400–3403.
[51] B. Staels, J. Auwerx, Role of PPAR in the pharmacological regulation of lipoprotein metabolism by fibrates and thiazolidinediones, Curr. Pharm. Des. 3 (1997) 1–14.
[52] T. Rubic, M. Trottmann, L. Lorenz Reinhard, Stimulation of CD36 and the key effector of reverse cholesterol transport ATP-binding cassette A1 in monocytoid cells by niacin, Biochem. Pharmacol. 67 (2004) 411–419.
[53] S. Herzig, S. Hedrick, I. Morantte, S.-H. Koo, F. Galimi, M. Montminy, CREB controls hepatic lipid metabolism through nuclear hormone receptor PPAR-gamma, Nature 426 (2003) 190–193.
[54] M. Xing, S. Post, R.S. Ostrom, M. Samardzija, P.A. Insel, Inhibition of phospholipase A2-mediated arachidonic acid release by cyclic AMP defines a negative feedback loop for P2Y receptor activation in Madin–Darby canine kidney D1 cells, J. Biol. Chem. 274 (1999) 10035–10038.
[55] S.R. Post, J.P. Jacobson, P.A. Insel, P2 purinergic receptor agonists enhance cAMP production in Madin–Darby canine kidney epithelial cells via an autocrine/paracrine mechanism, J. Biol. Chem. 271 (1996) 2029–2032.
[56] Y. Chen, M. Hughes-Fulford, Prostaglandin E2 and the protein kinase A pathway mediate arachidonic acid induction of c-fos in human prostate cancer cells, Br. J. Cancer 82 (2000) 2000–2006.