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# Molecular Identification of High and Low Affinity Receptors for Nicotinic Acid*

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**Nicotinic acid has been used clinically for over 40 years in the treatment of dyslipidemia producing a desirable normalization of a range of cardiovascular risk factors, including a marked elevation of high density lipoprotein and a reduction in mortality. The precise mechanism of action of nicotinic acid is unknown, although it is believed that activation of a $G_i$-G protein-coupled receptor may contribute. Utilizing available information on the tissue distribution of nicotinic acid receptors, we identified candidate orphan receptors. The selected orphan receptors were screened for responses to nicotinic acid, in an assay for activation of $G_i$-G proteins. Here we describe the identification of the G protein-coupled receptor HM74 as a low affinity receptor for nicotinic acid. We then describe the subsequent identification of HM74A in follow-up bioinformatics searches and demonstrate that it acts as a high affinity receptor for nicotinic acid and other compounds with related pharmacology. The discovery of HM74A as a molecular target for nicotinic acid may facilitate the discovery of superior drug molecules to treat dyslipidemia.**

Nicotinic acid has been used in the treatment of dyslipidemia for many years, producing a very desirable modification of multiple cardiovascular risk factors, increasing high density lipoprotein, and decreasing very low density lipoprotein, low density lipoprotein, triglycerides, and lipoprotein (a), which results in a reduction in mortality (1). Despite its long history of clinical use, the precise mechanism of action of nicotinic acid is unknown, although it is believed that inhibition of adipocyte lipolysis via the activation of a $G_i$-coupled receptor may contribute (2–4). It has been postulated that a reduction in free fatty acids liberated from adipose tissue results in a reduction of hepatic triglycerides available for very low density lipoprotein and low density lipoprotein synthesis, which in part explains the hypolipidemic effects observed during nicotinic acid therapy. Because the identification of a molecular target for nicotinic acid would facilitate our understanding of its mode of action and potentially enable the discovery of superior drug molecules, we instigated a strategy to identify this receptor. To identify the $G_i$-G protein-coupled receptor for nicotinic acid, orphan receptors were selected based on their tissue expression profiles for a rational screening exercise. Recently, the pharmacological sites of action of nicotinic acid were shown to be largely restricted to adipose tissue and spleen (5). Therefore, to identify this nicotinic acid receptor, we selected a subset of 10 orphan G protein-coupled receptors, which by mRNA distribution analysis (TaqMan) exhibited significant expression levels in both adipose tissue and spleen. These receptors were then expressed in an appropriate mammalian cell line to allow measurement of a functional response (GTPγS[1] binding) following nicotinic acid treatment. This paper describes the identification of HM74 as a low affinity receptor for nicotinic acid and the subsequent indication of HM74A, a high affinity receptor for nicotinic acid. The identification of HM74A has allowed us to test additional compounds that have been reported to possess a similar pharmacology to nicotinic acid.

## EXPERIMENTAL PROCEDURES

*Materials*—Nicotinic acid, nicotinuric acid, and nicotinamide were obtained from Sigma-Aldrich, 5-methyl nicotinic acid was from Maybridge, and pyridine-3-acetic acid was from ICN. LipofectAMINE, Dulbecco's modified Eagle's medium, and fetal calf serum were from Invitrogen. [$^{35}$S]GTPγS (1160 Ci/mmol) and [5,6-$^{3}$H]nicotinic acid (50–60 Ci/mmol) were purchased from Amersham Biosciences and Biotrend, respectively. Pertussis toxin was from Sigma-Aldrich. Acipimox, Acifran, and 5-methyl pyrazole-3-carboxylic acid were synthesized by chemists within GlaxoSmithKline.

*Molecular Biology*—The HM74 expressed sequence tag was identified from the public data base as a potential seven transmembrane-spanning receptor, and the predicted open reading frame was amplified using human placenta cDNA as template. Comparison of the nucleotide sequence of HM74 with that of the published sequence revealed 15 nucleotide differences as well as a 5-nucleotide insertion at the 3′ end of

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*The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AY148884 and EMM_patAR098624.*

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[1] The abbreviations used are: GTPγS, guanosine 5′-(γ-thio)triphosphate; GIRK, G protein-regulated potassium channel; CHO, Chinese hamster ovary.

the clone that resulted in a different 3′ coding sequence. The cloning procedure was performed twice more to confirm the changes in the amino acid sequence. To confirm the correct initiation methionine, a cDNA clone containing the entire coding region and the 5′-untranslated region was isolated using human placenta cDNA library. Sequence analysis of the clone, which we termed HM74A, showed the presence of a stop codon prior to the first initiation methionine. A murine sequence with significant homology to human HM74 was identified by searching public domain data bases with the peptide sequence for human HM74 taken from GenBank™ accession number D10923. A TBLASTN search produced significant alignment with accession numbers AJ300198 and AJ300199, which encode the *Mus musculus* PUMA-G gene for a putative seven transmembrane-spanning receptor (termed mHM74A). Using the human and murine sequence information, the PCR was used to amplify the corresponding rat gene. The accession number for human HM74A is AY148884. The cDNA sequence of rat HM74A is partially represented by EMM_patAR098624.

*TaqMan mRNA Analysis*—Poly(A)+ RNA from 20 tissues of four different individuals (two males, two females except prostate) was prepared, reverse transcribed, and analyzed by TaqMan quantitative PCR as described previously (6). Briefly, 1 μg of poly(A)+ RNA was reverse transcribed using random priming, and the cDNA produced was used to make up to 1,000 replicate plates with each well containing the cDNA from 50 ng of poly(A)+ RNA. TaqMan quantitative PCR (Applied Biosystems, Warrington, UK) was used to assess the level of each gene relative to genomic DNA standards. The data are presented as the means of mRNA copies detected per ng of poly(A)+ RNA from four individuals ± S.E. ($n$ = 4). The gene-specific reagents were: HM74, forward primer, 5′-ACTACTATGTGCGGCGTTCAGAC-3′, and reverse primer, 5′-GGCGGTTCATGGCAAACA-3′; TaqMan probe, 5′-ACCAGCCGGCAAGGGATGTCC-3′; HM74A, forward primer, 5′-ACAACTATGTGAGGCGTTGGGA-3′, and reverse primer, 5′-TGGCGGTTCATAGCCAACA-3′; TaqMan probe, 5′-ATCAGCCGGCAAGGGATGTCC-3′; GPR81, forward primer, 5′-TCGGATGAAGAAGGCGACC-3′, and reverse primer, 5′-GCTGGGCAGGTAGCATGTG-3′; and TaqMan probe, 5′-TGAACACAATTGCCACCACCATGTG-3′.

*Cell Biology*—For transient transfections, HEK293T cells (HEK293 cells stably expressing the SV40 large T-antigen) were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum and 2 mM glutamine. The cells were seeded in 90-mm culture dishes and grown to 60–80% confluence (18–24 h) prior to transfection with vectors containing the relevant DNA inserts using LipofectAMINE reagent. For transfection, 9 μg of DNA was mixed with 30 μl of LipofectAMINE in 0.6 ml of Opti-MEM (Invitrogen) and was incubated at room temperature for 30 min prior to the addition of 1.6 ml of Opti-MEM. The cells were exposed to the LipofectAMINE/DNA mixture for 5 h, and 6 ml of 20% (v/v) fetal calf serum in Dulbecco's modified Eagle's medium was then added. The cells were harvested 48 h after transfection. Pertussis toxin treatment was carried out by supplementation into the medium at 50 ng $ml^{-1}$ for 16 h. All of the transient transfection studies involved co-transfection of receptor together with the $G_{i/o}$ G protein, $G\alpha_{o1}$.

For the generation of stable cell lines, the above method was used to transfect CHO-K1 cells seeded in six-well dishes grown to 30% confluence. These cells were maintained in Dulbecco's modified Eagle's medium/Ham's F-12 medium containing 10% fetal calf serum and 2 mM glutamine. 48 h post-transfection the medium was supplemented with 400 μg/ml G418 for selection of antibiotic resistant cells. Clonal CHO-K1 cell lines stably expressing HM74A were confirmed by [$^{35}$S]GTPγS binding measurements, following the addition of nicotinic acid.

*P2 Membrane Preparation*—Plasma membrane-containing P2 particulate fractions were prepared from cell pastes frozen at −80 °C after harvest. All of the procedures were carried out at 4 °C. The cell pellets were resuspended in 1 ml of 10 mM Tris-HCl and 0.1 mM EDTA, pH 7.5 (buffer A), and by homogenization for 20 s with a Ultra Turrax followed by passage (5 times) through a 25-gauge needle. The cell lysates were centrifuged at 1,000 × $g$ for 10 min in a microcentrifuge to pellet the nuclei and unbroken cells, and P2 particulate fractions were recovered by microcentrifugation at 16,000 × $g$ for 30 min. P2 particulate fractions were resuspended in buffer A and stored at −80 °C until required.

*[$^3$H]Nicotinic Acid Binding*—Saturation binding assays were carried out on plasma membrane-containing P2 particulate fractions from HEK293T cells transiently co-expressing HM74A and $G\alpha_{o1}$ using $^3$H-labeled nicotinic acid as described (5). Briefly, the membranes (10 μg/point) were incubated with increasing concentrations of [5,6-$^3$H]nicotinic acid (60 Ci/mmol; Biotrend) for 3 h at room temperature with agitation. The assay was performed in 50 mM Tris-HCl pH 7.4 binding buffer containing 1 mM $MgCl_2$ in a total volume of 500 μl. Nonspecific binding was assessed in the presence of 1 mM nicotinic acid. Membrane-bound ligand was recovered onto presoaked GF/B filters using a Brandel 48-well harvester, washed four times with 1 ml of ice-cold binding buffer, and measured by liquid scintillation counting. [$^3$H]Nicotinic acid (20 nM) displacement assays were performed using plasma membrane-containing P2 particulate fractions, prepared from either a stable CHO cell line expressing recombinant human HM74A or human adipocytes (Zen-Bio) as described (5) and above.

*[$^{35}$S]GTPγS Binding*—[$^{35}$S]GTPγS binding assays were performed at room temperature in 96-well format as described previously (7). Briefly, the membranes (10 μg/point) were diluted to 0.083 mg/ml in assay buffer (20 mM HEPES, 100 mM NaCl, 10 mM $MgCl_2$, pH 7.4) supplemented with saponin (10 mg/l) and preincubated with 10 μM GDP. Various concentrations of nicotinic acid or related molecules were added, followed by [$^{35}$S]GTPγS (1170 Ci/mmol; Amersham Biosciences) at 0.3 nM (total volume of 100 μl), and binding was allowed to proceed at room temperature for 30 min. Nonspecific binding was determined by the inclusion of 0.6 mM GTP. Wheat germ agglutinin SPA beads (Amersham Biosciences) (0.5 mg) in 25 μl of assay buffer were added, and the whole was incubated at room temperature for 30 min with agitation. The plates were centrifuged at 1500 × $g$ for 5 min, and bound [$^{35}$S]GTPγS was determined by scintillation counting on a Wallac 1450 Microbeta Trilux scintillation counter.

*Oocyte Methods*—Capped cRNA (20–50 ng/oocyte) was injected into stage V–VI defolliculated oocytes (8), and two microelectrode voltage clamp recordings were made 3–7 days post-RNA injection from a holding potential of −60 mV. The oocytes were superfused with ND96 solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM HEPES, pH 7.5 at 25 °C) at a flow rate of 2 ml $min^{-1}$. To facilitate the recording of GIRK1/GIRK4 potassium currents, the extracellular solution was switched to a high potassium solution (90 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM HEPES). The recording electrodes had a resistance of 0.5–1.0 MΩ when filled with 3 M KCl. The measurements of potassium currents were made from two batches of oocytes harvested on different days from different toads. Nicotinic acid was applied by addition to the superfusate, and cumulative concentration response curves were constructed for each individual oocyte tested.

*Yeast*—Human HM74A was subcloned into p426GPD adjacent to the promoter (9), transferred to pRS306, and integrated into the *ura3* locus of MMY16 (10). β-Galactosidase assays to measure *FUS1-lacZ* reporter gene induction were performed as described (11) except that nicotinic acid was omitted from the assay mix, and the substrate fluorescein-β-D-galactopyranoside (Molecular Probes; final concentration, 20 μM) was used in place of chlorophenol red-β-D-galactosidase.

## RESULTS

Nicotinic acid-mediated stimulation of [$^{35}$S]GTPγS binding was observed only in membranes from HEK293T cells co-transfected with the cDNA for HM74 and the G protein $G\alpha_{o1}$ (Fig. 1*A*). The nicotinic acid-induced stimulation was concentration-dependent and was found to be abolished following pretreatment of cells with pertussis toxin (50 ng $ml^{-1}$ for 16 h), suggesting that the effect was $G_{i/o}$ G protein-mediated (Fig. 1*B*). However, the half-maximal effector concentration for nicotinic acid was estimated to be in excess of 1 mM, over 1000-fold higher than that previously reported in rat adipose tissue and spleen membranes (5). Subsequent to the identification of HM74 as a low affinity receptor for nicotinic acid, we utilized a molecular biology approach to identify a novel paralogue of HM74, termed HM74A. Comparison of the nucleotide sequences of HM74A and HM74 revealed 15 base changes as well as a 5-nucleotide insertion at the 3′ end of the clone resulting in HM74A possessing a shortened C-terminal tail (Fig. 2*A*). The two receptors are highly homologous, displaying 96% identity at the protein level and differing by only 15 amino acids. A third gene, GPR81, previously identified by customized searching of the GenBank™ high throughput genomic sequences data base (12), was also found to exhibit substantial homology to HM74 and HM74A (57 and 58% amino acid sequence identity, respectively). Despite their high degree of similarity, HM74 and HM74A are not simply polymorphic variants but are separate genes being co-located with GPR81 at chromosome

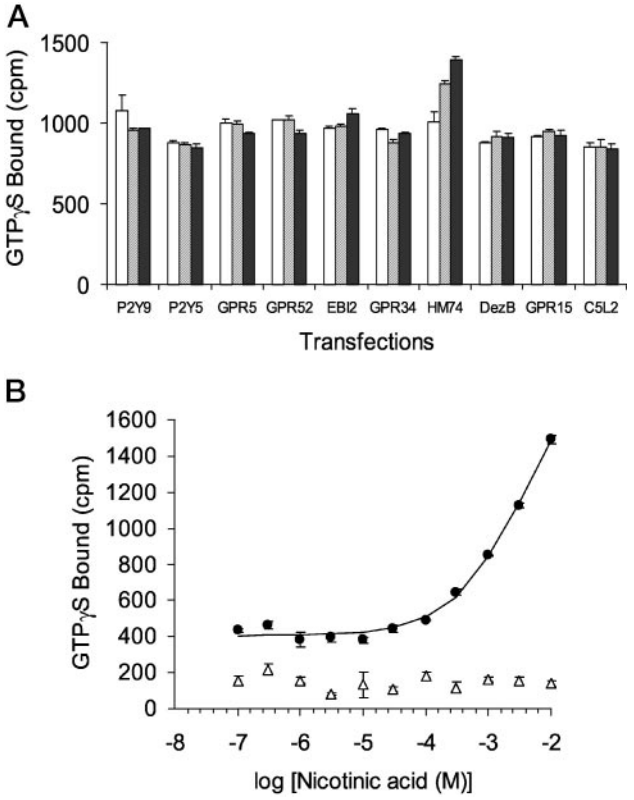


FIG. 1. **HM74 is a G protein-coupled receptor that responds to nicotinic acid.** *A*, application of 300 $\mu$M (*hatched columns*) and 1 mM (*filled columns*) nicotinic acid to membranes from HEK293T cells expressing a variety of orphan G protein-coupled receptors was found to stimulate [$^{35}$S]GTP$\gamma$S binding in membranes from cells expressing HM74 only (*open columns*, basal conditions). *B*, nicotinic acid stimulated a dose-dependent increase in [$^{35}$S]GTP$\gamma$S binding in cells expressing HM74 (*filled circles*) that was ablated by pretreatment with 50 ng ml$^{-1}$ pertussis toxin for 16 h prior to harvest (*triangles*). All of the transient transfection studies involved co-transfection of receptor together with the $G_{i/o}$ G protein, $G\alpha_{o1}$.

12q24.31 (part of this region of chromosome 12 is represented by accession number AC026362). Messenger RNA expression profiling of HM74 and HM74A using TaqMan quantitative reverse transcriptase-PCR analysis with probes designed and confirmed to discriminate between these two homologous receptors (data not shown) showed that both exhibited similar distribution patterns that were largely restricted to adipose tissue and spleen (Fig. 2*B*). Hence, the expression patterns of HM74A and HM74 are concomitant with that of a nicotinic acid receptor. Interestingly, GPR81 appears to be highly restricted to adipose tissue.

Expression of HM74A together with $G\alpha_{o1}$ in HEK293T cells gave robust concentration-dependent responses to nicotinic acid with a half-maximal concentration ($EC_{50}$ = 250 ± 27 nM) similar to that observed in rat adipose tissue and spleen membranes (5) (Fig. 3*A*). Conversely, GPR81 responded to nicotinic acid only at relatively high concentrations (10 mM). Expression of HM74A, but not HM74 or GPR81, also produced saturable specific binding of $^3$H-labeled nicotinic acid with an affinity ($K_d$ 95.8 ± 9.5 nM) similar to that recorded from rat adipose tissue and spleen membranes (5) (Fig. 3*B*). We have not been able to quantify the levels of expression of HM74 and GPR81 because of their low affinity for nicotinic acid. Next, we expressed HM74A and HM74 in *Xenopus* oocytes to study their coupling to the $G_i$-G protein-regulated potassium channels GIRK1 and GIRK4 (13). Concentration-dependent responses to nicotinic acid with a half-maximal concentration ($EC_{50}$) of 130 ± 50 nM were observed for HM74A (four oocytes). Responses to nicotinic

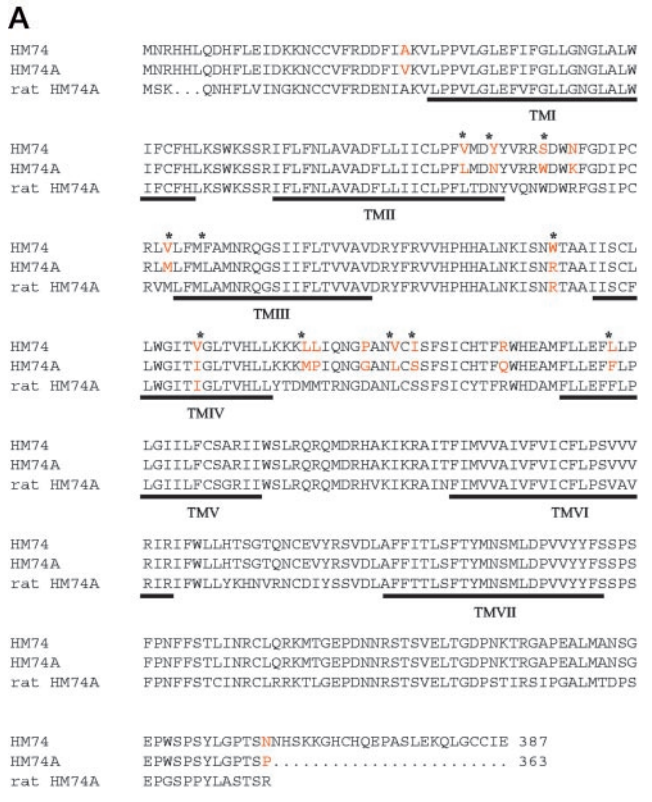


FIG. 2. **HM74 and HM74A are highly homologous proteins with similar mRNA tissue distribution patterns and chromosomal location.** *A*, amino acid sequences of human HM74 and human and rat HM74A aligned for comparison. Residues sharing identity between human and rat HM74A but not with HM74 are indicated with *asterisks*. *B*, TaqMan quantitative reverse transcriptase-PCR analysis of mRNA levels in human tissues. The cDNA from the reverse transcription of 1 ng of poly (A)$^+$ RNA from multiple tissues for four different nondiseased individuals was assessed for its HM74, HM74A, and GPR81 and housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), mRNA levels. The data are presented as the means (± S.E.) mRNA levels from four individuals for each tissue.

acid were also obtained at concentrations of 100 $\mu$M and above following expression of HM74 (5 oocytes) (Fig. 3*C*). Finally, we investigated coupling to the pheromone response pathway of

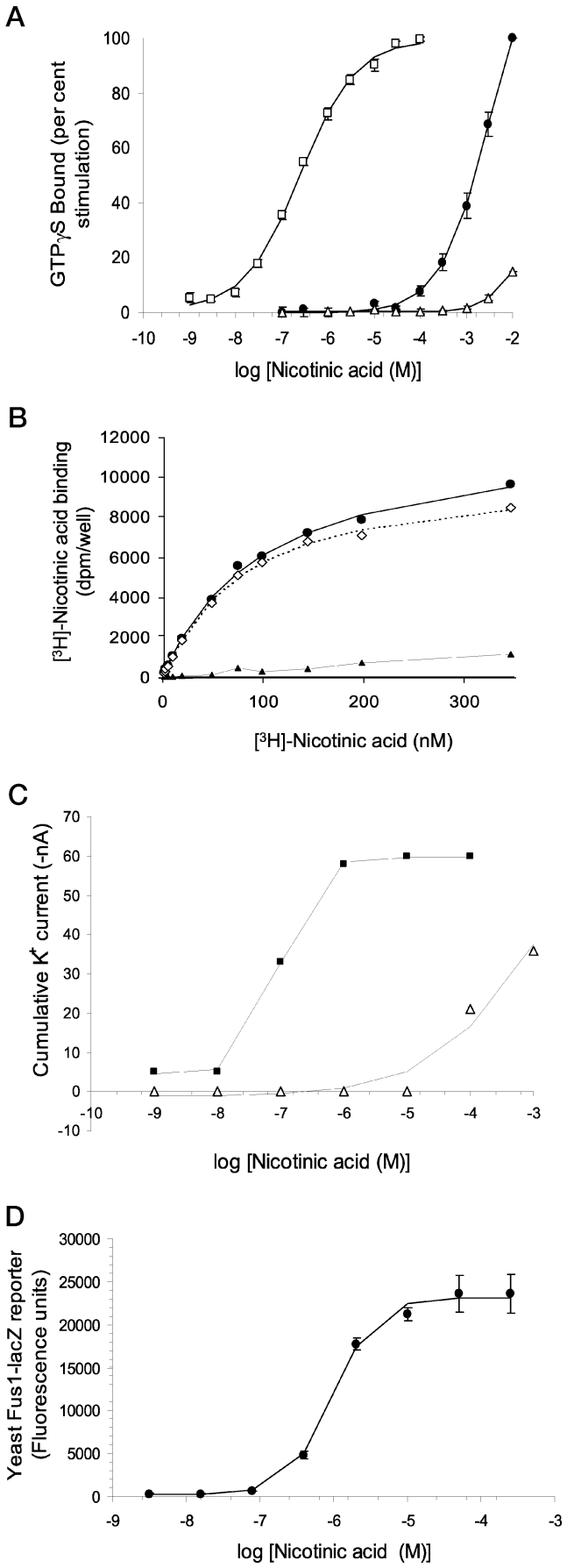


FIG. 3. **Radioligand binding studies and functional studies with nicotinic acid.** *A*, stimulation by nicotinic acid of [$^{35}$S]GTPγS binding in HM74-expressing (*filled circles*), HM74A-expressing (*squares*), and GPR81-expressing (*triangles*) membranes. The data are normalized to the peak response to nicotinic acid at HM74A. All of the transient transfection studies in HEK293T cells involved co-transfection of receptor together with $G\alpha_{o1}$. *B*, radioligand binding analysis with $^{3}$H-labeled nicotinic acid by saturation isotherm of human HM74A. *Filled circles*, total binding; *filled triangles*, nonspecific binding; *diamonds*, specific binding. The results shown exemplify $n = 3$, with each experiment performed in duplicate. *C*, oocyte data. Representative cumulative concentration response curves to nicotinic acid are shown for different individual oocytes expressing either HM74 (*triangles*) or HM74A (*filled squares*) in combination with the potassium channels GIRKs 1 and 4. *D*, activation of the yeast pheromone response pathway by nicotinic acid in yeast expressing recombinant human HM74A. The results show the means of two independent yeast isolates, each determined with $n = 6$.

TABLE I
*Comparison of functional activity of nicotinic acid analogues at human and rat HM74A*

Potencies are given as means of the $EC_{50}$ obtained from three separate experiments (± S.E.).

| Compound | $EC_{50}$ | |
|---|---|---|
| | Human HM74A | Rat HM74A |
| | μM | |
| Nicotinic acid | 0.25 ± 0.027 | 0.4 ± 0.05 |
| 3-Pyridine-acetic acid | 5.5 ± 0.95 | 13.7 ± 3.2 |
| 5-Methylnicotinic acid | 8.7 ± 4.0 | 22.4 ± 5.2 |
| Nicotinamide | >1000 | >1000 |
| Nicotinuric acid | >1000 | >1000 |
| 3-Cyanopyridine | inactive | inactive |

*Saccharomyces cerevisiae*, measuring receptor activation with a reporter gene. Using nicotinic acid-free growth medium, we demonstrated concentration-dependent activation of HM74A in response to nicotinic acid ($EC_{50}$ = 904 ± 28 nM; Fig. 3*D*). The optimal agonist responses were observed with chimeric yeast/mammalian Gα subunits having the C-terminal 5 amino acids of $G_i$ or the promiscuous Gα, $G_{16}$ (Fig. 3*D* and data not shown) (11). The yeast data confirm that HM74A is sufficient to confer the nicotinic acid response, because these cells lack endogenous G protein-coupled receptors capable of activating this pathway.

A number of nicotinic acid analogues were employed to characterize HM74A using the [$^{35}$S]GTPγS binding assay (Table I). Similar rank orders of potency were found at HM74A compared with those previously described in native rat tissue (5), whereas all of the analogues displayed either no or very weak activity at HM74 (data not shown). All of the analogues were also inactive at GPR81 (data not shown). Furthermore, we also cloned the rat orthologue of HM74A, which was found to exhibit 82% identity at the protein level with its human counterpart (Fig. 2*A*). As expected, no significant pharmacological differences were observed between recombinantly expressed rat and human HM74A (Table I).

Acipimox (Olbetam) and Acifran (AY-25,712) are two molecules that have been reported to produce a pharmacological profile resembling that of nicotinic acid in rat and human studies (14–17) (Fig. 4*A*). Using the [$^{35}$S]GTPγS binding assay, we found that Acipimox was a full agonist at HM74A ($EC_{50}$ = 6 ± 1 μM) and exhibited weak activity at HM74 and no activity at GPR81. Acifran acted as a full and relatively potent agonist at both HM74A ($EC_{50}$ = 2.1 ± 0.2 μM) and HM74 ($EC_{50}$ = 20 ± 4 μM) but showed no significant agonism at GPR81 up to 1 mM (Fig. 4*B*). Acifran also activated HM74A in yeast ($EC_{50}$ = 3.0 ± 0.08 μM). The fact that Acifran has been identified as a high affinity ligand for HM74 (Fig. 4*B*) strongly suggests that our transfection system is sufficiently efficient to allow agonist profiling. Furthermore, the signal to noise ratios observed with Acifran at both HM74A and HM74 are similar, which suggests

that these receptors are expressed at similar levels. In addition, we have expressed HM74A and HM74 in a range of different systems (mammalian, yeast, and oocyte), and in all of these expression systems there is an ~1000-fold separation in the potency of nicotinic acid, suggesting that this is a real observation.

Acifran and Acipimox were included in a group of molecules with structural or pharmacological similarities with nicotinic acid that were tested in a [$^3$H]nicotinic acid displacement assay performed in membranes from either a stable CHO cell line expressing recombinant human HM74A or human adipocytes (Table II). The rank order of potency for the displacement of [$^3$H]nicotinic acid binding was nicotinic acid > 5-methyl pyrazole-3-carboxylic acid = pyridine-3-acetic acid > Acifran > 5-methyl nicotinic acid = Acipimox ≫ nicotinuric acid = nicotinamide. This rank order of potency was the same in both the stable CHO cell line expressing recombinant human HM74A and human adipocytes.

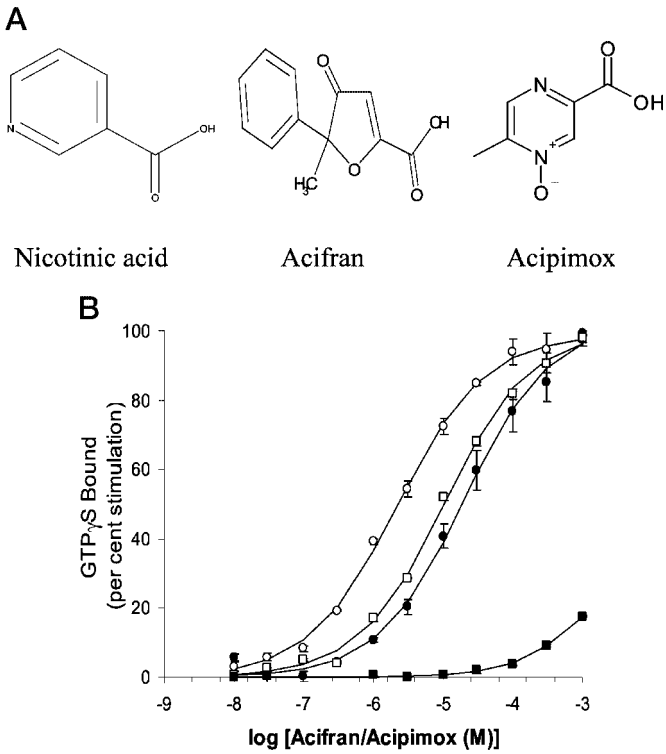


FIG. 4. **Acifran and Acipimox activity at HM74 and HM74A.** *A*, a comparison of the structures of nicotinic acid, Acipimox and Acifran. *B*, stimulation by Acifran (*circles*) and Acipimox (*squares*) of [$^{35}$S]GTPγS binding in HM74-expressing (*filled circles* and *filled squares*) and HM74A-expressing (*open circles* and *open squares*) membranes. The data are normalized to the peak responses to Acifran and Acipimox at HM74A. All of the transient transfection studies involved co-transfection of receptor together with the $G_{i/o}$ G protein, $G\alpha_{o1}$.

## DISCUSSION

HM74 was identified as a low affinity receptor for nicotinic acid following the screening of a panel of orphan receptors selected because of their tissue expression profile. HM74 is an orphan receptor that had been previously cloned from a cDNA library derived from human monocytes (18). The half-maximal effector concentration for nicotinic acid at HM74 was estimated to be in excess of 1 mM, ~1000-fold higher than that previously reported in membranes produced from rat adipose tissue or spleen (5). We considered three possible explanations for this discrepancy in nicotinic acid potency. First, close homologues of HM74 may act as higher affinity nicotinic acid receptors. Second, because G protein-mediated nicotinic acid effects on native tissue have almost always been recorded from rat, variation between human and rodent receptors may explain this phenomenon. Finally, differences in the pharmacological integrity of the recombinantly expressed receptor and its endogenously expressed counterpart may explain potency changes.

A molecular biology approach resulted in the identification of a novel paralogue of HM74, termed HM74A. Despite their high degree of similarity, HM74 and HM74A are not simply polymorphic variants but are separate genes being co-located with GPR81 at chromosome 12q24.31. TaqMan analysis confirmed that the expression pattern of HM74A was very similar to HM74. When expressed in a variety of test systems, HM74A was confirmed as a high affinity receptor for nicotinic acid. The activity and affinity of nicotinic acid was in good agreement with that previously reported in the literature (5). Furthermore, following the cloning of the rat orthologue of HM74A, we found no significant pharmacological differences between nicotinic acid derivatives tested against either human or rat HM74A. The murine variant of HM74A, PUMA-G, was recently reported to be an interferon γ-inducible gene in macrophages, suggesting a possible role in macrophage function (19). This finding is further supported by a recent report describing a nicotinic acid receptor in a murine macrophage cell line (20). Based on the TaqMan data generated for the distribution of human HM74A, there appears to be little or no expression in macrophages (Fig. 2*B*). This may indicate that species differences in the distribution of HM74A exist or is a reflection of the activation state of the macrophages used in this experiment. It will be of interest to determine whether the expression of HM74A can be up-regulated in human macrophages following incubation with interferon γ.

In the [$^3$H]nicotinic acid displacement assay, both the absolute potency and the rank order of potency of the HM74A ligands studied was the same, whether tested against the stable CHO cell line expressing recombinant human HM74A or human adipocytes. These data strongly suggest that HM74A is the $G_i$-G protein-coupled nicotinic acid receptor on human adipocytes. Acipimox and Acifran have also been identified as full

TABLE II

*A comparison of the potency of a range of HM74A agonists at displacing [$^3$H]nicotinic acid from either CHO membranes expressing recombinant human HM74A or human adipocyte membranes*

| | Mean $IC_{50}$ ± S.E. for displacement of [$^3$H]nicotinic acid from CHO membranes expressing human HM74A | Mean $IC_{50}$ ± S.E. for displacement of [$^3$H]nicotinic acid from human adipocyte membranes |
|---|---|---|
| | μM | μM |
| Nicotinic acid | 0.081 ± 0.003 ($n$ = 14) | 0.079 ± 0.003 ($n$ = 8) |
| 5-Methyl pyrazole-3-carboxylic acid | 0.525 ± 0.041 ($n$ = 11) | 0.518 ± 0.022 ($n$ = 4) |
| Pyridine-3-acetic acid | 0.535 ± 0.052 ($n$ = 6) | 0.553 ± 0.061 ($n$ = 4) |
| Acifran | 1.12 ± 0.052 ($n$ = 8) | 0.833 ± 0.050 ($n$ = 4) |
| 5-Methyl nicotinic acid | 4.12 ± 0.063 ($n$ = 7) | 3.68 ± 0.66 ($n$ = 3) |
| Acipimox | 5.10 ± 0.039 ($n$ = 6) | 4.34 ± 0.51 ($n$ = 4) |
| Nicotinuric acid | 70.5 ± 2.76 ($n$ = 7) | 63.8 ± 6.20 ($n$ = 3) |
| Nicotinamide | 92.3 ± 4.72 ($n$ = 7) | 75.3 ± 3.41 ($n$ = 3) |

agonists at HM74A. These compounds have also been reported to produce a pharmacological effect resembling that of nicotinic acid in rat and human studies (14–17). The other compounds identified that displace nicotinic acid from HM74A, 5-methyl pyrazole-3-carboxylic acid, pyridine-3-carboxylic acid, and 5-methyl nicotinic acid, have all previously been shown to inhibit adipocyte lipolysis (2, 23). Nicotinamide, which unlike nicotinic acid produces no alteration in lipoprotein profiles (22), acted only as a very weak agonist at HM74A. Indeed, nicotinamide was ~1000-fold less potent than nicotinic acid, a level of activity that could be due to contaminant nicotinic acid (*e.g.* 0.1%). It would appear that activation of HM74A would account for the inhibition of lipolysis observed with these compounds. Therefore, of the compounds that have been tested in man, it would appear that potency at HM74A is linked with their efficacy at normalizing lipoprotein profiles.

We have demonstrated that HM74A is a high affinity receptor for nicotinic acid and believe that this receptor is a likely candidate as a molecule target for the beneficial therapeutic effects observed with nicotinic acid. Nicotinic acid is an effective therapeutic agent; however, it has to be administered at high doses and has a characteristic side effect profile defined by intense, but transient, prostaglandin-mediated cutaneous vasodilation ("flushing") that affects patient compliance (21, 22).

Unlike HM74A, we were unable to identify rodent orthologues of HM74 using conventional gene cloning strategies and bioinformatics searches. This suggests that in humans HM74 may be the result of a relatively recent gene duplication event. Furthermore, of the compounds tested, only Acifran exhibited activity at HM74. In fact, Acifran is the first molecule that we have identified to date that exhibits significant potency at HM74. Because of the high degree of homology between HM74A and HM74 and the existence of highly selective ligands, site-directed mutagenesis may be a useful strategy in determining which amino acid residues play a key role in ligand binding. Indeed, 11 amino acid residues are conserved in human and rat HM74A but not in HM74 (Fig. 2*A*). Such residues may play key roles in determining the differences in ligand binding affinities between HM74A and HM74. The identification of HM74A as a molecular target for nicotinic acid will facilitate the discovery of potent and selective ligands for this receptor and may expedite the discovery of improved antihyperlipidemic drug molecules.

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