Cloning and characterization of the hamster and guinea pig nicotinic acid receptors

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Abstract In this study, we present the identification and characterization of hamster and guinea pig nicotinic acid receptors. The hamster receptor shares ~80–90% identity with the nucleotide and amino acid sequences of human, mouse, and rat receptors. The guinea pig receptor shares 76–80% identity with the nucleotide and amino acid sequences of these other species. [^{3}H]nicotinic acid binding affinity at guinea pig and hamster receptors is similar to that in human (dissociation constant = 121 nM for guinea pig, 72 nM for hamster, and 74 nM for human), as are potencies of nicotinic acid analogs in competition binding studies. Inhibition of forskolin-stimulated cAMP production by nicotinic acid and related analogs is also similar to the activity in the human receptor. Analysis of mRNA tissue distribution for the hamster and guinea pig nicotinic acid receptors shows expression across a number of tissues, with higher expression in adipose, lung, skeletal muscle, spleen, testis, and ovary.—Smith Torhan, A., B. Cheewatrakoolpong, L. Kwee, and S. Greenfeder. **Cloning and characterization of the hamster and guinea pig nicotinic acid receptors.** ***J. Lipid Res.*** **2007.** 48: **2065–2071.**

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Niacin (nicotinic acid) has been used to treat dyslipidemias since the 1950s, when it was discovered that the B-vitamin reduces plasma cholesterol levels (1). In the clinic, niacin has been used to reduce plasma LDL levels, increase HDL, decrease plasma triglycerides, and decrease lipoprotein [a] in patients with or at risk for cardiovascular disease (2–4). The benefits of niacin therapy are limited by poor patient compliance as a result of undesirable side effects. The most common side effect is flushing or cutaneous vasodilation accompanied by a burning sensation, itching, and occasionally hypotension. Pretreatment with aspirin or indomethacin reduces the incidence of flushing for most patients (5, 6).

In 2003, several groups reported the identification of a receptor for nicotinic acid, GPR109A (HM74A in humans, PUMA-G in mice) (7–9). GPR109A is a $G_{i\alpha}$-linked G protein-coupled receptor expressed primarily in adipose and immune cells. GPR109A inhibits cAMP production through the inhibition of adenylate cyclase and exhibits the expected pharmacological profile for a nicotinic acid receptor. Nicotinic acid, Acipimox, and Acifran bind to and activate GPR109A at physiologically relevant concentrations, whereas nicotinamide does not. In mice lacking GPR109A, niacin's ability to inhibit FFA release is lost (8). Additionally, in recent reports, GPR109A has been shown to mediate the flushing response caused by nicotinic acid in mice and to stimulate prostaglandin production in immune cells (10, 11). This indicates that GPR109A (HM74A/PUMA-G) is indeed the receptor through which therapeutic doses of niacin act.

GPR109A is closely related to two other G protein-coupled receptors that are also expressed in human adipose tissue, GPR109B (HM74) and GPR81 (9). All three genes are located on chromosome 12 in humans, 12q24.31 (9). GPR109B is a low-affinity receptor for nicotinic acid, with activation occurring in the 10–30 μM range. GPR109B is not found in rats or mice, whereas both GPR109A and GPR81 are (7, 9). The endogenous function and ligands for these three related receptors are unknown at present.

In this study, orthologs of GPR109A were cloned from the genomic DNA of hamster and guinea pig. The mRNA expression levels in different tissues were examined. The cloned receptors were also characterized by radioligand binding and functionally by measurement of adenylyl cyclase activity. No evidence of a GPR109B homolog in either hamster or guinea pig genomic DNA was found.

MATERIALS AND METHODS

Materials

Nicotinic acid and various analogs were purchased from Sigma (St. Louis, MO), Maybridge (Trevillet, Tintagel, Cornwall, UK), Onbio (Richmond Hill, Ontario, Canada), and Tocris Bioscience (Ellisville, MO).

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Abbreviations: NAR, nicotinic acid receptor.

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cDNA cloning of a full-length guinea pig and hamster GPR109A (nicotinic acid receptor) receptor

Guinea pig and hamster genomic DNA and cDNA from various tissues were purchased from Biochain Institute, Inc. (Hayward, CA). The Advantage 2 PCR kit was purchased from Clontech (Mountain View, CA). All primers were synthesized by Invitrogen. A series of primer pairs based on the consensus of human, mouse, and rat GPR109A failed to amplify a specific band from hamster or guinea pig genomic DNA. Therefore, selected primers in the conserved region upstream of the start codon of known GPR109A sequences were analyzed for specificity by performing a Basic Local Alignment Search Tool search against the GenBank nucleotide database. A consensus primer located 152 bases upstream of the human GPR109A start codon was chosen as a forward primer (GATTTCGTAGTTTCCTGGTAAC). Oligo 6.63 software (Cascade, CO) was subsequently used to search for appropriately matched reverse primers. One of the reverse primers, located 670 bases downstream of the human GPR109A start codon (GTGATGGCTCTCTTGATCTTGGC), yielded specific guinea pig and hamster GPR109A sequences in the 5′ region that include the start codons.

To obtain the 3′ end sequences of guinea pig and hamster GPR109A, a TOPO Walker kit (Invitrogen) was used. Three forward primers for each species were designed based on the known 5′ end sequence (**Table 1**, primer set A). Genomic DNA from each species was digested with *Pst*I enzyme and ligated to a linker provided with the kit. Nested PCR was subsequently carried out using GPR109A-specific primers together with linker primers supplied with the kit. The final PCR products were cloned into pCR4 TOPO for sequencing.

The sequences for both 5′ and 3′ fragments of GPR109A from various independent PCRs were assembled using Gene Jockey II software (Biosoft, Cambridge, UK) to obtain a consensus full-length sequence for both guinea pig and hamster nicotinic acid receptor (NAR). The CLUSTAL algorithm (12) was used to perform multiple sequence alignments of both cDNA and protein sequences using Lasergene software (DNASTAR, Inc., Madison, WI).

Construction of a plasmid for GPR109A expression

A set of primers spanning the coding sequence of either guinea pig or hamster GPR109A (Table 1, primer set B) was used to amplify genomic DNA to obtain a coding sequence. PCR products were cloned into pcDNA3.1D/V5-His TOPO (Invitrogen) and confirmed by double-strand sequencing (Sequetech).

Screening for the expression of a GPR109B (HM74) homolog

A set of primer pairs spanning the region surrounding the stop codon of either guinea pig or hamster GPR109A (Table 1, primer set C) was used to amplify cDNA and genomic DNA from both species. The PCR products were cloned into pCR4 TOPO and sequenced.

Generation of CHO-K1 cells stably expressing GPR109A (NAR)

CHO-K1 cells (American Type Culture Collection, Manassas, VA) were cultured in F12-K medium containing 10% fetal bovine serum (Invitrogen) and maintained at 37°C in a humidified incubator supplied with 5% CO_2. Cells at 90% confluence were transfected with guinea pig and hamster NAR plasmids using Lipofectamine 2000 (Invitrogen). Clonal cell lines were selected by limiting the dilution of transfected cells (48 h after transfection) using a growth medium supplemented with 4 mg/ml G418 (Invitrogen).

RT-PCR and real-time PCR

Total RNA was isolated from hamster (LVG strain from Charles River Laboratories, Wilmington, MA) or guinea pig (Charles River Laboratories) tissues using Totally RNA (Ambion, Austin, TX).

For RT-PCR, 2.5 μg of total RNA were reverse-transcribed using the SuperScript III First Strand Synthesis System for RT-PCR, oligo(dt) (Invitrogen). Five microliters of the first strand cDNA was used for real-time PCR using species-specific NAR primers (Table 1, primer set D). The cDNA and primers were added to the SYBR Green Master Mix (Applied Biosystems, Foster City, CA). The PCR program used was the following: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 min and 60°C for 1 min. GAPDH was used as an internal control for data normalization. The primers for GAPDH were purchased from Clontech. The concentration of each transcript (expressed as picograms of NAR per nanogram of GAPDH cDNA) was quantified using the comparative cycle threshold method on an ABI Prism 7700 Sequence Detection System (Applied Biosystems).

Northern blot analysis

Ten micrograms of total RNA from epidermal adipose tissue was loaded and electrophoresed onto an agarose/formaldehyde

TABLE 1. List of primers used

Primer	Sequence	
	Guinea Pig	Hamster
Group A		
Extension	CAGGCCCACCATGAGTTCC	GCTGAACCGCACGTT
First PCR	TGGCCTGGCGCTGTG	CCGGTGTTGGGATTGGA
Second PCR	GCCCTTCCTGACCGACA	ACATCCCTTGCCGTCTGATGCT
Group B		
Forward	CACCATGAGTTCCCAGGGTTACC	CACCATGACCAAGCAGAACCATTTTCTGGAG
Reverse	TTAACGCGAGCTGGGGTCC	GACTCCACCACGGCGTTTC
Group C		
Forward	CAGCTTCAGCATCTGCGACTC	GCACGACTGTGGCATCTACTC
Reverse	CTCGCTTCCTGGTGATCGTC	GCAGGCTTGGGAGACTAAGA
Group D		
Forward	GCCATCATCCTGTTCTGCTCGGTCAG	GCAGCCATCATCTCTTGCTTCTTG
Reverse	CCTCCGCAGGCAGCGGTTGAAGAGC	CTTCCGAAGGCAGCGGTTGATGCACG

Primer groups are as follows: A, 3′ end cloning primers; B, full-length expression cloning primers; C, GPR109B screening primers; D, real-time PCR primers. All sequences are listed in the sense direction.

gel and transferred onto a BrightStar-Plus membrane (Ambion; catalog number 10100-10104) according to the NorthernMax Kit (Ambion; catalog number 1940). RNA Millennium Marker (Ambion; catalog number 7151) was used as a size marker. The RNA was cross-linked to the membrane using a Stratalinker (Stratagene). Northern blots were prehybridized and hybridized according to the NorthernMax Kit with a full-length PCR product of either hamster or guinea pig cDNA. The cDNAs were labeled each with 50 μCi of [^{32}P]CTP Redivue (Amersham) according to the Rediprime II Random Prime Labeling System (Amersham; catalog number PRN1633). Hybridization was performed overnight, and low- and high-stringency washes were performed according to the NorthernMax Kit. The blots were wrapped in plastic wrap and exposed to Kodak Biomax XAR film.

Membrane preparation

Confluent cells from stably transfected cell lines were harvested with cell dissociation buffer (Invitrogen). All subsequent steps were carried out at 4°C. Cell pellets were suspended in a hypotonic buffer containing 10 mM Tris-HCl, pH 7.4, 1 mM $MgCl_2$, and 100 μM Pefabloc for 15 min on ice. The cells were homogenized using a glass Dounce homogenizer. The resulting homogenate was centrifuged at 100,000 *g* for 60 min. The pellet was resuspended in 10 mM Tris-HCl, pH 7.4, 1 mM $MgCl_2$, 125 mM sucrose, 100 μM Pefabloc, and 10% glycerol. The membrane suspension was homogenized again using a Dounce homogenizer. Small aliquots of membranes were frozen in liquid nitrogen and stored at −80°C.

Radioligand binding

For saturation binding, 5–300 nM [5,6-^{3}H]nicotinic acid (50 Ci/mmol; American Radiolabeled Chemicals, Inc., St. Louis, MO, or Amersham Biosciences, Piscataway, NJ, custom label) was incubated with 30 μg of human, hamster, or guinea pig GPR109A membranes for 3.5 h at room temperature with moderate shaking. Assays were carried out in 50 mM Tris-HCl, pH 7.4, 1 mM $MgCl_2$, and 0.02% (w/v) CHAPS (9) in a total volume of 200 μl. Nonspecific binding was determined in the presence of 1 mM nicotinic acid. Membrane-bound ligand was separated from unbound ligand by filtration through GF/B unifilter plates (Perkin-Elmer Life and Analytical Sciences, Wellesley, MA), presoaked for 1 h in cold 0.3% polyethyleneimine solution on a 96-well Brandel harvester. Filters were washed five times with 1 ml per well ice-cold 50 mM Tris-HCl, pH 7.4, and 1 mM $MgCl_2$ buffer. Filter plates were dried and Microscint-0 scintillant (Perkin-Elmer) was added. Samples were counted on a Packard TopCount (Perkin-Elmer). Data were analyzed using GraphPad Prism.

For competition binding, 60 nM [^{3}H]nicotinic acid was incubated with human, hamster, or guinea pig GPR109A membranes as described above in the presence of various concentrations of competing test compounds.

cAMP determinations

Intracellular cAMP levels were measured in stably transfected CHO-K1 cells expressing the human, hamster, or guinea pig GPR109A using the MSD cyclic AMP assay kit (Meso Scale Discovery, Gaithersburg, MD) according to the manufacturer's instructions. Briefly, cells expressing the human, hamster, or guinea pig GPR109A were incubated in PBS containing 3 μM forskolin and 0.25 mM 3-Isobutyl-1-methylxanthine (final concentrations) for 30 min in the presence or absence of various compounds. Plates were read on a SECTOR Imager (Meso Scale Discovery). The data were analyzed using GraphPad Prism.

RESULTS

cDNA cloning of a full-length guinea pig and hamster GPR109A (NAR) receptor

Using the strategies described above, two full-length cDNA sequences coding for guinea pig NAR and hamster NAR were obtained (GenBank accession numbers EF185820 and EF185821). For the guinea pig, a total of 1,290 bp of NAR sequence consisting of a 1,089 bp coding sequence, a 151 bp 5′ untranslated region, and a 51 bp 3′ untranslated region was isolated. For the hamster NAR sequence, a total of 1,510 bp of NAR sequence was obtained, 1,080 bp of which was a coding sequence; the lengths of the 5′ and 3′ untranslated regions were 157 and 273 bp, respectively. Based on multiple sequence alignment of the cDNA coding regions (data not shown), guinea pig cDNA has 78.9, 80.5, 78.4, and 79.3% identity with that of hamster, human, mouse, and rat, respectively. Hamster cDNA has 82.6, 89.3, and 89.2% identity with the sequences of human, mouse, and rat, respectively. Subsequent to our work, a tentative genomic sequence for guinea pig was submitted to GenBank in November 2005 (accession number AAKN01762938), which contains the GPR109A sequence as identified in this report.

Figure 1 shows an alignment of GPR109A proteins from various species. The prediction of transmembrane helices in each protein was carried out using the TMHMM algorithm at http://www.cbs.dtu.dk/services/TMHMM-2.0. The guinea pig NAR protein is more distantly related to that of other rodents and human (~76–77% identity), whereas the hamster NAR sequence is more highly related to the mouse, rat, and human sequences (82–90% identity). As with mouse and rat, both guinea pig and hamster have N termini that are three amino acids shorter than that of human (Fig. 1). Guinea pig has a two amino acid insertion (Y-171, L-172) in extracellular domain 2, and hamster has a deletion (P-323) in its C-terminal cytoplasmic domain (Fig. 1).

As shown in **Fig. 2** both guinea pig and hamster show an ~4.2 kb RNA species upon Northern blot analysis of RNA from adipose tissue. In guinea pig, it appears that this is the only species of RNA detected. Two additional bands are visible in the hamster RNA, in the range of 1–1.5 kb. The exact nature of these RNA species is unknown.

Tissue distribution of hamster and guinea pig GPR109A (NAR)

The tissue distribution of hamster and guinea pig GPR109A was determined by quantitative real-time PCR using hamster- or guinea pig-specific GPR109A primers (Table 1, primer set D) and mRNA isolated from various tissues. The distribution in both species is consistent with that seen in other species (7–9). Expression is fairly widespread, with highest expression in lung, spleen, testis, and adipose in hamsters (**Fig. 3A**) and in spleen, lung, stomach, skeletal muscle, ovary, and adipose in guinea pigs (Fig. 3B).

GPR109B (HM74) homolog

GPR109B is a highly related homolog of GRP109A (95% identity at the amino acid level) that to date has only been found in humans (7, 9) and chimpanzees (based on in

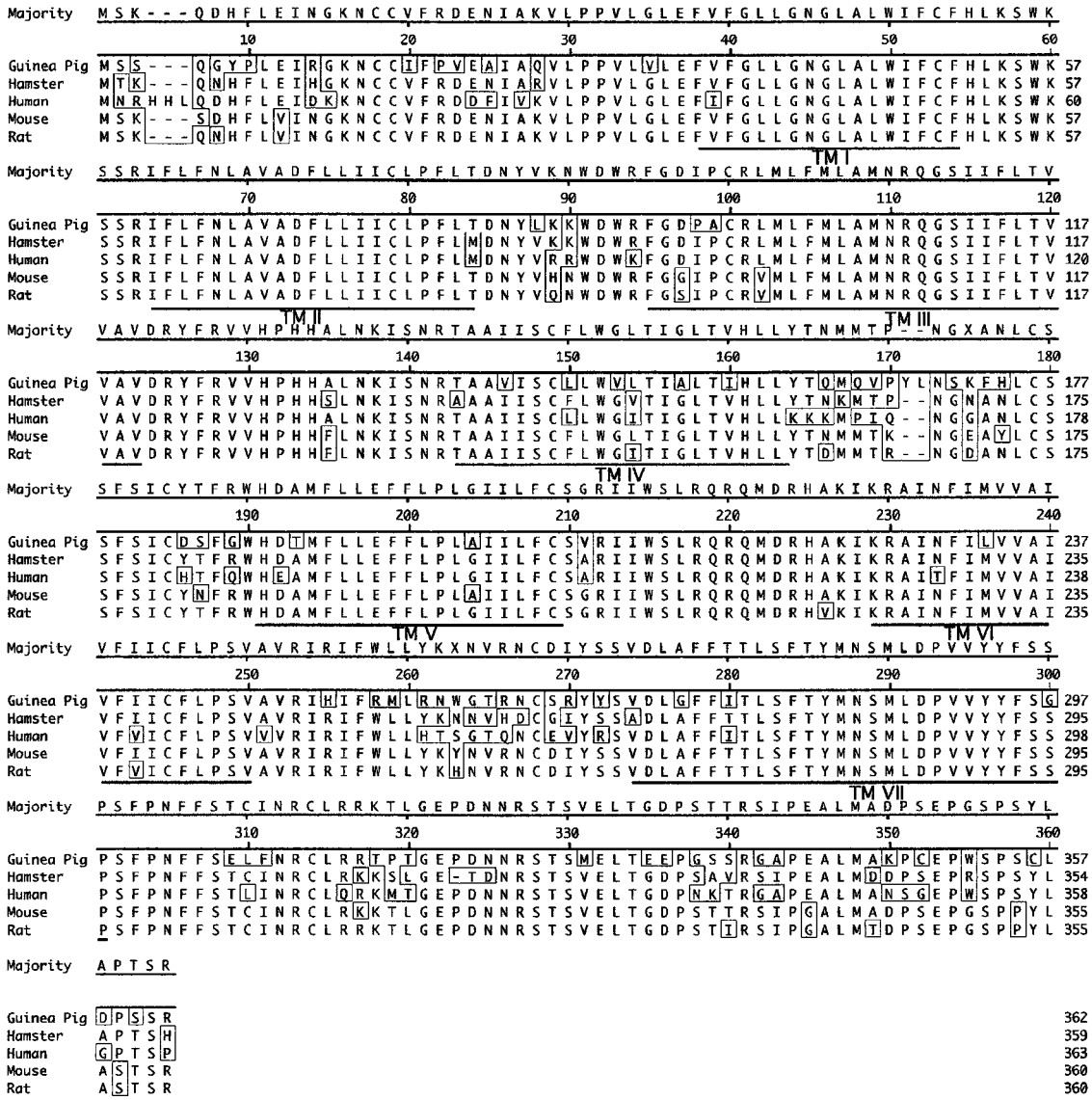

Fig. 1. Alignment of guinea pig, hamster, human, mouse, and rat GPR109A amino acid sequences. Transmembrane domains (TMI–TMVII) are underlined. Residues that differ from the consensus are boxed.

silico genomic sequence analysis; data not shown) and that apparently has arisen from gene duplication. GPR109B has a 24 amino acid extension on the C terminus that results from the deletion of 5 bp before the stop codon of GPR109A. The DNA sequences of human GPR109A and GPR109B around this 5 bp difference are nearly identical. Therefore, primers flanking the stop codons of the guinea pig and hamster GPR109A were used to generate PCR fragments from the corresponding genomic DNA and cDNA. Examination of PCR products by direct sequencing revealed no evidence of a GPR109B ortholog in either guinea pig or hamster. In addition, there is no GPR109B gene sequence found in the guinea pig genome sequence deposited in GenBank.

Radioligand binding

Membranes from stable cell lines expressing the hamster, guinea pig, or human GPR109A were used to compare saturation binding of [^{3}H]nicotinic acid and the relative affinities of nicotinic acid analogs between the three species (**Fig. 4A, B**). Saturation binding of [^{3}H]nicotinic acid yielded similar dissociation constants for the three receptors: 74, 72, and 121 nM for human, hamster, and guinea pig, respectively (**Table 2**, Fig. 4A). These results are similar to those previously reported for cloned receptors of human, mouse, and rat GPR109A, at 63.1, 45.3, and 60.8 nM, respectively (7). In competition binding experiments, the hamster and guinea pig nicotinic acid receptors again showed similar binding affinities to those of human for nicotinic acid and related compounds (Fig. 4B, **Table 3**). The exceptions were 5-methyl-2-pyrazine carboxylic acid, 5-methyl nicotinic acid, and 6-methyl nicotinic acid, which were less potent at the guinea pig receptor. The inhibition constants (K_i) for nicotinic acid were 110, 97, and 177 nM for human, hamster, and guinea pig, respectively. The human and hamster GPR109A

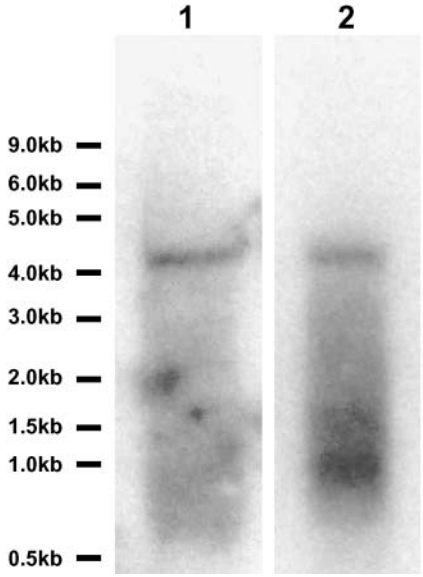

Fig. 2. Northern blot analysis of total RNA isolated from guinea pig (lane 1) and hamster (lane 2) adipose tissue. Molecular mass size standards are indicated.

K_i values for 5-methyl-2-pyrazine carboxylic acid were 3.53 and 2.99 μM, respectively, whereas the guinea pig K_i was 30.2 μM (Table 3). Nicotinamide, which has none of the lipid-modifying effects of niacin (13), binds poorly, with a K_i of 30 μM or greater in all three species.

Functional analysis

Nicotinic acid and related compounds were tested for their ability to inhibit forskolin-stimulated cAMP production in stably transfected cells expressing the hamster, guinea pig, or human GPR109A (**Table 4**). The human and hamster EC_{50} values for nicotinic acid were very similar, at 50 and 74 nM, respectively. For the guinea pig receptor, which is more distantly related to the other two, the EC_{50} for nicotinic acid was 329 nM (Fig. 4C, Table 4). Acifran was slightly more potent than nicotinic acid in hamster (EC_{50} = 46 nM) and guinea pig (EC_{50} = 249 nM), but not in human (EC_{50} = 123 nM). The other compounds tested showed a similar order of potency between the three species, with the exceptions of 5-methyl-2-pyrazine carboxylic acid, 5-methyl nicotinic acid, and 6-methyl nicotinic acid. As in the binding studies, these compounds were much less potent in the guinea pig than in hamster or human (Table 4). Nicotinamide had no effect on forskolin-stimulated cAMP accumulation.

DISCUSSION

This study presents the cloning of the hamster and guinea pig nicotinic acid receptors (GPR109A) from genomic DNA and the subsequent characterization of these receptors expressed in stable cell lines. The GPR109A

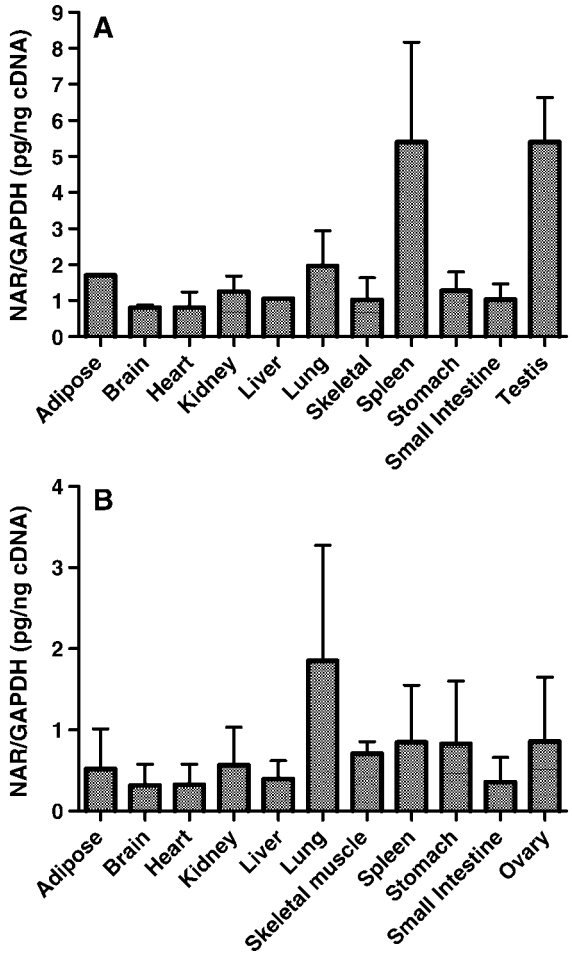

Fig. 3. Tissue distribution of hamster (A) and guinea pig (B) GPR109A was determined by quantitative real-time PCR using species-specific primers. Data were normalized to GAPDH levels. Error bars indicate SEM.

ortholog in hamster is closely related to its human, rat, and murine counterparts based on cDNA and amino acid sequence comparisons, whereas that of the guinea pig is more distantly related. As has been reported for rat and mouse, GRP109A appears to be the sole nicotinic acid receptor in both hamster and guinea pig, because no evidence of a GPR109B homolog was found in either species (7, 9). This is not surprising, because an examination of genomic sequences of various species available to date indicates that GPR109B exists only in humans and chimpanzees and thus may be a Hominoidea-specific receptor.

In humans, mice, and rats, GPR109A is expressed in adipose, spleen, lung, and immune cells (7, 9). Expression in hamster and guinea pig is fairly widespread, with higher expression of hamster GPR109A in lung, spleen, testis, and adipose. The guinea pig GPR109A exhibited higher expression in spleen, lung, skeletal muscle, stomach, ovary, and adipose. Because the physiological role of the nicotinic acid receptor is unknown at present, the significance of these findings, compared with the expression patterns of GPR109A in human, rat, and mouse, cannot be fully determined.

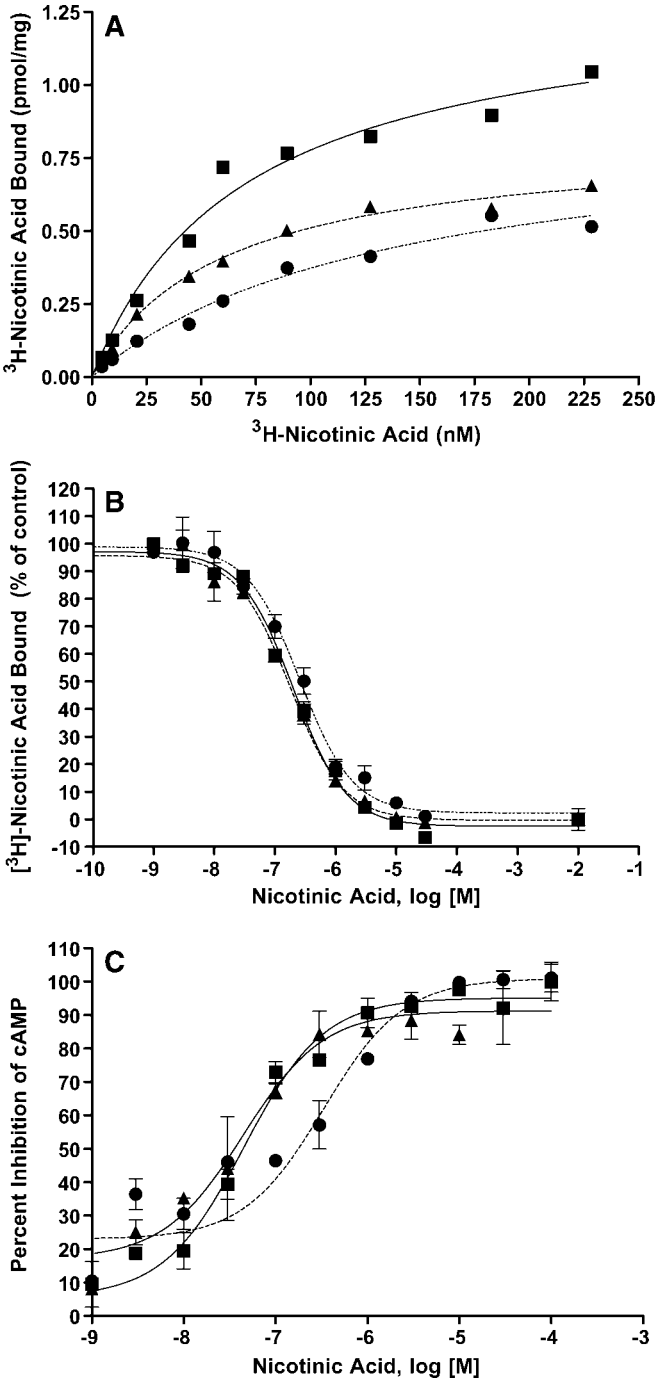

Fig. 4. Pharmacological characterization of guinea pig and hamster GPR109A. A: Saturation binding of [^{3}H]nicotinic acid to human, hamster, or guinea pig GPR109A. B: Competition binding analysis of human, hamster, and guinea pig GPR109A. C: Inhibition of forskolin-stimulated cAMP production by nicotinic acid in cells expressing human, hamster, or guinea pig GPR109A. For all graphs, data are from one representative experiment performed in duplicate. Squares, human; triangles, hamster; circles, guinea pig. Error bars indicate SEM.

To explore the pharmacology of these receptors, binding and functional activity were studied in cells expressing recombinant hamster, guinea pig, or human GPR109A. Binding affinities for [^{3}H]nicotinic acid were similar for all three receptors. In competition binding, the K_i values and the order of potency for nicotinic acid, Acipimox, Acifran, and related analogs were again similar among the three species. The sole exception was the K_i for 5-methyl-2-pyrazine carboxylic acid, which was 10-fold higher in guinea pig than in hamster and human.

In a recent paper, Tunaru et al. (14) described the ligand binding site for nicotinic acid, with R111 in transmembrane domain III (TMIII) as the critical docking point for the carboxylic group of nicotinic acid and with N86/W91 (TMII/ECL1), F276,Y284 (TMVII), and S178 (ECL2) interacting with the heterocyclic ring. These residues are conserved in hamster and guinea pig. However, the guinea pig NAR has two additional amino acid residues (Y168/L169) inserted in its ECL2 compared with the human, hamster, mouse, and rat sequences (Fig. 1). It is possible that these extra amino acids have altered the putative nicotinic acid binding pocket in the guinea pig. Interestingly, removal of these two residues in the guinea pig NAR causes a decrease in the functional response to nicotinic acid (data not shown). The physiological function of NAR in vivo is currently unknown; therefore, the significance of these additional amino acids in the guinea pig cannot yet be determined.

The EC_{50} values for cAMP inhibition by nicotinic acid and related analogs were slightly higher in the guinea pig NAR compared with the hamster and human, although the order of potencies was approximately the same in the three species. Again, the exceptions to this rank order

TABLE 2. Saturation binding of [^{3}H]nicotinic acid in transfected CHO cells expressing the human, hamster, or guinea pig GPR109A

Species	Dissociation Constant	Binding Sites
	nM	*pmol/mg*
Human	74 ± 3.7	1.4 ± 0.3
Hamster	72 ± 13	0.7 ± 0.1
Guinea pig	121 ± 5.0	0.6 ± 0.1

Data represent mean values ± SEM of at least three independent experiments.

TABLE 3. Affinities of nicotinic acid and related analogs to the human, hamster, and guinea pig GPR109A determined by competition binding with [^{3}H]nicotinic acid

Compound	Inhibition Constant		
	Human	Hamster	Guinea Pig
		nM	
Nicotinic acid	110 ± 5	97 ± 10	177 ± 19
Isonicotinic acid	9,178 ± 895	12,983 ± 971	14,760 ± 1,797
5-Methyl nicotinic acid	2,229 ± 174	1,705 ± 175	6,523 ± 1,208
6-Methyl nicotinic acid	4,429 ± 764	4,423 ± 437	10,648 ± 2,728
5-Methyl-2-pyrazine carboxylic acid	3,531 ± 674	2,990 ± 284	30,217 ± 338
Acipimox	2,206 ± 166	1,120 ± 68	2,020 ± 401
Acifran	885 ± 131	253 ± 78	1,757 ± 281
Nicotinamide	29,607 ± 393	>30,000	>30,000
2-Picolinic acid	>30,000	>30,000	>30,000

Data represent mean values ± SEM of at least three independent experiments.

TABLE 4. Potencies of nicotinic acid and its analogs at human, hamster, and guinea pig GPR109A in the cAMP assay

Compound	EC_{50}		
	Human	Hamster	Guinea Pig
		nM	
Nicotinic acid	50 ± 7	74 ± 18	329 ± 57
Isonicotinic acid	9,000 ± 3,154	26,388 ± 15,631	50,196 ± 35,617
5-Methyl nicotinic acid	6,005 ± 2,112	5,887 ± 3,203	36,562 ± 11,279
6-Methyl nicotinic acid	14,855 ± 7,772	40,278 ± 18,931	28,027 ± 10,953
5-Methyl-2-pyrazine carboxylic acid	2,950 ± 849	6,700 ± 2,589	83,067 ± 16,933
Acipimox	1,431 ± 485	2,136 ± 931	7,157 ± 2,794
Acifran	123 ± 40	46 ± 11	249 ± 91
Nicotinamide	>100,000	>100,000	>100,000
3,4-Pyridinedicarboxylic acid	3,168 ± 1,111	1,314 ± 343	26,140 ± 17,170

Data represent mean values ± SEM of at least three independent experiments.

were 5-methyl-2-pyrazine carboxylic acid, 5-methyl nicotinic acid, and 6-methyl nicotinic acid. Acifran was more potent than nicotinic acid in both rodents, but not in the human receptor, in the cAMP assay. However, the binding studies indicate that nicotinic acid binds with greater affinity to the receptor compared with Acifran in all three species. It may be that Acifran is more effective than nicotinic acid at signaling through GPR109A in these rodents.

Given the apparent differences in both receptor expression and in the binding and functional activation of the guinea pig and hamster receptors, it would be of interest to examine the effects of nicotinic acid on the lipid profile of these species. Data regarding the effect of nicotinic acid on lipolysis in guinea pigs are lacking. However, several studies document the antilipolytic effect of nicotinic acid on adipose tissue and isolated adipocytes from hamsters (15–17).

GPR109A, recently identified as the target of the antilipolytic agent niacin, appears to be highly conserved throughout mammalian species. This study presents the identification and characterization of the high-affinity nicotinic acid receptors in hamster and guinea pig. It is hoped that these observations regarding the subtle differences in receptor architecture and ligand binding may add to the understanding of this receptor and will facilitate the development of better treatments for dyslipidemias.

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