International Union of Basic and Clinical Pharmacology. LXXXII: Nomenclature and Classification of Hydroxy-carboxylic Acid Receptors (GPR81, GPR109A, and GPR109B)

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This article is available online at http://pharmrev.aspetjournals.org.
doi:10.1124/pr.110.003301.

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Abstract—The G-protein-coupled receptors GPR81, GPR109A, and GPR109B share significant sequence homology and form a small group of receptors, each of which is encoded by clustered genes. In recent years, endogenous ligands for all three receptors have been described. These endogenous ligands have in common that they are hydroxy-carboxylic acid metabolites, and we therefore have proposed that this receptor family be named hydroxy-carboxylic acid (HCA) receptors. The HCA1 receptor (GPR81) is activated by 2-hydroxy-propanoic acid (lactate), the HCA2 receptor (GPR109A) is a receptor for the ketone body 3-hydroxy-butrylic acid, and the HCA3 receptor (GPR109B) is activated by the β-oxidation intermediate 3-hydroxy-octanoic acid. HCA1 and HCA2 receptors are found in most mammalian species, whereas the HCA3 receptor is present only in higher primates. The three receptors have in common that they are expressed in adipocytes and are coupled to G1-type G-proteins mediating antilipolytic effects in fat cells. HCA1 and HCA2 receptors are also expressed in a variety of immune cells. HCA2 is a receptor for the antidysslipidemic drug nicotinic acid (niacin) and related compounds, and there is an increasing number of synthetic ligands mainly targeted at HCA2 and HCA3 receptors. The aim of this article is to give an overview on the discovery and pharmacological characterization of HCAs, and to introduce an International Union of Basic and Clinical Pharmacology (IUPHAR)-recommended nomenclature. We will also discuss open questions regarding this receptor family as well as their physiological role and therapeutic potential.

I. Introduction

In 1955, Rudolf Altschul discovered that the administration of relatively high doses of the vitamin nicotinic acid (niacin) leads to a decrease in plasma cholesterol levels by approximately 10% in healthy volunteers and by more than 20% in patients with hypercholesterolemia (Altschul et al., 1955). The fact that nicotinamide, which is equivalent to nicotinic acid as a vitamin, had no effect on plasma cholesterol levels indicated that this effect was unrelated to the function of nicotinic acid as a vitamin. Meanwhile, nicotinic acid has been well established as an antidyslipidemic drug (Carlson, 2005).

Soon after the discovery of the pharmacological effects of nicotinic acid, research efforts to understand the mechanism of action were directed at adipose tissue, because the most immediate effect of nicotinic acid on lipid metabolism is a decrease in free fatty acid levels that results from an antilipolytic effect (Carlson and Orio, 1962; Carlson, 1963). Thereafter, nicotinic acid was shown to reduce cAMP levels in adipose tissue (Butcher et al., 1968), an effect that resulted from G1-mediated inhibition of adenylyl cyclase (Aktories et al., 1980a). At that time, the hypothesis was formulated that nicotinic acid acts through a G1-coupled receptor (Aktories et al., 1980b), a concept that was later supported by the demonstration of specific binding sites for nicotinic acid on plasma membranes of adipocytes and spleen cells (Lorenzen et al., 2001).

In 2003, the human and mouse orphan G-protein-coupled receptors HM74A and PUMA-G, respectively, were discovered to be receptors for nicotinic acid (Soga et al., 2003; Tunaru et al., 2003; Wise et al., 2003); shortly thereafter, the ketone body 3-hydroxy-butylate was shown to be an endogenous ligand for GPR109A1 (Taggart et al., 2005). Since then, many synthetic ligands of GPR109A have been developed. In addition, for the closely related receptors GPR81 and GPR109B (see Table 1 and Figs. 1 and 2), endogenous and synthetic ligands have been discovered and generated.

In this review, we introduce a new nomenclature for the receptors GPR81, GPR109A, and GPR109B. Because all known endogenous ligands of GPR81, GPR109A, and GPR109B are hydroxy-carboxylic acids, the receptor family is called hydroxy-carboxylic acid receptors with
the individual names HCA₁ (GPR81), HCA₂ (GPR109A), and HCA₃ (GPR109B) (see Table 1). We also describe the cellular, physiological, and pathophysiological role of the receptors, their pharmacological characterization, and their therapeutic potential.

II. Molecular Basis for Receptor Nomenclature

A. Cloning of cDNAs Encoding Hydroxy-carboxylic Acid Receptors

The HCA₃ receptor (GPR109B) was the first hydroxy-carboxylic acid receptor whose cDNA was cloned and analyzed (Nomura et al., 1993). In search of new putative leukocyte chemotactic peptide receptors, Nomura et al. (1993) used degenerate oligonucleotide primers representing conserved cDNA regions encoding transmembrane domains of the human receptors for interleukin-8, complement factor 5a and N-formyl peptides to perform polymerase chain reactions on a human monocyte cDNA library. Full-length clones were isolated from the same cDNA library using the obtained polymerase chain reaction amplificate. One of the cDNAs encoding a new G-protein-coupled receptor was called HM74 (HCA₁), and the receptor was shown to be expressed in monocytes and neutrophils. It was also noted that the receptor had a different ligand.

The HCA₁ receptor (GPR81) was first found by a database search in which expressed sequence tag and high-throughput genomic sequences databases were analyzed for homologies to sequences of various known G-protein-coupled receptors using the TBLAST algorithm (Lee et al., 2001). The HCA₁ receptor cDNA was then cloned from a human bacterial artificial chromosome genomic clone localized to chromosome 12q. Lee et al. (2001) noticed the high homology to the HCA₂ receptor (GPR109B, HM74) and also observed that the genes encoding HCA₁ and HCA₃ receptors are near each other on the same human bacterial artificial chromosome.

The HCA₂ receptor (GPR109A) was originally identified in mice in a search for genes differentially expressed in interferon-γ (INF-γ)/tumor necrosis factor-α (TNF-α)-stimulated macrophages (Schaub et al., 2001). Schaub et al. (2001) used a subtractive hybridization strategy to identify cDNAs present in a cDNA library generated from the murine macrophage cell line ANA-1 stimulated for 16 h with INF-γ and TNF-α but absent from cDNA libraries derived from untreated ANA-1 cells or from IFN-γ-stimulated embryonic fibroblasts. The expression of the HCA₂ receptor in response to INF-γ and TNF-α was verified subsequently in various mouse macrophage cell lines, and the murine HCA₂ receptor was therefore designated PUMA-G (protein up-regulated in macrophages by IFN-γ). Expression of HCA₂ could also be induced in the spleens of mice after infection with Listeria monocytogenes. Human and rat HCA₂ receptors were cloned after the discovery of HCA₃ as a low-affinity receptor for nicotinic acid during a search for novel paralogs of the HCA₃ receptor (Soga et al., 2003; Wise et al., 2003).

B. Sequence Alignment and Phylogenetic Tree

The HCA₁ receptor (GPR81) has been shown to be expressed in humans and rodents, and, based on genomic sequences of various species, seems to be present in most mammals as well as in fish (C. Kuei, J. Yu, J. Zhu, J. Wu, T. Lovenberg, and C. Liu, manuscript in preparation). Likewise, the HCA₂ receptor (GPR109A), which exhibits substantial homology to the HCA₁ receptor (approximately 50% amino acid sequence identity), is

<table>
<thead>
<tr>
<th>Properties</th>
<th>HCA₁</th>
<th>HCA₂</th>
<th>HCA₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous names/aliases</td>
<td>GPR81, GPR104, TA-GPCR, LACR, FKSG80</td>
<td>GPR109A, HM74a (human), PUMA-G (mouse), HM74b, NIACR1</td>
<td>GPR109B, HM74, NIACR2</td>
</tr>
<tr>
<td>Genomic location</td>
<td>12q24.31 (human), 5F (mouse)</td>
<td>12q24.31 (human), 5F (mouse)</td>
<td>12q24.31 (human)</td>
</tr>
<tr>
<td>Amino acid length</td>
<td>346 (human), 343 (mouse)</td>
<td>363 (human), 360 (mouse)</td>
<td>387 (human)</td>
</tr>
<tr>
<td>Naturally occurring specific agonists (pEC₅₀)</td>
<td>2-OH-propanoate (2.5–2.8)</td>
<td>3-OH-butyrate (3.1), nicotinic acid (6.6–7.2)</td>
<td>3-OH-octanoate (5.1), 2-OH-octanoate (5.4), D-phenylalanine (5.0), D-tryptophan (5.4)</td>
</tr>
<tr>
<td>Specific synthetic agonists (pEC₅₀)</td>
<td>acipimox (5.2–5.6)</td>
<td>1-Isopropylbenzene-triazole-5-carboxylic acid (6.4), 5-methyl-5-(5-methyl-thio-phen-3-y1)-4-oxo-4,5-dihydro-furan-2-carboxylic acid (6.7)</td>
<td></td>
</tr>
<tr>
<td>G-protein coupling</td>
<td>G/Gₐ</td>
<td>G/Gₐ</td>
<td>G/Gₐ</td>
</tr>
<tr>
<td>Expression in human tissue</td>
<td>Adipocytes</td>
<td>Adipocytes, macrophages, neutrophils, keratinocytes, intestinal epithelial cells</td>
<td>Adipocytes, neutrophils, macrophages, intestinal epithelial cells</td>
</tr>
<tr>
<td>Cellular function</td>
<td>Inhibition of lipolysis</td>
<td>Inhibition of lipolysis, activation of immune cells</td>
<td>Inhibition of lipolysis, activation of immune cells</td>
</tr>
<tr>
<td>Phenotype of mice lacking receptor</td>
<td>Reduced insulin-induced antilipolysis</td>
<td>Lack of nicotinic acid effects on lipid plasma levels and of nicotinic acid-induced flushing</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 1

Current HCA receptor nomenclature and receptor properties

For further details, see IJzerman et al. (2010).
present in mammalian species. HCA2 and HCA3 receptors are highly homologous, being 95% identical on the protein level (see Figs. 1 and 2). The receptors differ by 15 amino acids that cluster in the first and second extracellular loops as well as in the outer parts of transmembrane regions 2 and 3. In addition, the HCA3 receptor has an extended C terminus containing 24 additional amino acids. In contrast to HCA1 and HCA2 receptors, the HCA3 receptor is not present in rodents. The analysis of available genomic sequences shows an ortholog of the human HCA3 receptor gene only in chimpanzees, whereas lower primates, such as rhesus monkeys, do not carry a gene encoding an HCA3 receptor. Thus, the HCA3 receptor is obviously the result of rather recent gene duplication.

Several single-nucleotide polymorphisms in the coding regions of genes encoding HCA2 and HCA3 receptors occur with heterozygosity ratios of 0.14 to 0.46 (Zellner et al., 2005). It is not clear, however, whether any of these variations alters the physiological or pharmacological functions of the receptor proteins.

C. Deorphanization of Hydroxy-carboxylic Acid Receptors

1. HCA1. The receptor HCA1 (GPR81) remained classified as an orphan receptor until 2008, when lactate was identified as a ligand of the HCA1 receptor (Cai et al., 2008). Lactate was shown to stimulate GTP\textsuperscript{\gamma}S-binding in a HCA1 receptor-dependent fashion with an EC\textsubscript{50} of 1.3 mM. Inhibition of forskolin-stimulated cAMP production in CHO cells transfected with HCA1 occurred with an EC\textsubscript{50} of 2.1 mM. Lactate did not activate the HCA2 or HCA3 receptor, and L-lactate was twice as potent and efficacious as D-lactate. Although various other short-chain fatty acids had no effect on the HCA1...
partial agonists of the HCA1 receptor, whereas nicotinic caproic acid, maleate, tartrate, and propionate are weak agonists of the HCA1 receptor. The basic pharmacological properties of the HCA1 receptor were later confirmed independently (Ahmed et al., 2010). The low ligand affinity of lactate for HCA1 is not without precedent. The low ligand affinity of the human hydroxy-carboxylic acid receptors HCA1, HCA2, and HCA3 as well as of the most related human receptors GPR31 and the 5-oxo-ETE receptor OXER1 (GPR170, TG1019). Data are based on an alignment by the Clustal W method. B, schematic representation of the genomic organization of the genes encoding hydroxy-carboxylic acid receptors.

An increase in lactate levels is seen in various conditions, such as under conditions of increased oxidative flux, during exercise, after fasting, and in patients with diabetic ketoacidosis, where lactate is a specific agonist of the HCA1 receptor. Because of the relatively low plasma levels of propionate, this activity is unlikely to be of physiological relevance. In a parallel study, Liu et al. (2009) identified lactate as an agonist of the HCA1 receptor by testing extracts from different rat tissues and by subsequent chromatographic purification and identification of lactate from porcine brain extracts. The reported EC50 values for lactate-induced GTPγS-binding and inhibition of cAMP formation were slightly higher (5 and 4.2 mM, respectively). Liu et al. (2009) also showed that lactate is a specific agonist for the human HCA1 receptor and that HCA1 receptors cloned from various mammalian species, including mouse, rat, dog, pig, cow, and monkey, responded in a comparable manner to lactate. Furthermore, the zebrafish HCA1 receptor seems to have a higher affinity for lactate at 1 mM (C. Kuei, J. Yu, J. Zhu, J. Wu, T. Lovenberg, and C. Liu, manuscript in preparation). In addition to L-lactate, α-hydroxybutyrate, glycolate, α-hydroxyisobutyrate, and γ-hydroxybutyrate were shown to activate HCA1 with low potency (EC50 values 5–15 mM). In contrast, D-lactate, α-hydroxycaproic acid, maleate, tartrate, and propionate are weak partial agonists of the HCA1 receptor, whereas nicotinic acid, pyruvate, β-hydroxybutyrate, acetate, GABA, and butyrate are not active. In addition, Liu et al. (2009) found that dichloroacetate and trifluoroacetate are partial agonists of the HCA1 receptor. The basic pharmacological properties of the HCA1 receptor were later confirmed independently (Ahmed et al., 2010). The low ligand affinity of lactate for HCA1 is not without precedent. The Ca2+-sensing receptor is also activated by millimolar concentrations of its agonist; however, this receptor is a class C GPCR with somewhat different activation mechanisms compared with class A, to which HCA receptors belong (Hofer and Brown, 2003).

Of all the reported naturally occurring agonists of HCA1, only L-lactate reaches levels sufficient to activate the receptor. Basal plasma concentrations of lactate are in the range of 0.5 and 2 mM (Huckabee, 1958; Marbach and Weil, 1967; Niessner and Beutler, 1973) and would be too low to lead to full HCA1 activation. However, under certain conditions, systemic lactate concentrations as well as localized concentrations (e.g., in fat tissue) increase severalfold (Kreisberg, 1980; DiGirolamo et al., 1992; Hagström-Toft et al., 1997). During intense exercise, lactate plasma levels are as high as 10 to 20 mM (Turrell and Robinson, 1942; Osnes and Hermansen, 1972).

2. HCA2. Taggart et al. (2005) reported the dephosphorylation of the HCA2 receptor (GPR109A) as a receptor for the ketone body 3-hydroxybutyrate. Racemic (DL)-3-hydroxybutyrate activated GTPγS binding through the human and mouse receptors with an EC50 values of 0.7 and 0.8 mM, respectively. The EC50 for the L-enantiomer was approximately 2-fold higher than that for the physiologically relevant D-enantiomer, which stimulated GTPγS-binding with an EC50 of 1.6 mM through the human receptor (Taggart et al., 2005). Other ketone bodies, such as acetoacetate or acetone, were without activity. Several short-chain fatty acids, such as pentanoic, hexanoic, octanoic, and butyric acids, were also shown to activate the human HCA2 receptor with EC50 values of 0.4 to 1.6 mM. However, physiological concentrations are unlikely to reach sufficient levels to activate the receptor. 3-Hydroxybutyrate had no activity on HCA1 or HCA3 receptors. 3-Hydroxybutyrate levels are between 20 and 200 μM under normal conditions. However, after fasting for 2 to 3 days, ketone body levels in the plasma increase to approximately 2 mM and can reach up to 6 to 8 mM after prolonged starvation (Owen et al., 1969), levels sufficient to fully activate the HCA2 receptor.

3. HCA3. Despite the high homology of HCA2 and HCA3 receptors, they show quite different binding properties for nicotinic acid and related substances, and various synthetic ligands specific for the HCA3 receptor have been generated (see section V). 2- and 3-Hydroxylated medium-chain fatty acids, in particular 3-hydroxyoctanoate, have been shown to be specific agonists of HCA3, acting through the receptor with an EC50 of 4 to 8 μM (Ahmed et al., 2009a). 3-Hydroxyoctanoate was not able to activate HCA1 or HCA2 receptors, and a shortening or extension of the chain length of 3-hydroxyoctanoate by more than two carbon atoms resulted in a complete loss of activity at the HCA3 receptor. 3-Hydroxyhexanoate, 3-hydroxyoctanoate, and 3-hydroxydecanoate are intermediates of fatty acid β-oxidation. Under conditions of increased β-oxidation flux, during starvation, under ketogenic diet, or in patients with diabetic ketoacidosis, 3-hydroxyoctanoate plasma con-
It is noteworthy that various aromatic \textit{D}-amino acids, such as \textit{D}-phenylalanine, \textit{D}-tryptophan, and \textit{D}-kynurenine, were shown to decrease cAMP levels by acting through the HCA3 receptor with EC\textsubscript{50} values between 10 and 100 \(\mu\text{M}\) (Irukayama-Tomobe et al., 2009). Aromatic \textit{D}-amino acids had no effect on the HCA2 receptor. The physiological enantiomers \textit{L}-phenylalanine, \textit{L}-tryptophan, and \textit{L}-kynurenine did not activate HCA3 at concentrations of 100 \(\mu\text{M}\) (Irukayama-Tomobe et al., 2009). Because \textit{D}-amino acids are extremely rare, it is unclear whether the ability of aromatic \textit{D}-amino acids to activate the HCA3 receptor is of physiological or pathophysiological significance.

### D. Proposed Nomenclature

In the past, various names were given to the receptors HCA\textsubscript{1} (GPR81), HCA\textsubscript{2} (GPR109A), and HCA\textsubscript{3} (GPR109B) (see Table 1). After the identification of the HCA2 receptor (GPR109A) as a receptor for nicotinic acid, this receptor family has been called the “nicotinic acid receptor family” or “niacin receptor family.” However, the name nicotinic acid receptor family is not appropriate because nicotinic acid (niacin) is not an endogenous ligand and because only HCA2 (GPR109A) and not HCA3 (GPR109B) nor HCA1 (GPR81) is a receptor for nicotinic acid. Endogenous ligands for all three members of the family have been described (see section II.C). Because all these endogenous ligands are hydroxy-carboxylic acids, we propose that this receptor family be called the “hydroxy-carboxylic acid (HCA) receptor family,” which currently has three members: HCA\textsubscript{1}, HCA\textsubscript{2}, and HCA\textsubscript{3} (see Table 1).

The G-protein-coupled receptors most closely related to the HCA receptor family are the orphan receptor GPR31 and the 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE) receptor OXER1 (Fig. 1 and 2) (Zongoni et al., 1997; Hosoi et al., 2002; Bjarnadóttir et al., 2006). An arginine residue in transmembrane helix 3, which has been proposed to represent an anchor point of the carboxylic group of HCA receptor ligands (Tunaru et al., 2005), is conserved not only among HCA receptors but also in GPR31 and the 5-oxo-ETE receptor (Fig. 1). These homologies suggest that GPR31 and OXER1 ligands may structurally resemble HCA receptor ligands. Although endogenous ligands for GPR31 are unknown, OXER1 binds not only 5-oxo-ETE but also, with lesser affinity, 5-hydroxy-eicosatetraenoic acid and 5-hydroperoxy-eicosatetraenoic acid (Hosoi et al., 2002; Jones et al., 2003; Brink et al., 2004). Thus, the fact that OXER1 is activated by a polyunsaturated fatty acid substituted in the 5-position with an oxo, hydroxy, or hydroperoxy group resembles HCA receptors also with regard to its agonistic ligand.

### III. Receptor Mutagenesis and Modeling Studies

Although the sequence similarity between HCA2 and HCA3 receptors is very high, nicotinic acid is recognized with only low affinity by the HCA3 receptor. In contrast, the antidyslipidemic agent acifran (Cayen et al., 1982) stimulates both the HCA2 and HCA3 receptors, although it has a predilection for the former (EC\textsubscript{50} values of 1.9 and 90 \(\mu\text{M}\), respectively). This intriguing observation was the starting point for mutagenesis studies in which chimeras between the two receptors as well as point mutations were introduced (Tunaru et al., 2005). A chimera linking the N-terminal part of the HCA3 receptor and the C-terminal part of the HCA2 receptor at the interface between intracellular loop 2 and TM4 responded well to acifran (EC\textsubscript{50} = 2 \(\mu\text{M}\)) but was insensitive to nicotinic acid. The same chimera, studied in a later article, responded well to 2-oxo-octanoic acid, a close analog of the endogenous HCA3 receptor ligand 3-hydroxy-octanoic acid, in a manner identical to the wild-type HCA3 receptor (Ahmed et al., 2009a). The “reverse” chimera, in which the N-terminal half was from HCA\textsubscript{2}, did not respond to 2-oxo-octanoic acid. The second approach of site-directed mutagenesis was applied to both the HCA2 and HCA3 receptors. In the HCA2 receptor, Asn86 and Trp91 (at the interface of TM2 and extracellular loop 1) and Ser178 (in extracellular loop 2) were mutated to Tyr, Ser, and Ile, respectively, which are the corresponding amino acids in the HCA3 receptor. This yielded receptor constructs that did not respond to nicotinic acid any more; acifran kept HCA3 receptor-like potency. All arginine residues in the HCA2 receptor TM domains, as putative counterparts for the negatively charged carboxylic function in both nicotinic acid and acifran, were also examined. Of the four arginines available, Arg111 present in TM3, when mutated to alanine, rendered the HCA2 receptor completely insensitive to nicotinic acid. This position is equivalent to the well known Asp residue in biogenic amine receptors. Eventually, it was deduced that three more residues may constitute the binding site of nicotinic acid on the HCA2 receptor, Phe180 (extracellular loop 2) and Phe276 and Tyr284 (both present in TM7). A receptor homology model was presented based on rhodopsin’s crystal structure (Palczewski et al., 2000). Because the endogenous ligand in rhodopsin, retinal, is quite large compared with nicotinic acid, there is some uncertainty as to how to dock nicotinic acid or other small HCA receptor ligands into the putative binding site. The authors postulated that the pyridine ring of nicotinic acid is embedded between Trp91 and Phe276/Tyr284, and hydrogen-bonded via its nitrogen atom to Ser178. Deng et al. (2008) docked an anthranilic acid derivative (Fig. 6a), considerably larger than nicotinic acid, inside an independently constructed HCA2 receptor homology model, also based on the rhodopsin/retinal homology model, the ligand was positioned in an extended conformation and sur-
rounded by residues mainly from TM3, TM5, TM6, and the second extracellular loop. The residues in the binding pocket represent three groups of interactions. First, there is a triad of two arginines and one serine. Arg111 (TM3) and Arg251 (TM6) are thought to form salt bridges with the ligand’s carboxylate moiety. Ser178 (extracellular loop 2) is close to the carboxamide fragment in the ligand, forming a hydrogen bond. Secondly, hydrophobic residues coordinate the anisole moiety of the ligand, including Ile254 (TM6), Phe255 (TM6), and Phe276 (TM7). The third group of interactions involves several polar residues at the entrance of the binding pocket, including Asn171 (extracellular loop 2), Ser179 (extracellular loop 2), and His259 (extracellular loop 3). This latter group of amino acids was also postulated to be involved in the interaction with an optimized lead molecule (Fig. 6d; see also Shen et al., 2007b), in which the hydroxyl substituent on the quinoxaline moiety is hydrogen-bonded to Asn171. The recently determined crystal structures of other GPCRs may be of further help in the modeling of the HCA receptors and their binding sites (Cherezov et al., 2007; Jaakola et al., 2008; Warne et al., 2008).

Ahmed et al. (2009a) introduced further mutations into the HCA3 receptor, focusing on positions 86, 91, 103, and 107. Mutation of three of these positions in the HCA2 receptor to the corresponding residues in the HCA3 receptor (N86Y, M103V, L107F) made the receptor fully respond, such as the wild-type HCA3 receptor, to 2-hydroxyoctanoic acid, yet another close analog of the endogenous HCA3 receptor ligand. A very similar mutant (N86Y, W91S, M103V) did not respond at all to 2-hydroxyoctanoic acid. In a separate, single HCA3 receptor mutant (R111A), the response to 2-hydroxyoctanoic acid was completely abrogated too, suggesting that this amino acid is vital for anchoring the acidic group in HCA3 receptor ligands.

The HCA1 receptor was the subject of two recent mutagenesis studies (Ge et al., 2008; Liu et al., 2009). In the first (Ge et al., 2008), a chimeric receptor was constructed that consisted of the N terminus, all three extracellular loops, and all seven TM domains from the cysteinyl-leukotriene 2 (CysLT2) receptor and all three intracellular loops and the C terminus from the HCA1 receptor. Leukotriene D4, the cognate ligand for the CysLT2 receptor, was capable of activating this receptor construct through a pertussis toxin-sensitive inhibitory receptor. Leukotriene D4, the cognate ligand for the HCA2 receptor, focusing on positions 86, 91, 103, and 107. Mutation of three of these positions in the HCA2 receptor to the corresponding residues in the HCA3 receptor (N86Y, M103V, L107F) made the receptor fully respond, such as the wild-type HCA3 receptor, to 2-hydroxy-octanoic acid. In a separate, single HCA3 receptor mutant (R111A), the response to 2-hydroxy-octanoic acid was completely abrogated too, suggesting that this amino acid is vital for anchoring the acidic group in HCA3 receptor ligands.

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IV. Gene Structure, Expression, and Regulation

The genes encoding HCA1, HCA2, and HCA3 receptors are next to each other in tandem on human chromosome 12q24.31 in a configuration in which the gene encoding the HCA3 receptor is flanked by the two other genes (see Fig. 2). In all species known to carry genes for HCA receptors, the coding sequence of the receptors is present on a single exon.

A. HCA1

The HCA1 receptor was originally reported to be expressed in human pituitary tissue (Lee et al., 2001). However, this has never been confirmed. Meanwhile, evidence has been provided that the receptor is primarily expressed in adipocytes (Wise et al., 2003; Ge et al., 2008; Jeninga et al., 2009; Liu et al., 2009; Ahmed et al., 2010). In humans, the HCA1 receptor was found to be most abundantly expressed in brown adipose tissue with also rather high expression in white adipose tissue (Liu et al., 2009). Similar expression patterns were found in mice and in rats. In addition, receptor expression was markedly induced during differentiation of 3T3-L1 preadipocytes (Ge et al., 2008; Jeninga et al., 2009; Liu et al., 2009). Very low levels of HCA1 receptor expression were seen in several other tissues. However, it is not clear whether this is due to specific expression of the HCA1 receptor or to the presence of adipocytes in these tissues. It is noteworthy that in both mouse and human models, it could be shown that thiazolidinediones acting via the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) increase the expression of HCA1 receptor mRNA 4- to 5-fold (Jeninga et al., 2009). This effect was shown to be mediated by the binding of the PPARγ/retinoid X receptor heterodimer to a functional PPAR-response element present in the proximal promoter of the HCA1 receptor gene (Jeninga et al., 2009). Jeninga et al. (2009) provided additional data suggesting that the antilipolytic effect of thiazolidinediones is mediated in part through the up-regulation of the HCA1 receptor in adipocytes.
**B. HCA₂**

The receptor of HCA₂ shows highest expression levels in white and brown adipose tissue in both human and mouse (Soga et al., 2003; Tunaru et al., 2003; Wise et al., 2003). During the differentiation of 3T3-L1 preadipocytes, expression of the HCA₂ receptor is induced 1 to 2 days earlier than expression of the HCA₁ receptor (Ge et al., 2008; Jeninga et al., 2009). Similar to HCA₁, the expression of the HCA₂ receptor is increased by PPARγ activation (Jeninga et al., 2009). Because there is no functional PPAR-response element in the promoter of the HCA₂ receptor gene, it has been suggested that the PPAR-response element present in the proximal promoter of the HCA₁ receptor gene controls the HCA₂ receptor gene promoter (Jeninga et al., 2009).

Apart from adipocytes, expression of the HCA₂ receptor is also found in various immune cells, including neutrophils, macrophages, dendritic cells, and epidermal Langerhans cells (Schaub et al., 2001; Benyó et al., 2005; Maciejewski-Lenoir et al., 2006; Kostylina et al., 2008) but not in mouse and human B and T lymphocytes or in human eosinophils (Schaub et al., 2001; Kostylina et al., 2008; Tang et al., 2008). HCA₂ receptor expression was not detected in immature bone marrow neutrophils, indicating that induction of HCA₂ receptor expression occurs during the late stages of terminal differentiation of neutrophils (Kostylina et al., 2008). Expression of the HCA₂ receptor in macrophages can be increased by treatment of cells with IFN-γ, TNF-α, lipopolysaccharide, and CpG oligodeoxynucleotides, whereas IFN-α, IFN-β, granulocyte macrophage–colony-stimulating factor, interleukin-1, interleukin-12, and interleukin-18 had no effect (Schaub et al., 2001). In primary human monocytes and adhesion-differentiated macrophages, the HCA₂ receptor was up-regulated by hypoxia (Knowles et al., 2006).

Expression of the HCA₂ receptor has also been reported in the apical membrane of epithelial cells of the small and large intestine. Expression in intestinal epithelial cells seems to be reduced in colon cancer as well as under germ-free conditions (Thangaraju et al., 2009; Cresci et al., 2010). In addition, retinal pigment epithelial cells express the HCA₂ receptor, which appears primarily localized to the basolateral membrane of these cells (Martin et al., 2009). On the mRNA level, HCA₂ receptor expression has been shown in primary human keratinocytes (Maciejewski-Lenoir et al., 2006; Tang et al., 2008); however, immunohistochemistry failed to demonstrate HCA₂ receptor expression in keratinocytes (Maciejewski-Lenoir et al., 2006). Expression of the HCA₂ receptor in keratinocytes has recently been shown by genetic and functional approaches (Hanson et al., 2010), and strong HCA₂ receptor expression has also been described in the human epidermoid carcinoma cell line A431 (Maciejewski-Lenoir et al., 2006; Zhou et al., 2007). Similar to macrophages, expression of the HCA₂ receptor in keratinocytes and keratinocyte cell lines is induced by IFN-γ (Tang et al., 2008).

**C. HCA₃**

Expression of the HCA₃ receptor seems to be very similar to that of the HCA₂ receptor. The HCA₃ receptor is expressed in adipose tissue (Soga et al., 2003; Tunaru et al., 2003; Wise et al., 2003), and this expression can be increased by activation of PPARγ (Jeninga et al., 2009). In addition, the HCA₃ receptor is expressed in various immune cells, including neutrophils, monocytes, and macrophages (Nomura et al., 1993; Yousefi et al., 2000; Ahmed et al., 2009a; Irukayama-Tomobe et al., 2009). Expression in neutrophils has been shown to be stimulated by granulocyte macrophage–colony-stimulating factor (Yousefi et al., 2000), whereas expression in primary human monocytes and phorbol 12-myristate 13-acetate-differentiated THP1 and U937 monocytic cell lines can be increased by hypoxia (Knowles et al., 2006). Evidence has also been provided for the expression of the HCA₃ receptor in epithelial cells of the colon (Thangaraju et al., 2009).

**V. Receptor Classification with Pharmacological Tools**

Although nicotinic acid was introduced in humans in the 1950s (Altschul et al., 1955), structure-activity relationships for its target(s) were developed much later. Only in the 1980s did Aktories et al. (1980a, 1983) propose the existence of a specific receptor for nicotinic acid and a related compound, acipimox. Progress being slow, members of the same laboratory explored a few more compounds related to nicotinic acid in a number of receptor assays, using membranes from rat adipocytes and rat spleen (Lorenzen et al., 2001). A few years later, the human HCA₂ receptor was cloned (Wise et al., 2003). In that study, a number of nicotinic acid-like compounds were also tested. Therefore, we will use these two studies, the data from which are gathered in Table 2, as the starting point for this paragraph. In Fig. 3, the chemical structures of these compounds are represented. Because most efforts have been directed toward the HCA₂ receptor, we will discuss the synthetic ligands for this receptor first, followed by the more restricted information on the HCA₃ receptor. To our knowledge, synthetic ligands for the HCA₂ receptor have only been reported in the patent literature, which is beyond the scope of this review. Last but not least, antagonists have not been disclosed for any of the HCA receptors, which is currently hampering a full pharmacological characterization of these receptors.

**A. Structure-Activity Relationships for the HCA₂ Receptor**

1. **Nicotinic Acid-Like Compounds.** Lorenzen et al. (2001) observed that nicotinic acid increases [³⁵S]GTPγS
binding in rat epididymal adipocyte membranes in a concentration-dependent way. This provided a convenient assay format, with an EC$_{50}$ value for nicotinic acid (1.4 μM) that was in good agreement with the values found in earlier GTPase activation and adenylyl cyclase inhibition studies (Aktories et al., 1980a). A good window in the same assay format of [35S]GTP$_S$ binding was also observed in rat spleen membranes (EC$_{50}$ = 0.70 μM). No increase in binding was detectable in membranes from forebrain, liver, kidney, testis, heart, or lung. In a saturation radioligand binding assay with [3H]nicotinic acid, high affinity was also found in the same two tissues of rat epididymal adipocyte membranes (K$_d$ = 0.044 μM) and rat spleen membranes (K$_d$ = 0.023 μM). The latter, more readily available tissue was used for radioligand displacement studies. Nicotinic acid competitively displaced [3H]nicotinic acid from its binding sites (K$_i$ = 0.033 μM). The same two assays were used by Wise et al. (2003), but now on the cloned human HCA2 receptor and on human adipocytes. Similar potency and affinity values were found for nicotinic acid, suggesting that there are no huge species differences between rat and man for nicotinic acid itself. Two other marketed products, acifran and acipimox,

![Chemical structures](image)

**Fig. 3.** Chemical structures of nicotinic acid and related compounds (biological activities are in Table 1). CA, carboxylic acid. *+, chiral center.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rat$^a$ GTP$<em>S$ Binding Assay (EC$</em>{50}$)</th>
<th>[3H]Nicotinic Acid Binding Assay (K$_d$)</th>
<th>Human$^b$ GTP$<em>S$ Binding Assay (EC$</em>{50}$)</th>
<th>[3H]Nicotinic Acid Binding Assay (K$_d$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adipocytes</td>
<td>Spleen</td>
<td>Spleen</td>
<td>HCA2 (Cloned)</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>1.4</td>
<td>0.70</td>
<td>0.033</td>
<td>0.25</td>
</tr>
<tr>
<td>Acifran</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>2.1</td>
</tr>
<tr>
<td>Acipimox</td>
<td>10</td>
<td>6.6</td>
<td>0.31</td>
<td>6.0</td>
</tr>
<tr>
<td>3-Pyridine-acetic acid</td>
<td>16</td>
<td>17</td>
<td>0.40</td>
<td>5.5</td>
</tr>
<tr>
<td>Pyrazidine-4-COOH</td>
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<td>3.1</td>
<td>0.12</td>
<td>N.D.</td>
</tr>
<tr>
<td>Pyrazine-2-COOH</td>
<td>26</td>
<td>22</td>
<td>0.76</td>
<td>N.D.</td>
</tr>
<tr>
<td>5-Me-nicotinic acid</td>
<td>30</td>
<td>30</td>
<td>0.72</td>
<td>8.7</td>
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<tr>
<td>5-Me-pyrazine-2-COOH</td>
<td>52</td>
<td>15</td>
<td>0.65</td>
<td>N.D.</td>
</tr>
<tr>
<td>6-Me-nicotinic acid</td>
<td>73</td>
<td>54</td>
<td>1.7</td>
<td>N.D.</td>
</tr>
<tr>
<td>Nicotinic acid-1-oxide</td>
<td>80</td>
<td>74</td>
<td>3.2</td>
<td>N.D.</td>
</tr>
<tr>
<td>2-OH-nicotinic acid</td>
<td>132</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Furan-3-COOH</td>
<td>142</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>N.D.</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

N.D., not determined.  
$^a$ Lorenzen et al. (2001).  
$^b$ Wise et al. (2003).
were also tested. Acifran, on the human receptor, was
~10-fold less potent than nicotinic acid, as was acipimox
on the rat receptor. Acipimox was less active on the
human receptor. Changing the distance between het-
cyclic core and carboxylic moiety, as in 3-pyridine-acetic
acid, was not well tolerated, with a reduction in potency
and affinity of more than 10-fold in both species. Modif-
ying the heterocyclic core, either by substitution of the
pyridine ring or by introduction of other heterocyclic
structures, led to overall reduced activity. Best tolerated
was pyridazine-4-carboxylic acid, being ~5-fold less ac-
tive than nicotinic acid, whereas the simple substitution
pattern in 6-methylnicotinic acid rendered the com-
 pound 50-fold less active. It is noteworthy that all active
compounds in Table 2 were full agonists, in that they
stimulated \([35S]GTP^S\) binding to the same extent as
nicotinic acid. Nicotinamide was inactive, however, sug-
 gesting that the carboxylic acid group as a negatively
charged moiety is essential for activity. Gharbaoui et al.
(2007) provided further variations to the theme of different
heterocycles, confirming that changing the heterocycle in-
variably led to compounds with lower, if not negligible,
potency than nicotinic acid.

2. Pyrazoles. One compound, pyrazole-3-carboxylic
acid (Table 3, R1 = \(R2 = H\)), was a high-efficacy partial
agonist in the rat \([35S]GTP^S\) binding assay (relative
intrinsic activity of 85%) with submicromolar affinity
\((K_i = 0.59 \, \mu M)\) in the rat spleen radioligand binding
 assay (Lorenzen et al., 2001). This finding was taken as
 the starting point for a synthetic program, both in aca-
demia and industry. Partial agonists may display tissue
selectivity on the basis of differences in receptor expres-
sion in different tissues. This was thought to be poten-
tially beneficial in the case of the HCA2 receptor, as the
side effect of flushing might be separated from the de-
sired action in dyslipidemia. In the 1980s, Seki et al.
(1984) had reported on substituted alkylpyrazoles as
hypolipidemic agents; logically, Lorenzen et al. (2001)
 hypothesized that such compounds might act via the
HCA2 receptor. Van Herk et al. (2003) prepared two
series of alkyl- and benzyl-substituted pyrazole-3-car-
boxylic acid derivatives (Table 3). Partial agonism was
maintained throughout the series, and some compounds
had affinity values in the same range as nicotinic acid.
This was particularly true for the propyl- and butyl-
substituted derivatives as well as the 3-carbon atom
ring-closed compound, with \(K_i\) values of 0.14, 0.072, and
0.16 \(\mu M\), respectively, and relative intrinsic activities
of 70, 81, and 56% in rat tissue. The R1 = 3-chlorobenzyl
derivative had quite acceptable affinity \((K_i = 0.50 \, \mu M)\),
modest potency in the \([35S]GTP^S\) binding assay, and a
relative intrinsic activity of 39%. A more extended series
of pyrazoles, along the same vein of either alkyl or ben-
zyl substitution, was disclosed by Gharbaoui et al.
(2007), corroborating the findings by Van Herk et al.
(2003). The authors identified the 3-fluorobenzyl R1-
substituted pyrazole as slightly more active than the
3-chlorobenzyl derivative in a cAMP assay. Introduction
of a fluoro substituent on position R2 (Table 3) was
tolerated when in combination with an alkyl substituent
on R1 (Skinner et al., 2007b). In that same publication,
it was shown that the carboxylic acid could not easily be
replaced by a similarly acidic tetrazole moiety, because
most pairs differed 1 to 2 log units (or even more) in
activity. Later, Semple et al. (2008) reported on one
particular exception, in which the carboxylic acid/tetra-
 zole switch yielded a partial agonist with substantial
activity on the cloned human and mouse receptors in a cAMP
assay. In mice, this compound [3-(1H-tetrazol-5-yl)-1,4,5,6-
etrahydrocyclopentapyrazole (MK-0354)] (Fig. 4a) was as
active as nicotinic acid in reducing the amount of plasma
free fatty acids in vivo and had quite favorable pharma-
cokinetic properties, but did not cause vasodilation in
the mouse ear, a surrogate marker for flushing. This
differential behavior might indeed be due to the partial
agonistic nature of the compound, although other expla-

<table>
<thead>
<tr>
<th>(R_1)</th>
<th>(R_2)</th>
<th>(K_i)</th>
<th>(EC_{50})</th>
<th>RIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotinic acid 0.033</td>
<td>0.74</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>0.59</td>
<td>22</td>
<td>85</td>
</tr>
<tr>
<td>i-C(_3)H(_7)</td>
<td>H</td>
<td>0.68</td>
<td>22</td>
<td>68</td>
</tr>
<tr>
<td>C(_6)H(_5)</td>
<td>H</td>
<td>0.14</td>
<td>5.1</td>
<td>70</td>
</tr>
<tr>
<td>C(_6)H(_9)</td>
<td>H</td>
<td>0.072</td>
<td>2.3</td>
<td>81</td>
</tr>
<tr>
<td>-C(_6)H(_5)</td>
<td>H</td>
<td>0.16</td>
<td>7.0</td>
<td>56</td>
</tr>
<tr>
<td>-C(_6)H(_9)</td>
<td>H</td>
<td>3.5</td>
<td>85</td>
<td>47</td>
</tr>
</tbody>
</table>

TABLE 3
Affinities (\(K_i\) values in radioligand displacement study), potencies (\(EC_{50}\) values in \([35S]GTP^S\) binding assay), and relative intrinsic activities (RIA; \([35S]GTP^S\) binding assay) of pyrazole-derived partial agonists for the rat nicotinic acid receptor.
nations such as biased, ligand-directed signaling may be equally feasible (Richman et al., 2007; Walters et al., 2009). MK-0354 appeared very selective, in that it did not show activity in a panel of more than 120 other proteins, including the human ether-a-go-go-related gene channel. Imbriglio et al. (2009) extended on the scaffold of MK-0354 by introducing fluorinated phenyl substituents, the 2,3,5-trifluoro variant of which (Fig. 4b) was 2- to 3-fold more potent than nicotinic acid in vitro. It displayed an acceptable pharmacokinetic profile in vivo and, like MK-0354, caused no vasodilation in the mouse ear. Similar derivatives, now with a carboxylic acid function, were reported by Schmidt et al. (2009) and Imbriglio et al. (2010). One typical racemate example (Fig. 4c) and its most active enantiomer were as active as nicotinic acid in both the [3H]nicotinic acid binding assay and an EC50 value of 6 μM in the [35S]GTPγS binding assay, focusing on pharmacokinetic aspects, including the so-called serum shift. Although not addressed in all publications, the anthranilic acid derivatives, unlike the pyrazoles discussed above (Schmidt et al., 2009), seem to be prone to high plasma protein binding with a strong negative impact on the in vivo activity of the molecules. This was simulated by measuring the apparent affinities in the [3H]nicotinic acid binding assay in the absence and presence of 4% human serum. Serum shifts (IC50 shifts) of 1000-fold or more seemed to be typical for some of the anthranilic acid derivatives. A typical example is the biphenyl compound (Fig. 6b) with high affinity in the [3H]nicotinic acid binding assay in the absence of serum (IC50 = 4 nM) but with a serum shift of 8000. Changing one of the phenyl groups to a heterocyclic moiety as in Fig. 6c improved this profile (IC50 = 10 nM; serum shift = 40). This compound was tested in vivo, showing a favorable profile of a strong reduction of FFA levels in mice after oral dosing but failing to induce vasodilation in the mouse ear as a measure for the flushing side effect. The introduction of a piperazine ring as a linker between the aromatic fragments within the molecules yielded a series of urea analogs as in Fig. 6d (Shen et al., 2007b). This compound had an IC50 value of 140 nM in the [3H]nicotinic acid binding assay and an EC50 value of 470 nM in the [35S]GTPγS binding assay, displaying high-efficacy partial agonism (83% of the maximal nicotinic acid response). Its serum shift was not reported. A similar derivative without the hydroxyl group on the quinoxaline moiety was tested in vivo. After intraperitoneal administration, it reduced free fatty acid levels in mice at least as efficaciously as nicotinic acid and did not induce any vasodilation. Building further on the bicyclic quinoxaline moiety, the Merck researchers developed tricyclic derivatives as well (Shen et al., 2009). A typical example (Fig. 6e) was tested in vivo in rats rather than

HCA2 receptor, as was the case for the introduction of a similarly substituted thiophene moiety instead of phenyl (Fig. 5c). Again, little selectivity, if any, was observed with respect to the human HCA3 receptor. Some of the analogs were resolved into their individual stereoisomers, showing that the (+)-isomer was invariably the biologically active principle.

4. Anthranilic Acid Derivatives. Anthranilic acid derivatives emerged from high-throughput screening campaigns at a number of companies. Merck has been most prolific in bringing these results into the peer-reviewed public domain. Shen et al. (2007a) reported on the optimization of an early hit (Fig. 6a) with an EC50 value of 4% human serum. Serum shifts (IC50 shifts) of 1000-fold or more seemed to be typical for some of the anthranilic acid derivatives. A typical example is the biphenyl compound (Fig. 6b) with high affinity in the [3H]nicotinic acid binding assay in the absence of serum (IC50 = 4 nM) but with a serum shift of 8000. Changing one of the phenyl groups to a heterocyclic moiety as in Fig. 6c improved this profile (IC50 = 10 nM; serum shift = 40). This compound was tested in vivo, showing a favorable profile of a strong reduction of FFA levels in mice after oral dosing but failing to induce vasodilation in the mouse ear as a measure for the flushing side effect. The introduction of a piperazine ring as a linker between the aromatic fragments within the molecules yielded a series of urea analogs as in Fig. 6d (Shen et al., 2007b). This compound had an IC50 value of 140 nM in the [3H]nicotinic acid binding assay and an EC50 value of 470 nM in the [35S]GTPγS binding assay, displaying high-efficacy partial agonism (83% of the maximal nicotinic acid response). Its serum shift was not reported. A similar derivative without the hydroxyl group on the quinoxaline moiety was tested in vivo. After intraperitoneal administration, it reduced free fatty acid levels in mice at least as efficaciously as nicotinic acid and did not induce any vasodilation. Building further on the bicyclic quinoxaline moiety, the Merck researchers developed tricyclic derivatives as well (Shen et al., 2009). A typical example (Fig. 6e) was tested in vivo in rats rather than

![Fig. 4. Chemical structures of bicyclic pyrazole MK-0354, a highly selective partial agonist for the HCA2 receptor, and two later follow-up compounds.](image)

![Fig. 5. Novel acifran analogs. * chiral center.](image)
mice. Although not endowed with optimal pharmacodynamic and pharmacokinetic properties, the compound reduced free fatty acid levels as well as nicotinic acid, but its effect was longer lasting. Vasodilation in the rat ear was less than with nicotinic acid, yielding a therapeutic index (defined as EC$_{50}$, vasodilation/EC$_{50}$, free fatty-acid suppression) of 8 compared with 1 for nicotinic acid. In that study, it was also demonstrated that partial hydrogenation of the anthranilic acid phenyl ring yielded compounds that retained activity on the HCA$_2$ receptor. This finding was more elaborately explored in another article from the Merck group (Raghavan et al., 2008). The tetrahydro analog (Fig. 6f) had 2 nM affinity in the [$^3$H]nicotinic acid binding assay, an EC$_{50}$ value of 18 nM in the [$^{[35]}$S]GTP$_{y}$S binding assay, and very acceptable pharmacokinetics in mice, whereas a value for the serum shift and in vivo efficacy data were not presented. The authors concluded that the tetrahydro variants of anthranilic acid derivatives might fare better than the parent molecules because they seemed to yield improved oral bioavailability and better cytochrome P450 profiles. A recent publication (Shen et al., 2010) describes the discovery of MK-6892 (Fig. 6g), which resulted in a preclinical candidate from the Merck anthranilic acid effort. It was also found (Ding et al., 2010; Schmidt et al., 2010) that the cyclohexene ring system in such compounds can be further substituted (e.g., Fig. 6h).

5. Fumaric and Other Acids and Their Esters. A mixture of fumaric acid esters is on the market in Germany for the treatment of psoriasis. It has recently been shown that both the monomethyl (MMF) and monoethyl (MEF) esters of fumaric acid (Fig. 7), but not fumaric acid itself, have micromolar affinity for the HCA$_2$ receptor (Tang et al., 2008). MMF induced a concentration-dependent Ca$^{2+}$ signal in transfected cells with an EC$_{50}$ value of 9.4 μM (nicotinic acid, 2.0 μM). MEF was approximately 3-fold less potent than MMF with an EC$_{50}$ value of 26 μM, whereas dimethylfumarate was inactive. In a [$^3$H]nicotinic acid radioligand displacement assay, somewhat lower $K_i$ values were obtained: for MMF, 0.98 μM; for MEF, 1.3 μM. A number of “simple” acids were tested by Ren et al. (2009) in several HCA$_2$ receptor assay formats. The two most potent compounds were trans-cinnamic acid and para-coumaric acid (Fig. 7), with IC$_{50}$ values in a [$^3$H]nicotinic acid binding assay of 36 and 58 μM, respectively (nicotinic acid, 0.1 μM). In the [$^{[35]}$S]GTP$_{y}$S binding assay both compounds were somewhat less active, with EC$_{50}$ values of 240 and 310 μM, respectively. It is noteworthy that in a similar assay on the HCA$_3$ receptor trans-cinnamic acid was also active (EC$_{50}$, 180 μM), whereas para-coumaric acid was not. Oral administration of trans-cinnamic acid to wild-type mice led to a significant reduction in plasma free fatty acid levels, whereas the compound was without effect in HCA$_2$ receptor KO animals.

6. Pyridopyrimidinones. Peters et al. (2010) reported on a different and quite intriguing scaffold from which HCA$_2$ receptor agonists were derived. The pyridopyrimidi-
nones (Fig. 8) can be regarded as derivatives of nicotinamide (Fig. 3), a compound shown to be inactive at HCA₂ receptors. Nevertheless, submicromolar affinity and potency were observed in this series, although the compounds reported so far suffer from poor pharmacokinetics.

7. Pyrazolopyrimidines as Allosteric Agonists. Shen et al. (2008) discovered another series of agonists for the HCA₂ receptor with intriguing pharmacological activity. The chemical structure of the compound that was most thoroughly described is represented in Fig. 9. When tested alone it stimulated [³⁵S]GTP₇S binding to the human receptor to 75% of activation levels achieved with nicotinic acid, classifying it as a partial agonist. Its potency was higher than for nicotinic acid, with EC₅₀ values of 0.12 μM for the allosteric compound and 1.0 μM for nicotinic acid in this assay format. It is noteworthy that the presence of the pyrazolopyrimidine (500 nM–1 μM) shifted the concentration-effect curve of nicotinic acid significantly to the left, when measured at the level of cAMP in intact CHO cells expressing the human receptor, suggestive of its allosteric enhancing capability. In a radioligand binding assay, the pyrazolopyrimidine dose-dependently increased rather than displaced specific [³H]nicotinic acid binding to 400% of control levels with an EC₅₀ value of 0.17 μM, yet another token of its nature as an allosteric enhancer. In general, such compounds might be used to increase the potency and/or intrinsic activity of the natural ligands for the HCA receptors. This would provide a rather physiological way of intervening with receptor function, especially if the compounds were pure allosteric enhancers without agonist activity when tested alone.

8. Patent Literature. Many companies have published patents on ligands for the HCA₂ receptor. Because these publications are not peer-reviewed, we refrain from discussing them here. However, the most remarkable developments in this area have been published in four recent reviews, to which we refer the interested reader: Boatman et al., 2008; Martres, 2009; Shen, 2009; Shen and Colletti, 2009.

B. Structure-Activity Relationships for the HCA₃ Receptor

Because the HCA₃ receptor occurs only in higher primates, the development of (rodent) animal models is hampered, which may explain the limited medicinal chemistry efforts so far. Despite the high (>95%) homology between the two receptors, nicotinic acid is in fact very selective for the HCA₂ receptor. Acifran, however, is not very selective for the two receptors, with slightly lower micromolar affinity for the HCA₃ receptor (for example, Mahboubi et al., 2006; Ren et al., 2009). The most extensive structure-activity study with acifran analogs (Jung et al., 2007) showed that an ethyl rather than a methyl substitut at the chiral center in acifran (see Fig. 3) provided some (5-fold) selectivity for the HCA₃ receptor, whereas all other compounds were slightly selective for the HCA₂ receptor. Ren et al. (2009) identified ortho-coumaric acid (Fig. 5) as ~20-fold selective for the HCA₃ receptor in a [³⁵S]GTP₇S binding assay. EC₅₀ values for this compound were 70 and 1270 μM for the HCA₃ and HCA₂ receptors, respectively, whereas the isomer para-coumaric acid was inactive at the HCA₃ receptor but not at the HCA₂ receptor (see section V.A).

The first exclusive search for HCA₃ receptor ligands was reported by Semple et al. (2006). In a screening campaign using a forskolin-stimulated cell line expressing the human HCA₃ receptor, they discovered a benzotriazole compound (Fig. 10a) with r = isopropyl as a 400 nM hit, reducing the cAMP production; its activity was confirmed in an antilipolysis assay with human subcutaneous adipocytes. Further exploration of the R-substituent led to a number of other potent HCA₃ receptor agonists.

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**Fig. 8.** Pyridopyrimidinones as a new class of selective HCA₂ receptor agonists.

**Fig. 9.** Allosteric agonist for the HCA₂ receptor.
agonists [e.g., \( R = 2\)-butyl (EC\(_{50} = 330 \text{nM}) \), \( R = \text{CH}(\text{CH}_3)\text{CH}_2\text{OCH}_3 \) (EC\(_{50} = 200 \text{nM}) \) being the most potent compound. None of the compounds displayed any activity on the HCA\(_2\) receptor. A subtle change to \( R = \text{propyl} \) instead of isopropyl yielded significantly less activity (EC\(_{50} = 7.4 \text{\mu M}) \).

The same research team discovered that some 4-amino-3-nitrobenzoic acids, used as intermediates in the synthesis of the benzotriazoles, also displayed significant activity at HCA\(_3\) receptors and selectivity over HCA\(_2\) receptors (Skinner et al., 2007a). A wider range of these acids, now substituted at the 4-amino group (Fig. 10b), was synthesized and tested in the cAMP whole-cell assay mentioned above. Three compounds displayed full agonist responses with EC\(_{50}\) values below 100 nM, the most potent (\( R_1 = \text{propyl}, R_2 = \text{H} \)) with an EC\(_{50}\) value of 30 nM. In the same publication, it was hypothesized that a pyridine ring could substitute for the nitro-aryl moiety, avoiding the nitro group as a possible toxicophore. Indeed, the resulting 6-amino-substituted nicotinic acids (Fig. 10c) proved active as HCA\(_3\) receptor full agonists when tested in the cAMP whole-cell assay. The structure-activity relationships between the two series were very similar. Again, the \( n\)-propyl-substituted compound was the most active one (EC\(_{50} = 51 \text{nM}) \). Double substitution of the amino group, however, was less favored than in the 4-amino-3-nitrobenzoic acids.

The synthetic efforts were extended to the pyrazole carboxylic acids as a template for the HCA\(_3\) receptor (Skinner et al., 2009). Not surprisingly, the same substituted amino group was introduced to the pyrazole ring system (Fig. 10d). Here, double substitution to yield a tertiary amine was particularly explored, providing a typical pattern of thiophene derivatives, one of which (Fig. 10e) had high potency with an EC\(_{50}\) value of 3 nM and more than 1000-fold selectivity with respect to the HCA\(_2\) receptor. It is noteworthy that replacing -S- with -O- to yield 3’-furanyl substitution rendered the compound at least 1000-fold less active at the HCA\(_3\) receptor.

VI. Receptor Signaling and Regulation

A. G-Protein Coupling

The sensitivity of nicotinic acid-induced effects to the action of pertussis toxin (islet-activating protein) pointed to G-proteins of the G\(_i/G_o\)-family as the G-proteins coupled to the nicotinic acid receptor (Aktories et al., 1983). After the identification of the HCA\(_2\) receptor as a nicotinic acid and ketone body receptor and the discovery of ligands activating HCA\(_1\) and HCA\(_3\) receptors, it has been shown in numerous models that the effects mediated by all three receptors are inhibited by pertussis toxin (Soga et al., 2003; Tunaru et al., 2003; Wise et al., 2003; Cai et al., 2008; Ge et al., 2008; Ahmed et al., 2009a; Irukayama-Tomobe et al., 2009; Liu et al., 2009). Thus, HCA\(_1\), HCA\(_2\), and HCA\(_3\) receptors are G\(_i/G_o\)-coupled receptors.

B. Downstream Signaling

Consistent with the fact that all three receptors are G\(_i/G_o\)-coupled, agonists of HCA\(_1\), HCA\(_2\), and HCA\(_3\) receptors have been shown to inhibit adenyl cyclase activity and thereby to decrease cAMP levels in various cells after heterologous expression of the receptors as well as in primary adipocytes (Soga et al., 2003; Tunaru et al., 2003; Wise et al., 2003; Richman et al., 2007; Ahmed et al., 2009a, 2010; Liu et al., 2009). In adipocytes, a decrease in cAMP results in an antilipolytic effect because cAMP is the major intracellular regulator of lipolysis by stimulating cAMP-dependent kinase to activate lipolytic enzymes (Duncan et al., 2007). Also, in human neutrophils as well as in the human epidermoid cell line A431, activation of the HCA\(_2\) receptor has been shown to result in decreased cAMP levels (Zhou et al., 2007; Kostylina et al., 2008). Kostylina et al. (2008)
suggested that the decrease in cAMP levels induced by activation of the HCA$_2$ receptor in neutrophils induces apoptosis through a reduction in phosphorylation of the proapoptotic protein Bad via cAMP-dependent protein kinase.

In immune cells, activation of G$_i$ results in stimulation of phospholipase C $\beta$-isoforms, most likely through the release of G-protein $\beta\gamma$-subunits (Exton, 1996). Consistent with this, HCA$_2$ and HCA$_3$ receptors, which are expressed in neutrophils, macrophages, and other immune cells, have been shown to mediate ligand-induced increases in free intracellular Ca$^{2+}$ concentrations in a G$_i$/G$_o$-dependent manner (Benyó et al., 2006; Tang et al., 2006; Kostylina et al., 2008; Ahmed et al., 2009a; Irukayama-Tomobe et al., 2009). It has been suggested that the increase in intracellular Ca$^{2+}$ concentrations mediated by HCA$_2$ receptors in response to nicotinic acid results in the activation of Ca$^{2+}$-sensitive phospholipase A$_2$ and subsequent formation of prostanoids (Benyó et al., 2005, 2006; Tang et al., 2006). This would well explain the rapid formation of prostanoids such as prosta-glandins D$_2$ and E$_2$ in response to nicotinic acid (Morrow et al., 1989; Cheng et al., 2006; Maciejewski-Lenoir et al., 2006). Alternatively, phospholipase A$_2$ can also be phosphorylated and activated by ERK, which in turn can be activated through HCA receptors (Tunaru et al., 2003; Richman et al., 2007; Ahmed et al., 2009a; Liu et al., 2009; Walters et al., 2009). This effect is dependent on $\beta$-arrestin, and activation of ERK via the HCA$_2$ receptor has been shown to involve the recruitment of $\beta$-arrestin to the plasma membrane (Walters et al., 2009). In addition, Walters et al. (2009) showed that upon HCA$_2$ receptor activation, $\beta$-arrestin-1 binds to cytosolic phospholipase A$_2$ (PLA$_2$) and activates the enzyme. This results in the release of arachidonic acid and provides an alternative mechanism for the stimulation of PLA$_2$-dependent prostanoid formation through HCA receptors in immune cells. The HCA$_2$ receptor-mediated activation of PLA$_2$ and subsequent formation of prostanoids such as prostaglandins (PG) D$_2$, E$_2$ in epidermal cells is a critical mechanism underlying the nicotinic acid-induced flushing reaction (see section VII).

C. Receptor Desensitization

There is ample evidence that some of the effects mediated by the HCA$_2$ receptor are subject to desensitization (Stern et al., 1991). Consistent with this, nicotinic acid-induced flushing mediated by the HCA$_2$ receptor as well as nicotinic acid-induced increases in intracellular Ca$^{2+}$ concentrations are showing desensitization within minutes (Benyó et al., 2005; Kostylina et al., 2008). However, whether these desensitization phenomena are due to effects on the receptor itself or are at the level of downstream signaling processes is not clear. For heterologously expressed HCA$_1$ and HCA$_2$ receptors, internalization of the receptor in response to ligand application has been described previously (Richman et al., 2007; Liu et al., 2009; Li et al., 2010). In the case of heterologously expressed HCA$_2$ receptor, internalization is dependent on the type of ligand. The full agonist nicotinic acid induced internalization, whereas a partial agonist (which, unlike nicotinic acid, did not induce ERK phosphorylation) had no effect (Richman et al., 2007). Internalization of heterologously expressed HCA$_2$ activated by nicotinic acid was shown to occur in a manner dependent on G-protein-coupled receptor kinase 2 (GRK2) and arrestin 3, whereas the recycling of the internalized receptor was independent of endosomal acidification (Li et al., 2010).

VII. Biological Roles

A. HCA$_1$

Because the HCA$_1$ receptor is almost exclusively expressed in adipocytes and because it mediates lactate-induced, G$_i$-dependent inhibition of adenyl cyclase activity, the obvious biological role of the HCA$_1$ receptor is the inhibitory regulation of adipocyte lipolysis (Cai et al., 2008; Liu et al., 2009; Ahmed et al., 2010). The classic situation leading to elevated plasma lactate levels is intensive exercise during which the anaerobic degradation of carbohydrates to lactate is the major energy-providing pathway (Brooks and Mercier, 1994). It is plausible that under conditions of intensive exercise, when free fatty acid oxidation is strongly reduced, lactate would exert an inhibitory effect on the fatty acid release from adipocytes (Issekutz and Miller, 1962). Although lactate can inhibit lipolysis (Fredholm, 1971; Boyd et al., 1974; Cai et al., 2008; Liu et al., 2009; Ahmed et al., 2010), there is no proof for this concept, which has been rather controversial (Trudeau et al., 1999). When wild-type and HCA$_1$ receptor-deficient mice were trained to exercise at an intensity that resulted in plasma lactate levels sufficient to activate the HCA$_1$ receptor, no difference between the plasma concentrations of free fatty acids could be observed between wild-type and HCA$_1$ receptor-deficient mice (Ahmed et al., 2010). Therefore, lactate and its receptor do not seem to critically contribute to the regulation of lipolysis under conditions of intensive exercise.

Adipocytes are another source of lactate, in that they can convert more than 50% of the metabolized glucose to lactate, a process stimulated by insulin and glucose uptake (DiGirolamo et al., 1992). The liver takes up the lactate released from adipocytes and uses it for gluconeogenesis and glycogen synthesis. Because insulin-induced glucose uptake results in a severalfold increase in extracellular lactate levels in adipose tissue (Jansson et al., 1994; Qvisth et al., 2007; Ahmed et al., 2010), the hypothesis was formulated that lactate, acting through the HCA$_1$ receptor, contributes to the inhibition of lipolysis induced by insulin. In fact, studies in HCA$_1$ receptor-deficient mice and adipocytes clearly showed that insulin-induced inhibition of lipolysis and insulin-in-
duced decrease in adipocyte cAMP were strongly reduced in the absence of HCA1 (Ahmed et al., 2010). Thus, lactate acting through HCA1 functions in an autocrine and paracrine fashion to mediate insulin-induced antilipolytic effects (Fig. 11). Mice lacking HCA1 show a reduced weight gain under high fat diet, indicating that this mechanism may contribute to the increase in body weight under hypercaloric diet. Thus, blockade of the HCA1 receptor may be a strategy for treating obese patients.

B. HCA2

Although the HCA2 receptor is an important target for the drug nicotinic acid, its biological role is instead related to the ketone body 3-hydroxy-butyrate, which activates the receptor with an EC$_{50}$ of approximately 700 µM (Taggart et al., 2005). Under normal conditions, 3-hydroxy-butyrate plasma levels are 50 to 400 µM, but they increase after an overnight fast to 1 to 2 mM and reach 6 to 8 mM during prolonged starvation (Owen et al., 1969). Because the formation of 3-hydroxy-butyrate in the liver depends on the delivery of free fatty acids induced by lipolysis in adipocytes, the activation of the HCA2 receptor by 3-hydroxy-butyrate at millimolar concentrations and the subsequent antilipolytic effect represent a classic negative feedback mechanism that controls the lipolytic rate during starvation (Senior and Loridan, 1968) (Fig. 11). In isolated adipocytes from wild-type mice 3-hydroxy-butyrate inhibits lipolysis, an effect that is not seen in adipocytes from HCA2 receptor-deficient mice (Taggart et al., 2005), further supporting the notion that the HCA2 receptor functions as a metabolic sensor that regulates lipolytic activity during starvation to avoid excessive triglyceride degradation.

The HCA2 receptor agonist nicotinic acid has been used for decades as an antidyslipidemic drug to prevent and treat atherosclerosis (Meyers et al., 2004; Carlson, 2005). Its therapeutic potential is well established (see section VIII). Several mechanisms have been proposed to account for the antiatherosclerotic effects of nicotinic acid (Gille et al., 2008; Kamanna and Kashyap, 2008; Digby et al., 2009). Nicotinic acid reduces low-density lipoprotein (LDL) cholesterol, triglyceride, and lipoprotein(a) plasma levels and simultaneously increases levels of HDL cholesterol (Carlson, 2005). Among the mechanisms that have been proposed to account for the antidyslipidemic properties of nicotinic acid is the activation of HCA2 receptors expressed on adipocytes. The antilipolytic effect of HCA2 receptor activation on adipocytes results in a decreased release of free fatty acids from fat cells. This in return reduces the supply of free fatty acids to the liver, leading to a reduced synthesis of triglycerides and very-low-density lipoprotein as well as a subsequent drop in LDL cholesterol levels (Carlson, 1963). How nicotinic acid increases HDL cholesterol levels is less clear. The decrease in triglyceride content of apolipoprotein B-containing lipoproteins may result in a decreased exchange of triglycerides for cholesteryl esters from HDL particles mediated by the cholesterol transfer protein, eventually leading to increased HDL cholesterol levels (Kontush and Chapman, 2006; Offermanns, 2006; Joy and Hegele, 2008). This mechanism is supported by the observation that HDL-cholesterol elevation in response to nicotinic acid depends on the presence of cholesterol transfer protein (Hernandez et al., 2007; van der Hoorn et al., 2008). Whether the HCA2 receptor also mediates the increase in HDL-cholesterol levels in response to nicotinic acid, however, remains unclear.

![Fig. 11. Functions of the recently deorphanized receptors HCA1, HCA2, and HCA3. The lactate receptor HCA1 mediates the short-term anabolic effects of insulin on adipocytes and thereby helps to store energy after feeding (A). In contrast, HCA2 and HCA3 receptors are involved in the long-term regulation of lipolytic activity, being receptors for the ketone body 3-hydroxy-butyrate (HCA2) and the β-oxidation intermediate 3-hydroxy-octanoate (HCA3). In situations of increased β-oxidation rates (e.g., during starvation), 3-hydroxy-butyrate and 3-hydroxy-octanoate plasma levels are increased and result in the inhibitory regulation of lipolysis via HCA2 and HCA3 receptors, respectively, in the form of a negative feedback loop (B). Thereby, HCA2 and HCA3 receptors help preserve energy stores during starvation. AC, adenylyl cyclase; TG, triglycerides; HSL, homon-sensitive lipase; ATGL, adipocyte triglyceride lipase; FFA, free fatty acids; PKA, cAMP-regulated protein kinase.](image-url)
(Bodor and Offermanns, 2008; Kamanna and Kashyp, 2008). Because mouse lipoprotein metabolism is considerably different from that of humans, genetic mouse models are not helpful in analyzing this question. Selective antagonists of the HCA2 receptor tested in appropriate models may eventually be helpful in answering this question.

The physiological role of the HCA2 receptor in immune cells, such as macrophages or neutrophils, that express the receptor remains currently unclear. Given that plasma levels of 3-OH-butyrate, the endogenous ligand of HCA2, are highly elevated under starving conditions, one may speculate that the HCA2 receptor expressed on immune cells mediates anti-inflammatory effects that could be advantageous under conditions of starvation. In this respect, it is interesting to know that HCA2 expression in macrophages is up-regulated by various cytokines and activators of innate immunity (see section IV).

There is good evidence that expression of the HCA2 receptor in epidermal Langerhans cells and keratinocytes is responsible for one of the major unwanted effects of nicotinic acid, the flushing reaction. Flushing results from a cutaneous vasodilation associated with a sensation of tingling and burning that causes many patients to discontinue nicotinic acid therapy (Benyò et al., 2005, 2006; Maciejewski-Lenoir et al., 2006; Gille et al., 2008; Kamanna et al., 2009). The induction of a flush reaction by nicotinic acid or the antipsoriatic drug monomethyl fumarate is mediated by HCA2 and shows a biphasic increase in dermal blood flow (Hanson et al., 2010). The first phase is induced via activation of HCA2 on Langerhans cells, and HCA2 on keratinocytes is responsible for the late phase of the response. Whereas Langerhans cell-mediated flushing involves cyclooxygenase-1, PGD2, and PGE2, keratinocyte-mediated late-phase flushing involves cyclooxygenase-2 and PGE2 (Benyò et al., 2005; Cheng et al., 2006; Paolini et al., 2008; Hanson et al., 2010). The expression of HCA2 in various cells of the epidermis and the marked epidermal and dermal effects resulting from the activation of HCA2 has raised the question whether this receptor system has any physiological or pathophysiological role in the skin, besides its pharmacological function. HCA2-mediated prostaglandin formation in Langerhans cells and keratinocytes, for instance, may underlie some of the many forms of skin reactions in which alterations or traumata in the epidermis result in dermal vasodilation and various dermal sensations. It will be interesting to explore in the future the potential physiological and pathophysiological role of HCA2-mediated cellular effects in the epidermis.

The HCA2 receptor has also been shown to be expressed in intestinal epithelial cells where the receptor may respond to butyrate, which is present in millimolar concentrations in the gut lumen. It has been suggested that HCA2 thereby functions as a tumor suppressor and anti-inflammatory receptor (Thangaraju et al., 2009).

C. HCA3

The biological role of the HCA3 receptor seems to be quite similar to that of the HCA2 receptor with regard to the regulation of adipocyte lipolysis. The plasma concentration of the β-oxidation intermediate 3-hydroxy-octanoate, which activates HCA3 receptors at micromolar concentrations, increases substantially under conditions of increased free fatty acid oxidation during fasting, during diabetic ketoacidosis, in various mitochondrial fatty acid β-oxidation disorders, and under the influence of a ketogenic diet (Costa et al., 1998; Jones et al., 2002; Ahmed et al., 2009a). Similar to HCA2, the HCA3 receptor mediates a negative feedback mechanism under these conditions to counterbalance prolipolytic stimuli (Ahmed et al., 2009b) (Fig. 11). The fact that the HCA3 receptor is found only in higher primates suggests that the optimal regulation of lipolytic activity under conditions of increased free fatty acid release during starvation and other extreme conditions represents an important mechanism to use the energy stored in form of triglycerides economically and to avoid a waste of energy. The advantage of having additional regulatory mechanisms under these conditions was apparently sufficient to drive the evolution of additional lipolysis-controlling receptors in higher primates. Whether HCA3 has potential biological roles outside the adipose tissue is currently not known.

VIII. Therapeutic Potential of Hydroxy-carboxylic Acid Receptor Ligands

In this paragraph the effects of HCA receptor ligands in the clinic will be briefly discussed. Not surprisingly, nicotinic acid itself has been the best studied compound in this respect, either alone or in combination with other lipid-altering drugs.

A. Nicotinic Acid Alone

Nicotinic acid has been used in clinical practice for more than half a century now, ever since Altschul et al. (1955) pioneered its use as an antidysslipidemic drug in man. This finding was followed-up by a number of further clinical studies that all established the usefulness of nicotinic acid as a "broad-spectrum" lipid-modulating drug (for a recent review, see Carlson, 2005; Guyton, 2007), albeit with predominantly dermatological side effects (flushing). In a well defined early long-term trial, nicotinic acid treatment had no effect on total mortality compared with placebo, although it showed a modest benefit in lowering the incidence of recurrent myocardial infarction (Coronary Drug Project Research Group, 1975). It is noteworthy that 9 years after conclusion of this trial, mortality in the nicotinic acid-treated patient group was a significant 11% lower than placebo (Canner et al., 1986). The authors concluded that this delayed benefit of nicotinic acid, occurring after discontinuation of the drug, may relate to the early favorable effect of nicotinic acid on reinfarction incidents and/or may be
the result of the cholesterol-altering effect of nicotinic acid.

B. Nicotinic Acid with Antiflushing Strategies

The use of generic nicotinic acid in its crystalline form has always been associated with the common side effect of flushing, which had a strong negative impact on patient compliance. Strategies such as taking tablets during meals and/or taking acetylsalicylic acid concomitantly often render the flushing bearable; in addition, it seems to wane over time.

1. Modified Release Formulations. Formulation of nicotinic acid in slow- or extended-release forms also helps. In particular, the extended-release form has been clinically evaluated, also as monotherapy. Capuzzi et al. (1998) defined its efficacy and safety in a long-term study in over 500 patients for almost 2 years. A reduction in LDL cholesterol levels of $\approx 20\%$ was observed with a concomitant increase of $\approx 28\%$ in HDL cholesterol levels. Nevertheless, flushing continued to be a significant side effect in 75% of the patients.

2. Combinations with Antiflushing Drugs. Acetylsalicylic acid can be combined with nicotinic acid to reduce flushing (Oberwittler and Baccara-Dinet, 2006). The rationale is that the compound inhibits prostaglandin synthesis, including the formation of prostaglandin D$_2$. This eicosanoid plays a major role in the genesis of the flushing syndrome in that it causes cutaneous vasodilation (see sections VI and VII). A more recent development is the addition of a selective PGD$_2$ receptor antagonist, laropiprant, to nicotinic acid therapy (Cheng et al., 2006). More than 400 patients were treated, a subpopulation of which received nicotinic acid in its extended release form in combination with various doses of laropiprant for at least 8 weeks. These patients experienced significantly less flushing than those on nicotinic acid alone. It is noteworthy that all doses of laropiprant tested were equally effective in inhibiting the flushing syndrome (Paolini et al., 2008). The data from this and a few more recent trials with similar outcomes have been summarized by McKenney et al. (2010). As a result, a fixed-dose combination formulation (1000 mg of nicotinic acid/20 mg of laropiprant) was allowed access to the market in Europe in 2008, whereas the U.S. Food and Drug Administration has asked for further studies.

C. Nicotinic Acid in Combination with Other Lipid-Altering Drugs

Many later clinical trials were designed to evaluate the use of nicotinic acid in combination with other lipid-altering drugs, particularly HMG-CoA reductase inhibitors (“statins”). On many occasions, a significant reduction of coronary events and mortality was observed. With statins emerging as a first-choice strategy for lipid lowering in the 1990s, it was only logical to examine the combined effects of nicotinic acid with one of the early HMG-CoA reductase inhibitors, simvastatin, in a double-blind trial with 160 patients with established coronary heart disease and low HDL cholesterol plasma levels observed for 3 years (Brown et al., 2001). The mean levels of LDL and HDL cholesterol were unaltered in the placebo group, but changed by $-42$ and $+26\%$, respectively, in the simvastatin-plus-nicotinic acid group. This was manifested clinically by a strong reduction in the frequency of a first cardiovascular event (24 versus 3%). Although the ideal control group receiving simvastatin alone was not tested, the reduction in cardiovascular events in patients treated with simvastatin and nicotinic acid was much larger than would have been expected from a treatment with simvastatin alone.

An important surrogate endpoint in many trials is the impact on the size of atherosclerotic lesions, which is not altered very much on statin monotherapy (Guyton, 2007). In all clinical trials, addition of nicotinic acid to statin therapy resulted in a reduced development of atherosclerotic lesions. For instance, in the ARBITER 2 study (Taylor et al., 2004), patients with coronary heart disease who were treated with statins and had low levels of HDL cholesterol received nicotinic acid as a supplementary drug regimen. The primary endpoint was the change in common carotid intima-media thickness after 1 year. After this period, compared with values at the start of the study, the thickness was significantly increased in the placebo group but unchanged in the patients treated with nicotinic acid. The overall difference in thickness progression between the nicotinic acid and placebo groups was not statistically significant, however (Taylor et al., 2004). A similar, more recent study (ARBITER6-HALTS) compared the addition of either nicotinic acid (extended release) or ezetimibe to statin monotherapy (Taylor et al., 2009). Niacin proved superior to ezetimibe on the basis of the same primary clinical endpoint of the mean common carotid intima-media thickness after 14 months of treatment. A total of 363 patients were enrolled, but after 208 patients had completed the study, it was decided to halt the trial because the use of ezetimibe led to a paradoxical increase in atherosclerosis, despite the significant (further) reduction in LDL cholesterol levels. In the nicotinic acid-plus-statins group, a significant regression of both mean and maximal carotid intima-media thickness was observed, in combination with a significant 18% increase in HDL cholesterol levels.

Two cardiovascular endpoint trials are currently under way to test whether the addition of nicotinic acid to statins has a beneficial effect on the progression of cardiovascular disease, incidence of major cardiovascular events, and cardiovascular disease-associated mortality. The Atherothrombosis Intervention in Metabolic Syndrome with low HDL/high triglycerides and Impact on Global Health Outcomes (AIM-HIGH) study compares simvastatin and extended-release nicotinic acid with simvastatin monotherapy in 3300 patients at high risk suffering from cardiovascular disease, low HDL chole-
terol, and high triglyceride levels (http://clinicaltrials.gov/ct2/show/NCT001202889). The Heart Protection study 2: Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE) compares simvastatin plus extended-release nicotinic acid plus the prostaglandin D2 receptor 1 antagonist laropiprant with simvastatin monotherapy in more than 20,000 patients with coronary heart disease (http://clinicaltrials.gov/ct2/show/NCT00461630). The results of these trials are expected to be reported between 2011 and 2013.

D. Fumaric Acid Derivatives

The use of fumaric acid esters for the treatment of psoriasis goes back to case reports in Germany as early as 1959 (Schweckendiek, 1959). A small randomized, double-blind, placebo-controlled study involving 39 patients showed that oral administration of a combination of fumarate esters (the monoethyl- and dimethyl analogs) led to a significant reduction of body surface affected with psoriasis with an average of 21% at the start of the study to 6.7% after 16 weeks. Almost all patients suffered from flushing and diarrhea (Nugteren-Huying et al., 1990). Prolonged use of fumaric acid esters up to 14 years did not seem to cause unacceptable or unbearable side effects, composed mostly of flushing, diarrhea, and lymphocytopenia (Hoefnagel et al., 2003). In a recent study, a retrospective analysis of close to 1000 patients treated in over 150 centers for at least 2 years yielded similar results (i.e., sustained clinical efficacy and an acceptable safety profile) (Reich et al., 2009). Among the many suggested molecular mechanisms of action, the fumarate esters have been shown to interact with the HCA2 receptor, which may in fact cause the flushing. A similar preparation (BG-12, consisting of dimethylfumarate only) was tested in a double-blind, placebo-controlled clinical phase Ib study in more than 250 patients with relapsing-remitting multiple sclerosis. Treatment with BG-12 (240 mg three times daily) significantly reduced the mean total number of new brain lesions by more than 70% compared with placebo. Again, adverse events included abdominal pain and flushing (Kappos et al., 2008). Given the expression of HCA2 receptors in various immune cells and the reported beneficial effects of fumaric acid esters, which are able to activate HCA2 receptors, it is tempting to speculate that HCA2 receptors may be interesting targets for anti-inflammatory or immunosuppressive therapeutic approaches.

E. Clinical Candidates

A partial agonist for the HCA2 receptor, MK-0354 (Fig. 4), was evaluated in both phase I and II clinical studies. The effects of single and multiple doses of MK-0354 (300 mg–4 g) were evaluated in two phase I studies conducted in healthy men. In a phase II study, the effects of MK-0354 (2.5 g once daily) on lipids were recorded during 4 weeks in 66 dyslipidemic patients. In the phase I study, it was noticed that a single dose (300 mg) of MK-0354 reduced free fatty acid levels comparable with a 1-g dose of extended-release nicotinic acid, which did not alter after 7 days of administration. In the phase II study, 2.5 g of MK-0354 produced little flushing; however, no clinically meaningful effects on lipids, especially on HDL levels, were observed (Lai et al., 2008).

IX. Conclusion

Despite the medical relevance of HCA2 as a receptor for nicotinic acid and the development of many synthetic ligands of HCA2 and HCA3 receptors, endogenous agonists of receptors of the HCA family have only recently been identified. This is most likely because their physiological ligands do not represent classic GPCR agonists such as hormones, neurotransmitters, or other mediators but are metabolites that have traditionally not been regarded as bona fide G-protein-coupled receptor agonists. Another unusual property of endogenous HCA receptor ligands that has made their identification difficult is their relatively low potency, especially in the case of HCA1 and HCA2, with EC50 values in the millimolar range. Based on the well known pharmacological properties of nicotinic acid and the fact that all three HCA receptors are expressed in adipocytes and signal through G1-type G-proteins, there has been great interest in the development of synthetic ligands that act through HCA receptors to induce antidyshlipidemic effects similar to those of nicotinic acid. Although this approach involved the generation of agonistic ligands, the analysis of HCA1-deficient animals suggests also that antagonists may have a pharmacological potential by reducing postprandial antilipolysis and reducing the weight gain under high-calorie diet. Future studies will have to clarify whether any of these strategies will be beneficial in defined groups of patients. In contrast to the HCA1 receptor, HCA2 and HCA3 receptors are also expressed in a variety of immune cells. Increasing evidence indicates that activation of HCA2 and HCA3 on immune cells may have beneficial effects on various inflammatory diseases, making both receptors attractive targets for atherosclerosis, chronic inflammatory, and autoimmune disorders. The development of selective high-affinity agonists and antagonists for these receptors will in the future help to not only better understand their physiological roles but also to further explore their potential as therapeutic targets.

Acknowledgments

This work was supported by the German Research Foundation (to S.O.) and the Dutch Top Institute Pharma [project D1-105] (to A.P.IJ.). We thank Svea Hümmer for excellent secretarial help in preparing the manuscript.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Offermanns, Colletti, Lovenberg, Semple, Wise, and IJzerman.
References


