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

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Abstract	270
I. Introduction	270
II. Molecular basis for receptor nomenclature	271
A. Cloning of cDNAs encoding hydroxy-carboxylic acid receptors	271
B. Sequence alignment and phylogenetic tree	271
C. Deorphanization of hydroxy-carboxylic acid receptors	272
1. HCA ₁	272
2. HCA ₂	273
3. HCA ₃	273
D. Proposed nomenclature	274
III. Receptor mutagenesis and modeling studies	274
IV. Gene structure, expression, and regulation	275
A. HCA ₁	275
B. HCA ₂	276
C. HCA ₃	276
V. Receptor classification with pharmacological tools	276
A. Structure-activity relationships for the HCA ₂ receptor	276
1. Nicotinic acid-like compounds	276
2. Pyrazoles	278
3. Acifran analogs	279
4. Anthranilic acid derivatives	279
5. Fumaric and other acids and their esters	280
6. Pyridopyrimidinones	280
7. Pyrazolopyrimidines as allosteric agonists	281
8. Patent literature	281
B. Structure-activity relationships for the HCA ₃ receptor	281
VI. Receptor signaling and regulation	282
A. G-protein coupling	282
B. Downstream signaling	282
C. Receptor desensitization	283
VII. Biological roles	283
A. HCA ₁	283
B. HCA ₂	284
C. HCA ₃	285

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VIII. Therapeutic potential of hydroxy-carboxylic acid receptor ligands	285
A. Nicotinic acid alone	285
B. Nicotinic acid with antilflushing strategies	286
1. Modified release formulations	286
2. Combinations with antilflushing drugs	286
C. Nicotinic acid in combination with other lipid-altering drugs	286
D. Fumaric acid derivatives	287
E. Clinical candidates	287
IX. Conclusion	287
Acknowledgments	287
References	288

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-butyric 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 G_i-type G-proteins mediating antilipolytic effects in fat cells. HCA₂ and HCA₃ receptors are also expressed in a variety of immune cells. HCA₂ is a receptor for the antidiabetic 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 antidiabetic 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 Oro, 1962; Carlson, 1963). Thereafter, nicotinic acid was shown to reduce cAMP levels in adipose tissue (Butcher et al., 1968), an effect that resulted from G_i-mediated inhibition of adenylyl cyclase (Aktories et al., 1980a). At that time, the hypothesis was formulated that nicotinic acid acts through a G_i-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-butyrate 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

¹Abbreviations: CHO, Chinese hamster ovary; CysLT₂, cysteinyl-leukotriene 2; ERK, extracellular signal-regulated kinase; ETE, eicosatetraenoic acid; GPR, G protein receptor; GRK, G-protein-coupled receptor kinase; GTP γ S, guanosine 5'-O-(3-thio)triphosphate; HCA, hydroxy-carboxylic acid; HDL, high-density lipoprotein; INF, interferon; LDL, low-density lipoprotein; MEF, monoethyl ester of fumaric acid; MK-0354, 3-(1H-tetrazol-5-yl)-1,4,5,6-tetrahydrocyclopentapyrazole; MMF, monomethyl ester of fumaric acid; PG, prostaglandin; PLA₂, phospholipase A₂; PPAR, peroxisome proliferator-activated receptor; TM, transmembrane; TNF, tumor necrosis factor.

TABLE 1
Current HCA receptor nomenclature and receptor properties
For further details, see IJzerman et al. (2010).

Properties	HCA ₁	HCA ₂	HCA ₃
Previous names/aliases	GPR81, GPR104, TA-GPCR, LACR, FKSG80	GPR109A, HM74a (human), PUMA-G (mouse), HM74b, NIACR1	GPR109B, HM74, NIACR2
Genomic location	12q24.31 (human), 5F (mouse)	12q24.31 (human), 5F (mouse)	12q24.31 (human)
Amino acid length	346 (human), 343 (mouse)	363 (human), 360 (mouse)	387 (human)
Naturally occurring specific agonists (pEC ₅₀)	2-OH-propanoate (2.5–2.8)	3-OH-butyrate (3.1), nicotinic acid (6.6–7.2)	3-OH-octanoate (5.1), 2-OH-octanoate (5.4), D-phenylalanine (5.0), D-tryptophan (5.4)
Specific synthetic agonists (pEC ₅₀)		acipimox (5.2–5.6)	1-Isopropylbenzo-triazole-5-carboxylic acid (6.4), 5-methyl-5-(5-methyl-thio-phen-3-yl)-4-oxo-4,5-dihydro-furan-2-carboxylic acid (6.7)
G-protein coupling	G _i /G _o	G _i /G _o	G _i /G _o
Expression in human tissue	Adipocytes	Adipocytes, macrophages, neutrophils, epidermal Langerhans cells, keratinocytes, intestinal epithelial cells	Adipocytes, neutrophils, macrophages, intestinal epithelial cells
Cellular function	Inhibition of lipolysis	Inhibition of lipolysis, activation of immune cells	Inhibition of lipolysis, activation of immune cells
Phenotype of mice lacking receptor	Reduced insulin-induced antilipolysis	Lack of nicotinic acid effects on lipid plasma levels and of nicotinic acid-induced flushing	

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 amplicate. One of the cDNAs encoding a new G-protein-coupled receptor was called HM74 (HCA₃/GPR109B), and the receptor was shown to be expressed in monocytes and neutrophils. It was also noted that the HCA₃ sequence differed considerably from that of leukocyte chemotactic peptide receptors, suggesting that this 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 HCA₃ 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 INF- 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 INF- 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

HCA ₁	MYNG-----S--	CCRIEGDT	ISQVMPPLLI	VAFLVLGALGN	GVALCGFCFH	43
HCA ₂	MNRHH-LQDH	FLEIDKKN--	CCVFRDDF	IVKVLPPVLG	LEFIFGLLGN	GLALWIFCFH 55
HCA ₃	MNRHH-LQDH	FLEIDKKN--	CCVFRDDF	IAKVLPPVLG	LEFIFGLLGN	GLALWIFCFH 55
OXER1	MLCHR* ⁽⁶⁻³⁹⁾ MELH	NLSSPSPS*	GPCHPTSSSL	VSAFLAPILA	LEFVLGLVGN	SLALFIFCIH 120
GPR31	MP-----	FPNCSAPSTV	VATAVGVLG	LECGLGLLGN	AVALTFLFR	42
	*	**	*	*	*	*
HCA ₁	MKTWKVPSTVY	LFNLAVADFL	LMICLPFRD	YYLRRRHWF	GDIPCRVGLF	TLAMNRAGSI 103
HCA ₂	LKSWKSSRI	LFNLAVADFL	LIICLPFLMD	NYVRRWDWKF	GDIPCRMLF	MLAMNRQSGI 115
HCA ₃	LKSWKSSRI	LFNLAVADFL	LIICLPFVMD	YYVRRSDWNF	GDIPCRVLV	MFAMNRQSGI 115
OXER1	TRPWTNTV	LVSLVAADFL	LISNLPRLVD	YYLLHETWRF	GAAACKVNLF	MLSTNRTASV 180
GPR31	VRVWKPYAVY	LLNLALADLL	LAACLPFLAA	FYLSLQAWHL	GRVGCWALHF	LLDLSRSVGM 102
	*	*	*	*	*	*
HCA ₁	VFLTVAADR	YFKVVHPHHA	VNTISTRVAA	GIVCTLWALV	ILGTVYLLLE	NHLCVQETAV 163
HCA ₂	IFLTVVAVDR	YFRVVHPHHA	LNKISNRTAA	IISCLLWGIT	IGLTVHLLKK	KMPIQNGGAN 175
HCA ₃	IFLTVVAVDR	YFRVVHPHHA	LNKISNWTAA	IISCLLWGIT	VGLTVHLLKK	KLLIQNGPAN 175
OXER1	VFLTAIALNR	YKVVVQPHHV	LSRASVGAA	RVAGGLWVGI	LLNGHLLLS	TF-----SGP 235
GPR31	AFLLAAVALDR	YLRVVHPRLK	VNLLSPQAL	GVSGLVWLLM	VALTCPGLLI	SEAAQNSTR 161
	*	*	*	*	*	*
HCA ₁	SCESFIM--	-ESANGWHD	MFQLEFFMPL	GIILFCSFKI	VWSLRRR-QQ	LARQARMKKA 218
HCA ₂	LCSSFSI--	-CHTFQWHEA	MFLLLEFFLPL	GIILFCSARI	IWSLRQR-Q-	MDRHAKIKRA 229
HCA ₃	VCISFSI--	-CHTFRWHEA	MFLLLEFFLPL	GIILFCSARI	IWSLRQR-Q-	MDRHAKIKRA 229
OXER1	SCLSYRVGK	PSASLRWHQA	LYLLEFFLPL	ALILFAIVSI	GLTIRNR--G	LGGQAGPQRA 293
GPR31	-CHSFYSRAD	GSFSI IWQEA	LSCLQFVLPF	GLIVFCNAGI	IRALQKRLRE	PEKQPKLQRA 220
	*	*	*	*	*	*
HCA ₁	TRFIMVVAIV	FITCYLPSVS	---ARLYFLW	TVPSA---C	DP--SVHGA-	--LHITLSFT 267
HCA ₂	ITFIMVVAIV	FVICFLPSVV	---VRIRIFW	LLHTSGTQNC	EVYRSVDLA-	--FFITLSFT 283
HCA ₃	ITFIMVVAIV	FVICFLPSVV	---VRIRIFW	LLHTSGTQNC	EVYRSVDLA-	--FFITLSFT 283
OXER1	MRVLAMVVAV	YTICFLPSII	FGMASMVAFW	L-----	SACRSLDLCT	QLFHGSLAFT 344
GPR31	QALVTLLVVVL	FALCFLPCFL	-ARVLMHIF-	-----QNL	GSCRALCAVA	HTSDVTGSLT 271
	*	*	*	*	*	*
HCA ₁	YMNSMLDPLV	YFSSSPSPFK	FYNKL--KIC	SLKPKQPGHS	KTQRPEEMI	SNLGRRSICIS 325
HCA ₂	YMNSMLDPVV	YFSSSPSPFN	FFSTL--INR	CLQRKMTGEP	DNNRSTVEL	TGDPNKTG-R 340
HCA ₃	YMNSMLDPVV	YFSSSPSPFN	FFSTL--INR	CLQRKMTGEP	DNNRSTVEL	TGDPNKTG-R 340
OXER1	YLNVSVDPLV	YCFSSPNFLH	QSRALLGLTR	GRQGPVSDES	SYQPSRQWRY	REASRKAEI 404
GPR31	YLHVSVDPLV	YCFSSPTFRS	SYRRVFHTLR	G-KGQAAEPP	DFNPRDSYS-	----- 319
	*	*	*	*	*	*
HCA ₁	VANSFQSQSD	GQWDPHIVEW	H-----	-----	-----	346
HCA ₂	APEALMANS	EPWSPSYLGP	TSP-----	-----	-----	363
HCA ₃	APEALIANSG	EPWSPSYLGP	TSNNHSHKKGH	CHQEPASLEK	QLGCCIE	387
OXER1	GKLKVQGEVS	LEKEGSSQG-	-----	-----	-----	423
GPR31	-----	-----	-----	-----	-----	319
	*	*	*	*	*	*

FIG. 1. Amino acid sequence alignment of HCA₁, HCA₂, and HCA₃ as well as of the related receptors GPR31 and OXER1 (TG1019, GPR170) based on the Clustal V method. Transmembrane regions are indicated by gray boxes. Two short sequences in the N-terminal part of OXER1 (residues 6–39 and 52–80) have been omitted to facilitate the alignment (gaps are indicated by asterisks). Positions with identical amino acids in HCA₁, HCA₂, and HCA₃ are marked by one asterisk (*), positions with identical residues in all five receptors are indicated by two asterisks (**).

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 GTPγS-binding in a HCA₁ receptor-dependent fashion with an EC₅₀ of 1.3 mM. Inhibition of forskolin-stimulated cAMP production in CHO cells transfected with HCA₁ occurred with an EC₅₀ 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

FIG. 2. A, phylogenetic tree and percentage amino acid sequence identity 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-EET 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.

receptor, sodium propionate activated the receptor with an EC₅₀ of 3 mM. 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 mech-

anisms 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 intensive 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 deorphanization of the HCA₂ receptor (GPR109A) as a receptor for the ketone body 3-hydroxy-butyrate. Racemic (DL)-3-hydroxy-butyrate 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-Hydroxy-butyrate had no activity on HCA₁ or HCA₃ receptors. 3-Hydroxy-butyrate 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-hydroxy-octanoate, 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-Hydroxy-octanoate was not able to activate HCA₁ or HCA₂ receptors, and a shortening or extension of the chain length of 3-hydroxy-octanoate by more than two carbon atoms resulted in a complete loss of activity at the HCA₃ receptor. 3-Hydroxy-hexanoate, 3-hydroxy-octanoate, and 3-hydroxy-decanoate 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-hydroxy-octanoate plasma con-

centrations reach levels sufficient to activate the HCA₃ receptor (Costa et al., 1998; Jones et al., 2002; Ahmed et al., 2009a).

It is noteworthy that various aromatic D-amino acids, such as D-phenylalanine, D-tryptophan, and D-kynurenine, were shown to decrease cAMP levels by acting through the HCA₃ receptor with EC₅₀ values between 10 and 100 μ M (Irukayama-Tomobe et al., 2009). Aromatic D-amino acids had no effect on the HCA₂ receptor. The physiological enantiomers L-phenylalanine, L-tryptophan, and L-kynurenine did not activate HCA₃ at concentrations of 100 μ M (Irukayama-Tomobe et al., 2009). Because D-amino acids are extremely rare, it is unclear whether the ability of aromatic 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 HCA₁ (GPR81), HCA₂ (GPR109A), and HCA₃ (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 neither 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 hydroxycarboxylic acids, we propose that this receptor family be called the “hydroxycarboxylic acid (HCA) receptor family,” which currently has three members: HCA₁, HCA₂, and HCA₃ (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) (Zingoni 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 (EC₅₀ values of 1.9 and 90 μ 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 (EC₅₀ = 2 μ 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 HCA₂, 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 complex. 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 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, 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-hydroxy-octanoic 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 G protein. This evidence suggests that the HCA₁ receptor, an orphan receptor at the time of the experiments, indeed signals via G_i proteins, because the wild-type CysLT₂ receptor is coupled predominantly to G_q. In adipocytes of transgenic mice expressing the chimeric receptor, leukotriene D₄ inhibited lipolysis, measured as a reduction in glycerol release. This effect was not observed in wild-type mice lacking the receptor construct. Nicotinic acid served as a control, administration of which led to an inhibition of lipolysis in all mice.

Liu et al. (2009) performed site-directed mutagenesis of the HCA₁ receptor, providing some evidence that conserved key residues in the TM domains make up the binding site for lactate. Four point mutations were separately introduced: R99A (TM3), Y233A (TM6), R240A (TM6), and T267A (TM7). The first amino acid is equivalent to Arg111 in the HCA₂ receptor, the putative anchor point for the ligand's carboxylic acid group. All four mutations led to inactive receptors, because lactate did not stimulate [³⁵S]GTPγS binding any of these. A receptor homology model based on the structure of rhodopsin structure was presented in which lactate was manually docked to interact with the four amino acids, somewhat at odds with the proposed orientation of nicotinic acid in the HCA₂ receptor.

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; Jenning 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; Jenning 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 (Jenning 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 (Jenning et al., 2009). Jenning 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. HCA_2

The receptor of HCA_2 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 HCA_2 receptor is induced 1 to 2 days earlier than expression of the HCA_1 receptor (Ge et al., 2008; Jeninga et al., 2009). Similar to HCA_1 , the expression of the HCA_2 receptor is increased by PPAR γ activation (Jeninga et al., 2009). Because there is no functional PPAR-response element in the promoter of the HCA_2 receptor gene, it has been suggested that the PPAR-response element present in the proximal promoter of the HCA_1 receptor gene controls the HCA_2 receptor gene promoter (Jeninga et al., 2009).

Apart from adipocytes, expression of the HCA_2 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). HCA_2 receptor expression was not detected in immature bone marrow neutrophils, indicating that induction of HCA_2 receptor expression occurs during the late stages of terminal differentiation of neutrophils (Kostylina et al., 2008). Expression of the HCA_2 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 HCA_2 receptor was up-regulated by hypoxia (Knowles et al., 2006).

Expression of the HCA_2 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 HCA_2 receptor, which appears primarily localized to the basolateral membrane of these cells (Martin et al., 2009). On the mRNA level, HCA_2 receptor expression has been shown in primary human keratinocytes (Maciejewski-Lenoir et al., 2006; Tang et al., 2008); however, immunohistochemistry failed to demonstrate HCA_2 receptor expression in keratinocytes (Maciejewski-Lenoir et al., 2006). Expression of the HCA_2 receptor in keratinocytes has recently been shown by genetic and functional approaches (Hanson et al., 2010), and strong HCA_2 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 HCA_2

receptor in keratinocytes and keratinocyte cell lines is induced by IFN- γ (Tang et al., 2008).

C. HCA_3

Expression of the HCA_3 receptor seems to be very similar to that of the HCA_2 receptor. The HCA_3 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 PPAR γ (Jeninga et al., 2009). In addition, the HCA_3 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 HCA_3 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 HCA_2 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 HCA_2 receptor, we will discuss the synthetic ligands for this receptor first, followed by the more restricted information on the HCA_3 receptor. To our knowledge, synthetic ligands for the HCA_1 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 HCA_2 Receptor

1. Nicotinic Acid-Like Compounds. Lorenzen et al. (2001) observed that nicotinic acid increases [35 S]GTP γ S

TABLE 2
 EC_{50} and K_i values of nicotinic acid and related compounds

Compound	Rat ^a			Human ^b		
	GTP γ S Binding Assay (EC ₅₀)		[³ H]Nicotinic Acid Binding Assay (K _i)	GTP γ S Binding Assay (EC ₅₀)	[³ H]Nicotinic Acid Binding Assay (K _i)	
	Adipocytes	Spleen			HCA2 (Cloned)	HCA2 (Cloned)
	μM					
Nicotinic acid	1.4	0.70	0.033	0.25	0.081	0.079
Acifran	N.D.	N.D.	N.D.	2.1	1.1	0.83
Acipimox	10	6.6	0.31	6.0	5.1	4.3
3-Pyridine-acetic acid	16	17	0.40	5.5	0.54	0.55
Pyridazine-4-COOH	3.8	3.1	0.12	N.D.	N.D.	N.D.
Pyrazine-2-COOH	26	22	0.76	N.D.	N.D.	N.D.
5-Me-nicotinic acid	30	30	0.72	8.7	4.12	3.68
5-Me-pyrazine-2-COOH	52	15	0.65	N.D.	N.D.	N.D.
6-Me-nicotinic acid	73	54	1.7	N.D.	N.D.	N.D.
Nicotinic acid-1-oxide	80	74	3.2	N.D.	N.D.	N.D.
2-OH-nicotinic acid	132	N.D.	N.D.	N.D.	N.D.	N.D.
Furan-3-COOH	142	N.D.	N.D.	N.D.	N.D.	N.D.
Nicotinamide	>1000	>1000	N.D.	>1000	92	75

N.D., not determined.

^a Lorenzen et al. (2001).

^b Wise et al. (2003).

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 adenyl cyclase inhibition studies (Aktories et al., 1980a). A good window in the same assay format of [³⁵S]GTP γ 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,

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

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 heterocyclic 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. Modifying 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 active than nicotinic acid, whereas the simple substitution pattern in 6-methylnicotinic acid rendered the compound 50-fold less active. It is noteworthy that all active compounds in Table 2 were full agonists, in that they stimulated [³⁵S]GTPγS binding to the same extent as nicotinic acid. Nicotinamide was inactive, however, suggesting 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 invariably 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]GTPγS binding assay (relative intrinsic activity of 85%) with submicromolar affinity (K_i = 0.59 μM) 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 academia and industry. Partial agonists may display tissue selectivity on the basis of differences in receptor expression in different tissues. This was thought to be potentially beneficial in the case of the HCA₂ receptor, as the side effect of flushing might be separated from the desired 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-carboxylic 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 μM, 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 μM), modest potency in the [³⁵S]GTPγS binding assay, and a relative intrinsic activity of 39%. A more extended series of pyrazoles, along the same vein of either alkyl or benzyl 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/tetrazole switch yielded a partial agonist with substantial activity on the cloned human and mouse receptors in a cAMP assay. In mice, this compound [3-(1*H*-tetrazol-5-yl)-1,4,5,6-tetrahydrocyclopentapyrazole (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 pharmacokinetic 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 3
Affinities (K_i values in radioligand displacement study), potencies (EC_{50} values in [³⁵S]GTPγS binding assay), and relative intrinsic activities (RIA; [³⁵S]GTPγS binding assay) of pyrazole-derived partial agonists for the rat nicotinic acid receptor

<div> </div>					<div> </div>			
R ₁	R ₂	K_i	EC_{50}	RIA	R ₁	K_i	EC_{50}	RIA
		μM		%		μM		%
Nicotinic acid		0.033	0.74	100	C ₆ H ₅ -CH ₂ -	1.3	87	50
H	H	0.59	22	85	C ₆ H ₅ -(CH ₂) ₂ -	1.6	N.D.	N.D.
i-C ₃ H ₇	H	0.68	22	68	C ₆ H ₅ -(CH ₂) ₃ -	6.3	N.D.	n.d.
C ₃ H ₇	H	0.14	5.1	70	3Cl-C ₆ H ₄ -CH ₂ -	0.50	47	39
C ₄ H ₉	H	0.072	2.3	81	4Cl-C ₆ H ₄ -CH ₂ -	3.6	N.D.	N.D.
-C ₃ H ₆ -		0.16	7.0	56	4-CH ₃ -C ₆ H ₄ -CH ₂ -	20	N.D.	N.D.
-C ₄ H ₈ -		3.5	65	47	4-OCH ₃ -C ₆ H ₄ -CH ₂ -	66	N.D.	N.D.

N.D., not determined.

FIG. 4. Chemical structures of bicyclic pyrazole MK-0354, a highly selective partial agonist for the HCA₂ receptor, and two later follow-up compounds.

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 and [³⁵S]GTPγS binding assays. Boatman et al. (2008) (Fig. 4d) reported a further derivatization of MK-0354 with a cyclopropane extension.

3. Acifran Analogs. Acifran (Fig. 3) was developed in the early 1980s as a lipid-lowering agent, but structure-activity relationships were not reported. Mahboubi et al. (2006) synthesized and evaluated a small number of acifran analogs to study phosphorylation of extracellular signal-regulated kinase (ERK) 1/ERK2 in CHO cells expressing the human HCA₂ receptor and to explore the triglyceride-lowering potential in fructose-fed rats. First, they identified the *S*-enantiomer of acifran as the active principle. Second, the introduction of a *para*-fluor substituent on the phenyl ring (Fig. 5a) preserved activity in the in vivo animal model, whereas other modifications were not allowed. Third, there was little selectivity with respect to the HCA₃ receptor. A further, more extensive study was performed by Jung et al. (2007). Other substituents on the phenyl ring were tried; a 3-chloro (Fig. 5b) or -bromo substituent yielded slightly higher potency than acifran in a cAMP assay of the human

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 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₊/IC₅₀₋) 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. 5. Novel acifran analogs. *, chiral center.

FIG. 6. Anthranilic acid derivatives and related compounds as high-affinity HCA₂ receptor full agonists.

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 $EC_{50, \text{vasodilation}}/EC_{50, \text{free fatty-acid suppression}}$) 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 HCA₂ 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 [³H]nicotinic acid binding assay, an EC_{50} value of 18 nM in the [³⁵S]GTP γ 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 HCA₂ receptor (Tang et al., 2008). MMF induced a concentration-dependent Ca²⁺ signal in transfected cells with an EC_{50} value of 9.4 μ M (nicotinic acid, 2.0 μ M). MEF was approximately 3-fold less potent than MMF with an EC_{50} value of 26 μ M, whereas dimethylfumarate was inactive. In a [³H]nicotinic acid radioligand displacement assay, somewhat lower K_i values were obtained: for MMF, 0.98 μ M; for MEF, 1.3 μ M. A number of "simple" acids were tested by Ren et al. (2009) in several HCA₂ receptor assay formats. The two most potent compounds were *trans*-cinnamic acid and *para*-coumaric acid (Fig. 7), with IC_{50} values in a [³H]nicotinic acid binding assay of 36 and 58 μ M, respectively (nicotinic acid, 0.1 μ M). In the [³⁵S]GTP γ S binding assay both compounds were somewhat less active, with EC_{50} values of 240 and 310 μ M, respectively. It is noteworthy that in a similar assay on the HCA₃ receptor *trans*-cinnamic acid was also active (EC_{50} , 180 μ M), 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 HCA₂ receptor KO animals.

6. Pyridopyrimidinones. Peters et al. (2010) reported on a different and quite intriguing scaffold from which HCA₂ receptor agonists were derived. The pyridopyrimi-

FIG. 7. Fumaric acid esters and aromatic acids as agonists for the HCA₂ and HCA₃ receptor.

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]GTPγS 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 substituent 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]GTPγS 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

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

FIG. 9. Allosteric agonist for the HCA₂ receptor.

FIG. 10. Various classes of HCA₃ receptor ligands.

agonists [e.g., R = 2-butyl (EC_{50} = 330 nM)], R = CH(CH₃)CH₂OCH₃ (EC_{50} = 200 nM) being the most potent compound. None of the compounds displayed any activity on the HCA₂ receptor. A subtle change to R = propyl instead of isopropyl yielded significantly less activity (EC_{50} = 7.4 μ 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 HCA₃ receptors and selectivity over HCA₂ 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 (R₁ = propyl, R₂ = 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 HCA₃ 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 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 HCA₃ 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 HCA₂ receptor. It is noteworthy that replacing -S- with -O- to yield 3'-furanyl substitution rendered the com-

pound at least 1000-fold less active at the HCA₃ 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 HCA₂ receptor as a nicotinic acid and ketone body receptor and the discovery of ligands activating HCA₁ and HCA₃ 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, HCA₁, HCA₂, and HCA₃ 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 HCA₁, HCA₂, and HCA₃ receptors have been shown to inhibit adenylyl 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 HCA₂ 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 HCA₂ 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 β -isoforms, most likely through the release of G-protein $\beta\gamma$ -subunits (Exton, 1996). Consistent with this, HCA₂ and HCA₃ receptors, which are expressed in neutrophils, macrophages, and other immune cells, have been shown to mediate ligand-induced increases in free intracellular Ca²⁺ 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 Ca²⁺ concentrations mediated by HCA₂ receptors in response to nicotinic acid results in the activation of Ca²⁺-sensitive phospholipase A₂ 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 prostaglandins D₂ and E₂ in response to nicotinic acid (Morrow et al., 1989; Cheng et al., 2006; Maciejewski-Lenoir et al., 2006). Alternatively, phospholipase A₂ 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 β -arrestin, and activation of ERK via the HCA₂ receptor has been shown to involve the recruitment of β -arrestin to the plasma membrane (Walters et al., 2009). In addition, Walters et al. (2009) showed that upon HCA₂ receptor activation, β -arrestin-1 binds to cytosolic phospholipase A₂ (PLA₂) and activates the enzyme. This results in the release of arachidonic acid and provides an alternative mechanism for the stimulation of PLA₂-dependent prostanoid formation through HCA receptors in immune cells. The HCA₂ receptor-mediated activation of PLA₂ and subsequent formation of prostanoids such as prostaglandins (PG) D₂ E₂ 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 HCA₂ receptor are subject to desensitization (Stern et al., 1991). Consistent with this, nicotinic acid-induced flushing mediated by the HCA₂ receptor as well as nicotinic acid-induced increases in intracellular Ca²⁺ 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 HCA₁ and HCA₂ 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 HCA₂ 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 HCA₂ 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. HCA₁

Because the HCA₁ receptor is almost exclusively expressed in adipocytes and because it mediates lactate-induced, G_i-dependent inhibition of adenylyl cyclase activity, the obvious biological role of the HCA₁ 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 HCA₁ receptor-deficient mice were trained to exercise at an intensity that resulted in plasma lactate levels sufficient to activate the HCA₁ receptor, no difference between the plasma concentrations of free fatty acids could be observed between wild-type and HCA₁ 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 HCA₁ receptor, contributes to the inhibition of lipolysis induced by insulin. In fact, studies in HCA₁ 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 EC₅₀ of approximately 700 μ M (Taggart et al., 2005). Under normal conditions, 3-hydroxy-butyrate plasma levels are 50 to 400 μ M, 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 to 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.

(Bodor and Offermanns, 2008; Kamanna and Kashyap, 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 (Benyó 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 (Benyó 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 prostanoid 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 antidyslipidemic 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 $\leq 20\%$ was observed with a concomitant increase of $\leq 28\%$ 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 1 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 dou-

ble-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-statin 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 choles-

terol, and high triglyceride levels (<http://clinicaltrials.gov/ct2/show/NCT00120289>). 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 IIb 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 G_i-type G-proteins, there has been great interest in the development of synthetic ligands that act through HCA receptors to induce antidyslipidemic 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.

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Authorship Contributions

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

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