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Chapter 2

Biological and pharmacological roles of hydroxy-carboxylic acid receptors

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Abstract

The hydroxy-carboxylic acid (HCA) receptors HCA_1, HCA_2 and HCA_3 were previously known as GPR81, GPR109A and GPR109B, respectively, or as the nicotinic acid receptor family. They form a cluster of G protein-coupled receptors with high sequence homology. Recently, intermediates of energy metabolism, all hydroxyl-carboxylic acids, have been reported as endogenous ligands for each of these receptors. The HCA receptors are predominantly expressed on adipocytes and mediate the inhibition of lipolysis by coupling to G_i–type proteins. HCA_1 is activated by lactate, HCA_2 by the ketone body 3-hydroxy-butyrate and HCA_3 by hydroxylated β-oxidation intermediates, especially 3-hydroxy-ocatanoic acid. Both HCA_2 and HCA_3 are part of a negative feedback loop which keeps the release of fat stores in check under starvation conditions, whereas HCA_1 plays a role in the antilipolytic (fat conserving) effect of insulin.

HCA_2 was first discovered as the molecular target of the anti-dyslipidemic drug nicotinic acid (or niacin). Many synthetic agonists have since been designed for HCA_2 and HCA_3, but the development of a new, improved HCA-targeted drug has not been successful so far, despite a number of clinical studies. Recently, it has been shown that the major side-effect of nicotinic acid, skin flushing, is mediated by HCA_2 receptors on keratinocytes, as well as on Langerhans cells in the skin. In this chapter, we summarize the latest developments in the field of HCA receptor research, with emphasis on (patho)physiology, receptor pharmacology, major ligand classes and the therapeutic potential of HCA ligands.

List of non-standard abbreviations

5-HpETE, 5-hydroperoxy-eicosatetraenoic acid; 5-oxo-ETE, 5-oxo-6,8,11,14-eicosatetraenoic acid; BAC, bacterial artificial chromosome; CETP, cholesterol ester transfer protein; COX-1, cyclooxygenase 1; COX-2, cyclooxygenase 2; $CysLT_2$, cysteinyl-leukotriene 2; DP_1, prostaglandin D_2 receptor 1; EC_{50}, half-maximal effective concentration; EL, extracellular loop; ER, extended release; ERK, extracellular signal-related kinase; GTPγS, guanosine 5'-O-[gamma-thio]triphosphate; HCA, hydroxy-carboxylic acid; HDL, high density lipoprotein; IFN-γ, interferon-γ; IL, intracellular loop; LDL, low density lipoprotein; LTD_4, leukotriene D_4; MEF, monoethyl ester of fumaric acid; MMF, monomethyl ester of fumaric acid; PGD_2; prostaglandin D_2; PGE_2, prostaglandin E_2; PLA_2, phospholipase A_2; PPARγ; peroxisome proliferator-activated receptor γ; PUMA-G, protein up-regulated in macrophages by INF-γ (murine HCA_2); TM, transmembrane domain; VLDL, very low density lipoprotein.

I. Introduction

In the past twenty years it has become clear that GPCR ligands include not only traditional hormones and neurotransmitters, but also ions and other endogenous molecules. In this chapter, we focus on a recently discovered GPCR family with affinity for several intermediates of energy metabolism. These metabolite ligands are all hydroxy-carboxylic acids, hence the novel nomenclature for this receptor family: hydroxy-carboxylic acid receptors [1]. HCA_1 (GPR81) is endogenously activated by lactate, HCA_2 (GPR109A) by 3-hydroxy-butyrate and HCA_3 (GPR109B) by 3-hydroxylated β-oxidation intermediates, especially 3-hydroxy-octanoic acid [2-5]. All three receptors are predominantly expressed in adipose tissue, where they couple to G_i proteins [1]. Activation of the receptors has an antilipolytic effect. Discovery of the endogenous HCA ligands has increased our understanding of the (patho)physiological roles of their receptors, and opens new avenues for research and drug discovery.

Of the HCA family, HCA_2 is most extensively studied since it is the target of the anti-dyslipidemic drug nicotinic acid (or niacin) [6-8]. Nicotinic acid has been used since the 1950's [9] and is still the most efficacious drug approved to raise HDL cholesterol plasma levels [10]. In HCA_2 knockout mice, the antilipolytic and triglyceride lowering effects of nicotinic acid are abolished [8]. Skin flushing, the major nicotinic acid side-effect, was also shown to be receptor dependent [11]. Separating this side-effect from the therapeutic effects of HCA_2 ligands is one of the major challenges in this field. In addition, it will be important to analyze the potential of HCA_1 and HCA_3 as therapeutic targets. This review will summarize current knowledge on the pharmacology and physiology of HCA receptors as well as the recent development of new synthetic ligands of this receptor class.

II. Identification and characterization of HCA receptors

A. Cloning of HCA receptors

The HCA_3 receptor (GPR109B) was first cloned from a human monocyte cDNA library and identified as an orphan G protein-coupled receptor, HM74 [12].

Lee et al. [13] discovered the HCA_1 receptor (GPR81) as another orphan GPCR by BLAST analysis. The cDNA of the HCA_1 receptor was then cloned from a bacterial artificial chromosome (BAC) clone carrying a region of human chromosome 12. The HCA_1 receptor cDNA showed high homology [11] to the HCA_3 receptor (GPR109B/HM74), and the genes encoding HCA_1 and HCA_3 receptors were localized in close proximity on the same BAC clone. The HCA_2 receptor (GPR109A) was originally identified in murine macrophages upon stimulation of cells with interferon-γ (INF-γ) and called "protein up-regulated in macrophages by INF-γ" (PUMA-G) [14]. In 2003, the human and rat HCA_2 receptors were cloned and shown to be highly homologous to the murine orthologue PUMA-G [6, 7].

B. Sequence alignment and phylogenetic tree

The HCA_2 and the HCA_3 receptor are highly homologous as they share 95% sequence identity on the protein level. In fact, HCA_3 differs from HCA_2 in only 16 amino acids of

which 12 are non-conservative changes which are clustered around extracellular loops 1 and 2. In addition, the HCA_3 receptor has an extended C-terminus of 24 amino acids. The HCA_1 receptor has almost 50% sequence homology with both HCA_2 and HCA_3. Most notably, HCA_1, HCA_2 and HCA_3 receptors share a conserved arginine residue in the third transmembrane helix which is supposed to be critically involved in ligand binding (see Section VI). While HCA_1 and HCA_2 receptors are present in the genome of numerous mammalian species including humans and rodents, the HCA_3 receptor is exclusively found in humans and higher primates like chimpanzee. The HCA_3 receptor obviously evolved through a relatively recent gene duplication, as indicated by its tandem location with HCA_2 on human chromosome 12 and its high level of sequence identity to HCA_2.
Several single nucleotide polymorphisms in the coding regions of genes encoding HCA_2 and HCA_3 receptors have been described [15]. The effects of these mutations on the physiological or pharmacological functions of the respective receptors are unknown.

C. Deorphanization of HCA receptors

1. HCA_1 receptor

In two recent studies it was shown that lactate was able to activate the HCA_1 receptor with half-maximal effective concentrations of 1.3 and 4.8 mM, respectively [5]. Lactate was a specific agonist of HCA_1 as it did not activate the closely related receptors HCA_2 and HCA_3. Various other hydroxy-carboxylic acids structurally related to lactate had a strongly reduced potency or were inactive towards HCA_1. For instance, 2- and 4-hydroxy-butyrate were weak agonists with an EC_{50} value of 8.5 and 15 mM, respectively, whereas 3-hydroxy-butyrate was completely inactive. Interestingly, the physiologically relevant stereoisomer (S)-lactate was much more potent and efficacious than (R)-lactate. Given the fact that plasma levels of (S)-lactate can reach concentrations sufficient to activate HCA_1, it is conceivable that lactate would be a physiologically relevant endogenous ligand of the HCA_1 receptor.

2. HCA_2 receptor

In 2005, the ketone body 3-hydroxy-butyrate was described as an endogenous ligand of HCA_2, the receptor of the anti-dyslipidemic drug nicotinic acid [3]. Racemic 3-hydroxy-butyrate activated human and mouse HCA_2 receptor with an EC_{50} value of 0.7 and 0.8 mM, respectively. 3-hydroxy-butyrate was a specific agonist of HCA_2 as it was inactive on the closely related receptor HCA_3. Other ketone bodies like acetoacetate or acetone had no activity on HCA_2. Short and medium chain fatty acids like butyrate, hexanoate and octanoate were also weak agonists on mouse and human HCA_2 with EC_{50} values ranging from 0.13 to 1.6 mM. While under physiological conditions plasma concentrations of short chain fatty acids would be too low to activate HCA_2, plasma levels of ketone bodies like 3-hydroxy-butyrate can increase during fasting and reach levels sufficient to activate the receptor.

3. HCA_3 receptor

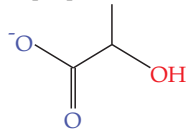
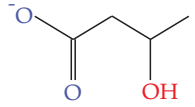
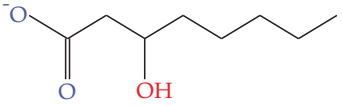
Despite the high homology of the HCA_3 receptor to the nicotinic acid and ketone body

receptor HCA_2, HCA_3 is not activated by nicotinic acid or 3-hydroxy-butyrate. Recently, 2- and 3-hydroxylated medium-chain fatty acids have been identified as endogenous ligands of the orphan HCA_3 receptor [4]. 2- and 3-hydroxy-octanoate were specific agonists of HCA_3 with EC_{50} values of 4 and 8 μM, respectively. Under certain conditions, which go along with increased fatty acid β-oxidation rates, plasma concentrations of 3-hydroxy-octanoate reach levels sufficient to activate the HCA_3 receptor [4, 16, 17].

Moreover, aromatic D-amino acids like D-phenylalanine or D-tryptophan were shown to specifically activate the HCA_3 receptor [18]. Given the extreme rare occurrence of D-amino acids, it is unclear whether the ability of aromatic D-amino acids to activate HCA_3 is of physiological or pathophysiological significance.

D. Novel nomenclature of HCA receptors

Table 1. HCA receptor nomenclature. The structures of 2-OH-propanoate, 3-OH-butyrate and 3-OH-octanoate are shown.

Receptor	Aliases	Naturally occurring ligands
HCA_1	GPR81, GPR104, TA-GPCR, LACR, FKSG80	2-OH-propanoate (lactate) 
HCA_2	GPR109A, PUMA-G, HM74A, HM746, NIACR1	3-OH-butyrate 
HCA_3	GPR109B, HM74, NIACR2	3-OH-octanoate  2-OH-octanoate, D-phenylalanine, D-tryptophan

In the past, various names were given to the receptors HCA_1 (GPR81), HCA_2 (GPR109A/HM74A/NIACR1) and HCA_3 (GPR109B/HM74/NIACR2) (see table 1). After the identification of HCA_2 (GPR109A) as the receptor of the anti-dyslipidemic drug nicotinic acid, HCA_1 (GPR81), HCA_2 (GPR109A) and HCA_3 (GPR109B) were often called the "nicotinic acid receptor family" or "niacin receptor family". This was, however, for two reasons misleading: firstly, nicotinic acid is unlikely to be the physiologically relevant ligand because its concentrations are too low to activate HCA_2 (GPR109A), and secondly, the two closely related receptors HCA_1 (GPR81) and HCA_3 (GPR109B) do not respond to nicotinic acid at reasonable concentrations and therefore are no nicotinic acid receptors. With the identification of hydroxy-carboxylic acids as the endogenous ligands of HCA_1, HCA_2 and HCA_3, the physiological and pathophysiological functions of these receptors could be clarified (see section IV). Based on sequence homology, ligand similarity and

their physiological role, HCA_1, HCA_2 and HCA_3 are now regarded as members of a novel subfamily of G protein-coupled receptors, the hydroxy-carboxylic acid (HCA) receptor family.

The orphan receptor GPR31 and the 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE) receptor OXER1 are the receptors most closely related to HCA_1, HCA_2 and HCA_3 [19-21]. Interestingly, the arginine residue in transmembrane helix 3, which is conserved among HCA_1, HCA_2 and HCA_3 and which has been suggested to serve as a molecular anchor of the carboxylic group of HCA_1 receptor ligands, is also present in GPR31 and the 5-oxo-ETE receptor. OXER1 binds 5-oxo-ETE as well as, with lesser affinity, 5-hydroxy-eicosatetraenoic acid and 5-hydroperoxy-eicosatetraenoic acid (5-HpETE). Thus, OXER1, a receptor for polyunsaturated fatty acids with an oxo, hydroxy or hydroperoxy substitution in the 5-position may well be regarded as another member of the hydroxy-carboxylic acid receptor family.

III. Gene structure and tissue distribution

A. Gene structure

The genes encoding HCA_1, HCA_2 and HCA_3 receptors are tandemly located on human chromosome 12q24.31 and have likely evolved from gene duplication (see figure 1). In humans and other mammals which express HCA receptors the genes for HCA_1, HCA_2 and HCA_3 consist of each a single exon.

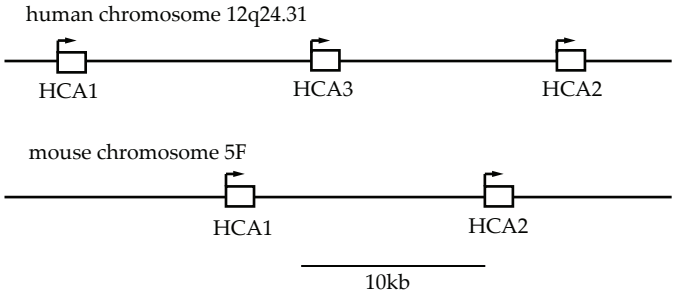


Figure 1. Gene structure of the HCA receptors. Schematic representation of the genomic organization of the genes encoding hydroxy carboxylic acid (HCA) receptors.

B. Tissue distribution

1. HCA_1 receptor

The HCA_1 receptor was originally reported to be expressed in human pituitary [13]. However, this has never been confirmed. Several studies have independently shown that HCA_1 is primarily expressed in white and brown adipose tissue of both humans and rodents [5, 7, 22-24]. Only minor amounts of mRNA of HCA_1 were detected in kidney, skeletal muscle or liver. In addition, expression of HCA_1 was increased during differentiation of 3T3-L1 preadipocytes [5, 22, 23]. In mouse and human adipocyte cell lines transcription of the HCA_1 gene was induced upon treatment with peroxisome proliferator-activated receptor γ (PPARγ) agonists.

2. HCA_2 receptor

Similar to the HCA_1 receptor, HCA_2 is highly expressed in human and murine white and brown adipose tissue [6-8]. Expression of HCA_2 was increased during differentiation of 3T3-L1 preadipocytes, as well as upon treatment with PPARγ agonists [23]. Moreover, the HCA_2 receptor is expressed in various immune cells including monocytes, neutrophils, macrophages, dendritic cells and epidermal Langerhans cells [14, 25-27]. In macrophages expression of HCA_2 was inducible by treatment with IFN-γ. A recent study demonstrated expression of the HCA_2 receptor in keratinocytes by utilizing advanced BAC-transgenic reporter mice for HCA_2 [28]. Similar to macrophages, expression of the HCA_2 receptor in keratinocytes and keratinocyte cell lines was induced by IFN-γ [29]. In addition, expression of GPR109A has also been reported in retinal pigment epithelium as well as in the intestinal epithelium [30, 31].

3. HCA_3 receptor

Expression of the HCA_3 receptor appears to be very similar to the expression pattern of the HCA_2 receptor and can be found to be highly expressed in white adipose tissue [6-8]. In addition, the HCA_3 receptor is expressed in various human immune cells including neutrophils, monocytes and macrophages [4, 12, 18, 32]. Evidence has also been provided for the expression of the HCA_3 receptor in epithelial cells of the colon [30].

IV. Physiological and pharmacological roles of HCA receptors

A. HCA_1 receptor

The HCA_1 receptor mediates the inhibitory regulation of adipocyte lipolysis by lactate [2, 5, 24]. Since plasma lactate levels are strongly increased under conditions of intensive physical exercise, it would be conceivable that lactate inhibits and thereby restricts the supply of fatty acids under anaerobic conditions. However, Ahmed et al. [24] studied wild-type and HCA_1 receptor-deficient mice which were trained to exercise at an intensity resulting in plasma lactate levels sufficient to activate the HCA_1 receptor and found that plasma concentrations of free fatty acids were not different between wild-type and HCA_1 receptor-deficient mice. Thus, there is so far no evidence for a role of lactate and its receptor in the regulation of lipolysis during intensive exercise.

Interestingly, the adipose tissue can convert more than 50% of the metabolized glucose to lactate, a process stimulated by insulin and glucose uptake [33]. Lactate is then released from adipocytes and taken up by the liver to serve as a substrate for gluconeogenesis and glycogen synthesis. Insulin-induced glucose uptake results in a several-fold increase in lactate levels in the adipose tissue [24, 34, 35]. In HCA_1 receptor-deficient mice as well as in HCA_1-deficient adipocytes, insulin-induced inhibition of lipolysis and insulin-induced decrease in adipocyte cAMP were strongly reduced [24]. This suggests that lactate acting through HCA_1 functions in an autocrine and paracrine fashion to mediate insulin-induced antilipolytic effects and thereby regulates lipolysis postprandially (figure 2A). When on a high fat diet, mice lacking HCA_1 showed a reduced weight gain (Ahmed et al., 2010). This

indicates that the lactate/HCA_1-mediated antilipolytic effects contribute to the increase in body weight under hypercaloric diet.

B. HCA_2 receptor

The ketone body 3-hydroxy-butyrate which activates the HCA_2 receptor with an EC_{50} of 0.7 mM has been described as the endogenous ligand of HCA_2 [3]. In fact, 3-hydroxy-butyrate plasma levels increase to 1-2 mM after an overnight fast and reach 6-8 mM during prolonged fasting [36]. It is very likely that the HCA_2 receptor activated by 3-hydroxy-butyrate at millimolar concentrations during starvation mediates a negative feedback regulation that controls the lipolytic rate [37] (figure 2B). This regulatory mechanism would help to avoid excessive triglyceride degradation and thereby save energy during food shortage.

The antidyslipidemic drug nicotinic acid activates HCA_2 receptors expressed on adipocytes resulting in a rapid decrease in the release of free fatty acids from fat cells. This in turn reduces the supply of free fatty acids to the liver, leading to a reduced synthesis of triglycerides and very low density lipoprotein (VLDL) as well as to a subsequent decrease of low density lipoprotein (LDL)-cholesterol levels [38]. It is less clear how nicotinic acid increases levels of high density lipoprotein (HDL)-cholesterol. It is possible that the decrease in triglyceride content of apolipoprotein B (ApoB) containing lipoproteins results in a decreased exchange of triglycerides for cholesteryl esters from HDL-particles mediated by the cholesterol ester transfer protein (CETP) eventually leading to increased HDL-cholesterol levels [39-41]. Consistent with this hypothesis, HDL-cholesterol elevation in response to nicotinic acid has been shown to depend on the presence of CETP [42, 43]. Whether the HCA_2 receptor mediates the increase in HDL-cholesterol levels in response to nicotinic acid is, however, currently not clear [44, 45]. It is also unknown whether the activation of HCA_2 receptors expressed by cells outside the adipose tissue plays a role during starvation. It is possible that elevated 3-hydroxy-butyrate levels during starvation activate HCA_2 receptors expressed on immune cells and thereby induce anti-inflammatory effects which could be advantageous under conditions of starvation.

Besides its antilipolytic effect, nicotinic acid has been shown to influence the function of the adipose tissue as an endocrine organ. Both in vitro and in vivo data indicate that nicotinic acid increases the release of adiponectin from adipocytes through HCA_2 [46, 47]. The anti-inflammatory and antidiabetic consequences of increased adiponectin plasma levels [48] may contribute to the beneficial effects of nicotinic acid.

Recently, studies in mice have shown that the anti-atherosclerotic effect of nicotinic acid is not only due to nicotinic acid-induced changes in lipid metabolism but also results from direct effects of nicotinic acid on HCA_2 expressed by immune cells. In contrast to atherosclerosis-prone LDL-receptor-deficient mice carrying the wild-type receptor, mice lacking HCA_2 or mice transplanted with HCA_2-deficient bone marrow showed strongly reduced anti-atherosclerotic effects in response to nicotinic acid. The nicotinic acid receptor HCA_2 is expressed by monocytes and macrophages including plaque macrophages, and nicotinic acid inhibits the recruitment of macrophages to atherosclerotic lesions in HCA_2-dependent manner. In addition, HCA_2 mediates a stimulatory effect of nicotinic

acid on the cholesterol efflux from macrophages. Thus, nicotinic acid appears to reduce the progression of atherosclerosis also through direct anti-inflammatory effects and stimulatory effects on the reverse cholesterol transport [49, in press].

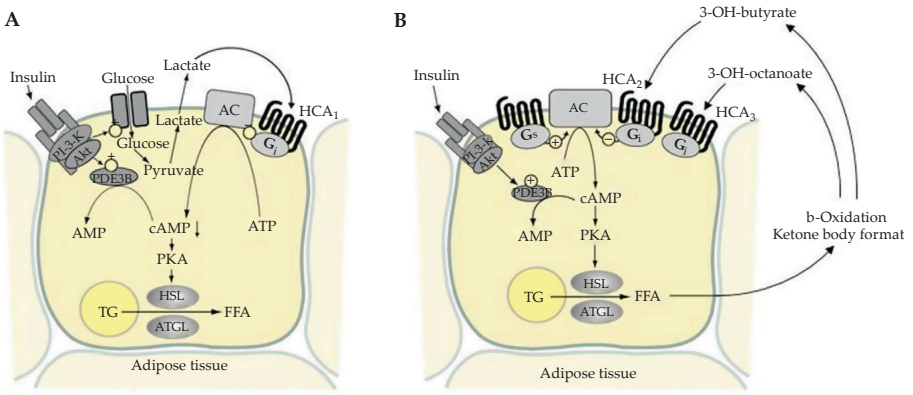


Figure 2. Biological roles of the HCA receptors. Functions of the recently deorphanized receptors HCA_1, HCA_2 and HCA_3. The lactate receptor HCA_1 mediates the acute anabolic effects of insulin on adipocytes and thereby helps to store energy after feeding (A). In contrast, HCA_2 and HCA_3 receptors are involved in the long-term regulation of lipolytic activity being receptors for the ketone body 3-hydroxy-butyrate (HCA_2) and the β-oxidation intermediate 3-hydroxy-octanoate (HCA_3). 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 HCA_2 and HCA_3 receptors, respectively, in form of a negative feedback loop (B). Thereby HCA_2 and HCA_3 receptors help preserve energy stores during starvation. AC, adenylyl cyclase; TG, triglycerides; HSL, hormone-sensitive lipase; ATGL, adipocyte triglyceride lipase; FFA, free fatty acids; PKA, cAMP-regulated protein kinase.

Evidence has been provided that HCA_2 receptors expressed by epidermal Langerhans cells and keratinocytes mediate the major unwanted effects of nicotinic acid, the flush reaction. The symptoms of flushing consist of a cutaneous vasodilation as well as sensations of tingling and burning which impair patients' compliance [11, 25, 27, 50, 51]. Nicotinic acid or the antipsoriatic drug monomethyl fumarate induce a biphasic increase in dermal blood flow which is mediated by HCA_2 [28]. While the first phase is due to activation of HCA_2 on Langerhans cells, the second phase of the flush depends on HCA_2 expressed by keratinocytes. The Langerhans cell-mediated flushing involves cyclooxygenase-1 (COX-1), prostaglandin D_2 (PGD_2) and prostaglandin E_2 (PGE_2). In contrast, keratinocyte-mediated flushing at later phases of the reaction involves cyclooxygenase-2 (COX-2) and PGE_2 [27, 28, 52, 53].

There is evidence indicating that the HCA_2 receptor expressed in intestinal epithelial cells responds to butyrate which is present in millimolar concentrations in the gut lumen, and that HCA_2 thereby functions as a tumor suppressor and anti-inflammatory receptor [30]. Furthermore, comparison of the HCA_2 potencies [3] and reported fecal concentrations of the short-and medium-chain fatty acids [54] suggests that pentanoate and hexanoate may also activate gut HCA_2.

C. HCA_3 receptor

Similar to HCA_2, the primary physiological role of the HCA_3 receptor appears to be the regulation of lipolysis. Plasma concentrations of the β-oxidation intermediate 3-hydroxy-

octanoate, which activates HCA_3 receptors at micromolar concentrations, are elevated under conditions of increased fatty acid oxidation such as starvation, diabetic ketoacidosis, various mitochondrial fatty acid β-oxidation disorders and under a ketogenic diet [4, 16, 17]. Under such conditions the HCA_3 receptor mediates a negative feedback loop to counterregulate pro-lipolytic stimuli in order to prevent excessive lipolysis which would result in the futile release and circulation of free fatty acids [1] (figure 2B). The fact that the HCA_3 receptor is only found in humans and chimpanzee suggests that a negative feedback loop of lipolysis mediated through 3-hydroxy-octanoate/HCA_3 has evolved in higher primates to economize the use of fatty acids during periods of starvation. Whether HCA_3 has potential physiological roles in immune cells or other organs is currently not known.

V. Receptor classification with pharmacological tools

Although nicotinic acid was introduced in man in the 1950s [9], structure-activity relationships for its target(s) were developed much later. Only in the 1980s Aktories and colleagues [55, 56] proposed 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 [57]. A few years later, the human HCA_2 receptor was cloned [7]. In that paper a number of nicotinic acid-like compounds were also tested. As most medicinal chemistry efforts have been directed towards 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 few years ago we published a review on the then available ligands, largely nicotinic acid-like compounds (Soudijn et al., 2007). The current review does not reiterate that but starts from there, and is organized according to chemical classes. We report representative structures of these classes in Figure 3.

A. Structure-activity relationships for the HCA_2 receptor

1. Nicotinic acid-like compounds

Lorenzen and colleagues [57] observed that nicotinic acid (1 in figure 3) displaces [^{3}H] nicotinic acid from and increases [^{35}S]GTPγS binding to rat epididymal adipocyte and spleen membranes with (sub)micromolar potency. The same two assays were used by Wise et al. [7] with similar results, now on the cloned human HCA_2 receptor. Apparently there are no huge species differences between rat and man for nicotinic acid itself. Two other marketed products, acifran (2 in figure 3) and acipimox (3 in figure 3), were also tested but showed lower potencies. Nicotinamide (4 in figure 3) was inactive, indicating that the carboxylic acid group is essential for activity. Gharbaoui et al. [58] evaluated other heterocyclic scaffolds, confirming that changing this moiety invariably led to compounds with lower potency than nicotinic acid, if at all.

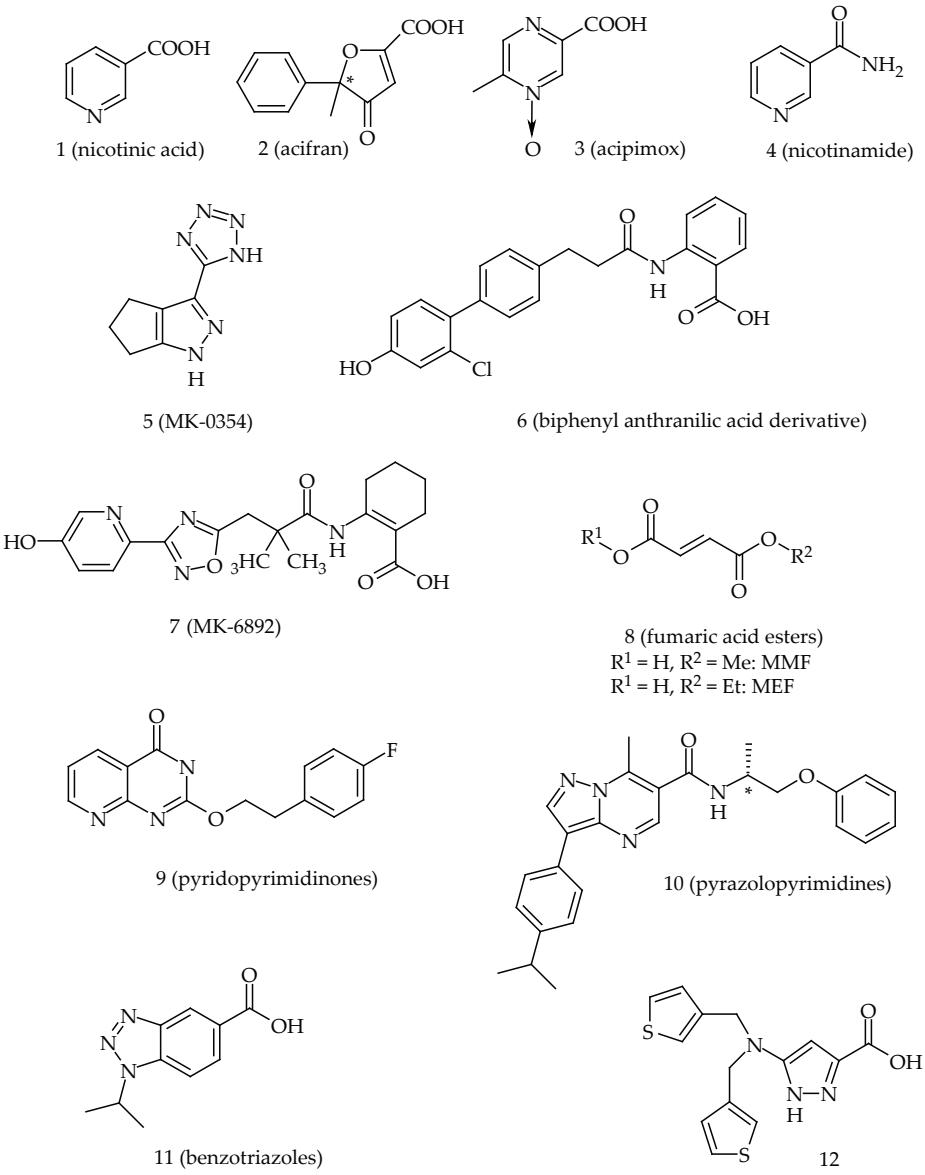


Figure 3. Representative compounds from various chemical classes as HCA receptor agonists. (* denotes chiral center).

2. Pyrazoles

Pyrazole-3-carboxylic acid acted as a high-efficacy partial agonist in a rat tissue [^{35}S]GTPγS binding assay [57]. This finding was taken as the starting point for a synthetic program, both in academia and industry. Partial agonists may display tissue selectivity, thought to be potentially beneficial in the case of the HCA_2 receptor, as the side effect of flushing

might be separated from the desired action in dyslipidemia. Van Herk et al. [59] prepared two series of alkyl- and benzyl-substituted pyrazole-3-carboxylic acid derivatives; they were all partial agonists, and some compounds had micromolar affinity. This was particularly true for the butyl-substituted derivative. Gharbaoui et al. [58] and Skinner et al. [60] reported on a more extended series of pyrazoles largely corroborating the findings of Van Herk et al. [59]. In the latter publication it was shown that the carboxylic acid could not easily be replaced by a tetrazole bioisostere. Later, Semple and colleagues [61] reported on one particular exception, in which the carboxylic acid/tetrazole switch yielded a partial agonist. In mice this compound (MK-0354, 5 in figure 3) was as active as nicotinic acid in reducing the amount of plasma free fatty acids in vivo and had quite favorable pharmacokinetic properties, whilst not causing vasodilation in the mouse ear, a surrogate marker for flushing (see also section VIII). Imbriglio et al. [62] synthesized further MK-0354 analogues by introducing fluorinated phenyl substituents, the 2,3,5-trifluoro variant of which was 2-3 fold more potent than nicotinic acid in vitro. Similar derivatives, now with a carboxylic acid function were reported by Schmidt et al. [63] and Imbroglio et al. [64]. A further derivatization of MK-0354 with a cyclopropane extension was reported by Boatman et al. [65].

3. Acifran analogues

Acifran (5 in figure 3) was developed in the early 1980s as a lipid lowering agent. Only in 2006 Mahboubi and coworkers [66] synthesized and evaluated a small number of acifran analogs. The introduction of a para-fluoro substituent on the phenyl ring preserved activity in the in vivo animal model, while other modifications were not allowed. There was little selectivity with respect to the HCA_3 receptor. A further, more extensive, study was performed by Arena Pharmaceuticals [67]. Other substituents on the phenyl ring (e.g., 3-chloro) or replacement by thiophene yielded some compounds that showed slightly higher potency than acifran, but without a significant degree of selectivity towards the HCA_3 receptor. Some of the analogs were resolved into their individual stereoisomers, showing that invariably the (+)-isomer was the biologically active principle.

4. Anthranilic acid derivatives

High-throughput screening (HTS) campaigns at a number of companies, in particular Merck, led to the discovery of anthranilic acid derivatives as HCA_2 ligands, first reported by Shen et al. [68]. Such compounds [63] appear prone to have high plasma protein binding with a strong negative impact on the in vivo activity of the molecules, e.g. the biphenyl compound 6 in figure 3. Other anthranilic acid derivatives were reported by Shen et al. [69]. Partial hydrogenation of the anthranilic acid phenyl ring yielded compounds that retained activity on the HCA_2 receptor, elaborately explored by Raghavan et al. [70]. The authors concluded that the tetrahydro variants of anthranilic acid derivatives show improved oral bioavailability and better cytochrome P450 profiles. A recent publication [71] describes the discovery of (pre)clinical candidate MK-6892 (7 in figure 3). It was also found (Ding et al., 2010; Schmidt et al., 2010) that the cyclohexene ring system in such compounds can be further substituted.

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. Interestingly, the monomethyl (MMF) and monoethyl (MEF) ester of fumaric acid (8 in figure 3), but not fumaric acid itself, have micromolar affinity for the HCA_2 receptor [29] (see also section VIII). A number of 'simple' acids rather than esters were tested by Ren et al. [72]. The two most potent compounds were trans-cinnamic acid and para-coumaric acid, although substantially less active than nicotinic acid. On the HCA_3 receptor trans-cinnamic acid was also active, while 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. Further SAR on the HCA_2 receptor for this ligand class was recently reported by us. A rather restricted binding pocket on the receptor was delineated with trans-cinnamic acid itself being the largest planar ligand with appreciable affinity for the receptor [73 in press].

6. Pyridopyrimidinones

Peters et al. [74] reported on a very different scaffold from which HCA_2 receptor agonists were derived. The pyridopyrimidinones (e.g., 9 in figure 3) can be regarded as derivatives of nicotinamide, but that compound is inactive at HCA_2 receptors. Nevertheless submicromolar affinity and potency were observed in this series, although the compounds behaved poorly in pharmacokinetic studies.

7. Pyrazolopyrimidines as allosteric agonists

Shen and coworkers [75] described another series of agonists for the HCA_2 receptor with intriguing pharmacological activity, in particular 7 in figure 3. When tested alone it behaved as a partial agonist with 8-fold higher potency than nicotinic acid. Interestingly, the presence of 10 shifted the concentration-effect curve of nicotinic acid significantly to the left, suggestive of an allosteric mechanism of action. In a radioligand binding assay the pyrazolopyrimidine dose-dependently increased rather than displaced specific [^{3}H] nicotinic acid binding, yet another token of its nature as an allosteric enhancer.

8. Patent literature

Many companies have published patents on ligands for the HCA_2 receptor. As 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 [76-79].

B. Structure-activity relationships for the HCA_3 receptor

Despite the high (>95%) homology between HCA_2 and HCA_3 receptors, nicotinic acid is very selective for the HCA_2 receptor, whereas acifran is not (see e.g. [66, 72, 80]. The most extensive structure-activity study with acifran analogs [67] showed that an ethyl rather than a methyl substituent at the chiral center in acifran provided some selectivity for the HCA_3 receptor, whereas all other compounds were slightly selective for the HCA_2

receptor. Ren et al. identified ortho-coumaric acid as approx. 20-fold selective for the HCA_3 receptor, while the isomer para-coumaric acid was inactive at the HCA_3 receptor but not at the HCA_2 receptor (see paragraph A.5. in this section).
New HCA_3 receptor ligands were reported by Semple and coworkers [81]. In a screening campaign the authors discovered a benzotriazole compound (11 in figure 3) with nanomolar activity. Further exploration, e.g. by replacing the isopropyl group by 2-butyl, led to even more potent compounds, but without effect on the HCA_2 receptor.
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 [82]. Further substitution of the 4-amino group led to compounds with potencies in the higher nanomolar range. In the same publication the nitro-aryl moiety was substituted by a pyridine ring like in nicotinic acid, yielding HCA_3 receptor-selective full agonists.
Recently, the synthetic efforts were extended to the pyrazole carboxylic acids as a template for the HCA_3 receptor [83]. A similar substituted amino group as mentioned above was introduced to the pyrazole ring system. A typical representative (12 in figure 3) displayed high potency with an EC_{50} value of 3 nM, and over 1000-fold selectivity with respect to the HCA_2 receptor.
An intriguing conclusion from this research is that 'on average' ligands for the HCA_3 receptors have higher affinity than ever met for the HCA_2 receptor, despite intensive efforts on the latter receptor.

VI. Mutagenesis and receptor modeling studies

A. HCA_1 receptor

Prior to the deorphanization of HCA_1, Ge and coworkers [22] constructed a chimeric cysteinyl-leukotriene 2 ($CysLT_2$) receptor where the intracellular domains (all ILs and the C-terminus) were replaced by the HCA_1 sequence. The endogenous $CysLT_2$ ligand, leukotriene D_4 (LTD_4), activated the receptor and this resulted in stimulation of G_i. Wild-type $CysLT_2$ predominantly couples to G_q, so these findings suggested that HCA_1 is a G_i coupled receptor. A first clue regarding the biological function of HCA_1 was obtained when LTD_4 was shown to inhibit lipolysis in primary mouse adipocytes expressing the chimera. After lactate was identified as the endogenous HCA_1 ligand, a homology model was constructed of the binding pocket [5]. Four conserved residues predicted to interact with lactate were mutated to alanine in separate mutant receptors: Arg99Ala (TM3), Tyr233Ala (TM6), Arg240Ala (TM6) and Thr267Ala (TM7). Stimulation of [^{35}S]GTPγS binding by lactate was absent in all four mutants, suggesting that these residues are all needed for receptor activation by lactate.

B. HCA_2 and HCA_3 receptors

The HCA_2 and HCA_3 receptors have different ligand repertoires and SAR, although there is some cross-selectivity (see section V). For example, nicotinic acid binds almost exclusively to HCA_2, whereas acifran [84] stimulates both HCA_2 (EC_{50} = 1.9 μM) and HCA_3 (EC_{50} = 90 μM). This is surprising since these receptors have > 95 % amino acid sequence

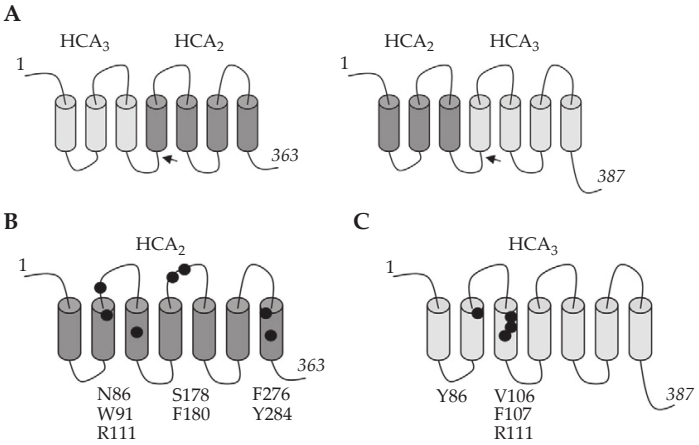


Figure 4. HCA_2/HCA_3 chimeras and residues involved in ligand binding. **A.** Schematic representation of HCA_2/HCA_3 chimeras discussed in Tunaru et al. [85] and Ahmed et al. [4]. The arrow indicates the juncture point. **B.** Schematic representation of HCA_2 indicating the positions of residues important for ligand binding (black dots). **C.** Schematic representation of HCA_3 indicating the position of residues important for ligand binding (black dots).

identity. To investigate which amino acids are involved in ligand selectivity, Tunaru and coworkers [85] constructed HCA_2/HCA_3 chimeras and introduced point mutations in both native receptors. For the chimeras, the receptors were cut at the interface of IL2 and TM4, and then the fragments were joined together at this point (see figure 4A). On the chimera with the HCA_2 receptor C-terminally, acifran had HCA_2-like potency (EC_{50} = 2 μM). At the same time, this receptor was insensitive to nicotinic acid. It was also HCA_3-like in its response to 2-oxo-octanoic acid, which is a close analogue of the endogenous HCA_3 ligand [4]. The HCA_3 N-terminal part seemed indeed responsible for the affinity of 2-oxo-octanoic acid, since the 'reverse' chimera with the HCA_2 sequence N-terminally did not respond to this compound. Using the site-directed mutagenesis approach, a HCA_2 mutant containing HCA_3 residues on three positions was constructed. The mutations Asn86Tyr, Trp91Ser (interface of TM2 and IL1) and Ser178Ile (EL2) prevented HCA_2 from responding to nicotinic acid and lowered the potency of acifran to HCA_3-like levels (see figure 4B). As a next step, the positively charged arginine residues in HCA_2 were investigated as likely counterparts for the negatively charged carboxylate function in nicotinic acid and acifran. All four arginine residues in the sequence were mutated to alanine, and only the Arg111Ala (TM3) mutant was rendered insensitive to nicotinic acid. The HCA_3 single mutant Arg111Ala, which was later constructed, also showed a deficient binding of the ligand 2-hydroxy-octanoic acid, which is another analogue of the endogenous HCA_3 ligand [4] (see figure 4C). This suggests that the acidic moiety of the ligands is anchored at the same position in both HCA_2 and HCA_3. Interestingly, the HCA Arg111 corresponds to the conserved Asp residue important for agonist binding in the biogenic amine receptors. In HCA_2, three more residues seem implicated in nicotinic acid binding: Phe180 (EL2), Phe276 and Tyr284 (both TM7) [85]. In a later paper, the roles of HCA_2 residues Asn86, Met103 and Leu107 were investigated further [4]. Mutations into corresponding HCA_3 residues (respectively Tyr, Val and Phe) conferred a full HCA_3-like response to 2-hydroxy-octanoic acid. Conversely, the highly similar mutant Asn86Tyr,

Trp91Ser, Met103Val showed no response to this ligand. In the paper describing the HCA_2/HCA_3 chimeras, a receptor homology model for HCA_2 was also presented [85]. The model was based on the rhodopsin crystal structure [86], although the binding mode of the large ligand retinal did not give many clues on how to dock the small HCA receptor ligands. It was proposed that Trp91, Phe276 and Tyr284 interact with the pyridine ring of nicotinic acid, whereas a hydrogen bond may be present between Ser178 and the nitrogen. An independently constructed HCA_2 homology model, also based on the rhodopsin structure, was used to dock an anthranilic acid derivative [87]. The proposed binding pocket was lined by residues mainly from TM3, TM5, TM6 and EL2, which could bind the ligand in an extended conformation. Three residues important for nicotinic acid binding, Arg111 (TM3), Ser178 (EL2) and Phe276 (TM7), were also implicated here. Recently the repertoire of available GPCR crystal structures has grown considerably [88-91]. These new templates have not been exploited yet for HCA receptor homology modeling, but may yield better models in the near future.

VII. Signal transduction and receptor desensitization

A. G protein coupling

It was previously shown that nicotinic acid-induced effects are sensitive to treatment with pertussis toxin which specifically inactivates α-subunits of G_i-type G proteins [56]. During the last decade numerous studies have shown that HCA_1, HCA_2 and HCA_3 receptor-mediated effects are sensitive to pertussis toxin [2, 4-8, 18, 22]. Thus, HCA_1, HCA_2 and HCA_3 receptors couple to G_i/G_o -type G proteins.

B. Downstream signaling pathways

Agonists of HCA_1, HCA_2 and HCA_3 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 [4-8, 24, 92]. Since cAMP is the major intracellular regulator of lipolysis by stimulating cAMP-dependent kinase to activate lipolytic enzymes, a decrease in cAMP results in an antilipolytic effect in adipocytes [93].
Activation of G_i-type G proteins in immune cells results in stimulation of phospholipase C β-isoforms most likely through the release of $\beta\gamma$-subunits of G proteins [94], and activation of HCA_2 and HCA_3 receptors in neutrophils, macrophages or other immune cells has been shown to result in increases in free intracellular Ca^{2+} concentrations [4, 11, 18, 26, 95]. An increase in the intracellular Ca^{2+} concentration induced by nicotinic acid via HCA_2 receptors may lead to the activation of Ca^{2+}-sensitive phospholipase A_2 (PLA_2) and subsequent formation of prostanoids [11, 27, 95]. PLA_2 can also be activated by phosphorylation through extracellular signal-regulated kinase (ERK) which can be activated via HCA receptors as well [4, 5, 8, 92, 96].

C. Receptor desensitization

Some of the effects mediated by the HCA_2 receptor are subject to desensitization [97]. For instance, nicotinic acid-induced flushing and nicotinic acid-induced increases in intracellular Ca^{2+} concentrations via the HCA_2 receptor desensitize within minutes [26, 27]. Whether

these desensitization phenomena are due to effects on the receptor itself or at the level of downstream signaling processes is unclear. There is some evidence that heterologously expressed HCA_1 and HCA_2 receptors internalize in response to full agonists [5, 92, 98]. In contrast, a partial agonist, which did not induce ERK phosphorylation, did not induce receptor internalization [92]. Ligand-dependent internalization of HCA_2 appears to involve G protein-coupled receptor kinase 2 (GRK2) and arrestin 3 [98].

D. Receptor oligomerization

It is generally accepted that some G protein-coupled receptors can form dimers or oligomers. In a recent study it was shown by bioluminescence resonance energy transfer that HCA_2 and HCA_3 receptor constructs can interact when heterologously expressed in human embryonic kidney cells [80]. However, the existence of homo-/heterodimers of HCA_2 and HCA_3 in native tissues and the implications of oligomerization for receptor function remain unknown.

VIII. Therapeutic potential of HCA receptor ligands

Although all members of the HCA receptor family are potentially interesting drug targets, only HCA_2 is currently exploited as such. Therefore, this section will focus on this receptor alone.

A. Nicotinic acid alone

The use of high-dose nicotinic acid in the clinic has a long history starting in the 1950's [9]. A number of clinical studies demonstrated the usefulness of this compound as a lipid-modulating drug, although patient comfort and compliance are compromised by the common skin flushing side-effect [for reviews see: 10, 99; see also section IV]. Nowadays anti-flushing formulations of nicotinic acid are usually chosen over the crystalline form of the drug.

B. Nicotinic acid with anti-flushing strategies

Flushing is a widely occurring side effect of high-dose nicotinic acid. It seems to decrease with continued nicotinic acid treatment, but still up to one in five patients stops the treatment due to this side effect [100]. Administration of nicotinic acid with a meal seems to attenuate the flushing, probably due to a slower absorption rate. Acetylsalicylic acid, which is an inhibitor of prostaglandin synthesis, is also effective against flushing [101]. The more elegant versions of these strategies are extended-release nicotinic acid [102] and a combined formulation of nicotinic acid with laropiprant [52]. Modern extended-release (ER) nicotinic acid has been proven efficacious and has overcome the initial hepatotoxicity problems by decreasing the half-life compared to earlier sustained-release preparations [103]. In all of the clinical trials discussed below, ER nicotinic acid was used instead of an immediate-release formulation. Laropiprant is a prostaglandin D_2 receptor 1 (DP_1) antagonist. Like aspirin, it can reduce the cutaneous vasodilatation which is at the core of the flushing syndrome (see sections IV and VII). Nicotinic acid with laropiprant and

nicotinic acid alone (4 weeks 1 g/day followed by 20 weeks 2 g/day) were shown to have comparable lipid-modifying effects and safety profiles except for flushing, which was less severe with laropiprant [104]. In a separate study, severity of flushing in the first 16 weeks of nicotinic acid treatment was evaluated in patients with dyslipidemia [105]. Despite higher nicotinic acid doses, patients receiving the combination treatment experienced significantly less flushing. Furthermore, a lower number of patients from the combination group discontinued the trial due to flushing (7.4% vs 12.4%). The data from these and all other trials evaluating nicotinic acid/laropiprant have recently been compiled by McKenney and coworkers [106]. A fixed-dose formulation (1000 mg nicotinic acid/20 mg laropriprant) is on the market in Europe since 2008, but the FDA has requested further studies before introduction in the USA.

C. Nicotinic acid in combination with other lipid-altering drugs

HMG-CoA reductase inhibitors ('statins') are the first choice for lowering cholesterol plasma levels. Therefore, it was a logical step to investigate if combination of statins with nicotinic acid had any added benefit. An early example of such a trial showed that combination treatment with simvastatin and nicotinic acid for 3 years significantly improved the lipid profile in patients with coronary heart disease and low HDL cholesterol [107]. Furthermore, the frequency of a first cardiovascular event was only 3% in the treatment group versus 24% in the placebo group. Overall, the therapeutic efficacy of the combination was much better than simvastatin alone [see for example: 108].

An important surrogate endpoint in the evaluation of lipid-modifying therapy is the effect on the size of atherosclerotic lesions, usually measured by carotid intima-media thickness (CIMT) [99]. Statin monotherapy does not greatly influence atherosclerotic plaque formation, whereas statin/nicotinic acid combination treatment has been shown to reduce lesion development in several clinical trials. In the ARBITER 2 study, patients on statins received supplementary nicotinic acid or placebo therapy during one year [109]. CIMT was significantly increased in the placebo group, whereas it was unchanged in the group receiving nicotinic acid treatment, although the difference between the groups did not reach significance. In the more recent ARBITER6-HALTS study the effects of ER nicotinic acid or ezetimibe as add-on therapy with statin treatment were assessed [110]. Ezetimibe inhibits cholesterol absorption from the gut. After 14 months of treatment, a significant reduction in mean and maximal CIMT was observed in the nicotinic acid/statin group; HDL cholesterol was significantly increased, whereas it was decreased in the ezetimibe/statin group. Although the majority of the patients completed the study, it was halted prematurely because of an unexpected increase in atherosclerosis in the ezetimibe/statin group [see also: 111, 112]. In another very recent clinical study, participants received ezetimibe/statin combination treatment with or without additional nicotinic acid [113]. This triple therapy significantly improved lipid levels including HDL, LDL and apolipoprotein AI, and was well tolerated overall. Small and transient side-effects on fasting glucose were seen with nicotinic acid use.

The nicotinic acid/statin combination therapy is currently being evaluated further in two large trials. The endpoints are progression of cardiovascular disease, incidence of

major cardiovascular events and cardiovascular disease-associated mortality. The AIM-HIGH study compares ER nicotinic acid/simvastatin with simvastatin monotherapy and enrolled 3300 patients suffering from cardiovascular disease, low HDL cholesterol and high triglyceride levels (see at http://clinicaltrials.gov/ct2/show/NCT00120289). The HPS2-THRIVE compares ER nicotinic acid/simvastatin/laropiprant with simvastatin alone in patients with coronary heart disease (N>20,000) (see at http://clinicaltrials.gov/ct2/show/NCT00461630). The results of these trials are expected between 2011 and 2013. If a significant benefit of nicotinic acid is shown, a much more widespread use of the drug can be expected.

D. Fumaric acid derivatives

Fumaric acid esters, which have now been identified as HCA_2 agonists [29], have been used for the treatment of psoriasis as early as the 1950's [114]. Although their mechanism of action is still poorly understood, good and prolonged clinical efficacy and an acceptable safety profile have been reported for the oral administration of a mixture of monoethyl- and dimethylfumarate [115, 116]. For a recent retrospective analysis of almost 1000 patients see Reich et al. [117]. Dimethylfumarate (DMF) is quickly metabolized in vivo to monomethylfumarate (MMF), which has a higher potency at HCA_2 [29; see also section V]. DMF was also evaluated in a phase II trial including 240 patients for the treatment of relapsing-remitting multiple sclerosis [118]. Administration of 3 times 240 mg/day reduced the mean number of new brain lesions emerging between the 12^{th} and 24^{th} week of treatment by 70%, compared to placebo. It has not been conclusively demonstrated that activation of HCA_2 is at the basis of the therapeutic effects of fumaric acid esters, but the receptor is a plausible mediator as it is definitely expressed in immune cells. Nicotinic acid itself has also been suggested for the treatment of multiple sclerosis [119]. If HCA_2 agonists can indeed act as anti-inflammatory or immunosuppressive drugs, the treatment of several auto-immune diseases could be improved with these relatively safe and inexpensive compounds.

E. Clinical candidates

An HCA_2 agonists, MK-0354, was selected on the basis of low or negligible ERK1/2 activation in vitro and greatly reduced flushing in animal studies [61]. In a phase II study, 2.5 g of MK-0354 lowered free fatty acid levels in dyslipidemic patients consistently, comparable to 1 g of ER nicotinic acid. Little flushing was observed with MK-0354; however, no clinically meaningful lipid modification occurred either [120]. The recently completed trial with another HCA_2 agonist, MK-1903 (structure not disclosed), seems to have yielded similar results (see at http://www.clinicaltrial.gov/ct2/show/NCT00847197). Merck announced that the HDL cholesterol elevation was not large enough to meet the objectives for efficacy, and no safety problems were mentioned (see at http://drugdiscovery.pharmaceutical-business-review.com/news/merck_to_discontinue_development_of_mk1903_091224/). It seems that clinical efficacy of HCA_2 agonists cannot (yet) be accurately predicted at the preclinical stage. A better understanding of the mechanism of action of nicotinic acid could decrease the risk of attrition at a late stage of drug development.

IX. Conclusion

The first steps towards understanding the physiological relevance of the HCA receptor family have only recently been taken. These receptors seem to have evolved to recognize hydroxylated intermediates of energy metabolism with a relatively low affinity, in order to regulate lipolysis. HCA_1 seems to contribute to insulin-induced anti-lipolysis, and also to the weight gain induced by a hypercaloric diet. HCA_2 is important for the conservation of adipose tissue under starvation conditions, next to its pharmacological role as the high-affinity nicotinic acid receptor. HCA_3 seems to be part of the same negative feedback loop to limit lipolysis during starvation. HCA_2 and HCA_3 may have additional roles in the immune system, but further studies are needed in that area.

Despite rather spectacular effects on lipid levels and also on atherosclerosis progression, and the introduction of reduced-flushing formulations, the use of the antidyslipidemic drug nicotinic acid is still stunted by its side-effects. Other HCA_2 ligands, including partial agonists and biased agonists, may be better tolerated. However, despite extensive efforts of the pharmaceutical industry, no new HCA_2 ligands have been successful as clinical candidates so far. More research is needed to enable the identification of genuinely promising molecules. Possibly the most important enigma is how nicotinic acid induces HDL elevation. Recent animal experiments suggest that nicotinic acid reduces the progression of atherosclerosis also via lipid-independent anti-inflammatory effects and by increasing cholesterol efflux from plaque macrophages. Lipid-independent beneficial effects of nicotinic acid, in particular anti-inflammatory effects, deserve further analysis in the future. HCA_1 also has potential as a drug target for antilipolysis, and it is very unlikely that agonists for this receptor cause flushing because no skin expression has been detected. HCA_1 antagonists may reduce weight gain, but no antagonists are known to date for any of the HCA receptors. Thus, studies in animals have revealed unexpected physiological and pharmacological roles of HCA receptors. Much more work on the generation of new agonistic and antagonistic ligands of HCA receptors and their analysis in in vitro and in vivo models is required to explore all options to harness HCA receptors as targets to prevent and treat a variety of diseases like dyslipidemia, adipositas, cardiovascular diseases or chronic inflammatory and immune diseases.

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