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Chapter 2

Biological and pharmacological roles of hydroxy-carboxylic acid receptors

Abstract

The hydroxy-carboxylic acid (HCA) receptors HCA₁, HCA₂, and HCA₃ 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 Gᵢ-type proteins. HCA₁ is activated by lactate, HCA₂ by the ketone body 3-hydroxy-butyrate and HCA₃ by hydroxylated β-oxidation intermediates, especially 3-hydroxy-octanoic acid. Both HCA₂ and HCA₃ are part of a negative feedback loop which keeps the release of fat stores in check under starvation conditions, whereas HCA₁ plays a role in the antilipolytic (fat conserving) effect of insulin.

HCA₂ was first discovered as the molecular target of the anti-dyslipidemic drug nicotinic acid (or niacin). Many synthetic agonists have since been designed for HCA₂ and HCA₃, 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 HCA₂ 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-hydroxy-6,8,11,14-eicosatetraenoic acid; BAC, bacterial artificial chromosome; CETP, cholesterol ester transfer protein; COX-1, cyclooxygenase 1; COX-2, cyclooxygenase 2; CysLT₂, cysteinyl-leukotriene 2; DP₁, prostaglandin D₁ receptor 1; EC₅₀, 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; LTD₄, leukotriene D₄; MEF, monoethyl ester of fumaric acid; MMF, monomethyl ester of fumaric acid; PGD₂, prostaglandin D₂; PGE₂, prostaglandin E₂; PLA₂, phospholipase A₂; PPARγ, peroxisome proliferator-activated receptor γ; PUMA-G, protein up-regulated in macrophages by INF-γ (murine HCA₂); 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]. HCA₁ (GPR81) is endogenously activated by lactate, HCA₂ (GPR109A) by 3-hydroxy-butyrate and HCA₃ (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 Gᵢ 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, HCA₂ 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 HCA₂ 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 HCA₂ ligands is one of the major challenges in this field. In addition, it will be important to analyze the potential of HCA₁ and HCA₃ 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 HCA₂ 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 HCA₁ receptor (GPR81) as another orphan GPCR by BLAST analysis. The cDNA of the HCA₁ receptor was then cloned from a bacterial artificial chromosome (BAC) clone carrying a region of human chromosome 12. The HCA₁ receptor cDNA showed high homology [11] to the HCA₃ receptor (GPR109B/HM74), and the genes encoding HCA₁ and HCA₃ receptors were localized in close proximity on the same BAC clone. The HCA₂ 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 HCA₂ receptors were cloned and shown to be highly homologous to the murine orthologue PUMA-G [6, 7].

B. Sequence alignment and phylogenetic tree

The HCA₁ and the HCA₃ receptor are highly homologous as they share 95% sequence identity on the protein level. In fact, HCA₃ differs from HCA₂ in only 16 amino acids of
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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
Biology and pharmacology of HCA receptors

Receptor HCA₂, HCA₃ 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 HCA₃ receptor [4]. 2- and 3-hydroxy-octanoate were specific agonists of HCA₃ with EC₅₀ 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 HCA₃ receptor [4, 16, 17]. Moreover, aromatic D-amino acids like D-phenylalanine or D-tryptophan were shown to specifically activate the HCA₃ receptor [18]. Given the extreme rare occurrence of D-amino acids, it is unclear whether the ability of aromatic D-amino acids to activate HCA₃ 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.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Aliases</th>
<th>Naturally occurring ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCA₁</td>
<td>GPR81, GPR104, TA-GPCR, LACR, FKSG80</td>
<td>2-OH-propanoate (lactate)</td>
</tr>
<tr>
<td>HCA₂</td>
<td>GPR109A, PUMA-G, HM74A, HM746, NIACR1</td>
<td>3-OH-butyrate</td>
</tr>
<tr>
<td>HCA₃</td>
<td>GPR109B, HM74, NIACR2</td>
<td>3-OH-octanoate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-OH-octanoate, D-phenylalanine, D-tryptophan</td>
</tr>
</tbody>
</table>

In the past, various names were given to the receptors HCA₁ (GPR81), HCA₂ (GPR109A/HM74A/NIACR1) and HCA₃ (GPR109B/HM74/NIACR2) (see table 1). After the identification of HCA₂ (GPR109A) as the receptor of the anti-dyslipidemic drug nicotinic acid, HCA₁ (GPR81), HCA₂ (GPR109A) and HCA₃ (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 HCA₂ (GPR109A), and secondly, the two closely related receptors HCA₁ (GPR81) and HCA₃ (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 HCA₁, HCA₂ and HCA₃, 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.

![Gene structure of the HCA receptors](image)

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 \(\gamma\) (PPAR\(\gamma\)) 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 PPAR$\gamma$ 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-$\gamma$. 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-$\gamma$ [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
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indicates that the lactate/HCA1-mediated antilipolytic effects contribute to the increase in body weight under hypercaloric diet.

B. HCA2 receptor

The ketone body 3-hydroxy-butyrate which activates the HCA2 receptor with an EC50 of 0.7 mM has been described as the endogenous ligand of HCA2 [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 HCA2 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 HCA2 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 HCA2 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 HCA2 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 HCA2 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 HCA2 [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 HCA2 expressed by immune cells. In contrast to atherosclerosis-prone LDL-receptor-deficient mice carrying the wild-type receptor, mice lacking HCA2 or mice transplanted with HCA2-deficient bone marrow showed strongly reduced anti-atherosclerotic effects in response to nicotinic acid. The nicotinic acid receptor HCA2 is expressed by monocytes and macrophages including plaque macrophages, and nicotinic acid inhibits the recruitment of macrophages to atherosclerotic lesions in HCA2-dependent manner. In addition, HCA2 mediates a stimulatory effect of nicotinic acid.
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].

Evidence has been provided that HCA2 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 HCA2 [28]. While the first phase is due to activation of HCA2 on Langerhans cells, the second phase of the flush depends on HCA2 expressed by keratinocytes. The Langerhans cell-mediated flushing involves cyclooxygenase-1 (COX-1), prostaglandin D2 (PGD2) and prostaglandin E2 (PGE2). In contrast, keratinocyte-mediated flushing at later phases of the reaction involves cyclooxygenase-2 (COX-2) and PGE2 [27, 28, 52, 53].

There is evidence indicating that the HCA2 receptor expressed in intestinal epithelial cells responds to butyrate which is present in millimolar concentrations in the gut lumen, and that HCA2 thereby functions as a tumor suppressor and anti-inflammatory receptor [30]. Furthermore, comparison of the HCA2 potencies [3] and reported fecal concentrations of the short- and medium-chain fatty acids [54] suggests that pentanoate and hexanoate may also activate gut HCA2.

**C. HCA3 receptor**

Similar to HCA2, the primary physiological role of the HCA3 receptor appears to be the regulation of lipolysis. Plasma concentrations of the β-oxidation intermediate 3-hydroxy-

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**Figure 2. Biological roles of the HCA receptors.** Functions of the recently deorphanized receptors HCA1, HCA2, and HCA3. The lactate receptor HCA1 mediates the acute 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 form of a negative feedback loop (B). Thereby HCA2 and HCA3 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.
octanoate, which activates HCA3 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 HCA3 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 HCA3 receptor is only found in humans and chimpanzee suggests that a negative feedback loop of lipolysis mediated through 3-hydroxy-octanoate/HCA3 has evolved in higher primates to economize the use of fatty acids during periods of starvation. Whether HCA3 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 HCA2 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 HCA2 receptor we will discuss the synthetic ligands for this receptor first, followed by the more restricted information on the HCA3 receptor. To our knowledge synthetic ligands for the HCA1 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 HCA2 receptor

1. Nicotinic acid-like compounds

Lorenzen and colleagues [57] observed that nicotinic acid (1 in figure 3) displaces [3H]nicotinic acid from and increases [35S]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 HCA2 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.
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 HCA3 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 HCA3 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 HCA2 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 HCA2 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 HCA₂ 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 HCA₁ 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 HCA₂ receptor KO animals. Further SAR on the HCA₂ 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 HCA₂ 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 HCA₂ 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 HCA₂ 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 [³H] 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 HCA₂ 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 HCA₂ receptor
Despite the high (>95%) homology between HCA₂ and HCA₃ receptors, nicotinic acid is very selective for the HCA₂ 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 HCA₃ receptor, whereas all other compounds were slightly selective for the HCA₂
receptor. Ren et al. identified ortho-coumaric acid as approx. 20-fold selective for the HCA3 receptor, while the isomer para-coumaric acid was inactive at the HCA3 receptor but not at the HCA2 receptor (see paragraph A.5. in this section).

New HCA3 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 HCA2 receptor. 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 [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 HCA3 receptor-selective full agonists.

Recently, the synthetic efforts were extended to the pyrazole carboxylic acids as a template for the HCA3 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 EC50 value of 3 nM, and over 1000-fold selectivity with respect to the HCA2 receptor.

An intriguing conclusion from this research is that ‘on average’ ligands for the HCA3 receptors have higher affinity than ever met for the HCA2 receptor, despite intensive efforts on the latter receptor.

VI. Mutagenesis and receptor modeling studies

A. HCA1 receptor

Prior to the deorphanization of HCA1, Ge and coworkers [22] constructed a chimeric cysteinyl-leukotriene 2 (CysLT2) receptor where the intracellular domains (all ILs and the C-terminus) were replaced by the HCA1 sequence. The endogenous CysLT2 ligand, leukotriene D4 (LTD4), activated the receptor and this resulted in stimulation of Gi. Wild-type CysLT2 predominantly couples to Gq, so these findings suggested that HCA1 is a Gi coupled receptor. A first clue regarding the biological function of HCA1 was obtained when LTD4 was shown to inhibit lipolysis in primary mouse adipocytes expressing the chimera. After lactate was identified as the endogenous HCA1 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 [35S]GTPγS binding by lactate was absent in all four mutants, suggesting that these residues are all needed for receptor activation by lactate.

B. HCA2 and HCA3 receptors

The HCA2 and HCA3 receptors have different ligand repertoires and SAR, although there is some cross-selectivity (see section V). For example, nicotinic acid binds almost exclusively to HCA2, whereas acifran [84] stimulates both HCA2 (EC50 = 1.9 µM) and HCA3 (EC50 = 90 µM). This is surprising since these receptors have > 95% amino acid sequence
identity. To investigate which amino acids are involved in ligand selectivity, Tunaru and coworkers [85] constructed HCA2/HCA3 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 HCA2 receptor C-terminally, acifran had HCA2-like potency (EC50 = 2 µM). At the same time, this receptor was insensitive to nicotinic acid. It was also HCA3-like in its response to 2-oxo-octanoic acid, which is a close analogue of the endogenous HCA3 ligand [4]. The HCA3 N-terminal part seemed indeed responsible for the affinity of 2-oxo-octanoic acid, since the ‘reverse’ chimera with the HCA2 sequence N-terminally did not respond to this compound. Using the site-directed mutagenesis approach, a HCA2 mutant containing HCA3 residues on three positions was constructed. The mutations Asn86Tyr, Trp91Ser (interface of TM2 and IL1) and Ser178Ile (EL2) prevented HCA2 from responding to nicotinic acid and lowered the potency of acifran to HCA3-like levels (see figure 4B). As a next step, the positively charged arginine residues in HCA2 were investigated as likely counterparts for the negatively charged carboxylate function in nicotinic acid. All four arginine residues in the sequence were mutated to alanine, and only the Arg111Ala (TM3) mutant was rendered insensitive to nicotinic acid. The HCA3 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 HCA3 ligand [4] (see figure 4C). This suggests that the acidic moiety of the ligands is anchored at the same position in both HCA2 and HCA3. Interestingly, the HCA Arg111 corresponds to the conserved Asp residue important for agonist binding in the biogenic amine receptors. In HCA2, three more residues seem implicated in nicotinic acid binding: Phe180 (EL2), Phe276 and Tyr284 (both TM7) [85]. In a later paper, the roles of HCA2 residues Asn86, Met103 and Leu107 were investigated further [4]. Mutations into corresponding HCA3 residues (respectively Tyr, Val and Phe) conferred a full HCA3-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 HCA₂/HCA₃ chimeras, a receptor homology model for HCA₂ 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 HCA₂ 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 G₁-type G proteins [56]. During the last decade numerous studies have shown that HCA₁, HCA₂ and HCA₃ receptor-mediated effects are sensitive to pertussis toxin [2, 4-8, 18, 22]. Thus, HCA₁, HCA₂ and HCA₃ receptors couple to G₁/Gᵦ-type G proteins.

B. Downstream signaling pathways

Agonists of HCA₁, HCA₂ and HCA₃ receptors have been shown to inhibit adenyl cyclase activity and thereby to decrease cAMP levels in various cells after heterologous expression of the receptors as well as in primary adipocytes [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 G₁-type G proteins in immune cells results in stimulation of phospholipase C β-isoforms most likely through the release of βγ-subunits of G proteins [94], and activation of HCA₂ and HCA₃ receptors in neutrophils, macrophages or other immune cells has been shown to result in increases in free intracellular Ca²⁺ concentrations [4, 11, 18, 26, 95]. An increase in the intracellular Ca²⁺ concentration induced by nicotinic acid via HCA₂ receptors may lead to the activation of Ca²⁺-sensitive phospholipase A₂ (PLA₂) and subsequent formation of prostanoids [11, 27, 95]. PLA₂ 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 HCA₂ receptor are subject to desensitization [97]. For instance, nicotinic acid-induced flushing and nicotinic acid-induced increases in intracellular Ca²⁺ concentrations via the HCA₂ 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 HCA₁ and HCA₃ 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 HCA₂ 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 HCA₂ and HCA₃ receptor constructs can interact when heterologously expressed in human embryonic kidney cells [80]. However, the existence of homo-/heterodimers of HCA₂ and HCA₃ 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 HCA₂ 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 D₂ receptor 1 (DP₁) 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 laropiprant) 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 HCA2 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 HCA2 [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 12th and 24th week of treatment by 70%, compared to placebo. It has not been conclusively demonstrated that activation of HCA2 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 HCA2 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 HCA2 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 HCA2 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 HCA2 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. HCA₁ seems to contribute to insulin-induced anti-lipolysis, and also to the weight gain induced by a hypercaloric diet. HCA₂ is important for the conservation of adipose tissue under starvation conditions, next to its pharmacological role as the high-affinity nicotinic acid receptor. HCA₃ seems to be part of the same negative feedback loop to limit lipolysis during starvation. HCA₂ and HCA₃ 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 HCA₂ ligands, including partial agonists and biased agonists, may be better tolerated. However, despite extensive efforts of the pharmaceutical industry, no new HCA₂ 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. HCA₁ 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. HCA₁ 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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Chapter 2


