



Universiteit
Leiden
The Netherlands

A quest for connections : ligands for the HCA2, adenosine A3 and GPR88 receptors

Blad, C.C.

Citation

Blad, C. C. (2012, November 15). *A quest for connections : ligands for the HCA2, adenosine A3 and GPR88 receptors*. Retrieved from <https://hdl.handle.net/1887/20128>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/20128>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/20128> holds various files of this Leiden University dissertation.

Author: Blad, Clara Catelijne

Title: A quest for connections : ligands for the HCA2, adenosine A3 and GPR88 receptors

Date: 2012-11-15

Chapter 8



General discussion and future perspectives

For the preparation of this thesis a ligand-based approach was used to investigate three different G protein-coupled receptors from class A, that are all (potentially) important drug targets. The most well characterized is the adenosine A₃ receptor, which was discovered by homology cloning in 1992 and readily recognized as an adenosine receptor [1]. The adenosine A₃ receptor has received attention as a potential target for anti-cancer medication. Approximately a decade later, the second receptor of interest, GPR109A, was deorphanized by pairing to endogenous ligand 3-hydroxybutyrate, prompting re-naming as the hydroxy carboxylic acid receptor 2 (HCA₂). This receptor is a target for anti-atherosclerotic drugs. The focus of the last research chapter is on the orphan receptor GPR88, which is still a mystery in many aspects, but it may play a role in the pathophysiology of the brain.

The hydroxy-carboxylic acid receptor 2 (HCA₂)

In **chapter 2**, an overview is presented of the literature on the 3-hydroxybutyrate receptor HCA₂ and the two other members of the subfamily, namely HCA₁ (for lactate) and HCA₃ (for β -oxydation intermediates). As the target of nicotinic acid, HCA₂ has been most widely studied, although HCA₁ and HCA₃ may present interesting future drug targets, for instance for the treatment of obesity and dyslipidemia, respectively. After their deorphanization, the physiological roles of the HCA receptors on adipocytes are now coming into focus: HCA₁ boosts the antilipolytic effect of insulin, and HCA₂ and HCA₃ inhibit excessive lipolysis during starvation. Especially for HCA₂, but also HCA₃, many synthetic agonists have been developed, with the aim of introducing a new and improved successor for nicotinic acid in the clinic. Hopes were high for MK-0354, a biased HCA₂ agonists with very low ERK1/2 activation *in vitro* and almost no flushing *in vivo*, but in phase II clinical trials the lipid modification targets were not reached [2]. Another HCA₂ agonist, MK-1903, followed a similar path. However, new findings concerning the mechanism of action of nicotinic acid beg the question whether other clinical endpoints are needed for clinical trials of HCA₂ agonists. A significant part of the anti-atherosclerotic effect of nicotinic acid appears to be mediated by activation of HCA₂ on immune cells, decreasing endothelial dysfunction and vascular inflammation, and increasing cholesterol efflux from plaque macrophages [3]. Therefore, instead of a complete focus on lipid modification, more attention should be paid to the anti-inflammatory effects of new HCA₂ agonists in (pre)clinical drug testing.

The skin flushing side-effect of nicotinic acid was recently shown to be partly mediated by HCA₂ on keratinocytes (through COX-2 and PGE₂), next to the well-known involvement of Langerhans cells [4]. Better understanding of flushing could lead to better prevention in the future, although advances have already been made. The use of extended-release formulations of nicotinic acid [5] and combination with the DP₁ prostaglandin receptor antagonist laropiprant [6] have significantly reduced the occurrence and severity of nicotinic acid-induced flushing.

In **chapter 3**, the *in vitro* and *in vivo* effects of two partial agonists for HCA₂, both pyrazole compounds, are presented. We have shown that these ligands have lower relative potency for ERK1/2 phosphorylation compared to nicotinic acid, a property that has been linked

to decreased skin flushing [7]. In mice, we confirmed that the pyrazoles did not increase cutaneous temperature. Although less precise than laser doppler velocimetry, we believe surface temperature recording is a good alternative for measuring blood flow in the skin, and thus flushing. The absence of a skin flushing response may be due to the moderate shift in the relative potencies on the G protein pathway and the ERK phosphorylation pathway, to the reduced intrinsic efficacy of these ligands, or to a combination of these two factors. Nicotinic acid and both partial agonists significantly reduced plasma VLDL concentrations. In the case of nicotinic acid, a 2-fold reduction of hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) in the adipose tissue seemed mainly responsible for this effect. Interestingly, the two pyrazole compounds did not affect gene expression in the adipose tissue. Instead, these compounds triggered a >40% reduction of apolipoprotein B (ApoB) in the liver, which indicates decreased hepatic VLDL production. The reduced ApoB expression could be downstream from suppressed hepatic PGC-1 β expression, which has been previously reported as an effect of nicotinic acid [8]. No effect was seen on HDL, but this is expected in wildtype mice that naturally lack a homolog to the human cholesteryl ester transfer protein (CETP), responsible for transfer of cholesterol from HDL to less dense particles [9].

Chapters 4 and 5 describe medicinal chemistry programs in which potential new ligands for HCA₂ were devised, synthesized and characterized in our laboratory. In **chapter 4** a series of propenoic acid derivatives and their affinity for HCA₂ is presented. Two agonists of this compound class, monomethyl- and monoethyl-fumarate (MMF and MEF), are used in the clinic as anti-psoriatic drugs [10]. We confirmed binding of these compounds and of cinnamic acid to HCA₂, and describe synthesis and affinity testing of a series of fumaric acid esters and amides, as well as cinnamic acid derivatives. The binding pocket for these ligands seems very restricted: in a series of 55 related compounds, only 15 had a K_i of ≤ 10 μ M, and none could be identified with higher affinity than MMF and MEF. On the basis of molecular modeling and analysis of the structure-activity relationships, we propose a pharmacophore featuring a planar *trans*-propenoic acid with a maximum length of 8 Å, with larger substituents oriented out-of-plane.

Chapter 5 revolves around a very different type of ligands, the pyrazolopyrimidines, which are much larger and bind at an allosteric site on the HCA₂ receptor protein. Starting from a previously published ligand series [11], we prepared a number of novel compounds and characterized them in radioligand binding and functional assays. Several compounds were potent and efficacious agonists on HCA₂ when tested alone, even matching nicotinic acid's potency, while at the same time enhancing the potency (up to 20-fold) and/or efficacy (up to +50%) of nicotinic acid when simultaneously present in the assay. We observed that the agonistic and potency-enhancing properties of the pyrazolopyrimidines were correlated, suggesting that the ability to stabilize an active receptor conformation contributed to the modulating properties of these ligands. Of special interest is our finding that some derivatives markedly enhance the efficacy and potency of the endogenous HCA₂ ligand 3-hydroxybutyrate, since this highlights the potential of this type of ligands for future drug development.

The adenosine A₃ receptor (A₃R)

In **chapter 6**, we report that the naturally occurring nucleosides N⁶-(2-isopentenyl)-adenosine (IPA) and racemic zeatin riboside are selective human A₃R ligands with affinities in the high nanomolar range, which is comparable with the affinity of adenosine. IPA also bound to the rat A₃R with good affinity. Both ligands could inhibit forskolin-induced cAMP formation with micromolar potency, and in the case of IPA we showed that an A₃R antagonist could block this effect. IPA was previously reported to inhibit tumor growth, but the A₃R was not implicated in this effect until now. We observed strong and similar anti-proliferative effects of IPA and reference A₃R agonist Cl-IB-MECA in two tumor cell lines. At low concentrations, the effect of IPA could be blocked by a selective A₃R antagonist, but at higher concentrations this was not the case. The anti-proliferative effect at higher concentrations of IPA seems to be independent of A₃R, which was previously observed for high concentrations of other A₃R agonists, for example for Cl-IB-MECA [12]. The existence of an additional endogenous ligand for A₃R, next to adenosine, with protective anti-cancer properties, was previously predicted [13]. Although IPA could fit this profile, we were unable to detect the ligand in fresh rat muscle using HPLC analysis. Thus, no definite proof could be found for a role of IPA as an endogenous agonist for A₃R.

Orphan receptor GPR88

Chapter 7 focuses on GPR88, which is one of the dozens of G protein-coupled receptors for which no ligand or function is known to date. Analysis of its expression pattern and changes in expression levels, as well as the phenotype of a mouse model lacking the receptor, suggest that GPR88 may be implicated in several diseases including schizophrenia, depression and bipolar disorder. We attempted to identify a ligand for this receptor by testing a library of 4131 small molecules and naturally occurring peptides, using changes in intracellular calcium levels as the readout. In native HEK293 cells, GPR88 may stimulate the calcium pathway via Gα_q, but since the coupling preference of the receptor is unknown, cell lines co-expressing chimeric Gα_{q15} or Gα₁₆ were also used (in case of natural G_i or G_s coupling, respectively). Our primary screen, performed using the Fluorescent Imaging Platerreader (FLIPR) in 384-well format, yielded 46 hits, with responses in all three cell lines but with poor reproducibility. In validation assays, dose-response curves could only be obtained for papaverine, but these seemed independent of GPR88, since calcium mobilization was also observed in control cell lines lacking the receptor. Thus, our efforts mainly served to confirm the challenging nature of receptor deorphanization programs.

Future perspectives

G protein-coupled receptors have become an important field of study within the life sciences. We now know that they make up the largest gene family in our genome, and are prominently involved in most major physiological processes in the body [14]. Since their discovery, new concepts have regularly emerged that refined our understanding of this fascinating protein family in general, or of specific receptors. Recent advances include,

but are not limited to, structural data on a number of receptors (reviewed by [15]), the realization that GPCRs function not only as receptors for transmitters, taste and odorants, but also as sensors for nutrients and intermediates of metabolism [16-18], and receptor-ligand pairing of a number of orphan receptors, including the three hydroxy-carboxylic acid receptors (reviewed by [19]). However, many unresolved questions remain in GPCR research. The current thesis focuses on three receptors: the hydroxy-carboxylic acid receptor 2 (HCA₂) (chapters 2-5), the adenosine A₃ receptor (chapter 6), and orphan receptor GPR88 (chapter 7). In this section I will discuss the open questions regarding these 3 receptors and possible routes towards their resolution. Some general remarks regarding the future of GPCR research will also be made.

Hydroxy-carboxylic acid receptors

All three hydroxy-carboxylic acid receptors have been deorphanized in the last few years [20-23]. It has been proposed that their roles in physiology are the conservation of fat stores during periods of starvation (HCA₂ and HCA₃), and under influence of insulin (HCA₁). In the case of HCA₂, the negative feedback loop which is formed when 3-hydroxy butyrate counteracts its own production by activating HCA₂, thus reducing lipolysis, may also be important as a break on the levels of acidifying ketone bodies in the blood. In primates HCA₃ may also play a role here, since it also forms a negative feedback loop on lipolysis with its ligands produced by β -oxydation of free fatty acid [20]. As yet, the feedback mechanism I propose has not been verified. It is well known that insulin keeps the plasma levels of ketone bodies in check, and even if the HCA₂ loop has a role here as well, it is apparently, in certain cases, not sufficient to prevent diabetic ketoacidosis when the insulin loop is non-functional. However, 3-hydroxy butyrate may not be a full agonist for HCA₂ (chapter 5), and thus have a limited effect even at high concentrations. Full agonists or allosteric enhancers of HCA₂ or HCA₃ may have therapeutic potential in acidosis. Acute nicotinic acid administration also diminishes gluconeogenesis in the liver [24], which would be of further benefit to counteract the hyperglycaemia in diabetic ketoacidosis. In addition, a recent publication suggests that elevation of 3-hydroxy butyrate in diabetic patients, with concomitant upregulation of HCA₂ expression, is a mechanism to protect the tissues from the damaging effects of the inflammation associated with the disease [25]. Even in absence of ketoacidosis, HCA₂ agonists may thus be valuable drugs in the treatment of diabetes. However, it has also been reported that nicotinic acid may decrease insulin sensitivity [26] and promote the onset of diabetes [27] so more research is needed into the role of HCA₂ and its ligands in this disease.

In intestine and skin, HCA₂ appears to prevent excessive inflammation and tumor formation [28-29]. In the intestine, the major ligand is likely not 3-hydroxy butyrate, but non-hydroxylated butyrate produced by bacterial fermentation. By comparing potency [23] and local concentration from the literature [30], it seems that pentanoate and hexanoate are also likely to activate HCA₂ in the intestine, but no published experimental work proves this. Although ligands derived from nutrition and bacterial fermentation in the intestine are not produced by the body itself, they could still be regarded endogenous ligands of GPCRs in the intestinal wall. Functionally they could be compared to the

receptors involved in taste, smell and vision, which also detect compounds and signals from the outside world. Several other GPCRs, for example the sweet taste receptor T1R2/T1R3, have already been implicated in nutrient sensing in the bowel [31].

In certain inflammatory diseases, like psoriasis and multiple sclerosis, HCA₂ has also been implicated. Psoriasis is already treated with topical application of HCA₂ agonist monomethylfumarate (MMF) [32], and a phase III trial has recently been favourably concluded with oral dimethylfumarate (BG-12) for multiple sclerosis (MS) [33-34]. *In vivo*, dimethylfumarate is rapidly metabolized to HCA₂ agonist MMF, and the side effect profile of BG-12, featuring mainly flushing and gastrointestinal complaints, is typical for a HCA₂ agonist. Inhibition of nuclear translocation of NF- κ B has been reported as the mechanism of action, and this is a known downstream effect of HCA₂ activation [29]. However, it has not been clearly demonstrated if HCA₂ is a key player in the therapeutic action of fumarates, so experimental confirmation in an animal model lacking HCA₂ would be valuable. Nicotinic acid itself has also been proposed as a potential anti-inflammatory therapy for MS [35], as well as sepsis [36], chronic renal failure [37] and arthritis [38]. For such acutely life-threatening or debilitating indications, the harmless side effect of skin flushing is less likely to affect patient compliance, and the side effect may be more acceptable if more efficacious or safer treatments are not available.

Adenosine A₃ receptor and isopentenyl adenosine

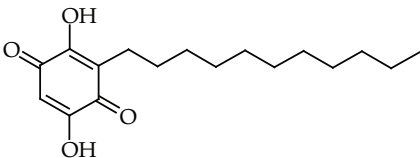
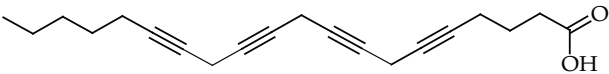
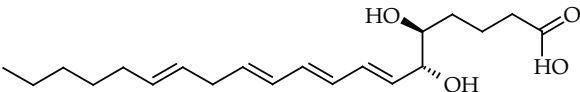
We identified N⁶-isopentenyl-adenosine (IPA) as a ligand for the adenosine A₃ receptor (A₃R). We also investigated whether IPA is an endogenous ligand for the A₃R. No evidence could be found for significant concentrations of IPA in muscle tissue, but it is still a possibility that IPA binds to the A₃R in certain (patho-) physiological conditions. The idea that one receptor can have more than one orthosteric ligand is well-known, especially when synthetic ligands are considered. In recent deorphanization efforts, receptors have regularly been found to have multiple endogenous ligands, that may or may not be chemically related (for example GPR17 with uracil nucleotides and cysteinyl-leukotrienes [39], and several receptors binding a range of free fatty acids (for a review see [40])). In some cases this could be a sign of erroneous receptor-ligand pairing (for example for GPR65/TDAG8, a proton sensor but not lysophosphatidylcholine receptor [41]), but in other cases the double or multiple pairing may be correct. It would be interesting to subject receptors that have known ligands (discovered by classical pharmacology or early screens) to a state-of-the-art receptor deorphanization screen. The possibility of additional endogenous ligands should also be kept in mind when investigating unexplained receptor activation or transmitter effects. Examples of GPCRs that were 'partial orphans' are the P2Y1 receptor which binds not only ADP but also ADP ribose [42] and β -NAD [43], and the CXCR2 receptor that binds 3 chemokines but was later shown to bind macrophage-derived lectin MNCf as well [44]. For adenosine receptors it was observed that endogenous adenosine concentrations could not always account for the activation levels [45-46], thus raising the question of alternative endogenous ligands. In the case of the A₃R, it has already been shown that it binds to inosine next to adenosine [47].

GPR88 and other orphan GPCRs

Since no endogenous ligand is known for GPR88, it is possible that this receptor functions independently from a ligand. Even if this is the case, the receptor remains a potential therapeutic target. Drugs targeting a ligand-less receptor could aim at changing its expression level, at blocking its interaction with other proteins in the cell, or synthetic ligands could be devised just as for a liganded receptor, even in absence of an endogenous ligand. For example, if a lower expression level or loss-of-function of GPR88 could be demonstrated in patients with schizophrenia, in parallel to the ‘schizophrenic’ GPR88 knock-out mice [48], treatment with synthetic GPR88 agonists or GPR88 up-regulating drugs could be effective.

The reported similarity of the GPR88 binding pocket to the class C glutamate and GABA_B receptor binding pockets [49] can be a starting point for the identification of a synthetic GPR88 ligand. An *in silico* prediction of GPR88 ligands (thesis J.K. Bray 2010 [50]) identified the lipids FFA, LPA and S1P as potential ligands. Functional or binding assays either confirming or refuting this prediction have not been reported. In the patent literature, ligands for medium-chain fatty acid receptor GPR84 were described with limited selectivity towards this receptor over GPR88 (see table 1) [51]. From the text it could be deduced that these ligands have a potency on GPR88 between 10 and 100 μ M (compounds 2 and 3), or between 100 μ M and 1 mM (compound 1), although this is not clearly stated. The reported potencies on GPR84 are in the nanomolar range, so these ligands are clearly not selective GPR88 ligands, but if their action on GPR88 were confirmed, they could be a starting point for further ligand optimization.

Table 1. Ligands with (high) micromolar potency at GPR88, next to nanomolar potency at GPR84. Adapted from (WO2007027661).

Compound	Chemical structure
1	
2	
3	

Ligand pairing of GPR88 and the other remaining orphan receptors will be a challenging task. A promising technique that may increase the chances of success is the use of a label-free biosensor [52]. When this is used as the screening assay, no knowledge is needed of the downstream coupling of the orphan receptor, because any change in the cells is captured [53]. This is useful even when a downstream signalling pathway is known, as

for constitutively active orphans, because binding of an agonist may activate a completely different pathway. Another advantage of label-free assays is the high sensitivity, which facilitates the use of a cell line endogenously expressing the GPCR of interest [54], instead of using heterologous receptor expression, ensuring that all components necessary for receptor function are present, next to the native receptor.

Final note

The work presented in this thesis revolves around the identification and characterization of novel ligands for G protein-coupled receptors: small molecule (partial) agonists and ago-allosteric modulators for HCA₂, naturally occurring nucleosides for A₃R, and a wide medium-throughput screen at GPR88. The model of lock and key, one receptor plus one ligand, opening the door to the same pathway every time, is too simple for GPCR reality. The receptor could be seen as a magical door, with multiple locks (orthosteric and allosteric sites), where different keys (ligands) in the same lock can open the door to different pathways, and where simultaneously inserting keys in multiple locks gives even more possible outcomes. In the body, a plethora of compounds is present at the receptor, and if there is sufficient affinity between receptor and ligand, binding will occur, possibly followed by an effect. The expectation that the physiological role of a GPCR is mainly determined by its interaction with a single ligand, may thus be somewhat simplistic. Even for receptors that are not orphans anymore, additional endogenous ligands may still be found. The interest in receptor deorphanization has prompted the development of highly sophisticated screening methods, and it could be profitable to use them on non-orphan receptors as well. Even though GPCRs can be considered established drug targets, novel concepts, such as those presented in this thesis, are needed to unravel their function in physiology, and to optimise their exploitation in medicine.

References

1. Zhou, Q.Y., et al., *Molecular cloning and characterization of an adenosine receptor - the A₃ adenosine receptor*. Proc Natl Acad Sci U S A, 1992. **89**(16): p. 7432-7436.
2. Lai, E., et al., *Effects of a niacin receptor partial agonist, MK-0354, on plasma free fatty acids, lipids, and cutaneous flushing in humans*. J Clin Lipidol, 2008. **2**(5): p. 375-83.
3. Lukasova, M., et al., *Nicotinic acid inhibits progression of atherosclerosis in mice through its receptor GPR109A expressed by immune cells*. J Clin Invest, 2011. **121**(3): p. 1163-73.
4. Hanson, J., et al., *Nicotinic acid- and monomethyl fumarate-induced flushing involves GPR109A expressed by keratinocytes and COX-2-dependent prostanoid formation in mice*. J Clin Invest, 2010. **120**(8): p. 2910-9.
5. Morgan, J.M., D.M. Capuzzi, and J.R. Guyton, *A new extended-release niacin (Niaspan): efficacy, tolerability, and safety in hypercholesterolemic patients*. Am J Cardiol, 1998. **82**(12A): p. 29U-34U; discussion 39U-41U.
6. Cheng, K., et al., *Antagonism of the prostaglandin D2 receptor 1 suppresses nicotinic acid-induced vasodilation in mice and humans*. Proc Natl Acad Sci U S A, 2006. **103**(17): p. 6682-7.

7. Walters, R.W., et al., *beta-Arrestin1 mediates nicotinic acid-induced flushing, but not its antilipolytic effect, in mice*. J Clin Invest, 2009. **119**(5): p. 1312-1321.
8. Hernandez, C., et al., *Regulation of hepatic ApoC3 expression by PGC-1beta mediates hypolipidemic effect of nicotinic acid*. Cell Metab, 2010. **12**(4): p. 411-9.
9. van der Hoorn, J.W., et al., *Niacin increases HDL by reducing hepatic expression and plasma levels of cholesteryl ester transfer protein in APOE*3Leiden.CETP mice*. Arterioscler Thromb Vasc Biol, 2008. **28**(11): p. 2016-22.
10. Tang, H., et al., *The psoriasis drug monomethylfumarate is a potent nicotinic acid receptor agonist*. Biochem Biophys Res Commun, 2008. **375**(4): p. 562-565.
11. Shen, H.C., et al., *Discovery of pyrazolopyrimidines as the first class of allosteric agonists for the high affinity nicotinic acid receptor GPR109A*. Bioorg Med Chem Lett, 2008. **18**(18): p. 4948-4951.
12. Kim, S.G., et al., *p53-Independent induction of Fas and apoptosis in leukemic cells by an adenosine derivative, Cl-IB-MECA*. Biochemical Pharmacology, 2002. **63**(5): p. 871-880.
13. Bar-Yehuda, S., et al., *Resistance of muscle to tumor metastases: A role for A3 adenosine receptor agonists*. Neoplasia, 2001. **3**(2): p. 125-131.
14. Bockaert, J. and J.P. Pin, *Molecular tinkering of G protein-coupled receptors: an evolutionary success*. EMBO J, 1999. **18**(7): p. 1723-9.
15. Congreve, M., C. Langmead, and F.H. Marshall, *The use of GPCR structures in drug design*. Adv Pharmacol, 2011. **62**: p. 1-36.
16. Ahmed, K., S. Tunaru, and S. Offermanns, *GPR109A, GPR109B and GPR81, a family of hydroxy-carboxylic acid receptors*. Trends Pharmacol Sci, 2009. **30**(11): p. 557-62.
17. Wellendorph, P., L.D. Johansen, and H. Brauner-Osborne, *The emerging role of promiscuous 7TM receptors as chemosensors for food intake*. Vitam Horm. **84**: p. 151-84.
18. Chen, X., et al., *TGR5: a novel target for weight maintenance and glucose metabolism*. Exp Diabetes Res, 2011. **2011**: p. 853501.
19. Offermanns, S., et al., *International Union of Basic and Clinical Pharmacology. LXXXII: Nomenclature and Classification of Hydroxy-carboxylic Acid Receptors (GPR81, GPR109A, and GPR109B)*. Pharmacol Rev, 2011. **63**(2): p. 269-90.
20. Ahmed, K., et al., *Deorphanization of GPR109B as a receptor for the beta-oxidation intermediate 3-OH-octanoic acid and its role in the regulation of lipolysis*. J Biol Chem, 2009. **284**(33): p. 21928-33.
21. Cai, T.Q., et al., *Role of GPR81 in lactate-mediated reduction of adipose lipolysis*. Biochem Biophys Res Commun, 2008. **377**(3): p. 987-91.
22. Liu, C., et al., *Lactate inhibits lipolysis in fat cells through activation of an orphan G-protein-coupled receptor, GPR81*. J Biol Chem, 2009. **284**(5): p. 2811-22.
23. Taggart, A.K., et al., *(D)-beta-Hydroxybutyrate inhibits adipocyte lipolysis via the nicotinic acid receptor PUMA-G*. J Biol Chem, 2005. **280**(29): p. 26649-52.
24. Pinter, E.J. and C.J. Pattee, *Biphasic nature of blood glucose and free fatty acid changes following intravenous nicotinic acid in man*. J Clin Endocrinol Metab, 1967. **27**(3): p.

- 440-3.
25. Gambhir, D., et al., *GPR109A as an anti-inflammatory receptor in retinal pigment epithelial cells and its relevance to diabetic retinopathy*. Invest Ophthalmol Vis Sci, 2012.
26. Kahn, S.E., et al., *Increased beta-cell secretory capacity as mechanism for islet adaptation to nicotinic acid-induced insulin resistance*. Diabetes, 1989. **38**(5): p. 562-8.
27. Henkin, Y., et al., *Niacin revisited: clinical observations on an important but underutilized drug*. Am J Med, 1991. **91**(3): p. 239-46.
28. Bermudez, Y., et al., *Nicotinic acid receptor abnormalities in human skin cancer: implications for a role in epidermal differentiation*. PLoS One, 2011. **6**(5): p. e20487.
29. Thangaraju, M., et al., *GPR109A is a G-protein-coupled receptor for the bacterial fermentation product butyrate and functions as a tumor suppressor in colon*. Cancer Res, 2009. **69**(7): p. 2826-32.
30. Tiihonen, K., A.C. Ouwehand, and N. Rautonen, *Effect of overweight on gastrointestinal microbiology and immunology: correlation with blood biomarkers*. Br J Nutr, 2010. **103**(7): p. 1070-8.
31. Tolhurst, G., F. Reimann, and F.M. Gribble, *Intestinal sensing of nutrients*. Handb Exp Pharmacol, 2012(209): p. 309-35.
32. Tang, H., et al., *The psoriasis drug monomethylfumarate is a potent nicotinic acid receptor agonist*. Biochem Biophys Res Commun, 2008. **375**(4): p. 562-5.
33. *Trial watch: Phase III success for Biogen's oral multiple sclerosis therapy*. Nat Rev Drug Discov, 2011. **10**(6): p. 404.
34. Papadopoulou, A., et al., *Dimethyl fumarate for multiple sclerosis*. Expert Opin Investig Drugs, 2010. **19**(12): p. 1603-12.
35. Penberthy, W.T., *Nicotinic Acid-Mediated Activation of Both Membrane and Nuclear Receptors towards Therapeutic Glucocorticoid Mimetics for Treating Multiple Sclerosis*. PPAR Res, 2009. **2009**: p. 853707.
36. Kwon, W.Y., et al., *Niacin attenuates lung inflammation and improves survival during sepsis by downregulating the nuclear factor-kappaB pathway*. Crit Care Med, 2011. **39**(2): p. 328-34.
37. Cho, K.H., et al., *Niacin ameliorates oxidative stress, inflammation, proteinuria, and hypertension in rats with chronic renal failure*. Am J Physiol Renal Physiol, 2009. **297**(1): p. F106-13.
38. Mitrofanov, V.A., et al., *Inflammatory degeneration of joint tissue in adjuvant arthritis after intraarticular treatment with the mixture of silver drug and nicotinic acid*. Bull Exp Biol Med, 2005. **140**(6): p. 702-4.
39. Ciana, P., et al., *The orphan receptor GPR17 identified as a new dual uracil nucleotides/cysteinyl-leukotrienes receptor*. EMBO J, 2006. **25**(19): p. 4615-27.
40. Wellendorph, P., L.D. Johansen, and H. Brauner-Osborne, *Molecular pharmacology of promiscuous seven transmembrane receptors sensing organic nutrients*. Mol Pharmacol, 2009. **76**(3): p. 453-65.
41. Seuwen, K., M.G. Ludwig, and R.M. Wolf, *Receptors for protons or lipid messengers or both? J Recept Signal Transduct Res*, 2006. **26**(5-6): p. 599-610.

42. Gustafsson, A.J., et al., *ADP ribose is an endogenous ligand for the purinergic P2Y1 receptor*. *Mol Cell Endocrinol*, 2011. **333**(1): p. 8-19.
43. Mutafova-Yambolieva, V.N., et al., *Beta-nicotinamide adenine dinucleotide is an inhibitory neurotransmitter in visceral smooth muscle*. *Proc Natl Acad Sci U S A*, 2007. **104**(41): p. 16359-64.
44. Moreno, A.N., et al., *The macrophage-derived lectin, MNCF, activates neutrophil migration through a pertussis toxin-sensitive pathway*. *J Histochem Cytochem*, 2005. **53**(6): p. 715-23.
45. Nilsson, K.F., et al., *Estimation of endogenous adenosine activity at adenosine receptors in guinea-pig ileum using a new pharmacological method*. *Acta Physiol (Oxf)*, 2010. **199**(2): p. 231-41.
46. Wiklund, N.P. and L.E. Gustafsson, *On the nature of endogenous purines modulating cholinergic neurotransmission in the guinea-pig ileum*. *Acta Physiol Scand*, 1987. **131**(1): p. 11-8.
47. Jin, X., et al., *Inosine binds to A3 adenosine receptors and stimulates mast cell degranulation*. *J Clin Invest*, 1997. **100**(11): p. 2849-57.
48. Logue, S.F., et al., *The orphan GPCR, GPR88, modulates function of the striatal dopamine system: a possible therapeutic target for psychiatric disorders?* *Mol Cell Neurosci*, 2009. **42**(4): p. 438-47.
49. Surgand, J.S., et al., *A chemogenomic analysis of the transmembrane binding cavity of human G-protein-coupled receptors*. *Proteins*, 2006. **62**(2): p. 509-38.
50. Bray, J.K., *The development and application of computational methods for the prediction of G protein-coupled receptors*. 2010, California Institute of Technology.
51. Hakak, Y., et al., *WO2007027661: Human G protein-coupled receptor and modulators thereof for the treatment of atherosclerosis and atherosclerotic disease and for the treatment of conditions related to MCP-1 expression* 2007.
52. McGuinness, R.P., et al., *Enhanced selectivity screening of GPCR ligands using a label-free cell based assay technology*. *Comb Chem High Throughput Screen*, 2009. **12**(8): p. 812-23.
53. Schroder, R., et al., *Deconvolution of complex G protein-coupled receptor signaling in live cells using dynamic mass redistribution measurements*. *Nat Biotechnol*, 2010. **28**(9): p. 943-9.
54. Fang, Y., G. Li, and A.M. Ferrie, *Non-invasive optical biosensor for assaying endogenous G protein-coupled receptors in adherent cells*. *J Pharmacol Toxicol Methods*, 2007. **55**(3): p. 314-22.

