Opinion

# GPR109A, GPR109B and GPR81, a family of hydroxy-carboxylic acid receptors

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**G-protein-coupled receptors (GPCRs) are the most versatile receptor family as they have the ability to respond to chemically diverse ligands. Despite intensive efforts during the past two decades, there are still more than 100 orphan GPCRs for which endogenous ligands are unknown. Recently, GPR109A, GPR109B and GPR81, which form a GPCR subfamily, have been deorphanized. The physiological ligands of these receptors are the ketone body 3-hydroxy-butyrate, the metabolite 2-hydroxy-propanoate (lactate) as well as the β-oxidation intermediate 3-hydroxy-octanoate. Thus, this receptor subfamily is activated by hydroxy-carboxylic acid ligands which are intermediates of energy metabolism. All three receptors are predominantly expressed in adipocytes and mediate antilipolytic effects. In this article, we propose that the hydroxy-carboxylic acid structure of their endogenous ligands is the defining property of this receptor subfamily and that hydroxy-carboxylic acid receptors function as metabolic sensors which fine-tune the regulation of metabolic pathways.**

## Introduction

Traditionally, classic hormones and neurotransmitters have been regarded as the quintessential endogenous G-protein-coupled receptor (GPCR) agonists. However, the deorphanization efforts of the past two decades have revealed that GPCRs are activated by many other endogenous diffusible molecules such as ions or metabolites. Recently, a subfamily of GPCRs consisting of GPR81, GPR109A and GPR109B has emerged as a new group of metabolite receptors. GPR109A, GPR109B and GPR81 by homology represent a subfamily of GPCRs (Figure 1), and their genes most probably evolved by duplication as indicated by their tandem location on human chromosome 12q24. GPR109B (HM74), which is found only in higher primates, and GPR81 were originally identified as orphan GPCRs [1,2], whereas GPR109A was first described in mice as a "protein upregulated in macrophages by interferon γ" [3]. In 2003, several groups identified GPR109A as the receptor of the antidyslipidemic drug nicotinic acid [4–6]. Using a mouse knockout model, GPR109A was demonstrated to be responsible for the antilipolytic and triglyceride-lowering effects of nicotinic acid as well as for the major side effects of nicotinic acid, a cutaneous vasodilation called flushing [5,7]. The ketone body 3-hydroxy-butyrate was identified as an endogenous ligand of GPR109A [8]. More recently, GPR81 was shown to be activated by lactate [9,10], whereas GPR109B is a receptor for 3-hydroxylated β-oxidation intermediates, in particular 3-hydroxy-octanoate [11] (Table 1). Here, we briefly summarize current knowledge on members of this GPCR subfamily which function as metabolic sensors being activated by intermediates of energy metabolism. We propose that this receptor subfamily is defined by the hydroxy-carboxylic acid structure of its endogenous ligands.

## Pharmacology

The discovery of GPR109A as a receptor for the antidyslipidemic drug nicotinic acid has included this receptor and its relatives GPR109B and GPR81 as part of many pharmaceutical research programs, and many new synthetic ligands with activity on this receptor family have been developed. In addition to nicotinic acid, acipimox, a drug with pharmacological effects similar to those of nicotinic acid as well as monomethylfumarate, an antipsoriatic drug, and a variety of newly synthesized compounds have been shown to be selective GPR109A agonists [12–14]. Several groups have developed partial agonists for GPR109A such as derivatives of pyrazole-3-carboxylic acid or cyclopentapyrazole (MK-0354) [15–17]. Recently,

### Glossary

**3-Hydroxy octanoic acid**: an intermediate of the β-oxidation of fatty acids. Its plasma level is elevated under conditions of increased β-oxidation rates.
**β-Hydroxy-butyrate (3-hydroxy-butyrate)**: together with acetone and acetoacetate, β-hydroxy-butyrate is referred to as a ketone body and is produced by the liver from acetyl-CoA during conditions of increased β-oxidation such as fasting.
**Lipolysis**: the hydrolysis of triglycerides by lipases in order to release free fatty acids and glycerol. It is regulated by hormones and metabolites, and its net rate is increased, for example during fasting.
**Nicotinic acid**: together with nicotinamide, nicotinic acid is a precursor of the cofactors nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) and belongs to the vitamin B complex. Nicotinic acid, but not nicotinamide, has pharmacological effects which are in part mediated by the G-protein-coupled-receptor GPR109A.
**β-Oxidation**: the process of enzymatic oxidative degradation of fatty acids in which two-carbon units are sequentially removed from the substrate to finally generate acetyl-CoA.
**Orphan G-protein-coupled-receptors (GPCRs)**: GPCRs for which endogenous ligands have not yet been identified.
**Receptor deorphanization**: identification of an endogenous ligand of an orphan receptor.

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0165-6147/$ – see front matter  doi:10.1016/j.tips.2009.09.001

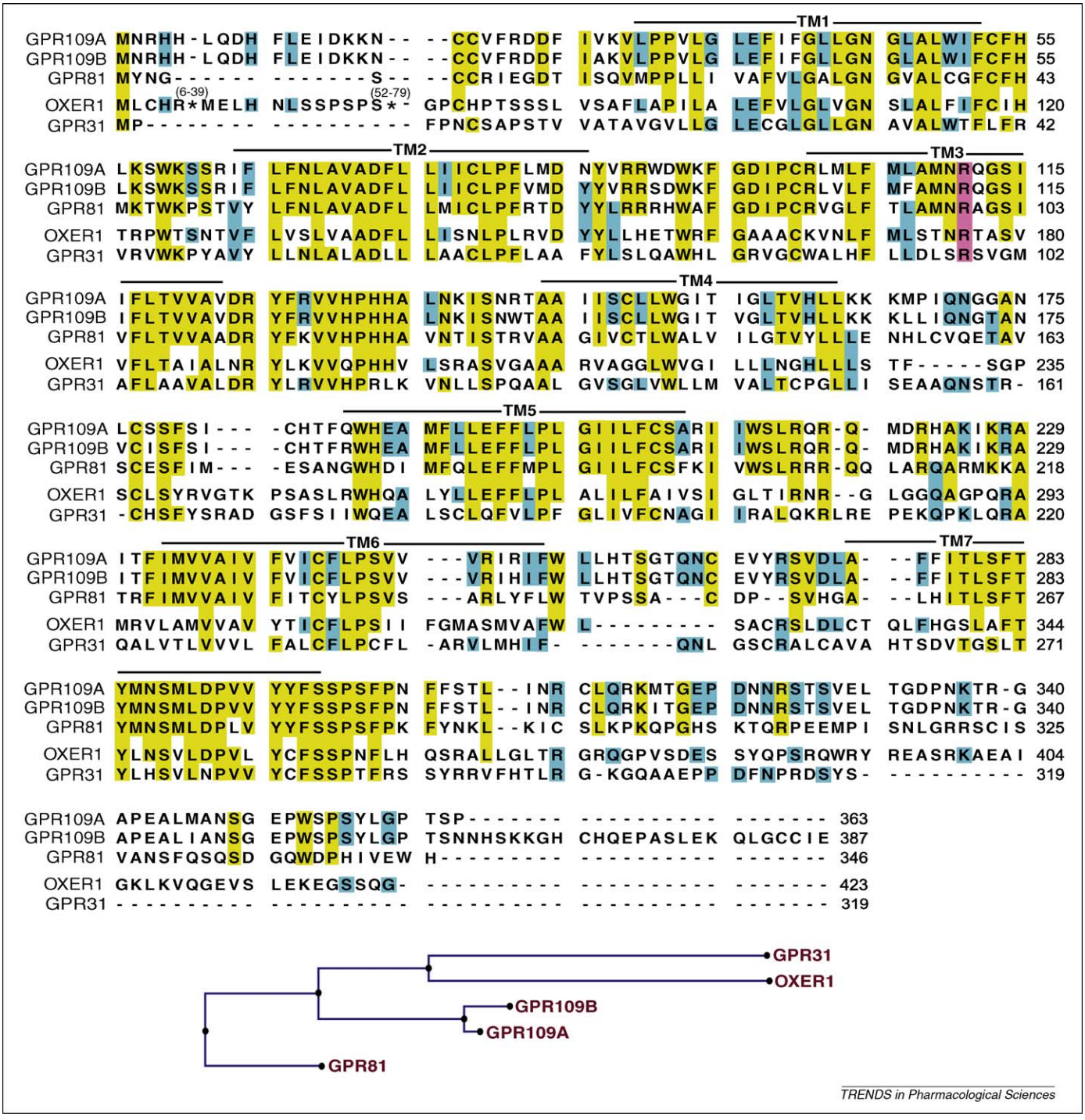
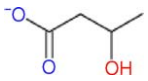
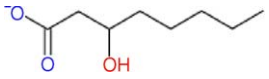
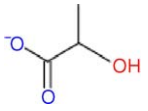



**Figure 1**. Alignment and phylogenetic tree based on the primary structure of GPR81, GPR109A, GPR109B as well as of the related receptors GPR31 and OXER1. Highlighted in yellow are residues which are identical in GPR109A, GPR109B and GPR81. Residues not identical in GPR109A, GPR109B and GPR81 but identical in at least three of the five receptors are marked in blue. An arginine residue in transmembrane helix 3, which has been proposed to bind the carboxylic group of GPR109A ligands is shown in magenta. Position of transmembrane helices 1–7 are indicated (TM1–TM7). Two short sequences in the N-terminal portion of OXER1 (residues 6–39 and 52–80) have been omitted to facilitate the alignment (gaps are indicated by stars).

allosteric agonists of GPR109A have been generated [18]. Also, selective agonists for GPR109B have been described such as 1- and 2-substituted benzotriazole-5-carboxylic acids, 6-amino nicotinic acids and benzoic acid derivatives as well as 5-*N*,*N*-disubstituted 5-amino-pyrazole-3-carboxylic acids [19–21]. It has recently been shown that aromatic D-amino acids are specific agonists of GPR109B [22]. Given the extreme rare occurrence of D-amino acids, it is unclear whether the ability of aromatic D-amino acids to activate GPR109B is of physiological significance. There are also synthetic ligands which are able to activate both GPR109A and GPR109B [6,23].

Despite the rapid development of synthetic ligands, the endogenous ligands of GPR109A, GPR109B and GPR81 remained elusive until recently. This is probably the result of their relatively low potencies and their metabolite character (Table 1). Traditionally, agonists of GPCRs were believed to belong to the group of hormones, mediators

**Table 1. $EC_{50}$ values (μM) and structures of endogenous hydroxy-carboxylic acids acting on GPR109A, GPR109B and GPR81**

| | Structure | GPR109A | GPR109B | GPR81 | Reference |
|---|---|---|---|---|---|
| 3-hydroxy-butyrate |  | 770 | >25,000 | Inactive | [8] |
| 3-hydroxy-octanoate |  | Inactive | 8 | Inactive | [11] |
| 2-hydroxy-propanoate (lactate) |  | Inactive | Inactive | 1500, 3000 | [9,10] |

$EC_{50}$ values are determined by ligand-induced GTPγS binding.

or transmitters, whereas only recently it has become clear that metabolites also use GPCRs to regulate body functions. In this regard, the hydroxy-carboxylic acid receptor subfamily of GPCRs shares similarities with the free fatty acid receptor family or with receptors for citric acid cycle intermediates [24,25].

A striking similarity between the natural ligands of GPR109A, GPR109B and GPR81 is their carboxylic acid function as well as the presence of a hydroxy group in the 2- or 3-position. Thus, this GPCR subfamily can be regarded as a family of hydroxy-carboxylic acid receptors. 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 GPR109A, GPR109B and GPR81 (Figure 1) [26,27]. Interestingly, the arginine residue in transmembrane helix 3, which has been suggested to serve as an anchor point of the carboxylic group of GPR109A ligands [28] is not only conserved among GPR109A, GPR109B and GPR81 but also in GPR31 and the 5-oxo-ETE receptor (Figure 1). OXER1 binds 5-oxo-ETE as well as, with lesser affinity, 5-hydroxy-eicosatetraenoic acid and 5-hydroperoxy-eicosatetraenoic acid (5-HpETE) [29]. Thus, OXER1, a receptor for a polyunsaturated fatty acid with an oxo, hydroxy or hydroperoxy substitution in the 5-position might well be regarded as another member of the hydroxy-carboxylic acid receptor family.

## Tissue distribution and signaling

GPR109A, GPR109B and GPR81 are predominantly expressed in adipocytes of both white and brown adipose tissue [5,6,10]. The expression of the lactate receptor GPR81 appears to be restricted to adipose tissue with only minor amounts of mRNA also found in kidney, skeletal muscle and liver [6,10,30]. In contrast, both expression and functional data clearly indicate that GPR109A is also expressed in various immune cells including monocytes, macrophages, neutrophils, dendritic cells and epidermal Langerhans cells [3,31,32]. In addition, expression of GPR109A has also been reported in retinal pigment epithelium as well as in the intestinal epithelium [33,34]. GPR109B appears to share with GPR109A its expression in adipose tissue as well as in various immune cells [4,6,11,22,35].

Analysis of the signaling mechanisms induced by the natural agonists of GPR109A, GPR109B and GPR81 showed that they are pertussis toxin-sensitive, indicating that this receptor family couples to $G_i$-type G proteins [4–6,9,10,22,30]. In most cells, activation of $G_i$-type G proteins results in an inhibition of adenylyl cyclase. In some cells, especially those of the immune system, $G_i$-activation via Gβγ-subunits also leads to an activation of β-isoforms of phospholipase C.

## Physiological and pathophysiological roles

The coupling of GPR109A, GPR109B and GPR81 to $G_i$ and their predominant expression in adipose cells suggests that these receptors mediate an inhibition of adipocyte adenylyl cyclase resulting in an antilipolytic effect. Indeed, 3-hydroxy-butyrate, 3-hydroxy-octanoate as well as 2-hydroxy-propanoate (lactate) have been shown to inhibit lipolysis at concentrations sufficient to activate their respective GPCRs [8,10,11]. In the case of 3-hydroxy-butyrate and lactate, studies in mice lacking GPR109A or GPR81 have confirmed the involvement of these receptors in the antilipolytic effects of their ligands [5,9,10]. The activation of GPR109A and GPR109B on adipocytes by their endogenous ligand requires millimolar concentrations of 3-hydroxy-butyrate and micromolar concentrations of 3-hydroxy-octanoate. These concentrations are typically reached under conditions of increased free fatty acid oxidation and subsequent ketone body formation as it occurs during fasting, diabetic ketoacidosis, in mitochondrial fatty acid β-oxidation disorders or under the influence of a ketogenic diet [11,36–38]. Under these conditions, activation of GPR109A and GPR109B would inhibit free fatty acid release from adipocytes thereby providing a negative feedback mechanism to counterbalance prolipolytic stimuli in situations of strongly increased lipolysis and fatty acid oxidation to avoid excessive degradation of triglycerides and thereby a waste of energy (Figure 2).

The physiological role of lactate-induced antilipolytic effects is less clear. It has been speculated that GPR81 mediates the inhibition of lipolysis during intensive exercise, a condition which results in high systemic lactate plasma concentrations [9,10]. Owing to oxygen shortage resulting from intensive exercise, skeletal muscle cannot utilize free fatty acids as an energy substrate. Therefore, an inhibition of lipolysis mediated by lactic acid and GPR81 would be a plausible mechanism. However, the concept of a link between elevated lactate plasma levels and a decreased fatty acid formation during anaerobic

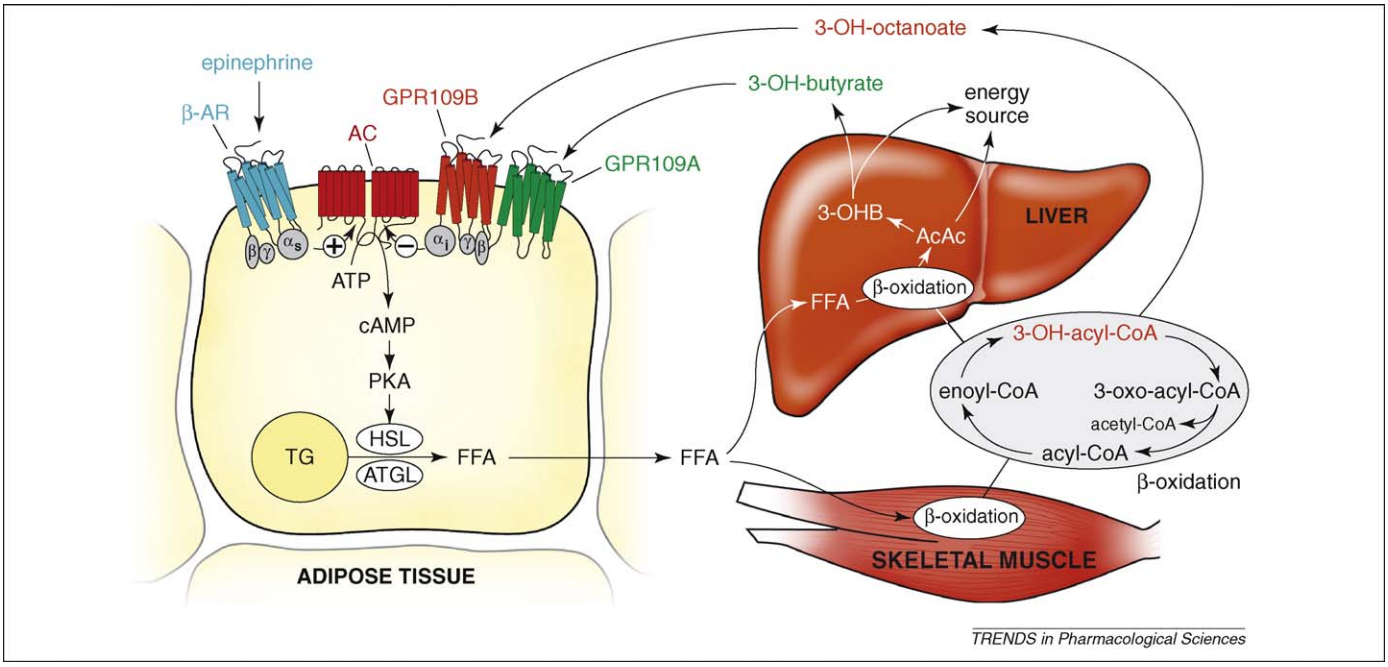



**Figure 2**. Model of the physiological function of GPR109A and GPR109B. During fasting or periods of increased energy demand such as exercise, the net rate of lipolysis is increased, and free fatty acids generated via lipolysis undergo β-oxidation in the muscle and liver to directly serve as a source of energy or as a precursor for ketone bodies. Ketone bodies such as 3-hydroxy-butyrate reach millimolar concentrations in the plasma during fasting. Increased β-oxidation rates are accompanied by micromolar plasma concentrations of some of the β-oxidation intermediates, in particular 3-hydroxy-octanoate. These plasma concentrations of 3-hydroxy-butyrate and 3-hydroxy-octanoate are sufficient to activate their respective receptors GPR109A and GPR109B which then mediate a negative feedback mechanism by inducing an inhibition of lipolysis through the inhibition of adenylyl cyclase. AC, adenylyl cyclase; β-AR, β-adrenergic receptor; PKA, protein kinase A; TG, triglyceride; HSL, hormone sensitive lipase; ATGL, adipocyte triglyceride lipase; FFA, free fatty acids; 3-OHB, 3-hydroxy-butyrate; AcAc, acetoacetate.

exercise has been somewhat controversial [39,40]. In light of the very specific expression of GPR81 in adipocytes, it is intriguing that adipocytes can produce and release considerable amounts of lactate [41]. Thus lactate may function in an autocrine manner to regulate triglyceride storage in adipocytes via GPR81.

The striking expression of GPR109A and GPR109B in various immune cells raises the question which physiological or pathophysiological role these receptors have in leukocytes. Although the expression of GPR109A in immune cells of the skin is responsible for the nicotinic acid-induced flushing phenomenon and potentially also for the beneficial effects of monomethylfumarate in the treatment of psoriasis [7,14,42], the physiological role of the receptors in immune cells is unclear. It is conceivable that GPR109A and GPR109B mediate the effect of an unknown epidermal mediator, which via activation of Langerhans cells and subsequent release of prostanoids induces under certain conditions a localized erythema and other inflammatory symptoms thereby regulating immunological responses of the skin as well as local dermal blood flow.

GPR109A is also activated by high concentrations of butyrate which is produced in large quantities by bacterial fermentation of dietary fibers in the colon. Recent data show that GPR109A is present in the apical membrane of intestinal epithelial cells and that it might function as a tumor suppressor and an anti-inflammatory receptor in the large intestine by mediating effects of butyrate [34].

## Therapeutic potential

The nicotinic acid receptor GPR109A is an established drug target, although it is not yet completely clear whether the receptor alone is responsible for the wanted antidyslipidemic effects of nicotinic acid [27,43]. Nevertheless, the major unwanted effect of nicotinic acid, the flushing reaction, is mediated by GPR109A [7]. The involvement of GPR109A in wanted and unwanted effects of nicotinic acid makes it difficult to develop drugs based on GPR109A agonism which have an improved ratio of wanted vs. unwanted effects. However, evidence has been provided that unwanted effects of nicotinic acid are mediated by the activation of G protein-independent pathways, whereas antilipolytic effects are mediated by $G_i$ [15,44]. Thus, differences in the post-receptor mechanisms mediating wanted and unwanted effects might be exploited to improve the pharmacological profile of drugs acting through GPR109A. In this regard, it is interesting that some partial agonists of GPR109A have full antilipolytic activity, whereas their ability to induce flushing appears to be reduced when compared with nicotinic acid [15,16]. Given that GPR109B has an expression pattern very similar to GPR109A, it is unlikely that selective agonists of GPR109B have a better profile than GPR109A agonists. Provided that the induction of antilipolytic effects via $G_i$-coupled receptors on adipocytes is the primary mechanism which mediates the beneficial effects of nicotinic acid, the receptor GPR81 might be an interesting drug target as its expression is restricted to adipose tissue. In this case, synthetic agonists of GPR81 are expected to lack unwanted effects of nicotinic acid such as the flushing response while preserving wanted effects mediated by their antilipolytic effects on adipocytes. Recently, monomethylfumarate, an established drug for the treatment of psoriasis, has been shown to be an agonist of GPR109A [14]. Because GPR109A is expressed in epidermal Langerhans cells, GPR109A will probably mediate the antipsoriatic effects

of monomethylfumarate. Given the broad expression of GPR109A and GPR109B in various immune and inflammatory cells, it will certainly be interesting to explore the potential of GPR109A and GPR109B agonists to treat immune disorders of the skin but also of other organs.

## Conclusion and future perspectives

The nicotinic acid receptor GPR109A and its close relatives GPR109B and GPR81 are primarily expressed in adipocytes and are coupled to $G_i$-type G proteins. Recently, the ketone body β-hydroxy-butyrate, the β-oxidation intermediate 3-hydroxy octanoic acid and lactate have been identified as endogenous ligands of these receptors. These ligands have in common that they are hydroxylated carboxylic acids for which plasma levels are subject to considerable changes depending on the metabolic state of the organism. Under various physiological and pathophysiological conditions, their plasma concentrations reach levels sufficient to activate the receptors and to inhibit lipolysis. Thereby, the receptors function as metabolic sensors to fine-tune the regulation of lipolytic activity in adipose tissue. In the future it will be interesting to explore the potential role of these receptors in metabolic disorders such as diabetes mellitus, dyslipidemia and obesity. In this regard, it will be important to search for other endogenous metabolic ligands of these receptors which might have remained elusive owing to their low concentration in body fluids. As GPR109A is the receptor for the established antidyslipidemic drug nicotinic acid, GPR109B and in particular GPR81 represent promising drug targets which can have advantages compared to GPR109A. The further development of subtype-specific and more potent ligands of this receptor family would be an important prerequisite to further evaluate these receptors as potential targets for new therapeutic strategies.

### Acknowledgement

The authors own work was supported by the German Research Foundation (DFG).

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