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# Overexpressing cell systems are a competitive option to primary adipocytes when predicting in vivo potency of dual GPR81/GPR109A agonists

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ABSTRACT

Mathematical models predicting in vivo pharmacodynamic effects from in vitro data can accelerate drug discovery, and reduce costs and animal use. However, data integration and modeling is non-trivial when more than one drug-target receptor is involved in the biological response. We modeled the inhibition of non-esterified fatty acid release by dual G-protein-coupled receptor 81/109A (GPR81/GPR109A) agonists in vivo in the rat, to estimate the in vivo $EC_{50}$ values for 12 different compounds. We subsequently predicted those potency estimates using $EC_{50}$ values obtained from concentration-response data in isolated primary adipocytes and cell systems overexpressing GPR81 or GPR109A in vitro. A simple linear regression model based on data from primary adipocytes predicted the in vivo $EC_{50}$ better than simple linear regression models based on in vitro data from either of the cell systems. Three models combining the data from the overexpressing cell systems were also evaluated: two piecewise linear models defining logical OR- and AND-circuits, and a multivariate linear regression model. All three models performed better than the simple linear regression model based on data from primary adipocytes. The OR-model was favored since it is likely that activation of either GPR81 *or* GPR109A is sufficient to deactivate the cAMP pathway, and thereby inhibit non-esterified fatty acid release. The OR-model was also able to predict the in vivo selectivity between the two receptors. Finally, the OR-model was used to predict the in vivo potency of 1651 new compounds. This work suggests that data from the overexpressing cell systems are sufficient to predict in vivo potency of GPR81/GPR109A agonists, an approach contributing to faster and leaner drug discovery.

## 1. Introduction

Elevated plasma and tissue concentrations of non-esterified fatty acids (NEFA) and triglycerides are often observed in patients with type 2 diabetes mellitus (T2DM) (DeFronzo, 2004; Paolisso et al., 1995; Reaven and Greenfield, 1981) and are strongly associated with insulin resistance (Boden, 1997; Groop et al., 1989; Groop et al., 1991). Reduction of NEFA concentration in the circulation improves insulin sensitivity and β-cell function, thereby reducing the risk for diabetes (Bajaj et al., 2004; Bergman and Ader, 2000; Dobbins et al., 2013; Fulcher et al., 1992; Kumar et al., 1994; Randle et al., 1963; Worm et al., 1994).

Suppression of lipolysis in the adipocytes by nicotinic acid or its analogues acutely improves glucose handling and insulin sensitivity in subjects with T2DM (Bajaj et al., 2004; Dobbins et al., 2013; Fulcher et al., 1992; Kumar et al., 1994; Liang et al., 2013; Santomauro et al., 1999; Worm et al., 1994). This effect is mediated by the G-protein-coupled receptor 109A (GPR109A, also called HCAR2 (hydroxycarboxylic acid receptor 2), Fig. 1) (Soga et al., 2003; Tunaru et al., 2003; Wise et al., 2003). However, GPR109A activation is associated with cutaneous vasodilatation manifested as flushing, due to release of vasodilatory prostanoids from epidermal Langerhans cells (Benyó et al., 2006; Benyó et al., 2005; Kamanna et al., 2009). Furthermore, the mechanism by which GPR109A reduces lipolysis also induces counter-regulatory processes manifested as tolerance development and rebound (Ahlström et al., 2011; Blackard and Heidingsfelder, 1969; Dobbins et al., 2015; Oh et al., 2011; Pereira, 1967; Worm et al., 1994). Thus, development of therapeutic agents that maintain the anti-lipolytic effect of GPR109A activation, but are not associated with the undesired effects of GPR109A, could significantly improve the treatment of T2DM.

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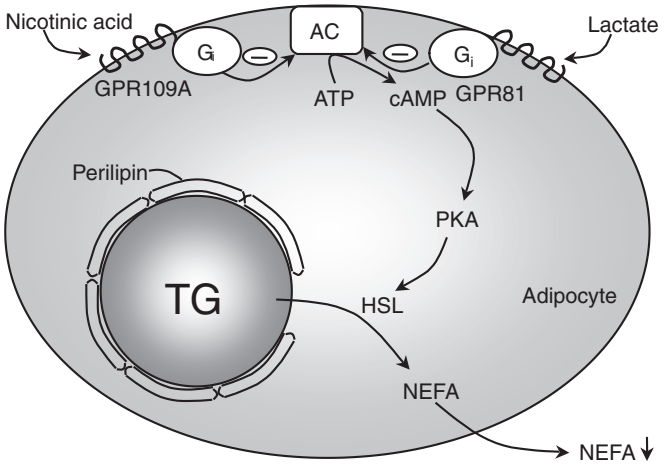


**Fig. 1.** Mechanism of GPR81- and GPR109A-mediated lipolysis in adipocytes. Nicotinic acid and lactate are endogenous ligands to the G-protein-coupled receptors GPR81 and GPR109A, respectively. Activation of GPR81 and GPR109A results in inhibition of adenylyl cyclase (AC) activity, leading to decreased formation of cyclic AMP (cAMP) from ATP. The reduction in cAMP results in less activation of protein kinase A (PKA) and in turn less phosphorylation of perilipin and hormone-sensitive lipase (HSL). Phosphorylation of perilipin enables HSL to hydrolyse triglycerides (TG) into non-esterified fatty acids (NEFA) and glycerol. The inhibition of lipolysis via activation of GPR81 or GPR109A results in decreased circulating NEFA levels.

Similar to GPR109A, activation of G-protein-coupled receptor 81 (GPR81, also called HCAR1 (hydroxycarboxylic acid receptor 1)) by lactate suppresses lipolysis (Fig. 1), suggesting GPR81 to be a potential drug target for treating T2DM (Boyd Iii et al., 1974; Cai et al., 2008; Gold et al., 1963; Houghton et al., 1971; Liu et al., 2009). GPR81 is primarily expressed in adipose tissue with no evidence of expression in epidermal Langerhans cells (Ge et al., 2008; Wise et al., 2003), indicating that a GPR81 agonist would not confer the flush associated with GPR109A. Indeed, selective GPR81 agonists indicating separation between lipolysis suppression and flush have recently been reported (Sakurai et al., 2014). GPR81 is 52% identical to GPR109A at the amino-acid level and belongs to the same subfamily of receptors as GPR109A and GPR109B (Ahmed et al., 2009; Blad et al., 2011). The high degree of receptor sequence identity suggests that compounds may have dual activity, and GPR109A agonism needs to be monitored during development.Partial agonism of GPR109A has been shown to be a way to avoid flush (Lai et al., 2008), but anti-lipolytic effects still seem to be transient.

An in vitro-in vivo correlation (IVIVC) between receptor potency and in vivo effect is fundamental to efficiently design new compounds in a chemical series by in vitro screening (Yamaguchi et al., 2013; Yu et al., 2006). A sufficiently good IVIVC confers reductions in cost and number of in vivo experiments. When dealing with multiple-target in vivo pharmacology where the targets are studied one at a time in vitro, the relationship between in vitro and in vivo effects may become difficult to discern. To some degree, this may be overcome by complex in vitro or ex vivo methods that increase confidence in the in vivo effect of the compounds before proceeding to in vivo experiments, but at the expense of being more resource-intensive.

In this study, we explore different mathematical models for describing the IVIVC of a preclinical dataset of 12 different dual GPR109A/GPR81 agonists. The objective is to establish a predictive model of in vivo lipolysis suppression in the rat based on in vitro potency data. First, a nonlinear mixed effects pharmacokinetic and pharmacodynamic (PKPD) model was applied to a longitudinal data set of rat in vivo lipolysis (Fig. 2, path 1). From that model, in vivo $EC_{50}$ values of NEFA inhibition were derived. These estimates subsequently served as the targets for a number of different in vitro-in vivo prediction models. Either primary adipocyte lipolysis potency data (Fig. 2, path 2), or in vitro cell assay potency data for GPR81 (Fig. 2, path 3) or GPR109A (Fig. 2, path 4) were used to fit simple linear regression models of in vivo $EC_{50}$. The in vitro potency data were acquired from overexpressing cell systems, i.e., cells that artificially express high levels of either GPR81 or GPR109A. The assay works by measuring decreases in cAMP, which occurs when GPR81 or GPR109A become activated and inhibits the production of cAMP via their effect on adenylyl cyclase (see Fig. 1). We also compared three simple methods of combining the in vitro cell assay data. Current biological understanding suggests that GPR81 and GPR109A function as metabolic sensors activated by different endogenous mediators, and that activation of either GPR81 or GPR109A is likely sufficient to deactivate the cAMP pathway (Cai et al., 2008; Ahmed, 2011). Based on this, a logical-circuit OR-model was our primary candidate (Fig. 2, path 5a). To challenge this model we also considered a logical-circuit AND-model (Fig. 2, path 5b), and a multivariate linear regression model (Fig. 2, path 5c).

We demonstrate that combining the in vitro data from the two cell assays improves prediction of in vivo effects for all three models, to the same level or better than using primary adipocyte data. Among these models the OR-model is most suitable for future in vitro screening due to its mechanistically inspired structure. Not only does this model provide adequate predictions of in vivo $EC_{50}$ but it also predicts the potential for a compound to selectively target GPR81. Finally, as an illustration of how to apply the OR-model in practice, we used it to predict in vivo potencies for 1651 other compounds whose potencies so far only have been determined in the two in vitro cell assays.

## 2. Methods

### 2.1. Experimental Methods

#### 2.1.1. Compounds

The compounds used in the studies were nicotinic acid, MK-0354, 3-chloro-5-hydroxy-benzoic acid (3-chloro-5-hydroxy-BA), SCH-900271 and AstraZeneca-synthesized compounds from three different chemical series. For in vitro tests, the compounds were solved in DMSO, while in vivo, compounds were formulated in saline or saline containing 5% DMA, 5% mannitol or 18% PEG400 depending on solubility.

#### 2.1.2. In vivo Rat Lipolysis in Anaesthetized Rats

All experimental methods were approved by the Local Ethics Review Committee on Animal Experiments (Göteborg Region, Sweden). In the afternoon of the day preceding the experiment male Wistar rats (Harlan, Netherlands) were moved to clean cages with free access to water but no food. On the experiment day the rats were initially anaesthetized with isoflurane followed by an intraperitoneal injection of Inactin® (135 mg/kg). The rats were tracheotomized (PE240) to secure spontaneous breathing. Catheters were inserted in the carotid artery or femoral artery for blood pressure measurement and blood sampling (PE50 or PE50/PE25), and in the jugular vein for infusion of compound (PE50). To prevent clotting of the arterial catheter it was flushed with a continuous infusion of saline/Na-citrate (0.02 M, 6 μL/min). After the preparation, the animals were left for 120 min before administration of compound or vehicle.

Twelve different compounds were administered intravenously and repeated blood samples were collected during infusion and washout to analyse NEFA and compound concentrations. The majority of experiments were run using a screening study design with escalating consecutive infusions, followed by a single long infusion at an efficacious exposure. For further description of the method see (Wallenius et al., 2017).

#### 2.1.3. Bioanalysis

Compounds were analysed using reversed-phase high-pressure liquid chromatography with rapid gradient elution. Mass spectrometry detection was performed on a triple quadropole instrument with

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