Original article

MOLECULAR METABOLISM

GPR40 (FFAR1) – Combined Gs and Gq signaling *in vitro* is associated with robust incretin secretagogue action *ex vivo* and *in vivo*

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ABSTRACT

Objectives: GPR40 (FFAR1), a clinically proven anti-diabetes target, is a Gq-coupled receptor for long chain fatty acids (LCFA) stimulating insulin secretion directly and mediating a major part of the dietary triglyceride-induced secretion of the incretins GLP-1 and GIP. In phase-II studies the GPR40 agonist TAK-875 decreased blood glucose but surprisingly without stimulating incretins.

Methods and results: Here we find that GPR40 can signal through not only Gq and IP3 but also Gs and cAMP when stimulated with certain agonists such as AM-1638 and AM-5262 in contrast to the endogenous LCFA ligands and agonists such as TAK-875 and AM-837, which only signal through Gq. In competition binding against [3H]AM-1638 and [3H]L358 the Gq + Gs and the Gq-only agonists either competed for or showed positive cooperativity by increasing the binding of the two different radio-ligands, in opposite ways. Nevertheless, both the Gq-only and the Gq + Gs agonists all docked surprisingly well into the binding site for TAK-875 in the X-ray structure of GPR40. In murine intestinal primary cell-cultures the endogenous LCFAs and the Gq-only agonists stimulated GLP-1 secretion with rather poor efficacy as compared with the high efficacy Gq + Gs GPR40 agonists and a prototype GPR119 agonist. Similarly, in fasting both male and female mice the Gq + Gs agonists showed significantly higher efficacy than the Gq-only agonists in respect of increasing plasma GLP-1 and plasma GIP in a GPR40-dependent manner.

Conclusions: It is concluded that stimulation of GPR40 by endogenous LCFAs or by Gq-only synthetic agonists result in a rather limited incretin response, whereas Gq + Gs GPR40 agonists stimulate incretin secretion robustly.

Keywords G protein-coupled receptor; Glucagon like peptide 1 (GLP-1); Biased signaling; Ago-allosteric agonist; Long chain fatty acids (LCFAs)

1. INTRODUCTION

GLP-1 mimetics, such as exenatide and liraglutide have over the last years become widely used in the treatment of type 2 diabetes. The success of GLP-1 mimetics is based on the fact that they function not only as incretins, i.e. compounds which stimulate insulin secretion in a glucose dependent manner, but that they also suppress glucagon secretion, delay gastric emptying, decrease appetite and promote β-cell survival [1,2].

However GLP-1 itself is just one out of a handful of gut hormones with similar beneficial metabolic properties, which all are released in response to food ingestion from closely related enteroendocrine cells scattered along the small and large intestine [3,4]. The co-released gut hormones act in symphony and in several cases even synergistically [5]. Thus, it could be tempting to try to develop compounds, which stimulate the secretion of the powerful mixture of endogenous gut hormones [6,7]; compounds, which may not even enter the body as such, but act locally in the gut [6].

Metabolites of dietary triglycerides, i.e. free fatty acids (FFA) and 2-monoacyl glycerol (2-MAG) are among the most efficacious gut hormone secretagogues [8,9] conceivably acting through the G protein-coupled receptors GPR40 [10–14] and GPR119 [9,15], respectively. Since these receptors are highly expressed on β-cells of the endocrine pancreas, they rapidly became high priority drug discovery targets for new anti-diabetes agents. A number of GPR119 agonists reached phase II [9,16], but, although the compounds stimulated GLP-1 and insulin secretion in preclinical models they apparently did not deliver the required clinical efficacy perhaps due to their ability to also stimulate the secretion of the counter regulatory hormone glucagon [9].

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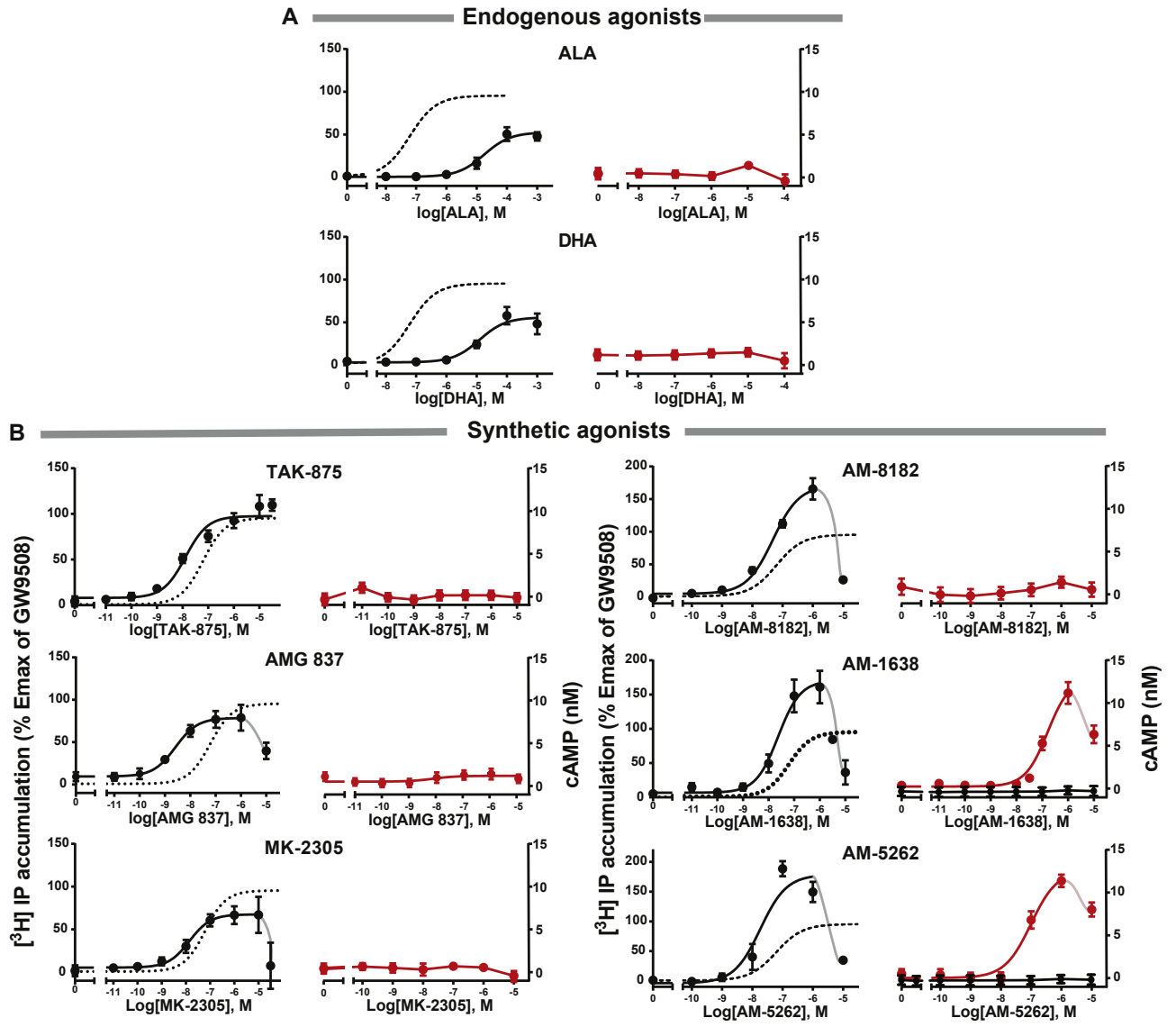

Figure 1: ***In vitro*** **signaling properties of GPR40 agonists in transiently transfected COS7 cells**. Effects on IP-turnover (black curves, left panels using GW9508 as positive control – stippled line) and cAMP accumulation (red curves, right panels) were determined for: (A) Endogenous lipid agonists α-linolenic acid (ALA) and docosahexaenoic acid (DHA); (B) synthetic agonists TAK-875, AMG 837, MK-2305, AM-8182, AM-1638 and AM-5262. Black curves in the cAMP accumulation panels for AM-1638 and AM-5262 represent empty vector control. Data are normalized to empty vector (0%) and Emax for GW9508 (100%) in IP-turnover. Concentrations of cAMP were interpolated based on cAMP standard curves for each experiment. Data represents mean ± SEM and represents a minimum of three experiments.

GPR40 was originally deorphanized as a receptor for medium to long chain fatty acids being almost exclusively expressed in the β-cells and potentially responsible for the glucose-dependent stimulation of insulin secretion by long chain fatty acids (LCFA) [10–12,17]. However, the notion that GPR40 potentially was involved in lipotoxicity made it unclear whether you wanted agonists or antagonists and whether GPR40 would at all be a good drug target [17,18]. However, transgenic overexpression of GPR40 and administration of selective agonists later demonstrated that GPR40 activation augments insulin secretion and improves glucose tolerance and that GPR40 may even protect islets from the toxic effect of fatty acids [19–21]. Importantly, GPR40 was shown to be highly expressed and enriched also on enteroendocrine cells and to mediate GLP-1 and GIP secretion [22,23].

In respect of clinical development Takeda has pioneered the field with the GPR40 agonist TAK-875 or fasiglifam (Figure 2A), which in phase-II studies decreased HbA1c as efficiently as sulfonylurea without signs of hypoglycemia [24]. This was obtained with surprisingly little effect on systemic insulin and no effect on plasma GLP-1 [25,26]. Recently the TAK-875 program was, however stopped in phase-III due to liver toxicity,[5] which probably is not GPR40-mediated as the receptor is not expressed in the liver. A new series of GPR40 agonists were by the Amgen group shown to be positive ago-allosteric modulators [27–29], i.e. compounds which both act as agonists and as allosteric modulators increasing the potency and/or the efficacy of other agonists, for example the endogenous agonists [30]. Interestingly, this new class of ago-allosteric modulators improved glucose tolerance not only through direct stimulation of insulin secretion but also through stimulation of GLP-1 [28,29,31].

In the present study we compare a series of GPR40 agonists in respect of their signal transduction and binding properties and their ability to

[5] Takeda press release, December 27, 2013; http://www.takeda.com/news/2013/20131227_6117.html.

