Original article

MOLECULAR METABOLISM

Incretin-like effects of small molecule trace amine-associated receptor 1 agonists

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ABSTRACT

Objective: Type 2 diabetes and obesity are emerging pandemics in the 21st century creating worldwide urgency for the development of novel and safe therapies. We investigated trace amine-associated receptor 1 (TAAR1) as a novel target contributing to the control of glucose homeostasis and body weight.

Methods: We investigated the peripheral human tissue distribution of TAAR1 by immunohistochemistry and tested the effect of a small molecule TAAR1 agonist on insulin secretion *in vitro* using INS1E cells and human islets and on glucose tolerance in C57Bl6, and db/db mice. Body weight effects were investigated in obese DIO mice.

Results: TAAR1 activation by a selective small molecule agonist increased glucose-dependent insulin secretion in INS1E cells and human islets and elevated plasma PYY and GLP-1 levels in mice. In diabetic *db/db* mice, the TAAR1 agonist normalized glucose excursion during an oral glucose tolerance test. Sub-chronic treatment of diet-induced obese (DIO) mice with the TAAR1 agonist resulted in reduced food intake and body weight. Furthermore insulin sensitivity was improved and plasma triglyceride levels and liver triglyceride content were lower than in controls.

Conclusions: We have identified TAAR1 as a novel integrator of metabolic control, which acts on gastrointestinal and pancreatic islet hormone secretion. Thus TAAR1 qualifies as a novel and promising target for the treatment of type 2 diabetes and obesity.

Keywords Pancreatic β-cell; Insulin secretion; Type 2 diabetes; Incretin hormones; Obesity

1. INTRODUCTION

TAAR1 is a G protein-coupled receptor (GPCR) belonging to the trace amine-associated receptor family [1–3]. It was identified in 2001 as a receptor for endogenous trace amines, i.e. p-tyramine, β-phenylethylamine, octopamine and tryptamine, which are metabolites of amino acids with structural similarity to biogenic amines [4,5]. Upon activation, TAAR1 signals via Gαs proteins leading to increased intracellular cAMP levels [1,4–6]. *Taar1* knockout mice expressing the *LacZ* gene under control of the *Taar1* promoter (*Taar1*$^{-/-}$*/LacZ*) demonstrated TAAR1 expression in restricted areas of the brain, where it modulates monoaminergic neurotransmission [2,7]. Therefore, TAAR1 recently emerged as a novel target for the treatment of psychiatric disorders [7–9]. In the periphery, TAAR1 is expressed in the stomach, the duodenum and pancreatic β-cells in mice [7,10,11]. In particular, in mouse islets, TAAR1 has been shown to be among the most highly expressed and enriched GPCRs as revealed by quantitative real time PCR analysis of 373 GPCRs [10]. However, little is known about the physiological effects of TAAR1 modulation in these tissues, which is attributed to the polypharmacology of trace amines and the hitherto lack of selective ligands for TAAR1. Recently, selective TAAR1 ligands have been described [8,9], allowing the exploration of the effects of specific TAAR1 activation in metabolic disease. We describe here for the first time that TAAR1 activation has beneficial effects on glucose control and body weight in animal models of type 2 diabetes and obesity.

2. METHODS

2.1. Generation of anti-human TAAR1-specific mouse monoclonal antibodies

An expression construct encoding Glutathione-S-Transferase (GST) fused in-frame to the N-terminal segment and all extracellular domains of human TAAR1 coupled with GSSG linkers was expressed in *Escherichia coli* [12]. Five mice were immunized by i.p. injection with recombinant protein. Animals were tail bled after two boosts, and sera

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Abbreviations

β gal	beta galactosidase
DIO	diet induced obesity
GI	gastrointestinal
GLP1	glucagon like peptide 1
GPCR	G-protein coupled receptor
ivGTT	intravenous glucose tolerance test
NASH	nonalcoholic steatohepatitis
oGTT	oral glucose tolerance test
PYY	peptide YY
TAAR1	trace amine associated receptor 1

were tested by ELISA on the immunogen to select the best candidate for hybridoma production. From the animals showing a specific immune response to human TAAR1, the spleens were removed and the cells were fused to Ag8 cells according to [13]. Positive hybridomas were selected by immunofluorescence and Western blotting using a recombinant human TAAR1 N-terminus/extracellular domain construct fused to His tag and expressed in HEK293 cells. Clonal purity was achieved in limited dilution conditions. Final selection of the anti-human TAAR1 (anti-hTAAR1) mAb was done by immunofluorescence on SF9 cells expressing full length human TAAR1 from baculovirus vector.

2.2. Gene expression analysis

mRNA purification from INS1E cells or human islets was performed with RNeasy Mini or Micro Kit (both Qiagen, Hombrechtikon, Switzerland) including an RNAse free DNAse I treatment according to the manufacturer's instructions. cDNA was synthesized using cDNA Synthesis System (Roche Applied Science, Rotkreuz, Switzerland). Quantitative real-time PCR assays (qRT-PCR) were performed using the QuantiFast SYBR Green PCR Kit and the Rotor-Gene 6000 (Qiagen, Hombrechtikon, Switzerland) with specific DNA primers. Analysis was done by the ΔCt threshold method to determine expression relative to GAPDH mRNA. Each analysis reaction was performed in duplicate, with two samples per condition.

2.3. Immunohistochemistry

Paraffin sections (4–5 μm) from adult normal human tissues (Asterand, Herts, UK; Cureline, San Francisco, USA), male C57BL/6J, or *Taar1*$^{-/-}$*/LacZ* mice tissues were incubated with following primary and secondary antibodies: mouse anti-hTAAR1 mAb (Roche clone 6/6); rabbit anti-chromogranin A and anti-peptide YY (Abcam, Cambridge, UK); guinea pig anti-swine insulin (Dako, Glostrup, Denmark); rabbit anti-GLP-1 (7–36) (Peninsula, San Carlos, USA), rabbit anti beta-galactosidase (MP Biomedicals Santa Ana, California, USA) and Alexa Fluor® 488- or 555-conjugated or peroxidase conjugated secondary antibodies (Invitrogen, Basel, Switzerland).

2.4. Insulin secretion

Experiments with INS1E cells were performed as described [14]. Experiments with transplantation-grade human islets (~80% purity, male donors 59–61 year old, BMI < 28) were performed using handpicked islets (10 islets/condition). Islets were starved for 2 h at 2.8 mM glucose, before insulin secretion was assessed by 1 h incubation with indicated glucose and compound concentrations. Insulin secretion was presented as % secreted insulin of total insulin content. Human islet experiments were approved by the University of Geneva ethics committee and were conducted in adherence to all relevant laws and ethical guidelines regulating the collection, transfer and use of human tissue.

2.5. Animals

All procedures were conducted in strict adherence to the Swiss federal ordinance on animal protection and welfare, according to the rules of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), and with the explicit approval of the local veterinary authority. Experiments using *Glp1r*$^{-/-}$ mice were performed following the approval and guidelines of the institutional animal care and use committees of the University of Cincinnati.

Male C57BL/6J and *db/db* mice (BKS. Cg-m$^{+/+}$ Leprdb/J) were purchased from Charles River Laboratories (Lyon, France). *Taar1*$^{-/-}$*/LacZ* mice are described elsewhere [2].

DIO mice were generated by placing C57BL/6J mice on SSNIFF diet (EF M D12492: 60% energy from fat, 21% from sugar) starting at 9 weeks of age at Charles River Laboratories (Lyon, France). The DIO mice were 39 weeks of age at the time of the experiment. *Glp1r*$^{-/-}$ mice were generated as previously described [15]. Animals were fed a HFD (Research Diets D12331: 58% energy from fat plus sucrose) starting at 8 weeks of age and maintained on a 12-light, 12-h dark cycle (lights on 6 am, lights off 6 pm).

2.6. Glucose tolerance tests

Animals were fasted for 10 h prior to glucose tolerance tests. For the oGTT, mice were treated per oral gavage with indicated doses of RO5166017 or vehicle (0.3% Tween 80 in water) 45 min prior to oral glucose challenge (2 g glucose/kg for C57BL/6J, *Taar1*$^{-/-}$/LacZ, DIO and *Glp1r*$^{-/-}$ mice and 1 g glucose/kg for db/db mice). For the ivGTT, mice were treated with RO5166017 (3 mg/kg s.c.), or vehicle (saline) 30 min prior to an i.v. glucose challenge (1 g glucose/kg). Blood was collected at indicated time points after glucose load.

Blood glucose levels were measured by tail vein sampling using a handheld glucometer (Accu-Chek Aviva, Roche) at indicated time points. Plasma insulin was determined by ELISA (Mercodia®, Uppsala Sweden), total GLP-1 by MSD mouse/rat total GLP-1 Assay kit (Mesoscale Discovery, USA) and total PYY with a rat/mouse radioimmunoassay kit (Millipore, MA. USA).

2.7. Gastric emptying

Gastric emptying was assessed using a carbohydrate-and protein-rich semisolid [16] and a liquid meal protocol. Overnight fasted animals were dosed p.o. with vehicle (0.3% Tween 80 in H_2O) or 0.3 mg/kg RO5166017 45 min prior to meal administration, or dosed s.c. with vehicle (saline) or propantheline at 30 min prior to meal. Gastric emptying rates were determined according to [16]. Liquid phase gastric emptying was determined as described [17] in overnight fasted animals treated with 0.3 mg/kg RO5166017 or vehicle (0.3% Tween 80 in H_2O) 45 min prior to oral glucose load containing 1% acetaminophen (Sigma Aldrich, Switzerland). Quantification of plasma acetaminophen levels was performed by LC-MS/MS.

2.8. Food intake and body weight studies

Taar1$^{-/-}$*/LacZ* mice or wild-type (wt) littermates were placed into an automated food monitoring system (TSE system©: TSE drinking and feeding monitor, TSE Systems GmbH, Bad Homburg, Germany). 10 h fasted animals were treated orally with 0.3 mg/kg RO5166017 or vehicle (0.3% Tween 80 in H_2O) 45 min prior to *ad libitum* food access.

