Biomedicine & Pharmacotherapy 98 (2018) 364–371

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Central administration of coagonist of GLP-1 and glucagon receptors improves dyslipidemia

Vishal Patel, Amit Joharapurkar*, Samadhan Kshirsagar, Brijesh Sutariya, Maulik Patel, Hiren Patel, Dheerendra Pandey, Dipam Patel, Rajesh Bahekar, Mukul Jain

Zydus Research Centre, Cadila Healthcare Limited, Sarkhej-Bavla N.H.No.8A, Moraiya, Ahmedabad, 382210, India

ARTICLE INFO

Keywords:
Coagonist of GLP-1/glucagon
Intracerebroventricular
Dyslipidemia
Hamsters

ABSTRACT

Coagonists of Glucagon-like peptide-1 (GLP-1) and glucagon receptors are under clinical investigation for treatment of obesity associated with diabetes. In addition to their role in glucose homeostasis, GLP-1 and glucagon modulate lipid metabolism. In this study, we have investigated the role of central GLP-1 receptor (GLP-1R) and glucagon receptor (GCGR) activation in regulation of lipid metabolism in cholesterol-fed hamsters. Hamsters were treated with coagonist alone (0.3 μg) or in combination with either GLP-1R antagonist (0.15 μg) or GCGR antagonist (0.3 μg) for 4 weeks by intracerebroventricular route (icv). A pair-fed control to coagonist was included in the experiment. In a separate experiment, vagotomized hamsters were treated with coagonist (0.3 μg) for four weeks. At the end of the treatment, plasma and hepatic lipids, bile homeostasis, and hepatic gene expression were determined. Coagonist treatment caused a reduction in plasma and liver lipids, and reduced triglyceride absorption from intestine. Also, hepatic triglyceride secretion, bile flow, and biliary cholesterol excretion were increased by the coagonist treatment. Coagonist treatment exhibited increased energy expenditure and reduced the expression of SREBP-1C, HMG-CoA reductase, SCD-1, FAS and ACC in liver. Increase in the expression of LDLR, ACOX1, CPT-1, PPAR-α, CYP7A1, ABCA1 and ABCB11 was also observed in liver. The effect of coagonist on lipids was partially blocked by either GLP-1R or GCGR antagonist. Coadministration of GLP-1R antagonist blocked the effect of coagonist on bile flow, while effect of coagonist on biliary cholesterol was blocked by co-administration of GCGR antagonist. Coagonist did not affect lipid metabolism in vagotomized hamsters. It appears that central administration of coagonist reduces dyslipidemia by activation of GLP-1R and GCGR, independent of its anorectic effect.

1. Introduction

GLP-1 R agonists are clinically used for the treatment of type 2 diabetes, and show modest weight loss [1,2]. Glucagon, another proglucagon-derived hormone, causes reduction in lipids and body weight in preclinical models. Combination of these two hormones was considered useful for treatment of obesity and type 2 diabetes, but the inherent risk of hyperglycemia in glucagon action hindered these efforts for a long time. However, recent discovery of coagonists of GLP-1 receptor (GLP-1R) and glucagon receptors (GCGR) has demonstrated that balanced coagonism of these two hormones can be a novel strategy for the treatment of type-2 diabetes associated with obesity, without the risk of hyperglycemia [3,4]. The proof of this concept was observed in clinical studies with oxyntomodulin (OXM), an endogenous coagonist of GLP-1R and GCGR, which can reduce obesity and diabetes, without hyperglycemia [5,6]. In addition to the effect on obesity and diabetes, coagonist treatment has been demonstrated to lower lipids in preclinical models of hyperlipidemia [4,7]. This effect was better than the effect of a single agonist (GLP-1 or GCGR) alone [7], and it was dependent on the activation of both GLP-1 and glucagon receptors [8].

GLP-1 and glucagon are gut derived hormones which are products of proglucagon gene. GLP-1R is mainly expressed in pancreas and brain [9], and major action of GLP-1 is considered to be through brain GLP-1R activation [1,10–12]. Glucagon mainly controls glucose metabolism, and glucagon receptor activation is linked to thermogenesis and increase in metabolism of lipids [13–15]. Similar to GLP-1R, GCGR is also abundantly expressed in brain [16]. Direct administration of glucagon in brain causes anorexia, hepatic glucose production, and increases metabolism of lipids [13,16]. Hence, it is suggested that central GLP-1R and GCGR can regulate feeding behavior, glucose and lipid metabolism.

Liver, intestine and adipose tissue, the primary regulators of lipid metabolism, are highly innervated by sympathetic and parasympathetic

* Corresponding author at: Department of Pharmacology & Toxicology, Zydus Research Centre, Cadila Healthcare Limited, Sarkhej-Bavla N.H.No.8A, Moraiya, Ahmedabad, India.
E-mail address: amitjoharapurkar@zyduscadila.com (A. Joharapurkar).

https://doi.org/10.1016/j.biopha.2017.12.068
Received 7 October 2017; Received in revised form 29 November 2017; Accepted 15 December 2017

nervous system [17]. Activation of sympathetic nerves in liver regulates triglyceride production [18,19], while sympathetic activation of adipose tissue increases lipolysis and thermogenesis [20]. Oxyntomodulin, a naturally occurring coagonist of GLP-1R and GCGR, suppresses appetite and increases energy expenditure when administered centrally [21]. We have previously observed that coagonist of GLP-1R and GCGR regulates lipid metabolism, by specific actions mediated through GLP-1 and glucagon receptors. These effects were found to be independent of the anorectic effect of the coagonist [8]. Whether these effects are mediated through central GLP-1R and GCGR remains to be investigated. In this study, we have investigated the effect of central administration of the GLP-1R/GCGR coagonist in hypercholesterolemic hamsters.

2. Materials and methods

2.1. Materials

Coagonist of glucagon and GLP-1 receptor, Aib2 C24 Chimera2 (H^1SQGT^{5}FTSDY^{10} SKYLD^{15} EQAAK^{20} EFIAW ^{25} LMNT-NH_2), GLP-1R antagonist (Exendin-9) and GCGR antagonist (DesHis1DesPhe6glucagonamide) were synthesized at Zydus Research Centre, Ahmedabad. Triglycerides, cholesterol, low-density lipoprotein (LDL), non-esterified fatty acid (NEFA) and glycerol were measured using kits purchased from RCFL (New Delhi, INDIA).

2.2. Animals

Male golden Syrian hamsters (6–8 weeks old) were obtained from the Animal Research Facility of Zydus Research Centre, Ahmedabad. The study protocol was reviewed and approved by Institutional Animal Ethics Committee (IAEC) of Zydus Research Centre (Ahmedabad, India), an AAALAC approved facility. Hamsters were fed on high fat high cholesterol diet (HFHC, 44% kcal fat and 0.5% cholesterol, D06050501; Research Diets, Inc., New Brunswick, NJ) or either chow diet (Teklad Global 18% Protein Rodent Diet-2018C, Harlan Laboratories, Inc, India).

2.3. Implantation of intracerebroventricular cannula

For intracerebroventricular (icv) injection, hamsters underwent stereotaxic surgery to place an indwelling guide cannula into third ventricle. Hamsters were anesthetized by pentobarbital (45 mg/kg, IP) and placed in stereotaxic apparatus. A permanent 7-mm guide cannula (23-gauge) was stereotaxically implanted into the third ventricle of hamsters (coordinates: AP-0 mm, ML-0 mm, DV-5 mm). It was secured to skull using stainless steel screws and dental cement, and closed with 30-gauge obturators. All animals were given analgesia (Meloxicam, 2mg/kg, SC) and antibiotic (teramycin, 60 mg/kg, IP), and were returned to home cage. Body weight and food intake were daily observed. Hamsters, which maintained their presurgery body weight, were used further in the study. Hamsters were fed on HFHC diet for two weeks to induce hypercholesterolemia. The location of the icv cannula in hamsters was verified by injecting 10 µL of 0.5% trypan blue dye at the end of the experiment. Brains were removed after 10–15 min of trypan dye injection and cut with a scalpel, and the spread of the dye within the ventricles was examined [22]. The animals with wrongly placed cannula were excluded from the study.

2.4. Repeated dose treatment in hypercholesterolemic hamsters

After 2 weeks of HFHC diet feeding, hamsters were randomized based on plasma cholesterol and triglyceride levels and assigned to a treatment group. HFHC diet was continued throughout the study treatment period. Animals were administered either vehicle (deionized water), or coagonist (0.3 µg/animal), or coagonist with either GLP-1R antagonist (0.15 µg/animal) or GCGR antagonist (0.3 µg/animal), by icv route (n = 12), once daily before onset of dark cycle, for 4 weeks. Dose volume was 10 µL. Control (chow-fed) animals were also administered vehicle once daily by icv route. Body weight and food intake were recorded daily before drug administration. Pair-fed controls were dosed with vehicle and given a daily food allotment equal to that consumed by a drug-treated counterpart over the previous 24 h period. After the treatment period was over, animals were randomized into two sets. One set was used for the estimation of bile flow and lipid content in bile, glycerol release from adipocytes, hepatic gene expression, and plasma biochemistry. Another set of hamsters (n = 6) was used for the estimation of post prandial lipemia and triton-induced hypertriglyceridemia. Animals were bled retro- orbitally under isoflourane anesthesia. Plasma samples were separated and stored for biochemistry. The liver samples were collected and stored at −70 °C for further analysis.

2.5. Truncal vagotomy in hamsters

Hamsters were anaesthetized using pentobarbital (45 mg/kg, IP) and the mid line incision was made in the abdomen. The anterior and posterior vagal trunks were identified with the aid of a dissecting microscope. Each vagal branch was then carefully separated from the esophageal wall and doubly ligated by 7-0 silk to leave 10 mm sections to be excised at a level sufficiently high to ensure transection of the stomach and hepatic branches. Truncal vagotomy was performed to elucidate the contribution of neural pathways in mediating metabolic effects. All animals were given analgesic (Meloxicam, 2 mg/kg, SC) and antibiotic (Terramycin, 60 mg/kg, IP) treatment, and were allowed to recover for a week [23]. After recovery, animals underwent icv cannula implantation as described above and were fed HFHC diet for 2 weeks.

2.6. Repeated dose treatment in vagotomized hypercholesterolemic hamsters

After 2 weeks of HFHC diet, vagotomized hamsters with icv-implants were randomized based on their plasma cholesterol and triglyceride levels and assigned to vehicle or coagonist (0.3 µg/animal) treatment, once a day, by icv route, for 4 weeks. After termination of treatment, animals were bled by retro-orbital puncture and plasma was collected for biochemistry.

2.7. Energy expenditure and respiratory quotient

Energy expenditure was measured using an indirect calorimeter (Oxylet; Panlab, Cornella, Spain). At the end of experiment, hamsters were acclimated in individual metabolic chambers, allowing free access to food and water. Oxygen consumption and carbon dioxide production were recorded for 3 min at 30 min interval using a computer-assisted data acquisition program (Metabolism V 2.1.01; Panlab, Cornella, Spain) over a 24-h period. Respiratory quotient (RQ) was derived from the ratio of VO_2 to VCO_2 and Energy expenditure (EE) was calculated according to the following formula:

EE [(Kcal/day) / (body weight) $\wedge$ 0.75] = [3.815 + (1.232 X VO_2/VCO_2)]

2.8. Postprandial lipemia

After chronic treatment, hamsters were fasted overnight. Next day, they were administered corn oil (6 mL/kg, PO). They were bled through retro-orbital puncture under isoflurane anesthesia 5 h after corn oil administration. Plasma samples were analyzed for triglyceride content.

2.9. Tyloxapol induced hypertriglyceridemia

After completion of treatment in the hamsters, jugular vein was cannulated using pentobarbital (45 mg/kg, IP) as an anesthetic and then cannulae were exteriorized at the back of the neck. Animals were