Acta Histochemica 116 (2014) 545–550

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Expression of glucose-dependent insulinotropic polypeptide and its receptor in the rat major salivary glands

Jutiporn Privatananupunt[a,*], Ippei Watari[a], Katarzyna Anna Podyma-Inoue[b], Mariko Kubono[a], Takashi Ono[a]

[a] Department of Orthodontic Science, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8549, Japan
[b] Department of Biochemistry, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8549, Japan

ARTICLE INFO

Article history:
Received 25 September 2013
Received in revised form
11 November 2013
Accepted 12 November 2013

Keywords:
Glucose-dependent insulinotropic polypeptide
Glucose-dependent insulinotropic polypeptide receptor
Expression
Major salivary gland
Rat

ABSTRACT

Glucose-dependent insulinotropic polypeptide receptors (GIPR) are expressed throughout the body. The expression of its ligand, glucose-dependent insulinotropic polypeptide (GIP) however, has only been reported in a limited numbers of organs. Although the rat submandibular salivary gland (SMG) has been found to express GIP, its biological role is still not understood. Moreover, nothing is known about the expression of GIP in other types of salivary glands, i.e. the parotid (PG) and sublingual (SLG) glands. We detected the expression of GIP mRNA in the rat PG, SMG and SLG. Immunohistochemical analyses revealed that GIP and GIPR were expressed only in the ductal area of all types of major salivary glands, and no immunostaining was found in the acini area. We also found GIP expression in the rat SMG to be age dependent, with 8-week-old rats showing 2–3-fold higher than those of 9- and 11-week-old rats, respectively. This is the first study to indicate both GIP and GIPR expression in the rat major salivary glands, as well as its variation in the rat SMG during the growth period. These findings are crucial for a better understanding of the physiological function of GIP in rat major salivary gland.

Introduction

Saliva, an exocrine secretion of the salivary glands, consists of water, electrolytes, enzymes, immunoglobulins, mucosal glycoproteins and numerous antimicrobial proteins, growth factors and regulatory peptides. Saliva plays a crucial role in digestion, mastication, oral microbial defense, gustation, lubrication, speech, deglutition, and preservation of mineralized and mucosal tissues (Mathison, 2009). This large variety of functions is essential for the maintenance of oral and pharyngeal health and a comfortable quality of life. Components of saliva are synthesized by three pairs of major salivary glands: the parotid (PG), submandibular (SMG) and sublingual (SLG) glands, as well as numerous minor salivary glands located throughout the oral cavity. Salivary glands are generally classified as exocrine glands that excrete their products externally via the gland duct system. However, evidence over the past few decades suggests that exocrine glands, like the salivary gland and pancreas, may also possess endocrine secretory capability (Isenman et al., 1999). Because a large number of physiologically active proteins and peptides important for maintaining proper oropharyngeal homeostasis are secreted by the salivary glands, more detail regarding the secretion and function of these products is required.

It is known that the first step of digestion occurs in the oral cavity, and that salivary components like amylase, lipase, mucin, insulin-like and glucagon-like peptides are necessary in digestive processes and glucose metabolism. Glucose-dependent insulinotropic polypeptide (GIP), a 42 amino acid long molecule, belonging to the secretin-vasoactive intestinal polypeptide (VIP) family of gastrointestinal (GI) hormones, is one of the incretin group of hormones found within the rat SMG. GIP has been reported to be expressed in intestinal K-cells and in the stomach, in both humans and rodents (Buffa et al., 1975; Fetner et al., 2005; Baggio and Drucker, 2007), in the rat SMG (Tseng et al., 1993, 1995), in the rat lens epithelial cells (Nakajima et al., 2002), in the rat brain (Nyberg et al., 2007) and in the human and mice pancreas (Fujita et al., 2010; Prasadan et al., 2011). GIP was initially isolated from a porcine intestinal extract and characterized for its ability to inhibit gastric acid secretion, hence its previous name: 'gastric inhibitory polypeptide' (Brown et al., 1969; Brown and Dryburgh, 1971; Pederson and Brown, 1972). However, subsequent studies evaluating wider physiological actions indicated that GIP stimulated glucose-dependent

* Corresponding author.
E-mail address: jutiorts@tmd.ac.jp (J. Privatananupunt).

0065-1281/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.acthis.2013.11.007

insulin secretion so that it is now referred to as 'glucose-dependent insulinotropic polypeptide' (Pederson et al., 1975; Pederson and Brown, 1976, 1978).

GIP exerts its effects by interacting with a specific receptor presented on the cell surface belonging to a group of the G protein-coupled receptors (GPCR). The glucose-dependent insulinotropic polypeptide receptor (GIPR) is expressed not only in the pancreas, but also in the stomach, small intestine, adipose tissue, adrenal cortex, pituitary, heart, testis, endothelial cells, bone, trachea, spleen, thymus, lung, kidney, thyroid, brain and human salivary glands (Boylan et al., 1999; Baggio and Drucker, 2007; Rudovich et al., 2007; Xie et al., 2007), which implies a physiological and pathological role for GIP in these tissues. Aside from its remarkable role in potentiating insulin secretion, the effects of GIP have been demonstrated on the facilitation of nutrient uptake into adipose tissue and bone, stimulation of bone formation (Bollag et al., 2001; Tsukiyama et al., 2006), activation of adipocytes and stimulation of lipogenesis (Hauner et al., 1988; Oben et al., 1991; Yip et al., 1998), glucocorticoid secretion (Mazzocchi et al., 1999), promotion of β-cell proliferation (Trumper et al., 2001; Ehses et al., 2003), proliferation of hippocampal progenitor cells (Nyberg et al., 2005), locomotor activity (Ding et al., 2006), modulation of the hepatic blood flow (Kogire et al., 1988), and stimulation of intestinal GLP-1 release (Simpson et al., 2007). Nevertheless, the action of GIP on many other organs is not well understood.

Although a few reports related to GIP in the salivary glands have been published, its secretion and function remain obscure and more information is crucial to better understand the role of this important regulatory peptide. Thus, we focused on GIP mRNA expression in the rat PG, SMG and SLG, as well as the distribution of GIP and GIPR proteins in the rat major salivary gland. In addition, we quantitatively compared the levels of GIP gene expression in the rat SMG at different ages during the growth period.

Materials and methods

Experimental model and tissue preparation

Animal protocols were approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University (0120237A, 0130060A), and experiments were carried out under the control of the University's Guidelines for Animal Experimentation.

Eight-week-old male Wistar rats ($n = 6$), weighing 230–260 g each, were used to investigate the expression and distribution of GIP in the rat major salivary glands. Rats were deeply anesthetized with an intraperitoneal injection of chloral hydrate 400 mg/kg body weight (Ishida et al., 2008; Mizumachi-Kubono et al., 2012) followed by dissection of the PG from surrounding adipose tissues. The SMG and SLG were then carefully separated from the encapsulated fascia. One side of each gland was used for the isolation of RNA, while the other side was immediately immersed in 10% formalin neutral buffered solution (Mildform 10N, Wako Pure Chemical Industries, Osaka, Japan) at 4 °C overnight and then embedded in paraffin wax, sectioned and subjected to histological analyses. To observe the alteration of GIP expression during different growth periods, the SMG of 8-, 9- and 11-week-old male Wistar rats ($n = 6$ for each age group) were collected and used for quantitative analysis.

Reverse transcription-polymerase chain reaction (RT-PCR)

GIP transcript levels in each major salivary gland were determined by RT-PCR. Briefly, total RNA was extracted from the PG, SMG and SLG tissues using RNA STAT-60 reagent (Tel-Test Inc, Friedenswood, TX, USA). Briefly, 5 μg of total RNA was converted to cDNA with an oligo(dT) primer using SuperScript III Reverse Transcriptase (Invitrogen, San Diego, CA, USA). Each PCR reaction was carried out in a total volume of 25 μl, containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP and 2.5 U Taq DNA polymerase (Takara Bio, Shiga, Japan). PCR was performed using GIP-specific primers (forward 5′-ACC ACG AGG CCC AAG GTA TG-3′; reverse 5′-CAG AGA CTT TGG ACC AGG GCA-3′) amplifying a 400 bp GIP cDNA fragment. The PCR conditions were as follows: initial activation of polymerase at 94 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 64 °C for 45 s, and extension at 72 °C for 1 min. After cycling, terminal elongation at 72 °C for 10 min was performed. To ensure the quality of the template, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene transcript was amplified for 30 PCR cycles using GAPDH-specific primers (forward 5′-ACC ACA GTC CAT GCC ATC AC-3′; reverse 5′-TCC ACC ACC CTG TTG CTG TA-3′) amplifying a 452 bp GAPDH cDNA fragment. The amplified PCR products were analyzed by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide and photographed under UV transillumination.

Immunohistochemistry

To evaluate the distribution of GIP and GIPR expression in the salivary tissues, immunostaining was performed using the three-step streptavidin–biotin-peroxidase method (Vectastain Universal Quick Kit, Vector Laboratories, Burlingame, CA, USA). Briefly, fixed specimens were embedded in paraffin and sectioned into 5-μm-thick sections (RM 2155, Leica, Nussloch, Germany). The sections were deparaffinized with xylene and rehydrated in a graded ethanol series. After antigen retrieval with sodium citrate buffer (pH 6.0), specimens were rinsed with TBST and treated with a solution of 0.3% H_2O_2 in methanol for 20 min to block endogenous peroxidase activity. The samples were then washed with TBST and blocked with blocking serum (Vectastain Universal Quick Kit) for 10 min followed by incubation overnight with primary rabbit anti-GIP (YII-Y103-EX, a dilution of 1:1000, Cosmo Bio, Tokyo, Japan) and anti-GIPR (LS-C138883, a dilution of 1:50, LSBio, Seattle, WA, USA) antibodies in a humidified chamber at 4 °C. After rinsing with TBST, the sections were incubated with a biotinylated universal secondary antibody (Vectastain Universal Quick Kit) for 10 min at room temperature, followed by incubation with streptavidin peroxidase complex (Vectastain Universal Quick Kit) for 5 min. The immunoreactive sites were visualized with 3,3-diaminobenzidine tetrahydrochloride, after which sections were counterstained with hematoxylin and mounted with Mount-Quick (Cosmo Bio). Negative controls were prepared by omission of the primary antibody.

Real-time quantitative reverse transcription PCR (qRT-PCR)

qRT-PCR was used to analyze the change in GIP expression level in the SMG of rats during the growth period. Reverse transcription was performed using 1 μg of total RNA per sample in a 20 μl reaction mixture using the high capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instruction. Next, 1 μl of cDNA along with a 20-μl reaction of 2× PCR master mixture and gene-specific Taqman assay mixture were loaded into the PCR reaction plate. Taqman assays (primer-probe sets) for GIP (Rn00571500_m1) and GAPDH (Rn99999916_s1) as an endogenous control were purchased from Applied Biosystems. The reaction was carried out in a StepOne™ Real-Time PCR detection system (Applied Biosystems) using the thermal conditions recommended by the manufacturer (10 min at 95 °C, and 45 cycles of 15 s at 95 °C and 1 min at 60 °C). The mean GIP mRNA expression