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Thyrotropin-releasing hormone in the dorsal vagal complex stimulates pancreatic blood flow in rats☆

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Abstract

Central administration of thyrotropin-releasing hormone (TRH) enhanced pancreatic blood flow in animal models. TRH nerve fibers and receptors are localized in the dorsal vagal complex (DVC), and retrograde tracing techniques have shown that pancreatic vagal nerves arise from the DVC. However, nothing is known about the central sites of action for TRH to elicit the stimulation of pancreatic blood flow. Effect of microinjection of a TRH analog into the DVC on pancreatic blood flow was investigated in urethane-anesthetized rats. After measuring basal flow, a stable TRH analog (RX-77368) was microinjected into the DVC and pancreatic blood flow response was observed for 120 min by laser Doppler flowmetry. Vagotomy of the several portions, or pretreatment with atoropine methyl nitrate or N^G-nitro-L-arginine-methyl ester was performed. Microinjection of RX-77368 (0.1 – 10 ng) into the left or right DVC dose-dependently increased pancreatic blood flow. The stimulation of pancreatic blood flow by RX-77368 microinjection was eliminated by the same side of cervical vagotomy as the microinjection site or subdiaphragmatic vagotomy, but not by the other side of cervical vagotomy. The TRH-induced stimulation of pancreatic blood flow was abolished by atropine or N^G-nitro-L-arginine-methyl ester. These results suggest that TRH acts in the DVC to stimulate pancreatic blood flow through vagal-cholinergic and nitric oxide dependent pathways, indicating that neuropeptides may act in the specific brain nuclei to regulate pancreatic function.

Keywords: Neuropeptide; Pancreatic microcirculation; Autonomic nervous system; Central nervous system

1. Introduction

Convergent neuroanatomical, electrophysiological, and neuropharmacological evidence has suggested a role for the central and autonomic nervous systems in the regulation of pancreatic functions [1,2]. Neuropeptides have recently been recognized as neurotransmitters in the central and peripheral nervous systems [3–5], and centrally acting neuropeptides have been reported to regulate a variety of physiological functions [3–6]. In particular, the effect of central thyrotropin-releasing hormone (TRH) and its analogs on physiological, pharmacological, and pathophysiological regulation of gastrointestinal tract functions has been reported [4,6]. We have recently found that intracisternal injection of TRH analog stimulates pancreatic blood flow mediated through vagal-cholinergic and nitric oxide dependent pathways [7], suggesting that pancreatic blood flow can be influenced by brain neuropeptides through modulation of autonomic nervous system. However, nothing is known about the central sites of action for TRH to elicit pancreatic blood flow. In the brain, TRH-immunoreactive nerve fibers

Abbreviations: DMN, dorsal motor nucleus of the vagus; NST, nucleus of the solitary tract; DVC, dorsal vagal complex; TRH, thyrotropin-releasing hormone.

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and terminals, and TRH receptors are localized in the dorsal vagal complex (DVC), which includes the dorsal motor nucleus of the vagus (DNM) and the nucleus of the solitary tract (NST) [8–10], which are important sites for the vagal nerve regulations [11]. Furthermore, pancreatic vagal nerves have been shown to originate from the DVC by retrograde tracing techniques [12–15]. These lines of evidence led us to speculate that TRH acts in the DVC to enhance pancreatic blood flow. The present study addressed this question by examining the effect of microinjection of TRH into the DVC on pancreatic blood flow in rats.

2. Material and methods

2.1. Animals

Male Wistar rats weighing 265–280 g (SLC Co., Shizuoka, Japan) were housed in group-cages under condition of controlled temperature (22–24 °C) and illumination (12-h light cycle starting at 6 AM) for at least 7 days before experiments. Animals were maintained on laboratory chow and tap water. Experiments were performed in rats deprived of food for 24 h but given free access to water up to the beginning of the study. Protocols describing the use of rats were approved by the Animal Care Committees of Dokkyo University School of Medicine and Asahikawa Medical College, and were in accordance with the Minister of Education, Culture, Sports, Science and Technology of Japan 'Guide for the Care and Use of Laboratory Animals'.

2.2. Chemicals

The following substances were used: a stable TRH analog, RX-77368, *p*-Glu-His-(3,3′-dimethyl)-Pro-NH_2 (Reckitt and Colman, Kingdom-upon-Hill, England) and urethane (Sigma Chemical, St Louis, MO). RX-77368 has similar binding characteristics as authentic TRH on rat brain [16,17]. This compound is characterized by higher stability and longer effective dilation compared with authentic TRH [16,17]. In fact, regarding gastric acid secretion, the analog is 22 times more potent than authentic TRH when given intracisternally [18]. RX-77368 was aliquoted in 0.5% bovine serum albumin (Sigma) and 0.9% saline at the concentration of 1.5 nmol/μl and kept frozen at −20 °C. The stock solution was diluted in 0.9% saline (pH 7.4) before the experiment.

2.3. Measurement of pancreatic blood flow

Rats were anesthetized with urethane (1.5 g/kg, ip) and underwent tracheotomy, and then PE-260 tubing (Clay Adams, B.D., Parsippany, NJ) was inserted into the trachea to ensure a patent airway. The femoral artery was cannulated with PE-50 tubing (Clay Adams, B.D.) to enable continuous monitoring and recording of arterial blood pressure using a pressure transducer (Uniflow, Baxter, Valencia, CA), a pressure amplifier (PA-001, Star Medical Co., Tokyo, Japan), and a computer (Macintosh G4, Apple Computer, Inc., Cupertino, CA) equipped with a data recording and analysis system (MacLab, AD Instruments Pty Ltd., Castle Hill, Australia). Rats were mounted on ear bars of a stereotaxic apparatus (Kopf model 900, David Kopf Instruments, Tujunga, CA), and each rat was positioned to expose the abdomen. After making a 3-cm midline abdominal incision, the pylorus was ligated and a cannula was placed into the nonglandular portion of the stomach to divert gastric secretion, so as to avoid the possibility of inducing duodenal acidification and gastric distention which may influence pancreatic functions. Then, a probe (diameter 6 mm, type H, Advance Co. Ltd., Tokyo Japan) of a laser Doppler flowmeter (ALF 21, Advance Co. Ltd.) was placed on the surface of pancreatic body. The flow signal was averaged with a 3-s time constant and recorded using a computer (Macintosh G4) equipped with a data recording and analysis system (MacLab). Pancreatic blood flow was expressed relative to the basal. Body temperature was kept at 37 °C by external heating and the pancreatic surface was continuously rinsed with 0.9% saline (37 °C, pH 7.4) to keep it moist.

2.4. Microinjection and experimental protocol

After the surgical preparation was completed, the animals were left undisturbed for 60 min for the stabilization of temperature, heart rate and arterial blood pressure, and basal pancreatic blood flow was observed for 30 min. Then, the rat head was fixed in a nose-down position (incisor bar set at −15 mm). The obex region of the dorsal medulla was exposed by resection of the dorsal cervical musculature and removal of the occipital skull plate and small pieces of the cerebellum. Then a glass micropipette (50–70 μm diameter) was positioned unilaterally (right or left side) into the DVC according to the following coordinates: 0.6 mm dorsoventral from surface, 0.3 mm, rostrocaudal from obex and 0.5 mm lateral from obex. RX-77368 (0.1, 0.3, 0.5, 1, 5 and 10 ng) or 0.9% saline vehicle was delivered in a 50-nl volume by pressure ejection over 1 min utilizing a 1-μl Hamilton syringe. The micropipettes were left in place for 3 min, and then withdrawn. After microinjection, changes of pancreatic blood flow were monitored for 120 min thereafter. At the end of the experiments, rats were killed by decapitation. Brains were removed and fixed in 10% formalin and 20% sucrose solution for at least 2 days. Frozen sections were sliced at 30 μm, mounted and stained with Toluidine blue. Histological sections were examined microscopically. The location of microinjection sites was identified by the visualization of the tip of the micropipette track and marked on plates reproduced from the atlas of Paxinos and Watson [19].