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Effects of Centrally Applied Serotonin in Ventromedial Hypothalamic Nucleus on Regulation of Bile Secretion and Lipid Metabolism in the Rat

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Abstract

Ventromedial hypothalamic nucleus (VMH) is one of the brain regions responsible for regulating gastrointestinal activities and lipid metabolism. Hepatic enzymes and serum lipids are controlled by the central nervous system and VMH is involved in the regulation of lipid metabolism. In the present study we sought to establish the role of serotonin to regulation of bile secretion, serum lipids level and hepatic enzymes in the rat. Rats were cannulated in the VMH for the administration of serotonin. After 1 week the common bile duct was cannulated and bile samples were collected after the administration of 5-HT, and biochemical analyses occurred on blood samples. Centrally applied 5-HT increased bile secretion at all studied periods and also decreased LDL-cholesterol (LDL-c) level. Present findings show that 5-HT participates in the central regulation of bile secretion in the rat, and VMH can be a special site for regulation of bile secretion and lipid levels.

Keywords: Serotonin, bile flow, VMH, serum lipids, hepatic enzymes.
Introduction

Serotonin, also known as 5-hydroxytryptamine (5-HT), is a monoaminergic neurotransmitter with activities that modulate central and peripheral functions (Watanabe et al. 2010). The central nervous system (CNS) stores close to 2% of the body’s serotonin. Serotonin plays a major role in neurotransmission within the CNS and the autonomic nervous system (ANS) to regulate feeding behavior, and meal size, and body weight (Ruddell et al. 2008) also affects sleep, anxiety, sexual behavior and mood. On the other hand, serotonin operates as a peripheral hormone, affecting vascular contraction and relaxation, gastrointestinal motility, primary hemostasis, liver repair, and the control of the T cell mediated immune system cell proliferation, apoptosis and platelet aggregation (Best et al. 2010; Ruddell et al. 2008; Watanabe et al. 2010). Furthermore, serotonin is thought not to be able to pass the blood-brain barrier. Thus two independent serotonin systems exist, one in the brain and the other in the periphery (Watanabe et al. 2010). The hypothalamus integrates various endocrine and autonomic physiologies and consists of anatomically distinct nuclei that together function to regulate sleep, circadian rhythm, energy homeostasis, sexual behaviors, and thermoregulation (Kurrasch et al. 2007).

LDL-c is cholesterol-rich particles. About 70% of plasma cholesterol occurs in this form. LDL-c is mainly involved in the transport of the cholesterol manufactured in the liver to the tissues, where it is used. Uptake of cholesterol into cells occurs when lipoprotein binds to LDL-c receptors on the cell surface. LDL-c is then taken into the cell and broken down into free cholesterol and amino acids (Boston et al. 1996). There is evidence for an association between serum lipid levels and 5-HT activity in the CNS (Goldstein et al. 2010; Steegmans et al. 1996).

Hypothalamic nuclei, such as lateral hypothalamic area (LHA, “hunger centre”) and ventromedial hypothalamic nucleus (VMN, “satiety centre”), as well as arcuate and paraventricular nuclei (PVN) have been shown to control food intake, energy homeostasis and body mass (Kubasik-Juraniec et al. 2003). There are two types of neurons connecting the hypothalamus and the liver: efferent and afferent nerves. Efferent neural pathways consist of sympathetic and parasympathetic nerves from three major areas in the hypothalamus which is involved in the autonomic regulation of the liver. These three major areas are the VMH, LHA and the PVN (Uyama et al. 2004). The ventromedial nucleus of the hypothalamus (VMH) is one of the brain regions responsible for regulating feeding behavior, autonomic nervous activity (Borg et al. 1994; Chen et al. 2010; Kitaoka et al. 2010; Kubasik-Juraniec et al. 2003; Kurrasch et al. 2007; Yadav et al. 2009) and also lipid metabolism (Takahashi and Shimazu 1981; Takahashi and Shimazu 1982). VMH neurons respond to glucose, free fatty acid, and other feeding-relevant agents that are involved in the regulation of feeding and energy metabolism.

Neuropeptides are widely distributed in the central nervous system as well as in peripheral nerves system, and act as neurotransmitters to regulate various physiological functions. The digestive organs are no exception, and several neuropeptides in the central nervous system are shown to act in specific brain sites and control gastrointestinal functions, such as gastric acid secretion, and gastrointestinal motility, through the autonomic nervous system (Yoneda et al. 2001). However, little is known about the central regulation of bile secretion and lipid levels by peptides as compared with the wide literature on the brain regulation of gastric secretion and gastrointestinal motor function. In the present study we sought to establish the effect of centrally applied 5-HT on bile secretion, serum lipids and hepatic enzymes.

Materials and Methods

Ethics

The protocol used in this study was approved by Ethics Committee of the School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Animals

Thirty male Wistar rats (280-320 g) (Razi Vaccine and Serum Research Institute, Shiraz, Iran)
were used. The rats were housed in group cages under conditions of controlled temperature (22±2 °C) and 12-h light/dark cycle (light from 7:00 to 19:00 h) for at least 7 days before the experiments.

**Animal preparation and experimental procedures**

**Stereotaxic surgery**

One week previous to bile secretion experiments, the rats were anesthetized with ketamine and xylazine (100 mg/ml and 8 mg/ml, respectively, i.p.), and were placed in a stereotaxic instrument (Stoelting Instruments, USA) and an external guide cannula was introduced into the VMH of the brain. A 22-gauge stainless steel cannula was placed in the VMH with the following coordinates: A/P: -2.8 mm from the bregma, L/M: 0.5 mm, D/V: -9.4mm, vertically (Paxinos and Watson 1997). Rats were placed in individual cages with food and water *ad libitum* and allowed one week for recovery.

**Bile secretion experiments**

Rats were fasted for 14 h in order to avoid possible changes in the release of different peptides and/or hormones that may eventually influence bile secretion, and were anesthetized with ketamine and xylazine (100 mg/ml and 8 mg/ml, respectively, i.p.). Through a 3-cm midline abdominal incision the common bile duct was exposed and cannulated with a polyethylene catheter (PC-10 Intramedic, USA) near its bifurcation to avoid contamination with pancreatic juice. Rats remained anesthetized during bile collection which was performed between 9:00 and 11:00 to avoid possible circadian changes. Body temperature was kept at 37 °C by external heating.

**Bile collection**

Bile volumes were determined using Hamilton microsyringe and bile flow was calculated as µl/min/100 g body weight.

At the end of the experiments, the animals were euthanatized by ether and the brain removed through the opening of the skull; the correct position of the cannula in the brain was verified by routine histology.

**Blood collection**

At the end of each experiment, blood samples were collected from the heart into dry centrifuge tubes and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 2500 rpm for 10 minutes and biochemical investigations were carried out.

**Biochemical Analyses**

The serum was analyzed for cholesterol by a modified Abell-Kendall/Levey-Brodie (A-K) method (Burtis and Ashwood 1994), triglyceride by the enzymatic procedure of McGowan et al. (1983). Lipoproteins were isolated using a combination of precipitation and ultracentrifugation. HDL-cholesterol was measured using the precipitation method. In the first step, the precipitation reagent (sodium phosphotungstate with magnesium chloride) was added to the serum to aggregate non-HDL lipoproteins, which were sedimented by centrifugation (10000 g for 5 min). The residual cholesterol was then measured by the enzymatic method (Burtis and Ashwood 1994). LDL-cholesterol was calculated as the difference between the cholesterol measured in the precipitate and in the HDL fraction. VLDL-cholesterol was estimated as one-fifth of the concentration of triglycerides (Friedewald et al. 1972). Total bilirubin, direct bilirubin and indirect bilirubin were measured according to modified Vandenberg method (Burtis and Ashwood 1994). Hepatic enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to Reitman-Frankel method (Burtis and Ashwood 1994).

**Statistical analysis**

The statistical analysis was performed using ANOVA followed by the Duncan test. Results are
expressed as the Mean ± SEM P-values of 0.05 or less were considered statistically significant.

Results and Discussion

**Bile Secretion**

Results showed that centrally applied 5-HT into VMH exhibited stimulatory effect on bile secretion, in compare with control and sham groups, from the catheter implanted into the common bile duct in ketamine-xylazine-anesthetized rats. Central injection of 5-HT at a dose of 0.5 µl increased bile secretion at all studied times during the first 15 min collection period (5, 10 and 15 min) after the injection, and also throughout the 60 min observation period thereafter to the end of the experiment (Fig. 1).

**Serum lipids profile and hepatic enzymes**

The results obtained from this study showed that injection of 5-HT in VMH had a decreased effect on the LDL-c level (Fig. 2). LDL-c was significantly decreased in the blood serum of the test group with central applied 5-HT compared to the control (no injection) and sham (saline injection) groups. Levels of cholesterol, triglyceride, VLDL-c, HDL-c, bilirubin and hepatic enzymes did not change (Figs. 2, 3, 4).

![Fig. 1: Effect of centrally applied 5-HT on bile flow (µl/min/100 g BW). Control; no injection, sham; 0.5µlNaCl, Test; 0.5 µl 5-HT. *: p<0.05. Number of cases: 10. BW: body weight.](image1)

![Fig. 2: Effect of centrally applied 5-HT on serum lipids concentrations. Control; no injection, sham; 0.5µlNaCl, Test; 0.5 µl 5-HT. *: p<0.05. Number of cases: 10. TG: triglyceride, VLDL: very low density lipoprotein, CHOL: cholesterol, HDL: high density lipoprotein, LDL: low density lipoprotein.](image2)
The major finding of the present study was that 5-HT (0.5 µl) injected into VMH increases bile secretion collected from the cannulated common bile duct and also decreases LDL-c level in ketamine-xylazine-anesthetized rats. The stimulatory action of 5-HT on bile secretion appeared during 5, 10 and 15 min periods, as the first 15 min collection period after basal secretion.

The liver is known to have a vast supply of autonomic nerves, which originate in the hypothalamus and enter the liver with the major vessels in the portahepatis. Nerves innervating the liver include afferent and efferent sympathetic, parasympathetic as well as peptidergic component (Gardemann et al. 1992). Efferent neural pathways from three major areas in the hypothalamus are involved in the autonomic regulation of the liver. These three major areas are the VMH in the medial hypothalamic area, the LHA and the PVN in the periventricular hypothalamic area (Uyama et al. 2004). Besides, adrenergic and cholinergic innervations, a peptidergic innervation has also been found in livers of several species (El-Salhy et al. 1993; Gardemann et al. 1992).

The ventromedial hypothalamic nucleus known as the "satiety center" was shown to control food...
intake, energy homeostasis, body mass (Borg et al. 1994; Chen et al. 2010; Kitaoka et al. 2010; Kubasik-Juraniec et al. 2003; Yadav et al. 2009) and regulation of lipid metabolism (Takahashi and Shimazu 1981; Takahashi and Shimazu 1982).

Serotonergic nerve fibers are included in the peptidergic family of the ANS and have been shown to be localized to the tunica media on branches of the hepatic artery and portal vein as well as bile ducts and the connective tissue of the interlobular septa (Ruddell et al. 2008).

Although the liver possesses many intrinsic and extrinsic nerve innervations, the functional role of these nerves in biliary physiology and lipid levels is poorly understood. The brain–liver relation has been widely studied with regard to the control of glucose metabolism, in which the hypothalamus plays an important role (Bianciotti et al. 2001). In addition, accumulated evidence has shown the stimulatory effects of hypothalamus on gallbladder and sphincter of Oddi (Shimazu 1987) and also hypothalamic regulation of lipid metabolism (Takahashi and Shimazu 1981; Takahashi and Shimazu 1982). However, little is known about the existence of a central regulation of lipids and bile secretion, although several peptides and neuropeptides have been shown to influence bile flow when they are applied to the brain (Sabbatini et al. 2002; Yoneda et al. 2001).

There are two serotonin systems, one in the brain and one in the periphery. These are independently regulated and have distinct functions. Peripheral serotonin stimulates the contraction of the gallbladder and accelerates the metabolism of lipids by increasing the excretion of bile (Watanabe et al. 2010). Previous findings by other authors have shown the stimulatory role of peripheral serotonin on bile secretion (Bogach and Liashchenko 1976; Uma and Sahin-erdemli 1991). However serotonin is not able to pass the blood-brain barrier (Watanabe, et al. 2010). We have demonstrated the central effects of serotonin on bile secretion. In the present work centrally applied 5-HT increased bile flow at 5 min after the injection of the peptide. This stimulatory action was revealed as a net increase of bile output throughout the 60 min observation period thereafter to the end of the experiment. There is evidence for an association between serum lipid levels and 5-HT activity in the CNS. Low serum cholesterol and LDL-c lead to a decrease in brain serotonin activity (Goldstein et al. 2010; Steegmans et al. 1996). Also, electrical stimulation of the VMH enhances lipogenesis in BAT and increases lipolysis in adipose tissue (Takahashi and Shimazu 1981; Takahashi and Shimazu 1982). In this study central 5-HT decreases serum LDL-c level. These findings suggest that 5-HT injected into the ventromedial hypothalamic nucleus acts to regulate lipid levels.

Previous investigations have reported 5-HT has an influence on the regulation of neuronal activity within the VMH, a structure which is involved in the mediation of signals for the state of satiety (Heidel and Davidowa 1998). Present results suggest that VMH can be a special site for the regulation of bile secretion and lipid metabolism in the brain.

Bile secretion is regulated by hormones and peptides, as well as by the autonomic nervous system, and electrical stimulation of the VMH through the activation of sympathetic nervous system also regulates lipogenesis and lipolysis (Takahashi and Shimazu 1981; Takahashi and Shimazu 1982). In addition, diverse peptides applied to the brain mediate their effect through the autonomic nervous system. Studies have shown that vagal stimulation tends to increase bile flow whereas noradrenergic stimulation reduces bile secretion (Yoneda et al. 2001). In this study the increased of bile flow induced by the centrally applied 5-HT raises the possibility that 5-HT may activate parasympathetic outflow to the gut.

This is further supported by anatomical studies that show 5-HT immunoreactive fibers and terminals in the dorsal vagal complex (Oskutyte et al. 2009). In addition, 5-HT receptors are also present in the dorsal vagal complex (Costedio et al. 2007; Ruddell et al. 2008). These neuroanatomical data, added to the possibility vagally mediated effect of central 5-HT to stimulate bile secretion suggest that 5-HT injected into VMH likely acts in the dorsal vagal complex.

To summarize, the present data indicate that the central administration of 5-HT into VMH acts in the brain to induce a stimulation of bile secretion and also has a significant decreased effect on the LDL-c
levels in rats. Central injection of 5-HT provides a useful tool to further investigate brain sites that influence the regulation of bile secretion and lipid metabolism. The present work further supports the physiological relevance of the action of neuropeptides on brain sites to control gastrointestinal function and reveals new insights into the brain–liver interaction.

References


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