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Short communication

Role of calcitonin gene-related peptide and capsaicin-sensitive afferents in central thyrotropin-releasing hormone-induced hepatic hyperemia

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Abstract

The involvement of capsaicin-sensitive afferent neurons and calcitonin gene-related peptide (CGRP) in the central thyrotropin-releasing hormone (TRH)-induced hepatic hyperemia was investigated in urethane anesthetized rats. Both systemic capsaicin pretreatment and intravenous administration of CGRP receptor antagonist, human CGRP-(8–37), completely abolished the stimulatory effect of hepatic blood flow induced by intracisternal injection of TRH analog (RX-77368; *p*-Glu-His-(3,3′-dimethyl)-Pro-NH2, 100 ng), assessed by the hydrogen gas clearance method. These data demonstrate the involvement of capsaicin-sensitive afferent neurons and CGRP in the central TRH-induced stimulation of hepatic blood flow.

Keywords: Neuropeptide; Vagus; Autonomic nervous system; Hepatic microcirculation

1. Introduction

It is well established that neuropeptides act in the central nervous system as a neurotransmitter or neuromodulator to regulate gastrointestinal functions through the autonomic nervous system (Taché et al., 1990). We have recently found that several neuropeptides act in the brain to control hepatobiliary physiologic functions and modulate experimental liver injury in animal models (Yoneda et al., 1996, 1997a,b, 1998; Yokohama et al., 1999). In particular, the central thyrotropin-releasing hormone (TRH) plays a variety of roles in the hepatic physiologic functions through the vagal-cholinergic pathways. We have found that intracisternal injection of the TRH analog enhances hepatic blood flow through nitric oxide synthesis as well as the vagal-cholinergic pathways in rats (Tamori et al., 1998).

In the rat skin microvasculature, acetylcholine-induced vasodilation involves both nitric oxide and capsaicin-sensitive components (Ralevic et al., 1992). Anatomic and functional observations are consistent with the possibility that hepatic hyperemia resulting from the central vagal-cholinergic activation may also involve the recruitment of capsaicin-sensitive afferent neurons. First, capsaicin-sensitive primary sensory neurons, which contain calcitonin-gene related peptide (CGRP), innervate the portal area which includes the hepatic artery and portal vein (Goehler and Sternini, 1996). Second, CGRP is well established as one of the most potent vasodilators of the hepatic artery and portal vein, and peripheral administration of CGRP stimulates hepatic blood flow (DiPette et al., 1987; Fletcher et al., 1990). Finally, recent reports indicate the involvement of CGRP and capsaicin-sensitive afferent neurons in the central TRH-induced gastric hyperemia (Király et al., 1994, 1997, 1998).

These lines of evidence have prompted us to examine a possible role for CGRP and capsaicin-sensitive afferent neurons on the central TRH-induced enhancement of hep-

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atic blood flow. Therefore, in this study, the effect of capsaicin pretreatment and CGRP receptor antagonist on a stimulation of hepatic blood flow by intracisternal injection of TRH analog was investigated in rats by using the hydrogen gas clearance method.

2. Materials and methods

The following chemicals were used: the stable TRH analog, RX-77368 (*p*-Glu-His-(3,3′-dimethyl)-Pro-NH2; Reckitt and Colman, Kingston-upon-Hill, UK), human CGRP-(8–37), CGRP receptor antagonist (Peptide Institute, Osaka, Japan), capsaicin (Sigma, St. Louis, MO, USA).

Male Wistar rats (240–280 g, Charles River Japan, Yokohama, Japan) were housed in group cages under condition of controlled temperature (22–24°C) and illumination (12-h light cycle starting at 6 a.m.) for at least 7 days before experiments. After overnight fasting, rats were anesthetized with urethane (1.5 g/kg, i.p.), a tracheotomy was performed and PE-260 tubing (Clay Adams, B.D., Parsippany, NJ) was inserted into the trachea to facilitate the administration of hydrogen. Rats were mounted on ear bars of a stereotaxic apparatus (model 900, David Kopf Instruments, Tujunga, CA) and each rat was positioned to expose the abdomen. A 3-cm midline abdominal incision was made, and the pylorus was ligated. Next, a cannula was placed into the nonglandular portion of the stomach to divert gastric acid secretion, avoiding the possibility of secondary influence of duodenal acidification and gastric distention to hepatic functions. PE-50 catheter was inserted into the jugular vein to deliver chemicals for bolus injection and continuous infusion. Then, a platinum needle type electrode (diameter = 0.3 mm, MH-50N, MT Giken, Tokyo, Japan) was inserted into the hepatic left lateral lobe and a reference electrode (Ag–AgCl) (MH-10, MT Giken) was placed inside of the peritoneal cavity. The platinum electrode and the reference electrode were connected to a polarographic and amplifying unit (Model DHM-3001, MT Giken) and a computer (PowerBook 150, Apple Computer) equipped with a data recording and analysis system (MacLab, AD Instruments). Body temperature was kept at 37°C by external heating and the liver surface was continuously rinsed with saline to keep moist. After a 60-min stabilization period, hepatic blood flow was measured by hydrogen gas-clearance technique as previously described (Tamori et al., 1998). After two consecutive measurements of basal hepatic blood flow, human CGRP-(8–37) (15 μg/kg i.v. bolus, followed by an infusion of 3 μg kg^{-1} h^{-1} through-

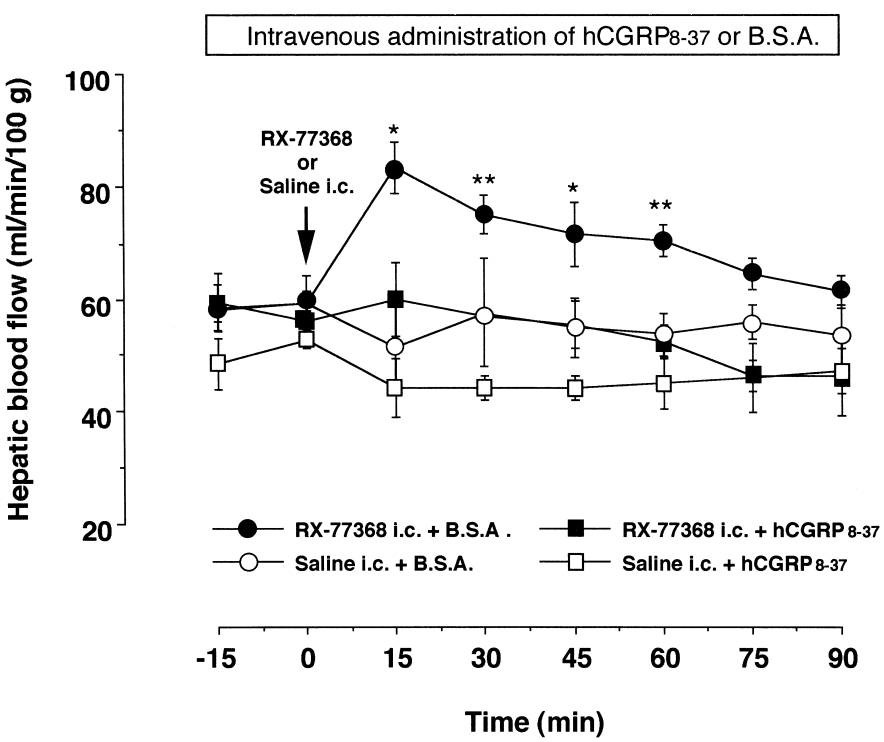

Fig. 1. The effect of intravenous administration of human CGRP-(8–37), CGRP receptor antagonist on hepatic hyperemic response to intracisternal thyrotropin-releasing hormone (TRH) analog, RX-77368, in urethane-anesthetized rats. After two consecutive measurements of basal hepatic blood flow by hydrogen gas clearance technique, either saline vehicle or RX-77368 (100 ng) was injected intracisternally. Human CGRP-(8–37) (hCGRP8-37) or 0.1% bovine serum albumin (B.S.A.) was injected i.v. bolus 15 min before intracisternal injection (i.c.), followed by a continuous infusion throughout the experiment. Hepatic blood flow was measured every 15 min for 90 min. Each point represents mean ± S.E. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the respective basal period.

out the experiment) or vehicle (0.1% bovine serum albumin) was administered. Hepatic blood flow was observed for 15 min after the induction of human CGRP-(8–37), and TRH analog, RX-77368 (100 ng) or 0.9% saline was injected intracisternally and observed a change of hepatic blood flow for 90 min. The dose of TRH analog was chosen from the results of our previous study (Tamori et al., 1998).

In another experiment, the response of hepatic blood flow to intracisternal injection of TRH analog was studied in systemic capsaicin-pretreated rats. Capsaicin treatment was performed 10 to 14 days before the experiment. Capsaicin was dissolved in absolute ethanol, Tween 80, and saline (10:10:80 vol/vol/vol), and injected subcutaneously under ether anesthesia three times at 12-h intervals. The control group received the same regimen of injection except that the vehicle instead of capsaicin was injected. Effect of capsaicin on afferent nerve was confirmed by the disappearance of the corneal chemosensory reflex to a drop of 0.1% NH_4OH.

Protocols describing the use of rats were approved by the Animal Care Committee of Asahikawa Medical College, and in accordance with the National Institute of Health ''Guide for the Care and Use of Laboratory Animals''.

All results are expressed as mean ± S.E. Comparison of the hepatic blood flow after peptide injection with the average of basal hepatic blood flow was calculated by analysis of variance (ANOVA)-repeated measurement followed by Fisher's protected least significant difference test. $P < 0.05$ was considered to be statistically significant.

3. Results

Although in rats intravenously administered with 0.1% bovine serum albumin which was a vehicle for human CGRP-(8–37), intracisternal injection of saline did not influence hepatic blood flow, intracisternal injection of TRH analog, RX-77368, at a dose of 100 ng enhanced hepatic blood flow by 44% during the first 15-min observation period after the injection, and enhanced hepatic blood flow returned to baseline at 90 min (Fig. 1). This stimulatory effect of TRH analog on hepatic blood flow was abolished by intravenous administration of human CGRP-(8–37) (Fig. 1). Intravenous administration of human CGRP-(8–37) per se did not modify basal hepatic blood flow assessed for 15 min and throughout a 105-min experimental period (observation in intracisternal saline injection animals; Fig. 1).

In rats pretreated with vehicle for capsaicin, intracisternal injection of saline did not have any effect on hepatic blood flow, but intracisternal RX-77368 at 100 ng stimulated hepatic blood flow by 46% at the first 15-min observation period. This stimulatory effect on hepatic blood flow by intracisternal TRH analog was completely reversed by systemic pretreatment with capsaicin (Fig. 2). Capsaicin pretreatment per se did not modify basal hepatic

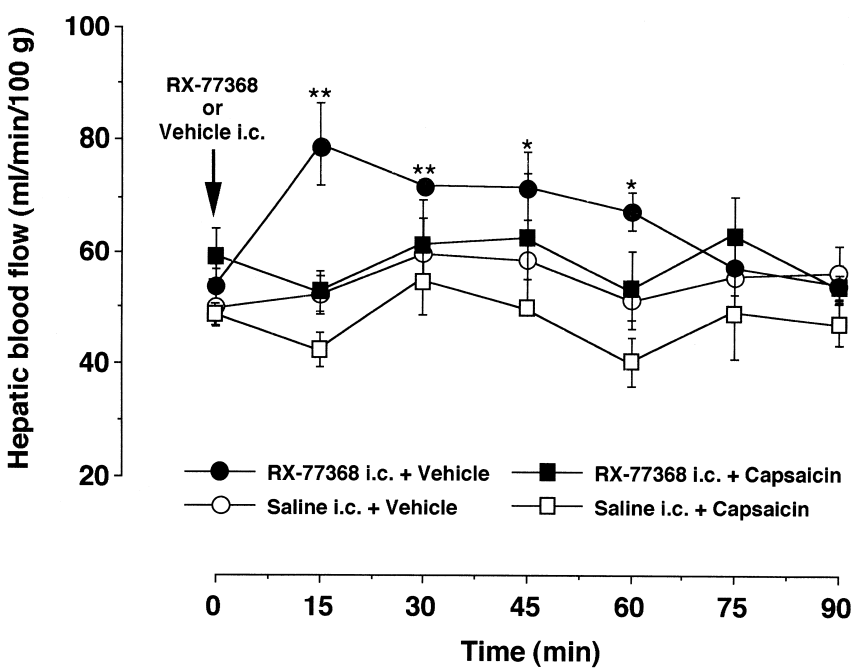

Fig. 2. The effect of systemic capsaicin pretreatment on hepatic hyperemic response to intracisternal thyrotropin-releasing hormone (TRH) analog, RX-77368, in urethane-anesthetized rats. Capsaicin (125 mg/kg) was subcutaneously injected 10–14 days before the experiment of hepatic blood flow. After two consecutive measurements of basal hepatic blood flow by the hydrogen gas clearance technique, either saline vehicle or RX-77368 (100 ng) was injected intracisternally (i.c.). Hepatic blood flow was measured every 15 min for 90 min. Each point represents mean ± S.E. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the respective basal period.

blood flow (ml/min/100 g: vehicle treatment, 52.1 ± 2.0; capsaicin treatment, 56.5 ± 4.2).

4. Discussion

Intracisternal injection of the stable TRH analog RX-77368 at 100 ng increased hepatic blood flow by 44–46% during the first 15-min period after the injection as measured by the hydrogen gas clearance technique in urethane-anesthetized rats. Thereafter, the stimulated hepatic blood flow decreased to reach basal levels within 90 min after peptide injection. Likewise, our previous report indicates that RX-77368 injected into the cisterna magna at 100 ng induced a robust increase in hepatic blood flow assessed by the hydrogen gas clearance technique (Tamori et al., 1998). Several evidence demonstrated that the hepatic hyperemic response to central injection of TRH is mediated by the vagal efferent cholinergic pathways. First, injection of TRH or TRH analog into the cerebrospinal fluid stimulates efferent activity of the vagal nerve (Somiya and Tonoue, 1984; O-Lee et al., 1997). Second, hepatic branch vagotomy completely abolished the rise in hepatic blood flow induced by TRH analog injected centrally (Tamori et al., 1998). Third, peripheral administration of muscarinic antagonist, atropine, completely suppressed the increase in hepatic blood flow induced by central injection of TRH analog (Tamori et al., 1998). Central administration of TRH is known to elevate systemic arterial blood pressure through sympathetic activation (Mattila and Bunag, 1986) and central TRH analog-induced increase in hepatic blood flow and systemic arterial blood pressure are not parallel with each other, indicating the increase in hepatic blood flow may be resulting from a decrease in vascular resistance and vasodilation rather than from secondary effect of increased systemic blood pressure (Tamori et al., 1998).

Several peptides exert a vasodilatory effect; however, CGRP is established to be one of the most potent vasodilators of vascular beds in many organs including the hepatobiliary system (DiPette et al., 1987; Fletcher et al., 1990). Calcitonin gene-related peptide is composed of 37 amino acids, the structure of which has been predicted on the basis of alternative processing of the primary transcript of rat calcitonin gene (Rosenfeld et al., 1983). Immunohistochemical analysis revealed a wide distribution of CGRP-like immunoreactivities in the central and peripheral nervous systems, including the hepatobiliary system (Goehler and Sternini, 1996). In the present study, intravenous infusion of CGRP receptor antagonist, human CGRP-(8–37), completely inhibited the stimulatory effect of intracisternal injection of TRH analog on hepatic blood flow, suggesting that peripheral CGRP plays a role in this stimulatory effect induced by the central TRH. These findings are very consistent with that in gastric hyperemic response to intracisternal injection of TRH analog (Király et al., 1994, 1997, 1998). We administered CGRP receptor antagonist with the same dose and manner as previously reported by Király et al. (1994, 1997, 1998), and we confirmed that intravenous administration of CGRP receptor antagonist does not affect basal hepatic blood flow such as gastric blood flow. These findings suggest no involvement of CGRP in the maintenance of basal hepatic and gastric microcirculations.

In the present study, the involvement of capsaicin-sensitive afferent neurons in central TRH-induced hepatic hyperemia was also investigated by systemic ablation of capsaicin-sensitive afferent neurons. Then, we found that systemic capsaicin pretreatment completely abolished the stimulatory effect of intracisternal injection of TRH analog on hepatic blood flow, such as that observed in acute effect of CGRP receptor antagonist on central TRH-induced hepatic hyperemia. Capsaicin acutely evokes and stimulates the release of CGRP from the afferent nerve terminals, and chronically diminishes CGRP immunoreactive nerve fibers after overstimulation of CGRP release inducing exhaustion of CGRP content (Holzer et al., 1990; Goehler and Sternini, 1996; Suzuki et al., 1997). Although the mechanisms responsible for releasing CGRP from capsaicin sensitive afferents after intracisternal injection are not known, a very recent study by Adelson et al. (1999) shows that intracisternal injection of TRH analog induces an excitation of gastric splanchnic afferent neurons. Afferent neurons of hepatic vagal or sympathetic nerve can be stimulated by central injection of TRH analog and it is of interest to confirm this phenomenon by electrophysiologic techniques. In our previous study, nitric oxide synthase inhibitor prevents central TRH-induced hepatic hyperemic response (Tamori et al., 1998), suggesting a possible interaction between nitric oxide and CGRP in the action of central TRH to induce stimulation of hepatic blood flow. From our present results, together with the previous report which presented the involvement of CGRP and capsaicin-sensitive afferent neurons in central TRH-induced gastric hyperemia (Király et al., 1994, 1997, 1998), the hepatic hyperemic response to central vagal activation induced by intracisternal TRH analog may be mediated by local effector function of capsaicin-sensitive afferent neurons releasing CGRP (Holzer, 1988).

In conclusion, our present studies suggest that CGRP-containing and capsaicin-sensitive afferent nerves are involved in the hepatic hyperemic response to central TRH, and that central vagal stimulation can activate the ''efferent function'' of capsaicin-sensitive afferent neurons in the liver.

Acknowledgements

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