Effect of intracisternal thyrotropin-releasing hormone on hepatic blood flow in rats

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Tamori, Keisuke, Masashi Yoneda, Kimihide Nakamura, and Isao Makino. Effect of intracisternal thyrotropin-releasing hormone on hepatic blood flow in rats. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G277–G282, 1998.—Central neuropeptides play a role in many physiological, regulatory processes through the autonomic nervous system. Thyrotropin-releasing hormone (TRH) is distributed in the central nervous system and acts as a neurotransmitter to regulate gastric functions through vagal-muscarinic pathways. The central effect of the TRH analog on hepatic blood flow was investigated in urethane-anesthetized rats. Hepatic blood flow was determined by the hydrogen gas clearance technique. Intracisternal injection of the stable TRH analog RX-77368 (5–100 ng) dose dependently increased hepatic blood flow with peak response at 15 min after the peptide was administered (net change from basal for vehicle and 5, 10, 100, and 500 ng RX-77368 was 2.0 ± 0.2, 8.9 ± 0.8, 19.4 ± 2.6, 32.6 ± 3.3, and 28.5 ± 6.8 ml·min⁻¹·100 g⁻¹, respectively), and this stimulatory effect returned to baseline at 90 min. The stimulation of hepatic blood flow by the intracisternally administered TRH analog was abolished by atropine methyl nitrate (0.15 mg/kg ip), indomethacin (5 mg/kg ip), N⁶-nitro-L-arginine methyl ester (10 mg/kg iv), and hepatic branch vagotomy but not by cervical spinal cord transection (C6 level). Intravenous injection of RX-77368 did not have any effect on hepatic blood flow. These results indicate that TRH acts in the central nervous system to stimulate hepatic blood flow through vagal-muscarinic and indomethacin- and nitric oxide-dependent pathways.

In the brain, TRH-immunoreactive nerve fibers and terminals and TRH receptors are localized in the hypothalamus and the dorsal vagal complex. The latter includes the dorsal nucleus of the vagus nerve and the nucleus of the solitary tract (11, 13), which are important sites for the autonomic nervous regulation of the digestive system, including the hepatobiliary system (3, 14). TRH causes a direct postsynaptic excitatory effect on preganglionic neurons in the dorsal nucleus of the vagus nerve and increases efferent vagal activity (12, 26). Moreover, the liver is richly innervated (14), and electrical stimulation of the hypothalamus and vagus nerve induces alterations in the hepatic circulation and microvascular filling pattern in the rat liver sinusoids (6, 27).

This evidence has prompted us to examine a possible role for TRH as a centrally acting neurotransmitter involved in the central nervous system regulation of hepatic microcirculation. Therefore, in this study, the effect of central TRH on hepatic blood flow was investigated in rats by the hydrogen gas clearance method.

MATERIALS AND METHODS

Animals. Male Wistar rats weighing 240–350 g (Charles River Japan, Yokohama, Japan) were housed in group cages under conditions of controlled temperature (22–24°C) and illumination (12:12-h light-dark 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.

Drugs. We used the following substances: the stable TRH analog RX-77368, p-Glu-His-(3,3-dimethyl)-Pro-NH₂, from Reckitt and Colman (Kingdom-upon-Hill, UK), and atropine methyl nitrate, indomethacin, and N⁶-nitro-L-argininemethyl ester (L-NAME), all from Sigma Chemical (St. Louis, MO). Although similar to natural TRH in binding characteristics on rat brain membranes, RX-77368 is more stable (4, 5). RX-77368 was aliquoted in 0.5% bovine serum albumin (Sigma Chemical) and 0.9% saline at a 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 and injected intracisternally in 10 µl using a 50-µl microsyringe (Hamilton, Reno, NV). Atropine methyl nitrate was dissolved in 0.9% saline and injected intraperitoneally (0.15 mg/kg), indomethacin was dissolved in 1% NaHCO₃ solution and injected intraperitoneally (5 mg/kg), and L-NAME was dissolved in 0.9% saline and injected intravenously (10 mg/kg) in a volume of 1.0 ml/kg.

Animal preparation. All experiments were done in rats anesthetized with urethane (1.5 g/kg ip). Tracheotomies were performed on the rats, and PE-260 tubing (Clay Adams, Parsippany, NJ) was inserted into the trachea to ensure an airway for administration of hydrogen in air. The right carotid artery was cannulated with PE-50 tubing (Clay Adams), and blood pressure was continuously monitored and...
recorded using a pressure transducer (Uniflow, Baxter, Valencia, CA), a pressure amplifier (PA-001, Star Medical, Tokyo, Japan), and a computer (PowerBook 150, Apple Computer, Cupertino, CA) equipped with a data recording and analysis system (MacLab, AD Instruments, Castle Hill, Australia). 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 secretion, avoiding the possibility of secondary influence of duodenal acidification and gastric distension to hepatic functions. 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 an Ag-AgCl reference electrode (MH-10, MT Giken) was placed into the lower peritoneal cavity against the lateral abdominal wall. The platinum contact electrode and a 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 0.9% saline (37°C, pH 7.4) to keep it moist.

Experimental design. After the surgical preparation was completed, the animal was left undisturbed for 60 min to allow temperature, heart rate, and blood pressure stabilization. Hepatic blood flow was determined by administering hydrogen gas in air through a tube placed over the tracheotomy tubing to achieve tissue saturation. The saturation of hydrogen gas required ~7–8 min and was ascertained by a plateau of the saturation curve. The basis for the measurement of blood flow by clearance of hydrogen gas is the Fick principle applied by Auckland et al. (1). The current is generated at the surface of the platinum electrode by oxidation of hydrogen to hydrogen ions and electrons. When rats breathe hydrogen, the current increases and reaches a plateau after the tissue becomes saturated with hydrogen. When the source of hydrogen is removed, the current slowly declines and returns to baseline. Because hydrogen can be removed only by blood flow perfusing the tissue, the rate at which desaturation occurs provides an estimate of blood flow. The desaturation curves were recorded and analyzed by a computer (Power Macintosh 8100, Apple Computer) equipped with an analog-to-digital conversion program (Chart V 3.0, AD Instruments). Hepatic blood flow was then calculated by a computer program that involved multiple iterations of a nonlinear least-squares regression and was expressed in milliliters per minute per 100 g. After two consecutive measurements of basal hepatic blood flow, either the TRH analog RX-77368 (5, 10, 100, or 500 ng) or 0.9% saline vehicle was injected intracisternally. The accuracy of the intracisternal injection was ascertained by the aspiration of cerebrospinal fluid before and after injection of the peptide. Hepatic blood flow was observed for 90 min after intracisternal injection every 15 min. In another experiment, RX-77368 (100 ng) or 0.9% saline vehicle was injected through the femoral wall. The platinum contact electrode and a reference electrode (MH-10, MT Giken) were placed into the lower peritoneal cavity against the lateral abdominal wall. The platinum contact electrode and a reference electrode were connected to a polarographic and amplifying unit (model DHM-3001, MT Giken) and a computer (PowerBook 150, Apple Computer, Cupertino, CA) 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 0.9% saline (37°C, pH 7.4) to keep it moist.

Results

Effect of intracisternal TRH analog on hepatic blood flow. Basal hepatic blood flow before peptide injection in urethane-anesthetized rats as measured by hydrogen gas clearance technique was 55.6 ± 1.1 ml·min⁻¹·100 g⁻¹ (n = 52). Although intracisternal injection of saline vehicle did not influence hepatic blood flow, when the stable TRH analog RX-77368 was injected intracisternally at a dose of 100 ng, it increased hepatic blood flow from the first 15-min observation period after the injection by 50 ± 11%, and the enhanced hepatic blood flow returned to baseline at 90 min (Fig. 1). The stimulatory action of RX-77368 on hepatic blood flow was dose related in doses ranging from 5 to 100 ng as observed at 15 min after peptide injection (net change from basal for vehicle and 5, 10, 100, and 500 ng RX-77368 was 2.0 ± 0.2, 8.9 ± 0.8, 19.4 ± 2.6, 32.6 ± 3.3, and 28.5 ± 6.8 ml·min⁻¹·100 g⁻¹, respectively) (Fig. 2). RX-77368 injected intravenously at 100 ng did not alter hepatic blood flow (Table 1).

Effect of atropine, indomethacin, L-NAME, hepatic branch vagotomy, and spinal cord transection on hepatic blood flow in response to intracisternal TRH analog. Hepatic branch vagotomy performed 120 min before the injection of the peptide or L-NAME (10 mg/kg) or 0.9% saline vehicle was injected intraperitoneally 15 min before, lindomethacin (5 mg/kg) or 1% NaHCO₃ vehicle was injected intraperitoneally 30 min before, L-NAME (10 mg/kg) or 0.9% saline vehicle was injected intravenously 15 min before, and hepatic branch vagotomy, spinal cord transection (C6 level), or respective sham operation was performed 120 min before the peptide was administered (23, 30). To evaluate the specificity of the effect of L-NAME on hepatic blood flow stimulated by central TRH analog, we administered L-arginine (800 mg/kg bolus iv followed by continuous 200 mg·kg⁻¹·h⁻¹ iv infusion throughout the experiment; Sigma Chemical) just before the administration of L-NAME to an additional group.

Statistical analysis. Results are expressed as means ± SE. Comparison of hepatic blood flow after peptide injection with the average of basal blood flow of two consecutive periods was calculated by analysis of variance (ANOVA) repeated measurement followed by Fisher’s protected least significant difference test. Multiple group comparisons were performed by ANOVA followed by Dunnett’s post hoc test. P < 0.05 was considered statistically significant.
before, atropine methyl nitrate (0.15 mg/kg) injected intraperitoneally 15 min before, indomethacin (5 mg/kg) injected intraperitoneally 30 min before, or L-NAME (10 mg/kg) injected intravenously 15 min before the peptide abolished the hepatic blood flow in response to intracisternal injection of RX-77368 (100 ng) (Fig. 3, A-D). Cervical cord transection at the C6 level performed 120 min before the peptide injection did not modify the stimulation of hepatic blood flow induced by intracisternal RX-77368 (100 ng) (Fig. 3E). L-Arginine (800 mg/kg bolus iv followed by continuous 200 mg·kg⁻¹·h⁻¹ iv infusion) abolished the inhibitory effect of L-NAME on central TRH analog-induced stimulation of hepatic blood flow (Fig. 3D). Although hepatic branch vagotomy, atropine, or indomethacin pretreatment did not influence basal hepatic blood flow before the intracisternal injection of the TRH analog, L-NAME pretreatment or spinal cord transection decreased it (Fig. 3).

Effect of intracisternal TRH analog on mean arterial blood pressure. Intracisternal injection of RX-77368 at 100 ng enhanced mean arterial blood pressure within 15 min, and this stimulatory effect was maintained over 90 min after the peptide injection (Table 2). Although atropine treatment 15 min before, indomethacin treatment 30 min before, L-NAME treatment 15 min before, or hepatic branch vagotomy 120 min before the peptide injection did not influence intracisternal TRH analog-induced increase of mean arterial blood pressure, spinal cord transection 120 min before the peptide injection inhibited the rise in mean arterial blood pressure induced by central injection of the TRH analog (Table 2). Although spinal cord transection suppressed and L-NAME treatment increased basal mean arterial blood pressure, hepatic branch vagotomy and atropine and indomethacin treatment did not modify basal mean arterial blood pressure (Table 2).

**DISCUSSION**

In the present study, we demonstrate that the stable TRH analog RX-77368 (5–500 ng) injected intracisternally stimulates hepatic blood flow in urethan-anesthetized rats as determined by the hydrogen gas clearance technique, which is known to measure only regional tissue blood flow. The stimulatory effect of intracisternal injection of RX-77368 is dose related in doses ranging from 5 to 100 ng. Intracisternal RX-77368 (100 ng) injection significantly increases hepatic blood flow with peak response at the first 15-min observation period, and thereafter the enhanced hepatic blood flow returns to baseline at 90 min. RX-77368 at a dose of 100 ng induces a change of 50 ± 11% (at 15 min after the peptide), which represents the maximal response because 500 ng of RX-77368 does not further stimulate hepatic blood flow. In contrast, when injected intravenously at the dose that was maximally effective when given intracisternally, RX-77368 did not influence hepatic blood flow. These results indicate that RX-77368, injected into the cisterna magna, acts in the central nervous system to stimulate hepatic blood flow and not through leakage into the peripheral circulation.

The pathways through which central administration of the TRH analog enhanced hepatic blood flow were investigated in the present study. The stimulated hepatic blood flow by intracisternal injection of TRH analog was abolished by hepatic branch vagotomy and atropine treatment, whereas cervical spinal cord transection had no effect. The results indicate that TRH acts centrally to stimulate hepatic blood flow in rats through vagal-muscarinic pathways. Previous reports also indicate that central TRH-induced stimulation of gastric acid, pepsin, and prostaglandin secretion and gastric mucosal blood flow is mediated through vagal-muscarinic dependent mechanisms (21, 25, 28). Furthermore, using an in vivo microscopic technique in rats, dilatation of the liver sinusoid after electrical vagal stimulation and acetylcholine application has been observed (6, 7). Taken together, these functional findings suggest that central TRH activates parasympathetic outflow to the digestive system. This is further supported by anatomic studies that show TRH immunoreactive fibers and terminals in the dorsal vagal complex (13). In addition, a high density of TRH receptors are also present in the dorsal vagal complex (11). Moreover, electrophysiological studies have provided...

**Table 1. Effect of intravenous injection of TRH analog RX-77368 on hepatic blood flow in urethan-anesthetized rats**

<table>
<thead>
<tr>
<th>Time after Intravenous Injection, min</th>
<th>-15</th>
<th>0</th>
<th>15</th>
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<th>60</th>
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<th>90</th>
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<td><strong>Hepatic blood flow, ml·min⁻¹·100 g⁻¹</strong></td>
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<td>Vehicle</td>
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<td>55±4</td>
<td>54±6</td>
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<td>52±4</td>
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<tr>
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<td>51±5</td>
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<td>53±5</td>
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Values are means ± SE of 5 rats per group. After 2 consecutive basal observations, vehicle or RX-77368 (100 ng) was injected intravenously. Hepatic blood flow was monitored thereafter for 90 min.
evidence that central TRH administration activates vagal efferent discharge in rats (18).

In regard to the stomach, central administration of TRH increases nitric oxide and prostaglandin release through activation of the vagus nerve (15, 29), and central TRH-induced stimulation of gastric mucosal blood flow is mediated through the nitric oxide pathway (23). In the present study, the role of prostaglandin and nitric oxide was investigated using the synthesis blockades indomethacin and L-NAME, respectively. Both indomethacin and L-NAME reversed the stimulatory effect of central TRH analog on hepatic blood flow, suggesting involvement of prostaglandin and nitric oxide in central TRH-induced modulation of hepatic

Fig. 3. Effect of hepatic branch vagotomy (A), atropine (B), indomethacin (C), N^6^-nitro-L-arginine methyl ester (L-NAME) (D), and spinal cord transection (E) on intracisternal TRH analog (100 ng)-induced enhancement of hepatic blood flow in urethan-anesthetized rats. Hepatic branch vagotomy and spinal cord transection at C6 level were performed 120 min before, atropine methyl nitrate (0.15 mg/kg ip) and L-NAME (10 mg/kg iv) were injected 15 min before, and indomethacin (5 mg/kg ip) was injected 30 min before intracisternal injection of RX-77368 (100 ng). L-Arginine (800 mg/kg bolus iv followed by 200 mg·kg^{-1}·h^{-1} continuous iv infusion) was administered just before L-NAME injection. For details, see Fig. 1 legend. Each point represents mean ± SE. *P < 0.05, **P < 0.01 compared with respective basal period. #P < 0.01 compared with vehicle-treated group.
pressure, but not hepatic blood flow, is blocked by over, central TRH-induced stimulation of systemic blood flow. It would be interesting to know whether vagal activation by central TRH stimulates hepatic nitric oxide and prostaglandin synthesis by direct measurement of hepatic prostaglandin and nitric oxide.

Hepatic blood flow is regulated by multiple factors (8). The liver is known to be richly innervated (14), and there has been abundant evidence indicating important roles for the central and autonomic nervous systems in hepatic function, including hepatic microcirculation (27). Very little is known about central neuropeptides acting as neurotransmitters to induce modulation of hepatic function. To date, only relation of central TRH to bile secretion has been reported (30, 31). In the present study, we have found that central administration of the TRH analog induces the stimulation of hepatic blood flow through vagal-muscarinic and prostaglandin- and nitric oxide-dependent pathways, and it is speculated that TRH is one of the most likely candidates to act in the brain as a neurotransmitter in the central regulation of hepatic circulation.

In summary, the present data indicate that the TRH analog injected intracisternally acts in the brain to stimulate hepatic blood flow in rats. Peptide action is mediated through vagal-muscarinic and prosta-
din- and nitric oxide-dependent pathways. The TRH analog provides a useful tool to further investigate brain sites of action to increase hepatic blood flow. In addition, it is of interest to know the role of endogenous TRH in hepatic blood flow by using the TRH antibody to elucidate the physiological relevance of central TRH in hepatic function.

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