Enhancement of Gastric Acid Output and Mucosal Blood Flow by Tripeptide Thyrotropin Releasing Hormone Microinjected into the Dorsal Motor Nucleus of the Vagus in Rats

Yasunobu OKUMA, Yoshitsugu OSUMI, Toshio ISHIKAWA* and Terunori MITSUMA**
Department of Pharmacology, Kochi Medical School, Nankoku, Kochi 781-51, Japan
*Department of Psychosomatic Medicine, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan
**Fourth Department of Internal Medicine, Aichi Medical University, Nagakute, Aichi, Aichi 480-11, Japan

Accepted November 1, 1986

Abstract—Central effect of thyrotropin releasing hormone (TRH) on gastric acid output and mucosal blood flow (MBF) was examined in urethane anesthetized rats. TRH, microinjected into the dorsal vagal complex [the dorsal motor nucleus of the vagus (NDV), the nucleus tractus solitarius and area postrema] induced dose-dependent (0.5–50 pmole) increases in gastric acid output and MBF. In contrast, 5 pmole TRH microinjected into various hypothalamic regions had no effect on these gastric parameters. Administration of TRH intraventricularly (i.c.v.) also increased these gastric parameters; however, about a 10 times higher dose of TRH was required to obtain the same order of excitatory effects seen with microinjection to the dorsal vagal complex. Application of anti-TRH serum to the dorsal vagal complex inhibited the increases in gastric acid output and MBF induced by TRH (i.c.v.). The effect of TRH applied to the dorsal vagal complex and i.c.v. were not modified by a concomitant administration of atropine. These results suggest that NDV is probably the site of action of TRH in inducing gastric hyperfunctions. The mode of action of TRH seems to be independent of cholinergic muscarinic mechanisms present in the NDV.

The tripeptide thyrotropin releasing hormone (TRH) is widely distributed in the central nervous system. (1–6). Centrally applied TRH produces various behavioral and pharmacological effects, independent of its action on pituitary TSH secretion. Included are increased locomotor activity (7, 8), analeptic properties in regulating levels of consciousness and arousal (9), suppression of feeding and drinking activity (10), alteration of sleep patterns (11), and centrally-mediated vasopressor response (12).

Tâché et al. (13, 14) found that intracisternally applied TRH caused a vagus-dependent stimulation of gastric acid secretion. Intracerebroventricular administration of TRH also elicited similar central effects on gastric acid secretion (15). However, sites and modes of action of this peptide in inducing vagally mediated gastric hyperfunctions remain obscure.

In the present study, therefore, effects of TRH microinjected into the dorsal vagal complex and various hypothalamic regions on gastric acid output and mucosal blood flow (MBF) were studied.

Materials and Methods

Male Wistar rats weighing 220–250 g were maintained in a room at 22–24°C under a constant day-night rhythm for 7–10 days and given food (laboratory chow, CE-2, Japan Clea Co.) and tap water, ad libitum. Prior to each experiment, all food but not water was withheld for 16 hr. With the rats...
under urethane anesthesia (1.1 g/kg, i.p.),
the femoral vein and femoral artery were
cannulated. The abdomen was opened by a
mid-line incision and a round-tip cannula (5
cm long, 0.5 cm outer diameter) connected
to a polyethylene tube was inserted into the
stomach via an incision into the duodenum
(1 cm distal from the pyloric sphincter).
The tip of the cannula lay just above the
pyloric sphincter and was held in place by
two ligatures around the duodenum, one at
the point of incision and the other close to
the pylorus, as described by Main and
Whittle (16). To remove the solid contents,
the stomach was flushed with saline, taking
care to avoid distention. After repeated
washings, two ml of solution prewarmed to
38°C was placed in the stomach at the
beginning of each 15 min collection period.
The composition of this solution was a 1.5
(v/v) mixture of glycine and mannitol
adjusted to 300 mosM and pH 3.5 by ad-
dition of 0.1 N HCl, according to Blair et al.
(17).

Acid output was determined by titration
of gastric samples to pH 7.0 with 0.01 N
NaOH, using a pH meter. MBF was measured
by the aminopyrine clearance technique
developed by Jacobson et al. (18) as based on
the pH partition theory of Shore et al. (19).
Thirty min after the priming dose of amino-
pyrine (30 mg/kg, i.p.), 6.6 mg/kg/hr infusion
through the femoral vein was started and was
continued throughout the experiment to
maintain a constant blood level of amino-
pyrine.

The animal was placed in a stereotaxic
instrument. Forty-five min were allowed for
stabilization of acid and aminopyrine con-
tents in the gastric juice after the onset of
aminopyrine infusion. To ensure that the rats
remained in good condition, the total volume
of blood samples was kept to a minimum.
Samples of arterial blood (0.5 ml) were
collected via a cannula inserted into the
femoral artery, once 60 min after the onset of
aminopyrine infusion and again immediately
after the end of the experiment. The plasma
level of aminopyrine at each 15 min interval
during the experimental period was estimated
by interpolation between two measured
points and was paired with each amino-
pyrine determination in the gastric juice. The
contents of aminopyrine in the plasma and
gastric juice sample were assayed according
to Brodie and Axelrod (20). The gastric MBF
was calculated from these measurements
(21).

Drugs were dissolved in saline and solution
containing the test substances was applied to
the lateral cerebral ventricle (AP: 7.5, L: 1.1,
H: 3.5 mm from the cortical surface) in a
volume of 10 μl through a stainless steel
micropipette (0.35 mm outer diameter). A
glass micropipette (70 μm outer diameter)
was used for the microinjections (in a volume
of 0.5 μl) of test substances into various
hypothalamic regions and an area adjacent
to the dorsal motor nucleus of the vagus
(NDV) (AP: −6.0, L: 0.5, H: 0.6), within the
dorsal vagal complex.

Microinjection of anti-TRH serum into the
dorsal vagal complex was performed
bilaterally (0.5 μl x2). Anti-TRH serum was
obtained as described (22). The antibody
which we used did not cross-react with TRH
analogues, amino acids, human pituitary
hormones or synthetic LH-RH (23).

After the experiment, the brain was
removed, fixed in 10% formalin, and the
frozen sections cut at 30 μm were stained
with cresyl violet for microscopic study of
microinjection sites. Results were expressed
as the mean±S.E. Statistical significance was
compared with the corresponding values of
control rats using Student's t-test for
unpaired comparisons.

Results
The mean basal gastric acid output and
MBF obtained from rats under urethane
anesthesia were 3.41±0.34 μEq/15 min
(n=56) and 1.81±0.13 ml/15 min (n=56),
respectively. Intraventricular administration
of TRH (0.5–500 pmole) induced dose-
dependent increases in both gastric acid
output and MBF, as previously noted (13, 15)
(Fig. 1). Microinjection of TRH (0.5–50
pmole) into the dorsal vagal complex induced
a dose-dependent increase in these gastric
parameters (Fig. 2). With this microinjection
of TRH into the dorsal vagal complex, doses
as little as one tenth of those given by the
i.c.v. route were adequate to obtain the same
order of responses (Fig. 3). On the other hand, 5 pmole of TRH given into various regions of the hypothalamus, such as the anterior hypothalamus, lateral hypothalamic area, ventromedial nucleus, paraventricular nucleus and dorsomedial nucleus did not...
Fig. 5. Effect of simultaneous administration of atropine with TRH on the gastric acid output. Test substances were microinjected into the dorsal vagal complex. Because of a slight increase in gastric acid output with vehicle (saline) alone, results were expressed as the net increase in gastric acid output from the respective basal levels with and without atropine. ○: 5 pmole TRH alone (n=5). ●: 5 pmole TRH plus 3 nmole atropine (n=4).

significantly affect the gastric acid output (data not shown). Pretreatment of anti-TRH serum microinjected into the bilateral dorsal vagal complex significantly inhibited the effects of TRH (50 pmol, i.c.v.) on gastric functions (Fig. 4).

As related to the induction of gastric hyperfunction by TRH, in another series of experiments, a possible interaction of TRH with the cholinergic muscarinic mechanism was examined at the level of the dorsal vagal complex. Microinjection of the vehicle (saline) into the dorsal vagal complex slightly increased the gastric acid output, as reported by Kadekaro et al. (24) and in our previous study (25), while microinjection of 3 nmole atropine alone into this region did not induce such an increase. Therefore, the results by administration of TRH were expressed as the net increase in gastric acid output from the respective basal levels, with and without atropine. The increasing effect of 5 pmole TRH microinjected into the dorsal vagal complex was not modified by a concomitant administration of 3 nmole atropine (Fig. 5). Similarly, the increasing effect of 50 pmole TRH applied i.c.v. was not modified by a concomitant administration of 30 nmole atropine (data not shown).

Discussion

In the present study, microinjection of TRH into the dorsal vagal complex induced marked increases in gastric acid output and MBF. These results correspond well with a recent finding by Rogers and Hermann that TRH injected into the NDV but not the area postrema produced significant increases in gastric secretion (26). Effective doses of TRH microinjected into the dorsal vagal complex, in the present study, were as little as one tenth compared with those given i.c.v. On the other hand, microinjections of this peptide into various regions of the hypothalamus were without effect. The application of anti-TRH serum into the bilateral dorsal vagal complex significantly increased in gastric acid secretion and MBF by i.c.v. applied TRH.

Immunohistochemical and immunochemical studies clearly demonstrated the existence of TRH in the medulla oblongata, particularly in parts of the NDV, hypoglossal nucleus and the nucleus tractus solitarius (4, 5). Therefore, the site of action of TRH causing gastric hyperfunctions is probably the NDV and/or an area within the dorsal vagal complex.

Yarbrough (27) suggested that some of the pharmacological actions of TRH can be accounted for through interactions with central cholinergic mechanisms by following observations: 1) the reduction of pentobarbital sleeping time by TRH was reduced by intracisternally administered atropine (28), 2) scopolamine completely blocked the EEG activation caused by TRH (29), and 3) TRH enhanced uptake of $^3$H-choline and its conversion to $^3$H-acetylcholine in striatal slices (30). In addition, Lahann and Horita (31) reported that centrally applied TRH increased colonic motor activity in rabbits. This effect of TRH was reduced by atropine given i.c.v. We recently found that bethanechol (cholinergic muscarinic agonist) microinjected into the dorsal vagal complex increased gastric acid secretion (25). Therefore, a possible interaction of TRH with cholinergic muscarinic mechanisms in inducing gastric hyperfunctions was examined.

The effects of TRH applied to the dorsal vagal complex or i.c.v. were not modified by a concomitant administration of atropine.
The dose of atropine used in the present study was 3 nmol, and this dose microinjected into the dorsal vagal complex completely blocked the increase in gastric acid secretion induced by electrical stimulation of the lateral hypothalamic area (25). Therefore, TRH probably acts in the NDV, independent of cholinergic muscarinic mechanisms and stimulates gastric acid secretion. Microinjection of test substances in the present experiments was given into an area as adjacently as possible to the NDV within the dorsal vagal complex. However, the possibility that the site of action was the nucleus tractus solitarius but not the NDV cannot be ruled out, since the NDV and the nucleus tractus solitarius are in close proximity, and there is a functional circuit between these two nuclei.

Acknowledgements: This work was supported in part by Grant-in-Aid for Scientific Research No. 59570085 from the Ministry of Education, Science and Culture, Japan.

References


