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Methiothepin attenuates gastric secretion and motility effects of vagal stimulants at the dorsal vagal complex

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Abstract

Methiothepin, a nonselective 5-HT receptor antagonist was utilized to explore the 5-HT modulation of dorsal vagal complex–TRH (thyrotropin releasing hormone) analogue stimulated gastric functional parameters. Intracisternal methiothepin pretreatment (200, 0.1 nmol) produced significant inhibition (70%, 44%, respectively) of the TRH analogue [*p*-Glu-His-(3,3′ -dimethyl)-Pro NH2; RX 77368 (12 pmol)]-induced gastric acid output compared to vehicle pretreatment. Intracisternal pretreatment with methysergide (nonspecific 5-HT receptor antagonist) or combined cyanopindolol (5-$HT_{1A\ and\ 1B}$ receptor antagonist) + ritanserin (receptor antagonist of the 5-HT_2 family) did not alter the dorsal vagal complex–RX 77368 response. Unilateral dorsal vagal complex pretreatment with methiothepin (50 nmol/50 nl) attenuated ipsilateral dorsal vagal complex–TRH analog (12 pmol) induced gastric secretory response by 57%. The gastric secretagogue response to stimulation of the raphe obscurus (mediated by TRH release into the dorsal vagal complex) was inhibited 50% by pretreatment with intracisternal dorsal medullary methiothepin (0.1 nmol/10 μl). Intracisternal methiothepin (200 nmol/20 μl) also attenuated (a) dorsal vagal complex–glutamate (60 nmol/30 nl) stimulated gastric acid secretion and (b) gastric motility stimulated by dorsal vagal complex–RX 77368 (12 pmol/30 nl). The data suggest that other properties of methiothepin, alone or in addition to its 5-HT receptor antagonist effect, mediate its inhibitory actions at the dorsal vagal complex.

Keywords: Vagus; Raphe nucleus; Brain stem; Autonomic nervous system; 5-HT (5-hydroxytryptamine, serotonin)

1. Introduction

A large body of evidence from neuroanatomical and physiological approaches suggest that thyrotrophin releasing hormone (TRH) is a neuronal chemical messenger acting at the dorsal vagal complex to regulate gastric function via vagal-mediated mechanisms (Yang et al., 1993; Somiya and Tonoue, 1984; Rinaman and Miselis, 1990; Rinaman et al., 1989; Kaneko and Tache, 1995; Kaneko et al., 1995a). TRH immunoreactive terminals in the dorsal vagal complex originate from the caudal raphe nuclei (nucleus raphe obscurus and nucleus raphe pallidus) and the parapyramidal region (Palkovits et al., 1986; Lynn et al., 1991). These fibers co-localize serotonin (5-hydroxytryptamine; 5-HT) with TRH among other chemical messengers (Kachidian et al., 1991; Johansson et al., 1981). TRH microinjection into the dorsal vagal complex produces changes in gastric functional parameters such as increases in gastric acid secretion, motility, pepsin and mucous secretion (Tache et al., 1995). TRH immunoreactive terminals have been shown to form asymmetric (excitatory) synapses with dendrites and cell bodies of gastric motoneurons, whereas the synapses with nucleus tractus solitarius neurons are symmetric (inhibitory) (Rinaman et al., 1989). It has been shown in vivo that TRH inhibits the firing rate of identified solitary tract cells, whereas it stimulates the firing rate of dorsal motor nucleus cells (Rogers and McCann, 1989; McCann et al., 1989). It is hypothesized that the effects of TRH on gastric function are produced by excitation of dorsal motor nucleus neurons coupled with the disfacilitation of the normal inhibition of solitary tract neurons (McCann et al., 1989).

Stimulation of the caudal raphe nuclei produces changes in gastric functional parameters that are likely mediated by TRH release at the dorsal vagal complex (Yang et al., 1993; Okumura et al., 1995a,b; Kaneko and Tache, 1995; Garrick et al., 1994). Similarly, 5-HT is released in the dorsal vagal

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complex upon electrical or chemical stimulation of the nucleus raphe obscurus (Mohammed et al., 1995; Brodin et al., 1990). Co-injection of 5-HT with TRH in the dorsal vagal complex augments the TRH-induced increase in gastric acid secretion (Yoneda and Tache, 1995; Chi et al., 1996). Recent work has shown that activation of receptors of the 5-HT_1 and 5-HT_2 family may be involved in the augmentation of the TRH analog stimulated gastric acid secretory response at the dorsal vagal complex (Yoneda and Tache, 1995; Varanasi et al., 1997). This study characterized the modulation of the stimulated gastric functional parameters by the 5-HT receptor antagonist, methiothepin (Barnes and Sharp, 1999).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 400–500 g (20–24 weeks) were fasted overnight for a period of 18–24 h. During this time, they had free access to water. All animal experimentation protocols were in compliance with the rules of the Institutional Laboratory Animal Care and Use Committee of the Ohio State University, Columbus, OH.

2.2. Drugs

The TRH analogue *p*-Glu-His-(3,3′ -dimethyl)-Pro NH2 (RX 77368) was obtained from Reckitt and Coleman (Kingston-upon-Hull, UK) and a stock solution was prepared in normal saline at a concentration of 12 pmol/30 nl. 1-[10,11-dihydro-8-(methylthio)dibenzo[b,f]thiepin-10-yl]-4-piperazine mesylate [methiothepin] and (−)-9,10-didehydro-*N*-[1-(hydroxymethyl)propyl]-1,6-dimethyl-ergoline-8-carboxamide maleate (methysergide) were obtained from Research Biochemicals (Natick, MA). 6-[2-[4-[bis(4-Fluorophenyl)methylene]-1-piperidinyl]ethyl]-7-methyl-5*H*-thiazolo[3,2-a]pyrimidin-5-one (ritanserin) was obtained from Research Biochemicals. (−)4-(3-t-Butylamino-2-hydroxypropoxy) indol-2-carbonitrile hemi fumarate (cyanopindolol) was obtained from Biomol Research laboratories (Plymouth meeting, PA). L-Glutamic acid (glutamate) was obtained from Sigma (St. Louis, MO). Methiothepin and cyanopindolol were prepared fresh everyday by dissolving in normal saline. Methysergide and ritanserin were suspended in 0.1% Tween 80.

2.3. Surgery

2.3.1. Gastric acid studies

2.3.1.1. Intracisternal pretreatment studies. Overnight-fasted, urethane-anaesthetized rats were equipped with a tracheal tube and their esophagus was ligated. Through a midline abdominal incision, the stomach was exposed, the pylorus ligated and a double lumen cannula inserted into the forestomach. The rat was then put on a stereotaxic instrument (David Kopf, Tujunga, CA) and a midline incision was given on the back of the neck and the atlanto-occipital membrane exposed after retracting the overlying muscles. Gastric acid collections were made every 10 min by flushing the cannula with 5 ml of water followed by 5 ml of air. The acid content was titrated to pH 7.00 by 0.05 N NaOH utilizing an autotitrator (Radiometer, Copenhagen). After a stable baseline of gastric acid secretion was obtained, the atlanto-occipital membrane was pierced with a beveled Hamilton syringe and 20 μl of either methiothepin (200 nmol–1 pmol) or vehicle were delivered in the intracisternal (i.c.) space. A separate set of studies was conducted assessing the effect of i.c. methysergide (200 nmol) or the combination of cyanopindolol/ritanserin (200 nmol each) due to the different vehicle necessary to prepare the drugs. Two minutes after the i.c. injection, the atlanto-occipital membrane was incised and the dorsal brain stem exposed by removing the meninges and the occipital plate. Twenty minutes after the i.c. injection, RX77368 (12 pmol/30 nl) was microinjected into the right dorsal vagal complex by standard pressure microinjection techniques. The co-ordinates utilized were 0.2 mm (right), 0.2 mm (anterior), 0.6 mm (ventral) to the calamus scriptorius. In one set of studies, 20 min after i.c. injection (vehicle or methiothepin), glutamate (60 nmol/30 nl) was microinjected into the right dorsal vagal complex.

2.3.1.2. Dorsal vagal complex pretreatment studies. The surgery for this set of studies was similar to that described for the intracisternal pretreatment studies. After a stable baseline of gastric acid secretion was obtained, the dorsal brain stem was exposed and the pretreatment with vehicle (saline) or methiothepin (50 nmol) was performed by microinjection into the dorsal vagal complex (50 nl). Twenty minutes after the pretreatment, a second ipsilateral dorsal vagal complex microinjection was performed with the TRH analog (12 pmol/30 nl).

2.3.1.3. Effect of intracisternal methiothepin on raphe obscurus stimulated gastric acid output. Urethane-anesthetized rats were equipped with a double lumen cannula in the forestomach for collecting acid. The dorsal brain stem was exposed after placing them in a stereotaxic frame, as described previously. Basal measurements of three 15-min gastric secretory measurements were performed. Topical application (10 μl) of either vehicle or methiothepin (0.1 nmol) was made just above the exposed area postrema, and one 15-min period later, kainic acid (100 pmol/10 nl) was administered into the nucleus raphe obscurus using previously described methods (Mohammed et al., 1995). Gastric secretion was collected every 15 min for eight periods after nucleus raphe obscurus stimulation in both groups.

2.3.1.4. Statistics. After dorsal vagal complex (or nucleus raphe obscurus) microinjection, a 2-h cumulative gastric

acid output was obtained. The acid output was expressed in μmol as mean ± S.E.M. To assess differences between groups, the methiothepin dose response data was analyzed by a one-way analysis of variance followed by a post hoc Neumann–Keuls test. All other comparisons were analyzed via Student's *t*-test.

2.3.2. Gastric motility studies

Overnight-fasted, urethane-anaesthetized male Sprague–Dawley rats (400–500 g) had strain gauges (RB products, Madison, WI) sewed on the anterior part of their stomach in the region of the antrum, and oriented to monitor the contractions of the circular muscles. The strain gauge signals were amplified by a Wheatstone bridge-based amplifier. The motility signals were then recorded on a polygraph (model 7D, Grass instruments), from which the motility index was calculated to quantitate the motility. The rats were then placed on a stereotaxic instrument and after a stable baseline motility was obtained, the atlanto-occipital membrane was pierced with a Hamilton syringe and 20 μl of vehicle or methiothepin (200 nmol) were delivered intracisternally. Two minutes after the intracisternal pretreatment, the hindbrain was exposed by incising the atlanto-occipital membrane and the arachnoid mater. Twenty minutes after the i.c. injection, RX77368 microinjection (12 pmol/30 nl) was made in the left dorsal vagal complex by standard pressure microinjection techniques. The co-ordinates utilized were 0.2 mm (left), 0.2 mm (anterior), 0.6 mm (ventral) to the calamus scriptorius. The left dorsal vagal complex was chosen in the study involving gastric motility since the anterior surface of the stomach, on which the strain gauge was sewn, is innervated by the left vagus (Ewart et al., 1988).

2.3.2.1. Protocol for recording motility. A 20-min baseline motility was recorded followed by pretreatment with vehicle or methiothepin. After recording motility for 20 min, RX 77368 was injected into the left dorsal vagal complex. The motility was recorded for a further 20 min. The motility index was calculated and used to compare the results of treatment with baseline motility. The motility index was calculated according to the formula of Ormsbee and Bass (1976).

$$\text{Motility index (MI)} = nA_{1-2} + 2nA_{2-4} + 4nA_{4-8} + 8nA_{8-16} + 16nA_{>16}$$

where nA_{1-2} denotes the number of contractions that is just detectable (at least one division) to that which is twice (two divisions) that of just detectable, etc.

2.3.2.2. Statistics. The motility indices were calculated for the 20-min periods representing the baseline, intracisternal (i.c.) pretreatment and the treatment-induced motility. The baseline motility indices were normalized to a value of 100 and the i.c. pretreatment and treatment (dorsal vagal complex–RX 77368)-stimulated motility indices were expressed as a percentage of the baseline motility. The data was expressed as mean ± S.E.M. The Student's *t*-test was applied to compare the motility index after RX 77368 injection versus basal motility index in both vehicle- and methiothepin-pretreated animals.

3. Results

3.1. Intracisternal pretreatment studies

Basal acid secretion was low (approximately 2 μmol/10 min) in all groups (Fig. 1). Injection of the TRH analogue RX 77368 (12 pmol/30 nl) into the dorsal vagal complex, 20 min after intracisternal vehicle pretreatment, produced an immediate enhancement in gastric acid secretory response, peaking at 30 min postinjection with a value of 33 μmol/10 min (Fig. 1). The net cumulative gastric acid output was 210 ± 28; (μmol/2 h, $n = 14$) in the vehicle pretreatment group (Fig. 2). Intracisternal methiothepin pretreatment with 200 nmol or 0.1 nmol, attenuated dorsal vagal complex–TRH analogue-stimulated gastric acid output by 70% and 45%, respectively, as compared to vehicle pretreatment [net cumulative gastric acid output (μmol/2 h): methiothepin (200 nmol) + RX 77368, 63 ± 13, $n = 5$; methiothepin (0.1 nmol) + RX 77368, 116 ± 30, $n = 6$]. Lower doses of intracisternal methiothepin (10 or 1 pmol) did not significantly alter the TRH analogue-elicited response (Fig. 2). In contrast, intracisternal methysergide at a high dose (200 nmol) or combined cyanopindolol + ritanserin (200 nmol each) were ineffective to significantly alter the net gastric secretory response to RX 77368 (12 pmol/30 nl) at the dorsal vagal complex (Fig. 3).

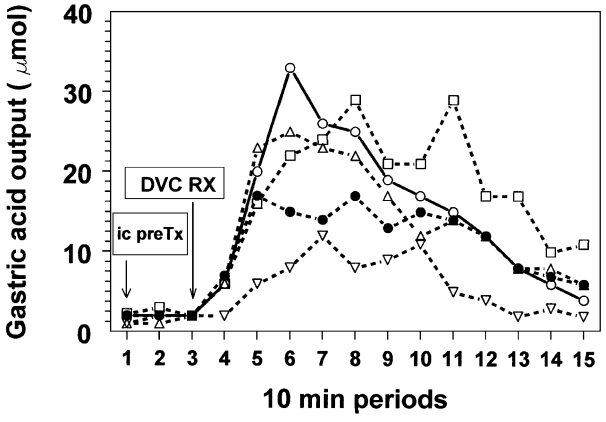

Fig. 1. Time course of the effect of intracisternal methiothepin (MT) pretreatment on dorsal vagal complex administered RX 77368 (RX; 12 pmol) stimulated gastric acid secretion. The values represent the mean responses at each time point. Standard errors of the mean were removed to enhance clarity (averaged 27% of the mean). -○- vehicle + RX, -□- MT (0.001 nmol) + RX, -△- MT (0.01 nmol) + RX, -●- MT (0.1 nmol) + RX, -▽- MT (200 nmol) + RX.

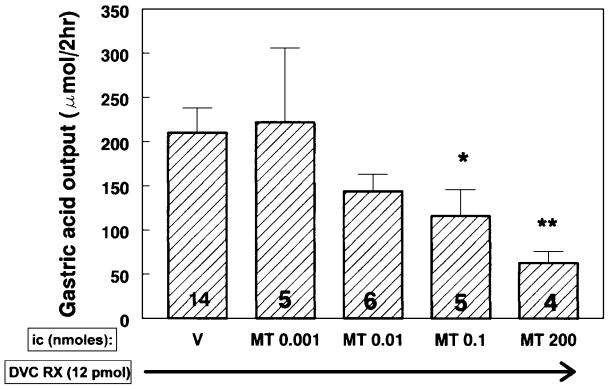

Fig. 2. The effect of intracisternal pretreatment with methiothepin on dorsal vagal complex administered RX 77368 (12 pmol) stimulated net gastric acid output. The intracisternal injections were in a 20-μl volume at the doses indicated on the abscissa. The 2-h net cumulative gastric acid output, after dorsal vagal complex microinjections are expressed as mean ± S.E.M. (μmol/2 h) for the number of animals indicated within the bar. ** $P<0.01$, * $p<0.05$.

To study the specificity of the inhibitory effect of methiothepin, its effect on glutamatergic stimulation of vagal-dependent gastric function was examined. L-Glutamate injected into the right dorsal vagal complex, following vehicle pretreatment, stimulated net gastric acid secretion and produced a cumulative acid output of 82 ± 21, $n=7$ (μmol/2 h; Fig. 4). Intracisternal methiothepin (200 nmol/20 μl) pretreatment significantly attenuated the L-glutamate stimulated gastric acid secretion by 63%.

3.2. Gastric motility studies

The average 20-min basal motility index for rats pretreated with vehicle was 82 ± 7 ($n=4$), which was normalized to a value of 100 (Fig. 5). Intracisternal pretreatment

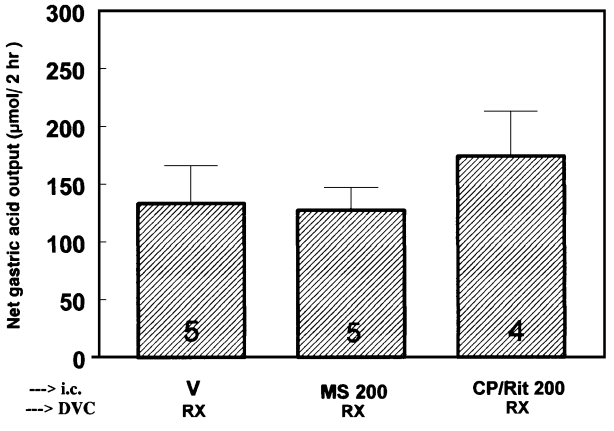

Fig. 3. The effect of intracisternal pretreatment with methysergide (MS 200) or cyanopindolol/ritanserin combination (CP/Rit 200) on dorsal vagal complex–RX 77368 (12 pmol) stimulated net gastric acid output. The intracisternal injections were 200 nmol of each agent in a 20-μl volume. The 2-h net cumulative gastric acid output, after dorsal vagal complex microinjections are expressed as mean ± S.E.M. (μmol/2 h) for the number of animals indicated within the bar.

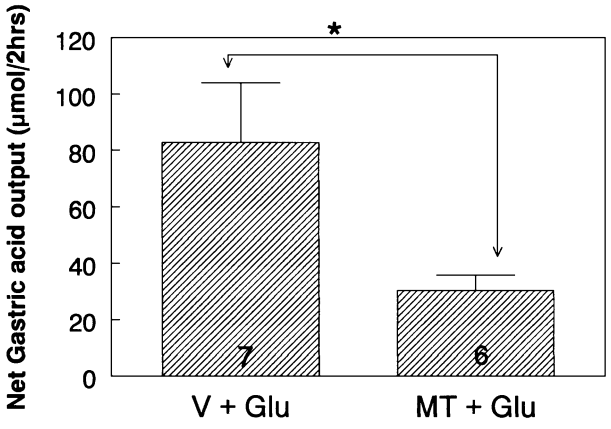

Fig. 4. The effect of intracisternal pretreatment with methiothepin (200 nmol) on dorsal vagal complex glutamate (60 nmol/30 nl) stimulated net gastric acid output. The intracisternal injections were in a 20-μl volume. The 2-h net cumulative gastric acid output, after dorsal vagal complex microinjections are expressed as mean ± S.E.M. (μmol/2 h) for the number of animals indicated within the bar. * $p<0.05$.

with vehicle did not significantly effect the motility index (106 ± 14% of baseline). RX 77368 (12 pmol/30 nl) microinjection into the dorsal vagal complex significantly increased the motility index to 249 ± 52% of baseline (Fig. 5). For the animals in the test group, i.c. methiothepin pretreatment (200 nmol/20 μl) did not change the motility index significantly (94 ± 18% of baseline, $n=5$) as compared to the basal (100; Fig. 5). However, RX 77368 (12 pmol/30 nl) microinjection into the dorsal vagal complex

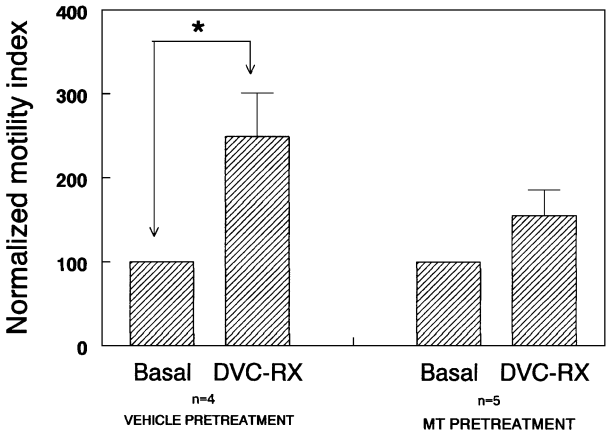

Fig. 5. The effect of intracisternal pretreatment with methiothepin (200 nmol) on dorsal vagal complex–RX 77368 (12 pmol/30 nl) stimulated gastric (antral) motility. The 20-min motility indices during basal (basal) and dorsal vagal complex–RX 77368 (dorsal vagal complex–RX) stimulation are expressed as normalized values for vehicle and methiothepin pretreated groups. The basal motility index was normalized to a value of 100 and the motility indices during pretreatment (vehicle or methiothepin) and dorsal vagal complex–RX 77368 stimulation were expressed as a percentage of the basal value for each rat. The bars denote normalized 20 min basal (mean ± S.E.M.) and dorsal vagal complex–RX 77368 stimulated (mean ± S.E.M.) motility index for the rats pretreated with vehicle and methiothepin ($P<0.05$; comparison between basal and dorsal vagal complex–RX 77368 stimulated motility).

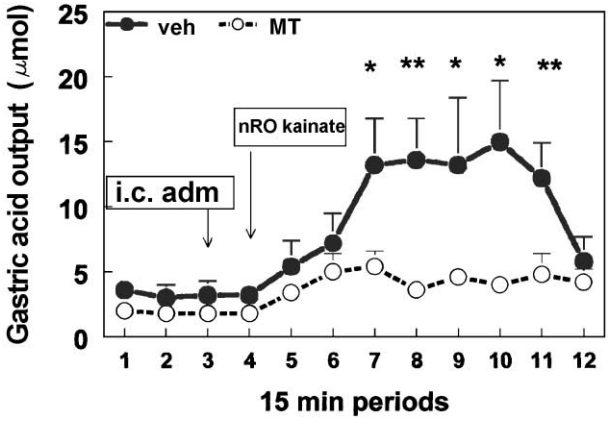

Fig. 6. The effect of topical pretreatment with methiothepin (MT; 0.1 nmol/10 nl) on nucleus raphe obscurus kainate (100 pmol/10 nl) stimulated gastric acid output. The 15-min gastric acid measurements in each group are expressed as mean ± S.E.M. (μmol) for the five animals in each group. ** $p < 0.01$, * $p < 0.05$.

after methiothepin pretreatment failed to significantly increase the motility index as compared to baseline motility (motility index = 155 ± 31%, $n = 5$; Fig. 5).

3.3. Effect of intracisternal methiothepin on nucleus raphe obscurus-stimulated gastric acid output

Several lines of evidence suggest that the gastric effects of raphe stimulation are mediated by TRH release at the dorsal vagal complex (Kaneko et al., 1995a,b; Kaneko and Tache, 1995; Chi et al., 1996). Thus, the effect of intracisternal methiothepin (100 pmol/10 μl) on nucleus raphe obscurus-stimulated gastric acid was assessed. The stimulatory effects of kainate (100 pmol/10 nl) injection into the nucleus raphe obscurus on gastric acid output was inhibited 58% by i.c. methiothepin administration (Fig. 6) [net cumulative gastric acid output; μmol/2 h: i.c. vehicle + nucleus raphe obscurus kainate, 88 ± 13 ($n = 5$); i.c. methiothepin + nucleus raphe obscurus kainate, 37 ± 4 ($n = 5$), $p < 0.01$].

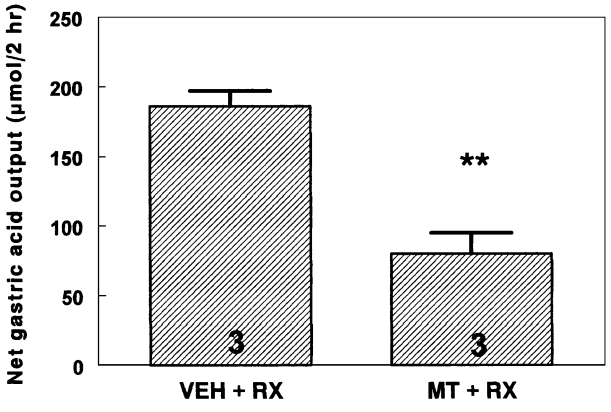

Fig. 7. The effect of dorsal vagal complex pretreatment with methiothepin (MT; 50 nmol/50 nl) on dorsal vagal complex–RX 77368 (12 pmol/30 nl) stimulated gastric acid output. The net 2-h cumulative gastric acid output, after dorsal vagal complex microinjections are expressed as mean ± S.E.M. (μmol/2 h) for the animals indicated within the bar. * $P < 0.05$.

3.4. Dorsal vagal complex pretreatment studies

Unilateral dorsal vagal complex pretreatment with vehicle (50 nl), followed 20 min later with ipsilateral dorsal vagal complex–RX 77368 (12 pmol/30 nl) microinjection produced a net gastric acid output of 186 ± 11 μmol/2 h, $n = 3$ (Fig. 7). Dorsal vagal complex pretreatment with methiothepin (50 nmol/50 nl), produced a 57% inhibition of ipsilateral TRH analogue-stimulated gastric acid output [80 ± 15 μmol/2 h, $n = 3$, Fig. 7, $p < 0.05$].

4. Discussion

The principal finding of this study was that methiothepin antagonized the effect of two gastric stimulants whose effects are mediated via the dorsal vagal complex. This inhibitory effect of methiothepin was achieved by intracisternal as well as dorsal vagal complex pretreatment, suggesting that the dorsal vagal complex is a site of the inhibitory action of methiothepin.

Methiothepin is a nonselective 5-HT receptor antagonist with moderate to high affinity for receptors of the 5-$HT_{1,2,5,6 \text{ and } 7}$ families (Barnes and Sharp, 1999). Methysergide parallels the profile of methiothepin with regard to exhibiting moderate to high affinity for each of the aforementioned 5-HT receptor families (Barnes and Sharp, 1999; Hoyer et al., 1994) (Table 1). The lack of effect of methysergide at a high dose (200 nmol), which produces millimolar concentrations in the cerebral spinal fluid, provides compelling evidence that the 5-HT receptor ligand property of methiothepin alone may not explain its TRH analogue antagonistic action. The lack of effect of combined cyanopindolol/ritanserin at high dose (200 nmol each; Fig. 3) lends further support to the notion that inhibition of receptors of the 5-HT_1 or 5-HT_2 family alone do not mediate the inhibitory action of methiothepin on gastric stimulants acting at the dorsal vagal complex. In agreement with these findings are data from a previous study showing the lack of effect of ketanserin (5-HT_2 receptor antagonist) or spiperone (5-HT_{1A} and 5-HT_2 receptor antagonist) to inhibit dorsal vagal complex-injected RX 77368 (25 pmol)-stimulated gastric acid secretion (Yoneda and Tache, 1995).

It is unclear which property of methiothepin mediates the inhibitory action at the dorsal vagal complex. The minimal effective dose of intracisternal methiothepin (100 pmol)

Table 1
Receptor affinities (pK_I) of methiothepin and methysergide for 5-HT receptor subtypes (references—Barnes and Sharp, 1999; Hoyer et al., 1994)

	5-HT_1	5-HT_2	5-HT_5	5-HT_6	5-HT_7
Methiothepin	7.7–8.1	8.2–9.0	7.0–8.0	8.7	9.0–9.4
Methysergide	6.5–7.0	9.0	7.0	6.4	8.0

would result in initial micromolar concentrations in the cerebral spinal fluid. Methiothepin possesses nanomolar affinity for histamine H_1, dopamine D_2 and α_1 and α_2 adrenoceptors (Hoyer et al., 1994; Leysen et al., 1981). Previous study of TRH-mediated central nervous system effects on gastric or cardiovascular function suggest no interaction between intracisternal TRH and systemic dopamine D_2 or histamine H_1 receptor antagonists (Tachibana et al., 1995; Tache et al., 1985; Nurminen et al., 1991), however, interactions with catecholamine systems have been described. Catecholamine depletion abolished intracisternal TRH stimulated gastric secretion (Tache et al., 1985). However, phentolamine (Maeda-Hagiwara and Watanabe, 1985), yohimbine (Maeda-Hagiwara et al., 1984; Maeda-Hagiwara and Watanabe, 1985) or prazosin (Maeda-Hagiwara and Watanabe, 1985) do not inhibit intracisternal TRH-induced gastric effects. Interpretational limitations exist with these studies involving adrenergic receptor antagonists because of the systemic administration of the drugs and the intracisternal administration of TRH (TRH has several medullary sites of action to stimulate gastric function (Ishikawa et al., 1988; Garrick et al., 1992)). In sum, the effects of modulation of noradrenergic systems on TRH effects at the dorsal vagal complex has not been thoroughly examined. It is also plausible that a combination of effects on several receptor systems is responsible for the inhibitory methiothepin effect. In a recent study, methiothepin also reversed the effects of TRH on pudendal motorneurons (Holmes et al., 2001). Further work will be required to elucidate the mechanism of methiothepin to attenuate stimulatory responses mediated by agents acting at the dorsal vagal complex.

The present study showed that the effect of methiothepin in attenuating the gastric acid secretory response is not specific for the dorsal vagal complex–TRH analog stimulated acid secretory response. Intracisternal methiothepin (200 nmol) pretreatment also attenuated the dorsal vagal complex–glutamate (60 nmol/30 nl) stimulated gastric acid secretion. Glutamate acts on neuronal cell bodies to increase their firing rate. Therefore, one of the potential sites of action of glutamate (and possibly methiothepin) are the cell bodies in the dorsal motor nuclei. This suggests that stimulation of gastric acid secretion mediated through the dorsal vagal complex by TRH or glutamate, involves interaction with one or more systems which are modified by methiothepin.

Moreover, the effect of methiothepin in attenuating the dorsal vagal complex–TRH analog response is not specific for the gastric acid secretory effect. Methiothepin also attenuates the dorsal vagal complex–TRH analog stimulated antral motility. An interesting, unresolved issue is the effect of methiothepin on vagally mediated gastroprotective effects mediated by low-dose TRH or nucleus raphe obscurus-stimulation acting at the dorsal vagal complex (Kaneko et al., 1995b). This vagal cholinergic effect, which is mediated by dorsal vagal complex–TRH at doses lower than those involved in stimulating gastric acid, is prostaglandin, nitric oxide (NO) and calcitonin gene related peptide (CGRP)-dependent (Tache et al., 1995).

The site of action of methiothepin within the dorsal vagal complex to antagonize excitatory gastric responses emanating from it also remains an open question. Vago–vagal reflex circuits in the medulla are responsible for the overall coordination and control of "digestive processes" (Wood, 1994; Berthoud et al., 1991). These brainstem circuits, comprised of vagal afferent fibers from chemo- and mechano-receptive sensory endings, interneurons of the nucleus of the solitary tract and visceral efferents originating from the neurons of the dorsal motor nucleus of the vagus are responsible for the smooth coordination of the digestive processes carried out by the proximal digestive tract. Further experiments will be necessary to delineate the site of action of methiothepin within the dorsal vagal complex.

Related studies have shown that 5-HT augmentation of the TRH response at the dorsal vagal complex is mediated by receptors of the 5-HT_1 and 5-HT_2 family (Varanasi et al., 1997; Yoneda and Tache, 1995). An electrophysiologic explanation for this interaction was provided by recent work suggesting that activation of 5-HT_{1A} receptors, unmasked by TRH-evoked increased cAMP levels within presynaptic neurons of the dorsal vagal complex, decrease GABAergic inhibitory postsynaptic currents within the dorsal vagal complex (Browning and Travagli, 2001). However, the gastric stimulatory effects of dorsal vagal complex administered TRH agonists alone are not altered by 5-HT_1 or 5-HT_2 receptor antagonists other than methiothepin (present study and Yoneda and Tache, 1995).

This study provides evidence that methiothepin acts to antagonize excitatory vagal responses initiated at the dorsal vagal complex. Further work needs to be carried out to illuminate (1) the site of action of methiothepin within the dorsal vagal complex (2) the receptor system(s) mediating the inhibitory effect and (3) whether vagally mediated systems activating gastroprotection against necrotizing agents are also attenuated by methiothepin.

Acknowledgements

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