5-CT or DOI augments TRH analog-induced gastric acid secretion at the dorsal vagal complex

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Varanasi, Sridhar, Jinhan Chi, and Robert L. Stephens, Jr. 5-CT or DOI augments TRH analog-induced gastric acid secretion at the dorsal vagal complex. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1607–R1611, 1997.—Serotonin (5-HT) interacts with thyrotropin-releasing hormone (TRH) at the dorsal vagal complex (DVC) to augment TRH-induced stimulation of gastric acid secretion. To investigate the 5-HT receptor family involved in the augmentation response, prototypic 5-HT receptor-selective agonists (146 pmol) were coinjected with the TRH analog RX-77368 (RX; 12 pmol) into the rat DVC in a 30-nl volume. The DVC coordinates were 0.2 mm anterior, 0.2 mm right, 0.6 mm ventral with respect to the calamus scriptorius. Coinjection of RX with the 5-HT agonists 5-carboxyamidotryptamine (5-CT) or (-)-1-(4-iodo-2,5-dimethoxyphenyl)-2-amino propane hydrochloride (DOI; 5-HT2 agonist) produced a 183 or 103% increase in gastric acid output compared with the RX injection alone. In contrast, coinjection of 2-methyl-5-HT (5-HT3 agonist) with RX produced no effect on RX-induced increase in gastric acid secretion. Moreover, coinjection of SC-53116 (5-HT4 agonist) decreased the gastric acid output by 45% compared with the RX response itself. Examination of the RX/5-HT agonist coinjection response in more rostral regions of the DVC using the same doses (5-CT/RX or DOI/RX) revealed that only 5-CT was effective in producing the augmented response to TRH analog. The results suggest that activation of 5-CT- or DOI-sensitive receptors augments, and of 5-HT4 receptors inhibits, the gastric acid response to TRH analog injected into the DVC. Thus the integrated response to several serotonin receptor subtypes may mediate changes to the TRH response induced by 5-HT at the DVC.

SEVERAL LINES OF EVIDENCE implicate thyrotropin-releasing hormone (TRH) as an important chemical messenger in the dorsal vagal complex (DVC: dorsal motor nucleus and nucleus of the solitary tract). TRH has been demonstrated in the nerve terminals of the DVC by immunohistochemical techniques (25, 26). These neurons originate from caudal raphe nuclei (raphe obscurus and raphe pallidus) and from the parapyramidal region of the medulla (16, 24). Relatively high concentrations of TRH receptors have been identified in the DVC (17). TRH microinjection in the DVC produces increased acid secretion, gastric motility, and mucosal blood flow (28), in addition to gastric cytoprotective effects seen at doses subthreshold to stimulated acid output (13). The gastric effects of TRH applied to the DVC are abolished by vagotomy or systemic treatment with atropine (13, 20). A recent set of findings showing that gastric functional changes elicited by stimulation of the raphe pallidus or 2-deoxyglucose administration are reversed by pretreatment with TRH antibodies suggest that TRH-containing projections to the DVC from the caudal raphe are important components of the parasympathetic control of gastric function (7, 14, 22, 23, 32).

Serotonin (5-HT) has generated interest as a neuromodulator of the caudal raphe-DVC axis because it is highly colocalized with TRH in the nerve endings at the DVC (11, 12). With respect to gastric acid output (GAO), although 5-HT has no effects on gastric acid secretion when administered in the DVC alone, 5-HT and TRH coadministration leads to larger gastric secretory responses than that elicited by TRH alone (20). Because stimulation of the caudal raphe leads to 1) gastric effects that are sensitive to TRH immunoneutralization (14, 15, 32) and 2) 5-HT release from the DVC (3, 21), it is speculated that corelease of 5-HT and TRH from the raphe-DVC axis occurs physiologically, and the interaction of these two chemical messengers may be important in the ultimate control of gastric function. At present, the mechanism of interaction of these two chemical messengers at the DVC is unclear. The identification of receptors through which such interactions occur in vivo may provide important clues regarding the cellular processes mediating this interaction. Previous work suggests that the 5-HT augmentation of the TRH analog-induced gastric acid secretion is mediated by receptors of the 5-HT2 family (33). Because of the documented rostrocaudal gradient of TRH receptors in the DVC (17), the present study was performed to examine site specificity of 5-HT/TRH interactions in the DVC. Prototypic selective agonists of the 5-HT receptor families were utilized to delineate mechanisms involved in the interaction with TRH at the DVC.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing 450–500 g were fasted overnight for a period of 18–24 h. During this time, they had free access to water.

Drugs. Stable TRH analog RX-77368 was obtained from Reckitt and Coleman (Kingston-upon-Hull, UK). 5-HT creatine sulfate was obtained from Sigma Chemical (St. Louis, MO). 5-Carboxyamidotryptamine (5-CT) was a generous gift of the Glaxo Research Group (Greenford, UK). (-)-1-(4-iodo-2,5-dimethoxyphenyl)-2-amino propane hydrochloride (DOI), 3-(2-aminoethyl)-2-(2-methyl-5-HT), and SC-53116 were obtained from Research Biochemicals (Natick, MA). A stock solution of RX-77368 (8 pmol/10 nl in 0.9% saline) was aliquoted into collection vials and was diluted with equal volume of 0.9% saline to yield RX-77368 (4 pmol/10 nl) or mixed with equal volume of freshly prepared 5-HT agonists (97 pmol/10 nl) to produce a coinjection solution (RX-77368 [4 pmol/10 nl] + 5-HT agonist [48.5 pmol/10 nl]). 5-CT, DOI, or 2-methyl-5-HT were freshly prepared in 0.9% saline. The lipophilic compound SC-53116 was dissolved in 0.1% Tween 80 in normal saline.

Surgery. Rats were anesthetized with urethan (1.5 g/kg ip). After tracheal intubation and esophageal ligation, the stom-
ach was exposed through a midline abdominal incision. After a pyloric ligation, the stomach was cannulated with a double-lumen cannula via an incision in the forestomach. The animal was then placed on a stereotaxic instrument (David Kopf, Tujunga, CA), and the dorsal surface of the brain stem was exposed by retracting the dorsal neck muscles, incising the atlantooccipital membrane, and removing part of the occipital bone and dura. Dorsal medullary microinjections were performed by loading the drug into micropipettes with tip diameters of 10–15 µm and performing injections into the DVC by standard pressure microinjection techniques.

Protocol. The agonist study involved recording a baseline acid output every 10 min for 30 min and then microinjecting the drug into the DVC. The acid output was then recorded every 10 min for nine collection periods. The coordinates for caudal DVC microinjections were 0.2 mm right, 0.2 mm anterior, 0.6 mm ventral with respect to the calamus scriptorius, whereas the DVC injections for investigating more rostral DVC sites were 0.5 mm right, 0.5 mm anterior, and 0.6 mm ventral to the calamus scriptorius. The agonist studies at caudal DVC involved microinjecting 30 nl of each of the following: RX-77368 (4 pmol), 5-HT selective agonists alone (48.5 pmol), or a mixture of RX-77368 and 5-HT agonists at 4 and 48.5 pmol, respectively. The control injections consisted of vehicle (0.9% saline) for the 5-HT agonists 5-CT (5-HT4), DOI (5-HT2), and 2-methyl-5-HT (5-HT3), and in a separate set of studies, the vehicle for the 5-HT4 agonist SC-53116 (0.1% Tween 80 in 0.9% saline). The studies examining interactions at the more rostral DVC sites involved administration of RX-77368, RX-77368/5-CT, and RX-77368/DOI microinjections at the above concentrations and volume. Acid collections were made through this cannula by flushing with 5 ml saline (pH = 7) followed by 5 ml of air. The acid collected was titrated to pH 7 with the use of a Radiometer autotitrator (Copenhagen).

Statistics. After DVC microinjection, a 90-min cumulative gastric acid output was obtained. The acid output was expressed as means (µmol) ± SE. The groups were analyzed by a one-way analysis of variance followed by a post hoc Newman–Keuls test to assess differences between groups.

RESULTS

Agonist coinjection studies. 5-HT agonists were injected alone or were co-injected with RX-77368 into the DVC in a 30-nl volume, producing a 5-HT agonist/RX-77368 molar ratio of 12, as described in MATERIALS AND METHODS. Coinjection of 5-CT or DOI produced a 183 and 103% increase in GAO compared with RX-77368 (4 pmol) alone (Fig. 1). In contrast, coinjection of 2-methyl-5-HT with RX-77368 did not alter the RX-77368-induced response. Neither of the 5-HT agonists produced responses different from the vehicle when injected alone into the DVC (Fig. 1).

In a separate set of studies, co-injection of SC-53116 (5-HT4 agonist) with RX-77368 at a molar ratio of 12 produced a 45% inhibition of RX-77368-stimulated GAO (P < 0.05; Table 1).

5-HT/ TRH interactions in rostral DVC. Previous studies suggest that 5-HT augmentation of RX-77368-stimulated gastric acid response was mediated by activation of 5-HT2 but not 5-HT1 receptors (33). Since the previous work was performed in more rostral regions of the DVC, the relative role of activation of 5-CT- or DOI-sensitive receptors was explored. Identical doses of 5-CT or DOI in combination with the RX-77368 dose used in the present study at the caudal DVC were microinjected into the rostral DVC using the coordinates indicated in MATERIALS AND METHODS. The gastric acid response for RX-77368 injection into the rostral DVC was 96 ± 26 (µmol/90 min, n = 7; Fig. 2). The response to coinjection of 5-CT/RX-77368 or DOI/RX-77368 was 197 ± 22 (n = 5) and 83 ± 16 (n = 10) µmol/90 min, respectively (Fig. 2). Thus the 5-CT/RX-77368 response was significantly enhanced (a 105% increase in GAO) compared with the RX-77368 response (P < 0.001). In contrast, the DOI/RX-77368 response was not greater than that produced by RX-77368 alone. A representation of medullary sites reached by microinjection into the two different sites is presented in Fig. 3.

Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>n (µmol/90 min)</th>
</tr>
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<tbody>
<tr>
<td>Vehicle</td>
<td>3 23 ± 3</td>
</tr>
<tr>
<td>SC-53116 (146 pmol/10 nl)</td>
<td>3 26 ± 3</td>
</tr>
<tr>
<td>RX-77368 (12 pmol/10 nl)</td>
<td>4 118 ± 24*</td>
</tr>
<tr>
<td>SC-53116 (146 pmol/10 nl)/RX-77368 (12 pmol/10 nl)</td>
<td>5 65 ± 19†</td>
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Values are means ± SE; n = no. of rats. The 90-min cumulative gastric acid output for the different groups of the 5-HT4 agonist study are shown. The volume of dorsal vagal complex microinjection was 30 nl in all the groups. *P < 0.05 compared with vehicle injection; †P < 0.05 with respect to the group injected with RX-77368 alone.
DISCUSSION

The principal finding of this study was that 5-HT may act through 5-CT- and DOI-sensitive receptors to augment TRH analog-stimulated GAO at the DVC. The effect of DOI (selective 5-HT2 agonist) coadministration with the TRH analog into the DVC reveals site specificity; the augmented response after caudal DVC injections is lost after identical injection into a more rostral region of the DVC. In contrast, the augmentation response produced by 5-CT is maintained irrespective of the rostrocaudal level of injection into the DVC. Although studies describing the regional distribution of 5-HT2 receptors in the dorsal medulla are lacking, the data of the present study suggest that a lower density may exist in the more rostral extent of the DVC or that 5-HT2 receptors are not located on neurons regulating GAO in this region.

The lack of effect of DOI at the more rostral DVC coordinates contrasts with the findings of a recent report (33). Methodological differences between the two studies include the doses of drugs used, the injection volume, and the molar ratio of the DOI/RX-77368. The present study used lower concentrations of each drug and lower injection volumes (DOI/RX-77368 = 146 pmol/12 pmol in 30 nl, compared with previous work in which DOI/RX-77368 = 1,000 pmol/25 pmol in 50 nl). The larger dose and volume of DOI injected in the previous work may be a factor in the disclosed interaction with TRH analog. Injectate likely achieves higher concentration and reaches sites in the dorsal medulla outside the radius of the 30-nl injections of the present work. Also, the higher dose of TRH analog used (25 pmol) in the previous work may be important in disclosing an interaction by the 5-HT2 receptor agonist.

There is a demonstrated decrement of TRH receptors moving from the caudal to rostral extent of the DVC (17). The dose ratios of DOI/RX-77368 were also slightly different in the two studies: DOI/RX-77368 = 12 (present work) versus 40 (previous work). However, this may be of limited importance because augmentation of the TRH response occurs at 5-HT/TRH ratios ranging from 8 to 40 (33).

5-CT possesses nanomolar affinity for receptors of the 5-HT1 family, but a previous report suggested that 5-HT1 receptors were not involved in the augmentation response with TRH at the DVC (33). It is possible that the lower dose of TRH analog used in the present study may be important in disclosing an interaction with 5-HT1 agonists. Alternatively, other rat 5-HT receptor families (5-HT5, 5-HT6, 5-HT7) also bind 5-CT with high affinity (6, 27). Of these newly identified receptor subtypes, only 5-HT7 receptors have been localized to the DVC, albeit at low levels (27). The lack of effect of 7-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), which has moderate affinity for 5-HT7 receptors, in augmenting TRH analog-induced GAO (33) may provide evidence against a role of 5-HT7 receptors mediating enhancement of TRH responses at the DVC. However, other explanations certainly are possible, given the activity of 8-OH-DPAT on other receptor systems.
(5-HT<sub>1A</sub>, α<sub>2</sub>-adrenergic). Thus it remains an open question upon which receptor 5-CT is exerting its interaction with TRH.

The neuronal cell(s) within the DVC where the 5-HT/TRH interaction occurs is also unknown. Recent work examining the interaction between TRH and 5-HT have reported that TRH (29, 30) and 5-HT (29) increase the firing rate of DMN cells in in vitro medullary slices. TRH and 5-HT decrease the afterhyperpolarizing current, and 5-HT decreases the standing outward K<sup>+</sup> current in the DMN cell (29). This has the effect of increasing the firing rate of the DMN cell. However, the same group of investigators failed to show that perfusion of a solution containing a combination of TRH and 5-HT can produce an additive or synergistic effect on the firing rate of these cells in vitro (29). Several possible explanations exist for the lack of effect of 5-HT/TRH combinations in the previous work. The combinations of 5-HT and TRH were examined at concentrations producing submaximal increases in firing rate of the DMN neurons, but at doses that produced clear effects. However, the 5-HT/TRH dose ratios used (0.01–0.03) were several orders of magnitude different from dose ratios examined in vivo that reveal an augmented vagally dependent gastric secretory response (4–400) (28). Alternatively, there may have been temperature effects (28). Finally, the interaction of 5-HT with TRH may not occur on the same cell in the DVC. For example, the 5-HT effect may occur on interneurons of the DVC containing 5-HT receptors (18) or on 5-CT-sensitive receptors of the nucleus of the solitary tract.

The inhibitory effect of the 5-HT<sub>4</sub> agonists on the RX-77368 response (Table 1) raises some important issues. 5-HT<sub>4</sub> binding sites of intermediate density compared with forebrain sites have been demonstrated in the DVC (10, 31). Receptors of the 5-HT<sub>4</sub> family are linked to a G protein positively coupled to adenylyl cyclase (5). The results of the present report suggest that elevation of intracellular cAMP reduces the TRH analog response if the interaction occurs on the same cell. Further work is needed to delineate the site(s) and intracellular mechanisms responsible for these interactions.

The presence of pathways from the raphe nuclei to the dorsal vagal complex forms the anatomic basis of this work. Neurons originating from the caudal group of raphe nuclei such as the nucleus raphe obscurus (nRO) and the nucleus raphe pallidus contain TRH and 5-HT (2, 12). Additionally, these two neurotransmitters are colocalized in the same neuron along with other neurotransmitters such as substance P and Leu- and Met-enkephalin (12). TRH is highly colocalized with 5-HT within neurons of the caudal raphe-DVC axis, although only 45–70% of the 5-HT neurons contain TRH (1). Other non-TRH-containing serotoninergic pathways from the dorsal or medial raphe also project to the DVC (8). Thus several factors may converge to control the relative levels of 5-HT and TRH in the DVC. The aforementioned non-TRH-containing serotoninergic neurons likely play a role in modulating the 5-HT/TRH molar ratio in the DVC independent of neurons colocalizing the two neurotransmitters. Moreover, the release pattern of 5-HT and TRH from neurons colocalizing them may be different. 5-HT is packaged in small synaptic vesicles that release 5-HT at lower levels of stimulation compared with TRH in descending bulbospinal projections from the caudal raphe (9). Thus a particular level of stimulation in various physiological or pathophysiological states could encode for varying ratios of 5-HT/TRH and consequently alter gastrointestinal function. A previous study demonstrates the effect of varying 5-HT/TRH ratios in the DVC; a cytoprotective response to DVC RX-77368 was converted to a gastric lesion-producing effect by elevating the 5-HT/TRH analog ratio in the DVC (4).

Other dose combinations of these 5-HT agonists and RX-77368 at the DVC may produce different results. Information concerning physiological relevance of the administered agents may be inferred from the release profile of 5-HT and TRH into the DVC in various physiological states. For example, there is evidence of increased TRH immunoreactivity at the DVC in the gastric adaptive cytoprotective response (13). Also of interest is possible modulatory effects of other colocalized substances in the caudal raphe-DVC axis (21).

This study provides evidence that 5-HT in the DVC may produce an augmentation of the TRH analog-induced increase in gastric acid response by activation of 5-CT- and/or DOI-sensitive (5-HT<sub>2</sub>) receptors. The study also demonstrates that 5-HT<sub>4</sub> receptor activation may inhibit the TRH analog response at the DVC. Further work will be required to delineate the relative role and sites of action of 5-HT receptor families in mediating interactions of 5-HT with TRH at the DVC.

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