Involvement of the 5-HT<sub>3</sub> receptor in CRH-induced defecation in rats

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Involvement of the 5-HT<sub>3</sub> receptor in CRH-induced defecation in rats. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G827–G831, 1998.—We evaluated the possibility that serotonin (5-HT) mediates defecation induced by corticotropin-releasing hormone (CRH) exogenously administered or released from the central nervous system by stress via the 5-HT<sub>3</sub> receptor in rats. Intracerebroventricular (ICV) injection of CRH (1, 3, and 10 µg/rat) dose dependently increased the number of stools excreted in rats, whereas intravenous (IV) injection of up to 100 µg/kg CRH did not affect defecation. α-Helical CRH-(9—41) and 5-HT<sub>3</sub> receptor antagonists ramosetron and azasetron inhibited CRH (10 µg icv)-induced defecation in a dose-dependent manner with ED<sub>50</sub> values of 4.3 µg/kg iv, 3.8 µg/kg po, and 70.4 µg/kg po, respectively. α-Helical CRH-(9—41) also inhibited CRH-induced defecation by ICV injection with an ED<sub>50</sub> value of 0.078 µg/rat. In contrast, ramosetron and azasetron injected ICV had no effect on CRH-induced defecation. α-Helical CRH-(9—41), ramosetron, and azasetron reduced defecation caused by restraint stress with ED<sub>50</sub> values of 0.32, 3.6, and 19.7 µg/kg iv, respectively. These results indicate that CRH exogenously administered or released from the central nervous system by stress peripherally promotes the release of 5-HT, which in turn stimulates defecation through the 5-HT<sub>3</sub> receptor.

MATERIALS AND METHODS

Animals. Male Wistar rats weighing 200–370 g were used. The animals were maintained on ordinary laboratory chow and tap water ad libitum under a constant 12:12-h light-dark cycle.

Intracerebroventricular cannulation. The rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and placed in a stereotaxic apparatus (Korf Instruments). The musculature on the skull was removed, and the skull was exposed. After a hole was drilled through the skull with a hand-operated drill (Lancelot; Tokyo Nakai, Japan), a cannula for ICV injection of drugs was inserted perpendicularly into the right lateral ventricle (coordinates: 0.8 mm caudal to bregma, 1.5 mm lateral from midline, 3.4 mm ventral from dura) and fixed to the skull with resin (Ortho Crystal; Rocky Mountain Morita). Experiments were performed at least a month after surgery. Rats with cannulas for ICV injection were used several times at an interval of a week or more. All drugs were administered in a volume of 10 µl over a period of 1 min under light anesthesia with ether.

Effect of CRH on defecation in rats. Initial experiments were conducted in the fed rat to determine the effects of ICV CRH administration on defecation. Preliminary experiments showed that CRH increased fecal pellet output. To determine doses for the following studies, the dose-response curve for CRH-induced fecal pellet output was therefore determined using approximately threefold increases in the CRH dose. Because the effect of CRH on defecation lasted for ~2 h, the number of fecal pellets expelled by each animal was measured 2 h after CRH injection. Inhibitory activity of the test drugs was evaluated in CRH (10 µg/rat icv)-induced fecal pellet output. α-Helical CRH-(9—41), which is a peptide and is seldom absorbed from the gastrointestinal tract, was administered intravenously (IV) 5 min before CRH administration under restraint condition, whereas ramosetron and azasetron were given orally to rats 1 h before CRH to avoid stress based on drug administration. In the case of ICV administration, test drugs were given just before CRH administration under light anesthesia with ether. In another experiment the effect of IV CRH on defecation was examined to confirm the effective site of CRH, either central or peripheral.

Effect of restraint stress on defecation in rats. The stress model used in the present study was restraint stress (17). Animals were stressed by placing them in individual compartments of special stress cages (Natsume Seisakusho; Tokyo, Japan; 265 mm wide × 95 mm long × 200 mm high) at room temperature (23°C). The effect of the test drugs on stress-induced increases in pellet output was determined 1 h after

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the beginning of the restraint stress. Test drugs were injected IV just after exposure to restraint stress.

Statistical evaluation. All values are means ± SE or the mean with 95% confidence limits (CL). The statistical significance of values for fecal pellet output was determined by Dunnett’s multiple-range test. Probabilities of <0.05 were considered significant. ED50 values with 95% CL were calculated as the dose causing 50% inhibition of the increase in stools excreted (control) by log-probit analysis from data obtained for three or four doses of each compound. All calculations were determined with reference to concomitantly tested control animals.

Drugs. Ramosetron hydrochloride (YM-060) and azasetron hydrochloride [Y-25130] were prepared by Yamanouchi Pharmaceutical (Tsukuba, Ibaraki, Japan). CRH and α-helical CRH-(9—41) were purchased from Peptide Institute (Osaka, Japan) and Sigma Chemical (St. Louis, MO), respectively. All drug doses were given as the free base. Ramosetron and azasetron were dissolved in physiological saline and injected in rats in volumes of 2 ml/kg. CRH and α-helical CRH-(9—41) were dissolved in distilled water and distilled water containing 3% DMSO, respectively. In the case of oral administration, ramosetron and azasetron were suspended in 0.5% methylcellulose solution and given to rats in volumes of 5 ml/kg.

RESULTS

Effect of CRH on defecation in rats. IV injection of CRH (1, 3, and 10 µg/rat) dose dependently increased the number of stools excreted (Fig. 1A), whereas IV CRH injection did not affect defecation up to 100 µg/kg (Fig. 1B). Because macroscopic findings of fecal pellets and the weight of each pellet were not affected by CRH at the highest dose of 10 µg/rat (data not shown), only the number of stools excreted by each animal was measured in the following experiments.

Blockade of CRH-induced increases in the number of stools excreted. In control animals, IV administration of CRH at 10 µg/rat resulted in an increase in defecation, with a 2-h pellet output count of ~6. As shown in Fig. 2A, α-helical CRH-(9—41) significantly inhibited CRH-induced increases in fecal pellet output at IV doses of 10 and 100 µg/kg, with an ED50 (95% CL) of 4.3 (3.4–5.6) µg/kg IV (Table 1). Ramosetron (10 and 100 µg/kg po) and azasetron (100 and 1,000 µg/kg po) also significantly inhibited CRH-induced defecation with ED50 values of 3.8 (0.7–19.4) and 70.4 (54.1–91.6) µg/kg po, respectively (Fig. 2, B and C; Table 1). α-Helical CRH-(9—41) (0.1 and 1 µg/rat) given IV just before CRH ICV injection also showed significant preventive effects on CRH-induced increases in the number of stools excreted in rats, with an ED50 value of 0.078 (0.01–0.52) µg/rat iv (Table 1). On the other hand, neither IV ramosetron (10 µg/rat) nor azasetron (100 µg/rat) affected CRH-induced defecation (Fig. 3).

Blockade of stress-induced increases in the number of stools excreted in rats. The changes in fecal pellet output in control rats during the observation time were negligible. Restraint stress resulted in increases in stools, with pellet output counts of 4.3 ± 1.1, 4.3 ± 0.6, and 5.7 ± 0.7 for the α-helical CRH-(9—41), ramosetron-, and azasetron-control groups, respectively (n = 10). As shown in Fig. 4, α-helical CRH-(9—41), ramosetron, and azasetron significantly inhibited restraint stress-induced increases in fecal pellet output, with ED50 values of 0.32 (0.22–0.46), 3.6 (3.2–4.1), and 19.7 (10.2–38.1) µg/kg IV, respectively (Table 1).

DISCUSSION

Endogenous CRH is thought to mediate stress-induced changes in colonic function, such as colonic motility, transit, and fecal excretion (3, 10, 19, 22, 24, 26, 30), and the action of CRH is suggested to be centrally mediated through CRH receptors and vagal efferent pathways (4, 10, 13, 25). In the present study, IV CRH dose dependently increased the number of stools excreted by Wistar rats, whereas IV injection of CRH did not affect defecation, confirming that CRH-induced changes in bowel function are evoked centrally. In contrast, according to our preliminary experiments using Sprague-Dawley rats, IV CRH as well as ICV CRH increased fecal pellet output in a dose-dependent manner (data not shown). The discrepancy of the effect of CRH when given IV between Wistar and Sprague-Dawley rats may be due to the difference in strain, especially in moving to the central nervous system through the blood-brain barrier, although the exact reasons and mechanisms are presently not understood.

A large portion of 5-HT in the body is found in gastrointestinal tissue, where it plays an important physiological role in producing smooth muscle contraction. Recently, 5-HT3 receptor cDNA of human and rat

![Fig. 1. Effect of intracerebroventricular (ICV; A) and intravenous (IV; B) injection of corticotropin-releasing hormone (CRH) on defecation in Wistar rats. CRH given ICV but not IV dose dependently increased number of stools excreted. Each bar represents means ± SE for 7–10 animals. *P < 0.05 and **P < 0.01 compared with the control group (Dunnett’s multiple-range test).](image-url)
was cloned (14). 5-HT₃ receptors have been found in the gastrointestinal tract by Northern blot and RT-PCR analysis. Ramosetron and azasetron used in our study are 5-HT₃ receptor antagonists (15, 21). These compounds inhibited CRH-induced defecation in rats by IV injection, as well as α-helical CRH-(9—41), a CRH receptor antagonist. According to the report by Miyata et al. (16), 5-HT released from enterochromaffin cells or enteric serotonergic neurons increases fecal pellet output excreted in rats through the peripheral 5-HT₃ receptor. Ramosetron suppressed CRH-induced fecal pellet output in doses to inhibit 5-HT-induced defecation. The inhibitory activity ratio of ramosetron and azasetron in CRH-induced defecation is approximately consistent with that in Bezold-Jarisch reflex mediated by 5-HT₃ receptors in anesthetized rats (17). The activation of 5-HT₄ receptors facilitates acetylcholine release from enteric nerve terminals and leads to the enhancement of gastrointestinal propulsion (27). Some of the gastrointestinal prokinetic benzamides possess affinities for 5-HT₄ as well as 5-HT₃ receptors (2, 7, 8). At least ramosetron, however, shows neither 5-HT₄ agonistic nor antagonistic properties (18). All considered, the inhibitory effects of ramosetron and azasetron on CRH-induced defecation are suggested to be based on their 5-HT₃ Receptor antagonistic properties. Furthermore, the site of action of ramosetron and azasetron appears to be peripheral, since ICV injection of ramosetron and azasetron did not affect defecation caused by CRH. Central 5-HT is known to be a potent stimulant of hypothalamic CRH secretion (5). In contrast, there is no report on the direct link between central CRH release and the peripheral 5-HT release. Diop et al. (6) have suggested that the release of central CRH by cold stress promotes the release of thyrotropin-releasing hormone (TRH), which in turn directly controls gastrointestinal function. TRH activates colonic transit via a vagally mediated serotonergic mechanism (11). Weiner (28) has reported that TRH increases 5-HT secretion into the stomach. Taken together, these findings suggest that CRH centrally released by stress mediates changes in colonic function through the peripheral 5-HT released from enterochromaffin cells or enteric serotonergic neurons.

Endogenous 5-HT is suggested to be one of the substances to mediate stress-induced responses of gastrointestinal function, such as defecation and diarrhea (16). In the present study, ramosetron inhibited CRH-induced defecation in doses to suppress restraint stress-induced defecation in rats. Furthermore, a CRH receptor antagonist α-helical CRH-(9—41) also inhibited fecal pellet output excreted by restraint stress. On the other hand, endogenous CRH is suggested to mediate stress-induced gastrointestinal dysfunction, including defecation, as mentioned previously. Altogether, these observations indicate that CRH exogenously administered or released from the central nervous system by

<table>
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<th><strong>Table 1.</strong> Effects of α-helical CRH-(9—41), ramosetron, and azasetron on CRH- and restraint stress-induced increases in number of stools excreted by Wistar rats</th>
<th><strong>ED₅₀ Values, 95% CL</strong></th>
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<tbody>
<tr>
<td><strong>Compound</strong></td>
<td><strong>CRH-induced defecation</strong></td>
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<tr>
<td>α-Helical CRH-(9—41)</td>
<td>4.3 (3.4—5.6) µg/kg iv</td>
</tr>
<tr>
<td>Ramosetron</td>
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<td>Azasetron</td>
<td>&gt;10 µg/rat iv</td>
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CRH, corticotropin-releasing hormone; CL, confidence limits.

![Fig. 2. Effects of IV α-helical CRH-(9—41) (A), oral ramosetron (B), and oral azasetron (C) on ICV CRH-induced increases in the number of stools excreted by Wistar rats. α-Helical CRH-(9—41), ramosetron, and azasetron significantly inhibited CRH-induced defecation, with ED₅₀ values of 4.3 µg/kg iv, 3.8 µg/kg po, and 70.4 µg/kg po, respectively. Each bar represents means ± SE for 7—10 animals. α-Helical CRH-(9—41) was given IV just before CRH (10 µg/rat) ICV injection, and ramosetron and azasetron were given orally 1 h before CRH. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with the control group (Dunnett’s multiple-range test).](http://ajpgi.physiology.org/content/early/2017/10/22033.4)
stress peripherally promotes the release of 5-HT, perhaps from enterochromaffin cells located in gastrointestinal mucosa, which in turn stimulates fecal pellet output through the 5-HT₃ receptor. Kawahito et al. (12) have reported that colonic mucosal enterochromaffin cells in normal humans produce CRH and have indicated that CRH in the colonic mucosa may play a role in modulation of the gastrointestinal functions basally during stressful conditions as well as the intestinal immune system. In addition to the central role of CRH therefore it is possible that stress-induced defecation might be mediated at least in part at peripherally located CRH receptors. This may be one possibility to explain the difference in potency for α-helical CRH-(9—41) in the CRH and stress models in the present study.

Gue et al. (9) have reported that peripheral 5-HT₁ but not 5-HT₂ and 5-HT₃ receptors are involved in the mediation of emotional stress-induced stimulation of colonic motility and that 5-HT₁ₐ receptor agonists inhibit stress-induced colonic dysfunction through the central activation of cholecystokinin neurons. These results (9) and our own indicate that endogenous 5-HT not only directly stimulates cholecystokinin neurons but also indirectly inhibits colonic hypermotility through the 5-HT₁ₐ receptor.

As described before, α-helical CRH-(9—41) given IV as well as ICV inhibited CRH-induced defecation, whereas CRH given ICV but not IV caused defecation in rats. Amtorp (1) has reported that substances with a molecular weight of less than 5,500 are subjected to restricted diffusion through the blood-brain barrier. The molecular weight of CRH is 5,000, indicating it to be borderline as to whether or not CRH diffuses through the blood-brain barrier. On the other hand, the molecular weight of α-helical CRH-(9—41) is four-fifths of that of CRH. Therefore, at least a small amount of α-helical CRH-(9—41) is expected to pass through the blood-brain barrier. In fact, the inhibitory effect of peripheral

Fig. 3. Effects of ICV injection of α-helical CRH-(9—41), ramosetron, and azasetron on ICV CRH-induced increases in the number of stools excreted by Wistar rats. α-Helical CRH-(9—41) given ICV significantly and dose dependently inhibited CRH-induced defecation with an ED₅₀ value of 0.078 µg/rat, whereas ramosetron and azasetron had no effect. Each bar represents means ± SE for 8 animals. Test compounds were given ICV just before CRH (10 µg/rat) ICV injection. ***P < 0.001 compared with the control group (Dunnett’s multiple-range test).

Fig. 4. Effects of IV α-helical CRH-(9—41) (A), ramosetron (B), and azasetron (C) on restraint stress-induced increases in the number of stools excreted by Wistar rats. α-Helical CRH-(9—41), ramosetron, and azasetron significantly inhibited restraint stress-induced defecation, with ED₅₀ values of 0.32, 3.6, and 19.7 µg/kg iv, respectively. Each bar represents means ± SE for 10 animals. Test compounds were given IV just after restraint stress. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with the control group (Dunnett’s multiple-range test).
α-helical CRH-(9–41) on CRH-induced defecation (ED_{50}, 4.3 μg/kg iv) was about 10 times less potent than that of central α-helical CRH-(9–41) (ED_{50}, 0.078 μg/rat iv), which corresponds to 0.2–0.4 μg/kg iv after body weight correction) in the present study.

In conclusion, centrally administered CRH increased fecal pellet output excreted by conscious Wistar rats. 5-HT_{3} receptor antagonists ramosetron and azasetron, similar to α-helical CRH-(9–41), inhibited not only restraint stress- but also CRH-induced defecation. Therefore, it is suggested that endogenous CRH centrally released by stress peripherally promotes the release of 5-HT, which in turn increases defecation through the 5-HT_{3} receptor.


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REFERENCES


