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Serotonin Depletion Potentiates Gastric Secretory and Motor Responses to Vagal but Not Peripheral Gastric Stimulants[1]

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ABSTRACT

Vagal stimulation is known to release gastrointestinal serotonin. The effect of depletion of serotonin stores on vagally stimulated gastric acid secretion and motility was studied in rats. Pretreatment of rats with parachlorophenylalanine (*p*-CPA) did not alter basal gastric acid and serotonin secretion but produced a 57% reduction in the intraluminal gastric release of serotonin and a 43 to 100% potentiation of the gastric acid secretory response elicited by intracisternal injection of the stable thyrotropin-releasing hormone analog, RX 77368, in conscious pylorus-ligated rats or urethane-anesthetized rats with an acute gastric fistula. Dose-response studies revealed that the gastric acid secretion induced by submaximal but not high doses of RX 77368 was elevated significantly by *p*-CPA pretreatment. *p*-CPA also enhanced the gastric acid output produced by submaximal, but not high doses of the vagal stimulant baclofen, [β-(*p*-chlorophenyl)-γ-aminobutyric acid]. In contrast, *p*-CPA pretreatment had no effect on gastric acid secretion stimulated by bethanechol, histamine or pentagastrin. Selective depletion of central serotonin stores by pretreatment with the neurotoxin 5,7-dihydroxytryptamine given alone, or combined with parachloroamphetamine did not alter RX 77368-stimulated gastric acid secretion. In addition, gastric contractility stimulated by intracisternal injection of RX 77368 was significantly enhanced by *p*-CPA but not by 5,7-dihydroxytryptamine pretreatment, whereas the contractile response to carbachol was not altered by *p*-CPA pretreatment. These results suggest that depletion of peripheral but not central serotonergic stores potentiate gastric acid secretion and contractility induced by vagally, but not peripherally acting gastric stimulants. Thus, peripheral serotonin may exert an inhibitory tone on vagally stimulated gastric acid secretion and motility in the rat.

Over 90% of the endogenous 5-HT is found in the gastrointestinal tract (Thompson, 1977). Stores of 5-HT are localized in endocrine (enterochromaffin and enterochromaffin-like cells), mast cells and enteric neurons (Inoue *et al.*, 1987; Rubin and Schwartz, 1983; 1984; Costa *et al.*, 1982). The stomach contains among the highest concentration of this biogenic amine relatively to other regions of the alimentary tract with the exception of the ascending colon (Thompson, 1977; Inoue *et al.*, 1987). Systemic administration of 5-HT produces inhibition of stimulated gastric acid secretion (Cho and Ogle, 1986; Bech and Andersen, 1985; Canfield and Spencer, 1983) and both inhibition or stimulation of gastric motility (Bech and Andersen, 1985; Bech, 1986). However, the role of endogenous 5-HT in the regulation of gastric function remains largely unclear. Recent studies which demonstrate that 5-HT is released into the portal blood (Horita and Carino, 1982; Gronstad *et al.*, 1984) or the gastric and small intestinal lumen (Stephens and Taché, 1989; Cho *et al.*, 1985; Ahlman and Dahlstrom, 1983; Ahlman *et al.*, 1981) after vagal stimulation in rats or cats have raised questions regarding its role in the regulation of normal and stimulated gastric function.

This study investigates the effects of depletion of 5-HT stores on gastric secretion and contractility stimulated by chemical vagal stimulants and other peripherally acting gastric secretagogues. Central and peripheral 5-HT depletion was achieved by pretreatment with an inhibitor of the rate-limiting enzyme of 5-HT synthesis, *p*-CPA (Koe and Weissman, 1966) and selective depletion of brain 5-HT neurons using the neurotoxins 5,7-DHT alone or in combination with *p*-chloroamphetamine. Part of this work was reported in abstract form (Stephens and Taché, 1988).

Methods

Animals. Male Sprague-Dawley rats (200–240 g, Simonsen Laboratory, Gilroy, CA and Harlan Laboratories, Indianapolis, IN) were maintained *ad libitum* on Purina Laboratory Chow and tap water. They were housed under controlled conditions of temperature (22 ± 1°C)

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ABBREVIATIONS: 5-HT, serotonin; *p*-CPA, parachlorophenylalanine; 5,7-DHT, 5,7-dihydroxytryptamine; TRH, thyrotropin-releasing hormone; i.c., intracisternal.

and illumination (6:00 A.M. to 6:00 P.M.). All experiments were performed in animals deprived of food but not water 18 to 24 hr before the beginning of the measurement of gastric function.

Measurement of gastric acid secretion. In one experiment, gastric acid secretion was measured in conscious pylorus-ligated rats. Laparotomy and pylorus ligation were performed in rats under light ether anesthesia. Conscious animals were decapitated 2 hr later and the stomachs were removed, the gastric contents collected on ice and centrifuged. A small aliquot of the gastric juice was separated for the measurement of 5-HT content (0.1–0.5 ml). The volume and pH of the remaining gastric juice were measured, the concentration of acid determined by titration with 0.1 N NaOH to pH 7, and the acid output determined by multiplication of the acid concentration by the total volume, correcting for the aliquot removed. In other studies, gastric secretion were collected from urethane-anesthetized rats (1.25 g/kg i.p.). A cannula was inserted into the trachea, and at this level the esophagus was ligated, sparing the vagi. The abdomen was then opened by a midline incision, the pylorus exposed and ligated and a double lumen cannula placed through a small incision in the forestomach. Gastric secretion were collected by flushing the lumen with a 3-ml bolus of normal saline and a 5-ml bolus of air at 15-min intervals for two periods before and 15- or 30-min intervals for 2 hr after drug injections. An aliquot (1 ml) of the collected secretion was separated for the determination of 5-HT content. Acid output was determined by titration of the flushed perfusate with 0.1 N NaOH to pH 7.0 on an automatic titrator (Radiometer, Copenhagen, Denmark).

Measurement of 5-HT secretion. The centrifuged aliquots of gastric secretion from pylorus-ligated or gastric fistula rats were filtered through a 0.45 micron Acro filter assembly (Gelman Sciences, Ann Arbor, MI) and a volume of 25 to 250 μl was injected into a high-performance liquid chromatograph. The liquid chromatograph was equipped with a LC 4B/17A electrochemical detector, a LC4B Amperometric Detector and a PM 30A Dual Piston Pump (Bioanalytical Systems, West Lafayette, IN). The system was connected to a Phase II, 100 × 3.3 mm ODS, 3 micron C_{18} column. The mobile phase consisted of the following components (grams per liter) dissolved in 10% absolute methanol and adjusted to pH 2.7: NaH_2PO_4, 24.6; sodium octane sulfonate, 0.2; and disodium EDTA, 0.04. The mobile phase was filtered through 0.45-μ filters (Millipore Filter Corporation, Bedford, MA) and degassed before use. The system was run at a flow rate of 1.0 ml/min, yielding a pressure of 3500–4000 psi, and the operating potential was +700 mV. Standard curves were made by injecting 1 to 10 ng of 5-HT dissolved in distilled water. Percentage of 5-HT recovery from the gastric samples was determined by measuring 5-HT after adding known amounts of 5-HT (0.5–2 ng) to 1 ml of flushed gastric perfusate, and subtracting from these values those obtained from the unaltered 1-ml sample of the same gastric perfusate. An average of 90 ± 5% 5-HT recovery was obtained.

Measurement of gastric contractility. Construction and implantation of force transducers was performed as described previously (Garrick *et al.*, 1986). Briefly, the transducers, which measured 5.5 × 5.0 × 1.0 mm, were sutured to the anterior serosal surface of the distal part of the corpus of the stomach and the wires routed through an abdominal incision to the recording equipment. The physiologic recorder (Gilson Model 5/6H; Middleton, WI) was set with the high frequency (low pass) filter at 0.1 Hz. Output from the recorder was routed into a personal computer through an analog-to-digital converter (DT2801, Data Translation, Marlboro, MA) and stored on diskette for subsequent analysis.

Basal gastric contractility was recorded in urethane-anesthetized rats (1.25 g/kg i.p.) for 15 min before drug injection (basal), and for 30 min thereafter. Contractility tracings were collected in 15-min blocks and were quantified using previously described methods (Garrick *et al.*, 1988). For each 15 min recording, the first 10-min block was analyzed for the area under the contractility curves, the frequency of contractions and the average duration of each contraction. Ten-minute segments were used in order to allow excision of the occasional artifact which entered in the recordings. Inasmuch as transducer measurements between animals are not reliable due to variation in surgical implantation, or state of contraction at the time of implantation, all recordings are standardized in each animal. Two successive blocks of data after drug injection (S_1 and S_2) were summed and divided by the basal (B) recording, establishing a relative motility index for the comparison of the gastric contractility effects of drug treatment ($(S_1 + S_2)/B$).

Measurement of tissue levels of 5-HT. At the end of the experiments, stomachs and brains were collected on ice and weighed. Tissues were homogenized in 10 volumes of 0.1 M perchloric acid using a glass homogenizer. The samples were then centrifuged at 15,000 × g at 4°C for 20 min, the supernatant filtered through 0.45-μ filters and 20 μl injected into the high-performance liquid chromatograph as described previously. The 5-HT concentration was calculated using a standard curve of four concentrations of the standard and expressed as nanograms per gram of wet weight of tissue. The recovery of 5-HT from the brain and the stomach after these procedures was 70 and 65%, respectively.

Drugs. The stable TRH analog, RX 77368 (Reckitt and Colman, Kingston upon Hull, England), obtained in powder form, was dissolved in 0.5% bovine serum albumen and 0.9% saline at an initial concentration of 20 μg/ml and kept at −20°C. The peptide was injected i.c. in a 10 μl volume in ether- or urethane-anesthetized rats. *p*-CPA methyl ester and *p*-chloroamphetamine (Sigma Chemical Co., St Louis, MO) were dissolved in distilled water at concentrations of 60 mg/ml (salt form) and 10 mg/kg, respectively, and injected i.p. −72 and −48 hr before the experiment. 5,7-DHT creatine sulfate (Sigma) was dissolved in a solution of 0.9% saline and 1% ascorbic acid. β-(*p*-chlorophenyl)-γ-aminobutyric acid (Baclofen, Lioresal, Geigy Pharmaceuticals, Summit, NJ) was dissolved in 0.9% saline. Desipramine (Sigma) was dissolved in distilled water.

Statistics. Statistical analyses were performed with one-way analysis of variance and post hoc Newmann-Keuls test, and by unpaired t test.

Results

Effect of *p*-CPA pretreatment on vagal stimulation of gastric secretion elicited by RX 77368 and the γ-aminobutyric acid$_B$ agonist, baclofen. Pretreatment with *p*-CPA (300 mg/kg i.p.; −72 and −48 hr) decreased by 65 and 95% 5-HT levels, respectively, in the stomach (table 1) and the brain (413 ± 37 ng/g in control group, n = 5 to 24 ± 5 ng/g in *p*-CPA-treated rats, n = 5). *p*-CPA produced no significant modifications of gastric acid output, secretory volume or luminal 5-HT content in conscious, 2 hr pylorus-ligated rats given an i.c. injection of vehicle, in comparison to vehicle-pretreated group (table 1). Intracisternal injection of the stable TRH analog, RX 77368, (100 ng) in vehicle-pretreated animals increased gastric acid output, secretory volume and markedly stimulated intraluminal 5-HT release (table 1). *p*-CPA pretreatment enhanced by 43% the gastric acid secretory response to i.c. injection of RX 77368 whereas it reduced by 57% the RX-77368-stimulated 5-HT output in gastric secretion (table 1).

In urethane-anesthetized rats with an acute gastric fistula, *p*-CPA pretreatment did not alter basal gastric acid secretion, but was effective in increasing by 249 and 100% the gastric secretory response to i.c. injection of 3 and 30 ng of RX 77368, respectively (fig. 1). However, the integrated 2-hr acid secretory response to higher doses of RX 77368 (100 and 300 ng) were not elevated significantly in this model. Levels of 5-HT in the stomach and brain measured at the end of the experiments were decreased by 45% ($P < .05$) and 94% ($P < .01$), respectively, in *p*-CPA-pretreated rats (fig. 1).

Time course and dose-response studies revealed that the

TABLE 1

Effect of *p*-CPA pretreatment on RX 77368-stimulated gastric acid output and luminal and tissue 5-HT levels in conscious pylorus-ligated rats

Treatment[a]	*n*	Gastric Secretion[b]			Stomach[b]
		Acid output	Volume	5-HT output	5-HT content
		μmol/2 hr	*ml/2 hr*	*ng/2 hr*	*ng/g tissue*
Sal + Sal	5	119 ± 43	1.8 ± 0.5	46 ± 12	3213 ± 782
p-CPA + Sal	5	59 ± 28	1.5 ± 0.5	39 ± 25	1128 ± 276*
Sal + RX 77368	9	333 ± 21*	5.0 ± 0.6*	403 ± 116*	2120 ± 202
p-CPA + RX 77368	8	477 ± 46*, **	5.6 ± 0.4*	175 ± 56*, **	1069 ± 231*, **

[a] Rats pretreated with vehicle or *p*-CPA (300 mg/kg; −78 and −48 hr before the experiment) were injected i.c. with RX 77368 (100 ng) and the pylorus was ligated under light ether anesthesia. Gastric secretion and stomach were collected 2 hr after pylorus ligation.

[b] Mean ± S.E.

* $P < .05$ with respect to the saline (Sal) + Sal group; ** $P < .05$ with respect to the Sal + RX 77368 group.

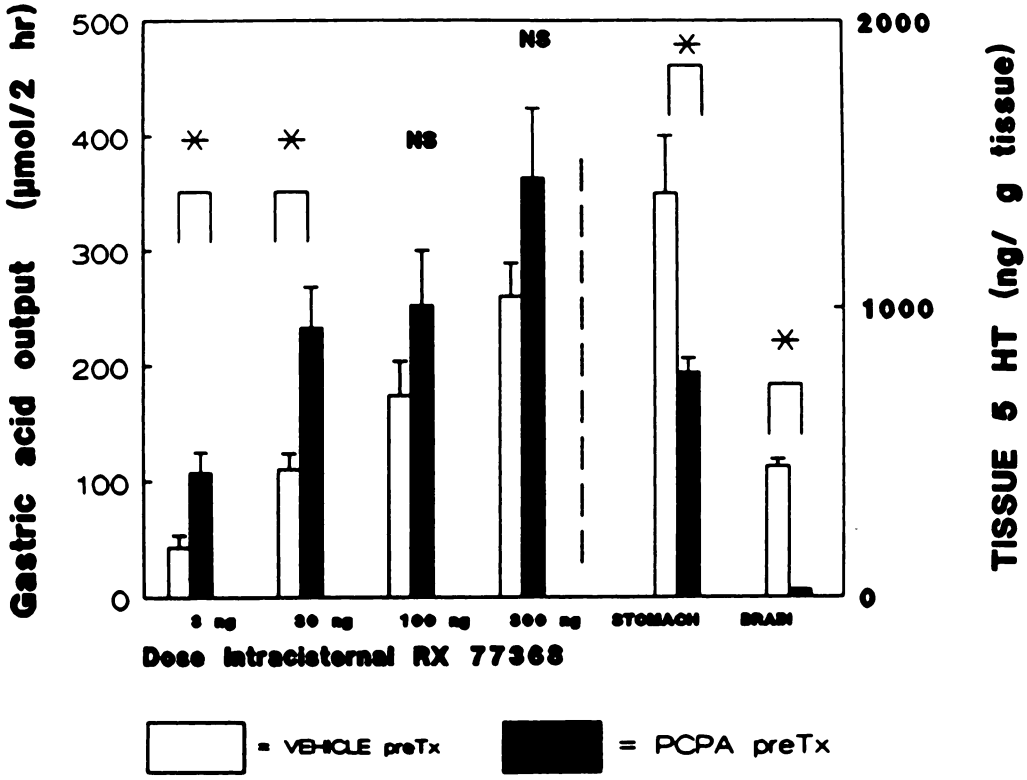

Fig. 1. Effects of *p*-CPA on i.c. RX 77368-induced increased in gastric acid secretion and on 5-HT levels in the brain and stomach of urethane-anesthetized rats. Rats were pretreated (pre-Tx) with vehicle or *p*-CPA (300 mg/kg i.p. −78 and −48 hr) and gastric acid secretion was measured for 2 hr after i.c. injection of RX 77368 (3, 30, 100 or 300 ng) in urethane-anesthetized rats. At the end of the experiments, brain and stomach of each animal were collected for determination of 5-HT content. Each column is the mean ± S.E. of 6 to 10 rats. * $P < .05$ as compared to vehicle.

significantly elevated gastric acid secretory response after i.c. injection of 3 and 30 ng of RX 77368 was first evident during the 15- to 30-min interval (3 ng) or the 30- to 60-min period after peptide injection (30 ng), and was maintained significantly elevated for two periods in each case during the remainder of the 120-min collection period (fig. 2). The gastric acid secretory response to higher doses (100 and 300 ng) of RX 77368 was elevated significantly at earlier time periods in *p*-CPA-pretreated animals as compared to the vehicle-treated groups (fig. 2). However, the early elevation of gastric acid secretion in *p*-CPA-pretreated groups at these higher doses of RX 77368 was not reflected in significantly elevated integrated 2-hr acid response (fig. 1). Thus, depletion of endogenous 5-HT stores is correlated with a potentiation of RX 77368-stimulated gastric acid secretion in both conscious pylorus-ligated rats and urethane-anesthetized rats with an acute gastric fistula, but the potentiation appeared to be diminished with higher doses of RX 77368.

Figure 3 illustrates that *p*-CPA pretreatment induced a 3-fold enhancement of the integrated 2-hr gastric acid secretory response to i.v. injection of baclofen (2 mg/kg) in urethane-anesthetized rats with a gastric fistula. A 67% ($P < .05$) and 93% ($P < .01$) decrease in 5-HT contents, respectively, in the stomach and brain was produced by the *p*-CPA pretreatment (fig. 3). The time course revealed significantly elevated acid secretory levels in the *p*-CPA-pretreated group *vs.* vehicle group at the first 15-min collection period after the baclofen injection. This elevation was maintained throughout the 2-hr experimental period (fig. 4). In contrast, the gastric secretory effect of a higher dose of baclofen (4 mg/kg) was not potentiated by the *p*-CPA pretreatment (fig. 3).

Effect of *p*-CPA pretreatment on bethanechol-, histamine- and pentagastrin-stimulated gastric acid secretion. The effect of *p*-CPA pretreatment on the gastric secretory responses produced by peripherally acting gastric secretagogues was determined in urethane-anesthetized, acute gastric fistula rats. Figure 5 reveals that *p*-CPA pretreatment was ineffective in altering gastric acid secretion stimulated by i.v. injection of bethanechol (0.1 or 1.0 mg/kg), s.c. injection of histamine (10 mg/kg) or i.v. infusion of pentagastrin (8 or 16 μg/kg/hr).

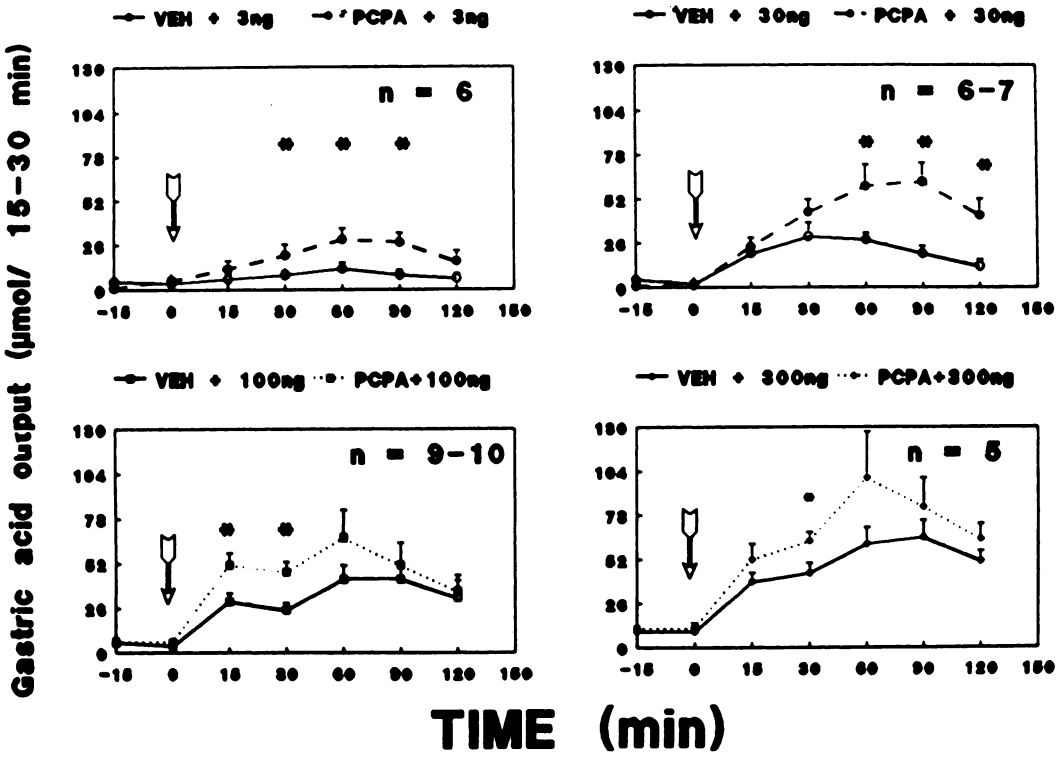

Fig. 2. Time course study of the enhanced gastric acid output in *p*-CPA-pretreated, urethane-anesthetized rats injected i.c. with RX 77368. The time of i.c. RX 77368 injection at the dose indicated in the legend is indicated by the arrow. Each point represents the mean ± S.E. of the gastric acid secretory response during the time interval and for the number of rats indicated in the graph. * $P < .05$ as compared to vehicle (VEH).

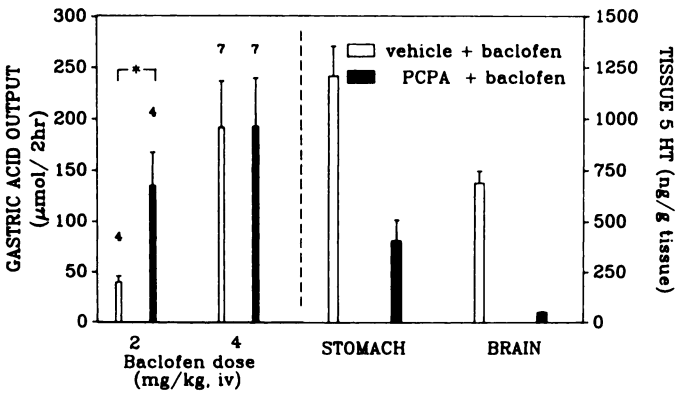

Fig. 3. Effect of *p*-CPA on i.v. baclofen-induced increase in gastric acid secretion and on 5-HT levels in the brain and stomach of urethane-anesthetized rats. Rats were pretreated with vehicle or *p*-CPA (300 mg/kg i.p., −78 and −48 hr) and gastric acid secretion was measured for 2 hr after i.v. injection of baclofen (2 and 4 mg/kg). At the end of the experiments, brain and stomach of each animal were collected for determination of 5-HT content. Each column is the mean ± S.E. of 6 to 10 rats. * $P < .05$ as compared to vehicle.

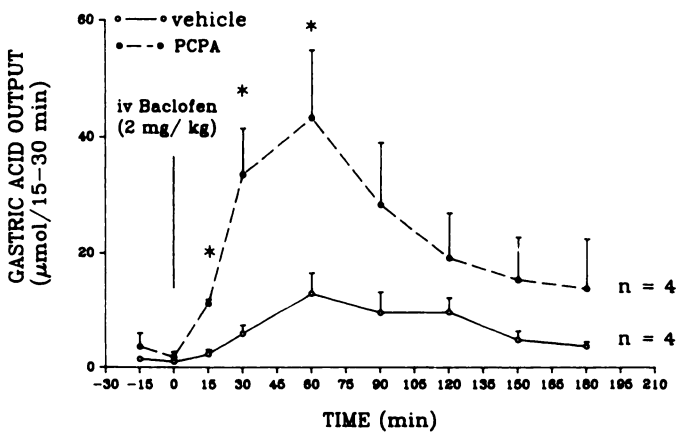

Fig. 4. Time course study of the enhanced gastric acid output in *p*-CPA-pretreated rats injected i.v. with baclofen in urethane-anesthetized rats. Each point represents the mean ± S.E. of number of rats indicated in the graph. * $P < .05$ with respect to animals pretreated with vehicle.

Effect of depletion of central serotonergic stores on the gastric secretory response to i.c. RX 77368. To assess whether depletion of brain 5-HT is responsible for *p*-CPA-induced potentiation of RX 77368-stimulated gastric acid secretion, animals were pretreated with the 5-HT neurotoxin, 5,7-DHT, in combination with desipramine, a substance that protects norepinephrine-containing neurons from the action of 5,7-DHT (Bjorklund *et al.*, 1975). 5,7-DHT injected i.c. (150 μg free base/20 μl, −10 days) in rats pretreated with desipramine, produced a 57% reduction in brain 5-HT (5-HT content in nanograms per gram of pooled brain samples was 417 ± 28 in vehicle-pretreated group and 178 ± 13 in 5,7-DHT-pretreated rats, $n = 8$, $P < .01$) whereas the levels of 5-HT in the stomach were not altered (vehicle: 1136 ± 40 ng/g, $n = 8$; 5,7-DHT: 1169 ± 123 ng/g, $n = 8$). This pretreatment did not modify basal gastric acid secretion significantly and did not alter the secretory response to i.c. RX 77368 (10 or 30 ng) in urethane-anesthetized rats with gastric fistula (table 2). Further selective depletion of brain serotonergic stores to 25% of control levels achieved by combined pretreatment with 5,7-DHT (200 μg, −10 days) and the neurotoxin *p*-chloroamphetamine (10 mg/kg ip; −78 and −48 hr) was still ineffective in altering the gastric acid secretory response to i.c. injection of 30 ng of RX 77368 (table 3).

Effect of serotonergic depletion on stimulated gastric contractility. In animals implanted with gastric strain gauge force transducers, *p*-CPA pretreatment produced a 2-fold increase in the stimulated gastric contractility response produced by i.c. RX 77368 (100 ng) as compared to vehicle-pretreated rats (fig. 6). *p*-CPA pretreatment alone resulted in a modest

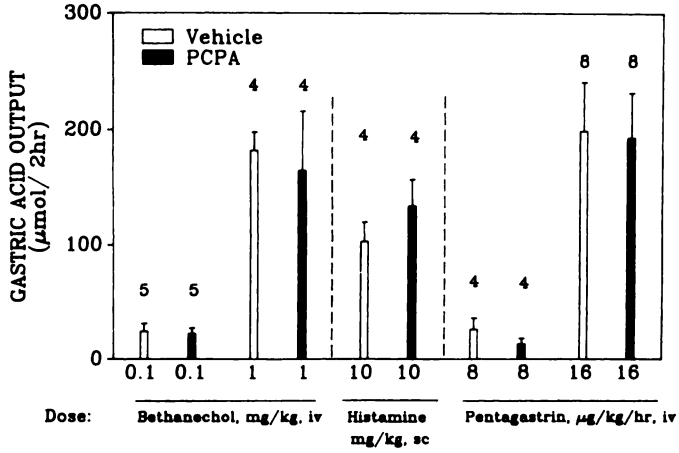

Fig. 5. Effect of *p*-CPA on bethanechol-, histamine- and pentagastrin-induced stimulation of gastric acid secretion in urethane-anesthetized rats. Each column represents the mean ± S.E. of number of rats indicated at the top.

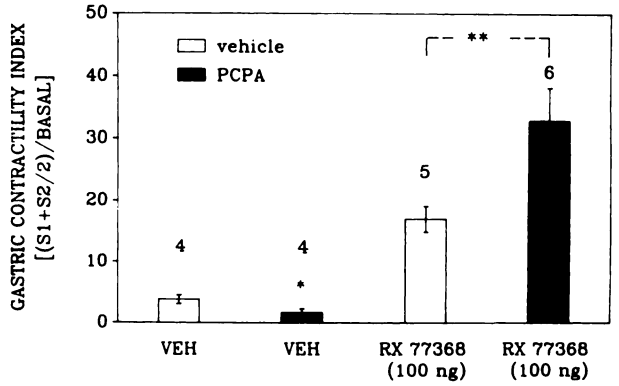

Fig. 6. Effect of *p*-CPA on basal and RX 7738-induced stimulation of gastric contractility in urethane-anesthetized rats. Each column represents the mean ± S.E. of number of rats indicated at the top of each graph. * $P < .05$ with respect to animals treated with vehicle (VEH) + VEH. ** $P < .05$ with respect to animals treated with VEH + RX 77368.

TABLE 2

Effect of 5,7-DHT pretreatment on RX 77368-stimulated gastric acid secretion in urethane-anesthetized rats with acute gastric fistula

Treatment[a]	RX 77368[b]	*n*	Gastric Acid Output
	ng		*μmol/2 hr*
Vehicle	10	4	124 ± 25
5,7-DHT	10	4	137 ± 25
Vehicle	30	4	167 ± 36
5,7-DHT	30	4	150 ± 35

[a] Ten days before the experiment the animals were injected i.p. with desipramine (50 mg/kg) and 1 hr later i.c. with 5,7-DHT (150 μg/20 μl) or vehicle (20 μl). Pretreated rats were anesthetized with urethane and a gastric fistula implanted. After 30 min for collection of basal gastric secretion, all groups were injected i.c. with RX 77368 and gastric secretion were collected for 2 hr.

[b] Mean ± S.E.

TABLE 3

Effect of pretreatment with *p*-chloroamphetamine combined with 5,7-DHT on the gastric acid secretory response to i.c. RX 77368 in urethane-anesthetized rats

Treatment[a]	*n*	Gastric[b] Acid Output	Tissue Content[b]	
			Brain	Stomach
		μmol/2 hr	*ng/g tissue*	
Veh + Veh + RX 77368	6	76 ± 19	426 ± 21	1165 ± 305
5,7-DHT + PCA + RX 77368	6	81 ± 14	107 ± 12*	1183 ± 273

[a] Animals were injected i.c. with vehicle (Veh, −10 days) and i.p. with Veh (−72 and −48 hr) or i.c. with 5,7-DHT (200 μg; −10 days) and i.p. with *p*-chloramphetamine (PCA, 10 mg/kg; −78 and −48 hr before the experiment). Pretreated rats anesthetized with urethane and implanted with a gastric fistula were injected i.c. with RX 77368 (30 ng) and gastric secretion were collected before and for 2 hr after RX 77368 injection.

[b] Mean ± S.E.

* $P < .05$ with respect to the Veh + Veh group.

inhibitory effect on basal contractility (fig. 6). In contrast, the stimulation of gastric contractility index produced by carbachol infusion (50 μg/kg/hr i.v.) was not altered significantly by *p*-CPA pretreatment (table 4). In addition, 5,7-DHT pretreatment (150 μg free base/20 μl i.c.) did not significantly alter the gastric contractility response to i.c. injection of RX 77368 (30 ng). The 30-min contractility index was 8.4 ± 1.7, $n = 5$, in vehicle-pretreated rats and 10.9 ± 3.2, $n = 6$, in the 5,7-DHT-pretreated group.

TABLE 4

Effect of *p*-CPA pretreatment on gastric contractility stimulated by carbachol infusion in urethane-anesthetized rats

Treatment[a]	*n*	Motility Index[b]
Veh + Carbachol	4	8.4 ± 2.2
p-CPA + Carbachol	6	5.0 ± 0.8 N.S.

[a] Carbachol was infused at a rate of 50 μg/kg/hr after a 15-min basal period. Animals were pretreated with vehicle (Veh) or *p*-CPA (300 mg/kg; −78 and −48 hr before the experiment).

[b] Results are expressed as the mean ± S.E. of the relative motility index (calculated according to the formula described under "Methods") for 30-min period recorded from 0 to 30 min after carbachol infusion.

Discussion

As established previously, we found that i.c. injection of the stable TRH analog, RX 77368, stimulated gastric acid and 5-HT secretion and motility in conscious pylorus-ligated or urethane-anesthetized rats (Taché *et al.*, 1984; Stephens and Taché, 1989; Garrick *et al.*, 1987). The gastric stimulatory effects of central RX 77368 or TRH are exerted through central vagal cholinergic pathways (Taché *et al.*, 1984, 1988; Stephens and Taché, 1989; Garrick *et al.*, 1987). The role of the endogenously released 5-HT in modulating the gastric response to RX 77368 was investigated using *p*-CPA. *p*-CPA has been characterized as an inhibitor of the rate-limiting enzyme for 5-HT synthesis, tryptophan hydroxylase. After treatment with this agent, levels of 5-HT and its principle metabolite 5-hydroxyindole-acetic acid are reduced markedly, whereas little effect on catecholamine systems or monoamine oxidase activity has been observed (Koe and Weissman, 1966). In the present study *p*-CPA pretreatment reduced markedly the content of 5-HT in both the brain and the stomach. *p*-CPA also inhibited by 57% the increase in intragastric release of 5-HT elicited by RX 77368 (table 1). Depletion of 5-HT by *p*-CPA did not modify basal gastric acid secretion in conscious or urethane-anesthetized rats. These results agree with other studies demonstrating that basal gastric acid secretion was not altered by *p*-CPA combined with the neurotoxin 5,6-DHT in conscious pylorus-ligated rats (Taché and Collu, 1982) or by the 5-HT antagonist methysergide in rat isolated stomach preparation (Cho and Ogle, 1986). In contrast, in this study *p*-CPA pretreatment increased significantly the stimulation of gastric acid secretion in response to i.c. injection of the TRH analog in conscious or urethane-anesthetized rats. An approximate 10-fold increase in potency of central RX 77368 to induce gastric acid secretion

was observed in p-CPA-pretreated animals (as indicated by the doses required to achieve approximately 100 and 230 μmol/2-hr gastric acid output in vehicle-treated animals compared with p-CPA-treated groups; fig. 1).

The 2-hr gastric acid secretion response produced in urethane-anesthetized rats by doses of RX 77368 which represents the peak of the dose-response curve (100 and 300 ng; Taché *et al.*, 1984) was not altered significantly by p-CPA pretreatment. Similarly, p-CPA also enhanced gastric acid secretion stimulated by a submaximal, but not a high dose of baclofen, another well established vagal stimulant of gastric secretion (Goto *et al.*, 1985). Thus, it appears that the gastric secretory capacity was not elevated by the p-CPA pretreatment.

The gastric acid response to the cholinergic agonist, bethanechol, histamine or pentagastrin was not modified by p-CPA pretreatment in urethane-anesthetized rats with gastric fistula. A difference in the magnitude of the increase in gastric acid output between the various secretagogues cannot account for the differential effects of p-CPA on vagal *vs.* peripheral gastric stimulants. The total acid outputs (micromole/2-hr) induced by pentagastrin (8 μg/kg/hr) was 26 ± 10, by histamine (10 mg/kg), 103 ± 17 and by bethanechol (0.1 mg/kg), 24 ± 7, which represents lower or equal gastric acid secretion compared to that elicited by the vagal stimulant, RX 77368 (30 ng; 110 ± 14 μmol/2 hr). Taken together these results demonstrate that the 5-HT depletion by p-CPA elicits a selective potentiation of vagally stimulated gastric acid secretion in conscious or anesthetized rats whereas it has no effect on basal gastric secretion or acid secretion stimulated by pentagastrin, histamine or cholinergic agonist.

The pool of 5-HT exerting an inhibitory effect on gastric acid secretion has been investigated. There is evidence that exogenous 5-HT acts in the brain to inhibit gastric acid secretion (Hierro *et al.*, 1980; Bugajski *et al.*, 1977; Lee *et al.*, 1969). However, it is unlikely that the potentiating effect of p-CPA on central vagal efferent stimulation of gastric acid secretion results from the removal of a central serotonergic inhibitory influence as selective depletion of brain 5-HT without alteration of gastric 5-HT content elicited by the neurotoxin, 5,7-DHT, given alone or combined with p-chloroamphetamine, did not potentiate the gastric response to i.c. RX 77368 (tables 2 and 3). This would imply that peripheral 5-HT is the physiological pool involved in producing the inhibitory tone on vagally stimulated gastric secretion. Vagal activation elicited by TRH or RX 77368 injected into the cerebrospinal fluid or by electrical stimulation of the vagus is well established to stimulate 5-HT release in the portal blood (Horita and Carino, 1982; Horita *et al.*, 1985; Gronstad *et al.*, 1987), and in the lumen of the stomach (Cho *et al.*, 1985; Stephens and Taché, 1989; and small intestine (Ahlman and Dahlstrom, 1983; Ahlman *et al.*, 1981, 1984; Gronstad *et al.*, 1985) of rabbits, rats and cats. The fact that p-CPA-induced decrease in the levels of 5-HT in the stomach and gastric secretion is associated with a selective potentiation of the acid response to vagal stimulation suggests that the vagally released peripheral 5-HT exerts an inhibitory effect on gastric acid secretion. This would also explain the selectivity of the response toward vagal stimulants as histamine, bethanechol or pentagastrin does not stimulate gastric release of 5-HT in the rat (Stephens and Taché, 1989).

The mechanisms through which p-CPA enhanced vagally stimulated gastric acid secretion is still unknown, although there are several pathways through which it may be acting. Several studies have established clearly that peripheral injection of 5-HT inhibited gastric acid secretion in conscious or anesthetized animals (Cho and Ogle, 1986; Bech and Andersen, 1985; Canfield and Spencer, 1983; Bech, 1986). The potentiation by p-CPA may be explained by the removal of the inhibitory effect of 5-HT on acid secretion, as the normally occurring release of 5-HT into the gastrointestinal lumen and the circulation elicited by vagal stimulation is altered. The exact mechanisms through which the inhibitory effect of 5-HT is exerted on gastric acid secretion is still unclear. It is unlikely that intraluminal release of 5-HT may act directly on the parietal cells as perfusion of the stomach with 5-HT in similar concentrations as those intragastrically release by vagal stimulation did not inhibit acid secretion stimulated by pentagastrin (unpublished observations).

The present study also demonstrates that p-CPA, unlike the neurotoxin, enhanced not only the stimulation of gastric acid secretion but also gastric contractility elicited by i.c. RX 77368 but not carbachol. The facts that the vagally mediated stimulation of both acid and contractility are enhanced by endogenous 5-HT depletion suggest the removal of serotonergic inhibitory pathways that may modulate the activity of vagal cholinergic neurons (Sanger and McClelland, 1986).

In summary, the present study established that endogenous gastrointestinal store of 5-HT exerts an inhibitory tone on gastric secretory and motor function stimulated by vagal activation and adds to the existing body of knowledge regarding 5-HT influence on gastric function.

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