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Serum Gastrin in the Rat: Cholinergic and Adrenergic Effects[1] (39680)

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Until recently neurochemical stimulation of gastrin secretion has been considered to be mediated largely by cholinergic mechanisms (1). However, in 1974 Kronborg *et al.* (2) reported that a β-adrenergic blocking agent, propranolol, could depress gastrin levels. More direct evidence for adrenergic involvement in gastrin secretion has emerged from the following observations: (a) patients with pheochromocytoma exhibited elevated blood gastrin levels which were reduced by either surgery or phenoxybenzamine administration (3); (b) direct iv infusion of epinephrine in dogs (3) or man (3-5) elevated blood gastrin levels; and (b) prior administration of a β-adrenergic blocking agent, pindolol, eliminated the serum gastrin stimulatory effects of iv epinephrine in normal subjects (5).

Previously, we found pronounced effects of β-adrenergic drugs on both blood calcium and thyrocalcitonin secretion (6). Since secretion of gastrin and thyrocalcitonin appear interrelated in certain species (7), we decided to explore possible effects of β-adrenergic drugs on gastrin secretion. The findings presented here suggest that, in the rat, β-adrenergic amines can exert striking effects on blood levels of immunoreactive gastrin.

Materials and methods. Animals. Male Holtzman rats (Madison, WI) 8-12 wk old and weighing 200-400 g were used for all experiments except that shown in Fig. 3 (32-week-old rats). Upon receipt they were maintained on Purina Laboratory Chow and tap water. In each experiment, all rats were from a single shipment and had been born on the same day. For a period ranging from 18 to 48 hr before each experiment, food was withheld and animals were allowed only drinking water. In experiments involving feeding, Purina Chow pellets were offered after the fasting period, and the weight of food consumed was monitored.

Experimental procedures. Surgical procedures were conducted under ether anesthesia. Truncal vagotomy plus pyloroplasty were performed through a midventral incision. Both trunks of the vagus nerve were transected at the distal end of the esophagus, a 3-mm longitudinal incision was made at the pylorus through the mucosa, and the incision closed with 6-0 nylon sutures. Control rats were subjected to pyloroplasty only.

Injections were given either ip or iv (lateral tail vein) using a 27-gauge, 3/4-in. needle, and all injections were given in a total volume of either 0.5 or 1 ml. Control animals received injections of appropriate vols of vehicle (0.15 *M* NaCl or propylene glycol). Oral administration of drugs was by gavage using a 16-gauge curved metal feeding tube. The total vol of solution given by gavage ranged from 3-6 ml, and control rats received an appropriate vol of vehicle.

Test preparations. The drugs tested and their sources were as follows: *l*-epinephrine bitartrate and *l*-phenylephrine hydrochloride (Winthrop Laboratories, New York, N.Y.); d, *l*-isoproterenol hydrochloride (donated by Winthrop Labs); propranolol hydrochloride (donated by Ayerst Laboratories, New York, N.Y.); phenoxybenzamine hydrochloride (donated by Smith, Kline, and French Labs, Philadelphia, Pa.); atropine sulfate (Mallinckrodt Co., St. Louis, Mo.); acetylcholine bromide (Eastman Kodak Co., Rochester, N.Y.).

Most of the drugs were dissolved in 0.15

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M NaCl with the doses calculated on the basis of weight of salt; the dose of epinephrine was calculated on the basis of free base. Acetylcholine bromide was dissolved in distilled water; phyenoxybenzamine hydrochloride was dissolved in propylene glycol.

Blood collection and analyses. Either one or two blood samples (2 ml volume) were obtained from each rat either from the tail or by cardiac puncture. When two samples were obtained, one was taken at zero time before treatment and a second at the desired time interval after treatment. For analysis, sera were quickly obtained by centrifugation and kept frozen at −20° until assayed. Gastrin was determined using radioimmunoassay procedures described previously in detail (7, 8). A guinea pig antiserum to porcine gastrin (final dilution 1:100,000) was used, and synthetic 1-17 human gastrin (ICI, England) was used for both iodination with ^{125}I and as unlabeled reference standard. As reported previously (7), intra- and interassay variations in the range of serum gastrin measured in this study were no more than 15 and 20%, respectively.

Statistical analysis. In experiments where rats were fasted 24 hr or longer and some serum gastrin levels were below the limits of detectability of the immunoassay, individual values for each rat are presented; in these experiments, significance of differences between groups was assessed using the nonparametric test of Wilcoxon (9). In experiments where rats had been fasted for only 18 hr and all levels were detectable (Table I, Expt 2, and Fig. 1), the changes in gastrin for each group were subjected to analysis of variance, the standard errors were calculated from the residual error term of the appropriate analysis, and the significance of differences between groups was determined by the multiple comparisons test of Hartley (9).

Results. Table I, Expt 1, shows one representative experiment of several conducted where a very small dose of epinephrine (1 μg/kg iv over a 5-min period) was administered to intact rats. The results show that this low dose of epinephrine raised serum gastrin levels rapidly (within 10 min). Similarly, as shown in Expt 2, a significant ($P < 0.05$) increase in serum gastrin levels also was observed in rats injected with a large dose of an α-adrenergic blocking drug, phenoxybenzamine, while no effect was observed in rats injected with an α-adrenergic agonist, phenylephrine.

The results in Fig. 1 illustrate that like epinephrine, a β-adrenergic agonist, isoproterenol, given iv greatly raised the serum level of gastrin within 60 min. In this experiment, a β-adrenergic blocking drug, propranolol, itself produced a small fall in serum gastrin ($P < 0.05$) compared with the initial value, and of greater interest, it was able to prevent completely the 60 min rise in gastrin when given 10 min before the injec-

TABLE I. SERUM GASTRIN IN RATS INJECTED WITH EPINEPHRINE OR PHENOXYBENZAMINE AND IN RATS GIVEN ISOPROTERENOL BY GAVAGE AFTER PYLORIC LIGATION.[d]

Treatment	Serum gastrin, pg/ml	
Expt 1:		
A. Control	N.D.*	
B. EPIN[a]	78 ± 8.9**	
	Basal value 0 Time	1 or 2 Hr change (mean ± SE)
Expt 2:		
A. Control	96 ± 28.1	−8 ± 13.1
B. PBZ	80 ± 20.4	+177 ± 76.8**
C. Phenylephrine[b]	86 ± 12.6	−2 ± 10.0
Expt 3:		
A. Control (Gastric pH = 2.9 ± 0.36)	N.D.*	
B. ISOP (Gastric pH = 2.0 ± 0.27)[c]	204 ± 55.9***	

Collection of blood by cardiac puncture was:

[a] In Expt 1 10 min after iv injection of epinephrine (EPIN) at a dose of 1 μg/kg given over a 5-min period (Control rats received 0.15 *M* NaCl).

[b] In Expt 2 at 0 time and again 2 hr after ip injection of phenoxybenzamine (PBZ, 10 mg/kg) or 1 hr after iv injection of phenylephrine (1 mg/kg). Control rats received propylene glycol ip and were bled 2 hr later.

[c] In Expt 3 30 min after concurrent pyloric ligation and gavage of isoproterenol (ISOP, 3 mg/3 ml 0.01 *N* HCl). Gastric fluid at 30 min also was collected by aspiration. Control rats received 3 ml 0.01 *N* HCl. pH in gastric fluid was determined with a Beckman digital pH meter.

[d] Rats were 8-9 wk old and weighed 240-300 g. In Expt 1 rats had been fasted for 48 hr; in Expt 3, for 24 hr. In Expt 2, rats were fasted only 18 hr, and basal levels were measurable. In each experiment all values are presented as mean ± SE for a separate group of 4-6 rats.

* N.D. = All were undetectable, i.e, <48 pg/ml.

** $P < 0.05$.

*** $P < 0.01$.

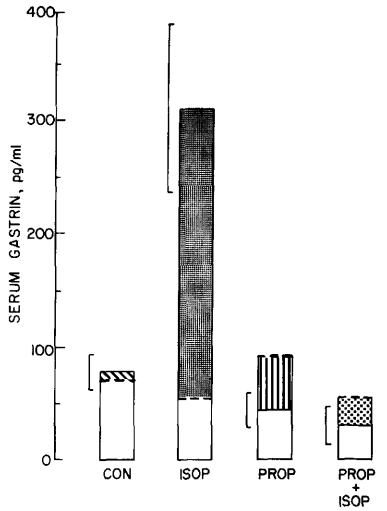

FIG. 1. Serum gastrin in rats injected with isoproterenol (ISOP) or propranolol (PROP) alone or in combination. Each bar represents serum gastrin from a separate group of four to five rats. Each rat was bled twice. Dashed lines with each bar show the mean 0 time value while the shaded areas show the 70-min change and brackets show the SE of the mean change. PROP (10 mg/kg) was given immediately after the initial bleeding, and ISOP (1 mg/kg) was given 10 min later. The second bleeding was 60 min after ISOP. Control rats received 0.15 *M* NaCl iv. Rats were 9 weeks old, weighed 250–300 g and had been fasted for only 18 hr. Significant difference in changes: ISOP vs. Con or PROP, $P < 0.05$; ISOP vs PROP + ISOP, $P < 0.02$; PROP vs Con, $P < 0.05$.

tion of isoproterenol. In addition to blocking isoproterenol-induced increases in serum gastrin (Fig. 1), injection of propranolol also restricted or prevented the rise in serum gastrin produced by feeding, as illustrated in Fig. 2.

Figure 3 illustrates that bathing the antral mucosa with isoproterenol, like iv isoproterenol, also increased serum gastrin in the rat. Here, isoproterenol given by gavage to rats with the pylorus ligated resulted in a consistent increase in gastrin 30 min later. Control rats with the pylorus ligated and gavaged with 0.15 *M* NaCl showed no demonstrable change in serum gastrin. In order to find out whether the effect of isoproterenol was due to increased antral pH, isoproterenol was dissolved in 0.01 *N* HCl and given by gavage to rats with the pylorus ligated. Control rats received 0.01 *N* HCl only. As shown in Table I, Expt 3 isoproterenol still produced a large increase in serum gastrin after oral administration despite the fact that the pH of the stomach contents was kept low (pH = 2.0 ± 0.27) and did not differ significantly from that of control rats.

Possible interactions between cholinergic and adrenergic influences on serum gastrin responses in the rat were studied in the experiments shown in Figs. 4–6. As shown in Fig. 4, a large dose of acetylcholine given by gavage to rats produced a large increase in serum gastrin. In this experiment atropine, the postanglionic cholinergic blocking agent, given orally itself raised serum gas-

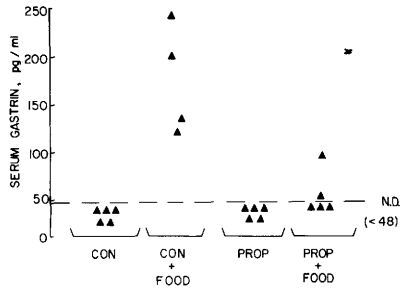

FIG. 2. Serum gastrin in rats pretreated with PROP and subjected to feeding. Each rat was fasted for 48 hr and then injected ip with 0.15 *M* NaCl (controls) or with PROP (10 mg/kg). Food (Purina Lab Chow) was presented immediately after PROP for a 1-hr period before blood collection; quantity of food consumed for all rats was 5.12 ± 0.35 g (mean ± SE). Each point represents an individual rat before or after feeding. Rats were 32 wk old and weighed 400–500 g. Significant difference: CON + Food vs CON, $P < 0.05$.

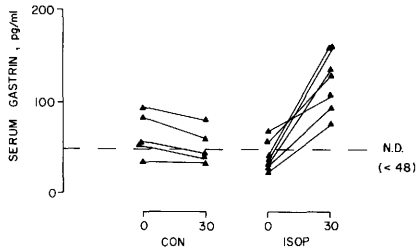

FIG. 3. Serum gastrin in rats with the pylorus ligated and gavaged with ISOP. Each rat was bled twice. After initial blood collection each rat underwent surgery and pyloric ligation. Then each animal was gavaged with 3 ml of 0.15 *M* NaCl (controls) or with ISOP (1 mg/kg in a 3-ml volume) and bled again 30 min later. Each pair of points represents an individual rat. Rats were 9 wk old, weighed 250–300 g and had been fasted for 24 hr. Significant change: ISOP vs CON, $P < 0.01$.

trin levels somewhat as reported by others (10) and, not unexpectedly, atropine also abolished or greatly restricted the ability of acetylcholine to increase serum gastrin. More surprisingly, a β-adrenergic blocking agent, propranolol, given orally, also appeared to reduce the serum gastrin response to acetylcholine, although two of the five rats tested still showed large responses to acetylcholine. Thus atropine restricted the response to acetylcholine, and propranolol also appeared effective in this regard (Fig. 4). Conversely, however, pretreatment of rats orally with atropine did not noticeably influence the serum gastrin elevation produced by administration of isoproterenol by gavage (Fig. 5).

The experiment illustrated in Fig. 6 shows basal (0 time) serum gastrin levels and 60 min responses to isoproterenol or propranolol in rats subjected to truncal vagotomy and pyloroplasty. Basal serum gastrin levels were highly variable in vagotomized rats but characteristically were higher than in control rats for all but three of 15 rats shown in Fig. 6. Injection of isoproterenol markedly raised the serum gastrin levels in vagotomized rats even further while injection of propranolol either reduced or did not affect

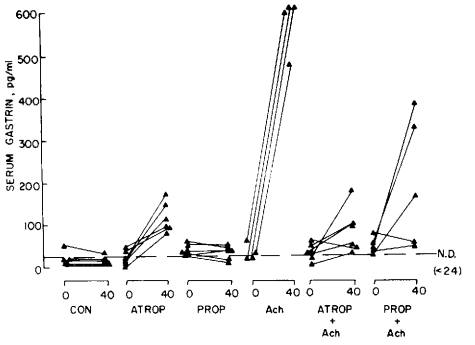

FIG. 4. Serum gastrin in rats gavaged with acetylcholine (Ach), atropine (ATROP) or PROP. Each rat was bled twice, once at 0 time and again 40 min later. ATROP, PROP (10 mg/kg in a 3-ml volume) or 3 ml 0.15 *M* NaCl (controls) was given immediately after initial blood collection; Ach (3 ml of 5% (w/v)) or vehicle (3 ml H_2O) was given 10 min later. Each pair of points represents an individual rat. Rats were 8 weeks old, weighed 220–280 g and had been fasted for 24 hr. Significant difference in changes: Ach or ATROP vs CON, $P < 0.01$; Ach vs ATROP + Ach or PROP + Ach, $P \leq 0.01$.

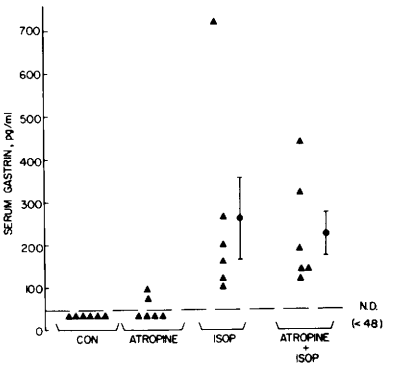

FIG. 5. Serum gastrin in rats gavaged with ISOP and ATROP alone or in combination. ATROPINE (10 mg/kg in 3 ml) or 3 ml 0.15 *M* NaCl (controls) was given and ISOP (1 mg/kg in 1.5 ml) or 1.5 ml 0.15 *M* NaCl was given 10 min later. Each point represents the serum gastrin level of an individual rat. Serum gastrin levels in both ISOP, and ATROPINE + ISOP are also presented as mean ± SE. Rats were 9 weeks old, weighed 220–320 g and had been fasted 48 hr. Significant difference: ISOP or ATROP + ISOP vs Con, $P < 0.05$; ISOP vs ATROP + ISOP, N.S.

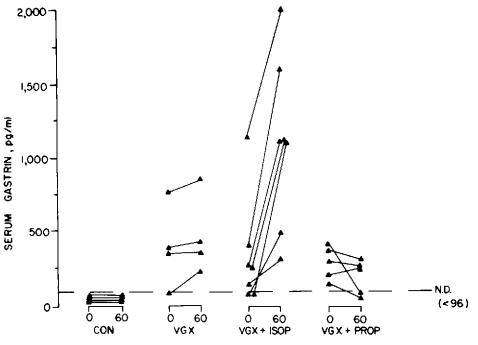

FIG. 6. Serum gastrin in rats after vagotomy (see Methods) and injection of ISOP or PROP. Vagotomy (VGX) was performed 30–60 days before the experiment. 0.15 *M* NaCl (controls), ISOP (1 mg/kg) or PROP (10 mg/kg) was injected immediately after bleeding at 0 time and rats were bled a second time 60 min later. Each pair of points represents an individual rat. Rats were 10–12 weeks old, weighed 250–325 g and had been fasted for 24 hr. Significant difference in changes: VGX + ISOP vs CON, VGX, or VGX + PROP, $P < 0.01$.

the elevated basal gastrin level in these animals.

Discussion. The present findings clearly show that in the rat catecholamines can markedly influence secretion of gastrin. In agreement with earlier observations by oth-

ers in man and the dog (3–5), administration of a small dose of epinephrine to rats produced a detectable increase in serum gastrin (Table I). The catecholamine-induced increase in serum gastrin appears to be mediated by β-adrenergic receptors. In support of this idea are the present findings showing that (a) two β-agonists, isoproterenol and epinephrine, raised serum gastrin levels (Table I, Expt 1 and 3; Figs 1 and 3) even in vagotomized rats with already high basal gastrin levels (Fig. 6); and (b) a β-adrenergic blocking agent, propranolol, abolished and hypergastrinemic effect of isoproterenol (Fig. 1) and reduced or abolished increases in serum gastrin produced by feeding (Fig. 2) and acetylcholine gavage (Fig. 4). As reported for man (2), we also observed in one experiment (Fig. 1) that iv propranolol appeared to reduce even basal serum gastrin levels. We found that an α-blocker, phenoxybenzamine, but not the α-agonist, phenylephrine, increased serum gastrin levels (Table I, Expt 2).

The effect of catecholamines on serum gastrin appears to be a direct one i.e., an effect directly on the antral G-cell. In support of this idea are the observations (a) that isoproterenol effectively increased blood gastrin after introduction into the stomach of rats with the pylorus ligated (Table I, Expt 3; Fig. 3); and (b) that parenteral administration of isoproterenol to acutely gastrectomized rats did not increase serum gastrin (unpublished results). The fact that exposure of the antral mucosa to isoproterenol given by gavage in 0.01 *N* HCl still elevated serum gastrin also supports the view that isoproterenol acted directly and not, as suggested by earlier work showing suppression of gastric acid secretion (11), indirectly by elevation of intragastric pH. It is of interest that isoproterenol and epinephrine, like atropine (10), paradoxically can act to both inhibit gastric acid secretion (11) and to increase blood gastrin. This occurs despite the fact that the function of gastrin is considered to be promotion of gastric acid secretion and cannot be explained by an increase in gastrin release secondary to a rise in intragastric pH (Table I, Expt 3; 10). Farooq and Walsh (10) have suggested that their findings with atropine alone may be best explained by the existence of cholinergic mechanisms which act to inhibit gastrin release as well as to promote gastric acid secretion. Similarly, β-adrenergic mechanisms may exist which act to promote gastrin release as well as to inhibit acid secretion. Whether endogenous catecholamines normally exert important effects on gastrin release in the rat remains to be established. Nevertheless, the present findings in the rat agree with earlier findings in the dog (3) and man (3–5) in suggesting that adrenergic mechanism may play a larger and more important role in mediating gastrin secretion than heretofore suspected.

Summary. Effects of vagotomy, acetylcholine and various adrenergic drugs on serum immunoreactive gastrin levels were studied in the rat. In rats fasted 24 hr or longer serum gastrin concentrations generally were <50 pg/ml; 60 min after the onset of feeding, serum gastrin concentrations reached levels >100 pg/ml. In rats fasted overnight, injections of epinephrine, isoproterenol and phenoxybenzamine all raised the serum gastrin level. Pretreatment with propranolol, a β-adrenergic blocking agent, abolished the hypergastrinemic affect of isoproterenol. Administration of propranolol also lowered basal serum gastrin levels and reduced the increase in serum gastrin produced by feeding. In vagotomized rats, isoproterenol futher increased the already elevated serum gastrin levels while propranolol alone slightly reduced the elevated basal gastrin levels in four or five vagotomized rats. Bathing the antral mucosa by gavage with isoproterenol (even in 0.01 *N* HCl) also increased serum gastrin levels, indicating that isoproterenol acted directly on the antrum and not indirectly by elevating intragastric pH.

Possible interactions between cholinergic and adrenergic agents on serum gastrin responses also were examined. Acetylcholine given by gavage produced its well known large increase in serum gastrin. An anticholinergic drug, atropine, given orally, greatly restricted the ability of acetylcholine to raise serum gastrin, and propranolol given orally also inhibited the increase in serum gastrin levels produced by oral acetylcholine. The findings support the idea that both choliner-

gic and adrenergic systems influence gastrin secretion in the rat.

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