

EFFECT OF SECRETIN ON SERUM GASTRIN AS MEASURED BY IMMUNOASSAY

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The effect of intravenous secretin on serum immunoreactive gastrin was studied in 34 subjects during routine secretin tests. Taking the lowest level to which gastrin fell in each individual patient, it was shown that Boots secretin (2 U per kg of body weight) caused a significant fall in serum gastrin from a mean (\pm SEM) fasting level of 44 ± 7.2 pg per ml to a level of 17 ± 3.8 pg per ml at a mean time of 25 min after injection. Secretin obtained from the Gastrointestinal Hormone Research Unit, Karolinska Institutet, Stockholm (1 U per kg of body weight) produced a fall in serum gastrin from a mean fasting level of 60 ± 9.8 pg per ml to a level of 15 ± 5.6 pg per ml at a mean time of 25 min after injection. Control injection of saline produced no significant change in gastrin level. This indicates that secretin may act not only as an inhibitor of gastrin action at a receptor site, but also to suppress the release of gastrin from gastrin-secreting cells or to promote the excretion or increased metabolism of gastrin.

Studies in dogs indicate that gastrin-stimulated acid secretion is inhibited by endogenous and exogenous secretin.¹⁻⁴ This does not hold in the cat,^{4, 5} while in man, basal secretion of acid is inhibited in some subjects^{6, 7} but not in others.⁸ These findings suggest that in some species, including man, secretin may be one of the duodenal mechanisms evoked in the inhibition of both basal and gastrin-stimulated gastric acid secretion. The mechanism of this inhibition is not known, but the theory has been proposed of noncompetitive inhibition at a receptor site.⁹ Other possible mechanisms

may be interference with release of gastrin, neutralization of gastrin after its release, or increased excretion of gastrin. This study has explored the effect of exogenous secretin injection on circulating immunoreactive gastrin to ascertain whether there is any change in serum gastrin.

Materials and Methods

Thirty-four patients, 18 males and 16 females whose ages ranged from 18 to 64 years, were studied after an overnight fast. A double lumen Dreiling tube was positioned under fluoroscopic control to obtain proper separation of gastric and duodenal contents. Aspiration was performed by continuous suction at 5 mm Hg, with frequent interruptions, by means of a Clements pump (Clements, Sydney, Australia) and the contents were collected into flasks under ice for 30 min before and for four consecutive 15-min periods after the injection of secretin.

Twenty-six patients had an intravenous injection of 2 U of Boots secretin (Boots, Nottingham, United Kingdom) per kg of body weight, and 8 patients had an intravenous injection of 1 U of pure natural secretin [(GIH) Gastrointestinal Hormone Research Unit, Karolinska

Institutet, Stockholm, Sweden weight. Twelve subjects had of 0.9% sodium chloride.

A 19-gauge needle was in arm vein and patency ens flushing with a solution of he ml of 0.9% sodium chloride. been shown not to affect se Blood was drawn for gastrin a at the time of, and 5, 10, 15, min after the injection of s chloride.

Duodenal contents were an for volume, and bicarbonate analyzed with a Van Slyke m.

Gastrin concentration in 0 estimated in duplicate by i since the original report some improved sensitivity and req current method of immuno Antibodies to synthetic hu perial Chemical Industries, were obtained from rabbits munization. Synthetic huma was iodinated¹¹ and the la rated from free iodide on column.¹² A volume of 200 dilution of antiserum was : microliters of ¹²⁵I-HG I tracer (3 and 0.5 ml of serum at 4 C. l tion period was 24 hr and th in the absence of unlabele cently a 72-hr incubation p binding in the absence of Standard curves (fig. 1) w saying from 0 to 500 pg of serum freed of gastrin by p tion.¹⁰ After incubation, tl was adsorbed to dextran-co Norit-A charcoal, 0.5% de phosphate buffer), the anti I was separated by aspirat ¹²⁵I-HG I was counted in a r (Nuclear-Chicago Corpora Ill.).

The sensitivity of this ass ml, that is, 2 to 2.5 pg car and this level produces a percentage of bound radioa trin.

Reproducibility of det assessed by freezing aliquo gastrin concentration in th mal range (\approx 50 pg per ml serially in lots of five tripl of weeks so that eight sets each were made. For each

Received September 8, 1970. Accepted February 22, 1971.

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The support of the National Health and Medical Research Council of Australia is gratefully acknowledged.

Tables showing complete data in each individual subject are available from the author upon request.

Institutet, Stockholm, Sweden] per kg of body weight. Twelve subjects had a control injection of 0.9% sodium chloride.

A 19-gauge needle was inserted into a forearm vein and patency ensured by frequent flushing with a solution of heparin, 1000 U in 20 ml of 0.9% sodium chloride. This solution has been shown not to affect serum gastrin levels. Blood was drawn for gastrin assay 30 min before, at the time of, and 5, 10, 15, 20, 30, 45, and 60 min after the injection of secretin or sodium chloride.

Duodenal contents were analyzed individually for volume, and bicarbonate concentration was analyzed with a Van Slyke manometer.

Gastrin concentration in 0.5 ml of serum was estimated in duplicate by immunoassay,¹⁰ but since the original report some modifications have improved sensitivity and reproducibility so the current method of immunoassay is described. Antibodies to synthetic human gastrin I (Imperial Chemical Industries, United Kingdom) were obtained from rabbits 3 months after immunization. Synthetic human gastrin I (HG I) was iodinated¹¹ and the labeled peptide separated from free iodide on a Sephadex G-10 column.¹² A volume of 200 μ liters of a 1:1000 dilution of antiserum was incubated with 200 μ liters of ¹²⁵I-HG I tracer (3000 counts per min) and 0.5 ml of serum at 4 C. Initially, the incubation period was 24 hr and this gave 40% binding in the absence of unlabeled gastrin. More recently a 72-hr incubation period has given 50% binding in the absence of unlabeled gastrin. Standard curves (fig. 1) were obtained by assaying from 0 to 500 pg of HG I in 0.5 ml of serum freed of gastrin by prior charcoal adsorption.¹⁰ After incubation, the "free" ¹²⁵I-HG I was adsorbed to dextran-coated charcoal (2.5% Norit-A charcoal, 0.5% dextran 500 in 0.1 M phosphate buffer), the antibody-bound ¹²⁵I-HG I was separated by aspiration, and the "free" ¹²⁵I-HG I was counted in a γ scintillation counter (Nuclear-Chicago Corporation, Des Plaines, Ill.).

The sensitivity of this assay system is 5 pg per ml, that is, 2 to 2.5 pg can be readily detected and this level produces a change of 2% in the percentage of bound radioactivity from "0" gastrin.

Reproducibility of determinations was assessed by freezing aliquots of serum with a gastrin concentration in the middle of our normal range (\approx 50 pg per ml). These were assayed serially in lots of five triplicates over a number of weeks so that eight sets of 18 determinations each were made. For each group of five tripli-

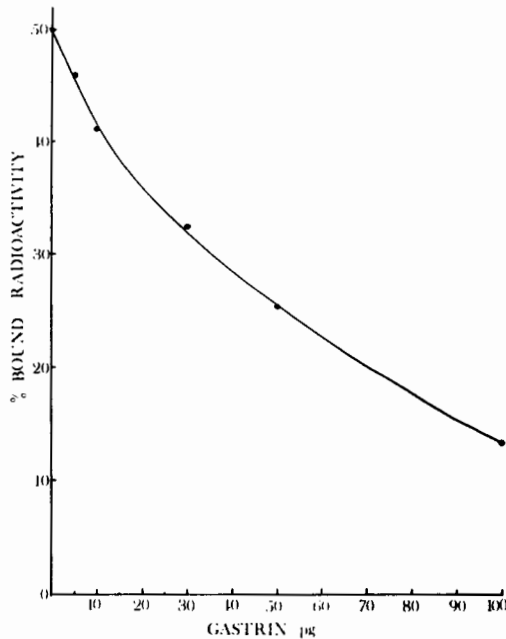


FIG. 1. Calibration curve for radioimmunoassay with antibody diluted 1:5000 showing percentage of antibody-bound ¹²⁵I-gastrin plotted against increasing amounts of unlabeled gastrin.

cates, coefficient of variation in serum gastrin level was 2.6% (within assay variation) and for the eight sets of sera assayed at 3-day intervals the coefficient of variation in serum gastrin level was 7.3% (between assay variation).

Cross reactivity with cholecystokinin (CCK) occurs above levels of CCK of 10⁸ pg, which is presumably well above fasting levels in human serum,¹³ and no cross reactivity has been demonstrated with other peptide hormones including secretin.

Statistical analysis of group means was performed by Student's *t*-test on an Olivetti computer using standard formulas.¹⁴ Coefficient of correlation (*r*) was determined using standard formulas.¹⁴

Results

Boots secretin: The effects of Boots secretin on serum immunoreactive gastrin are shown in table 1. There is a fall in serum gastrin from a mean basal level of 44 pg per ml to 28 pg per ml at 10 min, followed by a slight rise to 33 pg per ml and then a further fall to 25 pg per ml at 45 min (difference significant at *P* < 0.025).

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TABLE 1. Serum gastrin after intravenous Boots secretin

Time after secretin (min)	0	5	10	15	20	30	45	60
Serum gastrin (pg/ml)								
Range	0-160	0-110	0-100	0-96	0-140	0-78	0-100	0-82
Mean	44	40	28	29	33	30	25	28
Standard error	7.2	5.7	4.6	5.5	6.1	4.8	4.8	4.9
No.	26	26	26	25	26	26	26	26
P ^a		>0.1	>0.05	>0.05	>0.1	>0.05	<0.025	<0.05

^a Difference from fasting basal level (< 0.05 significant).

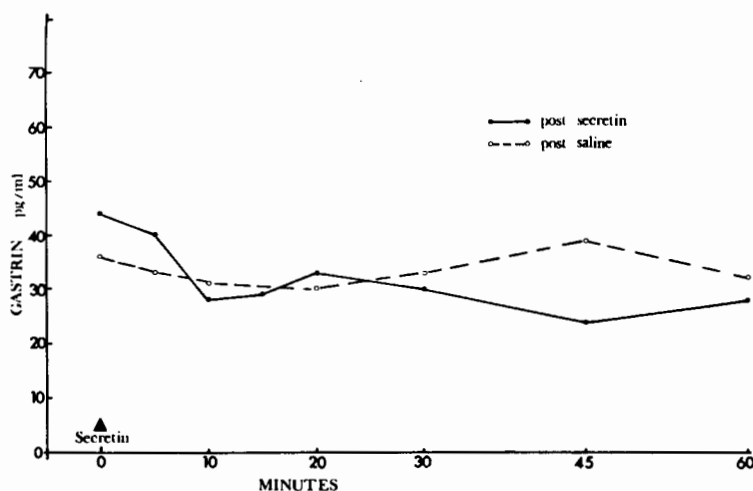


FIG. 2. Serum gastrin response to the intravenous injection of 2 U of Boots secretin per kg of body weight or 0.9% sodium chloride.

Figure 2 compares these results with those obtained in 7 subjects given a control injection of saline. Fasting gastrin levels in the two groups are different, but, whereas there is a significant fall in gastrin after secretin, the levels after saline are not significantly different from basal levels at any time during the hour postinjection and range from 35 to 33, 31, 30, 33, 39, and 32 pg per ml.

GIH secretin: The effects of GIH secretin on serum immunoreactive gastrin are shown in table 2. There is a fall in serum gastrin from a mean basal level of 60 pg per ml to 22 pg per ml at 15 min and 19 pg per ml at 30 min, these levels being significantly different from basal levels at $P < 0.01$ and $P < 0.02$, respectively.

Figure 3 compares these results with those obtained in 5 patients from the group

given a control injection of saline. Fasting levels are similar, but, whereas secretin caused a marked fall in gastrin levels, the levels following saline are not significantly different from basal levels at any time during the hour postinjection and range from 65 to 65, 62, 62, 62, 60, 58, and 60 pg per ml.

The time at which serum gastrin fell to its lowest level varied from patient to patient. Four patients, all tested with Boots secretin, showed no significant change in gastrin levels. In the remaining 30 subjects, 1 had the maximal fall at 5 min, 5 at 10 min, 6 at 15 min, 5 at 20 min, 7 at 30 min, 5 at 45 min, and 1 at 60 min after injection.

Because of this variability in time of fall of serum gastrin to lowest levels after secretin, the results have been analyzed in a different manner to enable a more accurate

Time after secretin
Serum gastrin (pg/ml)
Range
Mean
Standard error
No.
P ^a

^a Obtained from

^b Difference from

FIG. 3. Serum gastrin response to the intravenous injection of 2 U of Boots secretin per kg of body weight or 0.9% sodium chloride.

assessment to be of comparison of lowest level of gastrin, and the time of fall for each patient.

Boots secretin: The mean age of the patients, lowest gastrin level, lowest gastrin level after secretin, time at which gastrin fell to its lowest level, and the time at which gastrin returned to its basal level for the group is divided into three groups: (A) those with juice bicarbonate (B) and those with juice bicarbonate (C).

The group with juice bicarbonate had a mean gastrin concentration which fell to a mean of 3.8 pg per ml at 15 min ($P < 0.005$). The

TABLE 2. Serum gastrin after intravenous GIH secretin^a

Time after secretin (min)	0	5	10	15	20	30	45	60
Serum gastrin (pg/ml)								
Range	28-98	20-112	10-110	0-60	0-56	0-40	0-56	2-94
Mean	60	54	44	22	23	19	29	42
Standard error	9.8	12.6	11.9	7.4	6.8	5.7	10.9	12.3
No.	8	8	8	8	8	8	8	8
P ^b		>0.3	<0.2	<0.01	<0.01	<0.002	<0.05	<0.15

^a Obtained from the Gastrointestinal Hormone Research Unit, Karolinska Institutet, Stockholm, Sweden.

^b Difference from fasting basal level (< 0.05 significant).

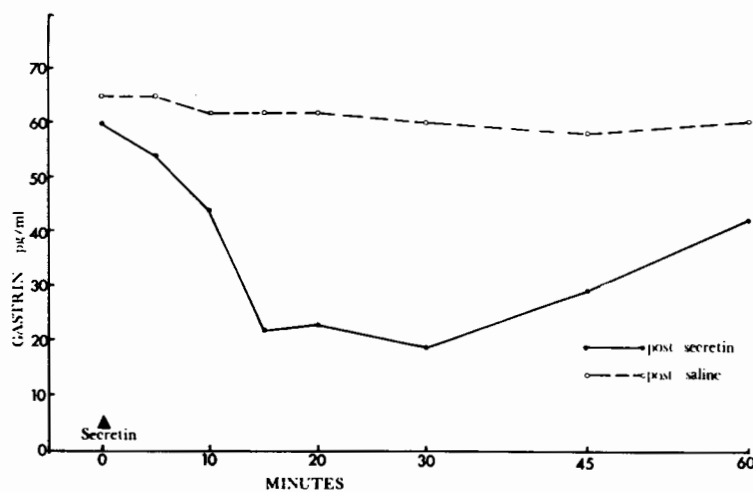


FIG. 3. Serum gastrin response to the intravenous injection of 1 U of secretin obtained from the Gastrointestinal Hormone Research Unit per kg of body weight, or 0.9% sodium chloride.

assessment to be made. Analysis is in terms of comparison of fasting gastrin levels, the lowest level of gastrin attained after secretin, and the time at which this occurred in each patient.

Boots secretin: Table 3 compares the mean age of the patient, fasting gastrin level, lowest gastrin level reached after secretin, time at which this lowest level occurred, and peak bicarbonate concentration for the group as a whole (A) and the group is divided into those with a pancreatic juice bicarbonate of over 80 mEq per liter (B) and those with a low bicarbonate concentration (C).

The group as a whole had a mean fasting gastrin concentration of 44 ± 7.2 pg per ml which fell to a mean concentration of 17 ± 3.8 pg per ml at a mean time of 25 min ($P < 0.005$). There were no significant dif-

ferences between patients with normal (B) or abnormal (C) pancreatic function with respect to age, fasting gastrin levels, lowest gastrin level reached after secretin or time to reach this lowest level.

GIH secretin: Table 4 compares the mean age of the patient, fasting gastrin level, lowest gastrin level reached after secretin, time at which this lowest level occurred, and peak bicarbonate concentration of pancreatic juice. Serum gastrin fell from 60 ± 9.8 pg per ml to 15 ± 5.6 pg per ml at a mean time of 25 min postinjection, and this fall is significant at $P < 0.0005$.

There was no correlation between peak bicarbonate concentration and fasting serum gastrin ($r = 0.26$) nor between bicarbonate concentration and lowest serum gastrin attained after secretin ($r = 0.03$).

Serum gastrin fell to levels which could

ated after boots secretin

of lowest gastrin	Peak HCO ₃
min	mEq/liter
5-60	17-98
25	70
2.9	7.5
16	26
0-45	80-98
23	90
3.3	1.4
5	15
-60	17-77
6	50
5.4	6.4
1	11

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fall in serum with GIH secretin but this may reveal levels in the group drug because the is similar in both been shown that times as powerful in terms of pan- may be another to immeasurable and did not fall in there may be ins of dose respon- study with higher olve this problem. in gastrin follow- subject to subject cretin it occurred ollowing injection. mean fall of 75% boots secretin pro- at 25 min if maxi- atients are com-

in serum gastrin om this study but exist. These are

inhibition of release of gastrin from gastrin-secreting cells, neutralization of gastrin in the blood, and the increased destruction or excretion of gastrin. Secretin added to gastrin-containing solutions does not alter the levels of gastrin, so neutralization is probably an unlikely mechanism. Urinary excretion of gastrin has not been studied in this project and, although the kidney is one of the sites of gastrin destruction,¹⁶ the effect of secretin on this aspect is as yet undetermined. Other sites of gastrin excretion are as yet unknown and the possibility exists that secretin may act on one of these sites to promote excretion of gastrin. The most probable mode of action of secretin under the conditions of this study is to inhibit the release of gastrin. Little information is available on the degradation and metabolism of gastrin but the half-life has been reported as varying from 7 min¹⁷ to 9 to 11 min.¹⁸ Boots secretin produced a fall of 38% in serum gastrin and GIH secretin a fall of 43% in serum gastrin in 10 min and this suggests that interference with release of gastrin is one of the mechanisms operative in diminution of gastrin following secretin.

The finding of a decrease in serum gastrin after secretin is not only seen in subjects with normal levels of gastrin as in the present group, but also in those with hypergastrinemia of pernicious anemia. In the latter group, significant decrease of serum gastrin occurs with both single injection and constant infusion of secretin¹⁹ and further strengthens the concept of a role of secretin in the inhibition of gastrin-stimulated acid secretion. The characteristics of the inhibition have been studied by Johnson and Grossman²⁰ and they suggest that secretin acts by noncompetitive inhibition at some receptor site. This effect of secretin, although applicable to the dog and perhaps to man, is not seen in the cat. This is not surprising, as the effects of various gastrointestinal hormones, particularly gastrin and cholecystokinin, are different in dogs and cats. Grossman⁹ has advanced the theory that gastrin, cholecystokinin, and secretin act on the receptor which has two interacting sites, one with an affinity for gastrin and cholecystokinin and one with an

affinity for secretin. With this hypothesis, simultaneous action of secretin with either gastrin or cholecystokinin leads to noncompetitive inhibition if one site is inhibitory or to noncompetitive augmentation if both sites are stimulatory. Secretin would inhibit the action of gastrin on the acid-producing cell in a noncompetitive manner.

This study has not explored this hypothesis but indicates that secretin may well have another inhibitory effect on gastrin. This is most probably interference with its release, but increased metabolism, degradation, or excretion cannot be completely discarded.

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OBSERVATION
ASPIRIN AND
COMPOSITE

In recent years there has been considerable interest in the use of aspirin in the treatment of peptic ulcers (especially in the case of the stomach).^{1,2} In this report we describe our experience with the administration of aspirin on the secretion of saliva (in the case of the dog when the tympani) and its effect on salivary gland function.

Experimental

In these experiments we used both male and female dogs weighing between 10 and 18.5 kg. We collected salivary secretions with intravenous administration of barbitol (Nembutal, Nembutal weight). For the control data, on its duct was a diaphragm catheter (inner diameter 0.5 mm).

Received December 18, 1971.

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This work was supported by the Carle Hospital Foundation.