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Potentiation Between Gastrin and Histamine in Stimulation of Gastric Secretion.* (28582)

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During studies on Heidenhain pouches in dogs we noted that the responses to certain combinations of gastrin extract plus histamine were greater than would be anticipated on the basis of summation of stimulatory effects(1). The study reported here was performed to determine whether the combination of gastrin plus histamine resulted in true potentiation of stimulation of secretion of acid.

Methods. Four dogs with Heidenhain pouches were fasted overnight before each study and studies were performed no oftener than twice a week. Gastrin and histamine were given by continuous intravenous injection using a calibrated peristaltic pump that delivered 20 ml/hr. An infusion of 0.15 M NaCl at this rate was maintained throughout the study and the concentration of gastrin and histamine in the infusion fluid was varied to give the desired dosage rates. Gastrin was prepared from mucosa of the pyloric gland area of the stomach of hogs by a method previously described(1). Doses of gastrin are expressed as the wet weight of mucosa from which the extract was derived. Doses of histamine are expressed as weight of the dihydrochloride salt. Collections of gastric juice were made every 15 minutes. The volume was measured and the acidity determined by titration with 0.2 N NaOH with phenol red indicator. Output of acid is expressed as mEq/hr for the last 60 minutes of each 75-minute period.

As pointed out previously, the simple criterion that response to a combination of agents given simultaneously is greater than the sum of the responses to the agents given separately is not reliable proof of potentiation(2). We have used here the same two criteria for potentiation as were used in a previous study of cholinergic agents with gastrin and with histamine(2). Potentiation was said to exist if 1) the response to the doses of the 2 agents given together exceeded the larger of the responses to twice the dose of each agent given separately, or if 2) the response to the combined agents exceeded the maximal response attainable with either agent alone.

Results. Two plans of study were used. In Series 1 the dose of gastrin was doubled every 75 minutes through 4 steps starting with 5 g per hour. A background dose of histamine was given at a constant level throughout each experiment, beginning 2 hours before the gastrin doses were started. The doses of histamine used as a background were 0, 0.25, 0.5, 1.0, and 2.0 mg of the dihydrochloride per hour. Most of the combinations of gastrin plus histamine resulted in potentiation of stimulation as judged by criterion 1 as indicated by the encircled values ($p<0.05$) in Fig. 1. Maximal responses occurred with the intermediate doses of gastrin; the largest dose of gastrin, 40 g per hour, always gave lower secretory rates than 20 g per hour when given with any of the doses of histamine used. Since we have shown that high dosage rates of gastrin extract can profoundly inhibit histamine-stimulated secretion(1), this inflection of the dose-response curve probably represents the beginning of the reversal of effect of gastrin from a stimulatory to an inhibitory action.

In Series 2 mixtures of gastrin extract and

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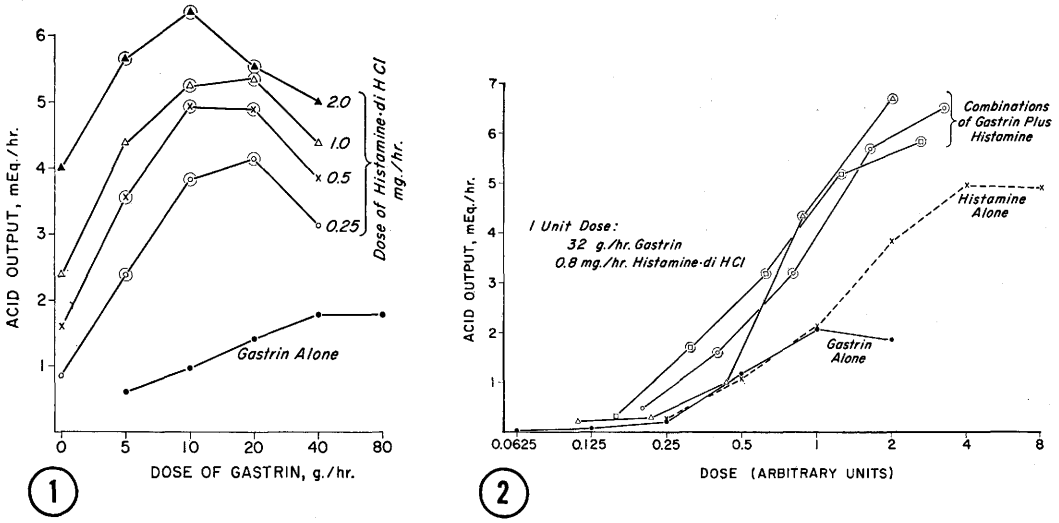

FIG. 1. Response to graded doses of gastrin alone and to graded doses of gastrin superimposed on background stimulation by histamine at various constant rates. Each point is the mean value for 8 tests, 2 on each of 4 dogs. Values that satisfy criterion 1 for potentiation (see text) are circled ($p < 0.05$).

FIG. 2. Response to graded doses of gastrin alone, of histamine alone, and of various combinations of gastrin plus histamine. Doses are expressed in arbitrary units as explained in text. For each set of symbols, successive points to the right represent doubling of doses. Lowest doses were: gastrin alone, 2 g/hr; histamine alone, 0.2 mg/hr; combinations of gastrin plus histamine, △ (0.05 mg/hr histamine plus 1.5 g/hr gastrin), □ (0.1 mg/hr histamine plus 1 g/hr gastrin), and ○ (0.15 mg/hr histamine plus 0.5 g/hr gastrin). Each point is the mean value for 8 tests, 2 on each of 4 dogs. Values that satisfy criterion 1 for potentiation (see text) are circled ($p < 0.05$).

histamine in various ratios were studied. The dose rate of the mixture was doubled every 75 minutes through 5 steps. In this series, in contrast with Series 1 in which the dose of histamine was constant during any one study, the doses of gastrin and histamine were raised simultaneously. For ease of visualization of the results, the doses of gastrin and histamine have been converted into arbitrary units, so selected that in the linear portion of the curve relating logarithm of dose to response one unit of either agent given alone gave essentially the same response. It is clear that most combinations of histamine plus gastrin extract gave larger responses per unit dose than did either agent alone as indicated by encircled values in Fig. 2. Furthermore, the highest response to a combination of gastrin and histamine (6.6 mEq/hr) was significantly greater ($p < 0.01$) than the maximal response to gastrin alone (2.1 mEq/hr) or to histamine alone (5.0 mEq/hr). Thus both criteria 1 and 2 for potentiation were satisfied.

Discussion. A number of different pairs of stimuli have been shown to act in a potentiated fashion in stimulation of gastric secretion of acid. These include cholinergic drugs plus histamine or plus gastrin(2) and methylated xanthines plus histamine or plus endogenous gastrin(3). In no instance is the mechanism of potentiation known. In the case of the combination gastrin-cholinergic stimuli, potentiation has been demonstrated without the use of exogenous agents, *i.e.*, by physiological release of gastrin from the pyloric mucosa combined with reflex vagal stimulation producing cholinergic effects(4).

The present study shows that exogenous gastrin in the form of gastrin extracts when given in appropriate combinations with exogenous histamine results in potentiation of stimulation of gastric secretion. Whether it will be possible to demonstrate potentiation between injected histamine and endogenous gastrin remains to be determined. Previous studies in man showing that acidification of the isolated pyloric gland area of the stomach

(6) or surgical resection of this part of the stomach(7) reduced the response to maximal stimulation with histamine, suggest that potentiation between endogenous gastrin and exogenous histamine may occur.

The finding that pairs of stimulants of which histamine is one member, such as histamine plus cholinergic stimulants and histamine plus gastrin, produce potentiation cannot readily be reconciled with the hypothesis that histamine is the final common mediator for all forms of stimulation of acid secretion (5). If all agents act through release of histamine, the simplest assumption would be that they should act additively with histamine in the degree of stimulation achieved.

Summary. Histamine and gastrin extracts were given separately and in various combinations of doses to dogs with Heidenhain pouches. Most of the combinations of histamine plus gastrin gave higher rates of secretion of acid than could be accounted for on the basis of summation of stimulatory effects. The maximal rate of secretion of acid was higher with the combination of agents than with either agent alone. It is concluded that there is potentiation between exogenous gastrin and exogenous histamine in stimulation of gastric secretion of acid.

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Properties of Guinea Pig 7S Antibodies IV. Antibody Response to *E. coli* Bacteria.* (28583)

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Previous reports(1-6) characterized 2 types of guinea pig 7S antibody differing in electrophoretic mobilities and biological properties. Guinea pig $7S\gamma_1$ antibodies sensitized guinea pigs for passive systemic and cutaneous anaphylaxis, $7S\gamma_2$ antibodies did not. Guinea pig $7S\gamma_2$, but not γ_1, antibodies fixed complement *in vitro* in the presence of antigen and sensitized antigen-coated cells for lysis in the presence of complement.

The influence of certain methods of immunization on production of these 2 types of antibody has been defined(1,3). Guinea pigs hyperimmunized with hapten conjugates, protein or tissue cells emulsified in complete Freund's adjuvant invariably produced both $7S\gamma_1$ and $7S\gamma_2$ antibodies. However, guinea pigs hyperimmunized with hapten conjugates (dinitrophenyl-bovine gamma globulin) administered intraperitoneally without adjuvants produced primarily γ_1 antibodies. The present report is concerned with the finding that guinea pigs hyperimmunized with a particulate antigen, *Escherichia coli* bacteria, emulsified in complete Freund's adjuvant produced predominantly $7S\gamma_2$ antibodies.

Methods. Immunization. E. coli 0 111:B4 bacteria were grown as described(7). Bacteria were killed by heating at 60°C for 30 minutes, washed twice in saline and suspended at a concentration of approximately 5×10^{11} bacteria per ml. Equal volumes of complete Freund's adjuvant and bacterial suspension were emulsified and 0.1 ml injected into each footpad of twenty-five 350-400 g Hartley-strain guinea pigs. Thereafter, animals were injected intradermally at 4

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