Heidenhain Pouch Response to Antral Stimulation before and after Antral Denervation in Dogs (34387)

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Release of gastrin from the mucosa of the pyloric gland area of the stomach is primarily under cholinergic control (1). Cholinergic release of gastrin may be evoked either by vagal stimulation, such as occurs during sham feeding, or by local reflexes (completed over nerves within the wall of the stomach) initiated by distention or by chemical stimulants acting topically on the mucosa of the pyloric gland area.

Extrinsic vagal denervation of the pyloric gland area of the stomach abolishes the gastrin release caused by vagal reflexes such as occur with sham feeding (2). Two previous studies on the question of the effect of extrinsic vagal denervation of the antrum on the release of gastrin by topically applied chemical stimulants have given conflicting results (3, 4).

In the present study, acid secretion from Heidenhain pouches in response to irrigation of antral pouches with graded concentration of acetylcholine or glycine was found to be greater before than after complete extrinsic denervation of the antral pouch.

Methods. Two dogs were prepared with Heidenhain pouches (HP) and isolated vagally innervated pouches of the pyloric gland area (antral pouches). The HP was drained by a Gregory cannula (5). The pylorus was brought through the abdominal wall to form a mucocutaneous fistula for the antral pouch. The duodenum was closed and continuity was restored by a gastrojejunostomy. While preparing the pouch of the pyloric gland area the border of the acid secreting oxyntic gland area was determined by pH indicator paper during histamine stimulation (6). A 1-cm rim of transitional mucosa was resected before a double mucosal wall was constructed between the pyloric and oxyntic gland areas. After completion of the first series of experiments the stomach was transected between the two mucosal walls. Also the omentums of the lesser and greater curvature of the separated antral pouch were divided and hence the pouch became autotransplanted to the abdominal wall. Secretory studies started 3 weeks after surgery and were carried out no oftener than twice a week after an 18-hr fast.

For perfusion of the antral pouch, a 3-ml balloon catheter (Foley) was inserted into the pouch and a polyethylene tube (1 mm o.d.) was introduced through the catheter. The pyloric pouch was perfused through the tube at a rate of 90 ml/hr (peristaltic pump, Harvard Apparatus Co., Dover, Mass.). The pouch was drained via the catheter into a reservoir at the level of the pouch to allow reflux back into the pouch. During collection of basal HP secretion the antral pouch was perfused with saline (0.15 *M* NaCl). The antral pouch was then irrigated with increasing concentrations of acetylcholine chloride or glycine dissolved in saline and with the pH adjusted to 7.0. The concentration was increased every hour by a factor of 2 or 4 (Fig. 1) until the increased concentration produced no further increase in volume flow rate from the HP. The pH of the fluid leaving the antral pouch was recorded for each 15-min sample.

For intravenous infusions, a constant rate of flow of saline (32 ml/hr) was maintained

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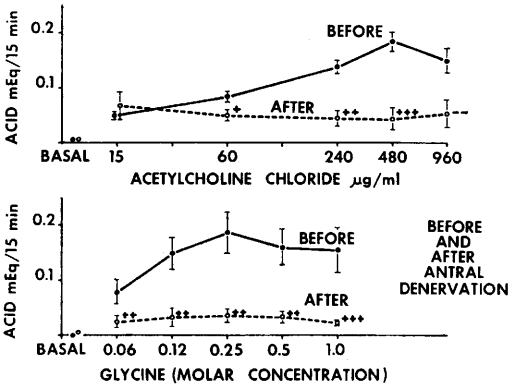

FIG. 1. Acid response from the Heidenhain pouch to perfusion of the antral pouch with increasing concentrations of acetylcholine (top); or glycine (bottom) before and after denervation of the antral pouch. For acetylcholine, 6 experiments on each dog before and 6 experiments after denervation of the antral pouch. For glycine, 6 experiments on each dog before and 5 experiments after denervation of the pouch. Vertical bars represent SE of the mean and plus signs the degree of significance of difference between response before and after denervation of the antral pouch, no +, $p>0.05$; +, $0.01<p<0.05$; ++; $0.001<p<0.01$; +++, $p<0.001$.

with a peristaltic pump. Stimulants were added to the saline to give the desired dose rate.

The acid secretory response of the HP to increasing doses of exogenous gastrin was determined before and after antral denervation as described previously (7). A single batch of gastrin extract was prepared from hog antral mucosa by the method of Gregory and Tracy (8) but carried only through the isopropanol stage; dose is expressed in grams wet weight of antral mucosa extracted. After collection of basal secretion from the HP for at least 30 min, 0.05 g/kg-hr was infused intravenously for 90 min. The gastrin dose was then doubled each hour until the increase in dose produced no further increase in volume flow rate from the HP. Throughout the experiment the antral pouch was perfused with saline at a rate of 90 ml/hr and the pH of the outflow was recorded.

To test vagal release of gastrin before and after antral denervation 200 mg/kg of body weight of 2-deoxy-D-glucose (2 DG) (Sigma Chemical Co., St. Louis, Mo.) was given as a single intravenous injection against a background stimulation with histamine (9). For the background stimulation a dose of 0.02 mg/kg-hr of histamine dihydrochloride was infused intravenously for 6 hr. Preliminary dose–response studies showed that this dose produced about 25% of the maximal HP response to histamine, confirming previous studies (10). A dose of 2 DG was injected 2 hr after the start of the histamine infusion. Throughout the experiments the antral pouch was perfused with saline and the pH of the antral pouch outflow was recorded.

Gastric juice was collected from the HP in 15-min samples and the volume was recorded. The acid concentration was determined by titration of 0.2-ml samples of gastric juice to pH 7.0 (Autoburette, Radiometer, Copenhagen, Denmark) with 0.2 *N* NaOH. In the dose–response curves the mean 15-min acid output during the last two 15-min periods at each dose level is given. The difference between the HP response before and after denervation of the pyloric pouch was evaluated for each dog using the U test for unpaired groups of different sizes (11). The probabilities of the differences for individual dogs were then combined to obtain the combined probability for the two dogs (12). In the experiments with 2 DG injected against background stimulation with histamine the mean 15-min acid output from the HP to histamine during the hour before the injection of 2 DG is referred to as the 100% level or the pre-injection level. The 15 min acid output during the 4 hours after the 2 DG injection is then expressed as percentages of the preinjection level.

Results. Perfusion of the antral pouch with solutions of acetylcholine in concentrations increasing from 15 to 480 μg/ml produced a gradual increase in the acid output from the HP (Fig. 1, top). The acid output in response to perfusion with 480 and 960 μg/ml did not differ significantly ($p>0.05$). The maximal mean response for the two dogs was 0.184 ± 0.019 (SEM) meq/15 min. Denervation of the antral pouch significantly ($p<0.05$) reduced the acid response to pouch perfusion with all but the lowest concentration of acetylcholine (Fig. 1, top). The pH of

the effluent from the antral pouch varied from 5.1 to 7.1.

Antral pouch perfusion with glycine in concentrations increasing from 0.06 to 0.25 *M* gradually increased the HP response (Fig. 1, bottom). The acid response to 0.5 and 1.0 *M* glycine did not differ significantly ($p>0.05$) from the response to 0.25 *M* glycine. The latter concentration produced the maximal mean response, 0.185 ± 0.037 meq/15 min which did not differ significantly ($p>0.05$) from the maximal mean response to acetylcholine. Denervation of the antral pouch markedly ($p<0.01$) reduced the HP acid response to perfusion with all concentrations of glycine tested (Fig. 1, bottom). In all experiments the antral effluent had a pH higher than 5.8.

With innervated antral pouches the maximal mean response of the HP to gastrin extract was 0.657 ± 0.099 meq/15 min and was produced by 1.6 g/kg-hr (Fig. 2). This response was more than 3 times higher than the response to endogenous gastrin released by antral perfusion with acetylcholine or glycine. After antral pouch denervation the highest mean response of the two dogs to gastrin extract, 0.876 ± 0.169 meq/15 min, was produced by 3.2 g/kg-hr. Whether a still larger dose would have produced a higher response was not investigated. The pH of the antral pouch effluent ranged from 5.2 to 7.4.

Before denervation of the antral pouch the

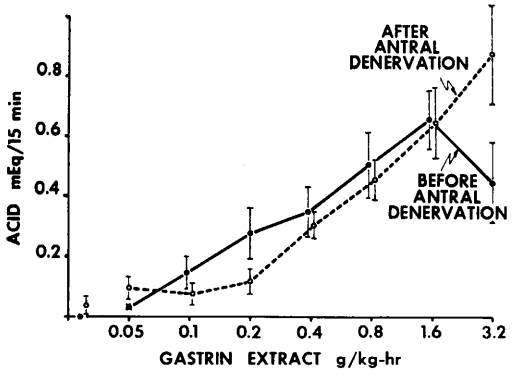

FIG. 2. Acid response from the Heidenhain pouch to graded doses of gastrin extract before and after denervation of the antral pouch. Three experiments on each dog before and after antral denervation. Vertical bars represent SE of the mean.

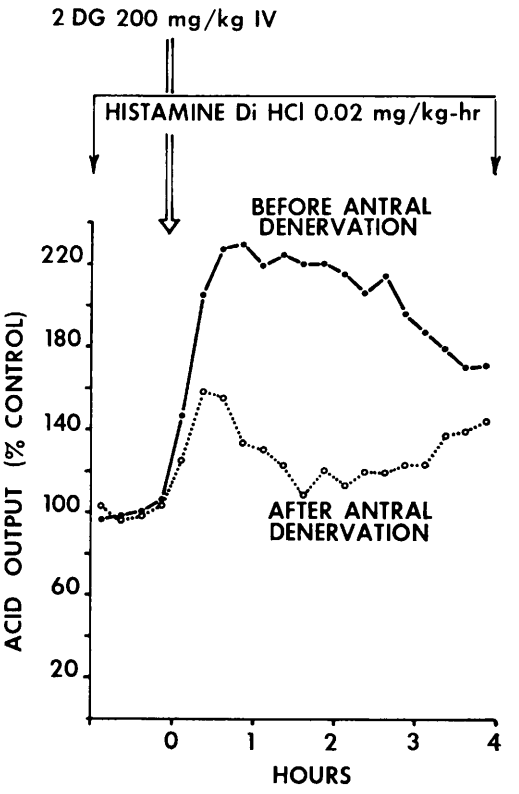

FIG. 3. Effect of an intravenous injection of 2 DG on histamine stimulated acid secretion from the Heidenhain pouch before and after denervation of the antral pouch. The 100% (preinjection) level before antral denervation was 0.541 meq/15 min and after antral denervation 0.434 meq/15 min. Number of experiments as in Fig. 2. The preinjection responses to histamine alone before and after denervation of the antral pouch were not significantly different ($p>0.05$).

intravenous injection of 2 DG against background stimulation with histamine produced a marked increase in acid output from the HP (Fig. 3). The highest mean acid output after 2 DG occurred in the fourth 15-min period and amounted to 229% of the mean preinjection level. After antral denervation the highest mean acid output after 2 DG was 158% of the preinjection level and occurred in the second 15-min period. The pH of the effluent from the antral pouch ranged from 5.5 to 6.8.

Discussion. Denervation of the antral pouch did not significantly change the response of the HP to submaximal doses of

exogenous gastrin (Fig. 2) or to histamine (Fig. 3, before injection of 2 DG). Therefore it is reasonable to conclude that the reductions found in the response of the HP to bathing the mucosa of the antral pouch with acetylcholine or glycine after antral denervation are attributable to changes in the release of gastrin resulting from the surgical procedure for denervation of the antral pouch. The surgical procedure used in denervating the antral pouch involved extensive transection of blood vessels to the pouch. Whether this devascularization was a significant factor in causing the observed decrease in HP response cannot be evaluated from the present studies. However earlier studies (13) have shown that antral pouches denervated in this way can still release enough gastrin to produce the same maximal response from gastric fistulas as exogenous gastrin. The present finding that denervation of the antral pouch decreased the HP response to antral stimulation suggests that tonic vagal impulses facilitate the local release of gastrin by acetylcholine and glycine.

Nyhus and co-workers (3) found that irrigation of antral pouches with ethanol produced as much acid response from Heidenhain pouches whether fully innervated antral pouches or pouches that had vagal interruption by antroneurolysis were used.

Dragstedt *et al.* (4) reported that denervation of the antral pouch by antroneurolysis did not reduce the HP response to antral perfusion with acetylcholine (50 μg/ml). Their results and ours are in agreement in regard to the finding that antral denervation did not alter the response to antral perfusion with low concentration (<60 μg/ml) of acetylcholine (Fig. 1 top); they did not use higher concentrations of acetylcholine.

The effectiveness of the antral denervation in greatly decreasing the vagal release of gastrin was shown by the marked lowering of the response to 2 DG. The small residual response to 2 DG after antral denervation (Fig. 3) could be caused by vagal release of gastrin from portions of the stomach or duodenum (14) not included in the antral pouch.

The findings of the present study confirm an earlier study from this laboratory (13) that the maximal response of HPs to exogenous gastrin was higher than to endogenous gastrin. The cause of this difference is not known and is particularly difficult to explain because gastric fistulas in the same animals showed the same maximal response to exogenous and endogenous gastrin (13).

In the present study the maximal response of the HP to irrigation of the antral pouch with acetylcholine or glycine were equal whereas in an earlier study (15) the response to acetylcholine was higher. We cannot explain this discrepancy.

Summary. In dogs with Heidenhain pouches and vagally innervated antral pouches, denervation of the antral pouch produced the following effects on secretion of acid from the Heidenhain pouches: (a) the responses to submaximal doses of exogenous gastrin extract and to histamine were not significantly changed, (b) the response to 2-deoxy-D-glucose, which presumably acts by vagal release of gastrin, was greatly decreased, and (c) the response to bathing the mucosa of the antral pouch with solutions of acetylcholine or glycine was greatly decreased. The results suggest that tonic vagal impulses facilitate the local release of gastrin by acetylcholine and glycine.

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