PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 163, 528–533 (1980)

Gastric Mucosal Damage in Rats Induced by Lateral Hypothalamic Lesions: Protection by Propantheline, Cimetidine, and Vagotomy (40809)[1]

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Grijalva and colleagues (1, 2) recently have shown that lateral hypothalamic (LH) lesions induce gastric mucosal damage in rats. Lateral hypothalamic lesions in dogs (3) and cats (4), and electrical stimulation of the LH in rats (5) are associated with increases in gastric acid secretion. However, Smith and Brooks (6) noted no significant change in basal acid output in rats after LH lesions. Since gastric acid secretion has been shown to be an important factor in the production of stress-induced gastric ulcers in rat (7, 8) and man (9), the first experiment examined whether the incidence of LH lesion-induced ulcers and erosions in the fundic (acid-secreting) gastric mucosa of rats could be altered by vagotomy, propantheline bromide (an anticholinergic), or cimetidine (a histamine H_2-receptor antagonist), all of which are known to reduce gastric acid output (10, 11). In a second experiment gastric mucosal barrier functions were tested very soon after producing LH lesions to determine if the barrier had been altered by the hypothalamic damage even before gross visible defects were present in the gastric mucosa.

Materials and methods. Experiment 1. Male Sprague–Dawley albino rats weighing 285–490 g were individually housed in wire mesh cages and given food (Purina Laboratory Chow) and water *ad libitum* for at least 1 week. At the beginning of the experiment they were deprived of food but not water overnight and then anesthetized with sodium pentobarbital (50 mg/kg body weight, ip). Groups of rats were then either given (i) bilateral subdiaphragmatic vagotomies ($N = 11$), or administered a single subcutaneous injection of (ii) propantheline bromide (10 mg/kg; $N = 10$), (iii) cimetidine (100 mg/kg; $N = 9$), or (iv) an equal volume of isotonic saline (5 ml/kg; $N = 18$). All four groups were then given bilateral LH lesions by the method described below. An additional control group ($N = 13$) was given a single injection of isotonic saline (5 ml/kg) followed by control brain surgery also described below. Only the cimetidine group received two additional injections (100 mg/kg) 8 and 16 hr following LH lesions.

Vagotomy and drug preparation. Bilateral subdiaphragmatic vagotomies were performed through a ventral midline incision made on the abdominal surface directly caudal to the xiphisternum. The lobes of the liver were gently deflected to locate the esophagus. The left gastric artery and vein were separated from the esophagus and were gently stripped of all connective and neural tissue. The anterior and posterior trunks of the vagus were then delicately teased away from the esophagus and a 1-cm section of each nerve removed. The esophagus was examined under a 40× dissection microscope and any remaining traces of neural or connective tissue were excised.

Cimetidine (Smith Kline & French Laboratories, Philadelphia, Pa.) solutions were prepared by dissolving the material in 0.5 *M* HCl, adjusting the pH to 6.0 with 1 *M* NaOH and diluting with normal saline.

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0037-9727/80/040528-06$01.00/0
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Propantheline bromide (Searle & Co., San Juan, P.R.) solutions were prepared by dissolving 30 mg of the material in 15 ml of normal saline.

Stereotaxic surgery. Rats were mounted in a Kopf Model 900 stereotaxic instrument. Bilateral electrolytic LH lesions were made with a lesion-generating device (C. H. Stoelting Co.) by passing a 1.2 mA direct anodal current for 15 sec through stainless steel electrodes (insect pins size 00 insulated with Epoxylite except for the cross section of the cut tip). A cathode clamped to a saline-soaked gauze pad wrapped around the tail completed the circuit. The electrodes were placed 2.4 mm posterior to bregma, 1.9 mm to each side of the midline, and 7.8 mm below the dura of the brain with the skull oriented in a horizontal position. Control brain surgery was performed by lowering the electrodes 6.5 mm below dura but no current was passed.

Postoperative procedure. All groups were food and water deprived for 24 hr following LH lesions or control brain surgery. Observations were made for presence of hypersalivation (12) (as indicated by saturations of saliva under the chin and around the mouth) and chromodacryorrhea (as indicated by the occurrence of reddish tearing around the orbits of the eyes), and then all groups receiving LH lesions were allowed access to food for 1 hr. This was done to obtain a preliminary indication that the brain lesions were placed within the LH area and, therefore, aphagia inducing. After the initial feeding test all groups were sacrificed with an overdose of sodium pentobarbital. The stomachs were removed quickly and opened along the greater curvature. The stomachs were gently rinsed with water, any abnormalities were noted, they were spread on a flat surface, and then fixed with 10% formalin. All stomachs were stored in 10% formalin for at least 1 week, after which each stomach was examined for gastric pathology with a binocular dissection microscope at 10×. One eyepiece was fitted with a reticle permitting gastric lesions to be quantified in terms of total area (mm^2) and, as in previous studies (1) any discontinuity in the gastric mucosa was considered a gastric lesion.

The brains of all animals sustaining LH lesions immediately were removed and fixed in 10% formalin for several weeks. They were then sectioned at 50 μm and stained with thionin. Extent of brain damage was determined with reference to the König and Klippel atlas (13). Briefly, the mounted and stained sections were superimposed with a microprojector onto corresponding structures representing tracings from coronal atlas plates. Magnification was adjusted until projected structures adjacent to the lesion corresponded well with the atlas structures. Outlines of the damage observed in representative sections through the lesion were drawn to scale onto tracings of the brain sections taken at appropriate anterior–posterior levels. Anterior–posterior extent of brain damage was estimated by tabulating the divisions of the atlas figures that were incorporated within the lesion outlines. Dorsoventral and mediolateral extent of the lesions was measured using the atlas scale.

Statistical analysis. One-way analysis of variance was used to compare extent of gastric ulceration for all groups and attainment of the 0.05 α level was accepted as grounds for rejection of the null hypothesis. The unpaired t test was used to determine the significance of differences between groups.

Results. Three rats in the LH lesion–saline group, one rat in the LH lesion–propantheline group, and one rat in the LH lesion–vagotomy group were eliminated from the experiment because histological analysis of the brains revealed that these animals sustained asymmetrical lesions which spared most of the LH. The stomach of these rats appeared normal. Final group samples were as follows: LH lesion–saline, $N = 15$; LH lesion–propantheline, $N = 9$, LH lesion–cimetidine, $N = 9$; LH lesion–vagotomy, $N = 10$; and control brain surgery–saline, $N = 13$.

No systematic differences were found in either brain lesion size or placement between the groups receiving LH lesions. Rats included in the study had bilaterally symmetrical lesions which were generally placed in the dorsolateral extent of the LH and centered at the approximate middle

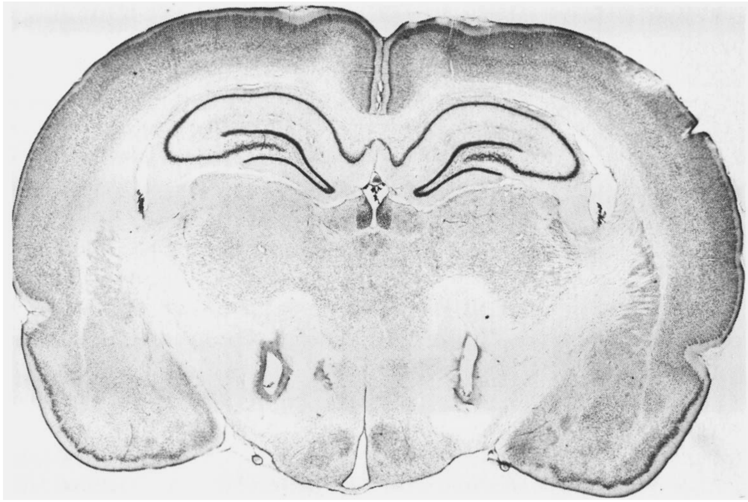

FIG. 1. Photomicrograph of a representative bilateral LH lesion (frozen section, thionin stained).

anterior–posterior extent of the ventromedial hypothalamic nucleus. The lesions frequently involved the ventromedial tip of the internal capsule, the fields of Forel, and the zona incerta. A representative histological section of the LH lesions is shown in Fig. 1.

In gross appearance, gastric lesions were typically circular or slightly oblong and superficially covered with blood and clearly distinguishable against the background of healthy tissue. Superficial hemorrhages were frequently observed to accompany the gastric lesions in freshly excised stomachs. However, these superficial defects sometimes dissappeared after fixation in formalin. Only discontinuities in the gastric mucosa remaining after formalin fixation were scored as gastric lesions. The gastric lesions were confined to the fundic mucosa.

Analysis of variance for total area of gastric pathology was significant ($P < 0.001$), and as depicted in Fig. 2, the LH lesion–saline group displayed significantly more fundic gastric mucosal damage than any of the other groups ($P < 0.01$) which did not differ significantly from each other.

Although the incidence of gastric ulceration was significantly reduced by propantheline, cimetidine, and vagotomy, only propantheline also reduced the occurrence of hypersalivation and chromodacryorrhea (Table 1).

Materials and methods. Experiment 2. Male Long–Evans rats weighing 350–400 g were deprived of food but not water overnight. After anesthetization with 50% urethane solution (3 ml/kg, ip) a neck incision was made and the esophagus ligated. A ventral midline incision was made on the abdominal surface and then through a slit in the duodenum two catheters were passed into the stomach and held in place by a ligature around the pylorus. One catheter containing saturated KCl in agar gel served

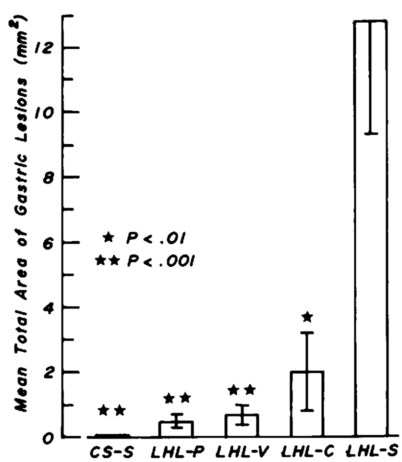

FIG. 2. Mean total area of gastric (fundic) lesions following lateral hypothalamic lesions or control brain surgery. (Abbreviations: LHL: = lateral hypothalamic lesion; CS = control brain surgery; S = saline, P = propantheline; V = vagotomy; C = cimetidine).

TABLE I. PERCENTAGE OF ANIMALS PER GROUP DISPLAYING VISIBLE SIGNS OF HYPERSALIVATION AND CHROMODACRYORRHEA

Group[a] (*N*)	Hypersalivation (%)	Chromodacryorrhea (%)
LHL–S (15)	80	80
LHL–C (9)	89	89
LHL–V (10)	70	70
LHL–P (9)	0	0
CS–S (13)	0	0

[a] Abbreviations: LHL = lateral hypothalamic lesion; CS = control brain surgery; S = saline; C = cimetidine; V = vagotomy; P = propantheline.

as the detecting electrode for electrical potential difference (PD) determination. The circuit for recording PD was completed with an indifferent KCl-agar electrode placed under the skin of the abdomen. These electrodes led to a pH meter–millivoltmeter (Beckman Model 3500) through two separate beakers each filled with saturated KCl solution and each containing a balanced calomel electrode. The changes in PD were continuously monitored using a Sargent Model SLR recorder connected to the pH meter. The second gastric catheter led to a three-way tap and was used for intragastric instillation and removal of the test solution. The test solution was 150 m*M* HCl and [^{14}C]polyethylene glycol (PEG), 2×10^{-3} μCi ml^{-1} was added to the test solution as a marker for determination of residual volumes.

Thirty minutes after abdominal surgery and cleansing rinses of the stomach, gastric transmucosal PD and net fluxes for H^+, Na^+, and K^+ ions were determined during seven consecutive 30-min test periods. To determine net fluxes 5 ml of 150 m*M* HCl containing [^{14}C]PEG was instilled into the stomach, mixed with the residual content, and then a 2 ml sample withdrawn. Thirty minutes later, the stomach was emptied by gentle aspiration, the volume of this sample was measured, and saved for analysis. The stomach was then washed three times with 150 m*M* HCl without [^{14}C]PEG and the ^{14}C activity in the combined rinses was determined and used to calculate residual volume. H^+ titration to pH 7 was done using an automatic titrator (Radiometer) and Na^+ and K^+ concentrations were measured by flame photometry (Radiometer FLM 3). With these values and the corrected estimates of volume the net fluxes for each of the ions were calculated.

The first 30-min test period (Period 1) occurred prior to stereotaxic surgery. Following the first test period one group of rats ($N = 7$) was given bilateral LH lesions and the second group ($N = 5$) was given control brain surgery employing the same methods described in Experiment 1. The remaining six consecutive 30-min tests were conducted immediately after stereotaxic surgery. Differences for net fluxes and PD values for each group were determined separately by comparing the results of preoperative period 1 to the mean values for the six postoperative test periods. A paired *t* test was used for statistical analysis.

Results. Changes in PD values and ionic fluxes are represented in Table 2. In the control group no significant changes occurred between the initial preoperative period and the mean values for the six periods following control brain surgery. In contrast, the group receiving LH lesions exhibited a small but significant decrease in PD ($P < 0.01$), significant increases in H^+ loss ($P < 0.01$) and Na^+ gain ($P < 0.01$), but no significant change in K^+ gain. At the termination of the experiment stomachs of all rats were examined postmortem. Control animals exhibited normal stomachs, however,

TABLE II. GASTRIC TRANSMUCOSAL POTENTIAL DIFFERENCE AND IONIC FLUXES FOLLOWING LATERAL HYPOTHALAMIC (LH) LESIONS OR CONTROL BRAIN SURGERY

30-min periods	PD (−mV)	Ionic fluxes (μeq/30 min) H$^+$ loss	Na$^+$ gain	K$^+$ gain
Control brain Surgery ($N = 5$)				
1	42 ± 3	25 ± 10	10 ± 5	2.1 ± 0.8
2 to 7	43 ± 2	15 ± 6	8 ± 5	2.0 ± 0.5
LH Lesion ($N = 7$)				
1	44 ± 2	20 ± 6	6 ± 2	2.2 ± 0.5
2 to 7	41 ± 2[a]	40 ± 9[a]	18 ± 3[a]	2.6 ± 0.9

[a] $P < 0.01$, paired *t* test. Difference between period 1 and mean values for periods 2 through 7 for each group.

petechial lesions and erosions and superficial bleeding were noted in two of the seven rats with LH lesions. Since only two of the seven test animals had gastric lesions but even those without lesions in the stomach showed significant reductions in PD and increases in net H^+ and Na^+ fluxes, we concluded that a reduction in barrier function occurred in the gastric mucosa of animals with LH lesions even before gastric erosions and hemorrhages were grossly detectable.

Discussion. The results of Experiment 1 demonstrated that propantheline, cimetidine, and vagotomy, all of which are known to reduce gastric acid output, effectively reduced the incidence of gastric mucosal damage after LH lesions. Previous studies (3, 4) have shown that gastric acid output is increased after LH lesions. It is likely that at least part of the protective effects of propantheline, cimetidine, and vagotomy in our study were due to their antisecretory action. The present results also suggest that a primary effect of LH lesions is to produce an abrupt increase in parasympathetic activity as indicated by the occurrence of hypersalivation and chromodacryorrhea and their inhibition by the anticholinergic, propantheline.

Lindholm *et al.* (2) reported that within 24 hr LH lesions resulted in gastric erosions. Experiment 2 demonstrated that within 3 hr LH lesions induced small but significant changes in gastric mucosal barrier (GMB) functions which preceded or were concomitant with the occurrence of morphological damage in the gastric mucosa. These changes in GMB functions may be due to at least two factors. First, the increased H^+ and Na^+ ion fluxes and the modest reductions in PD could be due to increased permeability of the gastric mucosa (14–16). Secondly, the increase in H^+ ion loss from the gastric contents could be due to increased production of HCO_3^-.

The flux and PD changes observed in the present study are similar to those reported by Kelly *et al.* (14) during the early stages of progressive rupture of the gastric mucosal barrier in dogs. Kelly and colleagues (14) also found in dogs, as we did in rats, that at this early stage of barrier damage there is no significant increase in the gain of K^+ ion to the contents. This occurs later in the damaging process when mucosal cells have begun to rupture. Werther and Horowitz (17) have concluded that abnormal gastric mucosal permeability to H^+ ion may also play a pathogenic role in stress-induced gastric ulceration. Thus, the morphological similarities between stress-induced (18) and LH lesion-induced gastric ulceration may extend to similarities in the mechanisms of their production.

The present findings indicate that LH lesions result in a powerful outflow of parasympathetic (cholinergic) impulses. Altamirano (19) and Garner and Flemström (20) have demonstrated that cholinergic drugs can increase the production of HCO_3^- by the gastric mucosa. Although HCO_3^- production by the gastric mucosa was not tested in the present study, some of the H^+ which disappeared from the gastric contents of the rats examined may have been due to neutralization by HCO_3^- produced by the mucosa as well as back diffusion across the mucosa. It is suspected that both increased permeability and HCO_3^- production occurred in the mucosa following lateral hypothalamic lesions.

The results indicate that the changes in the gastric mucosa are the consequence of excessive neural outflow since the occurrence of gastric mucosal lesions were blocked by vagotomy and an anticholinergic. The suspicion appears justified that the neural outflow liberated a paracrine substance or substances which affected the mucosal cells directly or indirectly by causing changes in blood flow.

Summary. Experiment 1 demonstrated that gastric mucosal damage in rats induced by lateral hypothalamic (LH) lesions could be significantly reduced by antisecretory doses of propantheline, cimetidine, and by vagotomy, however, only propantheline also prevented hypersalivation and chromodacryorrhea. These results indicate that a primary effect of LH lesions is to produce an abrupt increase in parasympathetic activity. Experiment 2 showed that gastric mucosal barrier functions were altered by LH lesions even before visible defects of the mucosa were present. It is

suggested that an increase in permeability of the gastric mucosa may play an important role in the pathogenesis of gastric ulceration induced by hypothalamic damage.

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Received July 27, 1979. P.S.E.B.M. 1980, Vol. 163.