Decreased Incidence of Restraint-Stress Induced Gastric Erosions in Rats Treated with Bovine Growth Hormone[1] (35032)

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The use of restraint to induce gastric erosions in small animals has been shown to be a useful and reproducible experimental model for the study of the pathogenesis and treatment of stress ulcer in humans (1), the latter an important cause of morbidity and mortality in numerous clinical situations (2). Previous studies from this laboratory and elsewhere indicating decreased proliferation of gastric epithelial cells, a decrease in DNA synthesis (3, 4), and a loss of RNA from gastric tissue of animals under stress (5, 6), have suggested that an impairment in the regenerative ability of the gastric mucosa contributes to the development of the lesions. Hypophysectomy also causes atrophy of gastric mucosa and decreases secretion of acid, pepsin, and mucous (7, 8), which could be associated with alterations in RNA and mucoprotein synthesis. Since the administration of growth hormone restores structure and function of gastric mucosa after hypophysectomy (7), it might be useful in counteracting the effect of restraint-stress on the gastric mucosa. It was the purpose of this study to show that the administration of growth hormone to rats subjected to restraint-stress does have a beneficial effect on the incidence and severity of gastric erosions.

Materials and Methods. Female CFE rats, weighing between 150 and 200 g, were individually caged at a constant temperature of 24° and were given food and water *ad libitum* until the time of restraint. After a series of preliminary experiments to establish reproducibility, timing, dosage, and blood glucose levels, which are not reported in detail here, four experiments were carried out using 65 animals. Restraint was achieved by stapling the rats, between 9:00 and 11:00 a.m., into envelopes made of aluminum window screening (9). Immediately after restraint the experimental animals received 5 mg of bovine growth hormone, prepared in these laboratories by the method of Dellacha and Sonenberg (10), subcutaneously, dissolved in 1 ml of buffer (0.1 *M* $NaHCO_3$, 4 parts; 0.1 *M* Na_2CO_3, 1 part), pH 9.5. Controls received 1 ml of buffer subcutaneously. A second dose of hormone and buffer was given 10 hr later, at which time the envelopes were restapled to allow for shrinkage of animals. The rats were anesthetized with ether at 24 hr and their stomachs were removed and examined under a dissecting microscope. The lesions were counted and measured, using an arbitrary scale of 0–3+, where 1+ = punctate, 2+ = 2 mm, and 3+ = >2 mm in length. The ulcer index and the severity index were calculated according to the method of Robert and Nezamis (11). The index includes the number of animals developing erosions, number per animal, and severity of erosions. The results were analyzed using Student's *t* test.

Results. Figure 1 depicts our overall experience with control substances and various dosages of bovine growth hormone in terms of the average number of 3+ gastric erosions induced by restraint-stress. Each circle represents from 8 to 12 animals. While there is some spread in the incidence of erosions generated in the controls, in each individual experiment, designated by the lines connecting the circles, it is clear that even relatively

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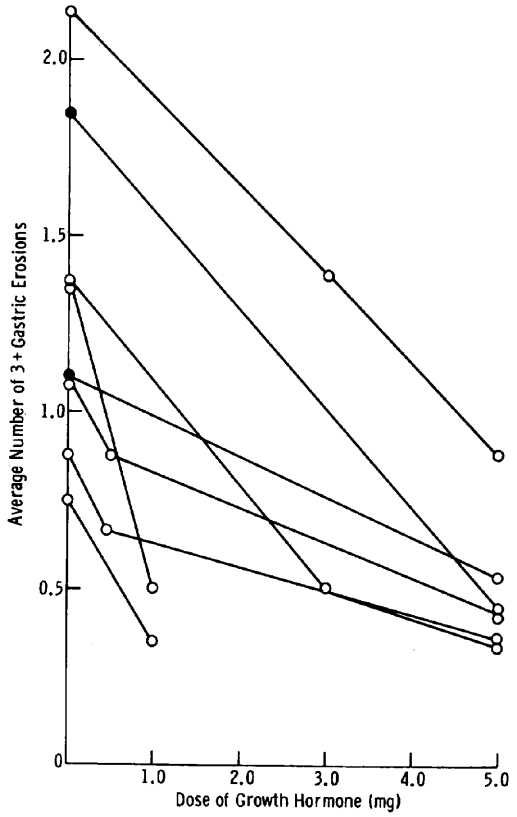

FIG. 1. The effect of various doses of growth hormone administered to rats on the incidence of 3+ (>2 mm in length) gastric erosions induced by restraint-stress. Each circle represents from 8 to 12 animals. Every experiment included a control group that received no hormone; (●) 5 mg of bovine serum albumin in buffer vehicle.

small doses of growth hormone decrease the incidence of 3+ erosions. There is suggestive evidence of a dose–response relationship.

Table I shows the average number of 3+ gastric erosions and the severity index in the four experiments. The controls (C) received buffer and the experimentals (GH) received 5 mg of growth hormone in buffer. Because the control group in one experiment had an unusually high number of erosions, this experiment could not be compared statistically with the remaining three experiments and is therefore shown separately as Expt. 1 in Table I. In the three remaining experiments statistical homogeneity in the controls permitted grouping and comparison with the treated animals (24 controls, 25 growth hormone). It is clear that incidence and severity of erosions are reduced by the administration of growth hormone.

These four experiments are reported in detail because they were carried out in an identical fashion, using similar amounts of growth hormone and control buffer. While p values are satisfactory, the number of animals is small. Twenty-three additional control animals received 5 mg of bovine serum albumin in the buffer vehicle (versus 11 animals receiving 5 mg of growth hormone) and 8 animals received buffer 4 hr after restraint without altering the incidence of erosions compared to other controls: ulcer index = 17.92, mean number 3+ erosions = 1.45. When the total number of controls (63 animals) is compared with the total experience using the 5-mg dose of growth hormone (44 animals), the p value for decreased incidence of 3+ erosions drop to $<.001$ and for severity to .001.

Discussion. It is apparent from these results that growth hormone provides partial but significant protection against the production of restraint-stress induced erosion, both in terms of incidence of erosions and their severity. The fact that equal amounts of bovine serum albumin did not alter the incidence of erosions while growth hormone did, argues against a nonspecific action of protein injection in decreasing erosion. Further support for the concept of a specific action of growth hormone on the gastric mucosa lies in the suggestive evidence that there is a dose–response relationship in the antiulcer effect of growth hormone.

An antiulcer effect of growth hormone has been noted in three other experimental systems: the formalin ulcer in rats (12), the histamine ulcer in guinea pigs (13), and the adrenal corticosteroid ulcer in rats (8). In the latter experiments, hypophysectomy, *per se*, decreased the incidence of steroid ulcers by half and the administration of growth hormone to these animals almost completely inhibited ulcer formation in spite of the fact that secretions of volume, acid, mucous, but not pepsin, were restored to normal. Re-

TABLE I. The Effect of the Injection of Bovine Growth Hormone (GH) Versus Buffer Alone (C) on the Mean Number of 3+ Gastric Erosions Induced by Restraint-Stress, Their Severity and the Ulcer Index.

Experiment 2 consists of three separate experiments which could be grouped together because of statistical homogeneity of the controls. Statistical analysis by Student's t test.

Exp.	No. of animals	No. 3+ erosions (mean ± 1 SE)	Severity index (mean ± 1 SE)	Ulcer index
1. C	8	2.13 ± 0.40	9.88 ± 1.03	24.38
GH	8	0.88 ± 0.30	4.00 ± 1.31	13.25
p value		<.05	<.02	
2. C	24	1.13 ± 0.15	5.88 ± 0.70	17.63
GH	25	0.52 ± 0.16	3.56 ± 0.64	13.52
p value		<.01	<.02	

straint-stress erosions would appear to be of a different etiology than those induced by steroids in that, according to Brodie (14), hypophysectomy does not protect against restraint-stress ulcers and may increase sensitivity to them. Also the administration of adrenal steroids diminishes susceptibility to erosions under restraint-stress (15). Presumably the formalin and histamine ulcers are also of different etiologies but since all four types respond favorably to growth hormone, it would be desirable to find a common pathway. An attractive hypothesis hinges on the ability of growth hormone to stimulate DNA, RNA, and protein synthesis. Whether a single parameter such as the stimulation of mucin production or "strengthening" of gastric tissue, as suggested by Robert *et al.* (8), or whether multiple and different factors play a role remains to be determined.

Since it has been demonstrated that hypoglycemia increases and hyperglycemia decreases the incidence of restraint-stress erosions (16), and since growth hormone has both hypoglycemic and hyperglycemic actions, consideration must be given to this relationship. While the rat does develop hypoglycemia following growth hormone administration (17, 18), any hypoglycemia that may have developed secondary to growth hormone administration should have increased the incidence of erosions rather than decreased it. In our experiments blood glucose was measured at the time of sacrifice in 15 control animals and 15 given growth hormone, without significant differences in the two groups. While this does not rule out an earlier hyperglycemic effect, the rat is said to be relatively insensitive to the hyperglycemic effect of growth hormone in the absence of force feeding or adrenal steroid administration (19, 20).

The results obtained do not confirm or deny our hypothesis that growth hormone prevents the previously observed decrease in gastric epithelial cell proliferation and DNA synthesis, etc., associated with restraint-stress erosion. Nor were the experiments designed to test the potential of growth hormone as a healing agent for previously induced erosions, as has been demonstrated for formalin ulcers (12). They do suggest, however, that further investigations along these lines are warranted.

Summary. Restraint-stress was induced in female CFE rats weighing between 150 and 200 g by stapling them in envelopes made of aluminum wire screening. Five mg of bovine growth hormone dissolved in 1 ml of buffer was administered subcutaneously immediately after stapling and again 10 hr later. Controls received injections of buffer or bovine serum albumin. The animals were sacrificed at 24 hr and the number of animals developing gastric erosions, the number per animal and the severity of erosions were determined with a dissecting microscope. Growth hormone provides partial but significant protection against the production of restraint-stress produced erosions, both in terms of

incidence of erosions and their severity.

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