

## Effects of ACTH and the Adrenals on Serum and Antral Gastrin Levels in the Rat<sup>1</sup> (40257)

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The growth and function of the gastric mucosa is intricately regulated by gastrointestinal as well as pituitary and adrenal hormones (1). The trophic action of gastrin is known to be a direct effect, not involving release of other hormones (2-4), while that of growth hormone (GH) may be partially indirect, since it releases endogenous gastrin (5).

Due to the increased incidence of ulcer disease associated with steroid therapy, adrenal glucocorticoids and ACTH have been studied largely for their effects on gastric acid secretion. These studies have led to controversial findings (1). Jacobson and Magnani (6) concluded that chronic cortisone or GH treatment in the hypophysectomized dog was permissive for acid secretory responses to other stimuli. In another study, intravenous ACTH increased acid secretion in the dog by 61%. This effect was diminished by antral resection and completely abolished by an-trectomy plus vagotomy (7), suggesting that the pituitary and adrenal hormones are acting via a secondary mediator, perhaps, gastrin. Watson *et al.* (8) reported an increase in basal and meal-stimulated serum gastrin levels in dogs following chronic injections of hydrocortisone. These increases preceded an increase in acid output. In man, multiple injections of either prednisolone or ACTH were associated with increased basal and reactive serum gastrin levels (9).

This study was designed to test the effects of ACTH and adrenal glucocorticoids or the absence of these hormones on both serum and antral gastrin levels in the rat.

**Methods.** Rats used in these studies were 200 g Sprague-Dawley males from Charles River Laboratories Inc., Wilmington, MA. Hypophysectomies were done by Charles Rivers Laboratories, Inc. Bilateral adrenalectomies were done in this laboratory through a dorsal incision under Ketamine anesthesia. All animals were allowed to recover from surgery for at least 2 weeks before the start of a study and were killed during week 4. Rats were maintained on standard Purina rat chow prior to the experiments. In some instances feeding was *ad libitum*. In experiments involving hypophysectomy all animals received a weighed portion of food equal to that normally consumed by the hypophysectomized control animals. The diets of all hypophysectomized animals were supplemented with 5% dextrose in water for 2 weeks after surgery. This treatment was continued throughout the experimental period. Adrenalectomized rats were maintained on isotonic saline. Animals with both hypophysectomy and adrenalectomy received 5% dextrose in saline following surgery. This supplement was continued throughout the experimental period. At sacrifice adrenalectomized animals were carefully inspected for regenerated cortical tissue. None was found.

Corticosterone acetate (Calbiochem) was suspended in saline immediately prior to injection. Hydrocortisone (Calbiochem) was suspended in 0.9% NaCl and 0.25% carboxymethyl cellulose and injected at the doses indicated. ACTH (Armour Pharmaceutical Co.) was injected as indicated. All hormones were given subcutaneously in volumes of 1 ml/kg.

All animals were fasted for 18 hr prior to sacrifice which always occurred between 9 and 11 AM. Drugs were also injected between these times. During ether anesthesia a ventral midline incision was made; blood was withdrawn directly from the heart and placed on ice to clot. Antrums were removed and homogenized in 2 ml boiling deionized water.

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The homogenate was boiled for an additional 20 minutes. Extracts were centrifuged for 30 min at 12,000 rpm and the supernatants recovered. Serum and antral extracts were frozen for subsequent gastrin radioimmunoassay. The gastrin radioimmunoassay was performed according to the method of Yalow and Berson (10). The antibody used was No. 1296 characterized by Dockray and Walsh (11). Synthetic human gastrin I (1-17), (Imperial Chemical Industries, Ltd., England), served as a standard.

Gastrin levels are expressed as pg/ml serum, and  $\mu\text{g/g}$  wet weight antrum. Statistical analysis was made using the Student's *t* test for unpaired data, and differences were considered significant if  $P < 0.05$ .

**Results.** Hypophysectomy resulted in dramatic and significant decreases in both serum and antral gastrin (Fig. 1). Five-day treatment of hypophysectomized rats with corticosterone (10 mg/kg once per day) significantly increased both serum and antral gastrin. Antral gastrin levels were completely restored to normal by corticosterone. ACTH (20 U/kg twice a day for 5 days) increased serum gastrin levels significantly over those found in hypophysectomized animals without altering tissue levels of the hormone (Fig. 1).

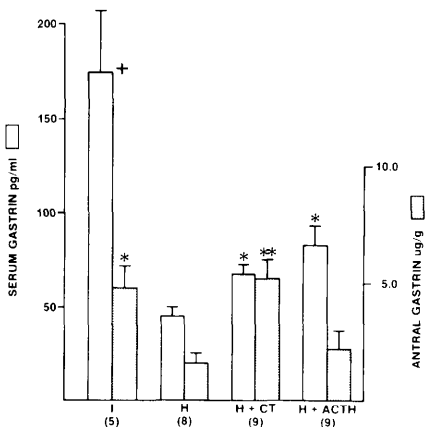


FIG. 1. Effect of hypophysectomy and 5 day treatment with corticosterone (10 mg/kg once per day) or ACTH (20 U/kg twice per day) on serum and antral gastrin levels. I = intact plus NaCl, H = hypophysectomized plus NaCl, H + CT = H plus corticosterone, H + ACTH = H plus ACTH. Bars represent means plus standard errors of means for *n* (numbers in parentheses) rats. + =  $P < 0.001$ , \*\* =  $P < 0.01$ , \* =  $P < 0.05$  compared to H group.

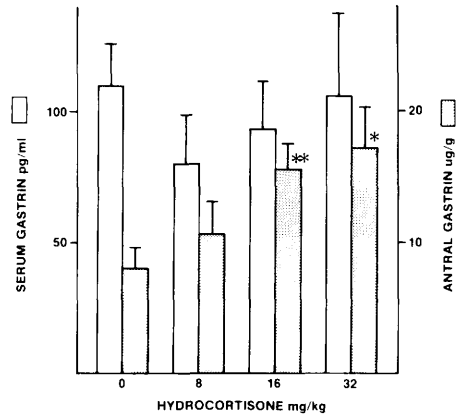


FIG. 2. Serum and antral gastrin levels in rats injected with different doses of hydrocortisone 90 min prior to sacrifice. Means and SEMs.  $n = 8$  for each group. \*\* =  $P < 0.025$ , \* =  $P < 0.05$  compared to 0 dose (NaCl).

In order to determine whether adrenal hormones would alter gastrin levels in intact animals varying doses of hydrocortisone or ACTH were injected once 90 min prior to sacrifice. Hydrocortisone (Fig. 2) at doses of 16 and 32 mg/kg significantly increased the antral content of gastrin without affecting release of the hormone into the serum. ACTH (Fig. 3), on the other hand did not alter either serum or antral gastrin.

The involvement of ACTH in altering gastrin levels and its relationship with the pituitary and adrenal glands was examined further in a large series involving intact, adrenalectomized, and adrenalectomized-hypophysectomized rats. Serum gastrin levels are shown in Fig. 4 and antral levels in Figure 5. ACTH was given once a day for 4 days at a dose of 40 U/kg. As was the case with single injections (Fig. 3) ACTH given chronically failed to alter either serum or antral gastrin levels in intact animals. Adrenalectomy by itself tended to increase serum gastrin levels but this change was not statistically significant (Fig. 4). Antral gastrin levels also remained unchanged from control levels after adrenalectomy and subsequent treatment with ACTH (Fig. 5). The combination of hypophysectomy and adrenalectomy resulted in changes in serum and antral gastrin levels similar to those found with hypophysectomy alone and depicted in Fig. 1. Serum gastrin levels in hypophysectomized-adrenalectomized

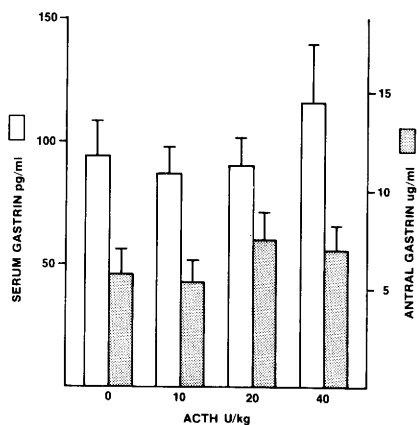


FIG. 3. Same as Fig. 2 except ACTH was injected.

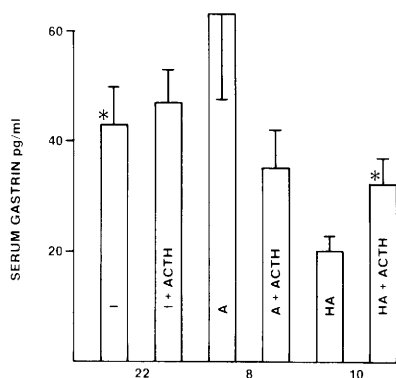


FIG. 4. Effect of ACTH (40 U/kg per day for 4 days), adrenalectomy (A), and hypophysectomy plus adrenalectomy (HA) on serum gastrin. I = intact. Means and SEM. N is shown below each group. \* =  $P < 0.05$  compared to HA.

tomized rats were significantly lower than intact controls (Fig. 4). ACTH prevented some of this decrease causing serum gastrin to return to near normal levels. This was again similar to the effect of ACTH in animals with hypophysectomy alone. Tissue gastrin levels (Fig. 5) in the hypophysectomized-adrenalectomized group were also significantly lower than control levels. ACTH had no effect on the antral levels of the hormone.

The gastric mucosa of each animal used in these studies was inspected for erosions. Some of the animals in the hypophysectomized and adrenalectomized groups had superficial mucosal erosions. Gastrin levels from the ulcerated animals were compared with hormone levels of nonulcerated animals within the same group. There was no indication that

mucosal erosions affected either serum or antral gastrin levels.

**Discussion.** Long considered functionally independent from other hormones, gastrointestinal hormones are now known to influence both the actions and release of these peptides. In turn growth hormone is necessary to maintain normal levels of both serum and antral gastrin (5). Watson *et al.* (8) in the dog and Raptis *et al.* (9) in man have shown that chronic administration of corticosteroids increases basal and stimulated serum gastrin levels. The purpose of this study was to provide information on the interrelationship of the pituitary and adrenals in regulating endogenous gastrin levels in the rat.

The only effect of ACTH or adrenal hormones on gastrin levels in intact animals found in the current study was a dramatic increase in antral gastrin 90 min after an injection of either 16 or 32 mg/kg of hydrocortisone (Fig. 2). The most important physiological determinant of gastrin levels is feeding, for in the rat a 2-day fast depresses serum gastrin by approximately 80% (12). In all the studies reported here food was removed 18 hr before an experiment, which usually began at 9 AM. Therefore, since rats are nocturnal feeders, they were effectively fasted for 30 hr. As such, serum and antral gastrin levels can be assumed to have been significantly depressed (5). The rapid increase in antral gastrin levels seen with hydrocortisone in our studies is also seen following feeding. Creutzfeldt *et al.* (13) fed 48 hr fasted rats and observed a threefold increase in antral gastrin concentration within 60 min. The entire response was blocked by cycloheximide. It is likely, then,

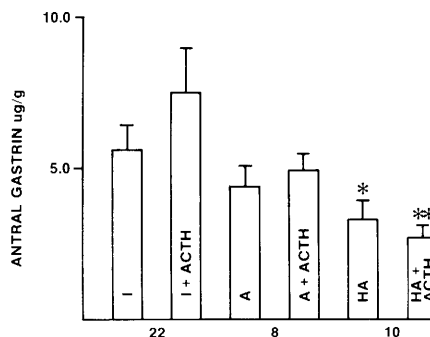


FIG. 5. Antral gastrin levels from same rats as Fig. 4. \*\* =  $P < 0.025$ , \* =  $P < 0.05$  compared to intact controls.

that the effect of hydrocortisone also depends on protein synthesis. No evidence however, is available to suggest an action of hydrocortisone on antral mucosal protein synthesis.

Growth hormone does not significantly increase gastrin levels in either fed or fasted intact rats (5). It will, however, significantly reverse the decrease in endogenous levels caused by hypophysectomy. To our knowledge this is the only prior study of the relationship between the pituitary and endogenous gastrin. As in that study hypophysectomy significantly lowered both serum and antral gastrin in the current experiments (Fig. 1). Corticosterone completely prevented the decrease in tissue gastrin and significantly increased serum gastrin. Adrenalectomy, however, did not result in decreased gastrin levels. It seems, therefore, that adrenal hormones do not play a major role in maintaining gastrin levels under physiological conditions. Increases in adrenal hormone levels, as shown in Fig. 2, however, may stimulate gastrin synthesis resulting in higher than normal tissue gastrin concentrations.

ACTH did not significantly change gastrin levels in intact animals. It did significantly increase serum gastrin levels in hypophysectomized animals (Fig. 1). Since rats have low levels of adrenal hormones in the absence of endogenous ACTH, the increase in serum gastrin stimulated by exogenous ACTH in the hypophysectomized rats was probably a direct effect.

Adrenalectomy which increases circulating ACTH as well as removing adrenal hormones from consideration had no significant effect on serum gastrin. Schwille *et al.* (14) had previously reported a 400% increase in serum gastrin after adrenalectomy. However, in that report the rats were deprived of water as well as food for 24 hr, were not maintained on saline and were not pair-fed. Whatever the reason, our study did not confirm this finding.

ACTH did significantly increase serum gastrin when administered to hypophysectomized-adrenalectomized rats indicating that this is a direct action of the hormone. The combination of hypophysectomy and adrenalectomy produced decreases in both serum and antral gastrin similar to those seen with hypophysectomy alone. The effect of ACTH on this group of rats was also identical to that

seen in the hypophysectomized group. These results suggest that ACTH directly stimulates an increase in serum gastrin. This effect was seen only in hypophysectomized animals indicating that it occurs only in the absence of either endogenous ACTH or growth hormone.

*Summary.* The effect of adrenal hormones and ACTH on serum and antral gastrin levels was examined in intact, adrenalectomized, hypophysectomized, and hypophysectomized-adrenalectomized rats. Hydrocortisone significantly increased antral gastrin within 90 min after injection of 16 or 32 mg/kg in intact rats. It failed to change serum levels. Adrenalectomy did not significantly change either serum or antral gastrin. Hypophysectomy decreased both antral and serum gastrin concentrations to a small fraction of normal. Corticosterone completely prevented the drop in tissue levels and significantly increased serum levels. These data indicate that adrenal hormones can increase tissue levels of gastrin but that this effect does not normally occur under physiological conditions with adequate supplies of growth hormone. ACTH increased serum gastrin levels in both hypophysectomized and hypophysectomized-adrenalectomized rats. Therefore, ACTH acts directly to release gastrin. However, this effect was noted only in the absence of the pituitary.

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