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MINIREVIEW

Regulation of the Mucosal Gastrin Receptor (41626)

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A receptor is a molecule that specifically binds a hormone, forming a hormone–receptor complex. This binding initiates cellular events that are ultimately manifested as the physiological effects of the hormone. Until recently information pertaining to gastrointestinal hormone receptors in mucosa has been garnered solely through the classical pharmacological methods of studying agonist–antagonist interactions. The heuristic advantage of studying properties of material hormone receptors, however, has been widely recognized by those interested in other areas of endocrinology.

A simplified scheme diagramming the release of a hormone and its combination with a receptor is illustrated in Fig. 1. In order to understand the regulation of this system one can use sensitive radioimmunoassay methods to measure the concentration of hormone in the blood. This same measurement allows the investigator to determine the stimuli and inhibitors of the release of that hormone. Since the overall effect depends on the concentration of the hormone–receptor complex much information can be acquired concerning the physiology of the system.

Recently, however, it has become apparent that the concentration of receptors can also vary, and, hence, impart a degree of regulation on the system. Regulation of hormone receptors is now recognized to play an important role in the hormone response of target cells (1). The concentration of receptors for a specific hormone may be regulated by other hormones. The levels of oxytocin receptors in the uterus and mammary gland, for example, are probably regulated by both estrogen and progesterone (2). In other cases a particular hormone may regulate the numbers of its own receptors. Insulin downregulates the number of its receptors (3), while prolactin increases its membrane receptors in both the liver and mammary gland (4, 5). Thus, the responses to several hormones are regulated not only by the amount of hormone reaching the receptor but also by the number of receptors present.

Physiological actions of gastrin. From the physiological viewpoint the purpose of hormone receptor binding studies is to relate that binding to the physiological effects of the hormone and, thus, learn more about the factors regulating function. Gastrin has a wide range of actions on epithelial tissue and smooth muscle of the gastrointestinal tract. Many of these actions, however, require pharmacological doses of the hormone (6). If one defines the physiological actions of a hormone as those which occur following an exogenous infusion of hormone which does not increase blood concentrations of hormone over the levels which result from the normal stimuli for release, then the physiological actions of gastrin are the stimulation of acid secretion (7), and the stimulation of mucosal growth of the oxyntic gland region of the stomach, colon, and duodenum (8, 9). It is not known whether the stimulation of pepsinogen secretion is a direct effect or whether it is related to increased output of gastric acid, since topical acid causes increased pepsinogen secretion via a local reflex mechanism (10). The cell types responsible for the various actions of gastrin and which would predictably contain gastrin receptors are the parietal cells in the case of acid secretion (11), the mucous neck cells (12) of the oxyntic gland mucosa and the crypt cells (13, 14) of the duodenal and colonic mucosa for the stimulation of growth, and the chief cells for pepsin secretion (15).

Gastrin receptor assays. Several laboratories have reported the development of gastrin receptor assays. Lewin *et al.* (16, 17) used tritiated gastrin having a specific activity of 60 Ci/mmole to demonstrate reversible binding to both partially purified plasma membranes and to intact gastric mucosal cells which had been separated by pronase digestion. This binding was time- and temperature-dependent and proportional to either the amount

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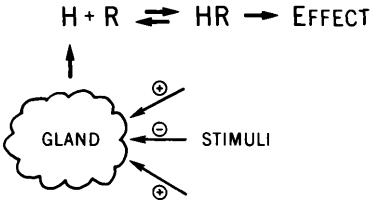

FIG. 1. Simplified scheme representing the major components involved in an endocrine response. H, hormone; R, receptor.

of membranes or the number of parietal cells present. Maximum binding was 50 fmole/mg protein or 12.5 fmole gastrin/million cells. The equilibrium K_d was 0.9×10^{-8} *M*, and Scatchard analysis suggested single class of binding sites. Unfortunately, the binding in these studies was not shown to be specific, and the binding interaction did not have an affinity in the physiological range of hormone concentration.

Baur and Bacon (18) have described an assay using ^{125}I-labeled synthetic human gastrin I to measure gastrin receptors present in membranes of canine antral smooth muscle cells. Maximal binding was about 50 fmole/mg protein and specific binding amounted to 53% of the total counts bound after binding to the filters was subtracted. Specific binding was equal to 0.12% of the 60,000 cpm of labeled gastrin added to the membranes. In other words, only about 72 counts were specifically bound out of a total of 60,000.

Using ^{125}I-labeled gastrin I, Brown and Gallagher (19) demonstrated a specific gastrin receptor in the 250–20,000*g* fraction of rat oxyntic gland membranes. Binding was reversible, temperature- and pH-dependent, and was inhibited by tryptic digestion of the membrane preparation. Cholecystokinin, gastrin and its analogues, and secretin inhibited binding, whereas cimetidine did not. Observed maximal binding of gastrin occurred at a concentration of label equal to 2.0×10^{-10} *M*, and there was no binding to membranes from antral mucosa.

Using radioiodinated 15-Leu gastrin 17-I with a specific activity of 2000 cpm/fmole we developed a gastrin binding assay (20, 21) that appeared to be quite similar to that of Brown and Gallagher (19). Maximal specific binding occurred in the 270–30,000*g* fraction of rat oxyntic gland mucosal membranes. Maximum binding was approximately 4 fmole/mg protein and had a K_a equal to 2.5×10^{11} M^{-1} and a K_d of 4×10^{-10} *M*. Optimal binding occurred after an incubation period of 30 min at 30°C and pH 7.0. Under these conditions binding could be reversed equally as well with cold gastrin or by dilution (21). Binding was totally prevented in the presence of trypsin. Specific binding of gastrin was present in duodenal mucosa as well as oxyntic gland mucosa, but was absent from antral mucosa, liver, kidney, and spleen (20). Thus, receptors were specifically localized in tissues known to respond to gastrin (acid secretion and growth of oxyntic gland mucosa and growth of duodenal mucosa) and absent from those tissues not known to be affected by gastrin. Unlabeled gastrin, CCK, caerulein, and pentagastrin competed for the gastrin receptor. Secretin also inhibited binding but did so in a noncompetitive manner. This was an interesting finding, since secretin is known to inhibit gastrin-stimulated acid secretion in the dog (22) and gastric mucosal growth in the rat (23) noncompetitively.

Biologically active labeled hormone and a standardized assay system are the first steps necessary for studying the interaction of a hormone with its receptor. To establish that the binding measurements obtained are a true reflection of hormone–receptor interaction in a pharmacological and a biological sense, five criteria must be satisfied. First, the receptor must exhibit a finite binding capacity. Second, the binding reaction should demonstrate a sufficiently high affinity, in keeping with the tissue sensitivity to the physiologically active concentration range of hormone in the blood. Third, the receptor must bind the hormone through a specific molecular interaction. Fourth, the hormone–receptor interaction should be restricted to organs or tissues with physiological sensitivity to the ligand. Fifth, a receptor-dependent hormonal response should be identified.

The first four of these criteria were satisfied by the binding described in our assay (20). However, it is difficult to correlate binding with a biological response when working with a membrane preparation. Since we were interested in the trophic response to gastrin rather than a secretory response to the hormone, this particular aspect of the study was

further complicated, because the trophic response is not immediate but occurs over a period of time. For these reasons and the facts that serum gastrin levels are easily manipulated and other trophic hormones are known to regulate their own receptors, we investigated the effect of altering serum gastrin levels on the number of gastrin receptors.

Autoregulation of the gastrin receptor. Most trophic hormones such as growth hormone (24, 25) and insulin (3, 26) downregulate the number of their receptors. Others, however, such as prolactin (4, 5) and angiotensin II (27) increase the number of their own receptors. Prolactin increases its receptors in both mammary gland (4) and liver (5) membranes, whereas angiotensin II upregulates its receptor numbers in the adrenal zona glomerulosa (27).

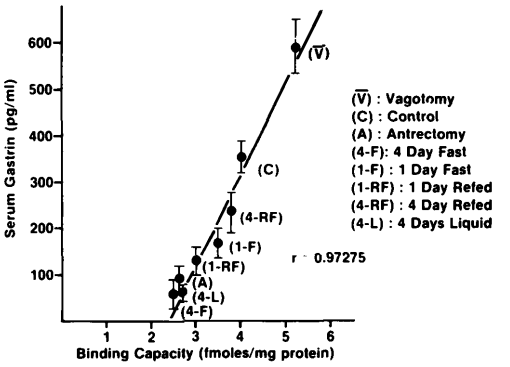

FIG. 2. Correlation of serum gastrin levels and binding capacities from 48 rats belonging to various groups described in the figure. SE values are shown for gastrin values. Reproduced with permission from Amer J Physiol **238**:G135–G140, 1980 (32).

If serum gastrin levels regulate the number of receptor sites for the hormone, then injection of exogenous gastrin should affect the number of binding sites, and alteration of endogenous gastrin levels should produce parallel changes in the number of gastrin receptors. One problem often encountered in working with gastrointestinal hormones is their distribution over wide areas of mucosa, making it difficult to remove the source of a hormone without compromising the health of the experimental animal. However, several established models produce significant changes in endogenous gastrin levels. Antrectomy with removal of the duodenal bulb results in a drop in serum gastrin to about 20% of normal (28). Vagotomy, on the other hand, increases serum gastrin due to both decreased acid secretion and removal of vagally mediated inhibition of gastrin release (28, 29). Serum gastrin levels also can be lowered by fasting or feeding a liquid diet (30, 31). The latter method has the advantage of not producing the metabolic and endocrinologic changes that occur during food deprivation.

In a series of experiments the results of which are summarized in Fig. 2 the number of gastrin receptors as determined by Scatchard analysis varied directly with serum gastrin levels (32). Intact, control rats had 4 fmole receptor/mg protein. In rats fasted, fed liquid diets for 4 days, or antrectomized, this value fell to 2.5, 2.6, and 2.8 fmole/mg protein, respectively. Treating fasted or liquid-fed rats with pentagastrin every 8 hr prevented these decreases. Refeeding 4-day fasted rats for various times between 1 and 4 days returned the number of receptors toward normal. Injection of fasted or liquid-fed rats with histamine instead of pentagastrin, in order to control for acid secretion, had no effect on receptor number (32). Vagotomy increased serum gastrin levels approximately 60% and resulted in a 25% increase in the gastrin binding capacity. In all groups of animals the K_d of the binding interaction remained relatively constant.

Because receptor numbers were expressed relative to the protein content of the membrane preparation, it was necessary to demonstrate that the autoregulatory effect of gastrin was exerted at the receptor level and not on the proportion of membrane protein in the preparation. Because gastrin is a trophic hormone for oxyntic gland mucosa, the possibility existed that it could increase membrane recovery or the number of cells responding to it (i.e., receptor-containing cells) out of proportion to the other types of cells making up the membrane preparation. Three pieces of evidence indicate that the changes in receptor levels represented true modulation of the receptor by gastrin. First, the effects of starvation and refeeding were even more dramatic if binding capacities were expressed per microgram DNA (20). Second, EGF, in a dose which has trophic effects on gastric mucosa equal to those of pentagastrin (33), had no effect on receptor numbers (32). Third, and

most important, the binding of choleratoxin to its receptors was unaltered by the liquid diet regimen and by pentagastrin injection (32). Choleratoxin specifically binds to monosialogangliosides of mammalian, avian, and amphibian cell membranes (34). The unusual rapidity of the binding of ^{125}I-labeled choleragen to plasma membranes, the stability of the resulting complex, and the fact that binding sites are ubiquitous and relatively abundant in most cell surfaces make these molecules ideal general cell membrane markers (34).

Although Scatchard analysis demonstrated that the above changes in receptor numbers were not accompanied by changes in binding affinities, the possibility existed that the decrease in binding capacity seen in fasted or antrectomized rats was caused by an increased rate of dissociation or gastrin from its receptor. This change would not be reflected in the calculation of K_d by Scatchard analysis if there were a simultaneous alteration in association rate. A subsequent analysis of both association and dissociation rates in fasted rats showed no significant changes in anything other than the binding capacity (35). Thus, it appears that the alterations observed in the binding capacity of gastrin to its receptor are true changes in receptor number as opposed to changes in association and dissociation rates.

In contrast to the stimulatory effect of gastrin on its receptor in gastric mucosa, elevated levels of many neurotransmitters, protein and glycoprotein hormones, polypeptides, and other endogenous substances are accompanied by declines in their respective receptor levels (36, 37). This has been termed downregulation, desensitization, or tachyphylaxis. While working with the insulin receptor of liver (24, 38) and cultured lymphocytes (39), Roth *et al.* originally demonstrated a decrease in receptor number with increasing hormone concentrations. Several investigators regard downregulation as an ubiquitous event closely related to the mechanism of hormone action (40, 41). In addition to its characteristic homospecific upregulation, prolactin has been reported to cause a brief and rapid downregulation of its receptor (42).

To test the possibility that gastrin, like prolactin, might cause downregulation of its receptors prior to producing an increase in receptor numbers, binding capacities were measured at relatively brief intervals following injection of 100 μg of gastrin-17 in the 12-hr fasted rat (43). Within 15 min of injection there was maximal occupancy of the gastrin receptor and receptor binding was reduced by 50%. At this time serum gastrin levels were significantly elevated. Receptor levels returned to normal between 1 and 3 hr after injection and significant upregulation had occurred by 6 hr (43). Receptor levels again were normal at 12 hr. The significant upregulation was not maintained because levels of serum gastrin were not chronically elevated. These data suggest that gastrin receptors have a short half-life. For other hormone receptors, having a longer half-life, the phenomenon of downregulation is more pronounced and of longer duration. Additional evidence for a short half-life of the gastrin receptor comes from experiments with the protein synthesis inhibitor, cycloheximide. Cycloheximide produced a maximal 60% decrease in the number of gastrin receptors only 3 hr after being injected. It also totally prevented the upregulation of the gastrin receptor which occurs following vagotomy (43).

One explanation for downregulation involves receptor mobility or internalization. Previously, internalization was considered to play a negligible role in protein hormone action, but recent findings implicate it in coupling mechanisms as well as in the regulation of receptor numbers (44–46). It is possible that following binding there is internalization of the gastrin–receptor complex. Gastrin induces cell growth in the oxyntic gland mucosa, and Edelman (47) has presented evidence that cell growth, recognition, and movement are coordinated by a plasma membrane receptor supramolecular complex and submembranous fibrillar structures. He suggests that surface changes in conformation, distribution, and mobility of the receptor precede alterations of the associated cytoplasmic events. In the case of gastrin cytoplasmic events may include the synthesis of new receptor molecules.

The gastrin receptor during development. Newborn humans and dogs have high serum gastrin levels, yet they do not secrete acid basally, and exogenous gastrin is an ineffective

stimulant despite the presence of parietal cells (48–51). These findings suggested end-organ insensitivity to gastrin in newborn animals. On the other hand, different data showed a dramatic change in mucosal growth parameters and enzyme patterns in the gastrointestinal tract at the time of weaning, when antral gastrin begins to increase (8, 52). A recent report by Malloy *et al.* (51) demonstrated that pentagastrin had no effect on gastric secretion or motility in newborn dogs until the second postnatal week. At this time pentagastrin decreased gastric pH from control levels of 5 or 6 to 2 or 3. After Day 9 pentagastrin decreased gastric contractile pressure to 50% of the control level.

In the newborn rat, serum gastrin levels remained between 600 and 1400 pg/ml until Day 18 which is when weaning took place (53). After Day 18, serum gastrin gradually decreased to adult levels on Day 30. Antral gastrin abruptly increased from 2 μg/g tissue on Day 18 to adult levels of 20 μg/g tissue on Day 22. The intragastric pH remained above 4.0 until Day 15 and by Day 20 it was between 2 and 3. Exogenous pentagastrin did not significantly stimulate acid secretion until Day 20 (53). Young rats (Days 5, 10, and 15) had high rates of DNA synthesis, and these were not altered by pentagastrin (Fig. 3). These high DNA synthetic rates were accompanied by rapid cell turnover with little subsequent mucosal growth (54). By Day 20 DNA synthetic rates decreased to those normally found in adult rats, and pentagastrin increased the rate of synthesis. By Day 25 pentagastrin caused a statistically significant increase in synthesis which was approximately 50% higher than control values. This response continued into adulthood (53).

In the same rats used to measure acid secretion there was no specific binding of gastrin to its receptor in oxyntic gland mucosal membranes until Day 18 (Fig. 4). By Day 22, significant levels of receptor were present in both weaned and unweaned rats. Receptor levels reached normal adult levels by Day 60 in weaned animals. The absence of weaning did not prevent or delay the induction of gastrin receptors. It did, however, significantly decrease the number of receptors present when compared with receptor levels in weaned rats of the same ages.

The developmental studies in newborn rats can be summarized by the following. There are no acid secretory and DNA synthetic responses to gastrin until shortly after the time of weaning. The absence of responses to gastrin is explained by the absence of receptors to the hormone in newborn animals. Both gastrin receptors and biological sensitivity to gastrin appear shortly after the start of weaning. The development of receptors at the time of weaning is not dependent on the presence

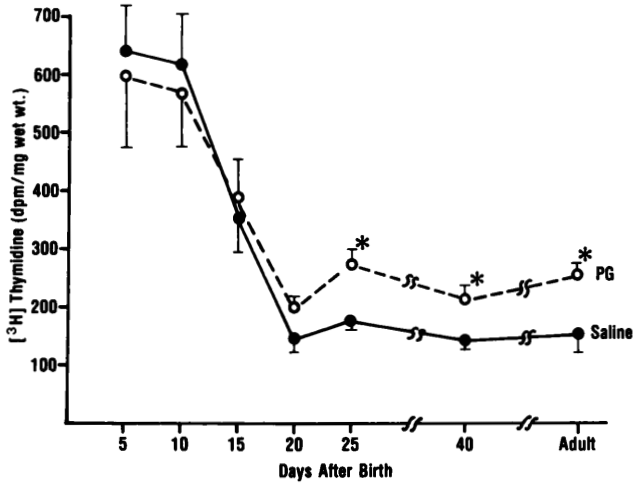

FIG. 3. DNA synthesis in oxyntic gland mucosa of rats of various ages in response to saline or pentagastrin (250 μg/kg). Each point represents the mean and SE of six determinations. * $P < 0.05$. Reproduced with permission from Amer J Physiol **240**:G163–G169, 1981 (53).

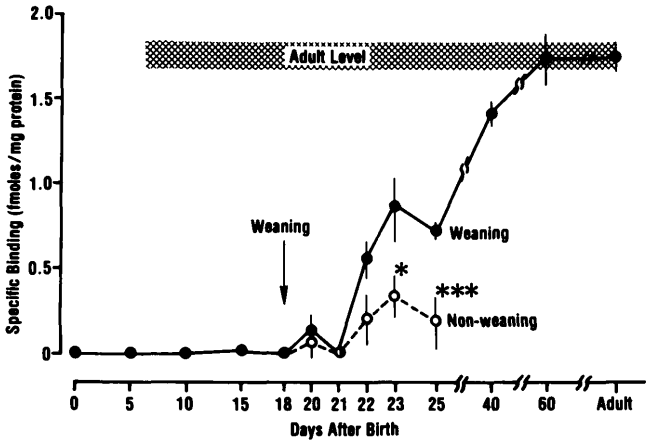

FIG. 4. Specific binding of ^{125}I-gastrin in weaned and nonweaned rats of various ages. * $P < 0.05$. *** $P < 0.001$. Reproduced with permission from Amer J Physiol **240**:G163–G169, 1981 (53).

of solid food in the diet. The enhancement of receptor levels in normally weaned rats is probably due to higher endogenous serum gastrin and its subsequent upregulation of the receptor.

There is considerable evidence that many of the changes in gastrointestinal function that occur at the time of weaning are mediated, at least in part, by adrenal corticosteroids. Administration of glucocorticoids during the second postnatal week causes precocious changes in enzyme activity patterns, and adrenalectomy delays the normal changes (55–57). Administration of hydrocortisone to suckling rats also decreases the rate of uptake of maternal antibody by the small intestine (58) and precociously increases gastric mucosal pepsinogen content (59).

A single injection of corticosterone acetate (250 mg/kg) administered to 7-day-old rats resulted in the premature appearance on Day 10 of both gastrin receptors and the acid secretory response to gastrin (60). The time course for the development of receptors and secretory response after corticosterone is shown in Fig. 5. The normal development is depicted in the same figure for comparison. In these same animals corticosterone also caused a premature increase in tissue gastrin levels (60).

Adrenalectomy prevented the development of gastrin receptors and tissue gastrin levels until Day 25. However, after this delay, receptor numbers and endogenous mucosal hormone levels increased at normal rates to adult levels. Administration of corticosterone prevented these developmental delays in the adrenalectomized rats. These results suggest that adrenal hormones may provide the trigger for the normal development of gastrin sensitivity and tissue hormone levels just as they do for other developmental changes in the gastrointestinal tract. Furthermore, the appearance of receptors on Day 25 in adrenalectomized rats and their subsequent normal development implies the involvement of another regulator(s) besides corticosterone.

Additional results in this study (60) confirmed those of our earlier experiments (53) that the nature of the diet (i.e., solid vs liquid

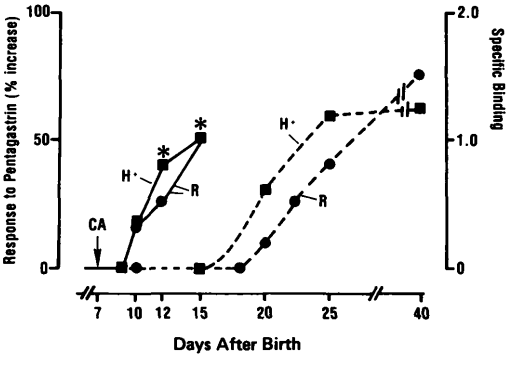

FIG. 5. Comparison of the time courses for the development of gastrin receptors (R) and the acid response to gastrin (H) during normal (dashed lines) development and after 250 mg/kg corticosterone acetate on Day 7 (solid lines). Binding is shown in fmole/mg protein.

food) was a modifier of, but did not provide the stimulus for, development of the gastrin receptor. If rats were forced to wean early on Day 13 rather than Day 18 there were no changes in receptor number (60). If rats were treated with corticosterone on Day 10 and subsequently on every third day, but were fed liquid diets instead of being weaned normally, receptor levels developed prematurely on Day 13 reaching adult levels on Day 18. However, in spite of repeated corticosterone injections, receptor levels then fell by Day 25 to those of normally developing animals being maintained on liquid diets without corticosterone. Thus, the presence of normal circulating levels of gastrin released by solid food is necessary to maintain normal receptor levels.

The role of corticosterone in gastric development appears to be nearly identical to that described for it in intestinal development. Henning and Sims (61) have divided the maturation of intestinal disaccharidase activity into three phases. The earliest occurs before weaning and is dependent only on corticosterone. The second, immediately after the start of weaning, depends both on corticosterone and the diet. The final phase is independent of corticosterone, and maintenance of the maturational changes depends on the nature of the diet.

Adult female rats have fewer gastrin receptors than adult males, which corresponds to the relative magnitudes of the maximal effects of gastrin on acid secretion and DNA synthesis in rats of different sexes (62). In pursuing the causes of these differences we found that binding capacities in male and female rats were equal until puberty, approximately Day 38. By Day 40 there were significantly more receptors present in males than females. Females had 2.4 fmole receptor/mg oxyntic gland mucosal protein by Day 40 and this value did not significantly change by adulthood. In the male the number of receptors continued to increase to 4.0 fmole/mg protein by Day 60. Castration did not significantly decrease the number of receptors in male animals, but treating the castrated rats with estrogen caused a significant decrease in receptor numbers. Ovariectomy increased the number of receptors in female rats to a value comparable to that of normal males. However, ovariectomized females also consumed more food and their serum gastrin increased to levels comparable to male values. Therefore, the increased receptor levels could have been due to upregulation by higher amounts of circulating gastrin. If ovariectomized females were pair-fed to intact females, the number of receptors was reduced but still remained significantly elevated over that of intact females. Adrenalectomy of ovariectomized females caused a further increase in receptor numbers (62). Thus, the development of gastrin receptors is repressed by estrogens in female rats but is apparently not influenced by androgens in the male rat.

Summary. The gastrin receptor in rat oxyntic gland mucosa is highly regulated. This regulation has so far only been found to be directed at the total numbers of receptors present. The affinity of the receptor is not altered significantly by agents which affect receptor numbers. Homospecific regulation occurs in that gastrin upregulates its receptor over long periods of time. Upregulation, however, appears to be preceded by a brief period of downregulation. During development corticosterone triggers the synthesis, or at least the appearance of gastrin receptors. Receptor development is maintained by solid food and presumably the gastrin it releases, but the change in diet which occurs at weaning does not in itself induce development. In female rats, estrogens prevent the increase of gastrin receptor levels to those found in males.

These results with the gastrin receptor emphasize the importance of studying receptor concentrations as well as hormone levels to the total understanding of an endocrine response. They also suggest that the receptors of the other gastrointestinal hormones are likely to be regulated and that this regulation will probably be important in understanding some of the diseases of the gastrointestinal tract.

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