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Pituitary-Induced Alterations in Gastrin Levels and Gastrointestinal Growth in Normal and Genetically Dwarf Mice (40538)

LENARD M. LICHTENBERGER AND ANDRZEJ BARTKE

Department of Medicine, Peter Bent Brigham Hospital, Harvard Medical School, Boston, Massachusetts 02115, and The Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

Evidence has accumulated over the past several years that the pituitary gland has a regulatory influence over the growth and functional activity of the gastrointestinal tract. This action was suggested by the findings in several animal species that hypophysectomy resulted in a reduction in pancreatic (1, 2) and gastrointestinal mucosal mass (2–5) as well as a decrease in gastric (6, 7) and pancreatic secretory capacity (8). The importance of growth hormone in regulating G.I. growth and function had been indicated by the finding that many of these changes associated with hypophysectomy can be partially reversed by growth hormone supplementation (2, 6, 9, 10). It has also been reported that in the pigeon, prolactin alone or in combination with other hormones will increase gastrointestinal mass after hypophysectomy (11).

It is presently uncertain whether the pituitary hormones directly stimulate the gastrointestinal mucosal cells or induce responses indirectly by secondarily releasing other hormones which in turn act on the G.I. tract. This latter possibility is supported by the finding that both serum and tissue levels of the G.I. trophic hormone, gastrin, are reduced in hypophysectomized rats (12) and that atrophic pancreatic changes associated with pituitary ablation can be reversed by exogenous pentagastrin supplementation (13). In addition, Enochs *et al.* has reported that growth hormone treatment effectively increases tissue and serum gastrin levels in male hypophysectomized rats (12).

In the studies presented here, we investigated the interrelationship among the pituitary hormones, gastrin, and G.I. cell growth in Snell dwarf mice (dw/dw) (14) which exhibit a hereditary deficiency of prolactin (15–17), growth hormone (17), and TSH (18, 19). These experiments were performed in the absence of surgical trauma normally associated with hypophysectomy. We found that antral gastrin concentration as well as various duodenal morphological dimensions were reduced below normal values in female dwarf mice. It was also determined that these changes can be partly reversed after ectopic transplantation of a normal pituitary gland which is known to be associated with the increased endogenous secretion of certain pituitary factors, notably prolactin. In contrast to these findings, exogenous prolactin supplementation was a less effective stimulant of either gastrin levels or duodenal growth in these mice.

Methods. Snell dwarf (dw/dw) female mice and their normal heterozygous female littermates, which were raised in the animal facility of the Worcester Foundation, were used in all studies. The animals were 60–90 days old at the time of the experiment. In the first study normal and dwarf mice were injected with either 125 μg of bovine prolactin (NIH-P-B4, 18.5 IU/mg, donated by the NIH Pituitary Hormone Distribution Program) or an equivalent volume (0.05 ml) of saline once a day for a 12-day period during which they had *ad libitum* access to chow and water. In the second study, pituitary glands excised from mature normal female mice of the same strain were transplanted beneath the renal capsule of anesthetized normal and dwarf mice. Each recipient mouse received a single pituitary homograft. After surgery, the animals were returned to their cages along with their unoperated littermates. All mice were given *ad libitum* access to chow and water for an additional 16 days.

At the termination of both studies the mice were sacrificed by cervical transection; the antrum was weighed and then extracted in boiling water as described previously (20). The gastrin content of the heat-extracted antral homogenate was determined by the radioimmunoassay technique of Yalow and

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Berson (21) as modified by Walsh (22). Antiserum 1296, kindly donated by Dr. Walsh, was used in all studies at a titer 1:300,000. The properties of this antiserum have been documented previously, and it has been reported to recognize all the known molecular forms of gastrin (23). Human gastrin G-17I, kindly donated by Dr. Morton I. Grossman (CURE), was used as both standard and label. Gastrin was iodinated and later purified by the method of Stadil and Rehfeld (24).

In addition to determining antral gastrin concentration, duodenal tissue (0.5 cm distal from the pylorus) was excised from five to six animals/group and then fixed in Bouins and infiltrated and embedded in paraffin. The tissue was then cut into 4-μm-thick sections and stained with hemotoxylin and eosin. Crypt and villus dimensions were measured on longitudinally cut sections using an eyepiece micrometer. Morphometric measurements were restricted to symmetrical finger shaped villi, and crypts sectioned along their entire length. The latter is indicated by the visualization of a lumen continuous from the crypt base to the crypt–villus junction which is bordered along its length by a single layer of cuboidal epithelial cells. These measurements were taken blind (on slides coded and identified by a number only) and the dimensions of 6–10 villus–crypt units were quantitated for each duodenal biopsy. In addition, the number of mitotic figures were blindly scored in a minimum of 10 well-oriented crypts.

A Student's t test for unpaired values was performed in all statistical comparisons and a P value of 0.05 or less was considered statistically significant.

Results. Antral gastrin concentration. Figure 1 demonstrates that the antral gastrin concentration of dwarf mice was approximately one-third of the values recorded in normal females of the same strain ($P < 0.001$). Transplantation of a normal pituitary under the renal capsule of dwarf mutants increased the antral gastrin concentration 1.7-fold to a value midway between the mean levels of the two groups ($P < 0.01$). Similarly, normal mice which received an extrasellar pituitary graft had an antral gastrin concentration which was significantly higher than the mean normal value.

In a separate study it was determined that exogenous administration of prolactin over a 12-day period, at a dose (125 μg/day) previously demonstrated to maintain pregnancy in dwarf mice (25), failed to increase antral gastrin concentration in either normal or dwarf mice (Fig. 2). Once again antral gastrin was approximately three times higher in normal mice than in mutants.

Duodenal structure. It can be seen in Table I that the dwarf mice had significantly shorter villi than their normal littermates. Neither exogenous prolactin administration or ectopic pituitary transplantation had a significant effect on villus height in the normal or mutant mice. Similarly, crypt length was decreased in the dwarf mice to 56% of normal values. Crypt length remained significantly below normal levels in dwarf mice chronically in-

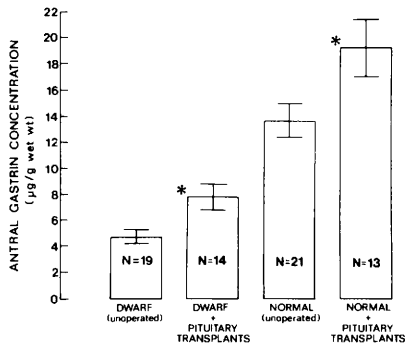

FIG. 1. Antral gastrin concentration in unoperated normal and dwarf mice and in animals which received a graft of a normal pituitary gland underneath their renal capsule 16 days prior to sacrifice. Asterisk indicates a significant difference between grafted and unoperated (control) groups at $P < 0.01$.

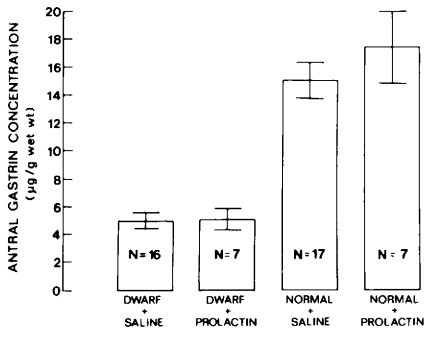

FIG. 2. Antral gastrin concentration of normal and dwarf mice which were subcutaneously injected with prolactin (125 μg/day) or an equivalent volume of saline once a day for a 12-day period.

TABLE I. EFFECTS OF ALTERATIONS IN PROLACTIN LEVELS BY INJECTIONS OF EXOGENOUS HORMONE OR BY ECTOPIC PITUITARY TRANSPLANTATION ON DUODENAL MUCOSAL STRUCTURE AND GROWTH IN NORMAL AND DWARF MICE[a]

Group	n	Villus height (μm)	Crypt length (μm)	Villus height / crypt length	Mitoses / crypt
Normal + saline	5	373 ± 22	123 ± 6	3.13 ± 0.14	1.84 ± 0.13
Normal + prolactin	6	354 ± 18	97 ± 5$^{\nabla}$	3.70 ± 0.26	1.29 ± 0.17$^{\nabla}$
Normal + pituitary transplant	5	400 ± 14	137 ± 5	2.94 ± 0.14	1.32 ± 0.10$^{\nabla}$
Dwarf + saline	6	296 ± 9$^{\nabla}$	70 ± 7$^{\nabla}$	4.41 ± 0.33$^{\nabla}$	1.29 ± 0.17$^{\nabla}$
Dwarf + prolactin	5	291 ± 4.5$^{\nabla}$	85 ± 10$^{\nabla}$	3.67 ± 0.54	1.27 ± 0.17$^{\nabla}$
Dwarf + pituitary transplant	6	273 ± 33$^{\nabla}$	105 ± 9*	2.75 ± 0.45*	2.11 ± 0.22*

[a] $^{\nabla}$ Indicates a significant difference ($P < 0.05$) in comparison to values for normal + saline group. * Indicates a significant difference ($P < 0.05$) between dwarf mice bearing pituitary grafts and saline-treated mutants.

jected with either saline or prolactin. It, however, should be noted that the mean crypt length of mutants with an ectopic pituitary graft was not different from normal values and was significantly greater than the crypt dimensions of saline-treated dwarf mice. One paradoxical finding was that crypt length was significantly reduced in normal mice chronically treated with prolactin, and not significantly different from the values obtained in prolactin-treated mutants.

The villus/crypt ratio is known as one of the more sensitive indices of intestinal growth, being inversely related to epithelial proliferative activity, since crypt length more closely parallels the proliferative rate than does the length of the villi (26). The data in Table I indicate that the villus/crypt ratio is significantly greater in saline-treated mutants than in normal mice. In contrast, the villus/crypt ratio of both mutants chronically injected with prolactin and those which received ectopic pituitary grafts was not significantly different from normal values. The lowest mean value for the villus/crypt ratio of all groups was, in fact, measured in the duodenal biopsies of mutants with pituitary grafts. Neither exogenous prolactin administration nor pituitary transplantation significantly influenced the crypt/villus ratio of normal mice.

The last column indeed indicates that there were significantly fewer mitoses per crypt in the mutant's duodenal mucosae than in normal mice. The number of mitoses was not increased in mutants injected with prolactin but was significantly increased and achieved normal levels in mutants bearing ectopic pituitary grafts. One unexplained finding is that the number of mitoses per crypt was significantly decreased in normal mice which either were injected with prolactin or received a pituitary transplant.

Discussion. In 1929, Snell (14) initially reported the characteristics of a strain of dwarf mice which carried the homozygous recessive gene dw/dw. Since then, the endocrine abnormalities of these mice have been studied fairly extensively by several groups of investigators (15–19). It has been demonstrated that the pituitary gland in these mutants is markedly reduced in size and is deficient in acidophils (mammotrophs and somatotrophs) (27) and thryrotrophs (28) which are known to synthesize prolactin, growth hormone, and thyroid-stimulating hormone, respectively. These morphological findings were then supported by the finding that tissue and serum levels of these hormones are either abnormally low or undetectable in the mutants (15–18). Sinha *et al.* (17) reported that in female dwarf mice, pituitary prolactin and growth hormone were reduced to one-twentieth and one-half of normal values, respectively.

In the study presented here we have determined that female dwarf mice have a deficiency in the hormone gastrin, having one-third the antral gastrin concentration found in normal female littermates. In addition, we have investigated the role of prolactin in influencing gastrin levels since it is the pituitary hormone most markedly reduced in female dwarf mice. In these studies it was demonstrated that exogenous administration of ovine prolactin (125 μg/day) was essentially ineffective, whereas sustained secretion of murine prolactin as well as other pituitary factors by an ectopic pituitary graft resulted in an 170 and 140% increase in antral gastrin

concentration in mutant and normal mice, respectively. Serum gastrin levels were not determined due to the technical difficulties involved in obtaining sufficient samples of blood from 10- to 12-g dwarf mice for reliable gastrin radioimmunoanalysis. Nevertheless, since under most chronic conditions the changes in serum gastrin concentration parallel those recorded in tissue hormone levels (20, 40, 42), it can be assumed that in the above groups serum gastrin concentration would be altered to a comparable extent to the reported changes in antral gastrin concentration.

In these studies it was also established that dwarf mice had a thinner duodenal mucosal lining, having shorter villi and shallower crypts, with a fewer number of mitoses than recorded in normal animals. In addition, it was demonstrated that the number of mitoses per crypt and crypt length in mutants were increased after the mice received an ectopic pituitary transplant but not after chronic exogenous prolactin supplementation. The villus/crypt ratio also was sensitive to the hormonal status of the animal and was significantly higher in mutants than in normals and this difference between groups was abolished by either exogenous prolactin administration or ectopic pituitary transplantation.

It is fairly well documented that gastrin trophically influences the mammalian gastrointestinal mucosae from the stomach to the colon and this may play an important physiological role in the normal regulation of growth of these tissues (12, 13, 42). In this study, we have demonstrated that various parameters of duodenal mucosal mass and cell proliferation were reduced below normal values in dwarf mice who had abnormally low antral gastrin concentration. Conversely, both gastrin levels and crypt cell proliferation increased in parallel after dwarf mice received an ectopic pituitary transplant. These results, therefore, support the possibility that pituitary-induced gastrointestinal growth may be mediated by changes in gastrin levels. The relationship between tissue gastrin levels and G.I. cell proliferation was less clear-cut in normal mice, where crypt cell proliferation was not increased in response to ectopic pituitary transplantation even though tissue gastrin levels were raised above normal values. The reason for this discrepancy is uncertain, but it may be due to the fact that the G.I. tract of gastrin-deficient dwarf mice was more sensitive to the trophic action of gastrin than tissue from normal mice. It also is possible that in the animal model studied here, gastrin and gastrointestinal growth are not causally related but instead both properties are dependent on an undefined pituitary factor not present in the dwarf mouse.

In the experiments just described it has been assumed that transplantation of normal pituitary glands to the renal capsule of either normal or dwarf mice results in an increase in endogenous prolactin levels from the extrasellar pituitary graft. This is due to the removal of the pituitary from the inhibitory influence of hypothalamic factors (29). This assumption is based on reports in the literature that serum prolactin levels are markedly raised when normal pituitary glands are transplanted beneath the renal capsule in hypophysectomized rats (29, 30). In addition, pregnancy can be maintained in dwarf mice with an ectopic pituitary graft and not in sham operated mutants (31). This indicates that significant quantities of biologically active prolactin are released by normal pituitary glands when transplanted to an extrahypothalamic site in these mutants. The observation that gastrin levels and G.I. growth were more responsive to ectopic pituitary transplantation than to exogenous prolactin treatment, may reflect the fact that elevations in serum prolactin levels would be chronically sustained in the former but not the latter preparation since prolactin has a very short circulatory half-life of 2–3 min (32). Another possible explanation is that the transplanted pituitary also may release another hormone—i.e., growth hormone and/or TSH, which in turn may influence these gastrointestinal properties. The evidence in the literature, however, is conflicting whether serum growth hormone and TSH levels are significantly increased by ectopic pituitary transplantation (33–36). Future studies will be needed to investigate whether these hormones may play a role in the mediation of these pituitary-induced gastrointestinal changes.

There are several reports in the literature that suggest that gastrointestinal functional capacity and growth are altered in parallel

with conditions associated with marked changes in endogenous prolactin levels. For example, gastrointestinal mucosal growth (37, 38) and gastric acid secretion (39) as well as gastrin levels (40) are enhanced during lactation in the rat when prolactin levels are elevated (41), and these changes are abolished by removal of the suckling stimulus. In this study, we have found that gastrointestinal growth and gastrin levels are abnormally low in dwarf mice and both properties are increased after ectopic pituitary transplantation. These conditions are associated with negligible and high endogenous prolactin level, respectively. We, however, were unable to establish the importance of prolactin in mediating these gastrointestinal responses, since exogenous administration of the hormone could not duplicate the changes seen after ectopic pituitary transplantation. This may suggest that other pituitary factors may be involved.

Summary. The antral gastrin concentration of female Snell dwarf mice (dw/dw) was 30% the concentration of normal female littermates. In addition, the mutants had an abnormally thin duodenal mucosa, having significant reductions in the number of mitoses/crypt, crypt length, and villus height. Because crypt length was reduced to a greater extent than villus height, the villus/crypt ratio was significantly higher in mutants than in normal mice. It was determined that antral gastrin concentration in both mutants and normal mice could be significantly increased over a 16-day period by transplantation of a normal pituitary under their renal capsule. Ectopic pituitary transplantation in mutants also significantly increased the number of mitoses per crypt as well as crypt length but did not influence villus height, resulting in a decrease in the villus/crypt ratio to normal levels. Administration of exogenous prolactin to mutants (125 μg/day) over a 2-week period did not significantly influence antral gastrin levels and had a marginal growth-promoting effect on the duodenal mucosae.

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