Effect of hypophysectomy on endocrine cell types in rat gastrointestinal mucosa

G. M. PORTELA-GOMES1, J. P.-G. ALBUQUERQUE1, M. A. FERRA2 AND L. GRIMELIUS3

1 Department of Medicine II and Centre of Gastroenterology, University Hospital of Santa Maria, and 2 Department of Nuclear Medicine, Cancer Institute, Lisbon, Portugal, and 3 Department of Pathology, University of Uppsala, Uppsala, Sweden

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ABSTRACT

The effect of hypophysectomy on the gastrointestinal tract was studied in the rat 8 wk after operation, particularly regarding the frequency and distribution of serotonin, somatostatin and gastrin-immunoreactive cells. Body weight, the length of the intestine and the thickness of the mucosa of the antrum and small intestine were all reduced in the hypophysectomised rats compared with sham-operated and untreated controls. In the hypophysectomised animals the serotonin-immunoreactive cells were fewer in the antrum and caecum, whereas they were more numerous in the proximal large intestine. There were fewer gastrin-immunoreactive cells in the antrum, while the somatostatin-immunoreactive cells were more numerous in the antrum and caecum. The significant influence of hypophysectomy on the gastrointestinal tract could be direct, but could also be associated with the marked effect of pituitary deficiency on endocrine cells, known to exert both trophic and antitrophic actions. However, it could also be an indirect effect on metabolism, resulting in lower food intake, other endocrine cell systems, and growth factors.

Key words: Pituitary gland; serotonin cells; somatostatin cells; gastrin cells.

INTRODUCTION

Most hormones released by the anterior pituitary gland exert trophic effects that stimulate secretion from the peripheral endocrine glands such as the thyroid, adrenal cortex and gonads, as well as having direct metabolic effects on tissues associated with hormones under control of the pituitary gland.

The influence of pituitary hormones on the gastrointestinal (GI) tract has been reported in several animal species, including man. These studies have been carried out mainly by examining the effect of hypophysectomy with or without single or multiple hormonal substitution. The changes in the GI tract caused by hypophysectomy include mucosal atrophy, and a reduction of gastric secretion and intestinal absorption (cf. Schapiro et al. 1970). Little is known about the effects of hypophysectomy on the GI hormones. A reduction was observed in the amount of secretin extracted from the small intestine of hypophysectomised rats (Dorchester & Haist, 1952, 1953) and a decrease in the concentration of gastrin in serum and antral mucosa has been reported (Enochs & Johnson, 1976). However, to our knowledge, there are no published data on the endocrine cells of the GI tract following hypophysectomy.

The aim of the present study was to ascertain whether hypophysectomy would affect the GI tract and certain major endocrine cell systems (serotonin, somatostatin and gastrin-immunoreactive (IR) cells) in the alimentary mucosa in the rat. This is of particular interest, as hypopituitarism occurs in man. The results may advance our understanding of the regulatory effects of pituitary hormones on the GI tract.

MATERIALS AND METHODS

Eighteen male rats of the Sprague-Dawley strain, obtained from Charles River, Cléon, France, aged
~ 65 d (250–300 g), were divided randomly into 3 groups: 6 untreated control (C) rats, 6 sham-operated (Sham-op) controls, and 6 hypophysectomised (Hypox) rats.

Hypophysectomy was carried out by excision via the trans cervical route. The surgical procedures were performed by the supplier 1 wk prior to delivery. The animals were fed on a pelleted laboratory diet (Nute SA, Solsona, Spain) ad libitum, with free access to water; in addition, the Hypox animals were given NaCl ad libitum in salt licks and 5% glucose was added to the drinking water.

At 8 wk after the operation the animals were killed by intraperitoneal injection of sodium barbiturate (20 mg/kg) after overnight starvation but with water ad libitum. Blood was collected before death by cardiac puncture for serum ACTH determinations which were used as an indicator of the effect of hypophysectomy. These determinations were performed with a commercially available radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA, USA). The sensitivity of the assay was 3 pg/ml and the intra-assay coefficient of variation was 7% (Ekins, 1981).

The GI tract was removed and the length of the intestine measured by suspending it vertically along a ruler with the stomach downwards. Specimens were collected from the oxyntic gland area; antrum; proximal and distal parts of the duodenum, jejunum and ileum; caecum; and proximal and distal parts of the large intestine. The specimens were approximately 1 cm long, and from the intestinal tract the width corresponded to the complete transverse section. In the Hypox rats the skull was opened and the sella turcica was inspected for pituitary fragments; the examination did not reveal any glandular remnants.

The tissue specimens were opened longitudinally, stretched flat on a filter paper, fixed in Bouin’s fluid (containing 1% acetic acid) for 8 h, dehydrated and embedded in paraffin wax with the mucosal surface perpendicular to the cutting surface. From each specimen, 5 µm serial sections were cut and attached to 4 chrome–alum–gelatin coated glasses, 2 sections separated by 50 µm on each glass.

The gut circumference was determined after fixation, by measuring with a ruler the width of the transverse section of the different parts of the intestinal tract.

Staining

The sections from each specimen were stained with haematoxylin-eosin and immunostained with the streptavidin–biotin complex (ABC) technique (Hsu et al. 1980, 1981) using rat monoclonal antibodies to serotonin (YC 5/45, Sera-Lab, Sussex, UK) and rabbit polyclonal antisera to C-terminal somatostatin and gastrin (Dako Corp., Santa Barbara, CA, USA, code nos A566 and A568 respectively).

Each antibody was used at a dilution of 1:1600 and the peroxidase activity was demonstrated by diamino-
Fig. 1. Rat antral mucosa immunostained for serotonin with the streptavidin–biotin complex method using diaminobenzidine as chromogen. Antrum of hypophysectomised rats (A) shows a reduction of mucosal thickness and of the number of serotonin-immunoreactive cells, compared with the control rats (B). Bar, 40 µm.

The controls for immunostaining were as recommended by Sternberger (1986) and included preincubation of the primary antisera with the relevant synthetic antigens (from Sigma Chemical Co., St Louis, Mo., USA).

Quantification
Counts of IR cells containing a visible nucleus were performed at a magnification of ×400, in well oriented parts of the section, i.e. with uninterrupted glandular lumina from the base to the top of the mucosa. The frequency of cells with their nuclear centre within the section was calculated by using the Floderus formula (Floderus, 1944), which takes into account factors that influence the counting results, i.e. nuclear size, smallest visible nuclear fragment and thickness of the section. Nuclear size was estimated by means of a scale in one of the oculars, and the thickness of the sections was measured by reading the micrometer screw scale when focusing on the upper and lower section surface (cf. Portela-Gomes et al. 1984). The number of IR cells was correlated to a mucosal volume of 1 mm³ (cell density). The mucosal areas were estimated with a television image analysis system (IMTEC, Uppsala, Sweden). The mean mucosal thickness was estimated by measuring the total mucosal area along 1 mm of the muscularis mucosae.

Statistical analysis
To test the significance of differences between means, a 2-way analysis of variance was used. All values in the following are means ± S.E.M.

RESULTS
During the experimental period, no deaths occurred in any of the 3 groups of animals.

At 8 wk after the operation the Hypox rats showed a decrease in weight, whereas the Sham-op and C animals had a regular increase in body weight. The
Figs 2(a–c). Frequency (mean ± S.E.M.) of serotonin (a), somatostatin (b), and gastrin-immunoreactive cells (c) in different parts of digestive tract from untreated control rats, sham-operated (Sham-op), and hypophysectomised (Hypox) rats. The frequency was determined as the number of cells in 1 mm² mucosal volume × 10⁻³. For abbreviations of the gastrointestinal levels, see Table. Asterisks: P < 0.02 (a), P < 0.001 (b), P < 0.002 (c).

Gastrointestinal tract

In the Hypox rats, both the small and the large intestine were shorter (small intestine: Hypox 97 ± 14, Sham-op 133 ± 12, C 135 ± 15 cm; large intestine: Hypox 12 ± 1, Sham-op 18 ± 1, C 17 ± 1 cm). The difference for both the small and the large intestine was significant (P < 0.001).

The intestinal circumference was the same in all 3 animal groups, except for the ileum where the Hypox rats showed a significantly shorter circumference than the controls (Table). The thickness of the GI mucosa was reduced in the Hypox rats compared with the 2 control groups, except in the caecum and large intestine. This difference was significant for the entire small intestinal mucosa (Table).

Microscopical examination of the GI mucosa showed no signs of abnormality, except for the differences in mucosal thickness and in the endocrine cells; nor did the intensity of the immunostaining of individual cells differ between the 3 groups.

Quantitative studies on the frequency and distribution of the serotonin, somatostatin and gastrin-IR cells revealed differences between the groups. In comparison with the control groups, the Hypox rats showed significantly reduced numbers of serotonin and gastrin-IR cells in the antrum, whereas somatostatin-IR cells were more numerous (Figs 1–2). In the duodenum and small intestine there were no obvious differences between the groups in the numbers of serotonin and somatostatin-IR cells. In the caecum, the Hypox rats had significantly fewer serotonin-IR cells, but significantly more somatostatin-IR cells, than had the controls (Figs. 2a,b). In the large intestine a significant increase in serotonin-IR cells was found in Hypox rats compared with the controls, although this difference was limited to the proximal part.

Concerning nuclear size, the nuclei in the gastrin cells in Hypox rats were significantly (P < 0.05) smaller (diameter 3.4 ± 0.4 µm) than those in Sham-op (5.2 ± 0.4 µm) and C rats (5.0 ± 0.4 µm). No differences in the nuclear size of the serotonin and somatostatin-IR cells could be discerned between the 3 experimental groups.
DISCUSSION

The present study showed that hypophysectomy, either directly or indirectly, affects body weight, the length of the intestine, the thickness of the GI mucosa, and the distribution of endocrine cells in the antrum, caecum and proximal large intestine.

The effectiveness of hypophysectomy was evidenced by the significantly reduced serum ACTH values, although the hormonal levels did not become immeasurable. This may have been due to the existence of ACTH-producing cells in the pars tuberalis (Stutinsky et al. 1964), in the parasellar regions, and in the brain where it is normally found (Krieger et al. 1977). The sella turcica was examined, but no pituitary remnants were found; furthermore it has been shown that in the rat, following hypophysectomy, any pituitary fragments accidentally left behind in the pituitary fossa rapidly atrophy (Smith, 1930). Moreover, the Hypox rats failed to grow, which is also evidence of a complete surgical procedure (Havivi et al. 1968).

The disector method developed by Gundersen et al. (1988) is certainly one of the most reliable quantification techniques available, but could not be used in the present study as we did not have access to the necessary microscopic equipment. Our quantification method for the estimation of numerical density has however taken into consideration various factors that influence the quantification results (Portela-Gomes et al. 1984).

Mucosal hypoplasia has been reported previously (Haeger et al. 1953; Havivi et al. 1968; Taylor et al. 1979) as evidence of the influence of pituitary gland function on the growth of the mucosa of the GI tract. The causes of these changes in the GI tract are not fully known, however. Metabolic alterations following hypophysectomy, which in the present study led to a reduced food intake, resulting in an almost 60% difference in body weight between the Hypox and the control animals, may have been involved. However, the reduced food intake was probably not the only factor responsible for the observed changes.

It seems likely that the reduced length and thickness of the gut is more related to a direct effect of decreased serum concentrations of pituitary hormones, as studies using administration either of growth hormone or ACTH, or prolactin, after hypophysectomy, have shown that these hormones prevent the trophic alterations in the mucosa of the GI tract (Dorchester & Haist, 1953; Campbell et al. 1959; Muller et al. 1977). Other hormonal effects of hypophysectomy may also be involved in the abnormal growth of the GI tract. The influence of the dysfunction of the thyroid and adrenal glands as well as alterations in the hypothalamopituitary axis must also be taken into consideration in these GI changes. In addition, the present study demonstrated that hypophysectomy markedly influences endocrine cell populations which are known to exert a trophic effect on the GI mucosa.

The results of endocrine cell counting, expressed as numerical density, are of course influenced by the mucosal volume. The thickness of the small intestinal mucosa, measured as the area in mm² of a mucosal segment 1 mm long, was significantly decreased in the Hypox rats compared with the 2 control groups. This reduced mucosal thickness means that the reduction in the estimated number of cells per unit segment is even greater than is suggested by numerical density data. Gut length (but not the gut circumference, except for the ileum) was also decreased in the Hypox animals. The higher cell frequency may be a reflection of the changes in the mucosal thickness; nevertheless the differences were so marked that they must denote a real change in cell density.

The nuclear size of endocrine cells is reportedly related to their functional state, i.e. increased nuclear size in hyperfunctional cells, the converse in hypo-functional cells (Hellman & Hellerström, 1959; Hultquist, 1965). Regarding the measurements in the present study, only in gastrin-IR cells were the nuclei significantly smaller. The nuclear size of the serotonin and somatostatin-IR cells, whether in larger or smaller numbers, was substantially the same in all 3 groups of animals, indicating that the individual cells are not necessarily hyper or hypofunctional.

The decrease in the number of serotonin-IR cells observed in the antrum and caecum is consistent with the absence of the growth stimulatory effect of ACTH observed on serotoninergic neurons in brain (Whitaker-Azmitia & Azmitia, 1991), although it has been reported that brain serotonin concentrations remain unaffected by hypophysectomy (Heal et al. 1983). The reason for the increase in serotonin-IR cells in the large intestine is therefore difficult to explain.

The decrease in the frequency of gastrin-IR cells is consistent with an earlier report where, following hypophysectomy, there was a decrease both in serum and antral gastrin concentrations, which returned to normal after growth hormone administration (Enochs & Johnson, 1976). The decrease in cell numbers may have been due to a decrease both in the stimulatory effect of corticosterone, secondary to the ACTH deficiency, and decreased food intake (Xynos et al. 1987). Gastrin has been considered to have a trophic effect on the mucosa of the GI tract (Castelyn et al. 1984).
1977; Johnson, 1977) and its decrease could therefore be an important factor in the development of the observed mucosal hypoplasia.

Somatostatin has been shown to have a direct inhibitory effect on cell proliferation in the GI mucosa (Lehy et al. 1979; Bosshard et al. 1980) hence the increased number of somatostatin-IR cells could be involved in this sense. Somatostatin may also exert an indirect antitrophic effect, as it has an inhibitory action on gastrin cells and it inhibits the secretion of epidermal growth factor (Kirkegaard et al. 1984), which has been known to have a trophic effect on the GI mucosa (Al-Nafussi & Wright, 1982).

The present study indicates that the hypophysectomised condition exerts a significant effect on the serotonin, somatostatin and gastrin-IR cells of the GI tract, more markedly apparent in the antrum. Which factors influence the endocrine cells in the rat after hypophysectomy is unclear; probably the reduced food intake plays a role, but the absence of pituitary hormones definitely contributed to a disturbed metabolism, which in turn influenced endocrine cell populations closely related to trophic and antiproliferative effects on the GI tract, especially the antral and intestinal mucosa.

It remains to be established whether hypophysectomy has any effect on other cell types producing peptides which may play a role in the GI changes, such as enteroglucagon (Jacobs et al. 1976; Sagar et al. 1982) or other peptides to which a putative trophic effect on the gut has been attributed, e.g. neurotensin (Feurle et al. 1987), CCK (Hughes et al. 1978), bombesin and endothelins (Rozengurt, 1994).

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