Upsala J Med Sci 84: 247–254, 1979

The Effects of Endogenous Hypergastrinemia and Hypogastrinemia on the Exocrine and Endocrine Rat Pancreas

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ABSTRACT

The effects of endogenous hypergastrinemia and hypogastrinemia on the exocrine and endocrine pancreas were studied in the rat. Hypergastrinemia was induced by antral exclusion, and hypogastrinemia by antral resection. The studies were made 14 weeks after surgery.

The total weight of the pancreas was increased both in hypergastrinemic and hypogastrinemic animals, due to hypertrophy of the exocrine cells. In contrast, the volume and total weight of the pancreatic islets were decreased.

There was no numerical difference in the A-, D-, PP-cells between the hyper- and hypogastrinemic animals, respectively, and the controls. The number of insulin-producing (B-) cells was certainly reduced after the induction of hypogastrinemia. There was, however, signs of increased B-cell activity, which might contribute to an underestimation of the number of B-cells with the technique used.

These findings do not support the hypothesis that antral gastrin has trophic influence on either exocrine or endocrine pancreas.

INTRODUCTION

Gastrin is known to influence the gastrointestinal tract in many ways. Its stimulatory action on the secretion of hydrochloric acid and pepsinogen and its trophic effect on the parietal cell area of the stomach are well defined (3,19, 20). Pentagastrin, administered in pharmacological doses, has also been reported to exert a trohpic action on the exocrine pancreas in rats (1,9). Hitherto, however, no such action has been found following various surgical procedures leading to an increased endogenous serum gastrin concentration (13). In the experiments by the latter authors no increase in the total dry weight of the pancreas was observed. A link between gastrin and the endocrine pancreas has been discussed by Larsson (10), who found islet cell hyperplasia in some patients with hypergastrinemia due to tumor production and in patients with atrophic gastritis. Furthermore, indirect evidence of a gastro-insular axis

mediated by gastrin has been produced, as an altered glucose tolerance has been found in patients with hypergastrinemia compared to normogastrinemic patients (16,17).

The present experiments were designed in order to further examine the effect of endogenous hypergastrinemia not only on the exocrine but also on the endocrine pancreas.

MATERIAL AND METHODS

Adult male Sprague-Dawley rats (body weight 250-300 g) were used. They were fed on laboratory food. The animals were randomly assigned to the following groups:

(1) 5 rats with exclusion of the antral part of the stomach (AE)
(2) 4 rats with resection of the antral part of the stomach (AR)
(3) 4 control rats - untreated (C).

Antral exclusion and antral resection were performed by techniques described previously (5).

Serum gastrin concentrations were determined by solid-phase radio-immunoassay (11) 12 weeks after operation, after a starvation period of 48 h.

The animals were killed 14 weeks after the stomach operation. The pancreas was extirpated, dissected free from fat tissue and weighed. The specimens were then fixed in Bouin's fluid for 18 h, dehydrated, cleared in xylene and embedded in paraffin. Deparaffinized 5 µm thick sections were stained with hematoxylin-eosin and by the van Gieson method.

To calculate the percentage volume of the pancreatic islets, the point sampling technique described by Chalkley (2) was employed. At least 3000 intersections (hits) were analysed on acinar or islet parenchyma of each pancreas. The weight of the endocrine parenchyma was calculated by multiplying its percentage volume by the weight of the entire pancreas. The densities of the exocrine and endocrine tissue were assumed to be equal.

The density of the exocrine cells per unit volume exocrine tissue was determined in a microscope equipped with a square grid placed in the focal plane of the eyepieces at a magnification of x 400. In each case three randomly selected areas of exocrine tissue were examined and the nuclei or nuclear fragments within the square grid were counted. The nuclear sizes of 50 randomly selected exocrine cells were also determined with the aid of a mm scale placed in one of the eyepieces. As the observed nuclear frequency is influenced by the nuclear size, the formula of Flodérus was used to correct these values (7). The relative number of exocrine cells was calculated by multiplying the corrected number of exocrine cells per unit volume by the exocrine pancreatic weight.

Gomori's aldehyde fuchsin technique (12) with ponceau fuchsin as a counterstain (8) was used to visualize B-(insulin)cells. D-(somatostatin)cells were

stained with the Davenport alcoholic silver nitrate technique as modified by Hellerström & Hellman (6). A-(glucagon)cells and PP-(pancreatic polypeptide) cells were identified with the immunoperoxidase (PAP) technique described by Sternberger (18). The sections were counterstained with Hernechtrot. Controls as described by Goldman (4) were used.

Differential counts were made in order to estimate the frequency of different types of endocrine pancreatic cells (except PP-cells). At least 2000 endocrine cells were counted in each animal and with each of the various staining procedures. Only cells with visible nuclei or nuclear fragments were included in the counts.

The nuclear size of B- and non-B-cells was estimated in aldehyde-fuchsin-stained sections (counterstained with ponceau fuchsin) as described above, at a magnification of 1250 x.

Statistics: Student's t-test was used.

RESULTS

The mean body weight at sacrifice was slightly higher in the control group than in the two experimental groups (Table 1).

The serum gastrin concentration was considerably increased in group AE and markedly decreased in group AR as compared with that in the controls (Table 2).

The mean pancreatic weight was much higher in both experimental groups than in the controls (Table 3).

The observed number of exocrine cell nuclei per unit volume was highest in the control group and lowest in group AR. After correction concerning the nuclear size the differences were more pronounced (Table 4). These findings indicate a hypertrophy of the exocrine pancreas both after antral exclusion and after antral resection. The relative values of the total number of the exocrine cells were also highest in the control group and lowest in group AR (Table 4), indicating a numerical decrease of the exocrine cells after antral resection.

The volume of the pancreatic islet tissue in relation to the total pancreatic volume was significantly lower in the experimental groups (Table 5). The calculated weight of the islets was significantly decreased in both experimental groups in comparison with the control group (Table 5). The islet weights of the two experimental groups did not differ from each other. The amount of non-parenchymal tissue (blood vessels, connective tissue, fat cells) was approximately equal in the three groups as judged by visual observation.

In group AE and the control group the B-cells showed a strong and even staining reaction, while this reaction was highly variable in group AR. No difference in the staining of A-, D- or PP-cells was found between the different groups. The frequency of B-cells in group AR was significantly lower than in the controls (Table 6), whereas the frequency of the other endocrine cell types

Table 1. Body weight at sacrifice

Group	Body weight (g) M ± S.D.
AE	417 ± 34
AR	411 ± 29
Controls	463 ± 35

Table 2. Serum gastrin

Group	Serum gastrin (mmol/l)
AE	287 ± 26
AR	138 ± 35
Controls	170 ± 19

Table 3. Weight of the pancreas

Group	Weight of pancreas (g) M ± SE
AE	2.297 ± 0.106
AR	2.319 ± 0.107
Controls	1.982 ± 0.115

(A- and D-cells) did not differ significantly between the three groups. PP-cells were only seen in some islets, mainly located in the periphery. They varied in number, but were most often sparse. No differences in the distribution were observed between the different groups.

The diameters of the B- and non-B-cell nuclei (Table 7) did not differ significantly between the three groups. However, in group AR the nuclear diameter of the weakly stained B-cells was significantly greater (6.05 ± 0.13) than that of the heavily stained B-cells (5.5 ± 0.10).

DISCUSSION

In these experimental models endogenous hypergastrinemia was induced by antral exclusion and hypogastrinemia by antral resection. The total pancreatic weight was higher in both experimental groups than in the control animals. This enlargement of the pancreas was due to hypertrophy and not to hyperplasia of the exocrine cells. The cellular hypertrophy was especially prominent in group AR. The hypertrophied cells were also characterized by nuclear enlargement,

Table 4. Diameters (μm) of the nuclei of exocrine cells and the observed and corrected (by the Floderus formula) number of exocrine cells per unit volume. The corrected values represent those nuclei whose centers lay within the section. The diameter of 50 randomly nuclei were estimated in each case.

Group	Nuclear diameter M ± SE	Significance	Observed number of exocrine cells per unit volume M ± SE	Corrected number of exocrine cells per unit volume M ± SE	Significance*
AE	5.37 ± 0.10	< 0.05	46 ± 4	31 ± 3	< 0.05
AR	5.62 ± 0.22	< 0.05	36 ± 1	23 ± 1	< 0.01
Controls	5.07 ± 0.05		59 ± 4	41 ± 3	

* P-value (t-test) for difference between the experimental groups and the control group

Table 5. Relative islet tissue volume per total pancreatic volume (%) and total calculated weight of islet tissue. The point sample technique was used in the volume estimation. The total islet tissue weight was calculated from the total pancreatic weight and islet tissue volume.

Group	Islet tissue volume (%) M ± SE	Significance*	Total islet tissue weight (mg) M ± SE	Significance*
AE	0.33 ± 0.06	< 0.005	8.80 ± 1.56	< 0.001
AR	0.36 ± 0.10	< 0.05	7.51 ± 2.33	< 0.001
Controls	0.77 ± 0.07		18.04 ± 1.08	

* See Table 4

Table 6. Relative frequency of islet B-, A- and D-cells per total number of islet cells

Group	B-cells M ± SE	Significance*	A-cells M ± SE	Significance*	D-cells M ± SE	Significance*
AE	77 ± 2	NS	21 ± 1	NS	12 ± 2	NS
AR	55 ± 3**	< 0.001	22 ± 2	NS	15 ± 3	NS
Controls	73 ± 1		21 ± 2		16 ± 3	

* See Table 4
** This value may be underestimated, for reasons given in the text

Table 7. Diameters (μm)* of B- and non-B-cell nuclei of pancreatic islets. 100 randomly selected nuclei were estimated in each cell type and group.

Group	B-cells M ± SE	Significance**	Non-B-cells M ± SE	Significance**
AE	5.12 ± 0.26	NS	4.23 ± 0.09	NS
AR	5.55 ± 0.12	NS	4.64 ± 0.14	NS
Controls	5.54 ± 0.13		4.41 ± 0.07	

* In nuclei with an ovoid shape the geometrical mean value $\sqrt{a \cdot b}$ was estimated, where a = the longest axis, b = the shortest axis
** See Table 4

probably reflecting increased cellular activity. On the other hand the total volume of pancreatic islet tissue was decreased both after antral exclusion and after antral resection. These findings do not indicate a trophic effect of antral gastrin on the exocrine or endocrine pancreas, since similar changes were observed in the two experimental groups.

When adding the percentages of the different cell types in the same group, the content exceeded 100 % for group AE and the controls. This was due to the fact that with the staining procedures used A-, B-, and D-cells are visualized in different ways. The reason for this was that the conditions for differential counting varied with staining technique, as not only the cell type in question but also other endocrine cells could appear differently depending on the background counterstain. Nevertheless, a comparison of the frequency of a particular type of cell between the individual groups is considered justifiable.

The only difference noted between the two experimental groups was a lower frequency of B-cells in group AR, but on the other hand, the B-cells in this group showed an increased rate of synthesis (nuclear enlargement) and release (decreased staining reaction to insulin). This lower frequency of B-cells might, however, be misleading, since some of the more or less degranulated cells might have been overlooked in the differential counting. The reason for this apparently altered function of B-cells after antral resection is not known.

It must be emphasized than in these experiments the duodenum was excluded from continuity with the gastrointestinal tract after both surgical procedures. It is therefore possible that some duodenal factor or factors might have been involved in the pathogenesis of the observed changes in both the endocrine and the exocrine pancreas. Such factors might well include hormones, e.g. cholecystokinin, secretin, somatostatin and duodenal gastrin. It has been suggested that exogenous cholecystokinin may exert a trophic effect on the exocrine pancreas (14,15), while secretin and somatostatin are believed to counteract the trophic action of gastrin (13,21). In our study the secretion of these hormones was not analysed. However, it may be assumed that the secretion of cholecystokinin and secretin was decreased, as stimulation by intraluminal contents of the duodenum is excluded. In order to test these speculations further, studies on the possible trophic action of duodenal hormones in the rat are planned. The findings of the present study with the experimental model used do not support the view that antral gastrin has a trophic effect on the rat pancreas.

ACKNOWLEDGEMENTS

Pure HPP and anti-HPP serum were a generous gift from Dr R.E. Chance, Lilly Research Laboratories, Indianapolis.

The expert technical assistance of Miss M. Kasper is gratefully acknowledged.

The study was supported by grants from the Swedish Medical Research Council (Nos 102, 4534 and 2297).

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Received August 10, 1979

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