STIMULATING EFFECT OF SEROTONIN ON MITOTIC ACTIVITY IN RAT PANCREAS IN PHYSIOLOGICAL AND REPARATIVE REGENERATION

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Injected serotonin stimulates mitotic activity in the regenerating rat liver [1, 2, 4, 5]. It is suggested that this stimulating effect is not due to serotonin itself, but to a product of its metabolism, oxyindole acetic acid, formed from serotonin by the action of monoamino oxidase [1, 2].

It has also been suggested that serotonin is connected in some way with the secretion of insulin by the β-cells of the islets of Langerhans. Vermel' and Kacharova [6] have shown in experiments on rats with part of the pancreas removed that there is some correlation between the number of Kulchitsky cells in the duodenum and the activity of β-cells in the pancreatic islets. These authors put forward the view that Kulchitsky cells produce a hormone which stimulates β-cells in the islets of the pancreas.

It was thought important to determine the effect of serotonin on cell proliferation in the pancreas, an organ in which cell proliferation is not normally a very prominent feature. The injection of serotonin is known to upset the carbohydrate balance for several hours [4] and it was therefore decided to estimate hepatic and pancreatic glycogen as an indirect index of carbohydrate balance in addition to examining the effect of serotonin on mitotic activity in the pancreas in the course of physiological and posttraumatic regeneration.

METHOD

Fifty-nine female rats of no particular strain, weighing between 180 and 210 g, were used. The effect of serotonin on mitotic activity in the normal pancreas was examined in a first series of experiments: 5-oxytryptamine hydrochloride 10 mg/kg (the preparation was produced in the laboratory of this Institute) was injected intraperitoneally to 19 rats over a period of 19 days; 12 intact rats formed the control group.

The animals in the second series of experiments (28 rats) were subjected to partial pancreatectomy under ether anesthesia, the splenic part of the gland, representing about half the entire organ by weight, being removed; 14 of these animals were given serotonin injections similar to those given in the first series of experiments from the third postoperative day; the other 14 rats served as a control group.

Animals were killed (4 or 5 at each time) and the pancreatic glands fixed 3, 11 and 22 days after the resection, i.e. 6 h, 8 and 19 days respectively after the start of serotonin injections. Rats from the first series were also killed at the same times.

Sections of pancreas, fixed with formalin, were stained with Feulgen stain and with Heidenhain's azan stain. In the first series of experiments three rats were also killed three and six hours after the first injection of serotonin, together with equal numbers of control animals, for estimation of hepatic and pancreatic glycogen (by the Shabadash method).

Mitoses were counted in the nuclei of acinar cells, about 5000 cells being examined in each case. The counts were made in acini close to the resection wound in the experimental rats and in corresponding areas in the controls. About 25,000 or 26,000 cells were counted at each period. As there are considerable numbers of binucleate cells in

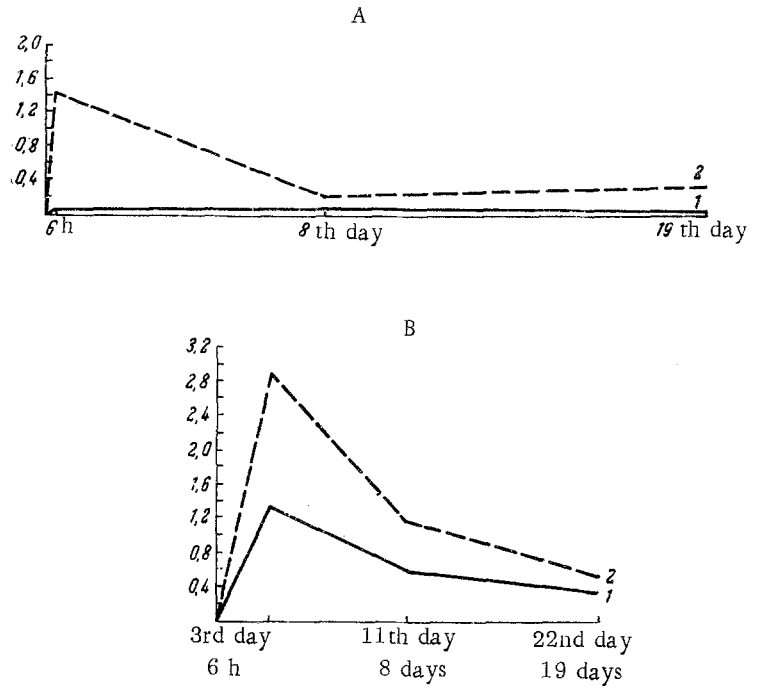

Fig. 1. Mitotic index for exocrine epithelium of rat pancreas. A (first series of experiments): Ordinate—average mitotic index (per 100 nuclei). Abscissa—time after first injection of serotonin. 1) Intact animals (controls). 2) Effect of serotonin on mitotic activity of exocrine epithelium in intact gland. B (second series of experiments): Abscissa—time after operation (above) and time after first injection of serotonin (below). 1) Reparative regeneration without serotonin. 2) Effect of serotonin on reparative regeneration.

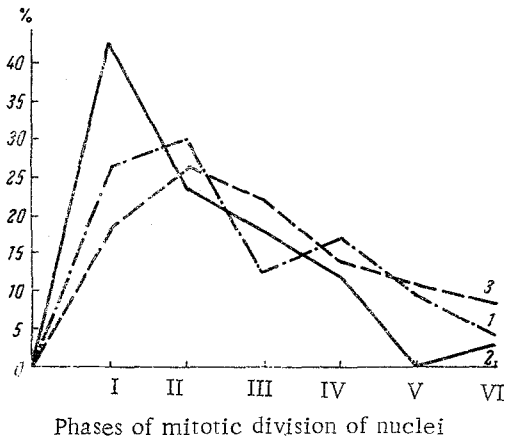

Fig. 2. Relative proportions (percentages) of different phase of mitosis in acinar cells of pancreas under various conditions. 1) Effect of serotonin on mitotic activity in course of physiological regeneration (6 h after first injection). 2) Mitotic activity in reparative regeneration (control, on third day after operation). 3) Effect of serotonin on reparative regeneration (third day after operation and 6 h after first injection). I) Early Prophase. II) Prophase. III) Metaphase. IV) Anaphase. V) Telophase. VI) Late Telophase.

the rat pancreas, the mitotic index employed was the number of mitoses per 1000 nuclei, not cells. Numerical data were processed (Student criterion).

RESULTS

Microscopic examination revealed no significant changes in the structure of the pancreas in normal rats as a result of the prolonged administration of serotonin.

Reparative regeneration proceeded in the same way when serotonin was being administered as in the controls [3].

The daily administration of serotonin produced considerable change in the mitotic activity of the acinar cells in the animals with intact pancreas (Fig. 1, A). Even six hours after the first secrotonin injection the mitotic index was 33 times greater than the index for the parenchyme of the pancreas in intact animals. The index had fallen considerably by the eighth day but it was again slightly higher on the nineteenth day of serotonin injections.

Serotonin also had a considerable effect on mitotic activity in the resected glands (Fig. 1, B). Three days after the operation (i.e. six hours after the first serotonin injection) the mitotic index for the acinar tissue was twice that found in the course of normal reparative regeneration. Thereafter

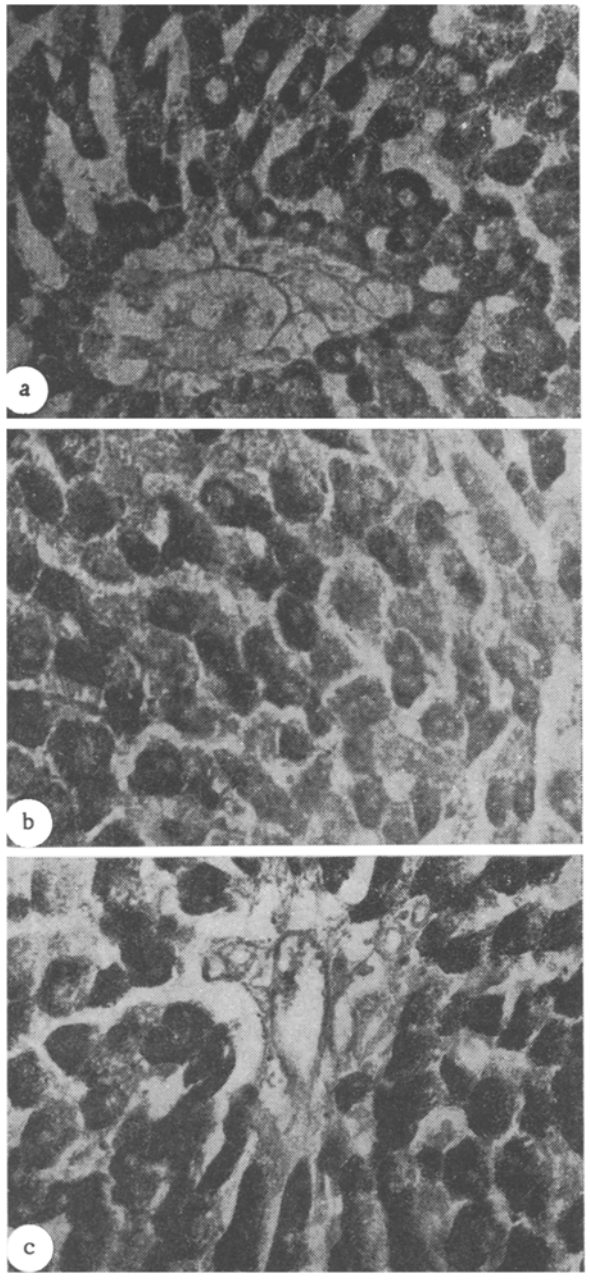

Fig. 3. Glycogen content of rat liver after injection of serotonin. a) Normal rat. b) 3 h after injection of serotonin; sharp reduction in quantity of glycogen in central part of lobule (right). c) almost complete restoration of glycogen content 6 h after injection of serotonin. Photomicrograph. Shabadash staining. x 400.

the mitotic indices for both groups of animals in the second series of experiments fell progressively to the end of the experiment, the decline being somewhat irregular up to and more even after the eleventh postoperative day (up to the twenty-second day). It will be seen from the graphs in Fig. 1, A and B, that mitotic activity in reparative regeneration without serotonin is almost the same as in physiological regeneration with daily injections of serotonin, as serotonin increased mitotic activity in the intact glands.

Fig. 1, B shows that the number of mitoses was greatest on the third postoperative day (i.e. six hours after the first injection of serotonin) in all cases. The relative proportions of different mitotic phases observed in the acinar cells of the pancreas were much the same for resected glands, fixed three days after operation, whether serotonin had been administered to the animals or not, and for intact glands fixed six hours after the first injection of serotonin (Fig. 2). In all cases the largest number of mitoses were in the first phases of division.

The difference between experimental and control values was found to be statistically significant (see Table 1).

Histochemical examinations showed that liver glycogen was much less three hours after the first serotonin injection than in controls (Fig. 3, a, b). The process of glycogen loss was more intense at the center than at the periphery of the hepatic lobules. The areas without glycogen were most extensive in the vicinity of large hepatic veins, probably because of more rapid removal of carbohydrates by the blood stream. The peripheral parts of the hepatic lobules stained a little less deeply than in the controls, however, even when the cytoplasm was filled with glycogen particles. This would indicate that the organ as a whole reacts to the injection of serotonin by loss of a considerable quantity of energy-producing material (glycogen). Although the original glycogen content of the cells was almost completely restored six hours after the first injection of serotonin, the parenchyma in the region of the central hepatic veins stained less intensely than controls, and some cells in the columns still contained no glycogen (Fig. 3, c).

The reduced glycogen content observed in the liver in the course of the first few hours after injection of serotonin in these experiments is in accord with observations [4] on adrenalectomized dogs in which the injection of serotonin was followed by first, transient hyperglycemia, and later, fairly persistent (for several hours after the injection) hypoglycemia in association with considerable reduction of glycogen content and increase of phosphorylase activity in liver and blood. Glycogen could not be demonstrated in the pancreas in either experimental or control material.

The results of experiments undertaken to determine the effect of serotonin on physiological and reparative regeneration in the pancreas and reparative regeneration in liver [2, 4] indicate that serotonin should be regarded as one of the important endogenous factors controlling cell proliferation in these organs.

Average Mitotic Index for Exocrine Epithelium of Pancreas in Physiological and Posttraumatic Regeneration and Changes Produced Therein by Serotonin

Index	Intact pancreas						Reparative regeneration					
	Time after first injection											
	6h		8 days		19 days		6 h		8 days		19 days	
	control	expt	control	expt	control	expt	control	expt	control	expt	control	expt
Average index (in %)	0,040	1,415	0,084	0,233	0,042	0,358	1,320	2,941	0,596	1,162	0,335	0,568
P . . .	—	0,0001	—	0,055	—	0,0001	—	0,0001	—	0,002	—	0,099

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