RHYTHMIC CHANGES IN PROTEIN SYNTHESIS INDUCED IN MOUSE PANCREATIC CELLS BY FOOD STIMULATION, ALLOXAN DIABETES, AND ISOPROTERENOL

R. Hamdan, E. A. Shubnikova, and L. S. Pogodina

UDC 591.437

KEY WORDS: pancreas; diabetes; isoproterenol; secretory cycle.

Rhythmic changes in the intensity of protein synthesis in different glands are well known [1]. Nevertheless, the effect of various physiological and pathological states, and also of certain chemical substances on the rhythm of protein synthesis in gland cells has hitherto received little study. The effect of isoproterenol (IPT) on the rhythm of protein synthesis in pancreatic cells, in particular, has not been investigated. Contradictory views have been expressed on the action of the β-adrenomimetic on pancreatic cell secretion. According to some workers [14] it inhibits the secretory process, whereas according to others [10, 13], it stimulates secretion. It has been shown that IPT increases the release of various pancreatic hormones, including insulin, into the bloodstream [11, 12]. Contradictory data also have been obtained on the effect of insulin insufficiency on the exocrine secretion of pancreatic cells. It is accepted that in diabetes mellitus, there is a deficiency of function [8]. We know [5] that the basal pancreatic secretion and cholecystokinin-stimulated amylase secretion are considerably depressed in diabetic rats [5]. Meanwhile, production of trypsinogen is distinctly increased. The inhibitory action of insulin on trypsinogen secretion also has been confirmed in normal animals. However, there is evidence that insulin potentiates zymogen secretion [15]. It has been shown that β-adrenergic sensitivity is reduced in diabetics, and for that reason they need a large dose of IPT [6].

The aim of this investigation was to study changes in the intensity of protein synthesis in mouse pancreatic cells during the secretory cycle and to compare these data with changes in size of the cells; to discover how alloxan diabetes affects the secretory rhythm and the intensity of protein synthesis in pancreatic cells, and to study the effect of a single injection of IPT into healthy and diabetic mice on the secretory rhythm and intensity of protein synthesis in their pancreatic cells.

EXPERIMENTAL METHOD

The secretory process was studied on pancreatic cells of CBA/C57BL mice. After 24 h of fasting the animals were fed a standard diet for 2-3 min every 3 h for 2.5 days. The animals were then killed by decapitation 10 min before the next meal was due, then at the time of the meal, and thereafter every 10 min for 3 h in the case of the control (intact) animals and for 1 h in the case of the experimental animals. Three mice were killed each time. Diabetes was induced by injection of alloxan (0.2 mg/g body weight). The presence of diabetes was established on the basis of the appearance of glucose in the urine by means of "Labstix" indicator strips. The animals were killed on the 5th day after injection of alloxan. IPT (0.16 mg/g) was injected in a single dose into both healthy and diabetic mice 1 h before the last meal. ^{3}H-Leucine (370 kBq/g body weight, specific activity 1900 TBq/mole) was injected 10 min before the routine time of sacrifice of the animals. Autoradiographs were prepared by the standard method. The mean intensity of the label per unit area (100 μ^2), with deduction of the background level, was determined in the pancreatic cells. At each time of the experiment 75-150 cells from three animals were counted. The results were subjected to statistical analysis.

Department of Cytology, Faculty of Biology, M. V. Lomonosov Moscow State University. (Presented by Academician of the Academy of Medical Sciences of the USSR N. K. Permyakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 2, pp. 142-144, February, 1991. Original article submitted December 30, 1989.

0007-4888/91/0002-0162$12.50

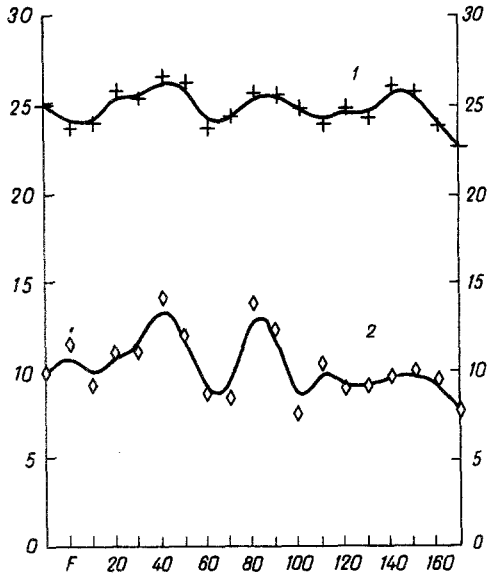

Fig. 1

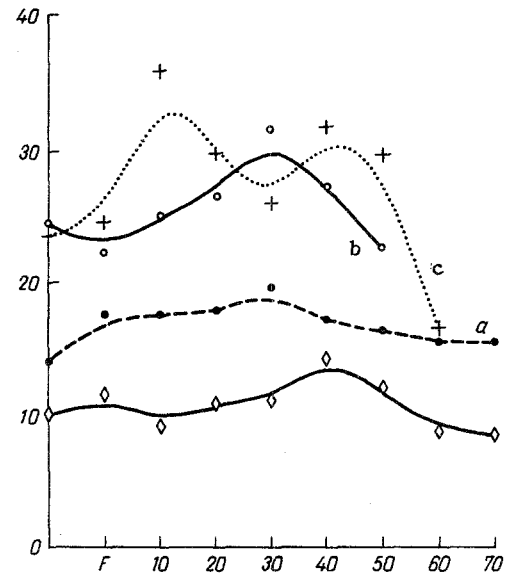

Fig. 2

Fig. 1. Incorporation of ^{3}H-leucine into pancreatic acinar cells and change in their area after food stimulation in normal mice. 1) Area of cell; 2) intensity of incorporation of ^{3}H-leucine in normal mice. Here and in Fig. 2: abscissa, time after feeding, min; ordinate, left — mean intensity of label per 100 μ^2 area of cell, right — mean area of cell (relative units). O) 10 min before feeding, F) feeding.

Fig. 2. Incorporation of ^{3}H-leucine into pancreatic acinar cells after food stimulation in normal mice receiving IPT (a), mice with alloxan diabetes (b), and mice with alloxan diabetes and receiving IPT (c).

EXPERIMENTAL RESULTS

The results showed (Fig. 1) that changes in the intensity of incorporation of ^{3}H-leucine took place in the control animals throughout the duration of the experiment. At the time of feeding, a very small increase was observed in uptake of the isotope, followed by a decrease 10 min after feeding, and then by a further increase in incorporation of the isotope, with a maximum after 40 min. Later the number of grains of silver per unit area decreased for 60-70 min after feeding. A second increase was observed 80 min, and a decrease 100 min after feeding. Later during the experiment the level of uptake of the isotope became more stable.

It was shown previously [4] that under similar experimental conditions the dimensions of the acinar cells (determined as the area of their cross section) in normal mice vary with a circumhoralian rhythm. Comparison of these data with the results of autoradiography showed that the dimensions of the acinar cells vary in phase with changes in their incorporation of ^{3}H-leucine. Only at the time of feeding did a decrease in area of the cells correspond to an increase in the intensity of labeling. Since an increase in size of the cells indirectly reflects the accumulation of secretion in them, whereas a decrease indicates extrusion, it can be postulated that at the time of feeding, previously synthesized (i.e., unlabeled) secretion is released, and protein synthesis is intensified. Extrusion is evidently determined by a reflex phase of gland function. The increase in the intensity of labeling 40-50 and 80-90 min after feeding is accompanied by an increase in size of the cells. Probably at this time regulation of secretion of the pancreas is mainly hormonal in nature (by gastrin or even by secretin), leading to intensification of protein synthesis and to the accumulation of labeled secretion in the cells. Our previous studies of ultrastructure of mouse pancreatic cells under similar experimental conditions [4] showed that during feeding and 40 and 90-100 min after feeding, i.e., at times of increased uptake of the isotope, the cisterns of the rough endoplasmic reticulum (RER) are considerably widened. This is in agreement with the view that widening of the RER in pancreatic cells characterizes the period of intensive protein synthesis [3]. Collapse of the cisterns

of RER 60 min after feeding is combined with a decrease in area of the cells and in the intensity of incorporation of ^{3}H-leucine. This characterizes the opposite state of the pancreatic cells, namely intensification of release of secretion and inhibition of protein synthesis.

Thus, the intensity of protein synthesis in intact mouse pancreatic cells undergoes circumhoralian fluctuations with peaks 40 and 80-90 min after food stimulation. This confirms data obtained on a culture of pancreatic cells [1] showing the existence of a circumhoralian rhythm in secretion of pancreatic cells.

In alloxan diabetes, injections of IPT and combinations of these procedures, this rhythmic activity of the cells is maintained. The general intensity of protein synthesis in this case was significantly greater than in the control (Fig. 2). In the course of 1 h, the maximum of incorporation of ^{3}H-leucine in animals receiving IPT and in mice with diabetes was observed 30 min after feeding, not 40 min thereafter as in the control. Under these circumstances, in mice receiving IPT, feeding caused increased uptake of the isotope, but a decrease in diabetic animals. When IPT was given to diabetic animals the first maximum of ^{3}H-leucine incorporation was observed earlier still, namely 10 min after feeding, whereas the second was observed 40 min after feeding. The general intensity of incorporation increased even more rapidly.

Under the influence of alloxan and IPT, separately and in combination, not only was the intensity of protein synthesis increased, but the duration of the secretory cycles was reduced, especially in the last case. These findings are contrary to the views expressed by Dandona et al. [8] on inadequate function of the exocrine part of the pancreas in diabetes, which these workers judged from a fall in the level of immunoreactive trypsin and of isoamylase activity in the blood serum of diabetic patients. However, the parameters they used only indirectly reflect the state of the secretory function of the pancreatic cells. In diabetes, lowering of the serum levels of pancreatic enzymes may perhaps be connected with a decrease in permeability of the cell membranes in insulin deficiency. The results obtained by these workers also contradict the results of a study of trypsinogen secretion [5].

Thus, we showed that a lesion of the endocrine part of the pancreas gives rise to reactive changes in its exocrine part, expressed as intensification of synthesis of cell proteins. During stimulation of β-adrenoreceptors by isoproterenol, protein synthesis in pancreatic cells also is activated.

LITERATURE CITED

1. V. Ya. Brodskii and N. V. Nechaeva, The Rhythm of Protein Synthesis [in Russian], Moscow (1988).
2. T. M. Inauri, Soobshch. Akad. Nauk Gruz. SSR, **121**, 617 (1986).
3. N. K. Permyakov, A. E. Podol'skii, and G. P. Titova, Ultrastructural Analysis of the Secretory Cycle of the Pancreas [in Russian], Moscow (1974).
4. R. Hamdan, Proceedings of the 19th Scientific Conf. of Junior Scientists of the Biological Faculty of Moscow Univ. [in Russian], Moscow (1988). Abstract Lodged with the All-Union Institute of Scientific and Technical Information, August 24, 1988, No. 6712, Bundle 88, p. 95.
5. A. Sofrankova, Acta Fac. Med. Univ. Brun, **92**, 15 (1985).
6. J. Berlin, A. Grimaldi, F. Bosquet, and A. Puech, J. Clin. Endocrinol. Metab., **63**, 262 (1986).
7. G. Boden, R. W. Master, M. A. Sattler, et al., Endocrinology, **111**, 1166 (1982).
8. P. Dandona, D. B. Freedman, Y. Foo, et al., J. Clin. Pathol., **37**, 304 (1984).
9. O. Flaten, T. Sand, and J. Myren, Scand. J. Gastroent., **17**, 283 (1982).
10. E. Heidbreder, P. Sieber, and A. Heidland, Res. Exp. Med., **171**, 79 (1977).
11. J. P. Palmer, J. Halter, and P. L. Werner, Metabolism, **28**, 237 (1979).
12. S. Rousseau-Migneron and T. Nadean, Can. J. Physiol. Pharmacol., **58**, 275 (1980).
13. C. Rose and J. de la Tour, Digestion, **11**, 458 (1974).
14. J. Rudick, Surg. Clin. N. Am., **61**, 47 (1918).
15. J. A. Williams and J. D. Goldfine, Diabetes, **34**, 980 (1985).