Pituitary growth hormone secretory granules in streptozotocin-induced diabetic rats

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Summary. The ultrastructural features of growth hormone (GH) secretory granules were observed by immuno-gold procedure in the anterior pituitary glands of streptozotocin (STZ) - induced diabetic rats. The diabetic state of rats induced by one injection of STZ was severely advanced at 10 months after the injection. The cytoplasmic organella were revealed to be poorly developed in GH-cells of the diabetic rats. The size of immunoreactive GH-granules of 10-months-old diabetic rats was smaller than that of sham-controls. The insulin therapy in the diabetic rats resulted in a considerable improvement in relation to the changes seen in the GHcells, but the therapy did not return the profile of the granule size distribution to that of the control group.

Key words: GH-cells, Anterior pituitary, Diabetes, Streptozotocin, Rat

Introduction

Not much information is available on the morphological changes in pituitary cells in diabetic animals. Deslex et al. (1976) described an increased number of somatotrophs in the pituitary gland of spontaneous diabetic Chinese hamsters. Yamada et al. (1967) stated that the acidophil cells of diabetic KK mice, when observed with the light microscope, tended to be hypertrophied and that the proportion of these cells was significantly lower than in normal C 57BL/6 mice. In electron microscopic observations, the secretory granules of GH-cells in diabetic KK mice exhibited a striking abundance, but the endoplasmic reticulum (ER) was far less in amount than in GH-cells of normal mice. However, it is still not clear whether pituitary GH-cells are altered into hypertrophic or hypotrophic states in diabetes.

Offprint requests to: Dr. Masataka Shiino, Department of Anatomy, Wakayama Medical College, 9-Kyubancho, Wakayama-shi, 640 Japan In the present study, we examined morphological alterations of GH-cells in the anterior pituitary gland of diabetic rats induced by the injection of STZ.

Materials and methods

Twenty female rats (Wistar strain) with body weights of about 70 g at 30 days old were given 80 mg/ kg of STZ following starvation for 12 h. The STZ was dissolved in 0.1 ml of 0.9% saline solution buffered to pH 4.5 with 10 mM citrate and injected into the tail vein. The STZ-injected rats were divided into 2 groups. Group 1 received subcutaneous injections of buffered saline solution twice a day for one month after the injection of STZ; group 2 received 2U monotard insulin (NOVO, Lente, Copenhagen) at 6:00 and 4U 18:00 hours in the same manner described in group 1. Ten age-matched female rats were used as sham-control animals and received vehicle injections only, and two uninjected-controls were used for comparison of ultrastructural observations. All animals were kept under controlled illumination (LD, 14:10) and given laboratory food and tap water ad libitum. Urinary glucose of experimental animals was measured by using Test-Tap (Elli-Lilly) and values of 1⁺ or higher were classified as diabetic. After weighing, the animals were sacrificed by decapitation during light anaesthesia with ether at 11 months old, and then blood glucose levels were estimated using a Dextrometer (Ames).

For light microscopic immunocytochemistry, some pieces of anterior pituitary glands were cut into small pieces and fixed in 2% acrolein-0.25% glutaraldehyde dissolved in 0.1 M cacodylate buffer (pH 7.4) for 2.5 h, postfixed in 1% osmium tetroxide in the same buffer for 1.5 h, and embedded in Epon-Araldite after routine dehydration, as described by Smith and Keefer (1982). The sections were reacted by the Biotin-Strept Avidin (BSA) method using an immunostaining Kit (Stravigen) obtained from BioGenex Laboratories, California, U.S.A. Other pieces of tissue were fixed in a mixture of 2% paraformaldehyde and 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 h, and postfixed in 1% osmium tetroxide in the same buffer. After routine procedure of dehydration, the tissues were embedded in Spurr's plastic mixture and thin sections were reacted by the protein A-gold procedure as described by Bendayan and Zollinger (1983). Protein A-gold complex was obtained from Janssen Products, Belgium. Primary antiserum to rat GH was diluted to 1:1000 for use. Control immunocytochemical tests were carried out by substituting normal rabbit serum or PBS for the specific antiserum.

To compare the size of secretory granules in the GHcells of each group, 80-120 GH-cells were selected at random from tissue sections of each group. The diameters (the long-axis) of all secretory granules in each cell were measured on the photographs.

Results

In the present study, the data of uninjected control rats were omitted since we could not recognize any differences between sham-control (only vehicleinjected) and uninjected-control groups in the experimental results. One injection of STZ 30-day-old rats (Wistar strain) induced «hyperglycemia»-diabetes in them. The diabetic state of rats, induced by the injection of STZ-injection. Some animals suffered from cataracts. As shown in Fig. 1, the body weights of diabetic rats were lower than those of the sham-control group (P < 0.001), but the insulin-therapy considerably increased the body weights of these animals as compared with those of untreated diabetic rats (P < 0.001). The serum glucose levels of diabetic rats were remarkably higher than those of controls but the injection of insulin into these diabetics significantly reduced the plasma rats glucose concentration as illustrated in Fig. 2. Although the size of immunoreactive GH-cells of diabetic rats was smaller than that of sham-controls, the insulin-therapy considerably increased the cell-size to near normal in many animals, as shown in Fig. 3. As shown in Fig. 4, we could see various sizes of secretory granules, but these granules may be divided roughly into three types, e.g., approximately 300, 200, or 100 nm in diameter respectively, with a rather uniform distribution of different sized granules in GH-cells of sham-control rats. However, most of the GH-cells of diabetic rats contained an uneven distribution of small secretory granules. Some GH-cells contained a much larger proportion of miniaturized secretory granules (50-100 nm, often) as shown in Fig. 5A. Others contained a reduced number of small secretory granules ranging from 100-150 nm (Fig. 5B). Unlike GH-cells of controls, most of the GH-cells of diabetic rats revealed poorly developed cytoplasmic organelles and granule-extrusion was completely prevented. The insulin-therapy considerably increased the number of large secretory granules in the GH-cells, and prompted the development of the Golgi apparatus and ER in many GH-cells (Fig. 6). Nevertheless, the



Fig. 1. Body weights of experimental animals. The bars indicate standard errors. Insulin: Insulin-treated.



Fig. 2. Serum glucose concentration of experimental animals. The bars indicate standard errors. Insulin: Insulin-treated.



recovery toward normal morphology in the GH-cells was never complete. Judging from the histograms of Fig. 7, the GH-cells of diabetic rats contained an increased proportion of small secretory granules (mostly, 100-200 nm) and the insulin-therapy induced an increase in the distribution of larger sizes of secretory granules. However, the insulin-therapy did not return the profile of the granule size distribution to that of the control group.

Discussion

There are conflicting results in previous reports concerning growth hormone (GH) secretion in diabetic subjects, e.g., some investigators demonstrated that plasma GH levels were enhanced in juvenile diabetes (Johansen and Hansen, 1969; Hansen and Johansen, 1970) and others could not demonstrate such enhancement of GH-secretion (Parker and Rossman, 1971; Garlaschi et al., 1970). Blue-Pajot et al. (1983) and González and Johin (1985) demonstrated a remarkable reduction of serum and pituitary GH in diabetic rats induced by the injection of STZ.

Above all, González and Johin (1985) reported that pituitaries from diabetic rats incorporated (³H)-leucine into GH at a greatly reduced rate, indicating a diminished GH storage in the pituitary gland of diabetic rats. In our study, we observed in the anterior pituitary gland of STZ-induced diabetic rats a reduction of both in the size of the GH-cells and of their secretory granules. The observation that secretory granules of GH-cells were generally of smaller size and that small granules were distributed throughout the cytoplasm, and also that they showed no exocytosis in STZ-induced diabetes may suggest that newly formed secretory granules were inhibited in their growth and in their release from the cell. Also, the fact that many GH-immunoreactive cells contained scanty numbers of secretory granules in 11month-old diabetic rats may indicate that the deficiency of insulin diminished the secretory of hypothalamic hormones as described by Debons et al. (1970) and Mitsuma and Nogimori (1982). While Sönsken (1975) stated that insulin might directly stimulate the metabolism of anterior pituitary cells only in diabetes, his results suggest that the deficiency of insulin could disturb the metabolism of the gland, followed by disturbance of hormone-synthesis and release. In similar observations, Drummond (1975) reported that a lowprotein diet reduced the amount of GH in the rat pituitary in a way similar to that of a protein-free diet. This result is definitely due to the disturbance of proteinous hormone synthesis which occurs with proteininsufficiency.

Previously, we observed a remarkable reduction of size and number of secretory granules in prolactin secreting cells in STZ-induced diabetic rats (Yamauchi

Fig. 3. Immunoreactive GH-cells in anterior pituitary gland. A: GHcells in sham-control rat. B: GH-cells in untreated diabetic rat. C: GHcells of diabetic rat treated with insulin. × 400



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Fig. 4. An example of an immuno-gold reactive GH-cells in control rat. One can see various size of secretory granules. \times 8,000. Insert shows immuno-gold particles on GH secretory granules. \times 25,000

Fig. 5. Immuno-gold reactive GH-cells in diabetic rats. **A:** An example of GH-cell in which small secretory granules are distributed excentrically in the mass \times 8,000. Insert is higher magnification of enclosed area of GH-cell. \times 25,000. **B:** An Example of GH-cell in which middle-sized secretory granules are located in one side of cytoplasm. \times 8,000. Insert shows higher magnification of enclosed area. One can see clear distribution of immuno-gold particles on GH-secretory granules. \times 25,000



Fig. 6. An example of immuno-gold reactive GH-cell of diabetic rat treated with insulin. Note an appearance of large-sized secretory granules and well-developed Golgi zone. × 25,000



and Shiino, 1986). We assumed that this reduction might be due to the disturbance of hormone-synthesis in prolactin cells as well as in the case of GH-cells. Reversely, however, Yamada et al. (1967) reported that strikingly increased secretory granules in the α -cells of hereditary diabetic KK mice clearly indicate an accumulation of the hormone in the cytoplasm. However, it is generally known that these hereditary diabetic rodents are characterized by showing spontaneous hyperglycemia with hyper-insulinemia and obesity (Hunt et al., 1976), whereas, our diabetic rats induced by the injection of STZ are hyperglycemic, insulin-deficient and lean (Tancrede et al., 1983). Consequently, one cannot compare directly the present results with the results reported by Yamada et al. Generally, it is known that insulin and glucose play a significant role directly in the modulation of GHsecretion (Goodman and Freinkel, 1961). Our results show that insulin-treatment in the diabetic rats results in a considerable improvement in relation to the changes seen in the GH-cells.

Kurosumi et al. (1986) have described three sub-types of GH-cells in the rat anterior pituitary glands owing to differences in the size of their secretory granules. Most of the GH-cells observed in our diabetic rats seemed to correspond to their type III GH-cell, but it is clear that the distribution of these tiny secretory granules in GHcells of the STZ-induced diabetic rats was induced by metabolic disturbance due to insulin deficiency. Consequently, it was not possible to directly apply the sub-classification scheme described by Kurosumi et al. to the GH-cells.

Acknowledgements. The authors wish to express their thanks to Dr. Edward G. Rennels for his kind advice in preparing the manuscript, and to Dr. Katsumi Wakabayashi, Gunma Institute of Endocrinology, Gunma, Japan for his generous gift of antiserum to rat GH. The authors also wish to express their thanks to Miss Kyoko Yamada for her technical assistance. This study was supported by Grant-in-Aid for General Scientific Research from the Ministry of Education, Science and Culture, Japan No. 62570016.

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Accepted January 29, 1988