Effects of streptozotocin-induced experimental diabetes on the morphology and function of the zona fasciculata of rat adrenal cortex

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Summary. The effects of a severe streptozotocin (STZ)-induced diabetes on the morphology and function of the adrenal zona fasciculata were examined in rats with intact or pharmacologically interrupted hypothalamic-hypophyseal-adrenal axis. In animals with an intact hypothalamic-hypophyseal axis, STZ-diabetes induced hypertrophy of the cells of the zona fasciculata and a rise in the plasma corticosterone concentration. Conversely, in rats in which the hypothalamic-hypophyseal axis had been interrupted, experimental diabetes provoked atrophy of the zona fasciculata cells, and a lowering in the plasma corticosterone level. The effects of STZ-diabetes were completely reversed by insulin infusion in both groups of rats. The hypothesis is discussed that the chronic lack of insulin may directly inhibit the growth and steroidogenic capacity of the rat zona fasciculata and that this effect of experimental diabetes may be masked in rats with an intact hypothalamic-hypophyseal axis by the concurrent enhancement of ACTH release due to chronic stress resulting from the metabolic consequences of prolonged diabetes.

Key words: Experimental diabetes – Streptozotocin – Adrenal cortex – Rat – Morphometry

Introduction

Much evidence indicates the existence of a functional interrelationship between insulin and the adrenal cortex. Chronic experimental diabetes has been shown to induce adrenal hypertrophy (Deverskis and Frawley 1963; Rakoto-Ratsimamanga et al. 1969; Kraus 1973; De Nicola et al. 1976, 1977), coupled with a significant rise in the blood (Kraus 1973; De Nicola et al. 1976, 1977) and intra-adrenal levels of corticosterone (Tornello et al. 1981). Morphological studies of the adrenal cortex in diabetic rats are very sparse (Holmberg 1974; Wexler and Lutmer 1975), and none of them employed morphometry. It seemed worthwhile, therefore, to study the effect of streptozotocin (STZ)-induced diabetes on the function and morphology of the rat adrenal zona fasciculata by combined stereological and biochemical techniques.

Materials and methods

Animal treatment

Young male Wistar rats (140–160 g body weight) were employed. They were maintained on purina rat-mouse chow (Zoofarm, Padua, Italy) and tap water ad libitum. All the animals were killed by decapitation between 10:00 and 11:00 a.m. and their trunk blood was collected and frozen.

Experiment I. Twenty-four rats were divided into three equal groups. Two groups were given a single i.p. injection of STZ (60 mg/kg, dissolved in 0.5 ml pH 4 citrate buffer; Sigma, St. Louis, Missouri) (Funakawa et al. 1983), and were infused subcutaneously for 2 weeks (Alzet osmotic pumps Mod. 2002; Alza, Palo Alto, California) with insulin (1 U/kg/day; human, biosynthetic; Sigma) (Karteszi et al. 1982) or saline. The third group served as a controls. These rats received an i.p. injection of 0.5 ml citrate buffer and were infused with saline for 2 weeks.

Experiment II. Twenty-four rats were divided into three equal groups and were infused subcutaneously for 2 weeks (Alzet pumps) with dexamethasone (10 μg/kg/h; Decadron, Merck, Milan, Italy) and ACTH (0.1 IU/kg/h; Sigma), in order to interrupt the hypothalamic-hypophyseal-adrenal axis (Robba et al. 1987). Routine RIA showed that this dose of dexamethasone induced an 80% decrease in the level of circulating ACTH (35.8 ± 7.1 against 7.2 ± 3.2 pg/ml), coupled with an 85% decrease in the plasma corticosterone concentration (8.2 ± 2.4 against 1.2 ± 0.3 μg/dl). ACTH infusion completely reversed these effects of dexamethasone (ACTH: 33.7 ± 6.8 pg/ml; corticosterone 7.8 ± 2.3 μg/dl). Two groups of rats were treated with STZ on the first day of infusion and one of these groups had insulin in the infusion vehicle. The doses of STZ and insulin

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were those employed in Experiment I. The third group of animals served as a controls and these were given an i.p. injection of 0.5 ml citrate buffer on the first day of infusion.

**Biochemical dosages**

The plasma glucose concentration was determined by colorimetry (Diagnostic Kit No 115; Sigma). Corticosterone was extracted from plasma, purified (Sippell et al. 1978) and its concentration was measured by competitive protein binding (Spät and Jozan 1972).

**Light and electron microscopy**

The adrenal glands were promptly removed, freed of adherent fat and weighed. The left adrenals were fixed in Bouin’s solution, embedded in paraffin and serially cut at 6–7 μm. Sliced fragments of the right adrenals were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer, and embedded in an epoxy resin. Thick and thin sections were cut with LKB III ultramicrotomes at the level of the zona fasciculata. Thin sections were counterstained with lead hydroxide and examined in a Hitachi H-300 electron microscope.

**Morphometry**

**Stage I: volume of zona fasciculata.** Using a magnification of 100 X and a square lattice test system of type A (Weibel 1979), the volume density of the zona fasciculata related to the entire gland was evaluated. The measurement was performed on every fifth paraffin section of the gland. The volume of the adrenal gland was calculated from its weight, by assuming an average specific gravity of 1.039 (Swinyard 1938).

**Stage II: size and number of zona fasciculata cells.** The volume densities (VvZF) of the nuclei and cytoplasm of parenchymal cells and stroma of the zona fasciculata, as well as the numerical density (NaZF) of the nuclear profiles, were estimated on a screen at 3000 X, using the multipurpose test system M42 (Weibel 1979). VvZF and NaZF were expressed as mm³ or number per mm³ of zona fasciculata. Holme’s correction for section thickness (Weibel 1979) was not performed. For each adrenal gland, a single paraffin section containing zona medullaris was selected and in this section, 30 test areas of the zona fasciculata were counted. The number of nuclei of parenchymal cells per mm³ of the zona fasciculata (NVZF) was estimated according to the formula of Weibel and Gomez (Weibel 1979):

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NVZF = \frac{K}{B} \times \frac{NaZF^2}{VvZF}. \]

The shape coefficient B, which depends on the axial ratio of estimated nuclear profiles, was calculated from the curve for ellipsoids of Weibel (1979) and found to be about 1.350. The size distribution coefficient K was assumed to be 1 (Weibel 1979). Since rat adrenocortical cells are mononucleated, Nv of nuclei corresponds to the number of cells per mm³ of the zona fasciculata. Subsequently, the average volume and the number of zona fasciculata cells were computed.

**Stage III: organelles of zona fasciculata cells.** On electron micrographs at a final magnification of 21000, the volume density (μm³/μm³ of zona fasciculata cell) of mitochondria and lipid droplets were estimated, using a square lattice test system of type A (Weibel 1979). On electron micrographs at a final magnification of 42000, the surface densities (μm²/μm³ of zona fasciculata cell) of mitochondrial cristae and smooth endoplasmic reticulum (SER) tubules were evaluated (Loud 1962). Section

**Statistical analysis**

The data obtained from each rat were averaged per experimental group and the standard deviation of the mean (SD) was calculated. As revealed by the χ² test, the data were not different from the normal distribution (P < 0.05). After testing the equality of variances, the statistical comparison of the data was performed by the multiple range test of Duncan (Bliss 1967).

**Results**

**Experiment I**

A six-fold rise in plasma glucose levels was found 15 days after STZ administration. Basal plasma corticosterone levels displayed a two-fold rise (Table 1). STZ caused a notable reduction in the body weight of rats (−21%), and a significant increase in both the absolute (27%) and relative adrenal weights (60%) (Table 2).

STZ provoked a striking increase in the volume of zona fasciculata (51%) and its parenchymal cells (24%) and nuclei (23%). The number of parenchymal cells in the zona fasciculata underwent a small but significant increase (17%) (Table 3). No striking qualitative ultrastructural alterations were noted in the zona fasciculata of STZ-treated rats, with the exception of an evident decrease in the number of lipid droplets (Figs. 1 and 2). However, stereology showed that STZ-induced cell hypertrophy was associated with significant increases in the volume of mitochondrial compartment (28%), and in the surface area per cell of mitochondrial cristae (28%) and SER profiles (37%). The volume of the lipid-droplet compartment displayed a notable drop (−40%) (Table 4).

Insulin infusion completely reversed all the STZ-induced changes (Tables 1–4).

**Experiment II**

Plasma glucose levels displayed a six-fold increase and basal plasma corticosterone concentrations a 23% decrease (Table 5). Body and adrenal weights underwent a notable reduction (−26%), while relative adrenal weights remained unchanged (Table 6).

The volume of zona fasciculata and its parenchymal cell and nuclei showed conspicuous de-