EFFECT OF STREPTOZOTOCIN-INDUCED EXPERIMENTAL DIABETES ON THE STEROIDOGENIC CAPACITY OF RAT ADRENAL ZONA FASCICULATA

GIUSEPPINA MAZZOCCHI¹, LUDWIK K. MALENDOWICZ², LETIZIA CAVALLINI¹, VIRGILIO MENEGHELLI¹ and GASTONE G. NUSSDORFER¹

¹Department of Anatomy, University of Padua, 35121 Padua, Italy, and ²Department of Histology and Embryology, Academy of Medicine, Poznan, Poland

ABSTRACT

Streptozotocin-induced experimental diabetes significantly lowered both basal and ACTH-stimulated corticosterone production in rats, whose hypothalamo-hypophyseal-adrenal axis had been pharmacologically interrupted. Basal and ACTH-stimulated activity of two key enzymes of steroid synthesis (Δ^5 -3 β -hydroxysteroid dehydrogenase and 11 β -hydroxylase) were also notably reduced in the zona fasciculata of diabetic rats. Insulin infusion completely reversed all these streptozotocin-induced effects. The conclusion is drawn that the chronic lowering in the level of circulating insulin impairs the steroidogenic capacity of rat zona fasciculata.

Evidence is available that chronic experimental diabetes stimulates the secretory activity of the inner adrenal layers in rats (2, 3, 9, 19). This effect was ascribed to the activation of the hypothalamo-hypophyseal-adrenal axis due to the chronic stress resulting from the metabolic consequences of the prolonged diabetic status (2, 3).

It therefore seemed worthwhile to investigate the effect of streptozotocin (STZ)-induced diabetes on the secretory activity of adrenal zona fasciculata of rats, whose hypothalamo-hypophyseal-adrenal axis had been pharmacologically interrupted.

MATERIALS AND METHODS

Young male Wistar rats (160-180 g body weight) were subcutaneously infused for two weeks (Alzet pumps Model 2002; Alza, Palo Alto, CA, U.S.A.) with dexamethasone ($10 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$; Decadron, Merck, Milan, Italy) and ACTH ($0.1 \text{ IU} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$; Sigma, St.

Louis, MO, U.S.A.), in order to interrupt their hypothalamo-hypophyseal-adrenal axes (12). Fig. 1 shows that basal and ACTH-stimulated ($5 \text{ IU} \cdot \text{kg}^{-1}$ ACTH, 30 min before the sacrifice) plasma concentrations of corticosterone of these rats did not differ significantly from those of intact animals.

The rats were divided into three groups. One group was given a single intraperitoneal injection of STZ ($60 \text{ mg} \cdot \text{kg}^{-1}$, dissolved in 0.5 ml of citrate buffer, pH 4; Sigma) (4), on the day of beginning of the dexamethasone/ACTH infusion. The second group was treated with STZ and had in the infusion vehicle insulin ($0.04 \text{ U} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$; human, biosynthetic; Sigma) (8). The third group served as a control and received an intraperitoneal injection of citrate buffer (0.5 ml). Each experimental group was divided into two subgroups, which were given an intraperitoneal injection of ACTH ($5 \text{ IU} \cdot \text{kg}^{-1}$) or 0.9% NaCl (0.5 ml), 30 min before the sacrifice.

All the rats were killed by decapitation

Fig. 1 Basal (A) and ACTH-stimulated (B) plasma concentration of corticosterone in intact (void bars) and dexamethasone/ACTH-treated (dotted bars) rats. SD are indicated.

between 1000 and 1030, and their trunk blood was collected. Plasma glucose concentration was determined by colorimetry (Diagnostic kit No. 115; Sigma). Corticosterone was extracted from plasma and purified (15), and its concentration was measured by competitive protein binding (16), using mouse serum as a source of binding protein.

The adrenal glands of each rat were promptly removed, decapsulated (to eliminate zona glomerulosa) and then bisected and enucleated (to remove the zonae medullaris and reticularis) under a dissecting microscope. Adrenal halves were homogenized in buffered 0.25 M sucrose. Δ^5 -3 β -Hydroxysteroid dehydrogenase (3 β HSD) activity was assayed on the whole homogenate by measuring the rate of conversion of exogenous pregnenolone (Sigma) to progesterone (13). Newly formed progesterone was estimated by radioimmunoassay (Diagnostic Products, Los Angeles, CA, U.S.A.). 11 β -Hydroxylase (11 β OH) activity was evaluated on a mitochondrial fraction obtained by differential centrifugation at 10,000 g for 15 min, by measuring the rate of conversion of exogenous 11-deoxycorticosterone (Sigma) to corticosterone (5). Details of these enzymatic assays were given in our previous paper (11).

The data obtained from each rat were averaged per experimental subgroup and SD was

calculated. As revealed by the χ^2 test, the data were not different from normal distribution ($P < 0.05$). After testing the equality of variances, the statistical comparison of the means was performed by the multiple range test of Duncan. A P value < 0.05 was considered statistically significant.

RESULTS

A six-fold rise in blood glucose (81.4 ± 14.7 vs. 492.7 ± 101.2 mg/dl; $P < 0.001$) and a significant loss in the body weight (230 ± 29 vs. 188 ± 22 ; $P < 0.01$) were found in STZ-treated rats, and were annulled by insulin administration.

Basal plasma corticosterone concentration displayed a 24% decrease (Table 1), and basal activities of 3 β HSD and 11 β OH were significantly reduced (−18% and −15%, respectively) (Table 2) in diabetic rats. Plasma corticosterone response to ACTH evidenced a significant lowering (−52%; $P < 0.01$) (Table 1) in STZ-treated rats, and the same occurred for the activities of 3 β HSD and 11 β OH (decreases of −32% and −30%, respectively; $P < 0.01$) (Table 2).

Insulin infusion completely reversed all the STZ-induced changes (Tables 1 and 2).

DISCUSSION

A single administration of STZ, a drug which is specifically toxic for β -cells of the pancreatic islets (7), causes a severe diabetes mellitus in young male rats, as evidenced by the loss in the body weight and hyperglycemia.

Our data indicate that prolonged experimental diabetes provokes a notable impairment of the secretion of corticosterone, the main glucocorticoid produced in *Rodentia* (14), in rats whose hypothalamo-hypophyseal-adrenal axis had been pharmacologically interrupted by the simultaneous administration of dexamethasone and maintenance doses of ACTH. Conceivably, the lowering in basal and maximally stimulated production of corticosterone is due to the reduction in the activity of 3 β HSD and 11 β OH, two key enzymes of steroid synthesis (10) in the zona fasciculata, which is the main site of glucocorticoid production (18).

These effects of experimental diabetes are

Table 1 *Effect of Experimental Diabetes and Insulin Infusion on the Corticosterone Response to an Acute Stimulation with ACTH*

Experimental groups (n=16)	Plasma corticosterone concentration ($\mu\text{g/dl}$)	
	None (n=8)	ACTH (n=8)
Control rats	7.6 $\pm$ 1.6	38.9 $\pm$ 8.4 ^a (411 ^d)
Streptozotocin-treated rats	5.8 $\pm$ 1.4 ^c	17.2 $\pm$ 4.9 ^{a,c} (196 ^d)
Streptozotocin-treated rats, infused with insulin	8.1 $\pm$ 2.2	41.5 $\pm$ 8.7 ^a (412 ^d)

^a $P < 0.01$ from the 'none' subgroup; ^c $P < 0.01$ from the respective 'control' subgroup; ^dpercent increase after ACTH

Table 2 *Effect of Experimental Diabetes and Insulin Infusion on Two Adrenal Enzymes of Steroid Synthesis*

Experimental groups (n=16)	Δ^5 -3 β -Hydroxysteroid dehydrogenase activity ($\text{pg} \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$)		11 β -Hydroxylase activity ($\text{pg} \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$)	
	None (n=8)	ACTH (n=8)	None (n=8)	ACTH (n=8)
Control rats	26.9 $\pm$ 6.4	68.1 $\pm$ 16.3 ^a (153 ^d)	69.5 $\pm$ 13.7	219.6 $\pm$ 52.7 ^a (216 ^d)
Streptozotocin-treated rats	22.1 $\pm$ 5.2 ^b	37.7 $\pm$ 9.4 ^{a,c} (71 ^d)	59.3 $\pm$ 10.3 ^b	130.9 $\pm$ 28.8 ^{a,c} (121 ^d)
Streptozotocin-treated rats, infused with insulin	28.3 $\pm$ 6.2	63.5 $\pm$ 14.9 ^a (124 ^d)	65.4 $\pm$ 12.2	215.2 $\pm$ 54.1 ^a (229 ^d)

^a $P < 0.01$ from the 'none' subgroup; ^b $P < 0.05$ and ^c $P < 0.01$ from the respective 'control' subgroup; ^dpercent increase after ACTH

completely annulled by insulin infusion, so that they can be reasonably ascribed to the chronic lack of this hormone and not to a direct toxic effect of STZ on the zona fasciculata.

A direct positive effect of insulin on the growth of many cell types was observed *in vitro* (17). Therefore, it is possible to suppose that the lowering in the level of circulating insulin may directly depress the *de novo* synthesis of the enzymes of steroid synthesis and, consequently, the steroidogenic capacity of rat zona fasciculata cells. This contention accords well with the autoradiographic demonstration of the presence of specific receptors for insulin in adrenocortical cells (1), as well as with the finding that insulin stimulates steroid release by bovine adrenocortical cells cultured *in vitro* (6).

The physiological meaning of this adreno-

corticotrophic action of insulin requires further investigation, inasmuch as, in rats with intact hypothalamo-hypophyseal axes, the adrenal effects of the lack of this hormone were reported to be completely reversed by the diabetes-induced chronic enhancement of ACTH release (2, 3).

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