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Effect of Streptozotocin-Induced Diabetes on Insulin Binding Parameters in Adult Rat Testis

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Summary: Insulin binding parameters have been measured in testicular membranes of streptozotocin diabetic male rats. Insulin binding decrease was ascribed to the well-known depressing effect of diabetes mellitus on circulating luteinizing hormone (LH). Because both LH and insulin receptors are modulated by pituitary LH and because of their reduction in testes of diabetic rats, we conclude that Leydig cell dysfunction is a secondary disorder associated with this complex metabolic condition.

Der Effekt eines durch Streptozotocin induzierten Diabetes mellitus auf die Insulinbindungskapazität beim ausgewachsenen Rattenhoden

Zusammenfassung: An den testikulären Membranen von männlichen Ratten wurden Insulinbindungsparameter untersucht, die mittels Streptozotocin in eine diabetische Stoffwechsellage gebracht worden waren. Die Verminderung der Insulinbindungskapazität wurde dem negativen Effekt des zirkulierenden LH auf den Diabetes mellitus zugeschrieben. Sowohl die LH- als auch die Insulinrezeptoren werden durch hypophysäres LH moduliert. Aus der Verringerung dieser Rezeptoren im Hoden der diabetischen Ratte wurde geschlossen, daß die Leydigzell-Insuffizienz ein sekundäres Phänomen dieser komplexen Stoffwechselstörung ist.

Introduction

Since preliminary report of Hunt and Bailey in 1961 with regard to the deleterious effects of diabetes on the reproductive system of young male rats, several works have described abnormalities at the pituitary and gonadal level. Paz et al. (1978) have explained the reduced fertility of rats by a partial block of secretion of luteinizing hormone (LH) or LH-releasing hormone (LH-RH) and found that accessory gland atrophy was much improved following treatment with insulin and human chorionic gonadotropin (HCG) instead of insulin alone. All along the years, convincing evidences have been gathered to firmly demonstrate that serum levels of LH were significantly lower in diabetic than in control animals (Howland and Zebrowski - 1976; Paz et al. - 1979; Jackson and Hutson - 1984) and that the decreased sex accessory organ weight was secondary to a lower production

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Table 1
Effect of streptozotocin-induced diabetes on body weight, liver and reproductive organ weights (grams ± standard error of the mean (S.E.M.)

Treatment	Body Weight	Liver	Testis	Epididymes
Control	353 ± 8.8	13.8 ± 0.7	2.9 ± 0.1	0.53 ± 0.03
Streptozotocin	281 ± 8.0**	13.1 ± 0.3	2.9 ± 0.1	0.51 ± 0.02

** $p < 0.01$

rate of testosterone (Foglia et al. - 1963). Leydig cell insensitivity to LH has been evoked as one possible explanation for the low androgen levels in diabetic rats (Murray et al. - 1981). In addition to these hormonal variations, several degenerative changes in seminiferous tubules leading as far as total cessation of spermatogenesis have been described (Foglia et al. - 1969; Oksanen - 1975) and later on associated with a decreased production of $^{14}CO_2$ from D [U-^{14}C] glucose by spermatozoa or seminiferous tubules (Ford and Hamilton - 1984). In light of our most recent works establishing the importance of pituitary LH in regulating testicular insulin receptors (Abelé and Tremblay - 1985) we have checked whether insulin binding was decreased in streptozotocin-induced diabetes in order to challenge the hypothesis that altered Leydig cell function of diabetic male rats is rather due to factors involved in gonadal response to LH instead of a lack of pituitary gonadotropins (Tesone et al. - 1977).

Material and Methods

Animals: Adult male Wistar rats (200–215 g B.W.) were purchased from Canadian Breeding Farm and Laboratory, Montreal, Canada. They received water and food (Purina rat chow) ad libitum and were individually housed at 23°C under standard lighting (05.00 to 19.00). Animals were weighted once a week. They were divided into two groups: control and diabetics (N = 7–8 rats/group). Diabetes mellitus was induced by intravenous administration of streptozotocin (a gift of Upjohn, Kalamazoo, Mi) dissolved in 0.2 ml of an acidified citrate buffer (pH 4.5). One week later, streptozotocin-treated rats were fasted for four hours and glucose was assessed in their tail blood with Dextrostix strips read with a Glucometer (Ames Division, Miles Laboratories, Rexdale, Ontario) and only those presenting a value between 250 and 400 mg/dl were kept in the protocol. Control rats received an equivalent volume of citrate buffer.
Hormones and Assays: Insulin binding from testes and liver was performed as described by Saucier et al. 1981. The intra-assay coefficient of variation was less than 10%. Plasma glucose was measured with an enzymatic method (Richterich and Dauwalder - 1971), while plasma insulin was determined (40 µl of plasma samples) by radioimmunoassay with rat insulin as standard and polyethylene glycol separation (Desbuquois and Aurback - 1971). Statistical analysis was performed by a student's 't' test.

Effect of diabetes mellitus on body and selected organ weights: The disease caused a significant decrease ($p \leq 0.01$) of body weight of the rats at sacrifice. Similar results were observed by Tancrède et al. (1982). As illustrated in Table 1, no significant variations in livers, testes and epididymis weights were recorded among the two groups.

Table 2
Serum concentrations (Mean ± S.E.M.) of insulin and glucose after 11 weeks of induced-diabetes in adult male rats

Treatment	Insulin (μU/ml)	Glucose (mg/dl)
Control	50 ± 3	134 ± 3
Streptozotocin	22 ± 3**	490 ± 23**

** $p \leq 0.01$

Table 3
Insulin binding parameters in liver and testicular membranes (Results are expressed as Mean ± S.E.M. N = 8 observations).

Treatment	Liver		Testis	
	^{125}I-Binding %	Ka^+ ($10^9 \times m^{-1}$)	^{125}I-Binding %	Ka^+ ($10^9 \times m^{-1}$)
Control	26.2 ± 1.2	0.78	8.4 ± 0.7	0.88
Streptozotocin	32.4 ± 1.7	0.66	5.7 ± 0.5	0.65

+ Corresponds to the high affinity binding sites
** $p < 0.05$

Blood chemistry and insulin binding in testis membranes: The administration of streptozotocin led to a drastic increase in plasma glucose which was the reflect of a low circulating concentration of immunoreactive insulin at sacrifice (Table 2). Opposite variations in insulin binding ($p \leq 0.05$) were recorded in liver and testicular membranes of diabetic rats (Table 3). For both tissues, curvilinear plots were interpreted as an indication of the presence of two binding sites and were resolved as such by computer analysis according to the method of Feldman (1972). Under these conditions similar affinity constants (Ka) were recorded in liver and testicular tissues in control and diabetic rats.

Epididymal spermatozoa and testicular morphology: After sacrifice, one epididymis of each animal in the 2 experimental groups was used to evaluate the number of spermatozoa in all three segments of the organs according to Saksena et al. 1979. A significant decrease ($p \leq 0.01$) was observed in diabetic ($2.17 \pm 0.12 \times 10^8$/g of tissue (S.E.M.) vs control rats ($2.76 \pm 0.21 \times 10^8$/g of tissue). Morphological examination of testes revealed scattered degenerative changes in seminiferous tubules of diabetic animals. These lesions in adult rats have been reported in connection with streptozotocin diabetes (French and Ritzen - 1973) and their presence explains reduction of spermatozoa number in epididymis.

Discussion

The present study has provided some confirmatory data on the influence of diabetes mellitus in adult male rats namely the decreased body mass and the altered testicular function reflected by an oligozoospermia. As expected our experimental approach was adequate to induce a moderate state of diabetes mellitus without affecting epididymal

and gonadal weights (Murray et al. - 1981). Degenerative changes in seminiferous tubules explained however the decreased number of spermatozoa in epididymis of diabetic rats. In fact, the central goal of this work was to reconcile pieces of unexplained information gathered in an abundant literature of the past ten years about the influence of diabetes mellitus on gonadal function of adult male rats. There is indeed a general agreement about a primary dysfunction of the hypothalamic-pituitary axis and of a primary disorder of the Leydig cells in diabetic male rats. Where is however the initial aggression in the system? The finding of abnormal LH secretory spikes in anovulatory diabetic female rats (Kirchick et al. - 1978; Weisenberg et al. - 1983) and of low LH circulating levels in diabetic male rats (Howland and Zebrowski - 1976; Paz et al. - 1978) suggests strongly a neuro-endocrine disorder secondary to streptozotocin administration. The marked reduction of LH (Charreau et al. - 1978) and insulin binding sites (present study) in Leydig cells of diabetic rats lend support to the hypothesis that LH represents a key factor to explain the abnormalities in reproductive organs of the rat. Both membrane receptors are indeed highly dependent of pituitary LH whose release is impaired by a yet unknown mechanism of accumulation of LH-RH in the hypothalamus (Rossi and Bestetti - 1981). Our previous observations with respect to the factors influencing in an opposite manner insulin binding in liver and gonads have essentialy shown that testosterone administration, hypophysectomy and chronic treatments with an LH-RH agonist lead to a 50% decrease of testicular insulin binding, while this binding is restored to control values in hypophysectomized rats by Human Chorionic Gonadotropin administration (Saucier et al. - 1983); this study reveals once more that a given stimulus (diabetes) initiates opposite variations in insulin binding in liver and gonadal tissues. Thus a severely lowered populations of LH and insulin receptors at gonadal level may then explain the well-known physiological alterations of the Leydig cell which result in decreased serum androgen levels (Paz et al. - 1978b; Murray et al. - 1981) and/or disturbances in testosterone/dihydrotestosterone metabolism in target tissues (Oksanen - 1975; Tesone et al. - 1976). In light of these altered biological functions of the Leydig cell and eventually some degenerative changes in ultrastructure (Murray et al. 1981), one can easily understand that ovarian and testicular tissue of diabetic animals are much less sensitive in vivo and in vitro to LH stimulation than control tissues. In fact, LH binding sites in Leydig cells from diabetic rats can be restored by insulin administration (Charreau et al. - 1978) and the interplay between both receptors under primary LH influence determines the functional integrity of the cell. Paz et al. (1978a) reached the right conclusion in stating that simultaneous administration of Human Chorionic Gonadotropin and of insulin was required for a full restoration of the reproductive function in diabetic rat.

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