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PROSTATIC ANDROGEN RECEPTOR AND PLASMA TESTOSTERONE LEVELS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

SHUK-MEI HO
Department of Biology, Tufts University, Medford, MA 02155, U.S.A.

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Summary—Diabetes was induced in male Sprague–Dawley (S–D) rats by streptozotocin (STZ) administration. Following STZ injection, plasma glucose levels in the treated rats were significantly elevated from values of untreated controls. Over the experimental period (140 days) plasma testosterone (T) levels, prostatic nuclear androgen receptor (AR) contents and prostatic weights declined with increasing age in the rats. The declines in both STZ-treated and untreated rats were similar in manner and no notable differences were discerned in the data obtained from the two groups. On the contrary, prostatic cytosolic AR contents in untreated rats remained unchanged with advancing age, but was reduced to 50% of normal control values in diabetic rats following STZ treatment. Correlation analyses revealed that prostatic nuclear AR contents correlated positively with plasma T levels while prostatic cytosolic AR contents correlated negatively with plasma glucose levels. These data support former claims that prostatic nuclear AR content is dependent on circulating T level and suggest a possible link between prostatic cytosolic AR content and plasma glucose concentrations.

INTRODUCTION

Although a large body of literature has related diabetes to reproductive dysfunction in men [1–3] and in experimentally-induced diabetic animals [4] the causes of this dysfunction are unclear. Psychologic impotence [5], vascular disturbances [6], diabetic neuropathy [1, 7] and endocrine disorders may all play a role in reducing the reproductive ability of the diabetic male. Among the endocrine disorders, the most widely studied aspect is the hypothalamus–hypophysis–gonadal axis [3, 8–13]. Studies on this topic focus primarily on circulating testosterone (T) and gonadotropins levels in circulation, testicular T output, and the pituitary response to gonadotrophin releasing factors.

By comparison, only a handful of investigations have examined diabetes-induced disturbances in the function of sex accessory organs as plausible causative factors of reproductive dysfunction in the diabetic male. Abnormalities were found in the semen of diabetic males [14–17]. Semen volume was low [14]; seminal fructose concentration was significantly elevated [18, 19]; but sperm count, sperm motility, as well as semen pH were similar to controls [15–17]. Information on the impact of diabetes on prostatic function is limited. Recently, Tesone and co-workers [20, 21] have studied the effect of streptozotocin (STZ)-induced diabetes on androgen receptor (AR) levels in the prostates of Wistar rats. Significant reduction in cytosolic (87%) and nuclear (38%) AR contents were accompanied by drastic decline in plasma T levels in the diabetic animals. T replacement therapy, but not insulin treatment, effectively restored prostatic nuclear AR content in diabetic Wistar rats. These findings support the argument that a reduction in AR content in the prostate is indirectly caused by T deprivation in the diabetic animals.

In the present study, we have examined the effect of STZ-induced diabetes on prostatic AR contents in the Sprague–Dawley (S–D) rats. In this rat strain, plasma T levels remain normal even though diabetes develops following STZ-treatment [22]. The effect of diabetes on prostatic AR levels can therefore be evaluated directly under normal circulating T environment.

MATERIALS AND METHODS

Animal procedure

Male Sprague–Dawley rats weighing 250–300 g (10 weeks old) were purchased from Charles River Breeding Laboratory (Wilmington, Mass). They were housed in animal facility

under a 12-h light–12-h dark cycle. All animals received standard laboratory chow (Purina) and water *ad libitum* except when indicated. After an overnight fast, (STZ-) treated rats were given a single injection (i.p.) of STZ (40 mg per kg body wt) in 0.1 M citrate saline pH 4.5. Untreated (control) rats received citrate saline vehicle. Animals were sacrificed at various time intervals following streptozotocin or vehicle injection. Six animals were used in each group for biochemical studies.

Buffers and chemicals

Buffer A contained 20 mM Tris–HCl, pH 7.5 (Schwartz/Mann, Orangeburg, N.Y.), 1 mM phenylmethylsulfonyl fluoride, 1.5 mM EDTA tetra-sodium salt (Sigma), 1 mM dithiothreitol (DTT; Sigma), 10% (v/v) glycerol (Fisher) and 20 mM sodium molybdate (Sigma). Buffer B contained 10 mM Tris–HCl, pH 7.5, 0.3 M sucrose and 5 mM $MgCl_2$. Buffer C contained 0.6 M KCl in Buffer A. Buffer D contained 50 mM phosphate-buffered saline containing 1 mg/ml gelatin. [^{3}H]R1881 or [^{3}H]methyltrienolone (87 Ci/mmol) was obtained from New England Nuclear Corp. (Boston, Mass). Compositions of other buffers and solutions were reported previously by Ho *et al.* [23].

Preparation of tissues

Rats were sacrificed by decapitation. Blood was collected into ice-chilled heparinized tubes and plasma separated from blood cells by low-speed centrifugation. Prostate lobes, including ventral, lateral and dorsal prostatic lobes, were removed from each animal and stripped of adhering fat, washed, weighed and rinsed in an ice-cold culture medium. All subsequent procedures for preparation of cytosol and nuclear extract were carried out according to procedures described in Ho *et al.* [23]. We have previously studied the effects of sodium molybdate on cytosolic and nuclear AR measurement. Sodium molybdate has been shown to have stabilizing effects on cytosolic AR [23–25] and thus is commonly included in homogenizing medium for preparation of subcellular tissue fractions for AR measurement. However, according to Thompson and Chung [25] sodium molybdate can extract AR from prostatic nuclei in a concentration-dependent manner. We have re-examined this possibility under our experimental conditions [23] and did not observe an extraction effect of nuclear AR by this anion. Thus, in all subsequent tissue preparations 20 mM sodium molybdate has been included in the homogenizing buffer (Buffer A).

Minced tissues were homogenized in 3 vol (per g tissue wet wt) of Buffer A with a Tissumizer (Tekmar, Cincinnati, Ohio) and the tissue homogenate centrifuged at 800 *g* for 15 min. The low-speed supernatant was then centrifuged at 130,000 *g* for 1 h to yield the cytosolic fraction. The nuclear fraction (pellet from the low-speed spin) was resuspended in 10 volumes of Buffer B (per original tissue wet wt), filtered once through double-layered cheesecloth and washed twice with 10 volumes of Buffer B. The washed nuclear pellet was resuspended in 3 volumes of Buffer C (per g original tissue wet wt) and incubated for 1 h with frequent mixing. At the end of the incubation, the nuclear extract was obtained by ultracentrifugation at 113,000 *g* for 1 h. Aliquots of cytosol and nuclear extract are stored at −80°C until used.

Determination of cytosolic and nuclear androgen receptor level

Cytosolic (CAR) and nuclear (NAR) androgen receptor levels were determined by incubating 0.4 ml of diluted sample with 10 nM of [^{3}H]R1881 plus 1 μM of triamcinolone acetonide to prevent binding of [^{3}H]1881 to progesterone receptor [26]. Nonspecific binding of the radioligand was determined by parallel incubation in the presence of a 100-fold excess of unlabelled R1881. Incubation at 4°C for 24 h was used for cytosolic receptor measurements, and 15°C 24 h incubation for nuclear binding study. These incubation conditions have been shown to be effective in allowing maximal exchange of the labelled ligand with endogenously bound androgens in a previous study of ours [23]. After the incubation, bound and free steroids were separated by a hydroxylapatite procedure as described by Ho *et al.* [23]. Duplicate incubations were used to obtain mean receptor values for each tissue sample. Radioactivity bound to the androgen receptor was extracted from the hydroxylapatite pellet by ethanol extraction and counted in scintillation fluid using a Packard Scintillation Counter. Androgen receptor levels were expressed as fmol [^{3}H]R1881 bound per mg DNA of the samples.

Plasma testosterone determination

Plasma samples were diluted to 0.5 ml with ice-cold distilled water and extracted with 10 volumes of anhydrous diethyl ether from freshly opened cans. The aqueous phase was frozen and

the organic phase removed, evaporated to dryness, and resuspended in 0.3 ml of Buffer D and stored at −80°C until used.

Plasma samples were diluted to appropriate concentrations and the amount of testosterone (T) in each sample was determined by radioimmunoassay (RIA) using a rabbit anti-testosterone-7α-BSA serum (Miles-Yeda Ltd., Assay kit No. 61-315, Elkhart, Ind.). Cross-reaction for 5α-dihydrotestosterone and androstenedione are 17 and 6% respectively.

Plasma glucose level

Plasma glucose level was determined using the glucose oxidase method (Sigma glucose kit No. 115, St Louis, Mo.).

DNA determination

DNA content of the tissue homogenate or nuclear fraction was determined by the diphenylamine procedure as described by Burton [27].

Statistical analyses

The significance of differences between the streptozotocin-treated and untreated groups was tested by two-way analyses of variance, and a Tukey test procedure was used for multiple range comparisons of the individual groups. Correlation studies were analyzed by linear repression analyses.

RESULTS

Plasma glucose level (Fig. 1)

STZ treatment significantly elevated plasma glucose levels in male rats ($P < 0.001$). Plasma glucose level rose from a control value of 180 ± 8 mg/100 ml to a peak value of 360 ± 70 mg/100 ml in 30 days and remained elevated for the remaining portion of the monitoring period a total of 140 days). Meanwhile, plasma glucose levels in untreated, eye-matched controls remained unchanged at 180 mg/100 ml throughout the entire experimental period.

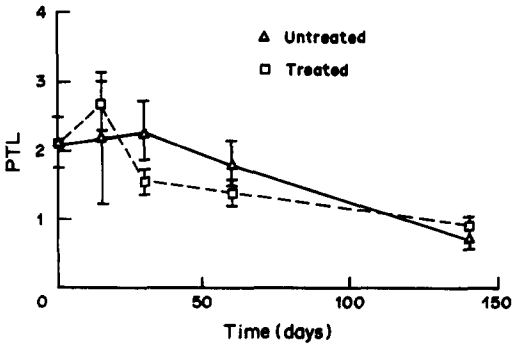

Fig. 2. Changes in plasma testosterone levels (PTL, in ng/ml plasma) in Sprague–Dawley rats after a single injection of STZ at Day 0 (10 week-old rats) to Day 140 (30 week-old rats) post-treatment (Time). Data points are means ± SEM ($n = 6$). Δ, untreated controls; □, STZ-treated rats.

Plasma testosterone level prostatic weight (Fig. 2)

Plasma T levels in STZ-treated and untreated rats declined in a similar manner with advancing age of the animals from a value of 2.10 ± 0.14 ng/ml (in 10 week-old rats) to approximately 0.7–0.9 ng/ml in 140 days (in 30 week-old rats). However, plasma T levels in STZ-treated animals were not different from those of untreated rats ($P > 0.05$). Although the relative organ weight of the prostate (prostatic wt/body wt) decreased slightly with advancing age of the animal, STZ-treatment did not cause any changes in this parameter (data not shown).

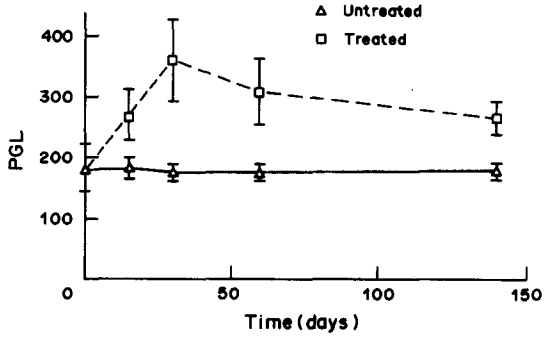

Fig. 1. Changes in plasma glucose levels (PGL, in mg/100 ml plasma) in Sprague–Dawley rats after a single injection of STZ at Day 0 (10 week-old rats) to Day 140 (30-week-old rats) post-treatment (Time). Data points are mean ± SEM ($n = 6$). Δ, untreated controls; □, STZ-treated rats.

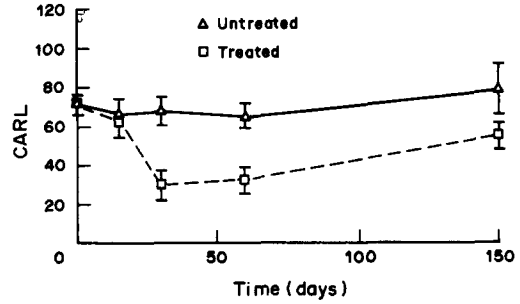

Fig. 3. Changes in cytosolic androgen receptor level (CARL, in fmol/mg DNA) in the prostate gland of Sprague–Dawley rats after a single injection of STZ on Day 0 to Day 140 post-treatment (Time). Data points are mean ± SEM ($n = 6$). Δ, untreated; □, STZ-treated rats.

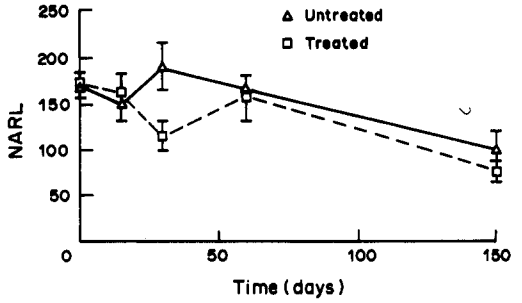

Fig. 4. Changes in nuclear androgen receptor level (NARL, in fmol/mg DNA) in the prostate gland of Sprague–Dawley rats after a single injection of STZ on Day 0 to Day 140 (Time). Data points are mean ± SEM ($n = 6$), Δ, untreated; □, STZ-treated rats.

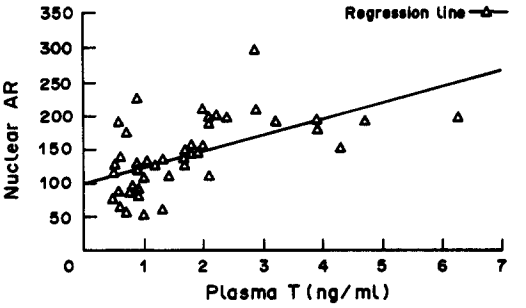

Fig. 6. Correlation of nuclear androgen receptor levels (NARL, in fmol/mg DNA) in the prostate gland with plasma testosterone levels. Linear regression analysis gives a value of 0.54 for the correlation coefficient between the two parameters ($P < 0.01$).

Cytosolic AR level (Fig. 3)

Administration of STZ significantly lowered cytosolic AR levels in the prostate glands of treated rats when compared to values of untreated animals ($P < 0.05$). Prostatic cytosolic AR level declined to a minimal on Day 30 following STZ treatment but demonstrated signs of partial recovery on Day 140 following STZ treatment. In contrast cytosolic AR levels in the prostate glands of untreated rats showed little variation throughout the 20 weeks monitoring period.

Prostatic nuclear AR level (Fig. 4)

STZ treatment had little effect on nuclear AR levels in the prostates of S–D rats; nuclear AR levels in STZ-treated and untreated rats were similar throughout most of the study period ($P > 0.05$). However, a transient decline in prostatic nuclear AR levels was observed on Day 30 in rats treated with STZ. As a general trend, nuclear AR levels in the prostates of both STZ-treated and untreated rats declined gradually from 170 ± 25 fmol/mg DNA to approx. 80–110 fmol/mg DNA tissue in 20 weeks time.

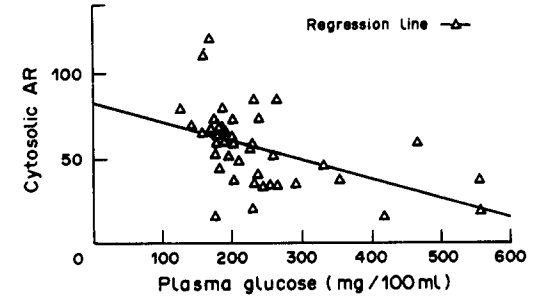

Fig. 5. Correlation of cytosolic androgen receptor levels (cytosolic AR, in fmol/mg DNA) in the prostate gland with plasma glucose levels. Linear regression analysis gives a value of −0.47 for the correlation coefficient between the two parameters ($P < 0.01$).

Relationships between prostatic AR contents and plasma glucose and T levels

Correlation analyses revealed that prostatic cytosolic AR contents were negatively correlated with plasma glucose levels (Fig. 5, correlation coefficient = −0.47, $P < 0.01$), while prostatic nuclear AR contents were positively correlated with plasma T levels (Fig. 6, correlation coefficient = 0.54, $P < 0.01$). STZ treatment did not change the relationships among these parameters.

DISCUSSION

Diabetes was induced chemically in male S–D rats by a single injection of 40 mg/kg body wt of STZ which is a known toxic chemical to B-cells in a variety of species including the rats. Signs of diabetes were evident from the elevated levels of plasma glucose in STZ-treated animals. As previously reported [22], plasma T levels in STZ-treated S–D rats were comparable to those of untreated controls although plasma T levels in both groups declined with age. This is in contrast to findings in Wistar and Long–Evans rats, in which STZ-induced diabetes caused marked reduction of plasma T levels [21, 28, 29]. Similar strain-related variation in susceptibility to the induction of diabetes by STZ was also observed in mice [30, 31]. In the human, susceptibility of B-cells to various diabetogens is also dependent on the genetic makeup of the individual [32]. Worthnoting is the pronounced decline in plasma T levels in S–D rats with the advancing age of the animals, reaching a 56% reduction by the 30th week of age. This age-dependent decline in plasma T levels was previously reported in rats [33, 34] as well as in some human studies (for a review see Ref. [35]).

The major question we attempted to address with this study is whether diabetes affects prostatic AR content directly in the absence of a diminished plasma T environment. So far, we have demonstrated a lack of impact with the diabetic condition on nuclear AR levels in the prostate glands of S–D rats. Correlation analyses indicates a tight association between nuclear AR content in the rat gland and plasma T level of the animal. Thus suggesting a strong dependency of prostatic nuclear AR content on T level in circulation. If nuclear AR population, which are considered as the biologically active receptors, remained unchanged in the rat prostate, then one would expect little decline in prostatic function following STZ treatment. Indeed, prostatic weights of STZ-treated rats were found to be comparable to those of controls throughout the experimental period. Although both plasma T levels and prostatic nuclear AR content declined with age, STZ treatment did not accelerate or slow-down the rates of these age-associated changes in S–D rats. Again, these data argued for a close relationship between prostatic nuclear AR and circulating T levels in the male rat [21].

Contrary to nuclear AR contents, cytosolic AR contents in the prostates of S–D rats remained unchanged with age in the control rats. STZ treatment severely reduced cytosolic AR contents in the diabetic animals. The extent of cytosolic AR loss in the prostates of STZ-treated rats was shown to be dependent on the severity of the diabetic condition which was reflected by the degree of glucose elevation in the plasma. Since diabetes developed in the absence of T decline in the plasma of the treated animals one can argue that the inhibitory effect of diabetes on cytosolic AR in the prostate is direct and not induced by a diminution of circulating androgen. Studies in Wistar rats had shown that diabetes selectively inhibited AR expression in these animals but have no impact on other steroid hormone receptors [36]. Cytosolic AR contents in both the prostate and the liver were depressed in diabetic Wistar rats while estrogen receptor and glucocorticoid receptor levels in the livers remained normal. The exact cause in the loss of cytosolic AR in the prostate of the diabetic rat is currently unknown. Cytosolic AR contents may be dependent on insulin or glucose availability in the tissue. Since a depressed cytosolic AR content has no sequelae on nuclear AR content in prostate of the diabetic rat, it remains to be determined as to whether it has any long-term physiological consequences on androgenic action in the rat gland.

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