Effect of streptozotocin-induced hyperglycaemia on androgen-binding protein in rat testis and epididymis

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Summary. In adult male rats treated with streptozotocin 6 weeks before the experiments, androgen-binding protein concentration was increased in testicular tissue by 33% ($p < 0.01$) and reduced in epididymal tissue by 25% ($p < 0.005$) in animals exhibiting severe hyperglycaemia as compared with animals remaining in normoglycaemia or moderate hyperglycaemia. Androgen-binding protein content was diminished in epididymal tissue by 40% ($p < 0.0005$) but not changed in testicular tissue. If related to constant body weight, the sum of

testicular and epididymal androgen-binding protein was identical in both normo- and hyperglycaemic animals. This disturbance in androgen-binding protein distribution may be the consequence of altered testicular secretion or impaired transport of androgen-binding protein from testes to epididymides.

Key words: Androgen-binding protein, testis, epididymis, streptozotocin-diabetes, Sertoli cells.

Numerous studies have demonstrated impairment of fertility [1] and testicular endocrine function [1–4] to be late complications of streptozotocin-treated hypoinsulinaemic, hyperglycaemic male rats. In addition to reports on defective spermatogenesis [5–6] and indications of disturbances of pituitary gonadotropin secretion [1–3, 5–7], dysfunction of the Leydig cells resulting in diminished testosterone formation in these diabetic animals seems to be the most extensively investigated problem in this field [1–4].

In contrast, less attention has been drawn to Sertoli cell function in diabetic animals. Although a specific androgen-binding protein (ABP) was shown several years ago to be a marker of Sertoli cell function [8–13], ABP measurements in diabetic rats have been reported in only one study [2]. A paradoxical increase of ABP content (expressed as μ l-equivalent of epididymal cytosol standard) of the testes (the site of production) but no change of ABP content of the epididymis (a possible site of action) has been found in rats 4 weeks after streptozotocin injection [2].

The aims of the present study on streptozotocin-treated male rats were to re-evaluate those results, to distinguish between ABP concentrations and ABP content of testes and epididymides by consideration of changes in organ weights and to make correlations between testicular or epididymal ABP and blood glucose levels in order to prove possible diabetes-induced changes in testicular ABP secretion.

Materials and methods

Eighteen male Han: Wistar rats, weighing 214 ± 5 g (mean $\pm$ SEM) at the beginning of the study, received 65 mg streptozotocin (2-desoxy-2-(3-methyl-3-nitrosurea)-1 α -glucopyranose; Calbiochem, Gießen, FRG)/kg body weight, i.p. (experimental group). One rat died during week 3. Five animals (205 ± 5 g initial body weight) were injected with citrate buffer only and served as a control group. All animals were allowed food (Altromin 1314; Altromin, Lage, FRG) and tap water ad libitum.

Post-prandial blood glucose levels were examined routinely (seven times during the 6-week period of the experiment) using the Eyetone reflectometer system (Dextrostix; Ames-Miles, Frankfurt/Main, FRG). Urinary excretion of glucose and ketone bodies was measured with the Keto-Diabur 5000 test (Boehringer, Mannheim, FRG).

The experimental group was divided into two subgroups. Group 1 consisted of nine animals exhibiting normo- or moderate hyperglycaemia (mean blood glucose levels < 15 mmol/l; glycosuria $> 3\%$ in one, ketonuria in none of the animals). Group 2 consisted of eight animals exhibiting severe hyperglycaemia (mean blood glucose > 15 mmol/l; glycosuria $> 3\%$ in all, ketonuria $++$ or $+++$ in five of the animals).

Animals were sacrificed 6 weeks after streptozotocin administration. Both epididymides and the left testis were removed for ABP measurement, decapsulated, trimmed of fat and weighed. Epididymides were minced with scissors and homogenized in six volumes, and testes in three volumes of Tris-HCl (50 mmol/l), Na₂ EDTA (1 mmol/l), KCl (80 mmol/l), 3-mercapto-1,2-propanediol (12 mmol/l), 10% glycerol (v/v) pH 7.4 (TEMG-buffer). All reagents were of analytical grade and purchased either from Merck, Darmstadt, FRG or from Sigma Chemie, München, FRG. A glass-glass Dual homogenizer was used (Kontes Scientific Glassware, Vineland, NJ, USA). Epididymis and testis homogenates were centrifuged at 150 000 g for 45 min, the lipid layer discharged and the cytosol mixed with the 4000 g pellet of an equal volume of charcoal (5 g/l), dextran T-70

Fig. 1A–L. Dependence of body and organ weights (A–C), epididymal (D–H), testicular (I–K) and total ABP (L) on mean blood glucose levels (A–E, H–L), organ weight (F) or body weight increase (G) in streptozotocin-treated rats 6 weeks after administration of the drug. Regression lines are drawn only if there was a significant correlation ($p < 0.05$)

Table 1. Control parameters and androgen-binding protein in streptozotocin-treated and control rats

	Control group (<i>n</i> = 5)	Group 1: normoglycaemia or moderate glycaemia (<i>n</i> = 9)	Group 2: hyperglycaemia (<i>n</i> = 8)	<i>p</i> (group 1 versus group 2)
Body weight increase (g/6 weeks)	132 ± 27	129 ± 23 (9)	24 ± 45	< 0.0005
Weight of two epididymides (g)	1.304 ± 0.17	1.219 ± 0.11 (9)	0.979 ± 0.19	< 0.005
Weight of left testis (g)	1.745 ± 0.19	1.621 ± 0.14 (8)	1.448 ± 0.29	NS
Epididymal ABP (pmol/g epididymal tissue)	53.70 ± 7.95	56.62 ± 4.64 (9)	42.60 ± 8.34	< 0.0005
Epididymal ABP (pmol/two epididymides)	69.24 ± 8.33	68.92 ± 7.63 (9)	41.45 ± 9.60	< 0.0005
Epididymal ABP (pmol/100 g body weight)	20.00 ± 2.85	20.39 ± 2.72 (9)	15.81 ± 3.45	< 0.005
Testicular ABP (pmol/g testicular tissue)	5.91 ± 1.32	5.93 ± 1.14 (8)	7.90 ± 1.50	< 0.01
Testicular ABP (pmol/testis)	10.25 ± 2.34	9.63 ± 2.10 (8)	11.58 ± 3.37	NS
Testicular ABP (pmol/100 g body weight)	6.46 ± 1.43	5.94 ± 1.17 (8)	9.36 ± 2.36	< 0.0025
Testicular + epididymal ABP (pmol/100 g body weight)	26.47 ± 2.85	26.12 ± 2.85 (8)	25.18 ± 3.62	NS

Results expressed as mean ± SEM with number of animals given in parentheses. NS = not significant

(0.5 g/l) (Pharmacia Fine Chemicals, Freiburg, FRG) in TEMG-buffer. After continuous shaking for 2 h at 2°C (in order to remove endogenous cytosolic steroids), the suspensions were centrifuged at 4000 *g* for 10 min. Aliquots (400 µl) of the supernatants were incubated for 15 h at 2°C with a mixture of 50 µl glycerol containing 2 pmol [1,2-³H(N)]-dihydrotestosterone (DHT; 5 α -androstane-17 β -ol-3-one; spec. act. 1.48 TBq/mmol; New England Nuclear, Dreieich, FRG) to achieve final ³H-DHT concentrations of 4 nmol/l.

ABP concentrations in these cytosols equilibrated with ³H-DHT were determined by steady-state polyacrylamide gel electrophoresis as described elsewhere [9, 14, 15] with the following modifications:

100 µl of the ³H-DHT-labelled cytosol were layered on 7% acrylamide rod gels (85 mm × 5 mm) containing glycerol (2.5%) and ³H-DHT 4 nmol/l. Electrophoresis was run for 4 h at 3 mA/gel. Following electrophoresis, gels were frozen in *n*-hexane on dry ice and then thawing gels were sliced into 1-mm-thick pieces. Slices were counted for radioactivity 48 h after addition of scintillation fluid (Quicksint 212; Zinsser, Frankfurt/Main, FRG) with an efficiency of 32% in a Tri-Carb model 2650 spectrometer (Packard, Frankfurt/Main, FRG). The ABP content was normally calculated from five fractions ($R_f = 0.46$) with elevated radioactivity (in comparison to the basal activity) and could be clearly distinguished [8] from a small peak representing se-

rum albumin ($R_t = 0.67$). A third peak of radioactivity reported to represent androgen receptor [15] could not be detected. The sensitivity of the method under these conditions is 15 fmol ABP/gel assuming one binding site/molecule.

Correlation of ABP with control parameters, e.g. mean blood glucose levels, was proved by linear regression analysis with the least squares method. Differences between groups were analyzed using Student's *t*-test.

Results

Body weight increase (Fig. 1 A), weight of epididymides (Fig. 1 B) as well as testicular weight (Fig. 1 C) showed negative correlations with mean blood glucose levels. Body weight increase and epididymal weight were reduced if group 2 was compared to group 1, but no difference existed between testicular weights in both groups (Table 1). The control group and group 1 did not differ significantly with regard to any of the variables tested.

A distinct reduction of both ABP concentration (Fig. 1 D, Table 1) and ABP content (Fig. 1 E, Table 1) in epididymides occurred with increasing mean blood glucose levels irrespective of the method of testing significant differences (regression analysis or *t*-test). Due to the positive correlation of epididymal ABP content with the epididymal weight (Fig. 1 F) the drop of ABP content in epididymides from hyperglycaemic rats resulted from both reduced ABP concentration and reduced epididymal weight. A positive correlation of epididymal ABP content with body weight increase (Fig. 1 G) was obtained. Nevertheless, epididymal ABP content was still significantly reduced in hyperglycaemic animals if related to constant body weight (Fig. 1 H, Table 1).

In contrast to the epididymis, ABP concentrations in the testes were higher in severely hyperglycaemic than in normo- or moderate hyperglycaemic rats (Fig. 1 I, Table 1). Due to slightly reduced testicular weight in hyperglycaemic animals (Fig. 1 C), the total ABP content of testicular tissue was not changed (Fig. 1 J, Table 1). However, testicular ABP content was increased in hyperglycaemic animals if related to constant body weight (Fig. 1 K, Table 1). The sum of ABP contents of both testes and epididymides was not influenced by hyperglycaemia (Fig. 1 L, Table 1). Therefore streptozotocin-induced hyperglycaemia resulted in a shift of ABP distribution from the epididymides back to the testes but not in a real increase or decrease of total ABP.

Discussion

In most of the previous studies on reproductive and especially endocrine testicular function of streptozotocin-diabetic male rats, animals not exhibiting glycosuria or other criteria of diabetes mellitus were excluded from the experiments and a control group was established

which received buffer injections only [1, 2, 4, 6]. The protocol chosen in the present study, however, excluded possible side-effects of the drug. With all streptozotocin-injected animals included in the study irrespective of the outcome of the treatment, it was possible to look into the dependence of reproductive function parameters on the degree of hyperglycaemia. On the other hand, the direct comparison of the untreated control group with group 1 one of the experimental group allows the conclusion that streptozotocin itself does not affect testicular or epididymal ABP (Table 1).

With the protocol chosen for the present study, the effectiveness of diabetogenesis was comparable to that achieved previously by other authors [1]. A marked reduction of epididymal weight and only a slight reduction of testicular weight occurred in streptozotocin-diabetic animals (Fig. 1). Under slightly different conditions, a 20% loss [1, 5, 6] or no change [2, 4, 7] of testicular weight has been reported, whereas epididymal weight has been shown to be reduced by 50% [1] or by 15% [2]. Whether this loss in epididymal weight can be attributed to decreased serum testosterone levels in the diabetic rats remains questionable [1, 4, 7]. Alternatively, a slower increase of epididymal weight during development of diabetic rats may be the simple consequence of the slower increase in body weight.

The reason why epididymal ABP concentration, as well as epididymal ABP content, were decreased in diabetic in comparison with normoglycaemic adult rats is at first unclear. Two hypotheses may be discussed: (1) is it possible that decreased epididymal ABP levels are due to decreased serum FSH levels? Serum FSH concentrations have been reported to be decreased in streptozotocin-diabetic animals [2], but this finding has not been confirmed by other authors [3, 7]. FSH has been proposed to enhance both testicular and epididymal ABP concentrations [8, 9, 11] as well as the ABP production by cultured Sertoli cells from immature rats [12]. Therefore possibly reduced circulating FSH concentrations in streptozotocin-diabetic male rats may result in a diminution of epididymal ABP but cannot explain increased testicular ABP concentrations. (2) Is it possible that decreased ABP levels are due to decreased serum testosterone levels? A reduction in circulating testosterone concentration has been observed in diabetic animals [1–4, 7]. Testosterone administration to normal rats results in elevated testicular and epididymal ABP levels either directly [10] or by stabilizing ABP activity [11]. But again, possibly reduced serum testosterone concentrations cannot explain the different development of testicular and epididymal ABP, so much the more as ABP production by Sertoli cells is unlikely to be affected in streptozotocin-hyperglycaemic rats (Fig. 1 L).

Therefore, a reduction of Sertoli cell ABP secretion or impaired ABP transport from the testes to the epididymides seem to be more probable explanations for the results presented in this study. This assumption

is supported by the observation that hypophysectomy, as well as administration of anti-LH serum, affects fluid secretion by the Sertoli cells and causes loss of ABP in the caput epididymidis in the long term [16]. A deficiency of LH might also be responsible for the impaired testicular ABP secretion in diabetes mellitus, although it cannot be excluded that testicular ABP secretion is affected via hormone-independent mechanisms.

In conclusion, it is suggested that streptozotocin-induced hyperglycaemia does not cause impaired ABP production by the Sertoli cells, but affects ABP secretion by, or ABP transport from, the testis, the result being a relative elevation of testicular and a relative diminution of epididymal ABP in diabetic animals.

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