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Inhibition of Testicular Testosterone Biosynthesis Following Experimental Varicocele in Rats

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ABSTRACT

In an attempt to determine whether defective testicular testosterone (T) biosynthesis may be associated with a varicocele, an experimental study was performed in adult rats whereby a unilateral left varicocele was surgically created. At 2, 4, 8, and 12 wk following the creation of the varicocele, intratesticular T as well as the activities of three (17α-hydroxylase, 17,20-desmolase, and 17β-hydroxysteroid dehydrogenase) of the five enzymes in the Δ4 pathway of testicular T biosynthesis were measured. Intratesticular T (ng/g testis ± SEM) in the left testis decreased significantly from 121 ± 21 in the control group to 59 ± 8 in the two-wk varicocele group ($p<0.01$), and remained significantly suppressed throughout the experimental period. The T concentrations in the right testis paralleled those in the left in both the control and varicocele animals. At 2 wk following the creation of the varicocele, the activity (nmol/min/testis ± SEM) of the 17,20-desmolase enzyme decreased significantly, from 115 ± 8 in the left testis of control rats to 87 ± 6 in the left testis of the varicocele animals ($p<0.025$), and remained low throughout the 12 weeks of the study. The activity of the 17α-hydroxylase enzyme was significantly decreased at the 8th and 12th weeks of the study, while the 17β-hydroxysteroid dehydrogenase activity did not show any significant change during the study period. The enzyme activities in the right testis paralleled those in the left testis. These data indicate that the surgical creation of a left-sided unilateral varicocele in the laboratory rat leads to a prompt and sustained diminution in the biosynthesis of T in both testes, and this effect seems to be primarily at the 17,20-desmolase step. This indicates that the bilateral anti-spermatogenic effect that occurs in the rat as a result of an experimental, unilateral varicocele may include a mechanism that involves defective T production in the testis.

INTRODUCTION

The male partner is believed to be the cause of the infertility in half (Simmons, 1956) of the approximately 15% of couples who have a barren marriage (MacLeod, 1971). In about 40% of these men, a varicocele, which is a dilatation of the pampiniform plexus of veins around the testis, may be diagnosed (Saypol, 1981), and most of these men demonstrate certain testicular and seminal fluid changes in physical and laboratory examination (Dubin and Hotchkiss, 1969; Zorgniotti and MacLeod, 1973; Dubin and Amelar, 1975; Cameron et al., 1980). The observation that these testicular and seminal fluid abnormalities may revert to normal after ligation of the varicocele would suggest a cause-and-effect relationship between a varicocele and infertility (Dubin and Amelar, 1975). While a variety of theories have been proposed to explain the etiology of the defective spermatogenesis seen in patients with a varicocele, the exact cause has not yet been completely elucidated. This has mainly been due to the lack of an appropriate animal model to study this abnormality. Recently, Saypol et al. (1981) used such a model in the laboratory rat and determined that testicular

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hyperthermia, increased testicular blood flow, and defective spermatogenesis resulted from the surgical creation of a varicocele.

Hyperthermia is known to be a potent inhibitor of enzyme activity, particularly in the testosterone (T) biosynthetic pathway in the testis (LeVier and Spaziani, 1968; Gomes et al., 1971; Wisner and Gomes, 1978). Since normal spermatogenesis requires a high intratesticular T concentration (Steinberger, 1971), this study was designed to determine whether the surgical creation of a varicocele, as described by Saypol et al., (1981), would lead to a diminution in T production by both testes. If this could be demonstrated, it would provide insight as to the cause of the defective spermatogenesis seen in men and in experimental animals with a varicocele.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (450 g) were obtained from institutional vivarium sources and acclimatized for 1 wk prior to experimental use. Animals were divided into 5 groups. Group 1, the control group, underwent a sham operation, without actual ligation of the left renal vein. Groups 2 to 5 underwent surgical creation of left varicocele, as described previously (Saypol et al., 1981; Turner et al., 1982; Green et al., 1984). Inspired halothane (1.2%) delivered via face cone was the anesthetic used for all five groups. At the appropriate time periods 2 to 12 wk later, the animals were killed by injecting potassium chloride into the left ventricle. Groups 1 and 2 were killed at 4 wk, Group 4 at 8 wk, and Group 5 at 12 wk. At the time of the killing, the diameter of the left spermatic vien as well as the weights of the seminal vesicles and right and left testes were recorded. The testes were snap-frozen on dry ice and stored at −70°C until performance of the intratesticular T and steroidogenic enzyme assays.

Tissue Preparation

The testes from each of the 5 groups were quickly thawed, decapsulated, and homogenized at 4°C, using a motor-driven glass homogenizer at approximately 2000 rpm with 15−20 strokes, in ice-cold 0.1 M phosphate buffer, pH 7.4 (1:5, w/v), containing 0.25 M sucrose and 1 mM dithiothreitol. The homogenate was then centrifuged at 800 × *g* for 20 min, and the supernatant was used for determining the enzymatic activities and T content.

Measurement of Intratesticular Testosterone

Intratesticular T concentrations were measured by using a radioimmunoassay (RIA) procedure described by Abraham et al. (1977). An aliquot (0.5 ml) of supernatant from testicular homogenate (equivalent to about 100 mg tissue) was used for extraction with diethylether after trace amounts (about 1000 cpm) of T were added to each sample to determine its recovery. Separation of T from the other steroids was achieved by celite column chromatography technique (Manlimos and Abraham, 1975). RIA procedure for T was carried out, using purified tritiated T, an authentic standard and specific antibody preparation raised employing the steroid-carboxyether:BSA conjugates (Radioassay System Laboratories, Carson, CA). The assay was performed after making appropriate dilutions of the recovered steroid samples. Dextran-coated charcoal was used to separte the free from the bound steroid. Specificity and accuracy of the assay were determined by taking different volumes of the extracts as well as by monitoring the recovery of the added steroid. Stripped testicular homogenate, with low, medium and high steroid pools along with commercial steroid pools (Radioassay System Laboratories) served as external controls for the assay. Recovery of the added steroid was between 80% and 90%, and no systematic deviation of results from linearity was observed. The cross reactivities for other steroids were less than 1%, using this specific T antibody. Sensitivity of the assay was 10 pg. The intraassay coefficient of variation was found to be 8%, while the interassay coefficient of variation was 12%.

Determination of Steroidogenic Enzyme Activities

An aliquot (100 μl) of the supernatant (about 1−2 mg protein) was used each time for the measurement of testicular enzyme activities of 17α-hydroxylase, 17,20-desmolase, and 17β-hydroxysteroid dehydrogenase, as previously reported (Sikka et al., 1985). For 17α-hydroxylase reaction, conversion of [^{3}H] progesterone (0.5 μM, 50−60 × 10^4 dpm) to [^{3}H] 17α-hydroxyprogesterone, [^{3}H] androstenedione and [^{3}H] testosterone was measured. For 17,20-desmolase activity, the conversion of 17α-[^{3}H] -hydroxyprogesterone (1 μM, 50−60 × 10^4 dpm) to

[^{3}H] androstenedione and [^{3}H] testosterone was measured. For 17β-hydroxysteroid dehydrogenase activity, the conversion of [^{3}H] androstenedione (5 μM, 40–60 × 10^4 dpm) to [^{3}H] testosterone was measured. The conversion of [^{3}H] dihydrotestosterone (DHT) and 3α,17β-[^{3}H] androstanediol (3α-diol), the distal metabolites of testosterone, was also measured for determination of all three enzymatic activities, which were expressed in terms of nmol of specific substrate converted per unit time per testis.

Data Analysis

Comparison of values between the control and varicocele groups was performed by analysis of variance, using the BMDP program implemented on a VAX 11/750 computer (Dixon, 1983), and the statistical significance was set at a two-tailed alpha level of 0.05.

RESULTS

The creation of a varicocele in these animals was confirmed by the marked three- to fivefold increase in the diameter of the left spermatic vein in the 2-wk animals; the spermatic vein remained dilated throughout the 12-wk experimental period (Table 1). Testicular and seminal vesicle weights were not significantly altered during the course of the experiment (Table 1).

Intratesticular T (ng/g testis ± SEM) was 121 ± 21 in the left testis of the control group (n = 18), and this decreased significantly ($p<0.01$) to 59 ± 8 in the left testis of the 2-wk varicocele group (Table 2). The intratesticular T of the left testis remained significantly low in the 4-wk (76 ± 17; $p<0.05$), 8-wk (42 ± 7; $p<0.0025$), and 12-wk (58 ± 14; $p<0.0125$) varicocele groups, respectively. The intratesticular T in the right testis of the control group was 119 ± 21. At 2, 4, 8, and 12 wk, this value was significantly reduced to 65 ± 9, 63 ± 15, 39 ± 7, and 40 ± 7, respectively.

The activity (nmol/min/testis ± SEM) of the 17,20-desmolase enzyme decreased significantly ($p<0.025$) from 115 ± 8 and 116 ± 9 in the left and right testes of the control group (n = 11), to 87 ± 6 and 92 ± 5, respectively, of the 2-wk (n = 10) varicocele group (Table 3). The activity of this enzyme remained significantly diminished throughout the experiment, when compared to the control group ($p<0.0005$). The activity of the 17α-hydroxylase enzyme did not change for the first 4 wk after surgery. However, by 8 and 12 wk after the creation of a varicocele, there was a significant ($p<0.002$) decline in the activity of this enzyme in both the left and right testes. In contrast, the activity of the 17β-hydroxysteroid dehydrogenase enzyme showed no significant change throughout the 12 wk of the study.

DISCUSSION

The results of this study demonstrate a marked inhibition in the production of T by the testis following creation of an experimental varicocele in the rat. This effect occurred by 2 wk and persisted for the duration of the experimental period. By using a sensitive assay system that measured the activities of the five enzymes in the Δ4 pathway of T biosynthesis in the rat testis, it was determined that T production was primarily inhibited at the 17,20-desmolase step. In addition, it appears that this seemingly unilateral condition of a left-sided varicocele led to defective T biosynthesis in both testes.

TABLE 1. Rat testicular and seminal vesicle (S.V.) weights and internal spermatic vein diameter (Int. Sp. Vein) 2 to 12 wk after creation of a unilateral, experimental left varicocele (n=5–8).

Group	Testis (g + SEM)		S.V. (g + SEM)	Int. Sp. Vein (mm + SEM)
	Right	Left		
1 (control)	1.94 ± 0.07	1.94 ± 0.06	1.20 ± 0.10	0.23 ± 0.08
2 (varicocele 2 wk)	1.95 ± 0.07	1.90 ± 0.10	1.56 ± 0.26	0.60 ± 0.12[b]
3 (varicocele 4 wk)	1.66 ± 0.17	1.89 ± 0.17	1.56 ± 0.19	0.82 ± 0.11[b]
4 (varicocele 8 wk)	2.03 ± 0.09	1.90 ± 0.08	1.13 ± 0.20	1.03 ± 0.33[b]
5 (varicocele 12 wk)	1.96 ± 0.13	1.97 ± 0.11	1.03 ± 0.10	0.85 ± 0.12[a]

[a] $p<0.005$.

[b] $p<0.025$.

TABLE 2. Intratesticular testosterone concentrations in right and left testes of rats two to twelve weeks following creation of a unilateral, experimental left varicocele.

Group	Intratesticular testosterone (ng/g testis + SEM)	
	Right testis	Left testis
1 (control; n = 8)	119 ± 21	121 ± 21
2 (2 wk; n = 10)	65 ± 9[b]	59 ± 8[b]
3 (4 wk; n = 8)	63 ± 15[c]	76 ± 17[d]
4 (8 wk; n = 9)	39 ± 7[a]	42 ± 7[a]
5 (12 wk; n = 8)	40 ± 7[a]	58 ± 14[b]

[a] $p<0.0025$.
[b] $p<0.01$.
[c] $p<0.025$.
[d] $p<0.05$.

A plausible explanation as to how the surgical creation of a varicocele may lead to defective T biosynthesis may be arrived at through a consideration of testicular temperature. In the rat, dog, monkey, and man, the presence of a varicocele results in an increase in the intratesticular temperature of both testes (Fussell et al., 1981; Saypol et al., 1981; Zorgniotti and MacLeod, 1973). The activity of any enzyme is known to be temperature-dependent, and an increase in intratesticular temperature is known, per se, to have an inhibitory effect on the activities of the enzymes involved in the conversion of cholesterol to T in the testis (LeVier and Spaziani, 1968; Bedrak et al., 1971; Gomes et al., 1971; Wisner and Gomes, 1978), resulting in a diminution in the production of T by that testis. This same effect of elevated temperature on testicular T biosynthesis may be seen in another clinical condition termed cryptorchidism, in which the testis remains relatively hyperthermic because of its location (Keel and Abney, 1980). In this latter disorder, the production of T by the testis is markedly inhibited, including the activities of 17α-hydroxylase, 17,20-desmolase, and 17β-hydroxysteroid dehydrogenase enzymes (Llaurado and Dominguez, 1963; Inano and Tamaoki, 1968; Farrer et al., 1985).

The present experimental findings (defective T production) in the rat model also provide insight into certain clinical observations in men with varicoceles. For example, Hudson and McKay (1980) demonstrated a hyperresponsiveness to exogenous gonadotropin-releasing hormone (GnRH) in patients with varicoceles who had abnormal semen analyses but a normal peripheral endocrine profile. Such a response to GnRH may be seen in patients with a Leydig cell defect. In addition, Rodriguez-Rigau et al. (1978) have demonstrated an abnormality in in vitro T biosynthesis by the testes of patients with bilateral varicoceles that are incubated with exogenous human chorionic gonadotropin (hCG). By measuring precursors of testosterone in the testes of these patients with varicoceles, the authors hypothesized that there may be a possible defect in the testicular 17,20-

TABLE 3. Effect of unilateral, experimental left varicocele on 17α-hydroxylase, 17,20-desmolase, and 17β-hydroxysteroid dehydrogenase enzyme activities (nmol/min/testis ± SEM) at 2, 4, 8, and 12 wk after creation of the varicocele in the laboratory rat (n=8–11).

Item	Control	Varicocele			
		2 wk	4 wk	8 wk	12 wk
17α-Hydroxylase					
Right testis	93 ± 7	85 ± 9	90 ± 6	47 ± 5[a]	65 ± 4[c]
Left testis	99 ± 7	87 ± 9	81 ± 7	51 ± 6[a]	63 ± 4[c]
17,20-Desmolase					
Right testis	116 ± 9	92 ± 5	67 ± 12[a]	66 ± 6[b]	89 ± 7[d]
Left testis	115 ± 8	87 ± 6	64 ± 11[a]	68 ± 10[b]	79 ± 3[d]
17β-Hydroxysteroid dehydrogenase					
Right testis	196 ± 29	196 ± 24	162 ± 30	141 ± 20	197 ± 41
Left testis	176 ± 24	195 ± 29	183 ± 32	119 ± 20	218 ± 52

[a] $p<0.0001$.
[b] $p<0.001$.
[c] $p<0.002$.
[d] $p<0.01$.

desmolase enzyme in these patients (Rodriguez-Rigau et al., 1978). This is the same enzyme defect found in the present experimental study in the rat.

If the abnormal spermatogenesis seen in patients with varicoceles is due to a lowered intratesticular T as a result of defective T biosynthesis, this theory may for the first time provide a scientific explanation for the efficacy of certain exogenously administered agents such as hCG and clomiphene citrate (both of which increase intratesticular T concentrations) in improving the sperm count and resultant pregnancy rates in this clinical condition in the human (Dubin and Amelar, 1975; Check, 1980).

More importantly, these results raise the question of whether patients with varicoceles and defective spermatogenic function should be treated initially with hormonal therapy (Check, 1980), rather than surgery (Dubin and Amelar, 1975) or radiological embolization of the internal spermatic veins (White et al., 1981), as is presently done. A study evaluating the efficacy of primary hormonal therapy versus these more common treatments in patients with varicoceles seems reasonable in view of the data presented herein.

Other studies have shown that hyperthermia of the testis results in a diminution of the activities of the intratesticular 17α-hydroxylase, 17,20-desmolase and 17β-hydroxysteroid dehydrogenase enzymes, resulting in a lowered intratesticular T production (LeVier and Spaziani, 1968). In the rat varicocele model, it is the 17,20-desmolase enzyme that is primarily affected by this condition. It is possible that the degree of hyperthermia that occurs with a varicocele is dependent on the severity of the varicocele, which may affect different enzymes to different degrees; since the 17,20-desmolase enzyme is believed to be the rate-limiting enzyme in the T biosynthetic pathway, it is this enzyme that will presumably demonstrate an abnormality before the others, as observed in the present study.

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