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Cyclosporine: Its Effects on Testicular Function and Fertility in the Prepubertal Rat

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The authors examined the effects of the immunosuppressive drug cyclosporine (CsA) on the male reproductive system in prepubertal rats. Twenty-one-day-old rats were subcutaneously injected with either cremaphor-saline vehicle or CsA (1 and 2 mg/kg/d). The animals were treated until they were 66 days old. Cyclosporine did not affect the weights of the body or testis but decreased the weights of all sex accessory organs. Quantitative analysis of the tubules in stage VII of spermatogenesis revealed a decline in the cell counts of pachytene spermatocytes and step VII spermatids. Testicular and epididymal sperm counts and motility were decreased by 50% and fertility by 60%.

Cyclosporine lowered serum testosterone despite an elevation of LH, indicating that the drug directly inhibited testosterone synthesis. Serum creatinine levels were normal in the treated animals, precluding renal failure as the cause for this impairment. Intratesticular concentrations of pregnenolone and 17-hydroxy progesterone were significantly elevated, while those of progesterone, androstenedione, and testosterone were markedly reduced. Determination of steroidogenic enzyme activities indicated that the administration of CsA inhibited the activity of Δ^5-3B-hydroxy steroid dehydrogenase-Δ^{5-4} isomerase (3beta-HSD). These results clearly indicate that CsA in the doses used is harmful to the male reproductive function in prepubertal rats.

Key words: cyclosporine, spermatogenesis, steroidogenesis, fertility.

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Cyclosporine (CsA), a cyclic undecapeptide of fungal origin, is a powerful immunosuppressive agent that has markedly improved graft survival rates in organ transplantation (Cohen et al, 1984) However, the drug has adverse side effects such as nephrotoxicity, hepatotoxicity, hirsutism, and gynecomastia (Hamilton et al, 1982; European Multicentre Trial Group 1982;1983). We recently reported that the administration of CsA at moderate to toxic doses to sexually mature rats caused a decline in testicular function, resulting in sterility within 14 days of treatment (Seethalakshmi et al, 1987). Rajfer et al (1987) also demonstrated that oral administration of CsA impaired the endocrine function of the testis and suggested that the impairment was secondary to pituitary dysfunction.

Many patients undergoing organ transplantation are prepubertal or in early puberty and will remain on CsA therapy for a long time. Yet, no studies have evaluated the effects of long-term administration of CsA on the male reproductive system in prepubertal subjects. To examine this, we have investigated the effects of pharmacological doses of CsA on testicular function and fertility in prepubertal rats.

Materials and Methods

Prepubertal Sprague Dawley rats (21 days old) weighing approximately 50 g were used for all experiments. The animals were divided into two groups: vehicle control and CsA-treated at 1 or 2 mg/kg/d. Cremaphor (650 mg diluted to 12.5 ml) was injected (100 μl) as the vehicle. Both the vehicle and CsA were injected subcutaneously until the animals were 66 days old. A total of 12 rats were used in each group. The animals in the 1 and 2 mg/kg/d CsA groups received a cumulative dose of 8.3 and 15.6 mg of CsA, respectively, over a period of 45 days. The animals were killed by an overdose of ether 24 h after the last injection. Blood was drawn by cardiac puncture, and the reproductive organs excised, blotted free of blood, and weighed immediately.

Sperm Counts and Motility

Immediately after excision, a portion of the testis and cauda epididymidis were weighed (100 mg) and minced in 2 ml of physiological saline solution (0.9% NaCl) (Taylor et al, 1985). The total number of spermatozoa, as well as the number of motile spermatozoa, were counted using a hemocytometer. Sperm counts and motility were expressed as 1×10^7/100 mg tissue and percentage, respectively.

Fertility Test

Two days before sacrifice, the fertility of male rats was tested by caging each male with an adult female in proestrus. The following morning, vaginal smears were taken and examined under the microscope for spermatozoa (Robaire et al, 1984). If spermatozoa were not present in the vaginal smears, the females were replaced with another set of proestrus females and the test was repeated. Subsequently, the females were separated and allowed to go to term. The data are presented as number of fertile male per number of treated male rats.

Histological Procedures and Quantitation of Spermatogenesis

The animals were sacrificed by a one-stage vascular perfusion of 2% paraformaldehyde-glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 (Karnovsky, 1965), and the testes were postfixed in epon (Chakraborty and Jhunjhunwala, 1982). Semithin (1 μm) sections were cut from the epon embedded blocks and stained with 1% toluidine blue for light microscopic examinations.

In the rat, stage VII (classification of Leblond and Clermont, 1952) is particularly androgen dependent. It is well known that pachytene primary spermatocytes and step VII spermatids are the germ cells most sensitive to androgen withdrawal (Russell and Clermont, 1977). Stage VII was therefore chosen for quantitation of spermatogenesis by germ cell counts. For each rat, 10 round cross sections of stage VII seminiferous tubules (324–380 μm in diameter) were counted per testis. The number of A-type spermatogonia, preleptotene and pachytene spermatocytes, and step VII round spermatids were counted and corrected according to the formula of Abercrombie (1946) using data for nuclear diameter derived by image analysis. Image analysis was carried out using software from Microscience Inc., Washington, DC.

Determination of Serum LH and Testosterone

Serum levels of LH and testosterone were determined with double antibody RIA methods. LH radioimmunoassays were done with a kit provided by NIAMD Pituitary Agency, (Bethesda, MD). The reference preparation and antiserum used for LH were LH-RP2 and anti-LH 8. Fifty to 100 μl were taken for the LH assay. The sensitivity of the assay was 100 pg/ml, and 50 μl samples were used. The intraassay coefficients of variation were 5.67% for LH and 3.5% testosterone, and the inter-assay coefficents of variation were 7.3% for testosterone and 10% for LH. The intraassay coefficients of variation were 5.67% for LH and 3.5% testosterone, and the inter-assay coefficents of variation were 7.3% for testosterone and 10% for LH.

Determination of Intra-Testicular Steroid Precursors of Testosterone Biosynthesis

Upon sacrifice of the rats, the testes were quickly decapsulated, weighed, and homogenized in an ice-cold potassium phosphate buffer (100 mM), pH 7.4, (1:5 w/v) containing 0.25 M sucrose and 1 mM dithiothreitol (DTT) in an Eberbach homogenizer at 4 C. The homogenate was centrifuged at 800 × g for 20 min and a 1 ml aliquot of the supernatant was frozen at − 80 C until assayed.

Pregnenolone

Briefly, a 1 ml fraction of testicular supernatant was used for the extraction with hexane after a known amount of the ^{3}H-tracer (1000 cpm) was added to determine the recovery. The steroid was then separated using a celite column chromatography technique described by Manlimos and Abraham (1975). The columns were prepared in our laboratory. Briefly, celite-545 was washed overnight with 6N HCl followed by distilled water and methanol, and dried in an oven at 1000 F. It was then mixed (2:1 w/v) with a mixture of ethylene glycol:propylene glycol (1:1v/v), and the columns were packed with this mixture. Pregnenolone was eluted by using system I. The maximum recovery was 80%. RIA was carried out by diluting the samples in 1:8 (v/v). The sensitivity of the assay was 25 pg/ml. Dextran coated charcoal was used to separate the free from the bound radiolabeled pregnenolone. The intra-assay variation for this steroid was 1.0%.

Progesterone

Progesterone was extracted with hexane from 1 ml of testicular supernatant after adding ^{3}H-progesterone tracer (1000 cpm) for recovery. The samples were extracted until the background count was achieved (recovery > 90%). They were then dried under nitrogen and constituted in 2 ml of assay buffer. The radio-immunoassay was carried out by using a ^{125}I progesterone kit (RSL, Carson, CA). The sensitivity of the assay was 200 pg/ml. One hundred

μl of samples were used for the determination. The intra-assay variation was 5.7%.

17hydroxy-progesterone

This steroid was assayed by using a ^{125}I RIA kit from RSL. The extraction procedure was similar to that for progesterone. The percentage recovery was greater than 90%. Ten μl of samples were used for the assay. The intra-assay coefficient of variation was 3.3%.

Androstenedione

Androstenedione was extracted from 300 μl of testicular supernatant with a mixture of ethylacetate:hexane (3:2) after the addition of ^{3}H-androstenedione (1000 cpm) to account for the recovery. After evaporation under nitrogen, the samples were reconstituted in 2.5 ml of diluent buffer and the assay was carried out using a ^{125}I-androstenedione kit from RSL. One hundred and twenty-five μl were used for the assay. The sensitivity of the assay was 100 pg/ml. The intra-assay coefficient of variation was 2.6%.

Testosterone

Testosterone from the testicular supernatant was extracted with hexane after the addition of ^{3}H-T(1000 cpm) for recovery, which was greater than 90%. After evaporation, the samples were reconstituted in 1 ml of diluent buffer and a 50 μl sample was taken for RIA by using a ^{125}I testosterone kit from RSL. The sensitivity of the assay was 100 pg/ml.

In order to determine whether CsA interfered with these assays, 500 ng/ml of CsA was added to the standards separately. Cyclosporine did not interfere with these asays.

Determination of the Activities of Steroidogenic Enzymes

The activity of cholesterol side chain cleavage enzyme activity (CSCCE) was determined by measuring the conversion of ^{3}H cholesterol into ^{3}H pregnenolone by the isolated mitochondria of interstitial cells from the testes of experimental rats. The interstitial cells were isolated according to the method of Wang et al (1983), and mitochondria were prepared by the conventional centrifugation technique (Sottocosa et al, 1967). Incubation was then carried out in 1 ml of Krebs-Ringer bicarbonate buffer, pH 7.2, containing 11 mg nicotinamide, 3.51 mg glucose-6-phosphate, 0.92 mg magnesium chloride, 6 mg glucose, 0.5 mM NADPH, a crystal of glucose-6-phosphate dehydrogenase and 7(n) ^{3}H-cholesterol (50 × 10^4 dpm, specific activity 9 Ci/mmol). Incubations were initiated after the addition of 13 μl (approximately 300 μg protein) of mitochondrial preparation and carried out at 32 C for 1 h in an atmosphere of 95% oxygen and 5% carbon dioxide in a shaking water bath.

The activities of steroidogenic enzymes, such as $\Delta^5$3beta-hydroxy steroid dehydrogenase- Δ^{5-4} isomerase (3beta-HSD), Δ^4-3keto-17alpha-hydroxylase (17alpha-hydroxylase, Δ^4-3 keto-17:20 lyase (17-20 lyase), and Δ^4—3 keto-17beta-hydroxy steroid dehydrogenase (17beta-HSD) were carried out according to the method of O'Shaughnessy and Payne (1982) and Sikka et al (1985). Upon sacrifice of the rats, the testes were quickly decapsulated, weighed, and homogenized in ice old potassium phosphate buffer (100 mM), pH 7.4, (1:5 w/v) containing 0.25 M sucrose and 1 mM DTT. The homogenization was carried out using an Eberbach homogenizer at 4 C. The homogenate was centrifuged at 800 × g for 20 min and 1 ml aliquots of the supernatant were frozen at −80 C until assayed for steroidogenic enzymes.

The activity of Δ^5-3beta-hydroxy steroid dehydrogenase-Δ^{5-4} isomerase (3beta-HSD) was determined by measuring the conversion of 4,7-^{3}H pregnenolone (110 Ci/mmol) to ^{3}H progesterone. Incubations were carried out in glass vials containing 50 mM phosphate buffer, pH 7.4, 0.5 mM NAD and NADP, and 200 μl of testicular supernatant (1–2 mg protein) at 32 C for 15 min in a shaking water bath.

The activity of Δ^4-3keto-17alpha-hydroxylase was determined by measuring the conversion of 1,2,6,7,16,17-^{3}H progesterone (50 × 10^4 dpm: 110 Ci/mmol) into ^{3}H-17alpha-hydroxy progesterone, androstenedione, and testosterone at 32 C in an atmosphere of 95% oxygen and 5% carbon dioxide in a shaking water bath. The incubation was carried out for 30 min in 50 mM phosphate buffer containing 0.5 mM NADPH and 200 μl of testicular supernatant.

The activity of Δ^4-3 keto17:20 lyase was assayed by measuring the conversion of 1,2,6,7-^{3}H 17alpha-hydroxy progesterone (74 Ci/mmol; 50 × 10^4 dpm) into ^{3}H-androstenedione and testosterone. The incubation conditions were the same as for 17alpha-hydroxylase.

The activity of Δ^4-3keto-17beta-hydroxy steroid dehydrogenase (17beta-HSD) was determined by measuring the conversion of 1,2,6,7-^{3}H androstenedione (84 Ci/mmol) into ^{3}H testosterone. The incubations were carried out at 32 C for 30 min. The incubation medium contained 50 mM phosphate buffer containing 0.5 mM NADPH and 200 μl of testicular supernatant.

The assay conditions were adopted from Sikka et al (1985) and O'Shaughnessy and Payne (1982) and were found to be linear with both time and enzyme concentrations in the system used. At the end of incubation, the reactions were stopped by the addition of 100 μl of 1N NaOH. ^{14}C-labeled cholesterol, pregnenolone, progesterone, androstenedione, and testosterone were added to account for their recovery.

Extraction, Separation and Crystallization

The samples were extracted repeatedly with chloroform:ether (1:3 v/v) until background counts were achieved in the aqueous phase. The samples were dried under nitrogen and 25 μg quantities of unlabeled steriods (cholesterol, pregnenolone, progesterone, 17-hydroxy-progesterone, androstenedione, and testosterone) were added to facilitate their detection on the chromatograms. The samples were chromatographed on thin layer chromatographic plates (LK6 D) coated with silica gel G (250 μm thick). The plates were then developed in a) chloroform:ether (7:1 v/v), initial run for 30 min; b) cyclohexane:ethylacetate:acetone (5:4:1), second run for 45 min; and c) benzene:methanol (9:2 v/v), third run for 40 min (Sikka et al, 1985). The efficiency of these solvent systems

in separating steroids in the incubation extracts was determined by a series of preliminary experiments in which authenic steroid reference compounds were chromatographed individually and in various combinations. After development of the chromatograms, the spots corresponding to all the steroids could be identified under iodine vapor. The individual areas were scraped from the plates and extracted with methanol (6 × 5 ml). Approximately 20 mg of unlabeled authentic steroids were added to each extract and filtered. The filtrates were then repeatedly crystallized using an acetone:hexane solvent system. Three to four crystals of constant specific activity and $^{14}C/^{3}H$ ratios were obtained as determined by counts in the liquid scintillations spectrometer (LKB model 1214 Rackbeta). Since ^{14}C-17 hydroxy-progesterone was not available, three crystals of constant specific activity were obtained without a $^{14}C/^{3}H$ ratio. The authenticity of the crystals were randomly tested by running TLC. The data were corrected for procedural losses and expressed as percentage conversion/mg protein. Protein was estimated by Lowry's procedure (1951).

Blood Levels of CsA

Cyclosporine levels were determined according to the method of Sawchuck and Cartier (1981). Briefly, CsA was extracted with methyl-t-butyl ether from a known amount of blood and dried under nitrogen at 40 C. The samples were dissolved in a mixture of methanol:0.05 M HCl: hexane (2:1:7), shaken and centrifuged. The hexane layer was aspirated and added to a mixture of 0.025 M HCl:dichloromethane (1:8), shaken and centrifuged. The organic layer was aspirated and dried under nitrogen at 40 C. Finally, the samples were reconstituted with 100 μl of methanol, and 40 μl aliquots were injected for HPLC separation using u-Bondapak-C18 columns, a Waters HPLC-M 45 solvent delivery system, and a Lambda Max Model 481 detector. Sodium phosphate buffer, pH 5.4, and acetonitrile were used in a ratio of 31:69 to elute cyclosporine from the column.

Determination of Serum Creatinine

Serum creatinine levels were determined using kits from the Sigma Chemical Company (St. Louis, MO)).

Statistical Analysis

All the data except fertility data were analyzed with one-way analysis of variance and the Neuman-Keul's multiple test at 5% level of significance. The data from the fertility test were analysed with the two-tailed Fisher's exact test with Bonferroni adjustment to compensate for additive type I error.

Results

Body and Organ Weights

Body weight was assessed to determine the effect of the treatment on the general health of the rats. The administration of cyclosporine did not affect body weight. Similarly, the weight of the testis was not altered after CsA treatment for 45 days. However, CsA administration resulted in a significant decline in the weights of the epididymis, seminal vesicle, and prostate (Fig. 1).

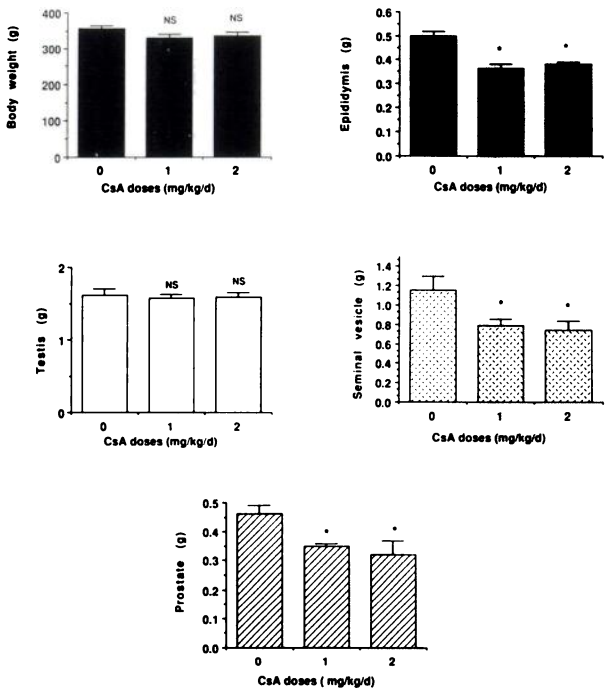

Fig. 1. Effect of CsA on body and reproductive organ weights. *P < 0.05; NS = not significant.

Quantitative Analysis of Spermatogenesis

In CsA-treated rats, the number of type A spermatogonia and preleptotene spermatocytes was not affected, while the counts of pachytene spermatocytes and step VII spermatids were significantly reduced (Fig. 2).

Sperm Counts, Motility, and Fertility Test

Table 1 illustrates the results of the effects of 1 and 2 mg/kg/d CsA administration on sperm counts, motility, and fertility. A 50–70% decrease was noted in testicular and epididymal sperm counts in the CsA-treated groups. Cyclosporine administration also resulted in a more than 50% decline in epididymal sperm motility.

Table 1 also demonstrates that 1 or 2 mg/kg/d CsA impaired fertility to the extent that only 40% of the treated males were fertile. Because spermatozoa were seen in the vaginal smear of all female rats, CsA appeared to inhibit fertility but not mounting ability.

Serum Levels of Testosterone and LH

The serum levels of testosterone were significantly lower in rats treated with 1 and 2 mg/kg/d CsA. This was associated with an elevation of the peripheral levels of LH (Table 2).

Intratesticular Levels of Steroids

The measurement of intratesticular levels of steroids revealed an almost two-fold elevation in the concentrations of pregnenolone and 17hydroxyprogesterone after CsA treatment. In contrast, the levels of progesterone, androstenedione, and testosterone were decreased significantly (Fig. 3).

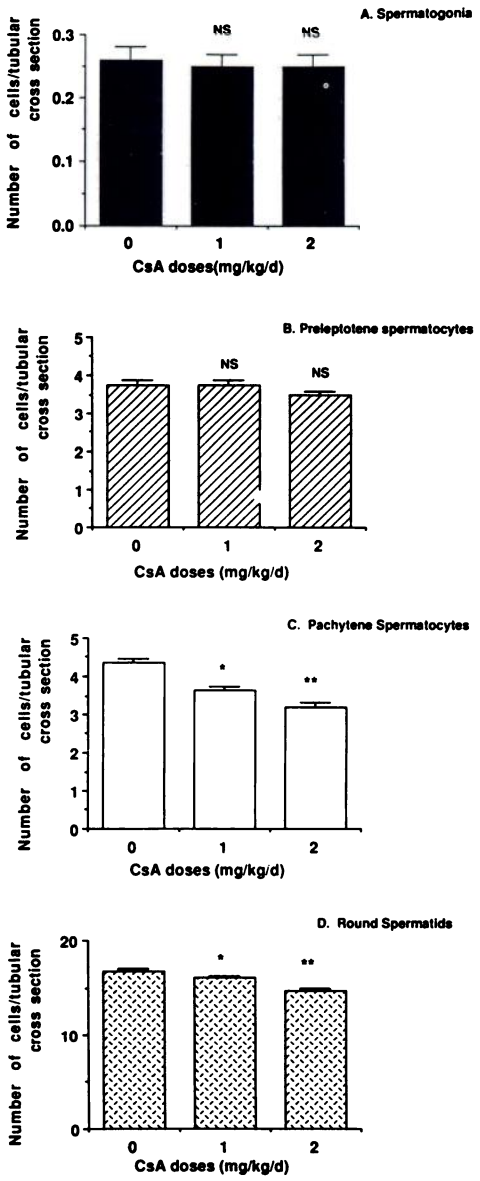

Fig. 2. Quantitative analysis of spermatogenesis in CsA treated rats. *P < 0.05 as compared to control. **P< 0.05 as compared to both control and CsA (1mg/kg/d) groups. NS = Not significant.

TABLE 1. Effects of CsA on Sperm Count, Motility, and Fertility

Parameter	Control	CsA 1mg/kg/d	CsA 2mg/kg/d
Sperm counts: (× 10^7/100mg tissue)			
Testicular	7.17 ± 0.12	3.67 ± 0.37*	3.21 ± 0.04*
Epididymal	38.00 ± 3.40	22.69 ± 4.99*	21.67 ± 1.70*
Sperm motility (%)	50.00 ± 1.70	25.69 ± 3.88*	24.36 ± 2.31*
Fertility (number of fertile males/ number of treated males)	12/12	5/12†	5/12†

The values are mean ± S.E.
*P < 0.05.
†P < 0.002.

Activities of Steroidogenic Enzymes

The administration of CsA reduced the activity of only 3beta-HSD. The activities of other steroidogenic enzymes were not significantly altered (Fig. 4).

Blood Levels of CsA

The determination of blood CsA by HPLC revealed that blood levels in the treated groups were 230–350 ng/ml. This concentration is lower than therapeutic levels used in human immunosuppression after organ transplantation (Table 2).

Serum Creatinine

The serum levels of creatinine in the 1 and 2 mg/kg/d CsA-treated rats were similar to the control values. This suggests that renal function was not significantly impaired in these animals (Table 2).

Discussion

This study demonstrates that CsA has a profound effect on the reproductive system of the male prepubertal rat. Although CsA did not decrease body weight and testicular weight, it did decrease the weights of the other reproductive organs, and arrest the spermatogenic process, resulting in a decline in pachytene spermatocytes. As a consequence, the number of spermatids and spermatozoa were

decreased. CsA also reduced the motility and the fertilizing ability of spermatozoa. Finally, CsA induced a decrease in serum testosterone and an increase in LH levels. Although, the circulating levels of CsA increased in a dose-dependent manner, the effects of both the 1 and 2 mg doses of CsA on the male reproductive tract were similar. This suggests that a certain critical threshold for CsA toxicity has been reached with the lower dose, and that small increments of CsA do not have proportionately increasing toxic effects on male reproduction.

In the present study, CsA did not alter the mean testicular weight. The fact that spermatogenesis decreased significantly with CsA treatment whereas testicular weight did not is somewhat puzzling. While there is a strong correlation between testicular weight and spermatogenesis, there is excellent evidence that toxic agents decrease spermatogenesis greatly before decreasing testicular weight (Van Kroonenburgh et al, 1986; Ratnasooriya and Sharpe, 1986). Our previous studies have shown that higher doses of CsA (20–40 mg/kg/d) decrease testicular weight and spermatogenesis in the adult rat (Seethalakshmi et al, 1987). The reason that testicular weight was not affected by CsA in this study may be a function of low doses of CsA used (1 and 2 mg/kg/d).

It is well known that spermatogenesis and sperm maturation are androgen-dependent processes, although the amount of androgen required for the quantitative maintenance of spermatogenesis is controversial (Cunningham and Huckins, 1979; Stevens and Steinberger, 1983; Sharpe et al, 1988).

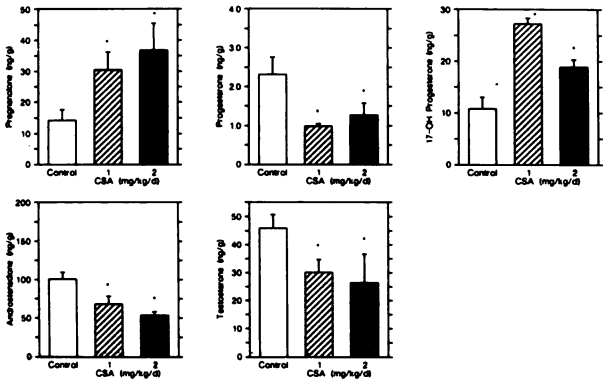

Fig. 3. Effect of CsA on intra-testicular levels of delta-4 steroids. The details of the methods are given in the text. *P < 0.05 as compared to the control group.

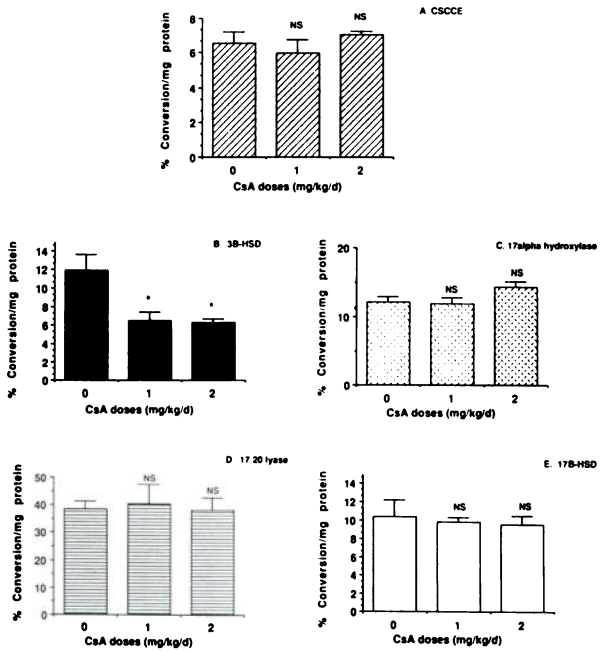

Fig. 4. Effect of CsA on the activities of steroidogenic enzymes. The details of the methods are given in the text. *P < 0.05 as compared to the control group. NS = not significant.

While Stevens and Steinberger (1983) reported that normal high intra-testicular levels of testosterone are necessary for quantitative maintenance of spermatogenesis, Cunningham and Huckins (1979) showed that spermatogenesis could be maintained with 10–20% of the normal testicular levels of testosterone, although subnormal numbers of spermatozoa were produced. In a more recent study, Sharpe et al, (1988) reported that the intra-testicular levels of testosterone need to be maintained at 24–46% of control values for quantitative maintenance

TABLE 2. Effects of Blood Levels of Testosterone, LH, Creatinine, and CsA

Parameter	Control	CsA 1mg/kg/d	CsA 2mg/kg/d
Testosterone (ng/ml)	2.29 ± 0.30	0.98± 0.13*	0.88 ± 0.14*
LH (ng/ml)	23.86 ± 2.11	66.23 ± 7.51*	77.75 ± 6.34*
Creatinine (mg/dl)	0.70 ± 0.03	0.75 ± 0.04†	0.74 ± 0.03†
CsA (ng/ml)		235.00 ± 13.20	333.00 ± 20.97

The values are mean ± S.E.
*P < 0.05.
†Not significant.

of spermatogenesis. In the present study, the intratesticular levels of testosterone in CsA-treated rats were 33–43% of the control; yet, the number of spermatocytes, spermatids, and spermatozoa was reduced. This suggests that an additional factor, such as a direct cytotoxic effect of CsA, may be involved in the arrest of spermatogenesis. CsA is known to be a potent inhibitor of cell proliferation (Kumagai et al, 1988) and exerts its effects in a fashion similar to that of steroid hormones, modulating DNA transcription and selectively inhibiting mRNA synthesis (Aiello et al, 1986).

The administration of CsA to prepubertal rats reduced the levels of testosterone. Therefore, in order to understand the mechanism by which CsA reduced testosterone levels, we examined testosterone biosynthesis. We first determined the intratesticular levels of steroids and discovered that the concentrations of pregnenolone and 17-hydroxy progesterone were elevated significantly, whereas those of progesterone, androstenedione, and testosterone were markedly reduced.

This data suggested that CsA administration to prepubertal rats may have caused a) an activation of CSCCE and 17alpha-hydroxylase resulting in an increased conversion of cholesterol into pregnenolone and progesterone into 17-hydroxy progesterone; b) an inhibition of 3beta-HSD and 17:20 lyase with a consequent increase in their substrates, namely pregnenolone and 17-hydroxy progesterone, respectively; or c) an inhibition of secretion of these steroids. In order to understand the mechanism(s) responsible for altered steroid levels, we measured the activities of the enzymes involved in the delta-4 pathway of testosterone biosynthesis. Our results indicate that long-term treatment of rats with very low doses of CsA significantly inhibits only the activity of 3beta-HSD, as evidenced by a very low percentage conversion of pregnenolone to progesterone. The testicular activity of 17alpha-hydroxylase and 17:20 lyase were not altered in CsA-treated animals. Therefore, the increase in the testicular levels of 17-hydroxy progesterone was not due to an inhibition of enzyme activity but possibly due to an inhibition of its secretion. Sikka et al, (1988) reported that CsA at higher doses inhibited CSCCE and 17:20 lyase *in vitro*. Our investigation on the administration of short-term effects of moderate to high doses of CsA (5–40 mg/kg/d) to sexually mature rats demonstrated that CsA inhibited the activities of all steroidogenic enzymes in a dose-dependent manner (not published). The present study seems to indicate that at much lower doses CsA has a different action on steroidogenic enzyme activity at least in prepubertal rats.

Nephrotoxicity is known to be a major side effect of CsA in animals as well as humans (Cohen et al, 1984; Whiting et al, 1985). Serum creatinine levels were normal in our CsA-treated animals, suggesting minimal or no damage to kidney function by the dosage of CsA used in the present study. However, since the serum level of creatinine is a less sensitive indicator of renal function than the inulin or creatinine clearance tests, subtle nephrotoxicity may have gone undetected in our animals. In the present study, we have not done the latter tests.

Renal failure is known to impair testicular function in both humans and in rats (Lim and Fang, 1975; Handelsman, 1985; Handelsman et al, 1985). Renal insufficiency is known to cause hypergonadotropic-hypogonadism in humans and hypogonadotropic-hypogonadism in rats (Handelsman, 1985; Handelsman et al, 1985). The hypogonadotropic-hypogonadism in uremic male rats is believed to be caused by a defect in the central nervous system that regulates LH secretion by the pituitary. In our CsA-treated animals, serum levels of LH increased, thereby ruling out uremia-induced hypogonadotropic-hypogonadism. Second, in an earlier study, we have demonstrated that in the 5/6 nephrectomy rat with serum creatinine twice the normal value, testicular function and fertility are relatively well maintained (Seethalakshmi et al, 1988). We, therefore, feel that the changes in reproductive function seen in the present study are not the result of subtle nephrotoxicity, if any, but more likely the result of a direct effect of CsA on the reproductive tract.

In conclusion, our results indicate that the chronic treatment of prepubertal rats with low doses of CsA impairs testosterone production, spermatogenesis, and fertility. These results were obtained at circulating levels of CsA well within the therapeutic levels (500 ng/ml) used in immunosuppression after organ transplantation. These observations may have significant clinical implications for the reproductive function of young men undergoing long term CsA administration.

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