Estrogenic inhibition of testicular growth by R2858 in preweanling rats

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Summary. Daily injections of physiologic doses of R2858 into preweanling rats from the 10th to the 19th day of age resulted in reduced testicular growth compared to controls. Body weights and other organ weights were unaffected. Leydig cells were smaller and less numerous, and showed distinct changes in ultrastructural morphology. Results of this study suggest that α-fetoprotein permits normal testicular development during postnatal life.

Administration of estrogenic compounds to adult and sexually immature male rats results in decreased testicular weight, altered Leydig cell morphology and reduced testosterone production[2-4]. Some information is available concerning effects of estrogen treatment in preweanling animals, but it comes from studies using pharmacologic doses of this steroid[5].

The endogenous estradiol in preweanling rats[6] is bound to α-fetoprotein, great quantities of which are found in plasma of young animals[7]. This protein has a great affinity for estradiol, a steroid which it retains for extended periods[8]. Thus, in preweanling rats, estradiol is prevented from leaving the circulation and entering the target cells. One possible role for α-fetoprotein would be to create an estrogen-free environment permitting normal development of the testis. This hypothesis was tested by injecting preweanling rats with low levels of R2858, an estrogen which lacks affinity for α-fetoprotein[7]. If low doses of R2858 in preweanling rats were to affect testicular development, the protective role of α-fetoprotein against estrogen in preweanlings would be confirmed.

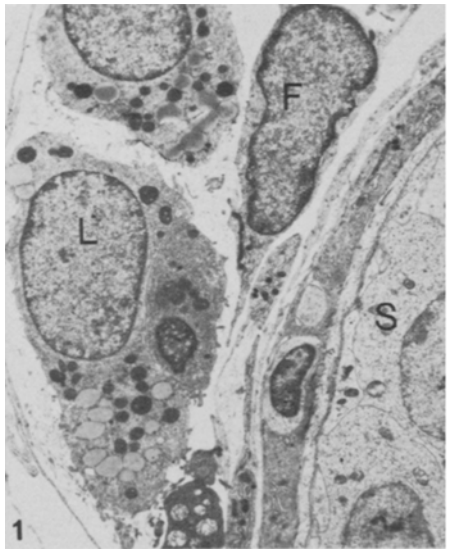
In this study, treatment with R2858 severely and selectively inhibited testicular growth. Ultrastructural examination of affected testes revealed marked changes in Leydig cell morphology, suggesting that as in adult rats, maintenance of the testis depends on normal Leydig cell function[9].

Method and materials. Male Sprague-Dawley rats, 10 days old at the beginning of this experiment were used in this study. Experimental and control rats were paired according to weight. Experimental rats received i.p. 1 of the following regimens of R2858: 0.01 µg or 0.1 µg in 2 µl propylene glycol daily between days 10–14 and 0.02 µg or 0.2 µg in 4 µl propylene glycol daily between days 15–19. The vehicle alone was administered to control animals. On the 20th day the animals were killed, and body weight and weights of testis, anterior pituitary and liver, were determined. Testes from both treatment groups and controls were immersed in paraformaldehyde-glutaraldehyde fixative immediately following excision. Tissues were sliced into 2–3 mm cubes and fixed for 30 min at room temperature and 60 min at 4 °C. After and overnight wash in 1% cacodylate buffer, the tissues were postfixed in 1% osmium tetroxide, dehydrated in ascending alcohols, embedded in araldite 502 and polymerized at 60 °C for 48 h. Thin sections were doubly stained with uranyl acetate and lead citrate before examination using a Philips 300 electron microscope.

The number of animals in the various groups ranged from 5 to 12. Significance of quantitative data was verified by using the analysis of variance and Scheffe's tests.

Results. Weight measurements are presented in the table. There was no significant difference between body weights of control and experimental groups. No differences in pituitary or liver weights were observed. Testicular weights of experimental animals were reduced significantly at both doses of R2858; the largest dose resulted in the greatest change ($p < 0.01$) and the smaller dose produced less of a reduction in the testicular weight ($p < 0.05$).

Ultrastructural features of Leydig cells in control rats were similar to those reported in the literature[3] (figure 1). These epitheloid cells were the largest cells in the interstitium. Numerous lipid droplets, round to ovoid mitochondria and abundant smooth endoplasmic reticulum were randomly dispersed in the cytoplasm. Leydig cells usually appeared in aggregates of 3–5 cells, although solitary cells were also found. In those animals treated with R2858 fewer Leydig cells were observed; generally they were smaller than those in controls. Those that were present had few lipid droplets and there was a concomitant reduction in mitochondria and smooth endoplasmic reticulum. The reduction in cytoplasmic organelles was so severe in the Leydig cells that at



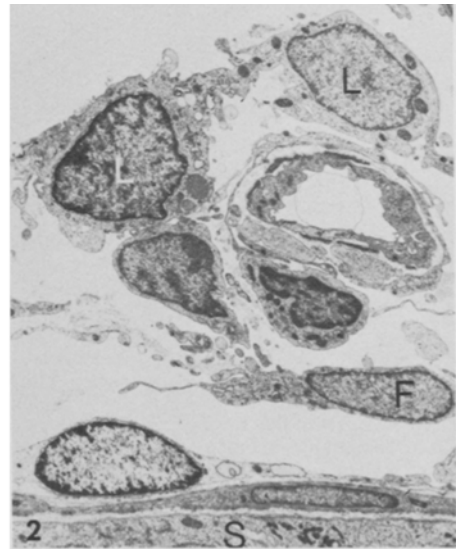


Fig. 1. Portions of several Leydig cells (L) from a sham-injected control. Note the numerous lipid droplets and round to ovoid mitochondria. Most of the cytoplasm is occupied by extensive smooth endoplasmic reticulum. Other components illustrated are fibroblasts (F) and seminiferous tubule (S). ×2700.

Fig. 2. A Leydig cell (L) cluster surrounds a small capillary (C) from an R2858-treated rat. Lipid droplets are absent and mitochondria appears reduced. Smooth endoplasmic reticulum is poorly developed. Fibroblast (F) retains its customary shape. ×2700.

Organ and body weights of preweanling rats treated with different doses of R2858

Treatment	Testis (mg)	Pituitary (mg)	Liver (mg)	Body weight (g)
Control	67.9±3.74	1.7±0.07	1277.2± 36.8	37.4±0.9
0.01 µg	*50.3±1.75	1.8±0.09	1412.0±115.0	39.4±3.28
0.1 µg	**40.2±2.16	1.7±0.11	1224.2± 43.92	35.1±0.52

Results are the mean± SEM of 5–12 determinations. Statistical difference compared to the control: * $p<0.05$; ** $p<0.01$.

times they were difficult to distinguish from adjacent fibroblasts (figure 2).

Discussion. Treatment with R2858 effectively retarded testicular growth in preweanling rats. Since body weights of treated rats were unchanged, testicular weight alterations were presumably not due to a pharmacologic effect of R2858. Because pituitary and liver weight were unaffected, these tissues are apparently unresponsive to estrogen at this time, unless more subtle changes have occurred. Pituitary estrogen receptors are few in young animals[9], but increase in number as the animal ages[10]. The response of the testis to estrogenic treatment in our experiments correlates well with known receptor activity in this organ[11]. Inhibition of testicular growth with the lower dose of R2858 (0.01–0.02 µg/day) indicates a remarkable sensitivity to this agent. Abetting this could be the immaturity of the steroid degrading system of the liver in preweanling animals which might allow accumulation of estrogen[12]. Our ultrastructural observations suggest that reduced testicular growth in treated rats reflects decreased Leydig cell activity. Decreased testosterone production and similar alterations of cellular morphology has been observed in older rats treated with estrogen[3,4].

The present study does not resolve whether R2858 has a direct effect upon Leydig cells or acts indirectly by decreasing pituitary gonadotrophic secretion. Diethylstilbesterol, another estrogenic substance with little affinity for α-fetoprotein, directly inhibits Leydig cell synthesis of testosterone in 28-day-old immature rats[13].

R2858 is a potent estrogenic substance in young female rats. Amounts comparable to those used in this study are uterotrophic in old animals, but similar doses of estradiol are ineffective[7]. The potency of this compound is attributed to its ability to bind with target cell receptors combined with its lack of affinity for α-fetoprotein. α-Fetoprotein is thought to protect the fetus against high levels of estrogen during pregnancy[14]. Injection of large amounts of R2858 into pregnant rats results in underdevelopment of the genital tract (seminal vesicles, prostate, genital tubercle) of the male fetuses[14]. Our study suggests that α-fetoprotein has a similar effect on preweanling animals. Although plasma estradiol is found in these animals, it is probably prevented from acting by α-fetoprotein. That estradiol is not effective in young animals is also evident from observations that a large dose of estradiol in sexually immature rats (21–28 days) had no effect on testicular growth[15].

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Effect of prolactin on human red cell sodium transport

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Summary. Incubation of red cells with higher concentrations of prolactin in vitro enhanced the cellular sodium level and produced a significant reduction in erythrocyte membrane adenosine triphosphatase activity. This effect was dose and time-dependent. It is the result of an inhibition of the active sodium pump similar to that produced by ouabain, suggesting altered red cell function and electrolyte balance in hyperprolactinemic states.

Prolactin is known to play a significant role in the regulation of the fluid and electrolyte balance, since its administration in human volunteers resulted in reduced renal excretion of sodium, potassium and water[2]. Parke and Horrobin[3] have observed a significant difference in plasma concentrations of prolactin when the same blood sample was estimated immediately on drawing and after keeping the sample for 6 h. The plasma prolactin concentrations estimated after 6 h of standing varied inversely with that of the initial concentration. This suggests the possibility that red cells may bind prolactin, exerting a sort of buffering action. Karmali et al.[4] have shown that in vitro addition of higher concentrations of prolactin (50 ng/ml) to blood samples for 6 h produced a small but very consistent fall in plasma sodium by 3% and a very significant increase in plasma osmolality with no change in potassium level. This indicates that prolactin at higher concentrations may enhance the movement of sodium into cells. Since no direct