PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 170-174 (1976)

Suppression of Postnatal Changes in Spermatogenesis, Size, and Nucleic Acid Content of Rat Testis by Chronic Administration of Isoproterenol (39504)

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Introduction. Chronic administration of large doses of the catecholamine isoproterenol can evoke marked proliferative activity in organs that normally exhibit low mitotic levels (1, 2). However, where mitosis is normally high, as in salivary glands of preweanling rats (3) or in intestinal mucosa of adult mouse (4), proliferation is not further increased but is either not affected at all (5, 6) or inhibited somewhat (7). These results suggest that isoproterenol cannot induce mitosis in tissues that are already dividing rapidly. However, the number of systems on which this effect has been examined is limited. Accordingly, it was considered important to examine the effects of chronic administration of isoproterenol on yet another dividing tissue (8). Testis was considered especially useful for this purpose, for while both the immature and mature organs exhibit high levels of mitosis, they also exhibit different levels of maturation (8). Consequently, this system can be used to distinguish between effects of isoproterenol on normal mitotic activity of the tissue and the influence exerted by degree of tissue maturation on such effects.

Materials and methods. Male Long-Evans rats, ranging in age from 10 days to 4 months, were maintained on lab chow and water *ad lib.* until just before sacrifice. A regimen of twice-daily ip injections of isoproterenol (ISO) in a dose previously shown to induce DNA synthesis (15 mg/kg) (2-6) was initiated 8-9 days before sacrifice of half of the males of each litter; the other half of the litter was untreated or injected with saline only. The age at which drug administration was initiated varied, so that groups receiving the drug for 8-9 days were 28, 35, 42-45, 50-51, and 4 months old at the end of the period of drug treatment. The drug did not significantly modify weight of the animals even though it was used in pharmacological doses. At the end of the period of drug administration, animals were anesthetized with 1% Nembutal and both testes were removed and individually weighed on a torsion balance; one was placed in ice-cold 0.4 *N* $HClO_4$ for subsequent nucleic acid extraction and the other was minced in isotonic Na-citrate with hyaluronidase (150 N.F. units/10 ml) for subsequent analysis of spermatogenesis. DNA was determined by the Burton modification of the diphenylamine reaction (9). RNA was determined by the orcinol reaction (10). The course of spermatogenesis was followed using the technique of Evans (11) with modifications as suggested by Schleirmacher (12). The minced testis preparation was placed on a magnetic stirrer for 10 min to release cells from the tubules. Following this, the suspension was allowed to stand a few minutes to allow the remaining tubules and connective tissue to settle to the bottom of the beaker. The supernatant was removed and centrifuged at 660 rpm, and the spermatogonia, spermatocytes, secondary spermatocytes, spermatids, and mature sperm were thus separated. These were treated with 3 ml of hypotonic (1% wt/vol) sodium citrate solution for 12 min, again centrifuged, and the supernatant was discarded. The cell pellet was suspended in a 3:1:0.025 absolute ethanol:acetic acid:chloroform fixative mixture. This suspension was again centrifuged and resuspended in 0.5 ml of fresh fixative.

The microscope slides were made by splashing a few drops of this suspension onto slides which had been chilled in distilled ice water. After drying, the slides were stained with Giemsa and mounted with Permount. Five slides were made from the testes of each rat, and 500 cells were counted. These were separated into appropriate phases of mitosis or meiosis on the basis of their chromosomal configuration.

The stages observed were spermatogonia; leptotene, zygotene, pachytene (L-Z-P); diplotene, diakinesis (D-D); metaphase I (M I) and II (M II); and mature sperm.

Results. From the data in Fig. 1, it is clear that between 10 and 51 days of age, weight, total DNA, and total RNA of rat testis increase progressively. Testis weight increased 66% between 28 and 35 days of age and approximately 55% at weekly intervals thereafter. Total RNA increased about 50% between 28 and 35 days of age, and the weekly increases thereafter were only slightly less than this (approximately 45%). DNA, on the other hand, increased about 35% between 28 and 35 days of age and also 35% between 35 and 42 days of age. The increase was significantly less ($P < 0.05$) at the next interval, and, thus, between 42 and 50 days of age, only a 14% increase appeared. The RNA/DNA ratio was less than 1 at 28 days of age, 1 at 35 days of age, and greater than 1 thereafter; by 50 days of age it was 1.47.

Chronic administration of isoproterenol for periods of 7–8 days did not prevent age-related changes in weight and nucleic acids but the course and characteristics of the changes were greatly altered. Thus, while weight and nucleic acid content of testes of isoproterenol-treated rats increased between 28 and 42 days of age, the magnitude of the increase in each age (28, 35, and 42 days) was less than that exhibited by testis of untreated littermates (Fig. 1). Thus, at 28 days of age, the weight of testis in isoproterenol-treated rats was 27% less than that of the testis of untreated littermates, and DNA and RNA levels were each 20% less than those of untreated animals. The differences at 35 days were somewhat greater: weight was 36% less, and DNA and RNA were from 25–30% less. The smallest difference was seen at 42 days, when weight and RNA of testis of isoproterenol rats were about 20% less than that of untreated rats, and DNA was only 10% less. The RNA/DNA ratio of testis of isoproterenol treated rats

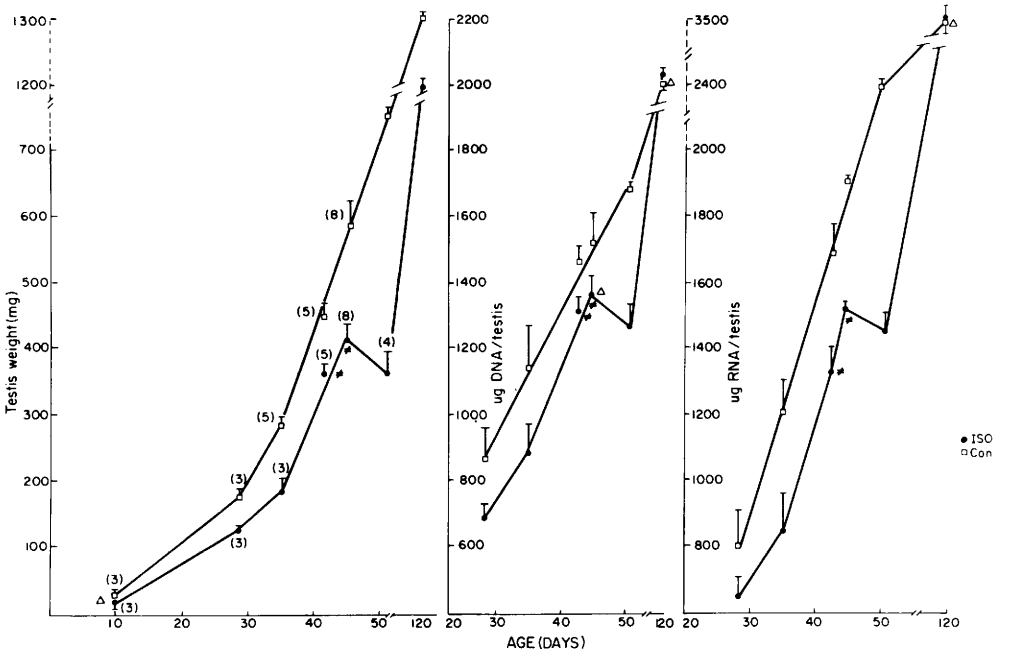

FIG. 1. Postnatal changes in weight, DNA, and RNA content of testes of untreated rats (Con) and of rats chronically injected with isoproterenol (ISO). Each point is the mean ± SE obtained from three to eight animals; equal numbers of littermates were untreated or injected with 15 mg/kg of isoproterenol, ip, twice daily for 7–8 days. Nucleic acids (DNA and RNA) are expressed as micrograms per testis, and wet weight of testis in milligrams. Changes from one age to the next that were not of statistical significance ($P > 0.05$) are designated with ≠; differences between Con and ISO-treated animals, at each age, were statistically significant ($P < 0.05$) except when designated with Δ.

TABLE I. POSTNATAL CHANGES IN NUMBER OF MATURE SPERM AND DISTRIBUTION OF MITOTIC AND MEIOTIC CELLS OF SEMINIFEROUS TUBULES OF RAT TESTIS, AND MODIFICATION BY CHRONIC ADMINISTRATION OF ISOPROTERENOL.[a]

Age (days)	Number of rats	Percentage spermatogonia[b] (mitotic)	Percentage (meiotic)				Total percentage of meiotic cells[c]	Number of sperm
			L-Z-P	D-D	M I	M II		
Control								
28	4	55.4 ± 0.9	36.5 ± 0.6	6.9 ± 0.05	0.8 ± .17†	0.2 ± 0.2	45.6	0
35	6	45.7 ± 1.0	49.1 ± 1.0	4.3 ± 0.4	0.6 ± 0.05	0.03 ± 0.03	54.3	0
42	14	38.2 ± 0.8	54.7 ± 1.1†	6.0 ± 0.4	0.8 ± 0.1	0.01 ± 0.01	61.8	146 ± 24
50	5	24.7 ± 0.8	55.5 ± 0.4	18.3 ± 0.8	1.2 ± 0	0.35 ± 0.1	75.3	454 ± 16
Adult	5	37.4 ± 1.3	53.5 ± 2.3	8.4 ± 1.5	0.7 ± 0.1	0	62.6	1103 ± 60
ISO-treated								
28	6	55.8 ± 1.1*	37.3 ± 0.9*	5.6 ± 0.2	0.9 ± 0.1	0.2 ± 0.1	44.2	0
35	6	52.3 ± 0.5	44.2 ± 0.7†	3.4 ± 0.3	0.4 ± 0.1†	0	47.7	0
42	12	43.5 ± 1.2†	46.6 ± 1.6†	7.7 ± 1.3*	0.5 ± 0.1	0.09 ± 0.06	56.5	54 ± 35
50	5	44.1 ± 1.2	44.8 ± 1.6	8.7 ± 0.2	1.5 ± 0.2*	0.7 ± 0.2*	55.9	141 ± 46
Adult	5	35.4 ± 2.6*	52.1 ± 1.2*	11.6± 3.5*	0.9 ± 0.1*	0.04 ± 0.04*	64.6	923 ± 84*

[a] Values are means ± SE. Changes from one age to the next that are not of statistical significance ($P > 0.05$) are designated with †; differences between control (untreated) and ISO-treated (twice daily ip injections of isoproterenol for 7–8 days) animals, at each age, were statistically significant ($P < 0.05$) except when designated with *. Stages as follows: spermatogonia (mitotic); L-Z-P, leptotene-zygotene-pachytene (meiotic); D-D, diplotene-diakinesis (meiotic); M I and M II, metaphase I and metaphase II (meiotic); sperm (mature sperm, and values are number of sperm counted in a field of 500 spermatogenic cells).

[b] Number of cells at each stage expressed as percentage of total number of spermatogenic cells (excluding sperm).

[c] Value in each case is sum of L-Z-P, D-D, M I, and M II percents.

was the same as that for controls at 28 days of age and 35 days of age; however, unlike the untreated rats, no change occurred thereafter and RNA/DNA was between 1.0–1.1.

Although administration of isoproterenol to adults for 7–8 days caused a statistically significant decrease in weight of testes ($P < 0.05$), the change was small in magnitude (7%), and the other parameters measured were not affected at all (Fig. 1).

As age of the animals increased, there was not only the prominent shift in pattern of increase in cell size and number but there was also a shift in the events of spermatogenesis. Isoproterenol effected changes in the course of spermatogenesis that were consistent with effects of this agent on size and nucleic acid content of the testes. The data in Table I show these effects, and proportions of cells found in seminiferous tubules for each age are shown for control as well as ISO-treated animals. As an untreated (control) animal aged, the number of cells found in the spermatogonial stage progressively decreased, and, at 28 days, this number represented 55% of all spermatogenic cells; at 35 days of age, 46%; at 42 days, 38%; and at 50 days of age, 25%. The change from one age to the next was in each case statistically significant ($P < 0.05$). As the percentage of spermatogonial cells decreased, the percentage of meiotic cells increased. Thus, at 28 days, meiotic cells comprised approximately 45% of all spermatogenic cells; at 35 days, 54%; and at 50 days, 75%. The distribution of cells at each meiotic stage also changed with increasing age of the animals and, for example, at 28 days of age, about 37% of the total spermatogenic cells were in L-Z-P stages of meiosis, and by 50 days of age, 56% were.

Mature sperm were not found as a consequence of spermatogenesis before 42 days of age, when approximately 142 sperm were found in a field of 500 spermatogenic cells; by 50 days, there was more than a threefold increase in this number (Table I).

In rats 21 days old at the time of initial injection of isoproterenol, the number of spermatogonial cells was not different from that of untreated rats, and approximately 56% of all spermatogenic cells were in the spermatogonial stage after 7 days of ISO treatment (Table I). As with untreated rats, the number of spermatogonia decreased with increasing age of the animals, but the extent of the decrease was much less. Thus, a statistically significant decrease ($P < 0.05$) was not observed until 42 days of age, at which time the number of spermatogonia had decreased to 44%. No further decrease

was seen at 50 days. Here also, the number of cells entering meiosis increased with age, but the magnitude of the age-related changes was much less than that exhibited by controls. Thus, at 28 days of age, 44% of all spermatogenic cells were in meiosis; this percentage did not change until 42 days of age when about 57% were in meiosis and remained at 57% at 50 days (Table I).

Some age-related changes in the percentage of cells at each meiotic stage were seen in the isoproterenol-treated testes also. However, only a few of the changes were significant. For example, the percentage of all spermatogenic cells in L–Z–P phases of meiosis was about 37 at 28 days of age, increased to 44 at 35 days, but remained at this level thereafter (Table I). Generally, the number of meiotic cells in the tubules of ISO-treated rats was, for each age except 28 days, less than the number found in tubules of untreated rats.

Mature sperm were first observed at 42 days of age in ISO-treated animals, the same time at which mature sperm were first observed in the testis of untreated animals. However, only 54 ± 35 were counted in a field of 500 tubular cells, in contrast to a level three times as great in the controls.

In the adult, the percentage of cells in the spermatogonial stage was about 37, 63% were thus in meiosis, and about 1100 mature sperm were consequently seen. Isoproterenol did not cause any statistically significant changes in any of these values (Table I).

Discussion. Growth and differentiation of testes normally continue postnatally (8, 13), but when isoproterenol is chronically administered to the immature rat, both processes are greatly retarded. On the basis of measurements of nucleic acids and weight, it was apparent that isoproterenol caused a decrease in cell number of rat testis at each postnatal age (28–50 days) examined. In the early phase (28 days old), the magnitude of the decrease in DNA, RNA, and weight were the same, and the RNA/DNA ratio remained at 1. Thus, only cell number, and not cell size, was affected by the drug. At later periods also (42 and 50 days), cell number, but not cell size, was reduced by the isoproterenol. From examination of the effects on spermatogenesis, the drug apparently acts to suppress cell proliferation by inhibiting the mitotic activity of spermatogonial cells. The normal age-related decrease in percentage of spermatogonial cells was inhibited by the isoproterenol and, as a consequence of this inhibition, fewer cells entered into meiosis. However, the distribution of cells among the various meiotic phases was not much modified by the isoproterenol regimen, and usually only the L–Z–P phases reflected the decrease in number of spermatogonia entering meiosis. Ultimately, the number of mature sperm formed also reflected the inhibitory influence exerted by isoproterenol on the spermatogonia, and the number in isoproterenol-treated rats was only one-third that of the control animals at 42 and 51 days of age.

The suppression of spermatogenesis was accompanied by a general suppression of maturation. For example, in preliminary experiments, it was found that the testosterone level in blood of a 42-day-old untreated male was 40/ng%, but no testosterone was measured in blood of the isoproterenol-treated littermates.

The inhibitory effects of the isoproterenol on testes were limited to the testes of immature animals. No significant changes in testes weight, DNA, or RNA were observed when adult animals were chronically injected with isoproterenol. Inhibitory effects of isoproterenol on cell proliferation have been found in salivary glands also, but here also only the undeveloped (15), rapidly dividing glands of the immature rat exhibit such a response (7), and the fully developed and mitotically quiescent glands of the adult animal (or even of older postnatal rats) (16) show a pronounced proliferative activity following isoproterenol administration (1, 2). The dividing cells of the fully developed intestinal mucosa of adult rats also show no response to isoproterenol (5, 14). Thus, the inhibitory effects on mitosis appear to be associated with already rapidly dividing systems, but only those of undifferentiated organs. The mechanism of these effects remains to be delineated.

Summary. Chronic administration of large doses of the catecholamine isoproterenol to immature rats suppressed the postna-

tal changes in testis size and spermatogenesis that normally occur. Nucleic acid content (total DNA and RNA) was reduced from controls at each weekly interval examined. With increasing age there is normally a decrease in number of spermatogonia observed in seminiferous tubules, and at 28 days of age, 56% remain, but by 50 days, only 25% remain. This change is altered by treatment with isoproterenol, and while the number at 28 days is the same, the number remaining at 50 days had decreased to a much smaller extent so that 44% remained. Isoproterenol thus greatly inhibited spermatogenesis, and the number of cells in meiotic stages and number of mature sperm formed were also greatly decreased from control. While mature sperm were first found in untreated and ISO-treated animals at the same age (42 days), the number in ISO-treated rats was only one-third that of controls. This same order of reduction remained evident at 50 days also. Treatment of adults with isoproterenol for 7 days did not alter nucleic acid content, course of spermatogenesis, or production of mature sperm.

This work was supported in part by National Institutes of Health Grant DE 02110. The authors wish to thank Dr. A. Siegel, Clinical Pathology, University of Alabama Medical Center, for determination of testosterone levels in serum.

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Received March 31, 1976. P.S.E.B.M. 1976, Vo 153.