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Interactions of Testosterone and Estradiol-17β on the Reproductive Tract of the Male Rat[1]

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ABSTRACT

The effects of subcutaneous sustained-release implants of testosterone and estradiol-17β, given either alone or in combination to adult male rats, on the weights of testis and sex accessory tissues, the testicular content of spermatids and spermatozoa and the serum concentrations of testosterone, estradiol-17β and gonadotropins were investigated. Increasing amounts of testosterone, in the absence of added estradiol, caused a biphasic response (decline followed by a partial recovery) in testicular weight and in the number of spermatids and spermatozoa/testis. Serum testosterone initially remained unchanged and subsequently rose. This pattern was reflected by the weights of sex accessory tissues. Before serum testosterone rose, serum LH decreased to undetectable levels. Serum estradiol-17β levels were not affected by increasing doses of testosterone. Low doses of estradiol-17β (0.1 cm and 0.3 cm implants), in the absence of added testosterone, had no significant effect on serum estradiol-17β levels, on testicular weights or on the testicular content of spermatids and spermatozoa, but did cause a fall in serum LH and testosterone levels and in the weights of sex accessory tissues. Higher doses of estradiol-17β resulted in increased serum estradiol-17β levels and either a decrease or a sustained low level for all other measured parameters (weights of testes and sex accessory tissues and testicular content of spermatozoa and spermatids). Depending on the doses, the combinations of testosterone and estradiol-17β resulted in 2 types of interaction: 1) at low doses, these 2 steroids acted synergistically to decrease testicular content of spermatids and spermatozoa and testicular weights but had little or no effect on serum testosterone and estradiol-17β or weights of sex accessory tissues; 2) at higher doses, there was an apparent direct antagonism between these 2 compounds on all tissue components measured.

INTRODUCTION

The effects of testosterone administration on spermatogenesis, serum levels of luteinizing hormone (LH) and testosterone and on accessory sex tissue weights in male rats have been studied extensively (Ludwig, 1950; Clermont and Harvey, 1967; Steinberger, 1971; Walsh and Swerdloff, 1973; Berndtson et al., 1974; Verjans et al., 1975; Albert, 1961 and others). These studies have established that the administration of "low" doses of testosterone to intact male rats diminishes spermatogenesis and markedly diminishes serum LH titers without influencing serum testosterone levels or weights of accessory sex tissues (seminal vesicle or ventral prostate). In contrast, the administration of "high" doses of testosterone results in a return of spermatogenesis, increased serum testosterone and proportionately increased weight of sex accessory tissues without a concomitant increase in serum LH.

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A number of studies have been done on the effects of estradiol-17β on the adult male rat (Lacy and Lofts, 1965; Steinberger and Duckett, 1965; Oshima et al., 1967; Swerdloff and Walsh, 1973; Danutra et al., 1973; Chowdhury et al., 1974; Verjans et al., 1974; Van Beurden et al., 1977; Sivella et al., 1978). It is clear that estradiol-17β is a potent inhibitor of gonadotropin release. Swerdloff and Walsh (1973), Van Beurden et al. (1977) and others have concluded that the observed regression of the reproductive tract of the male rat after estradiol-17β treatment is due only to this gonadotropin-release inhibitory potential whereas Chowdhury et al. (1974), Sivella et al. (1978), Steinberger et al. (1978) and others have presented evidence that there is a direct effect of estradiol-17β on the testis. In addition, Karr et al. (1974) and Andersson and Müntzing (1972) have found a direct effect of estradiol-17β on rat accessory sex tissues such as the ventral prostate. It should be noted, however, that the dose, regimen and route of administration of estradiol-17β differed markedly in most of these studies.

The interaction(s) of testosterone and estradiol-17β has not been studied as extensively (Jay and Dever, 1971; Grayhack, 1965; Briggs and Briggs, 1974; Ewing et al., 1977 and others). Ramirez and McCann (1965) suggest that these 2 compounds act synergistically on gonadotropin release whereas the results of Jay and Dever (1971) do not support this hypothesis. However, due to the different animal models and different doses and routes of drug administration, it is difficult to compare these studies.

We have recently shown that testosterone and estradiol-17β act synergistically to inhibit spermatogenesis (Ewing et al., 1977). In that study subdermal polydimethylsiloxane (PDS) implants of the 2 steroids were used to provide a sustained administration of the drugs. In the present communication, an extension of the above study is presented wherein a greater spectrum of doses of testosterone, estradiol-17β and of their combinations is utilized (via PDS implants) in order to understand further the nature of the interactions of these steroids. The 2 questions that we will attempt to answer here are: 1) Does estradiol-17β work solely via the inhibition of gonadotropin release or does it, in addition, have a direct effect on the testis and/or on the seminal vesicle and ventral prostate? 2) Can these effects be overcome by testosterone administration?

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (250–300 g) were randomly assigned to 1 of 42 treatment groups (5–6 animals/group). Six groups received subdermal polydimethylsiloxane (PDS, Silastic) testosterone implants measuring 0, 1.0, 2.5, 4.0, 6.0 or 12.0 cm, respectively. Seven groups received subdermal PDS estradiol-17β implants measuring 0, 0.1, 0.3, 0.5, 0.7, 1.0 or 6.0 cm, respectively. The remaining 30 groups received all possible combinations of these testosterone and estradiol-17β PDS implants to complete the 6 × 7 matrix.

All animals were weighed at the time of capsule implantation and again 3 months later, just prior to decapitation. After decapitation, trunk blood was collected and serum was prepared and stored at −20°C prior to measuring testosterone, estradiol-17β, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Testes, seminal vesicles and ventral prostate were removed, blotted and weighed. Fluid contained in the seminal vesicles was extruded by positive pressure prior to weighing.

The content, for each testis, of spermatids and spermatozoa was determined by hemocytometric counting of testicular homogenates under phase contrast microscopy according to the method of Robb et al. (1978).

Polydimethylsiloxane Implants

Polydimethylsiloxane (PDS) tubing (Dow Corning 602–305) and Silastic medical adhesive (Dow Corning 891) were used for the preparation of PDS implants according to a method previously described (Stratton et al., 1973). The release rates for testosterone and estradiol-17β were ∿30 μg/day/cm and 2.4 μg/day/cm, respectively.

Radioimmunoassay of Steroids

Testosterone. Testosterone concentrations in rat serum were measured by radioimmunoassay according to a method previously described by this laboratory (Schanbacher and Ewing, 1975). The antisera to testosterone-3-BSA was provided by Dr. S. A. Tillson. Testosterone obtained from Steraloids Inc. was recrystallized; [1,2,6,7^{3}H]-testosterone was purchased from New England Nuclear Corp. and repurified by thin layer chromatography at monthly intervals. The lower limit of detection of testosterone was ∿0.2 ng/ml. The intraassay coefficient of variance was found to be 14.4% while the interassay coefficient of variance was 19.4%.

Estradiol-17β. The concentration of estradiol in rat serum was measured according to a radioimmunoassay procedure described by England et al. (1974). Rabbit antisera to estradiol-6β-(0-carboxymethyl)oxime bovine serum albumin and [^{125}I]-tyrosine methyl ester-7-succinyl-estradiol-17β were obtained from Micromedic Systems Inc., Horsham, PA. Sheep antisera to rabbit gamma globulin was purchased from Antibodies, Inc., Davis, CA. Radioinert estradiol used for recovery determinations and standard curves and other steroids used to check the specificity of estradiol antisera were

purchased from Steraloids, Inc., Wilton, NH and recrystallized before use. We purchased [2,4,6,7,16,17-^{3}H]-estradiol (152 Ci/mmole from New England Nuclear Corp. and repurified it before use as described by Abraham et al. (1970). Standard curves were constructed for each assay using radioinert estradiol-17β diluted in 0.1% gel-PBS at 10 concentrations, in triplicate, to give a range from 1–1000 pg/assay tube. The results of each assay were analyzed together with a computer program which uses a logit-log transformation to obtain a linear inhibition curve (Duddleson et al., 1972).

The amount of estradiol-17β present in extracts prepared from 500 μl aliquots of water, pooled serum obtained from adrenalectomized and orchidectomized rats and pooled serum from adult male rats after treatment with 1% charcoal (1 h at 55°C) fell below the minimal level of estradiol-17β that could be detected with 95% confidence (<2 pg/assay tube). Addition of 100 pg of radioinert estradiol-17β to 500 μl aliquots of water and pooled serum (treated with 1% charcoal as above and containing 500 DPM of [^{3}H]-estradiol-17β) resulted in values of 98.6 ± 3.5 pg and 103.1 ± 3.4 pg (mean ± SEM; n = 12), respectively. Repeated determinations of the estradiol-17β content of aliquots from the same pool of serum from adult male rats averaged 34.6 ± 3.4 pg/ml (± SEM; n = 27) with intra-and interassay coefficients of variation of 3.7% and 4.9%, respectively.

Radioimmunoassay of Gonadotropins

Immunoreactive luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations were determined with reagents supplied, in part, by NIAMDD. These included NIAMDD:Rat-LH-1-4 Anti-Rat-LH-S-4, Rat-LH-RP-1, Rat-FSH-1-3, Anti-Rat-FSH-S6 and Rat-FSH-RP-1. Sheep anti-rabbit gamma globulin (SARGG) was obtained from Antibodies, Inc.

Double antibody radioimmunoassays were performed according to the general format described by Niswender et al. (1969). Serum samples were assayed at 2 dose levels selected to give activity that inhibited the binding between 20%–80%. Final values, expressed in terms of the indicated NIAMDD reference preparations, represent weighted mean potency estimates given by linear regression analysis of the combined log dose-logit response curves of the reference preparation and unknown samples obtained by using the computer program, RIANAL-6 (Duddleson et al., 1972). The minimal amount of hormone detected/assay tube (LH = 0.55 ng Rat-LH-RP-1; FSH = 12.3 ng Rat-FSH-RP-1) was determined by calculating the amount of hormone corresponding to the lower 95% confidence limits of the radioactivity present in buffer control tubes (no hormone).

The coefficient of variation, expressed in percent, for 15 determinations made on serum samples from control rat serum throughout the course of these studies averaged 6.4 for LH and 6.9 for FSH. Serum samples from rats hypophysectomized for 2–3 days consistently gave values that were indistinguishable from the buffer control tubes containing no hormone ($P>0.05$).

Statistical analyses. Data were analyzed using a two-way analysis of variance, regression analysis and unpaired Student's t test on a Hewlett-Packard 9830 according to procedures described by Snedecor and Cochran (1967).

RESULTS

Serum Hormones

Testosterone. As increasing doses of testosterone were administered alone to adult male rats (Table 1), the serum level of this hormone initially did not increase (1.0 and 2.5 cm PDS implants) but then rose significantly ($P<0.05$) with higher doses (4.0, 6.0 and 12.0 cm PDS implants). With increasing doses of estradiol-17β alone, the serum testosterone level fell rapidly. However, it is interesting to note that in the presence of exogenously administered testosterone, the serum testosterone levels in all animals were not significantly affected by the administration of increasing amounts of estradiol-17β (Table 1).

Estradiol-17β. The results shown in Table 2 demonstrate that serum estradiol-17β levels were not elevated by administration of low doses (0.1 and 0.3 cm estradiol-17β PDS implants) of this hormone, but rose significantly ($P<0.05$) when implants measuring 0.5 cm or longer were utilized. This observation is analogous to that of the effect of increasing doses of exogenous testosterone on serum testosterone described above. Furthermore, it is clear from Table 2 that exogenously administered testosterone, in the dose range used, had no measurable effect on serum estradiol-17β.

Gonadotropins. The serum level of LH in untreated adult male rats was 8.05 ± 0.70 ng/ml. The 2 lower doses of testosterone (1.0 and 2.5 cm PDS implants), when administered alone, diminished serum LH to 2.08 ± 1.16 and 2.88 ± 0.89 ng/ml, respectively; both values are significantly ($P<0.05$) lower than control values but do not significantly ($P>0.05$) differ from each other. Serum LH was brought to undetectable levels when higher doses of testosterone (4.0, 6.0 and 12.0 cm PDS implants) were administered. The lowest dose of estradiol-17β (0.1 cm implant), when used alone, led to a significant ($P<0.05$) decrease in serum LH to 2.66 ± 0.66 ng/ml. All higher doses of estradiol-17β alone resulted in a nondetectable serum LH level. Furthermore, serum LH was below the limit of detection in animals receiving any combination of testosterone and estradiol-17β implants.

Control animals had a serum FSH level of

TABLE 1. The effects of testosterone and estradiol-17β filled PDS implants on serum testosterone level in rats.[a]

Estradiol-17β implant length (cm)	Testosterone implant length (cm)					
	0	1.0	2.5	4.0	6.0	12.0
0	2.28 ± 0.18[b]	1.77 ± 0.12	3.00 ± 0.31	3.15 ± 0.28	4.80 ± 0.55	7.77 ± 0.95
0.1	0.92 ± 0.24	1.05 ± 0.16	2.20 ± 0.09	3.15 ± 0.16	4.00 ± 0.31	7.47 ± 0.50
0.3	0.58 ± 0.08	1.68 ± 0.40	2.63 ± 0.11	3.65 ± 0.20	3.88 ± 0.14	7.00 ± 0.34
0.5	0.50 ± 0.03	1.17 ± 0.14	2.57 ± 0.18	3.95 ± 0.34	4.52 ± 0.56	8.04 ± 0.94
0.7	0.62 ± 0.12	1.32 ± 0.14	2.23 ± 0.16	3.08 ± 0.19	4.30 ± 0.16	7.17 ± 0.61
1.0	0.55 ± 0.07	1.08 ± 0.05	2.35 ± 0.16	3.12 ± 0.15	4.30 ± 0.31	7.53 ± 0.78
6.0	0.58 ± 0.15	1.57 ± 0.10	2.32 ± 0.15	3.53 ± 0.11	4.47 ± 0.35	8.64 ± 1.16

[a]Capsules were made from Dow Corning #602–305 tubing (0.2 mm wall thickness, 3.18 mm o.d.) and were implanted subdermally for 3 months.

[b]Values are expressed as ng/ml and represent the mean ± SEM; n = 6.

410 ± 99 ng/ml. Although the serum FSH in all 41 treatment groups ranged from 185 ± 29 ng/ml to 408 ± 30 ng/ml, no single treatment group had a value for serum FSH that was significantly different ($P > 0.05$) from that of control animals. In addition, no significant trend, as analyzed by regression analysis, was observed for any group of treatments, e.g. increasing testosterone dosage alone.

Tissue Weights

Seminal vesicles. Like serum testosterone levels, the wet weight of seminal vesicles initially remained unchanged (1.0 and 2.5 cm PDS implants) and then increased (4.0, 6.0, 12.0 PDS implants) as increasing doses of testosterone were administered alone (Table 3). A progressive decline in the wet weight of seminal vesicles was observed when estradiol-17β PDS implants of increasing lengths were given alone. Note that the higher doses of estradiol-17β alone caused a decline in seminal vesicle weight (Table 3) whereas serum testosterone had already declined to undetectable levels (Table 1). The lowest dose of testosterone used (1.0 cm PDS implant) was sufficient to abolish the decrease in tissue weight due to estradiol-17β at all but the 2 highest (1.0 and 6.0 cm PDS implants) estrogen doses. At higher doses of testosterone (2.5, 4.0, 6.0 and 12.0 cm PDS implants) no tested dose of estradiol-17β caused a decrease in seminal vesicle wet weight.

Ventral prostate. Certain characteristics described above for the response of the seminal vesicles to testosterone and estradiol-17β are applicable to the observed changes in the

TABLE 2. The effects of testosterone and estradiol-17β filled PDS implants on serum estradiol-17β level in rats.[a]

Estradiol-17β implant length (cm)	Testosterone implant length (cm)					
	0	1.0	2.5	4.0	6.0	12.0
0	45.8 ± 10.7[b]	47.5 ± 13.9	48.0 ± 13.0	40.6 ± 5.0	57.8 ± 11.7	38.3 ± 4.8
0.1	46.2 ± 3.3	31.5 ± 7.7	42.7 ± 4.1	65.2 ± 4.0	79.6 ± 16.8	50.7 ± 9.0
0.3	49.9 ± 2.6	53.4 ± 6.8	51.7 ± 10.8	68.3 ± 8.7	50.8 ± 7.7	57.8 ± 2.3
0.5	116.5 ± 13.2	105.4 ± 15.3	235.6 ± 47.4	109.7 ± 12.2	99.8 ± 6.6	95.0 ± 12.9
0.7	148.5 ± 39.2	103.5 ± 12.8	92.3 ± 9.2	92.6 ± 4.4	112.6 ± 22.7	108.9 ± 10.4
1.0	103.7 ± 8.7	95.8 ± 17.2	108.9 ± 9.9	108.8 ± 9.0	101.6 ± 11.7	128.4 ± 11.3
6.0	397.9 ± 33.1	439.4 ± 25.3	369.6 ± 27.3	350.5 ± 18.0	384.7 ± 25.7	411.6 ± 38.3

[a]Capsules were made from Dow Corning #602–305 tubing (0.2 mm wall thickness, 3.18 mm o.d.) and were implanted subdermally for 3 months.

[b]Values are expressed as pg/ml and represent the mean ± SEM; n = 6.

TABLE 3. The effects of testosterone and estradiol-17β filled PDS implants on seminal vesicle weights (mg) in rats.[a]

Estradiol-17β implant length (cm)	Testosterone implant length (cm)					
	0	1.0	2.5	4.0	6.0	12.0
0	454 ± 8[b]	384 ± 15	464 ± 26	532 ± 25	559 ± 17	716 ± 28
0.1	258 ± 28	316 ± 22	451 ± 21	566 ± 22	617 ± 39	702 ± 57
0.3	248 ± 29	350 ± 12	508 ± 28	639 ± 44	616 ± 22	739 ± 61
0.5	169 ± 14	316 ± 21	579 ± 28	599 ± 16	597 ± 33	703 ± 15
0.7	199 ± 39	315 ± 4	493 ± 17	623 ± 15	680 ± 13	739 ± 23
1.0	95 ± 10	284 ± 17	417 ± 20	635 ± 23	674 ± 46	710 ± 25
6.0	74 ± 10	271 ± 7	422 ± 19	596 ± 35	723 ± 47	879 ± 87

[a]Capsules were made from Dow Corning #602–305 tubing (0.2 mm wall thickness, 3.18 mm o.d.) and were implanted subdermally for 3 months.

[b]Values represent the mean ± SEM; n = 6.

weight of the ventral prostate (Table 4): 1) increasing doses of testosterone alone, initially had no effect and then led to an increase in tissue weight; 2) increasing estradiol-17β doses alone caused a continuous progressive decline in the weight of the ventral prostate and it is of interest to note here that the decrease from control caused by the highest estradiol-17β dose was 13.1-fold for the ventral prostate while it was 6.1-fold for the seminal vesicles; 3) at the lowest testosterone dose (1.0 cm PDS implant), a significant decline in ventral prostate weight was observed as estradiol-17β doses led to a decrease in ventral prostate weight at testosterone doses ranging up to 6.0 cm PDS implant; at the highest testosterone dose (12.0 cm PDS implant), however, no significant change in this tissue's weight ($P>0.05$) was found as increasing doses of estradiol-17β were administered.

Testes. The paired testicular weights for the 42 treatment groups of rats are shown in Table 5. Increasing the dose of testosterone alone first caused no significant effect on the weight of this tissue (1.0 cm PDS implant), then led to a sharp decline ($P<0.05$) in testicular weight (2.5 and 4.0 cm PDS implants) and was subsequently responsible for a partial maintenance in testicular weight (6.0 and 12.0 cm PDS implants). This apparently complex response of a tissue to the administration of a single drug was also found in the response of the content of spermatids and spermatozoa in this tissue and will be analyzed in the Discussion. Increasing estradiol-17β dosage (0.1 and 0.3 cm PDS implants), initially resulted in no significant

TABLE 4. The effects of testosterone and estradiol-17β filled PDS implants on rat ventral prostate weight (mg).[a]

Estradiol-17β implant length (cm)	Testosterone implant length (cm)					
	0	1.0	2.5	4.0	6.0	12.0
0	577 ± 62[b]	513 ± 66	687 ± 57	814 ± 48	893 ± 52	812 ± 59
0.1	358 ± 36	433 ± 31	678 ± 34	946 ± 28	855 ± 76	847 ± 27
0.3	286 ± 35	431 ± 25	617 ± 42	692 ± 93	905 ± 80	951 ± 75
0.5	172 ± 31	356 ± 31	652 ± 59	799 ± 60	663 ± 58	947 ± 56
0.7	169 ± 48	303 ± 24	522 ± 32	671 ± 72	819 ± 40	876 ± 65
1.0	81 ± 6	250 ± 17	510 ± 27	649 ± 64	701 ± 46	1066 ± 44
6.0	44 ± 9	266 ± 15	436 ± 21	575 ± 48	739 ± 42	847 ± 73

[a]Capsules were made from Dow Corning #602–305 tubing (0.2 mm wall thickness, 3.18 mm o.d.) and were implanted subdermally for 3 months.

[b]Values represent the mean ± SEM; n = 6.

TABLE 5. The effects of testosterone and estradiol-17β filled PDS implants on rat paired testis weight (g).[a]

Estradiol-17β implant length (cm)	Testosterone implant length (cm)					
	0	1.0	2.5	4.0	6.0	12.0
0	3.35 ± 0.09[b]	3.18 ± 0.14	1.65 ± 0.30	1.53 ± 0.08	2.03 ± 0.11	2.24 ± 0.37
0.1	2.98 ± 0.13	1.36 ± 0.34	0.98 ± 0.02	1.12 ± 0.10	1.83 ± 0.06	2.16 ± 0.07
0.3	3.10 ± 0.11	0.67 ± 0.06	0.93 ± 0.04	1.17 ± 0.06	1.68 ± 0.15	2.25 ± 0.17
0.5	2.39 ± 0.20	1.19 ± 0.27	0.92 ± 0.06	1.14 ± 0.08	1.12 ± 0.13	2.24 ± 0.05
0.7	2.11 ± 0.42	1.09 ± 0.33	0.78 ± 0.06	0.95 ± 0.05	1.62 ± 0.08	1.98 ± 0.20
1.0	1.04 ± 0.12	0.61 ± 0.03	0.81 ± 0.02	0.75 ± 0.06	1.00 ± 0.88	2.08 ± 0.11
6.0	0.48 ± 0.03	0.58 ± 0.04	0.70 ± 0.05	0.94 ± 0.12	1.14 ± 0.09	1.57 ± 0.12

[a]Capsules were made from Dow Corning #602–305 tubing (0.2 mm wall thickness, 3.18 mm o.d.) and were implanted subdermally for 3 months.

[b]Values represent the mean ± SEM; n = 6.

change ($P>0.05$) in testicular weight and then led to a dramatic diminution in the size of the gonads of these animals, such that administration of 6.0 cm estradiol-17β PDS implant resulted in a 7-fold decline in testicular weight. At every dose of testosterone used, it may be noted that the simultaneous administration of at least some doses of estradiol-17β resulted in a significant ($P<0.05$) decrease in paired testicular weight. At the highest testosterone dose (12.0 cm PDS implant), this effect of estradiol-17β was significant ($P<0.05$) only with the highest estradiol-17β dose used (6.0 cm PDS implant). It should also be noted that, as for seminal vesicles and ventral prostate weights, at the highest estradiol-17β dose, a progressive increase in testicular weight was found with increasing testosterone dosage. There was also a supra-additive (synergistic) interaction between low doses of testosterone (1.0 and 2.5 cm PDS implants) and of estradiol-17β (0.1 and 0.3 cm PDS implants) on testicular weight.

Testicular Content of Spermatids and Spermatozoa

The biphasic response of the spermatid and spermatozoa content of rat testis to the administration of increasing doses of testosterone is shown in Table 6. It may be noted that this biphasic response, the trough at the 4.0 cm implant and the partial maintenance with 6.0 and 12.0 cm implants, is similar but more pronounced than that observed for the paired

TABLE 6. The effects of testosterone and estradiol-17β filled PDS implants on the number of spermatids and spermatozoa/rat testis ($\times 10^6$).[a]

Estradiol-17β implant length (cm)	Testosterone implant length (cm)					
	0	1.0	2.5	4.0	6.0	12.0
0	234 ± 12[b]	193 ± 9	61 ± 39	17 ± 6	80 ± 19	157 ± 11
0.1	194 ± 18	38 ± 26	<1[c]	3.8 ± 1.8	85 ± 7	130 ± 11
0.3	205 ± 15	<1	<1	3.5 ± 1.4	43 ± 11	95 ± 29
0.5	125 ± 23	<1	<1	3.9 ± 1.7	14 ± 10	130 ± 5
0.7	105 ± 36	<1	<1	1.0 ± 0.3	41 ± 8	93 ± 23
1.0	24 ± 10	<1	<1	1.0 ± 0.1	26 ± 6	126 ± 10
6.0	<1	<1	<1	<1	5.4 ± 2.5	76 ± 16

[a]Capsules were made from Dow Corning #602–305 tubing (0.2 mm wall thickness, 3.18 mm o.d.) and were implanted subdermally for 3 months.

[b]Values represent the mean ± SEM; n = 6.

[c]For the values <1, the mean is indistinguishable from 0.

testicular weights (Table 5). Increasing doses of estradiol-17β, given alone, (0.1 and 0.3 cm PDS implants) initially had no significant effect ($P>0.05$) on the testicular content of spermatids and spermatozoa and then caused a marked depression in this parameter, resulting in azoospermia at the highest estradiol-17β dosage. The synergism between low testosterone and estradiol-17β dosages noted above for testicular weights is apparent. In addition, it is clear that even at the highest testosterone dose used, estradiol-17β can exert a marked ($P<0.05$) effect on testicular content of spermatids and spermatozoa.

DISCUSSION

The results presented above indicate that testosterone and estradiol-17β interact with each other in the adult intact male rat in 2 different ways: 1) at low doses of both steroids, a supra-additive or additive interaction probably via the pituitary and/or hypothalamus is observed on testicular weight, testicular content of spermatids and spermatozoa and serum LH but not on serum testosterone concentration, estradiol-17β, seminal vesicle weight or ventral prostate weight; and 2) at higher doses of testosterone and estradiol-17β, an apparently direct competition at the target organ is observed between these 2 steroids on seminal vesicle, ventral prostate and testicular weights and content of spermatids and spermatozoa. Furthermore, a major difference exists in the relative sensitivity of the seminal vesicles, ventral prostate and testes to the capacity of testosterone to overcome the effects of estradiol-17β.

The synergistic aspect of the testosterone-estradiol-17β interaction has been discussed previously (Ewing et al., 1977). Though the mechanism by which this synergism occurs remains unclear, it most probably is mediated by a specific interference with the hypothalamo-pituitary-gonadal axis as opposed to by more general peripheral effects such as on the clearance or metabolism of steroids. The lack of effect of these low doses of steroid on either sex accessory tissues or serum levels of estradiol-17β or testosterone contrasts with the decrease in spermatogenesis and serum LH. This suggests that the hypothalamus and/or pituitary may be either sensing minute changes in steroid level, or, more probably, sensing the form of the steroid message: episodic steroid release in untreated animals (Bartke et al., 1973; Katongole et al., 1971; Kinson and Liu, 1973) vs relatively constant steroid release from PDS implants (Davidson et al., 1976). Thus, if this hypothesis were proven, the mode of administration as well as the dosage used would be critical in mediating a response from tissues such as the pituitary and hypothalamus. Recent observations by Plant et al. (1978) support this hypothesis. The potential contraceptive usefulness of a combination of testosterone and estradiol-17β has been suggested by Ewing et al. (1977), Briggs and Briggs (1974) and Lacy et al. (1973). The results shown here lend support to this suggestion.

The other type of interaction observed at higher steroid doses between testosterone and estradiol-17β has the characteristics of a direct competition between 2 drugs exerting opposite effects. By comparing Tables 3, 4 and 5, it becomes apparent that the atrophy of testes and sex accessory tissues induced by estradiol-17β may be countered by testosterone in sufficient quantity. The differences observed in the response of these 3 tissues, when the 2 steroids are administered simultaneously, indicate that the seminal vesicles are least affected by exogenous estradiol-17β, the ventral prostate is more responsive to this estrogen and the testes are most affected. Since it is well established that a large component of the testicular weight is associated with the spermatogenic function of this tissue, the changes observed in testicular weight (Table 5) with different hormone treatments may be due to the large changes observed in testicular content of spermatids and spermatozoa (Table 6). The competitive interaction of these 2 steroids is observed at doses at which serum LH is undetectable and serum FSH is not significantly affected, thus implying that this interaction is not mediated by the hypothalamus and/or the pituitary. In addition, the observed complete suppression of the production of spermatozoa without a significant reduction in serum FSH points to the presumably reduced testicular testosterone as the main, if not the only, mechanism of testosterone and/or estradiol-17β action in these animals. It would be of interest to compare in the same study, the relative binding affinities of testosterone and estradiol-17β to the androgen receptors in these 3 tissues. Thus, estradiol-17β can apparently exert a direct effect on male sexual organs in the rat by competing with testosterone. This effect,

however, can be detected only if serum estradiol-17β levels are raised substantially from the control serum level.

Some of the apparent discrepancies that may be found in the scientific literature (Chowdhury et al., 1974; Verjans et al., 1974; Van Beurden et al., 1977; Sivella et al., 1978) regarding the direct vs indirect effect of estradiol-17β in the male rat may simply be due to utilization of a single or a few doses of estradiol-17β, or to the different modes of steroid administration. The results presented above strongly suggest that detailed dose-response studies are essential to elucidate the action and interaction of steroids in male reproduction.

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