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# SHORT COMMUNICATION

# DIRECT INHIBITION OF TESTICULAR FUNCTION IN RATS BY ESTRIOL AND PROGESTERONE

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## SUMMARY

Polydimethylsiloxane implants containing progesterone or estriol, placed in close proximity to the left epidymis, released sufficient amounts of the hormone to induce atrophy of the left testis. The weight of the other testis was not influenced. Measurements of testosterone and FSH concentration in peripheral plasma and of fertility indicated that the function of the pituitary–hypothalamo axis was not inhibited. Progesterone and estriol were released in sufficient amounts to reduce 5α-reductase activity in the prostate. In progesterone treated animals the activity of 17β-dehydrogenase was increased.

## INTRODUCTION

To date male contraceptive methods based on systemic administration of hormones have not gained acceptance because the suppression of the hypothalamo-pituitary axis and atrophy of the gonads and accessory sex tissues are undesirable. The success achieved with intrauterine progesterone contraception [1] has led us to consider the possibility of designing a drug delivery system capable of inhibiting fertility in the male without interfering with the hypothalamo–pituitary function. To our knowledge attempts directed towards this goal have hitherto not been reported. The present communication describes experiments undertaken to explore this possibility.

## EXPERIMENTAL PROCEDURES AND RESULTS

Polydimethylsiloxane (PDS) implants made by filling tubing (O.D. 3.125 mm, I.D. 1.95 mm, length 20 mm) with the appropriate crystalline steroid were tied in close proximity to the left epididymis of adult male rats and left *in situ* for 7 or 28 days. Weights of proximal and distal testis, and of ventral prostate, expressed as tissue ratio (organ weight in mg × 100/body weight in g) were used to judge treatment effect. Prior to autopsy [$^3$H]-1,2,6,7-testosterone ($20.4 \times 10^6$ d.p.m. for 20 min pulse, and $51.1 \times 10^6$ d.p.m. for 60 min pulse) was injected i.v. Plasma protein were precipitated by adding sufficient methanol to 70% concentration, removed by centrifugation, washed once with 70% aqueous methanol and again centrifuged. The supernatants were combined, the solvent removed *in vacuo*, dissolved in ether and portioned between ether–water (three transfers). Ventral prostate was extracted with methanol (tissue grinder), the solvent was separated by centrifugation and purified as described below. Testosterone (T), 17β-hydroxy-5α-androstan-3-one (DHT) and 4-androstene-3,17-dione (A-dione) isolated from plasma and the prostate by paper and thin layer chromatography were crystallized to constant radiospecificity following addition of non-radioactive authentic specimens. A second experiment (controls and progesterone only) was performed employing the same protocol except that at the end of the experimental period (28 days) heparinized blood was drawn by heart puncture, plasma was separated, and testosterone and FSH were measured by radioimmunoassay. Testosterone antiserum was obtained from Endocrine Sciences, Tarzana, CA. All assays were in duplicate. Initial purification by ammonium sulfate precipitation in place of chromatographic procedures was used in one set of tests; the results were comparable to determinations using chromatographic purifications [2]. Follicle stimulating hormone (FSH) was determined using the procedures recommended by the National Institutes of Arthritis, Metabolism and Digestive Disease, National Institutes of Health, Reed Pituitary Hormone Distribution Program. All assays were in duplicate at two levels. A third group of animals (controls and progesterone implants) were carried for 28 days. At the end of this period each male was placed in individual cages with two adult females for 14 days. The females were then separated and observed until delivered.

Table 1 summarizes the effect of progesterone and of estriol (1,3,5(10)-estratriene-3,16α,17β-triol) on organ weights; a small group of animals with estradiol implants was included for control purposes. The data show that in both experiments progesterone and estriol diffused from PDS implants in sufficient amounts to induce significant atrophy of the proximal testis. It will be noted that the inhibition of testis growth (about 20% compared to controls) was about the same regardless of treatment duration. In animals having the estriol implant for 28 days testis atrophy was 57% compared to controls. The lack of ventral

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Table 1. Body and organ weights of male rats following treatment with different steroids in DPS implants

| Steroid used | No. of observations | Body wt. by ±S.E. | Tissue ratio ±S.E.[a] Prox. testis | Distal testis | Ventral prostate |
|---|---|---|---|---|---|
| Experiment 1 | | | | | |
| Control | 7 | 565 ± 23 | 364 ± 21 | 367 ± 22 | 87 ± 12 |
| Progesterone[b] | 11 | 475 ± 9 | 289 ± 21 | 421 ± 27 | 102 ± 11 |
| Estriol[c] | 8 | 377 ± 8 | 156 ± 11 | 467 ± 14 | 102 ± 6 |
| Estradiol[d] | 4 | 345 ± 19 | 73 ± 7 | 83 ± 9 | 4 ± 0.4 |
| Experiment 2 | | | | | |
| Control[c] | 8 | 534 ± 33 | 307 ± 33 | 358 ± 13 | 78 ± 3 |
| Progesterone[c] | 8 | 478 ± 27 | 256 ± 47 | 370 ± 18 | 79 ± 6 |

[a] Tissue ratio = (organ weight in mg/body weights in g) × 100; [b]7 days; [c]28 days; [d]60 days.

prostate inhibition indicates a lack of interference with the hypothalamo–pituitary–gonadal axis.

In contrast, in animals having the estradiol implant both testis and the ventral prostate were severely atrophied (80% and 95%, respectively) indicating that this steroid blocked the pituitary function. It is to be noted, however, that in this group the implants were left *in situ* for about twice as long (60 days) as the estriol implants.

In these experiments no attempt was made to determine the amount of steroids which were released from the implants. Based on our previous results [3] and those of others [4–7] it can be estimated that each progesterone implant released between 10 and 50 μg of progesterone per 24 h. The permeability of DPS to estradiol is much less than that to progesterone; we estimate that estradiol implants released between 2 and 10 μg of the hormone in 24 h; estriol was most likely released at an even lower rate (between 0.5 and 2 μg/24 h).

Measurements of testosterone and FSH in peripheral plasma showed no significant differences between controls and progesterone treated groups. Average values were 4.4 ± 1.0 ng/ml (±S.E.M.) in the control group and 4.6 ± 0.8 ng/ml in the group having progesterone implant; FSH concentration was 222 ± 9.1 ng/ml and 300 ± 16.4 ng/ml for the two groups, respectively. These findings further support the fact that hypothalamo–pituitary–gonadal axis was not inhibited by the progesterone treatment.

Fertility of males having a progesterone implant was not impaired; 5/6 males sired litters during the 14 days of observation. In the control group the number was 6/6. The number of pups born in both groups was average for our colony (8–16 pups per litter) and we noted no malformations.

Extraction of heparinized plasma obtained from rats in the control, estriol and progesterone groups followed by ether–water partition (three transfers) and radioactivity determination (Mark II Nuclear Chicago) revealed no marked differences in animals sacrificed 20 min after the i.v. injection. In control and progesterone treated animals (60 min pulse) there appeared to be an increase of radioactivity in the aqueous phase associated presumably with water soluble metabolites indicating an increase in conjugation and/or clearance rates (Table 2). Additional experiments would be needed to determine this.

Purification of the fractions isolated from the ventral prostate first by descending paper chromatography (propylene glycol-hexane) followed by purification on thin layer silica plates afforded three fractions corresponding in mobility to T, DHT and A-dione tracers. Addition of authentic specimens and crystallization to constant radiospecificity (ethyl acetate, aqueous methanol, and ethanol–water) established the authenticity of the isolated substances. The amount of radioactivity (expressed as d.p.m./mg steroid per 100 mg tissue) provided an estimate of the relative amounts of each steroid isolated (Table 3). The results obtained may serve only as a relative indication since we did not use a second tracer to calculate for losses and did not measure in this experiment endogenous hormonal production. Despite these limitations some conclusions can be drawn.

Both estriol and progesterone implants influenced the bioconversion of testosterone to 5α-dihydrotestosterone in the prostate. In control animals the ratio testosterone/DHT was 2.2 (20 min pulse) and 8.4 (60 min pulse) indicating increased 5α-reductase activity with time. In contrast the ratio was only about one-half that in estriol treated animals, while in progester-

Table 2. Distribution of radioactivity isolated from plasma of male rats pretreated with progesterone or estriol ([$^3$H]-testosterone by i.v.)

| Treatment group | 20 min pulse, d.p.m. Ether[b] | Water[c] | Ratio[d] | 60 min pulse, d.p.m. Ether | Water | Ratio[d] |
|---|---|---|---|---|---|---|
| Control | 10760 | 19730 | (0.55) | 5790 | 13340 | (0.43) |
| Estriol | 8240 | 17990 | (0.49) | 8940 | 43290 | (0.16) |
| Progesterone | 8150 | 21380 | (0.38) | 11780 | 94690 | (0.12) |

[a] d.p.m./ml of plasma; [b]ether extractable; [c]water soluble; [d]ratio d.p.m. ether/d.p.m. water.

Table 3. Radiospecificity of testosterone (T), dihydrotestosterone (DHT) and androstenedione (A) isolated from ventral prostate (animals pretreated with progesterone, or estriol)

| Treatment group | d.p.m./mg steroid per 100 mg tissue T | DHT | A |
|---|---|---|---|
| 20 min pulse | | | |
| Control | 260 | 570 | 64 |
| Estriol | 120 | 172 | 18 |
| Progesterone | 600 | 110 | 180 |
| 60 min pulse | | | |
| Control | 218 | 1820 | 50 |
| Estriol | 110 | 500 | 17 |
| Progesterone | 1200 | 560 | 295 |

one treated groups the ratio was only 0.2 and 0.5, respectively. The marked inhibition of 5α-reduction in progesterone implanted animals (left *in situ* for 7 days only) indicates the high anti-androgenic potency of this natural steroid [8]. Progesterone treatment also apparently increased 17β-dehydrogenase activity in the prostate. The accumulation of A-dione was significantly elevated in the prostates of progesterone treated males as compared to the other two groups. It has been shown by Bruchowsky and Wilson [9] that the conversion of testosterone to dihydrotestosterone is inhibited by estradiol, diethylstilbestrol and progesterone *in vitro*. The same group has also shown increases in 17β-dehydrogenase activity as a result of estrogen treatment. Dörner *et al.* [10] postulated a direct effect on testicular androgen secretion in men suffering from prostatic cancer given high doses (12 g) of diethylstilbestrol phosphate intravenously.

Our results are in support of these findings and show further that local inhibition of testosterone bioconversion to dihydrotestosterone can be achieved by microgram amounts of progesterone, or weak estrogens such as estriol, provided these are incorporated in a drug dosage form which permits a continuous release in a close proximity to the target tissue. Such a system should lend itself to treatment of prostatic hypertrophy without exposing the patient to systemic therapy.

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