EFFECT OF CHRONIC LOCAL ADMINISTRATION OF NORETHISTERONE ENANTHATE ON THE TESTES OF ADULT RAT

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Summary: Silastic implants containing crystalline Norethisterone Enanthate (NE) inserted into the epididymal fat-pads unilaterally were found to bring about a drastic reduction \( P<0.01 \) in the size and weight of the testis six weeks after implant insertion on the side bearing the progestin implant. The ipsilateral (treated side) testis, caput and cauda epididymis and the vas deferens showed either a complete absence of spermatozoa or occasional few immotile and decapitated sperms. In contrast, the smears obtained from the contralateral testis and the corresponding epididymal segments and the vas showed a population of entirely normal and motile spermatozoa. There was apparently no effect on the epididymis as the ipsilateral epididymis was almost similar (weight-wise) to that of the contralateral side. The results suggested an apparently local effect of NE implants inserted into the epididymal fat pads. The feasibility of the approach for long-term fertility regulation in males is discussed.

Key words: silastic implant epididymal fat-pad norethisterone enanthate ipsilateral (treated-side) contralateral (Sham-operated control-side)

INTRODUCTION

Unlike ovulation which is a cyclic reproductive phenomenon spermatogenesis is an arrhythmic process extending over about 42-72±2 days in different mammalian species. For any hormonal method employed for the regulation of male fertility, it thus, becomes necessary to sustain the effect over periods extending beyond the time required for the completion of at least one spermatogenic cycle before one could expect the manifestation of the antifertility effects of the method. With that pre-requisite fully realized, a variety of synthetic steroidal compounds administered orally, parenterally, and through slow-releasing implants have been investigated during the last few years (2,8,9,13,18,19,20). Eventhough quite a few of these proved fairly effective yet their use was found to be associated with a decrease in libido, one of the most important limiting factors in the development of an acceptable male contraceptive.

Since epididymis is critically involved in sperm maturation and the fact that the spermatozoa sojourn in the epididymis during their transit to the cauda epididymis for storage is of a relatively longer duration, efforts have been mainly directed at this organ as being perhaps, the most suitable locus for interrupting the sequela of male reproductive functions. Further, its delicate physiological equilibrium with the testicular androgen biosynthesis on
the one hand and the peripheral levels of circulating androgens, testosterone and dihydrotestosterone on the other, with a relatively higher threshold requirement for the two androgens (2) besides its own steroidogenesis potential (3,6,7,10,11,12) renders the epididymis as perhaps the most vulnerable locus for interruption of male reproductive functional coordination. It was, thus, conceivable that even slightest alteration in the biosynthetic equilibrium of the epididymis induced via a locally administered sustained-delivery steroidal preparation might produce the desired antifertility effects.

The present studies were undertaken to test the above hypothesis and for the purpose, effects of norethisterone enanthate chronically released from silastic capsules inserted in close proximity of the caput epididymis were investigated with a view to evaluate the feasibility and potential of such an approach for the long-term regulation of male fertility.

MATERIALS AND METHODS

Norethisterone Enanthate (NE) was prepared and purified in our own laboratories from Norethisterone (Schering AG, Berlin) by the standard methods. Crystalline NE was filled in 8-13 mm segments of the Silastic tubing no. 602-265 and 602-360 (Dow Corning Corporation, Michigan). The capsules were prepared as per the procedure already described elsewhere (21). The implants were dried down to constant weight before insertion and at removal, for calculating the 'average daily rate of release of the progestin'.

Colony-bred adult albino male rats (body weight 180-250 g) and with good collective fertility record were used for the study. The right testis along with the epididymal fat-pad was delivered into a wound made by a midventral supra-pubic incision under light ether anaesthesia. A single implant was inserted into the epididymal fat-pad through a small nick with the help of a thin-walled trocar and canula between the caput epididymis and the anterior end of the testis (Fig. 1). The implant was secured in place with the help of a short length nylon monofilament sutured through the fat. Complete asepsis was maintained as far as possible and the implants were dipped in 1% Cetavelon prior to insertion. The epididymal fat-pad of the contralateral side (Sham operated self-control) was similarly inserted with an empty silastic capsule (Gr.I). After 6 and 9 weeks of treatment (Gr. II & Gr. III respectively), the animals were killed by cervical dislocation and at autopsy the testis, epididymis and the vas deferens of the treated (ipsilateral) and control (contralateral) sides were quickly removed. The two epididymides were quickly weighed and immediately divided into three segments viz., caput, cauda and the corpus and the smears from each of the three epididymal segments, and the vas from the two sides were immediately examined under microscope for the relative quantity and quality of the sperm population. The weights of the testes were recorded, and the two testes were fixed in Bouin’s fluid. Paraffin sections (5 μ) were stained with haematoxylin and eosin for histological studies.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>6 weeks</th>
<th>9 weeks</th>
</tr>
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<tbody>
<tr>
<td>Gr. I</td>
<td>Control</td>
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<tr>
<td>Gr. II</td>
<td>Treatment</td>
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<td>Gr. III</td>
<td>9 weeks</td>
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* Average daily treatment dose
January-March 1980
Ind. J. Physiol. Pharmac.

Local Effects of Norethisterone Enanthate in Male

RESULTS

Extended treatment with Norethisterone enanthate released in chronic micro-doses locally at an average daily rate of 25-44 µg brought about drastic changes in the size, weight and gametogenic function of the ipsilateral testis of adult rat. As is evident from the results in Table I, while the treatment induced a significant reduction (P<0.01) in the weight

\[ \text{Average daily rate of release of NE as computed at the termination of the experiment.} \]

Number of animals in parentheses.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose of NE* (µg/24 hr)</th>
<th>No. of animals</th>
<th>Weight of testis (g)</th>
<th>Weight of epididymis (g)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control side</td>
<td>Treated side</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Control side</td>
<td>Treated side</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>(5)</td>
<td>1.786±0.235</td>
<td>1.762±0.185</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>24.4-25.2</td>
<td>(5)</td>
<td>1.756±0.245</td>
<td>0.641±0.070</td>
</tr>
<tr>
<td></td>
<td>6 weeks</td>
<td></td>
<td></td>
<td>0.47±0.01</td>
<td>0.47±0.02</td>
</tr>
<tr>
<td></td>
<td>9 weeks</td>
<td>17.5-44.06</td>
<td>(5)</td>
<td>1.817±0.142</td>
<td>0.717±0.081</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>0.43±0.02</td>
<td>0.45±0.03</td>
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Fig. 1: Line diagram showing the position of implant in situ.
of the testis on the side bearing the NE implant, the testis on the contralateral side remained unaffected after six weeks of treatment (Gr. II). Continuation of the treatment for another 3 weeks did not produce any further decrease in the weight of the ipsilateral testis (Gr. III). Similarly no significant differences were found between ipsi- and contralateral epididymis.

With regard to the germinal function of the testis, extended treatment with locally administered NE implants apparently exerted a selective local effect as indicated by the complete absence of spermatozoal cells in the smears taken from the ipsilateral testis and the entirely normal spermatozoal population in the smears of the contralateral testis. Further, the smears obtained from the ipsilateral caput- and cauda segments of the epididymis and the vas showed either a complete absence of spermatozoa or the presence of occasional dead, immotile, agglutinated and/or decapitated spermatozoa. In contrast, the smears taken from the corresponding epididymal segments and the vas deferens of the contralateral side showed completely normal population of motile spermatozoa.

Fig. 2a: 'Treated' Testis: Note the highly shrunken and desquamated seminiferous tubules devoid of spermatogenic cell populations except the occasional presence of spermatogonia in some tubules and spermatogenesis still on in isolated tubules. The interstitium is relatively large and loose containing Leydig cells which appear to be normal. HE (X 80).

Histologically the seminiferous tubules. All elements were present abundantly. Le testis showed marked decrease the gross decrease in testicular elements and the absence the occasional tubules (Fig. 2). Spermatozoa could be identified.
on the contralateral side remained intact. The treatment for another 3 months of the ipsilateral testis (Gr. III). Ipsilateral and contralateral epididymis.

extended treatment with locally effective effect as indicated by the presence of spermatozoa or the presence of motile spermatozoa. In contrast, the testis on the side bearing empty implants showed normal seminiferous tubules. All elements of the germinal series could be identified and for most part were present abundantly. Leydig cells too were normal (Fig. 2b). However, the treated-side testis showed marked decrease in size of seminiferous tubules, which, perhaps, accounted for the gross decrease in testicular weight. There was marked vacuolization in the chief germinal elements and the absence of secondary spermatocytes, spermatids and spermatozoa. In occasional tubules (Fig. 2a) spermatogenesis seemed to have continued unabated and spermatozoa could be identified.

DISCUSSION

The results of the present study indicate that sustained local administration of Norethisterone enanthate could provide effective long-term regulation of male fertility with perhaps no consistent adverse effect on the libido. The normal production of spermatozoa by the contralateral (control) testis in contrast to the absence of the spermatozoa in the testis...
on the side bearing the NE implant and the fact that there was no inhibitory effect of the treatment on the weight of the contralateral testis as observed in these studies, would empirically support a selective local action of NE. A systemic effect mediated via the hypothalamo-hypophysial axis, on the other hand, would have been reflected by an alteration in the size and weight of the testes on both the sides. Further, the accessory sex organs, notably the epididymis (Table I) were also found ponderally normal in weight and on gross visual examinations.

There was a drastic reduction in the weight of the ipsilateral testis six weeks after the initiation of the treatment, and continuation of the treatment for another 3 weeks did not produce any further reduction in the testicular weight suggesting that 6 weeks were, perhaps, sufficient to suppress the entire spermatogenic cell cycle altogether. The presence of few dead decapitated and occasionally agglutinated spermatozoa in the ipsilateral epididymal segments, in contrast to normal motile sperms in the contralateral epididymis, also suggest a selective local damaging effect of NE on the spermatozoa already present in the epididymis. Although the mechanisms underlying the relatively high antispermatogenic and anti-spermatozoal effects of NE as observed in the present study are not quite clear yet, a direct effect of NE on the functional integrity of the epididymis rendering the epididymal spermatozoa immotile and dead appears quite likely. It is possible that the metabolic equilibrium of the epididymis including its steroidogenic activity may be impaired due to an altered precursor bioavailability (5) under the influence of NE finding its way into the epididymis. The occurrence of dead spermatozoa in the ductus deferens could also be due to the possibility of NE binding to the epididymal spermatozoa thereby affecting the sperm metabolism directly (1,4,14,15,16,17).

The results of the present study suggest that Norethisterone enanthate released in chronic local microdoses through silastic implants administered into the epididymal fat-pads could be of great potential in the long-term regulation of male fertility.

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REFERENCES

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Kessuru, E., P. Comacho-Ortega, G. Laudhan and G. Schopflin. In vitro action of progestogens on sperm mig-
Rivarola, M.A., A.M. Camacho and C.J. Migeon. Effect of treatment with Medroxy-progesterone acetate (Pro-