THE INFLUENCE OF 17 α -METHYL-B-NOR-TESTOSTERONE (SK & F 7690) ON THE FERTILIZING ABILITY OF SPERMATOZOA IN HAMSTERS

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Summary. The effect of 17 α -methyl-B-nortestosterone (SK & F 7690) on spermatozoa in the isolated cauda epididymidis has been investigated in hamsters and it was found that the survival of the spermatozoa was impaired after treatment with this anti-androgen, which thereby caused rapid loss of fertility. Tests were carried out with entire animals so that endogenous androgens were present, and then with castrates in which exogenous androgens were administered by implant.

In castrated animals given testosterone, the anti-androgen competitively inhibited the stimulatory effects of androgens in the cauda epididymidis and impaired the fertilizing capacity of contained spermatozoa after 12 days. Loss of fertilizing capacity was not related to an increase in the proportion of dead spermatozoa and occurred before the live/dead ratio changed significantly. The anti-androgen appeared to impair an androgen-dependent aspect of epididymal function concerned with maintenance of the fertilizing capacity of contained spermatozoa.

In contrast to its effects on the cauda, SK & F 7690 had no effect on the concentration of fructose in the seminal vesicle. At each dose level, the Leydig tissue appeared to be histologically normal but spermatogenesis was always adversely affected. It seems that, in this species, the epididymis and seminal vesicles differ in their sensitivity to SK & F 7690.

INTRODUCTION

The synthetic compound, 17 α -methyl-B-nortestosterone (SK & F 7690), bears an overall structural resemblance to testosterone but it is not androgenic, oestrogenic or progestational (Saunders, Holden & Kerwin, 1964). Its anti-androgenic activity has, however, been demonstrated by a number of criteria including inhibition of comb-growth in testosterone-treated chicks (Dorfman, Fajkos & Joska, 1964; Saunders *et al.*, 1964), involution of accessory organs of

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reproduction in intact rats and mice (Saunders *et al.*, 1964; Saunders & Kerwin, 1966), in castrated rats treated with testosterone (Saunders *et al.*, 1964; Mahesh, Zarate, Roper & Greenblatt, 1966; Saunders & Kerwin, 1966) and by the reduction of sebum secretion in ovariectomized rats treated with testosterone (Ebling, 1967).

Hamster spermatozoa isolated in the cauda epididymidis have been found to lose their ability to fertilize 12 days after bilateral gonadectomy, but to retain a high fertilizing capacity in the presence of exogenous testosterone (Lubicz-Nawrocki & Glover, 1973a). The concept that circulating testicular androgens are required to prolong the viability of epididymal spermatozoa has now been tested by using the anti-androgen, SK & F 7690.

MATERIALS AND METHODS

Adult (10-week-old) male and female hamsters were maintained at an environmental temperature of approximately 26° C, and were exposed to 15 hr of light daily. They were allowed free access to Diet 41B (Dixon (Ware) & Sons Ltd) and water.

Treatment of animals

In both test and control animals, each cauda epididymidis was isolated by ligating the distal corpus epididymidis and the ductus deferens at its junction with the cauda epididymidis. Where possible, ligatures were placed beneath blood vessels to minimize damage to the blood supply.

Groups A, B and C consisted of male animals receiving a daily dose of 20, 40 or 60 mg SK & F 7690/100 g body weight, respectively, over a period of 12 days. Group D consisted of animals treated with testosterone (25-mg implant, Organon Laboratories Ltd) and twelve daily injections of 60 mg SK & F 7690/100 g body weight. Animals of Group E were subjected to the same treatment but were also bilaterally gonadectomized (the testes were removed and the ligated epididymides allowed to remain in the scrotum). The control group comprised three animals injected daily for 12 days with oil alone.

The anti-androgen was administered daily as a fine suspension in 0.2 ml arachis oil.

Experimental design

Animals in each group were killed after 12 days and the fertilizing ability of epididymal spermatozoa was tested (see below).

The viability of spermatozoa

Fertilization rate was used as a measure of the fertilizing ability of spermatozoa. Approximately 12×10^6 spermatozoa taken from the cauda epididymidis and suspended in Hanks' solution were injected into each uterine horn of three females in oestrus and eggs were flushed from the uterine tubes 30 to 36 hr after ovulation (Ward, 1946; Strauss, 1956). Whole mounts were prepared (Chang, 1952) and the eggs were examined under the phase contrast microscope and the presence of a headless spermatozoon in the vitellus of a cleaved

egg was taken as the sign of fertilization (Austin, 1956). The number of eggs fertilized, given as a percentage of those which were recovered, expressed the fertilization rate.

The percentage of live (partially stained) spermatozoa (see Lubicz-Nawrocki & Glover, 1973a) was estimated from smears of spermatozoa stained with aqueous nigrosin-eosin, by counting 100 spermatozoa in each of three separate smears. The concentration of spermatozoa injected into the uterine horns was estimated from haemocytometer counts and since the percentage of live spermatozoa in a sample was known from the smears, it was possible to estimate the number of live spermatozoa injected into each female.

Behavioural observations

In addition to examining its effects on the fertilizing capacity of epididymal spermatozoa, an additional series of tests was undertaken to examine whether SK & F 7690 had any effects on libido. For this, treated males were placed together with females in oestrus and the ability to mount females exhibiting lordosis was taken as evidence that libido was unimpaired.

Histological observations

Pieces of testis and cauda epididymidis were fixed for 24 hr in Bouin's fixative, dehydrated, embedded in paraffin wax and sectioned at 7 μ m. The sections were stained with haematoxylin and eosin and mounted in DPX.

Estimation of fructose in the seminal vesicles

A further test for the anti-androgenic effects of SK & F 7690 was the estimation of fructose concentration in the seminal vesicles (Mann, 1964), since this is well known to be androgen-dependent. The method was a modification of that of Lindner & Mann (1960) and has been previously described for the hamster by Lubicz-Nawrocki & Glover (1973a).

All data obtained in the experiment were analysed by the Student *t* test.

RESULTS

Table 1 and Text-fig. 1 show the fertilizing capacity of epididymal spermatozoa is inversely related to the dose of SK & F 7690. At the highest dosage (60 mg/100 g body wt), the mean fertilization rate was reduced to 17.9% of the control level (17.5 versus 97.6%) after 12 days of epididymal ligation. Although a uniform response was obtained with the 60-mg dosage, more variation (as indicated by the large standard error) was observed in fertilizing ability when lower doses were administered (Table 1). Thus, treatment with the 40-mg dose was necessary to produce a significant reduction of the mean fertilization rate ($P < 0.01$).

However, when testosterone-treated entire animals (Group D) received daily injections of 60 mg SK & F 7690, the epididymal spermatozoa retained a high fertilizing capacity ($85.2\% \pm 4.8$) whereas the same treatment in bilaterally gonadectomized animals also receiving testosterone replacement (Group E) reduced the mean fertilization rate to 21.6% (Table 1 and Text-fig. 2).

Table 1. The fertilizing capacity of hamster epididymal spermatozoa after treatment with SK & F 7690

Animal group	Treatment	Mean seminal vesicle fructose (mg/g dry wt $\pm$ S.E.)	Mean no. of live sperm. $\times 10^6$ /uterine horn $\pm$ S.E.	Total mean no. of eggs recovered (range)	Total mean no. of eggs fertilized	Mean fertilization rate in % $\pm$ S.E.
Control	Oil injection	162 $\pm$ 6	6.0 $\pm$ 0.5	29 (25 to 31)	28.3	97.6 $\pm$ 0.9
A	20 mg SK & F 7690	162 $\pm$ 2	5.9 $\pm$ 0.2	29.2 (22 to 35)	19.8	71.6 $\pm$ 13.9
B	40 mg SK & F 7690	160 $\pm$ 16	3.3 $\pm$ 0.5	29.4 (22 to 34)	11.0	45.9 $\pm$ 10.9
C	60 mg SK & F 7690	202 $\pm$ 24	4.3 $\pm$ 0.7	27.8 (26 to 32)	4.0	17.5 $\pm$ 2.0
D	Testosterone + 60 mg SK & F 7690	178 $\pm$ 8	3.8 $\pm$ 0.1	31 (28 to 33)	25.4	83.2 $\pm$ 4.8
E	Castration + testosterone + 60 mg SK & F 7690	188 $\pm$ 8	3.9 $\pm$ 1.2	28 (23 to 30)	6.0	21.6 $\pm$ 3.8

Effect on spermatozoa

Treatment with SK & F 7690 significantly reduced the proportion of live epididymal spermatozoa in some cases (Groups B and D; $P < 0.01$). There was, however, no correlation between the number of live spermatozoa placed in the female tract and fertilizing capacity (Table 1) so that the poor fertilizing capacity in spermatozoa from the animals in Groups C and E was not due to an increase in the proportion of dead spermatozoa.

Seminal vesicle fructose

Injections of SK & F 7690 had no effect on the mean fructose concentration in the seminal vesicles (Table 1 and Text-fig. 2) in animals from any experimental group.

TEXT-FIG. 1. The effect of graded doses of SK & F 7690 on the fertilizing capacity of hamster epididymal spermatozoa (Groups A, B and C).

Histological effects

Testis. In addition to its effect on sperm viability, a 10-mg dose of SK & F 7690 inhibited spermatogenesis at the primary spermatocyte stage (Group A). The severity of the effect was dose-dependent since with a 60-mg dose of SK & F 7690 most of the tubules were aspermic (Pl. 1, Fig. 2). These changes were not prevented by exogenous testosterone (Pl. 1, Fig. 3).

By contrast, the Leydig cells showed no change with any dose of the anti-androgen but appeared to have atrophied when both exogenous androgen and the anti-androgen were present (Group D).

Epididymis. Examination of material from animals in Groups A, B and C revealed that, within 12 days, treatment with anti-androgen resulted in a partial flattening of the principal cells of the tubular epithelium and a pronounced reduction in the number of clear cells which are normally frequently interspersed between the principal cells. A similar change in the distribution of these

TEXT-FIG. 2. (a) The mean fertilizing capacity of hamster epididymal spermatozoa and mean seminal vesicular fructose concentration following various treatments with SK & F 7690 (see text). (b) The mean fertilizing capacity of hamster epididymal spermatozoa and the mean number of live spermatozoa injected *in utero* 12 days after initiation of treatment with SK & F 7690.

cells was previously observed in the hamster 8 to 12 days after bilateral gonadectomy (Lubicz-Nawrocki & Glover, 1973a).

Effect on behaviour

In contrast to its effects on fertilizing capacity, SK & F 7690 did not impair

PLATE 1

FIG. 1. Section of hamster testis after 12 days of isolation of the cauda epididymidis (control). $\times 110$.

FIG. 2. Hamster testis after 12 daily subcutaneous injections of 60 mg SK & F 7690 (Group C). $\times 110$.

FIG. 3. Hamster testis after 12 daily subcutaneous injections of 60 mg SK & F 7690 following treatment with testosterone (Group D). $\times 110$.

libido. In each case, the animals retained the ability to mount females that exhibited lordosis.

DISCUSSION

These results show that the anti-androgen SK & F 7690 rapidly destroys the fertilizing capacity of mature epididymal spermatozoa in the golden hamster. Moreover, in gonadectomized animals, twelve daily doses of the steroid at a level of 60 mg/100 g body wt prevent the replacement effect of testosterone. This contrasts with the situation in entire animals with testosterone implants, in which SK & F 7690 appears to be relatively ineffective.

Whether or not the action of SK & F 7690 is mediated directly, say by affecting androgen receptor sites in the epididymis, or indirectly by influencing feed-back mechanisms, is a matter of conjecture and an effect on the spermatozoa themselves, whilst unlikely, cannot be entirely discounted.

The cauda epididymidis and the seminal vesicles are both functionally dependent upon androgen and, since epididymal function was so readily impaired by SK & F 7690, it is perhaps surprising that there was no effect on the seminal vesicles, particularly since this anti-androgen causes regression of all accessory organs in rats and mice (Saunders *et al.*, 1964; Saunders & Kerwin, 1966). It is possible that the anti-androgen is simply unable to interfere with the androgen receptors in the seminal vesicle so that fructose synthesis remains unimpaired. However, Tveter (1971) has shown that SK & F 7690 can reduce the uptake of 5α -dihydrotestosterone (thought to mediate the effects of testosterone) by prostatic cell nuclei, and the cells of the seminal vesicle might be expected to respond similarly. It appears that, in the hamster, there is a variation in the response of different accessory organs to anti-androgen and the epididymis seems to be more sensitive than the seminal vesicles.

Alternatively, like the anti-androgen, cyproterone acetate, SK & F 7690 might compete with androgen feedback on the hypothalamus and increase the secretion of testicular androgens in response to a resulting increase in LH secretion (Neumann, von Berswordt-Wallrabe, Elger, Steinbeck, Hahn & Kramer, 1970). This might then compensate for anti-androgenic activity in the seminal vesicle so that seminal vesicular fructose remains high. This is in line with the work of von Berswordt-Wallrabe & Neumann (1967, 1968) in rats, and that of Voigt, Apostolakis & Klosterhalfen (1968) in man following treatment with cyproterone, and parallels the results of Prasad, Singh & Rajalakshmi (1970), who obtained similar effects in rats with cyproterone acetate. If this were the only explanation of the mechanism of action, however, the response of the epididymis would seem to be paradoxical, so local effects are probably involved.

The changes observed in the tubular epithelium of the isolated cauda epididymidis after treatment with SK & F 7690 closely resemble those which follow androgen withdrawal after castration (Lubicz-Nawrocki & Glover, 1973a) and the almost complete absence of 'clear' cells supports the suggestion of Martan & Risley (1963a, b) that renewal of these cells is androgen-dependent.

In addition to its effects on the cauda epididymidis, SK & F 7690 had an

inhibitory effect on spermatogenesis, even at the lowest doses. This is surprising in view of the compound being generally regarded as a weak anti-androgen and at this stage, the mode of action on the spermatogenic epithelium can only be surmised. Synthesis of FSH might be inhibited, FSH release might be prevented, or both effects might occur. On the other hand, the drug might have a local anti-gonadotrophic action (Mahesh *et al.*, 1966) on the seminiferous tubules themselves. In this connection, it is interesting that PMSG maintains normal spermatogenesis in hamsters following hypophysectomy (R. P. Millar, personal communication) whereas HCG or testosterone alone will not (Lubicz-Nawrocki & Glover, 1973a, b).

An anti-fertility action of orally administered SK & F 7690 after 35 to 49 days has been reported in mice (Saunders, Tomaszewski, Pauls & Zuccarello, 1969), the suggestion being that decreased libido or failure to copulate properly were the underlying causes. In the present work, SK & F 7690 did not affect libido and although the effects of long-term treatment were not examined, initial loss of fertility was due rather to an effect on the spermatozoa.

The present findings show that anti-androgen impairs viability in mature epididymal spermatozoa and in this regard, SK & F 7690 simulates the effects of bilateral gonadectomy. The effects would appear to be due to a competitive inhibition of androgen upon which the cells lining the epididymis are known to be so dependent. Further investigation is needed to determine the precise mechanism of action of both androgen and anti-androgen on epididymal cells, for at the time of writing, there is no clear information on these subjects.

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