ANTAGONISTIC ACTION OF NORETHYNODREL
ON THE TESTOSTERONE-DEPENDENT PROCESS OF FRUCTOSE
FORMATION IN MOUSE SEX ACCESSORY ORGANS

By

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ABSTRACT

Norethynodrel antagonized the fructose stimulating effects of exogenous testosterone in sex accessory organs of castrate mice. It was antiandro-genic at both low doses (50 μg) and high doses (400 μg) of testosterone. Norethindrone and ethisterone suppressed fructose formation in the testosterone-treated castrate mouse, but not as effectively as norethynodrel. Norethandrolone exerted no antagonistic activity.

Several compounds have been shown to inhibit the typical growth response ordinarily produced by exogenous androgens in sex accessory organs of the rodent and the comb of the chick. Agents which are capable of suppressing the actions of androgen include progesterone (Pincus & Dorfman 1955; Mühlbock 1938), oestrogens (Mühlbock 1938; Hoskins & Koch 1939; Gley & Delor 1937), substituted anthrenes (Hertz & Tullner 1947; Eviatar et al. 1961) and certain other modified steroids (Bridge & Scott 1964; Uskokovic et al. 1958; Grauer et al. 1948; Neuman & Kramer 1964). The structural similarity of these compounds to naturally occurring steroids appears to afford the basis for hormonal antagonism.

Most earlier investigations have utilized reduced gravimetric responses in androgen sensitive end-organs as a criteria for hormonal antagonism or anti-
androgenic activity. Evaluation of androgenic activity by the application of the so-called 'chemical indicator tests' has been used extensively by Mann (Mann et al. 1949). Other investigators have demonstrated the fructose forming activity of various steroids in a variety of castrate species (Levey & Szego 1955; Mann et al. 1960; Price et al. 1949; Thomas & Strauss 1965).

Few investigations have studied the effects of simultaneously administered androgens and their antagonists on sex accessory organ fructose formation. The testosterone-dependent secretion of fructose in the male accessory gland secretions of the normal rabbit can be suppressed by the subcutaneous implantation of stilboestrol. Simultaneous implants of testosterone and stilboestrol in the castrate rabbit retard the formation of fructose by accessory organs (Parsons 1950). Conversely, small doses of oestradiol in combination with testosterone may augment the stimulatory effect of testosterone with respect to seminal plasma fructose in the castrate bull (Gassner et al. 1952). The present investigations were undertaken to study the process of hormonal antagonism with respect to fructose metabolism in sex accessory organs of the castrate mouse. Several synthetic steroids which were structurally similar to testosterone were examined for their ability to suppress fructose formation in the anterior lobes of the prostate gland and the seminal vesicles.

METHODS

Castrate mature male albino mice averaging 34.0 g and fed a standard diet of laboratory chow and water ad libitum were used in these studies. Subcutaneous injections of testosterone or testosterone and synthetic steroid (norethynodrel, norethindrone, norethandrolone or ethisterone) were initiated 7 days post-castration and were continued daily for a period of ten days. Experimental groups received daily simultaneous injections of testosterone and analogue while control mice received daily injections of only testosterone (50 or 400 μg). The selection of the testosterone dose schedules was based upon previous studies in the castrate mouse where a 50 μg dose schedule (daily × 10) was capable of returning fructose to about one-half normal while a 400 μg regimen restored sex accessory fructose to essentially pre-castration levels (Thomas & Strauss 1965). Steroids were suspended in a peanut oil vehicle and were given in a volume of 0.2 ml or less in doses of 100, 200, 400, or 800 μg/mouse/day × 10.

Animals were sacrificed approximately two hours after the tenth daily injection. Seminal vesicles and anterior lobes of the prostate gland were removed, rinsed in 0.9% sodium chloride and weighed. Contents of the seminal vesicles were carefully removed prior to weighing. Organs were immediately homogenized in 10% trichloro-

* Norethandrolone (17α-ethyl-19-nortestosterone) and norethynodrel (17α-ethynyl-17-hydroxy-5(10)-estren-3-one) in crystalline form were generously supplied by the G. D. Searle and Co., Chicago, Illinois. Crystalline norethindrone (17α-ethynyl-19-nortestosterone) was kindly donated by Ortho Research Foundations, Raritan, New Jersey. Other steroids were obtained commercially from General Biochemicals, Chagrin Falls, Ohio.

Acta endocr. 51, 2

225
acetic acid and aliquots measured for their fructose (Roe 1934). Fructose levels were expressed as µg/organ (contents) or as mg/100 mg (concentration). The means of control groups were compared with the means of experimental groups by the difference of means procedure (Snedecor 1956).

RESULTS

Fig. 1 shows the effects of simultaneously injected testosterone (50 µg) and varying amounts of norethynodrel on fructose levels in the anterior lobes of the prostate gland in castrate mice. The 100 µg dose of analogue caused about a 34% reduction in fructose contents. Norethynodrel significantly reduced mean fructose contents and concentrations at the 200, 400, and 800 µg increments (P ≤ 0.01).

Norethynodrel was not consistent in its ability to antagonize fructose formation in the seminal vesicles of the testosterone-treated castrate mouse (Fig. 2). Doses of 400 µg exhibited the most marked reductions (P ≤ 0.01). The 200 µg dosage was less effective in reducing fructose contents than the 400 µg dosage, yet the 200 µg amount was significantly lower than the control levels (P ≤ 0.05). An 800 µg dose schedule, despite repeated series of experiments, exhibited no evidence of antagonism.

With an increase in testosterone (400 µg), norethynodrel was still able to suppress fructose levels in sex accessory organs (Figs. 3 and 4). Regardless of

![Fig. 1.](image1)

![Fig. 2.](image2)

Fig. 1. The effect of simultaneously injected testosterone (50 µg) and varying amounts of norethynodrel on anterior prostate fructose levels in castrate mice. Controls received only testosterone (50 µg). Large bars (solid lines) represent mean (S.E.) fructose contents. Small bars (dashed lines) indicate mean fructose concentrations. Each bar depicts an average of 5 or more mice. See results section for statistical details.

Fig. 2. The effect of simultaneously injected testosterone (50 µg) and varying amounts of norethynodrel on seminal vesicle fructose levels in castrate mice. Controls received only testosterone (50 µg). Large bars (solid lines) represent mean (S.E.) fructose contents. Small bars (dashed lines) indicate mean fructose concentrations. Each bar depicts an average of 5 or more mice. See results section for statistical details.
The effect of simultaneously injected testosterone (400 µg) and varying amounts of norethynodrel on anterior prostate fructose levels in castrate mice. Controls received only testosterone (400 µg). Large bars (solid lines) represent mean (S.E.) fructose contents. Small bars (dashed lines) indicate mean fructose concentrations. Each bar depicts an average of 5 or more mice. See results section for statistical details.

Fig. 4. The effect of simultaneously injected testosterone (400 µg) and varying amounts of norethynodrel on seminal vesicle fructose levels in castrate mice. Controls received only testosterone (400 µg). Large bars (solid lines) represent mean (S.E.) fructose contents. Small bars (dashed lines) indicate mean fructose concentrations. Each bar depicts an average of 5 or more mice. See results section for statistical details.

dose, all mean fructose contents and concentrations were significantly lower than testosterone controls ($P \leq 0.01$). The magnitude of suppression of fructose levels was observed to be greater in the seminal vesicles (Fig. 4) than in the

Table 1.
The effect of simultaneously injected testosterone (50 µg) and either 400 or 800 µg of various synthetic steroids on sex accessory fructose levels in castrate mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mice/group</th>
<th>Anterior Prostate Fructose Content (µg/organ)</th>
<th>Seminal Vesicle Fructose Content (µg/organ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (Testosterone 50 µg)</td>
<td>13</td>
<td>59.3 ± 6.0a</td>
<td>92.7 ± 12.8</td>
</tr>
<tr>
<td>Testosterone (50 µg) + Norethandrolone (400 µg)</td>
<td>6</td>
<td>100.6 ± 10.2</td>
<td>100.4 ± 6.8</td>
</tr>
<tr>
<td>Testosterone (50 µg) + Norethindrone (400 µg)</td>
<td>5</td>
<td>53.7 ± 4.1</td>
<td>66.3 ± 11.2</td>
</tr>
<tr>
<td>Testosterone (50 µg) + Ethisterone (400 µg)</td>
<td>5</td>
<td>58.4 ± 8.2</td>
<td>70.9 ± 8.5</td>
</tr>
<tr>
<td>Testosterone (50 µg) + Norethandrolone (800 µg)</td>
<td>6</td>
<td>76.5 ± 4.9</td>
<td>95.9 ± 13.6</td>
</tr>
<tr>
<td>Testosterone (50 µg) + Norethindrone (800 µg)</td>
<td>6</td>
<td>54.9 ± 4.6</td>
<td>54.6 ± 5.6</td>
</tr>
<tr>
<td>Testosterone (50 µg) + Ethisterone (800 µg)</td>
<td>6</td>
<td>32.8 ± 2.8b</td>
<td>55.8 ± 4.1</td>
</tr>
</tbody>
</table>

a Mean ± S. E. of the mean.
b Statistically significant ($P \leq 0.01$).
prostate glands (Fig. 3). Even though exogenous testosterone was higher in these series (Figs. 3 and 4), the degree of fructose inhibition was still more marked than in the series receiving lower amounts of injected testosterone (Figs. 1 and 2).

In another series of experiments, the interaction of other testosterone related compounds (norethandrolone, norethindrone or ethisterone) and male sex hormone on fructose formation in sex accessory organs of castrate mice was investigated (Table 1). These analogues were generally less consistent in their ability to antagonize fructose formation. Norethandrolone actually enhanced fructose levels. Norethindrone was ineffective in reducing prostate fructose, but was able to reduce fructose contents in the seminal vesicles. Ethisterone (800 µg) significantly lowered prostatic fructose \( P \leq 0.01 \). It diminished amounts of fructose in the seminal vesicles at both the 400 and the 800 µg doses.

**DISCUSSION**

An antagonism between certain 19-nor steroids and testosterone in their effect on sex accessory fructose formation has been demonstrated. Previously this type of hormonal antagonism had been demonstrated by reduced gravimetric responses in sex accessory organs of the castrate rodent \( (\text{Randall} \& \text{Selitto} 1958) \) and in the chick comb \( (\text{Dorfman} \& \text{Dorfman} 1960; \text{Dorfman} \& \text{Nes} 1960) \). A recent report indicates an antagonism between testosterone propionate and a halogenated progesterone (6-chloro-\( \Delta^4 -1,2\alpha\)-methylene-17\( \alpha \)-hydroxyprogesterone acetate) on the histologic development of the genitalia of the female rat foetus \( (\text{Neumann} \& \text{Kramer} 1964) \). It has been suggested that this progesterone analogue exerts its antagonistic effect by partly occupying androgen receptors in female foetuses by inhibiting the masculinizing actions of testosterone propionate. The action of other antiandrogenic substances has been explained by competition for the receptors of male sex hormones at the target organs \( (\text{Randall} \& \text{Selitto} 1958; \text{Lerner} 1960) \).

Undoubtedly hormonal antagonism observed in gravimetric and histologic studies is somewhat analogous to these chemical studies. However, fructose metabolism in sex accessory organs of castrate mice was more sensitive in disclosing antagonism to exogenous testosterone than was weight response. If only gravimetric responses would have been observed in these studies, hormonal antagonism would only have been disclosed in the seminal vesicles of groups of mice receiving testosterone (400 µg) and varying amounts of nor-ethynodrel. All other series exhibited no demonstrable gravimetric alterations in either the prostate or seminal vesicles.

These studies relate some rather interesting structure-function activities.
For example, norethandrolone was the only steroid investigated that contained a saturated 17-alkyl side chain. Ethisterone, norethindrone, and norethynodrel possess certain structural differences, but all have unsaturated 17-alkyl side groups. In general, the fructose forming activity of these steroids in accessory organs of castrate mice is norethandrolone > ethisterone > norethindrone > norethynodrel (Thomas & Strauss 1965). The present studies indicate that those steroids previously reported to be more effective in stimulating fructose in the castrate mouse are less effective in causing antagonism of fructose in the testosterone-treated castrate mouse. In other words, the greater the hormonal antagonism, the less the inherent androgenicity of the particular synthetic steroid.

The failure of norethynodrel (800 µg) to reduce fructose levels remains unexplained (Figs. 1 and 2). Competition between testosterone and analogue for target organ receptors may explain the diminished fructose levels at lower dose levels. Competition between testosterone and analogue for hepatic degrading enzyme(s) may be partly responsible for the elevated fructose levels in these same sex accessory tissues. There is evidence to suggest that the 17-alkyl group may prevent complete conversion of testosterone to androst-4-ene-3,17-dione (Brendler & Winkler 1959). If the high doses of norethynodrel interfered with testosterone catabolism the biologic half-life of male sex hormone would be prolonged. This, coupled with non-specific alterations in hepatic function, may have accounted for elevated fructose levels.

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