ANDROGENIC PROPERTIES
AND ADRENAL DEPRESSANT ACTIVITY OF MEGESTROL ACETATE OBSERVED IN CASTRATED MALE RATS

By
Lars-Eric Tisell and Håkan Salander

ABSTRACT

Megestrol acetate (17α-acetoxy-6-dehydro-6-methylprogesterone), a synthetic steroid with high progesterational activity, is used in oral contraceptives but also in the treatment of prostatic diseases in man. To investigate whether megestrol acetate has any androgenic properties the growth of the ventral and dorsolateral prostate, the coagulating glands and the seminal vesicles was studied morphologically in castrated rats treated with megestrol acetate and in non-treated castrated rats. The effect of megestrol acetate on the body weight, the levator ani muscle and the adrenals was also studied. Megestrol acetate was administered in daily doses of 0.02 mg, 0.2 mg, 2.0 mg or 20.0 mg for a period of 21 days. Megestrol acetate in the two higher doses retarded growth and gave a low weight for the levator ani muscle at autopsy indicating an anti-anabolic or catabolic action of megestrol acetate in high doses. Megestrol acetate in daily doses of 0.2, 2.0 and 20.0 mg caused an involution of the adrenal glands. After the two higher doses the weight of the adrenals amounted to only about a third of that of the untreated rats.

Megestrol acetate in the lower doses had no demonstrable effect on the growth of the accessory reproductive glands. After the two higher doses of megestrol acetate some growth of the dorsal part of the dorsolateral prostate and of the coagulating glands was observed. Only the seminal vesicles exhibited complete morphological criteria of an androgenic stimulation and then only after the largest dose of megestrol acetate. The investigation shows that megestrol acetate has weak androgenic properties which are apparent at a dose per kg body weight approximately 200 times greater than that used in the treatment of prostatic diseases in man.
Megestrol acetate (17α-acetoxy-6-dehydro-6-methylpregesterone) is a synthetic steroid with high progestational activity (McKinney & Braselton 1970). This steroid is used in oral contraceptives (Goldzieher 1964; Asbjørn et al. 1971). Lately it has also been used in the treatment of benign prostatic hyperplasia (Lebech & Nordentoft 1967; Kollberg & Bäcklund 1969; Vernet 1970; Reichelt 1970) and prostatic cancer (Frick et al. 1971). As megestrol acetate is used with these indications it is of interest to know whether it has any androgenic activity. Androgenicity of a steroid can be tested by studying the ability of the hormone to induce normal growth in accessory reproductive glands of castrated male animals (Dorfman & Shipley 1956; Price & Williams-Ashman 1961). Megestrol acetate is claimed to be without androgenic activity and in experiments by David et al. (1963) it failed to increase the weight of the ventral prostate and the seminal vesicles of castrated male rats.

The ventral prostate in rat does not seem to be homologous with the ventral prostate in man, while the dorsolateral prostate and the coagulating glands in the rat may be homologous with corresponding lobes or regions in man (Price 1963). Moreover, the ventral prostate in man is absent or atrophic in postnatal life (Lowsley & Kirwin 1944). Hence it seems evident that when hormonal effects on the prostate are studied experimentally in the rat all the prostatic lobes should be examined simultaneously. This consideration appears still more justified as there are differences between androgens on the basis of their effect on the growth of different accessory reproductive glands in male rats (Tisell 1970). Since the cytological structure is a more sensitive indicator of androgenic stimulation than the weight of the organs (Price & Ingle 1957; Tisell & Angervall 1969) a histological examination of all the prostatic lobes and the seminal vesicles should be included in tests of androgenicity of steroids. The present communication is a quantitative and qualitative morphological study of the ventral and dorsolateral prostate, the coagulating glands and the seminal vesicles of castrated rats subjected to 21 daily injections of megestrol acetate.

MATERIAL AND METHODS

The experiment was performed on 44 male rats of the Spraque-Dawley strain supplied by Anticimex AB, Stockholm. When weighing 36 ± 0.3 g (mean and standard error of the mean) the rats were castrated by the transscrotal route, the epididymis being removed with the testis. Surgery was performed under ether anaesthesia.

Thirty days after castration the rats were divided into groups and were given 21 daily injections according to the following scheme.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Number of Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Castrated rats injected with 1 ml suspension medium once daily</td>
<td>8 rats</td>
</tr>
<tr>
<td>M₀.₀₂</td>
<td>Castrated rats injected with 0.02 mg megestrol acetate once daily</td>
<td>9 rats</td>
</tr>
<tr>
<td>M₀.₂</td>
<td>Castrated rats injected with 0.2 mg megestrol acetate once daily</td>
<td>9 rats</td>
</tr>
<tr>
<td>M₂.₀</td>
<td>Castrated rats injected with 2.0 mg megestrol acetate once daily</td>
<td>9 rats</td>
</tr>
<tr>
<td>M₂₀.₀</td>
<td>Castrated rats injected with 20.0 mg megestrol acetate once daily</td>
<td>9 rats</td>
</tr>
</tbody>
</table>
The megestrol acetate was supplied by NOVO AS, Copenhagen, in the form of specially prepared suspensions adjusted to permit the daily dose to be given as 1 ml. The suspension medium contained Tween 80, acetic acid, sodium acetate and mannitol.

Throughout the experiment all the rats were housed in temperature controlled and air-conditioned quarters (temperature 24°C and relative humidity 60%). An artificial lighting cycle which alternated 12 h of light and 12 h of darkness was employed. The rats were given unlimited tap water and food. The special commercial rat diet was supplied by Teknosan AB, Malmö.

On the day of the last injection the rats were exsanguinated through a large cardiac incision under ether anaesthesia. The rats were taken in sequence from the different groups. The adrenal glands, thymus, ventral prostate, dorsolateral prostate, coagulating glands, seminal vesicles and levator ani muscle were dissected free, while immersed in physiological saline, using a stereoscopic microscope. The organs were then blotted and weighed on an analytic balance with an accuracy of 0.01 mg.

Student's t-test was used for testing the differences between the means. For histological examination the ventral prostate, dorsolateral prostate, coagulating glands, seminal vesicles and adrenal glands were fixed in Bouin's solution for two hours. After dehydration the organs were embedded in paraffin and cut into 4-5 µm sections, which were stained according to Weigert- van Gieson or with PAS-staining (McManus 1948).

RESULTS

Food consumption

Before the period of injections the food consumption per 24 h of the castrated rat was about 21 g. The administration of megestrol acetate had no demonstrated tendency to change the food consumption.

Weights of body and levator ani muscle (Table 1)

All animals survived and gained weight during the experimental period. Administration of megestrol acetate in daily doses of 2.0 and 20.0 mg retarded growth significantly ($P < 0.001$). The weight of the levator ani muscle was also lower in the rats on these doses (groups $M_{2.0}$ and $M_{20.0}$) than in the controls ($P < 0.005$ and $P < 0.01$ respectively).

Weights of adrenal glands and thymus (Table 1)

Megestrol acetate administration in daily doses of 0.2 mg significantly ($P < 0.005$) decreased the mean weight of the adrenal glands as compared to that of the controls. After daily doses of 2.0 mg and 20.0 mg the mean adrenal weights were further decreased to about a third of that of the controls. Administration of megestrol acetate in daily doses of 2.0 mg decreased the mean weight of the thymus as compared to that of the controls ($P < 0.001$). After daily doses of 20.0 mg there was a further decrease in thymus weight ($P < 0.001$).
Table 1.
Mean body weights and mean weights of adrenal glands, thymus, ventral and dorsolateral prostate, coagulating glands, seminal vesicles and levator ani muscle in castrated rats injected with megestrol acetate in daily doses of 0.02, 0.2, 2.0 or 20.0 mg and in castrated control rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of rats</th>
<th>Body weight (g)</th>
<th>Ventral prostate (mg)</th>
<th>Dorsolateral glands (mg)</th>
<th>Coagulating glands (mg)</th>
<th>Seminal vesicles (mg)</th>
<th>Levator ani (mg)</th>
<th>Adrenal glands (mg)</th>
<th>Thymus (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At start of treatment</td>
<td>At autopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>159 ± 3</td>
<td>292 ± 4</td>
<td>9.5 ± 0.4</td>
<td>13.7 ± 0.8</td>
<td>2.9 ± 0.1</td>
<td>8.4 ± 0.3</td>
<td>88.3 ± 4.9</td>
<td>64.8 ± 1.9</td>
</tr>
<tr>
<td>M₀.₀₂</td>
<td>9</td>
<td>158 ± 3</td>
<td>287 ± 4</td>
<td>9.2 ± 0.3</td>
<td>13.4 ± 0.9</td>
<td>2.0 ± 0.1</td>
<td>9.0 ± 0.2</td>
<td>85.4 ± 1.8</td>
<td>62.5 ± 2.2</td>
</tr>
<tr>
<td>M₀.₂</td>
<td>9</td>
<td>158 ± 3</td>
<td>281 ± 5</td>
<td>9.5 ± 0.3</td>
<td>12.1 ± 0.6</td>
<td>3.0 ± 0.1</td>
<td>8.8 ± 0.2</td>
<td>79.7 ± 5.2</td>
<td>55.7 ± 1.7</td>
</tr>
<tr>
<td>M₂₀</td>
<td>9</td>
<td>158 ± 3</td>
<td>239 ± 6</td>
<td>9.0 ± 0.4</td>
<td>11.3 ± 0.3</td>
<td>3.2 ± 0.1</td>
<td>10.2 ± 0.3</td>
<td>68.4 ± 2.6</td>
<td>22.1 ± 0.8</td>
</tr>
<tr>
<td>M₃₀</td>
<td>9</td>
<td>167 ± 2</td>
<td>219 ± 5</td>
<td>9.4 ± 0.6</td>
<td>12.8 ± 0.9</td>
<td>3.5 ± 0.1</td>
<td>12.1 ± 0.3</td>
<td>67.8 ± 4.2</td>
<td>20.3 ± 0.5</td>
</tr>
</tbody>
</table>

The figures given in Table 1 are mean weight ± standard error of mean.
Weights of ventral prostate, dorsolateral prostate, coagulating glands and seminal vesicles (Table 1)

The various doses of megestrol acetate did not significantly change the mean weights of the ventral prostate as compared with that of the controls. After administration of daily doses of 2.0 mg megestrol acetate the mean weight of the dorsolateral prostate was lighter than that of the controls ($P < 0.02$). On the other hand administration of lower (groups M$_{0.02}$ and M$_{0.2}$) as well as higher (group M$_{20.0}$) doses resulted in mean weights for the dorsolateral prostate not different from that of the control rats.

The rats given megestrol acetate in daily doses of 2.0 and 20.0 mg had significantly higher mean weights of the coagulating glands ($P < 0.05$ and $P < 0.005$ respectively) and the seminal vesicles ($P < 0.001$) than those of the control rats.

Histological examination of the accessory reproductive glands

The ventral prostate, the dorsolateral prostate, the coagulating glands and the seminal vesicles in untreated castrated control rats exhibited an immature histological appearance. The acini were small and were surrounded by undeveloped smooth muscle cells. The epithelial cells were cubic to low columnar and the acini contained little or no secretion (Fig. 1). The administration of megestrol acetate in daily doses of 0.02 or 0.2 mg did not change the histological appearance of any of the accessory reproductive glands examined.

The appearance of the lateral part of the dorsolateral prostate was uninfluenced by megestrol acetate in daily doses of 2.0 or 20.0 mg. In the ventral prostate the acini were somewhat larger in rats given daily doses of 20.0 mg megestrol acetate, but the epithelium was low and there was no secretion. After administration of megestrol acetate in daily doses of 2.0 or 20.0 mg the dorsal part of the dorsolateral prostate had higher epithelial cells than the controls and the acini contained a small amount of secretion and exfoliated cells. This small stimulation of the growth of the dorsal part of the dorsolateral prostate was most apparent after the largest dose when the epithelium also formed some papillary formations. The two higher doses of megestrol acetate induced a small stimulating effect on the coagulating glands. The epithelial cells were higher than in the control rats but the nuclei still had basal positions and were not centrally placed as in the coagulating glands of intact rats and of castrated rats given testosterone (cf. Price & Williams-Ashman 1961). The acini were larger and contained more secretion and exfoliated cells than the controls.

In the seminal vesicles of rats on 2.0 mg megestrol acetate the acini were lined by higher epithelium than the controls. The number of papillary formations were increased and these were more gracile than in the control rats, and the acini contained more secretion. In the seminal vesicles of rats on 20.0 mg
megestrol acetate the signs of stimulated growth were still more apparent. After this treatment granules surrounded by light halos were observed in the columnar epithelial cells indicating secretory activity (Moore et al. 1930). The acini contained secretion and were surrounded by smooth muscle cells which had larger nuclei and more cytoplasm than in any of the other groups (Fig. 2).

_Histological examination of the adrenal glands_

In the adrenals of the control rats and of the rats given daily doses of 0.02 or 0.2 mg megestrol acetate the zona glomerulosa, the zona fasciculata and the zona reticularis were all well developed. The cortex of the adrenals in rats given 2.0 or 20.0 mg megestrol acetate daily was markedly reduced in width and the cells had smaller nuclei and less cytoplasm than in the control rats.

**DISCUSSION**

In the present study megestrol acetate in daily doses of 0.02 or 0.2 mg during three weeks failed to influence growth and the development of the prostatic lobes and the seminal vesicles in castrated male rats. Daily doses of 2.0 or 20.0 mg depressed growth and reduced the weight of the levator ani muscle.
compared with untreated castrated control rats. As megestrol acetate did not change the food consumption these observations indicate that in high doses it has an anti-anabolic or catabolic action in castrated male rats. The finding of small adrenals in the megestrol treated rats excluded the possibility that this action is mediated via an increased production of adrenal steroids.

Adrenal involution after megestrol acetate administration has previously been demonstrated in female rats (Elton et al., 1960; Arends, 1963) but not in intact male rats at dose levels comparable to those given to castrated rats in the present investigation (David et al., 1963; Karkun & Kar, 1965). Arends (1963) claimed that the adrenal depressant activity of megestrol acetate demonstrated in female rats is due to a direct action on the adrenal glands. The difference between our data from castrated male rats and those reported by David et al., (1963) and Karkun & Kar (1965) from intact male rats suggests that the presence of the testes may protect the adrenals from the depressant activity of megestrol acetate.

Megestrol acetate in the two higher doses had a slight tendency to stimulate epithelial growth in the coagulating glands, in the dorsal part of the dorsolateral prostate and in the seminal vesicles. Full restoration of a secretory epithelium was only observed in the seminal vesicles and then only after 20.0 mg
daily. The epithelial growth of the ventral prostate and of the lateral part of the dorsolateral prostate seemed uninfluenced by the various doses of megestrol acetate. This is in contrast to the effect of progesterone which in high doses stimulates the growth of the ventral prostate while its effect on the seminal vesicles is small and inconsistent (Greene et al. 1939; Price et al. 1955). These differences between megestrol acetate and progesterone may in part be explained by the fact that the former is not metabolized by the normal pathways of progesterone metabolism (Cooke & Vallance 1965).

That megestrol acetate could promote the growth of the seminal vesicles but not of the ventral prostate seems contradictory to the findings of Karkun & Kar (1965) who found unaltered seminal vesicles weight but a significant stimulation of ventral prostate weight after daily administration of 40 mg/kg for 7 days to castrated male rats. As they did not include any histological examination of the ventral prostate and seminal vesicles and furthermore used relative organ weight (organ weight/body weight) without reporting the body weight it is impossible to judge the significance of their results.

The definition of an androgen is a substance which is capable of stimulating the growth of accessory reproductive organs in castrated male animals and also of maintaining a normal histological structure and secretory activity in the epithelium (Dorfman & Shipley 1956; Price & Williams-Ashman 1961). According to this definition only the seminal vesicles in our investigation exhibited complete morphological criteria of an androgenic stimulation after megestrol acetate. This androgenic effect, was small and was only seen when megestrol acetate was given at a dose per kg body-weight approximately 200 times greater than that used in the treatment of prostatic diseases in man (Vernet 1970; Reichelt 1970).

Megestrol acetate has been reported to have an anti-androgenic effect in the rat (Karkun & Kar 1965). Such an effect has earlier been reported for other steroids with weak androgenic effects like progesterone (Dorfman 1963) and cortisone (Tisell 1972). The mechanism for this anti-androgenic action may be a competition for the site at which the strong androgen needs to be attached in order to produce its biological effect (Dorfman 1963). The finding in this study that megestrol acetate has a weak androgenic action may help in explaining the mechanism of its anti-androgenic action.

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REFERENCES


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