Antiandrogenic effect of delta-9-tetrahydrocannabinol in adult castrated rats

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Summary. In ventral prostate tissues of adult castrated rats, delta-9-tetrahydrocannabinol (THC) at 10 mg/kg for 7 days counteracted the testosterone-induced changes of (i) DNA, RNA and protein content, (ii) the tartrate-insensitive form of acid phosphatase, and (iii) the isoenzyme variant of acid phosphatase associated with the secretory type enzyme. These results indicate that THC acts anti-androgenically and directly at the level of male accessory sex organs.

Introduction

Marihuana and its psychoactive constituent delta-9-tetrahydrocannabinol (THC) have been implicated in the alteration of male reproductive functions. This drug reduces plasma testosterone concentrations in rats and men (Kolodny, Masters, Kolodner & Toro, 1974), inhibits testosterone secretion (Daltero, Barkte & Burnstein, 1977), alters glucose metabolism (Hussain & Lame, 1978) and marker proteins of testicular tissue (Scharz, Harclerode & Nyquist, 1978), suppresses spermatogenesis and produces change in sperm head proteins (Dixit, Sharma & Lohiya, 1974). In man, reduced sexual potency and gynaecomastia (Kolansky & Moore, 1972; Kolodny et al., 1974; Symons, Teale & Marks, 1976) have also been reported in heavy marihuana users. A reduction in certain androgen-dependent behavioural responses, copulatory behaviour in male rats and mice (Merari, Barak & Plaves, 1973) and weight of testes and accessory reproductive organs (Dixit et al., 1974; Hembree, Zeidenberg & Nahas, 1976; Rosenkrantz & Braude, 1976) has also been observed.

The effect of THC on accessory male reproductive organs has been considered as secondary to the primary effect of the drug on testicular testosterone production (Dixit et al., 1974; Kolodny et al., 1974; Daltero et al., 1977). But there has been no study on whether THC might act directly at the level of the target organs, apart from the observations of Purohit, Singh & Ahluwalia (1979) that THC counteracts androgen-induced stimulation of accessory sex organ weights. In the present paper a direct effect of THC on the accessory sex organs has been investigated.

Materials and Methods

Male albino rats (Charles Foster strain) weighing 175–200 g and maintained on a standard diet and water ad libitum were used. Castration was performed under light ether anaesthesia via the scrotal route. The control rats were sham operated. The animals were housed at 27°C with 12 h light (09:00–21:00 h)/(24 h).

Testosterone propionate (Ciba-Geigy, India Ltd) was injected subcutaneously at a dose of 2

0022-4251/81/040513-05$02.00/0
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mg/kg. The injection vehicle was 0.1 ml sesame oil and the injections were given daily for 7 days starting from the day of castration. THC (10 mg/kg) was also injected s.c. in 0.1 ml saline—Tween 80 suspension for 7 days, starting from the day of castration. This dose of THC was selected on the basis of previous studies (Collu, Letarto, Leboeuf & Ducharme, 1975; Dalterio, Barkte, Robertson, Watson & Burnstein, 1978). Control rats (castrated and sham-operated) received both vehicles, i.e. 0.1 ml sesame oil + 0.1 ml saline—Tween 80.

Rats were killed 24 h after the final dose and the ventral prostates were then carefully dissected out. Tissue was homogenized (10% w/v) in a glass homogenizer in 0.9% (w/v) NaCl at 0–4°C. The homogenate was centrifuged at 4000 g for 10 min at 0–4°C and the supernatant was used as the enzyme source in the assay of activity of acid phosphate (EC 3.1.3.2). This enzyme source was frozen and thawed twice and centrifuged again at 105,000 g for 1 h at 0–4°C; the final supernatant was used as the enzyme source in the gel electrophoresis study.

**Assay of enzyme**

The assay mixture (final volume 1.0 ml) contained 0.5 ml 100 mM-sodium acetate–acetic acid buffer, pH 4.85, 0.1 ml 5 mM-p-nitrophenyl phosphate (Sigma Chemical Co., St Louis, Missouri, U.S.A.) as substrate and 0.3 ml H2O. The reaction was initiated by the addition of 0.1 ml of the appropriately diluted enzyme source containing 200 µg protein. After incubation for 1 h at 37°C in a water bath with constant shaking the reaction was terminated by the addition of 2 ml 1 M-NaOH and the colour developed was measured spectrophotometrically at 415 nm 10 min after the addition of NaOH. Enzyme activity was expressed as mmol p-nitrophenol liberated/mg total tissue protein/h.

Total activity was indicated by the activity of the enzyme measured in the absence of DL-tartrate and tartrate-insensitive activity was measured in the presence of 20 mM-DL-tartrate. The fraction of the total activity that was inhibited by DL-tartrate was considered to be the tartrate-sensitive form.

**Polyacrylamide gel electrophoresis**

Polyacrylamide gel electrophoresis was performed essentially according to the method of Davis (1964) by using 7.5% polyacrylamide gel containing 0.5% Triton X-100. The gel was loaded with 100 µg protein in a 0.1 ml sample and electrophoresis was performed in 0.1 M-Tris–glycine buffer, pH 8.9, with a current of 4 mA per tube, for 45 min. At the end of electrophoresis the enzyme activity was located by incubating the gel with 0.1% a-naphthyl phosphate (Sigma) containing 0.1% Fast Garnet GBC salt (Sigma) to 0.1 M-sodium acetate–acetic acid buffer, pH 4.85. DNA was measured by the method of Burton (1956) using diphenylamine (Sigma) for the colour reaction and calf thymus DNA (Sigma) as standard. RNA was measured by the method of Mejbaum (1939) using orcinol (Sigma) for the colour reaction and *Torula* yeast RNA (Sigma) as standard. Protein in the samples for enzyme assay was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951). The protein content in the prostate tissues was estimated by the Biuret method of Gornall, Bardawill & David (1949) and by the folin phenol method (Lowry *et al*., 1951) using BSA (Sigma) as standard.

**Results**

As shown in Table 1, castration for 7 days caused decreases in DNA (45%), RNA (82%) and protein (84%) content in rat ventral prostatic tissue. Treatment with testosterone propionate resulted in massive stimulation of DNA (333%), RNA (1290%) and protein (1284%) content. However, when THC was also administered the stimulations were only 202% for DNA content, 929% for RNA content and 802% for protein content.
Table 1. Effect of delta-9-THC (10 mg/kg) and testosterone propionate (2 mg/kg) on nucleic acid and protein content of rat ventral prostate

<table>
<thead>
<tr>
<th>Treatment (daily for 7 days)</th>
<th>Protein (mg/organ)</th>
<th>RNA (mg/organ)</th>
<th>DNA (mg/organ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact (sham-operated) + oil + Tween 80–saline</td>
<td>11.37 ± 1.01</td>
<td>2.29 ± 0.21</td>
<td>0.77 ± 0.05</td>
</tr>
<tr>
<td>Castrated + oil + Tween 80–saline</td>
<td>1.85 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.41 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.41 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Castrated + THC + oil</td>
<td>1.78 ± 0.17</td>
<td>0.38 ± 0.06</td>
<td>0.39 ± 0.05</td>
</tr>
<tr>
<td>Castrated + testosterone propionate + Tween 80–saline</td>
<td>23.76 ± 2.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.29 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.40 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Castrated + testosterone propionate + THC</td>
<td>14.85 ± 1.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.81 ± 0.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.85 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m. for 6 different rats and were analysed by Student’s t test.

<sup>a</sup> Significantly different (P < 0.001) from values for intact control rats.
<sup>b</sup> Significantly different (P < 0.001) from values for rats castrated and given vehicle only.
<sup>c</sup> Significantly different (P < 0.005) from values for castrated rats treated with testosterone.

Castration caused loss of the tartrate-sensitive (56%) and -insensitive (67%) forms of acid phosphatase in the ventral prostate, but testosterone treatment gave increased activities of 379% and 1143% respectively (Table 2). Concurrent administration of THC and testosterone gave values only 253% and 631% stimulation of activity compared with castrated controls.

Ventral prostatic tissue of intact rats contained two variants of acid phosphatase (Text-fig. 1); the faster moving one, assumed to be secretory type (Tenniswood, Bird, Abrahams & Clark, 1978), disappeared after castration, but testosterone treatment restored this variant. Administration of testosterone and THC did not result in restoration of this acid phosphatase variant.

Administration of THC (10 mg/kg) alone to castrated rats gave no significant alteration in any of the responses examined from those in the untreated castrated animals.

Text-fig. 1. Diagram of the polyacrylamide gel electrophoretic pattern of rat ventral prostatic acid phosphatase. Enzyme activity was localized by using α-naphthyl-phosphate as substrate and Fast Garnet GBC as the coupling agent. (A) Intact rat; (B) castrated rat; (C) testosterone-treated castrated rat; (D) castrated rat treated with testosterone + THC; (E) castrated rat treated with THC. F indicates the faster moving variant.
Table 2. Effects of delta-9-THC (10 mg/kg) and testosterone propionate (2 mg/kg) on acid phosphatase activity in rat ventral prostatic tissue

<table>
<thead>
<tr>
<th>Treatment (daily for 7 days)</th>
<th>Acid phosphatase activity (mmol p-nitrophenol liberated/mg total tissue protein/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact (sham-operated) + oil + Tween 80–saline</td>
<td>Total 10.16 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>Tartrate insensitive 5.71 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>Tartrate sensitive 4.45 ± 0.29</td>
</tr>
<tr>
<td>Castrated + oil + Tween 80–saline</td>
<td>Total 3.85 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>Tartrate insensitive 1.86 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>Tartrate sensitive 1.97 ± 0.13</td>
</tr>
<tr>
<td>Castrated + THC + oil</td>
<td>Total 3.80 ± 0.28</td>
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<tr>
<td></td>
<td>Tartrate insensitive 1.85 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>Tartrate sensitive 1.96 ± 0.08</td>
</tr>
<tr>
<td>Castrated + testosterone propionate + Tween 80–saline</td>
<td>Total 28.75 ± 1.31</td>
</tr>
<tr>
<td></td>
<td>Tartrate insensitive 21.27 ± 1.75</td>
</tr>
<tr>
<td></td>
<td>Tartrate sensitive 7.48 ± 0.58</td>
</tr>
<tr>
<td>Castrated + testosterone propionate + THC</td>
<td>Total 16.96 ± 1.10</td>
</tr>
<tr>
<td></td>
<td>Tartrate insensitive 11.75 ± 1.09</td>
</tr>
<tr>
<td></td>
<td>Tartrate sensitive 4.98 ± 0.38</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m. for 6 different rats and were analysed by Student’s t test.

a Significantly different (P < 0.001) from values for intact control rats.
b Significantly different (P < 0.001) from values for rats castrated and given vehicle only.
c Significantly different (P < 0.005) from values for rats castrated and treated with testosterone.

Discussion

The present study presents evidence of the anti-androgenic potency of THC (10 mg/kg for 7 days), in relation to its effect in antagonizing the biochemical changes produced by testosterone in ventral prostate tissue of adult castrated rats.

The synthesis of nucleic acids and the protein content of rat ventral prostate tissue are completely dependent on androgen (Williams-Ashman, Liao, Hancock, Jurkowitz & Silverman, 1964; Rennie, Symes & Mainwaring, 1975). Testosterone stimulated these constituents in the ventral prostatic tissue of the adult castrated rats in this study but the stimulatory effect of androgen was significantly reduced when THC was also administered, indicating that THC has an anti-androgenic potency in relation to its counteracting effect on DNA, RNA and protein content in ventral prostatic tissue.

The acid phosphatase status of rat ventral prostatic tissue has been shown to be dependent on androgen (Tenniswood, Bird & Clark, 1976) and the stimulatory effect of testosterone on acid phosphatase in rat ventral prostate tissue was again reduced when THC was given with testosterone. The stimulatory effect of testosterone was relatively more marked on the tartrate-insensitive form of the enzyme and this was also more sensitive to the counteracting effect of THC.

The secretory type of acid phosphatase isoenzyme in rat ventral prostatic tissue is androgen-dependent (Tenniswood et al., 1978). A similar dependence of this isoenzyme of acid phosphatase of rat ventral prostatic tissue on the presence of testosterone was noted in our study. In the presence of THC, however, testosterone was unable to restore this androgen-dependent isoenzyme variant, further emphasizing the anti-androgenic potency of THC.

From the results of the present study, it is evident that THC can counteract the biochemical changes produced by testosterone in ventral prostatic tissue. Since THC alone has no effect on the ventral prostate of castrated rats and Purohit et al. (1979) have shown that under similar conditions of treatment THC cannot affect the conversion of testosterone to dihydrotestosterone in the ventral prostate, we conclude that THC acts directly in the prostatic tissue, perhaps by interaction with the androgen receptors.

We are grateful for financial assistance from the Indian Council of Medical Research; and thank Dr Olav J. Braenden, Narcotics Division, United Nations, Vienna, for the pure delta-9-THC.
References


Received 21 October 1980