ANTIOESTROGENIC ACTION OF CHLORMADINONE ACETATE

By

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ABSTRACT

The action of chlormadinone acetate (CAP) on the uptake of [6,7-3H]-17β-oestradiol in the castrated rat was studied. It was shown that when CAP is injected, the uptake of 3H-oestradiol diminishes significantly in the uterus and in the anterior pituitary (P < 0.01), whereas the uptake is not modified in the hypothalamus, posterior pituitary, skeletal muscle and plasma. A significantly (P < 0.01) smaller dose of CAP was required to inhibit the uptake of 17β-oestradiol by the anterior pituitary (27.2 ng) than by the uterus (1000 ng). Inhibition of oestradiol uptake can be demonstrated when CAP is administered before the oestrogen as well as when it is given subsequent to it (P < 0.01). This suggests the existence of a competitive mechanism between 17β-oestradiol and CAP for specific oestradiol receptors. When the action of CAP on different fractions of the uterine cell was studied, it was observed that the inhibition of oestradiol uptake occurs exclusively in the nuclear fraction (P < 0.01). Competition with oestrogens for specific protein receptors might be the common mechanism of action of the different manifestations of the effects of CAP.

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Chlormadinone acetate (CAP) is a progestagen which has been widely investigated as a contraceptive (Maqueo et al. 1963; Rudel et al. 1964; MacDonald et al. 1968; Zañarlu et al. 1968); nevertheless, its intimate mechanism of action is still unknown. Different loci of action have been described and several hypotheses have been suggested to explain the mode of action of CAP.

Döcke et al. (1968) suggested that CAP acts on the anterior pituitary by diminishing its sensitivity to the gonadotrophin releasing factors. Oestrogens are considered as being responsible for the lowering of the anterior pituitary threshold to the releasing factors.

Maqueo et al. (1963) postulated that CAP produces morphological modifications in the human endometrium which could be held responsible for a failure of implantation.

Martinez-Manautou et al. (1967) indicated that the primary action of CAP is due to its anti-oestrogenic effect on the cervical mucus.

The three mechanisms of action of CAP described seem to share an anti-oestrogenic effect. It is known that in order to exert its biological effects, oestrogens have to be taken up by specific receptors of the target cells (Jensen & Jacobson 1962). Therefore, the purpose of this work was to investigate whether different mechanisms of the actions of CAP could be interpreted as having a common factor, i.e. competition with oestradiol for its specific receptors.

**MATERIAL AND METHODS**

Female Wistar rats, average weight 200 g, spayed 6 weeks before the start of the experiments were used.

[6,7-3H] 17β-oestradiol, specific activity 40 Ci/mM (New England Nuclear Corporation, Boston, Mass.), was used after purification by paper chromatography in the benzene/formamide system, for 4 h. Rats were injected intravenously with different doses of chlormadinone acetate (6-chloro-17β-acetoxy-pregna-4,6-diene-3,20-dione, CAP) and with 2.72 µCi of the purified oestradiol dissolved in 0.5 ml of propylene glycol-ethanol 4:1 (v/v).

The animals were sacrificed by cervical dislocation 60 min after the administration of oestradiol.

In each experiment the organs were quickly removed, dissected out, weighed and digested in 5 N NaOH for 48 h. The radioactive material was extracted three times with 15 ml of ethyl acetate and the extracts were evaporated in small vials at 37°C. Ten ml of a scintillation liquid were added to each vial (4 g 2,5-diphenyloxazole and 40 mg p-bis 2-5-phenyloxazolyl/l (toluene) and the radioactivity was counted in a Packard Tri-Carb Liquid Scintillation Spectrometer; [4-14C] 17β-oestradiol (3.5 × 103 DPM) was added as an internal standard to correct for losses.

The results are expressed as DPM/mg of wet tissue.

The following four experiments were performed.

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Experiment No. 1

Objective. – To determine the modifications produced by CAP on the uptake of 17β-oestradiol by different tissues.

A dose of 100 µg of CAP was used and it was administered simultaneously with ³H-oestradiol.

The tissues studied were the uterus, the anterior and posterior pituitaries, the hypothalamus, the skeletal muscle and the plasma.

Experiment No. 2

Objective. – To determine the sensitivity of the uterus and the anterior pituitary to CAP.

The uptake of ³H-oestradiol by the uterus and the anterior pituitary was studied after the simultaneous injection of different doses of CAP: (equimolecular: 27.2 ng; 1, 5, 10, 100, 500 and 1000 µg).

Experiment No. 3

Objective. – To determine the existence of a competitive action between CAP and 17β-oestradiol.

One hundred µg of CAP were injected either 1 h before or 30 min after the injection of [6,7-³H] 17β-oestradiol.

Experiment No. 4

Objective. – To determine if CAP modifies the uterine subcellular distribution of 17β-oestradiol.

One hundred µg of CAP were injected simultaneously with [6,7-³H] 17β-oestradiol. For subcellular fractionation, the uteri were homogenized with sucrose 0.25 M (EDTA 10⁻³ M) and centrifuged for 10 min at 700 g in a Lourdes Beta Fuge refrigerated centrifuge. The supernatant was separated and the precipitate re-homogenized with 3.5 ml of cold sucrose solution. After repeated centrifugation, the precipitate (cellular walls and nuclei) was kept at 44°C; both supernatants were combined and centrifuged for 15 min at 10 000 g in a Beckman L2-50 preparative ultracentrifuge. The supernatant was separated and the precipitate re-suspended with 2 ml of cold sucrose and re-centrifuged. The precipitate (mitochondrial fraction) was kept at 4°C and both supernatants were centrifuged for 60 min at 105 000 g in order to complete the separation of the microsomal and soluble fractions. The purity of all the fractions was checked by electron microscopy. Oestradiol was extracted from the subcellular fractions using the previously described procedure.

RESULTS

Experiment No. 1

The administration of 100 µg of CAP produced a significant decrease in the uptake of oestradiol both by the uterus (P < 0.01) and by the anterior pituitary (P < 0.01). On the contrary, however, the uptake was not modified in the plasma, the skeletal muscle, the hypothalamus or the posterior pituitary (Table 1).
Table 1.

*In vivo* [6,7-\(^3\)H] 17\(\beta\)-oestradiol uptake in rats injected with 100 \(\mu\)g of chlormadinone acetate.

Chlormadinone acetate (CAP) and 2.72 \(\mu\)Ci of [6,7-\(^3\)H] 17\(\beta\)-oestradiol dissolved in 0.5 ml of propylene glycol-ethanol (4:1) were injected intravenously and simultaneously 1 h before sacrifice. Controls received only oestradiol.

<table>
<thead>
<tr>
<th></th>
<th>Controls DPM/mg wet tissue ± se</th>
<th>CAP DPM/mg wet tissue ± se</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>1.93 ± 0.54</td>
<td>2.34 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>3.60 ± 0.45</td>
<td>3.05 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>13.28 ± 1.35</td>
<td>15.90 ± 1.16</td>
<td></td>
</tr>
<tr>
<td>Anterior pituitary</td>
<td>389.50 ± 34.10</td>
<td>141.00 ± 4.56</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Posterior pituitary</td>
<td>93.62 ± 7.48</td>
<td>74.28 ± 4.57</td>
<td></td>
</tr>
<tr>
<td>Uterus</td>
<td>126.70 ± 6.97</td>
<td>64.04 ± 6.95</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

* Student's t test.

**Experiment No. 2**

It was observed that the various doses of CAP significantly diminished the uptake of oestradiol by the anterior pituitary \((P < 0.01)\). In the uterus, however, the lowest effective dose was 1 \(\mu\)g of CAP \((P < 0.01)\) (Table 2).

**Experiment No. 3**

CAP diminished the uterine uptake of oestradiol when injected both before as well as after oestradiol \((P < 0.01)\) (Table 3).

**Experiment No. 4**

The administration of CAP caused a significant decrease in the uptake of oestradiol only by the nuclear fraction of the uterus \((P < 0.01)\) (Table 4).

**DISCUSSION**

The results of the present work demonstrate that in spayed rats, CAP diminishes the uptake of oestradiol by the uterus and the anterior pituitary while it produces no modification in the uptake either by the hypothalamus or by the posterior pituitary.

The anterior pituitary is more sensitive to CAP than the uterus.

The inhibition of the anterior pituitary uptake of oestradiol is in accordance with the findings of Döcke *et al.* (1968) in that CAP implants in this gland prevent ovulation in the rat. These authors suggest that this might be due to
Table 2.
Effect of different doses of chlormadinone acetate on the in vivo uptake of [6,7-3H] 17β-oestradiol by the rat uterus and the anterior pituitary.
Chlormadinone acetate (CAP) and 2.72 µCi of [6,7-3H] 17β-oestradiol dissolved in 0.5 ml of propylene glycol-ethanol (4:1) were simultaneously injected intravenously 1 h before sacrifice. Controls received only oestradiol.

<table>
<thead>
<tr>
<th>CAP Doses</th>
<th>Uterus DPM/mg wet tissue ± se</th>
<th>Anterior pituitary DPM/mg wet tissue ± se</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>126.7 ± 7.0</td>
<td>389.5 ± 34.1</td>
</tr>
<tr>
<td>Equimolar (27.2 ng)</td>
<td>139.0 ± 7.6</td>
<td>250.0 ± 25.1</td>
</tr>
<tr>
<td>1 µg</td>
<td>99.7 ± 5.1</td>
<td>209.8 ± 15.3</td>
</tr>
<tr>
<td>10 µg</td>
<td>79.6 ± 3.1</td>
<td>213.0 ± 33.1</td>
</tr>
<tr>
<td>50 µg</td>
<td>72.0 ± 7.9</td>
<td>215.0 ± 19.2</td>
</tr>
<tr>
<td>100 µg</td>
<td>64.4 ± 7.0</td>
<td>141.0 ± 4.6</td>
</tr>
<tr>
<td>500 µg</td>
<td>58.5 ± 4.5</td>
<td>176.0 ± 14.4</td>
</tr>
<tr>
<td>1000 µg</td>
<td>62.7 ± 10.2</td>
<td>152.7 ± 13.5</td>
</tr>
</tbody>
</table>

All differences are significant in relation to the control at $P < 0.01$ except for the effect of the equimolar dose on the uterine uptake. Analysis of variance, Duncan test (Duncan 1955).

Table 3.
Effect of chlormadinone acetate injected before or after the administration of oestradiol.
Chlormadinone acetate (CAP) and 2.72 µCi of [6,7-3H] 17β-oestradiol dissolved in 0.5 ml of propylene glycol-ethanol (4:1) were injected intravenously. 3H-oestradiol was administered 1 h before sacrifice. Controls received only oestradiol.

<table>
<thead>
<tr>
<th>DPM/mg wet tissue ± se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>CAP 1 h before</td>
</tr>
<tr>
<td>CAP ⅓ h after</td>
</tr>
</tbody>
</table>

Analysis of variance (Duncan 1955) 1 vs. 2 $P < 0.01$
1 vs. 3
2 vs. 3

the fact that CAP implants inhibit the lowering of the sensitivity threshold to LH-RF, caused by oestradiol. Clomiphene produced similar effects in the rat's anterior pituitary (Kato et al. 1968). In the rat, clomiphene diminishes the sensitivity to gonadotrophins and acts as an anovulatory drug.

The decrease in the uptake of oestradiol occurs in the uterus when CAP is administered both before and after the injection of oestradiol. This suggests
Table 4.
Effect of chlormadinone acetate on the *in vitro* subcellular distribution of [6,7-3H] 17β-oestradiol in the rat uterus.

Chlormadinone acetate (CAP) and 2.72 μCi of [6,7-3H] 17β-oestradiol dissolved in 0.5 ml of propylene glycol-ethanol (4:1) were injected intravenously 1 h before sacrifice. Controls received only oestradiol.

<table>
<thead>
<tr>
<th></th>
<th>Nuclei dpm/mg ± SE</th>
<th>Mitochondria dpm/mg ± SE</th>
<th>Microsomes dpm/mg ± SE</th>
<th>Supernatant dpm/mg ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oestradiol</td>
<td>92.75 ± 4.00*</td>
<td>5.17 ± 0.65</td>
<td>3.43 ± 0.43</td>
<td>26.68 ± 1.82</td>
</tr>
<tr>
<td>Oestradiol + CAP</td>
<td>30.81 ± 6.63</td>
<td>4.40 ± 0.99</td>
<td>3.35 ± 0.48</td>
<td>21.21 ± 3.16</td>
</tr>
</tbody>
</table>

* P < 0.01 Student’s *t* test.

that there is a competitive mechanism between 17β-oestradiol and CAP for the oestrogen receptors.

The inhibition of the uptake of oestradiol by the uterus produced by the administration of CAP might account for the morphological changes in the human endometrium (*Maqueo et al.* 1963) and in the cervical mucus (*Martinez-Manautou et al.* 1967).

It is known that oestradiol interacts with a cytoplasmic protein to form the large 9.5 S receptor-oestrogen complex which results in a protein conformation capable of migrating into the nucleus. *Gorski et al.* (1968) demonstrated that more than 50% of the oestrogen was in the nuclear fraction and 30% in the cytosol; the remaining oestrogen was found in the mitochondrial and microsomal fractions. The increase in RNA and protein synthesis are early manifestations of oestrogen activity in the nucleus (*Gorski et al.* 1968).

By means of subcellular fractionation, it has been demonstrated that the decrease in the uptake of oestradiol takes place in the uterine cell nucleus.

The diminution in the uptake of oestradiol by the uterine nucleus could lead to modifications in the biological actions of oestradiol which, in turn, could be responsible for the described modifications in uterine function.

These results suggest that CAP competes with 17β-oestradiol for its specific receptors in the uterus in such a way that it produces a diminution in the oestradiol uptake by the nucleus.

REFERENCES


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