6-methoxy-tetrahydro-β-carboline (pinoline): effects on plasma renin activity and aldosterone, TSH, LH and β-endorphin levels in rats

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Abstract. Plasma renin activity and the plasma concentrations of aldosterone, TSH, LH and β-endorphin were radioimmunologically determined in rats after ip administration of 15 mg/kg of 6-methoxy-tetrahydro-β-carboline (6-MeO-THBC, pinoline), a compound found to occur normally in mammalian and avian pineal gland. A 2.6-fold increase of plasma aldosterone concentration was found and it was preceded by increase of plasma renin activity. An increase was also found in β-endorphin/β-lipotrophin concentration while TSH and LH concentrations did not change significantly. The present results do not prove nor exclude the concept that 6-MeO-THBC is the 'adrenoglotermulotropin'. The effects of 6-MeO-THBC on plasma β-endorphin/β-lipotrophin levels may be mediated by the dopaminergic or 5-HT-neurones.

Pineal gland has been shown to participate in the regulation of light-dependent biological rhythms and the function of endocrine glands (for ref. see Matthews & Seamark 1981; Motta 1982; Reiter 1981, 1982). Melatonin has often been thought to cause a major part of the endocrine actions of the pineal, but other biologically active compounds like other 5-methoxyindoles (McIsaac et al. 1965; Beck et al. 1982) and some peptides also occur in this gland (Reiter 1981; 1982). One pineal compound, thought to have a β-carboline structure, has been claimed to stimulate aldosterone secretion and was named 'adrenoglotermulotropin' by Farrell & McIsaac (1961). Recently we showed that 6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-THBC, pinoline, 5-methoxytryptoline) is a physiologically occurring compound in fowl and mammalian body with highest concentration in the pineal gland (Kari 1981; Kari et al. 1983). Both in pineal and in lesser degree in retina (Leino et al. 1983 and unpublished) its concentrations seemed to have a diurnal variation. It was also regularly present in human plasma and serum (Rimón et al. 1984).

6-MeO-THBC has been shown to increase the serum concentration of prolactin and corticosterone (Fang et al. 1978; Meyer & Buckholtz 1976), and to inhibit the oestrous cycle and the growth of genitals in rats (Airaksinen et al. 1970, 1978). In the present study we have measured plasma renin activity and the plasma concentrations of aldosterone, TSH, LH and β-endorphin in rats after a single dose of 6-MeO-THBC in order to evaluate the extent in which 6-MeO-THBC might participate in the regulation of the anterior pituitary hormones and the renin-angiotensin-aldosterone system.

Materials and Methods
Chemicals
6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-THBC) was synthetized according to Ho et al. (1968). The solution for injection was made by the following way. 6-MeO-THBC was first dissolved in some drops of N HCl. This was then diluted with 0.3% TRIS (Trizma®
hydrochloride, Sigma, St. Louis) in 2.5% glucose. The pH was adjusted to 7.2 with Sigma 7–9 biochemical buffer.

**Animals and treatment**

Male rats obtained from the animal colony of the University of Kuopio were used. They were Wistar origin (Wistar/Mol/Af/Han/Kuo) weighing 180–220 g. The rats were maintained under regularly alternating periods of light (14 h) and dark (10 h) and had free access to food (standard rat diet from Hankkuja Oy, Turku, Finland) and water. They were housed at 20 ± 0.3°C and 40–60% humidity in polyester cages containing wood shavings on the bottom.

6-MeO-THBC (15 mg/kg) was injected ip (1 ml/100 g) between 10 a.m. to 1 p.m. The control rats received an equal volume of the solvent by the same route. The rats were killed by decapitation. The blood was collected into cold glass tubes containing disodium EDTA in a 0.9% NaCl solution (about 3 mg EDTA/ml blood). The tubes were centrifuged refrigerated immediately and the plasma was stored at −20°C. About 100 rats were used, 8–12 in each treatment group and each plasma sample was usually used for the determination of three to four different hormones.

**Determinations**

The commercial aldosterone radioimmunoassay kit (ALDOCTK-125, International Cis, Paris) with antibody coated tubes was used for aldosterone determination. The sensitivity of this method of analysis was 10.5 ± 2.2 pg/ml and the percentage of cross-reaction with other steroids was highest with corticosterone (0.64%). The suitability of this test kit for rat plasma was studied by diluting various aliquots of rat plasma with human plasma (0-standard). The results obtained correlated well with the determinations with rat plasma without dilution.

Determination of the renin activity was based in the radioimmunoassay of angiotensin I which is generated during incubation of plasma at 37°C (RENK®, International Cis, Paris). Since the sensitivity of the test in relation to the activities was high, the incubation time used was 45 min instead of 1.5 h recommended for human plasma. This kit seemed to be suitable for rat plasma, although the pH optimum and the effect of some inhibitors of angiotensin I converting enzyme are different from those for human plasma (Menard & Catt 1972). The comparison of the measurements at pH 6.0 (optimum for human plasma) and pH 6.5 (optimum for rat plasma) showed 24% higher values when measurements were made at pH 6.5 (4.32 ± 0.56 at pH 6.0 and 5.36 ± 0.52 at pH 6.5, mean ± so for five measurement in each group). There is, however, good reasons to expect that the values measured at pH 6.0 correlate with those at the pH optimum for rat plasma. In addition to disodium EDTA, which is also an inhibitor of the converting enzyme, even the kit included two enzymatic inhibitors (8-hydroxyquinoline sulphate, 0.85 mmol/l and 2.3 mercaptopropanol, 1.29 μmol/l). According to Tikkanen et al. (1980) the combination of EDTA and 8-hydroxyquinoline appears the preferable enzyme inhibitor for renin assay in rat plasma. The sufficiency of inhibitor concentration was studied by adding EDTA (5 and 5 mmol/l) to the incubation mixture. Neither of these additions affected significantly the renin activity.

The plasma concentrations of TSH, LH and β-endorphin were determined by radioimmunoassays as follows. Purified rat LH and TSH (RPI) standards and rabbit anti-LH and -TSH sera were donated by NIMDHM, Bethesda, Md., USA. The hormones were iodinated and the plasma samples, buffers and antisera pipetted as presented in the instructions. The immunocomplexes were precipitated by sheep antirabbit serum and counted for radioactivity (Leppäluoto et al. 1974). β-endorphin was measured by the method described earlier (Vuotteenaho et al. 1981). Briefly, 100 μl plasma samples, iodinated porcine β-endorphin (a gift from N. Ling, The Salk Institute, Ca., USA) and diluted anti-β-endorphin (K2) were incubated at 4°C for overnight and precipitated by polyethylene glycol. The antisera detects β-endorphin and β-lipotrophin (β-LPH) at equal molar basis and hence the immunoreactivity was expressed as β-endorphin/β-LPH-immunoreactivity. 6-MeO-THBC added to the incubation mixtures was not found to interfere with any of the radioimmunoassays.

**Statistical analysis**

The one way analysis of variance was first carried out. Thereafter the multiple comparison test of Tukey was used to detect significant changes between the treated and non-treated groups and between groups with different sampling times.

**Results**

The results are presented in Table 1. Both the activity of renin and the plasma concentration of aldosterone increased highly significantly over the controls after the administration of 6-MeO-THBC. The maximum of renin activity (2.4 times the control) occurred already in the 15 min sample, while that of aldosterone (reaching 2.6 times the control) was found in the 30 min and 1 h samples. An increase was also found in serum β-endorphin/β-LPH level, while that of TSH tended to decrease (no significant changes were found in the analysis of variance or in Tukey test of TSH values although the 15 min values gave P < 0.05 in Stu-
Table 1.
The renin activity and the concentrations of aldosterone, thyrotrophin (TSH), luteinizing hormone (LH) and β-endorphin/β-lipotrophin (β-end/β-LPH) immunoreactivity in rat serum in different times after the administration of 6-MeO-THBC (15 mg/kg ip).

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Renin ng/ml/h</th>
<th>Aldosterone pg/ml</th>
<th>LH ng/ml</th>
<th>β-end/β-LPH ng/ml</th>
<th>TSH ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6-MeO-THBC</td>
<td>Controls</td>
<td>6-MeO-THBC</td>
<td>Controls</td>
<td>6-MeO-THBC</td>
</tr>
<tr>
<td>15 min</td>
<td>15.5 ± 1.9*</td>
<td>6.5 ± 1.1</td>
<td>1048 ± 136*</td>
<td>414 ± 100</td>
<td>36.3 ± 7.6</td>
</tr>
<tr>
<td>30 min</td>
<td>13.8 ± 1.2*</td>
<td>8.2 ± 0.9</td>
<td>1206 ± 148</td>
<td>457 ± 104</td>
<td>59.3 ± 10.9</td>
</tr>
<tr>
<td>60 min</td>
<td>9.3 ± 1.2*</td>
<td>6.4 ± 0.6</td>
<td>1198 ± 121*</td>
<td>619 ± 86</td>
<td>43.6 ± 4.1</td>
</tr>
<tr>
<td>120 min</td>
<td>6.9 ± 0.8</td>
<td>4.9 ± 0.4</td>
<td>490 ± 100</td>
<td>374 ± 109</td>
<td>53.8 ± 14.8</td>
</tr>
<tr>
<td>Non-treated</td>
<td>4.5 ± 0.3</td>
<td>262 ± 37</td>
<td>37.0 ± 6.0</td>
<td>269 ± 60</td>
<td>248 ± 90</td>
</tr>
<tr>
<td>(4)</td>
<td>(5)</td>
<td>(9)</td>
<td>(6)</td>
<td>(6)</td>
<td>(11)</td>
</tr>
</tbody>
</table>

Means ± SEM are given. Number of samples studied is in parentheses.

* P < 0.05 when compared with the corresponding controls (Tukey's test). In analysis of variance following F-values were obtained: aldosterone treated/untreated F = 28.3 P < 0.001, renin treated/untreated F = 18.2 P < 0.0001, β-endorphin treated/untreated F = 6.4 P < 0.016, TSH treated/untreated F = 3.84 P = 0.0543, LH treated/untreated F = 0.016 P = 0.90.
dent's non-dependent t-test). In LH no differences was found. The solvent (buffer of room temperature) tended to cause a small increase in the values of renin, aldosterone, LH and TSH (not significant with the analysis of variance).

Discussion

The present study shows that a single 15 mg/kg dose of 6-MeO-THBC significantly increased the secretion of aldosterone and β-endorphin/β-LPH. The mechanisms of its hormonal actions found in the present study and in the earlier ones are open and melatonin-like or other own specific actions of 6-MeO-THBC are by no means excluded. On the other hand, since 6-MeO-THBC by inhibiting MAO activity and 5HT uptake, increases tissue concentration of free 5HT (for ref. see Airaksinen & Kari 1981), the effects might be partly due to the increased 5HT (Airaksinen et al. 1978; Meltzer et al. 1982). This would mean a presence of a serotonergic link in the regulation of aldosterone secretion. At the dose level used 6-MeO-THBC is also a dopamine antagonist (Kari et al. 1980) and metoclopramide increases aldosterone secretion with a renin independent mechanism in several species including man and rat (Norbiato et al. 1977; Sowers et al. 1980; Aquilera & Catt 1984). The increase of renin activity comitantly with aldosterone concentration found in the present study suggest that the effects of 6-MeO-THBC on aldosterone secretion might go partly this way. Aldosterone level, however, increased so fast that a more direct effect on aldosterone secretion is also probable.

Although the results on the effects of both the pineal and 5HT on TSH are partly conflicting (Reichlin et al. 1978; Tuomisto 1981), a 5HT-ergic action generally seems to inhibit TSH secretion. Our present results, showing that 6-MeO-THBC may slightly decrease plasma TSH level, could thus be due to the increased 5HT effect.

Both 5HT and melatonin (perhaps acting through 5HT neurons) inhibit LH secretion (Franchini & Martini 1970; Müller et al. 1977; Nisticò & Scapagnini 1980), but component of pineal extract have shown both pro- and anti-gonadotrophic activity (Reiter 1974; Ebels et al. 1975). Our negative results on the plasma LH levels and those describing the decreased incidence of oestrus during ip 6-MeO-THBC treatment (Airaksinen et al. 1978) are somewhat controversial. The time of the day may not have been optimal for action on LH. On the other hand, the decreased incidence of oestrus may have been secondary to the increase of prolactin (Fang et al. 1978). The report on natural occurrence of a 6-MeO-THBC like compound in arcuate nucleus (Shoemaker et al. 1978) is interesting since the dopaminergic fibers from this nucleus may be essential inhibitors of the prolactin secretion.

The increased plasma β-endorphin/β-LPH levels after 6-MeO-THBC treatment is evidently due to increased release of hypothalamic CRF, since CRF also releases ACTH, and that has earlier been shown to increase after administration of 6-MeO-THBC (Buckholz 1980). CRF appears to be under an inhibitory dopaminergic tone (Jones 1978), since dopaminergic drugs have been observed to decrease the plasma levels of β-endorphin in rats (Locatelli et al. 1983). This might explain the stimulatory effects of 6-MeO-THBC on the secretion of ACTH (Buckholz 1980) and β-endorphin/β-LPH (this study).

In the present study a single but rather large dose of 6-MeO-THBC was given. It showed a significant effect on renin, aldosterone and β-endorphin/β-LPH. This does not show if the hormonal effects of 6-MeO-THBC have any physiological significance. In our preliminary studies a smaller dose (1 mg/kg) showed a rather weak effect on renin activity and corticosterone level. Thus, although the large response of plasma aldosterone in the present study seems to suggest that 6-MeO-THBC could be the compound which Farrell & McIsaac (1961) described as ‘adrenoglomerulotropin’, much more studies are needed to prove or disprove their hypothesis. However, even if this action will be shown to be of importance, 6-MeO-THBC seems to have effects on other function as well.

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References


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