CHANGES IN RAT ADRENAL MEDULLA FOLLOWING $\Delta^9$-TETRAHYDROCANNABINOL TREATMENT. A HISTOCHEMICAL STUDY

By

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ABSTRACT

The effects of acute (10 mg/kg) and chronic (10 mg/kg for 30 days) administration of delta-9-tetrahydrocannabinol ($\Delta^9$-THC) have been studied histochemically in the rat adrenal medulla, which include total catecholamines, noradrenaline, histometric measurements of adrenal medullary areas, calcium content of the medullary cells along with adenosine triphosphatase (ATPase), acetyl cholinesterase (AChE) and butyryl cholinesterase (BChE) activities.

Acute $\Delta^9$-THC treatment reduced the total catecholamine content (including noradrenaline) of the gland, was accompanied by increased ATPase, AChE, BChE activities and increased calcium distribution in the gland. Chronic $\Delta^9$-THC treatment caused significant hypertrophy of the chromaffin tissue, with decreased total catecholamine content, although noradrenaline containing areas exhibited no notable change. The calcium content and ATPase activity were increased along with a concomitant increase in AChE and BChE activities. Although the changes in adrenal medullary enzyme activities following both acute and chronic $\Delta^9$-THC treatment are qualitatively similar, marked quantitative increase is noted in the chronically treated groups. The results indicate an increased total catecholamine releasing activity of the adrenal medulla following acute $\Delta^9$-THC treatment, while chronic $\Delta^9$-THC administration produces a preferential release of adrenaline.

$\Delta^9$-Tetrahydrocannabinol ($\Delta^9$-THC), the major active component of cannabis (Mechoulam 1970) has been used as a tool by several investigators in their studies on the neurophysiological and neuropharmacological effects of cannabis
(Holtzman et al. 1969; Ho et al. 1970; Sofia et al. 1971; Scheckel et al. 1968). $\Delta^9$-THC induces an increased biogenic amine turnover in the CNS (Hollister et al. 1968; Sofia et al. 1971) and a rapid depletion of adrenomedullary catecholamines (Welch et al. 1971). Previous reports made from this laboratory (Biswas et al. 1974, personal communication) and of several investigators (Barry et al. 1971; Dewey et al. 1970) indicate that $\Delta^9$-THC has a marked activating effect on the adrenal cortex which bears a close functional interrelationship to the adrenal medullary activity. Most of the reported studies suggest that $\Delta^9$-THC exerts a considerable effect at the neuro-endocrinal level (Biswas et al. 1973; Biswas & Ghosh 1973), although little information is available on the $\Delta^9$-THC induced cytological changes of different endocrine glands.

Our present study describes the effects of acute and chronic administration of $\Delta^9$-THC on the histochemical changes of the adrenal medulla with regard to its total catecholamine (both adrenaline and noradrenaline), adenosine triphosphatase (ATPase) acetylcholinesterase (AChE), butyryl cholinesterase (BChE) activities, adrenal medullary areas and calcium content of the chromaffin tissue.

**MATERIALS AND METHODS**

*Animals*

Adult male albino rats weighing 100–120 g were used in this experiment. They were maintained on standard laboratory diet and water ad libitum. The animals were divided into two groups, i.e. Group I comprising of 48 animals for acute treatment and Group II comprising 48 animals for chronic treatment. Each group was again divided into two sub-groups consisting of an equal number of animals, one kept as experimental and the other as control.

*Drug treatment*

Pure synthetic $\Delta^9$-THC, stored in ethanol at $-5^\circ$C under nitrogen in sealed dark glass vials, was provided by the United Nations Narcotic Laboratory, Geneva. Before use the ethanol was evaporated out by bubbling through nitrogen, followed by suspensions of $\Delta^9$-THC in 0.9% saline–1% Tween-80 vehicle. For acute treatment a single injection of 0.5 ml $\Delta^9$-THC suspension in saline-tween 80 mixture (10 mg/kg) was given intraperitoneally, and the control groups received the saline-Tween vehicle in equivalent volume by the same route. The animals were sacrificed by rapid decapitation 6 h after the injection. The animals belonging to the chronic treatment group received a single intraperitoneal injection of 0.5 ml $\Delta^9$-THC in saline-Tween–80 vehicle (10 mg/kg) while the controls received the same volume of vehicle daily for 30 consecutive days. The animals were killed by rapid decapitation 24 h after the last injection.

*Histochemical procedure*

Adrenal glands were quickly removed and immediately processed for the histological and histochemical reactions.
(1) The chromaffin reaction was carried out essentially according to Hillarp & Hökfelt (1955) by fixing the tissue for 16 h in a 10.1 proportion of a 5% solution of K$_2$Cr$_2$O$_7$ for K$_2$CrO$_4$, adjusted to pH 5.6, to localize total catecholamines.

(2) Noradrenaline was localized by the iodate reaction according to Hillarp & Hökfelt (1955) by fixing the tissue in 10% neutral potassium iodate solution for 16 h at 10°C to increase specificity to the reaction.

(3) Noradrenaline and adrenaline cells were also identified by a glutaraldehyde-K$_2$Cr$_2$O$_7$ silver staining technique described by Jones (1967).

(4) Calcium was localized according to the method of Dahl (1952) by laking with Alizarin Red S.

(5) Fifteen µm cryostat of unfixed adrenal glands at -20°C were collected over pre-cleaned coverslips and incubated in the corresponding media for the demonstration of AChE, BChE by the method of Karnovsky & Roots (1964) and ATPase by the method of Padykula & Herman (1955). The onset of enzyme activities were noted and sections were fixed in 10% neutral formalin and finally mounted in glycerin jelly.

(6) Measurement of noradrenaline containing areas and adrenal medullary areas were done by Camera Lucida drawing method. Camera Lucida drawings were made of the optical sections of adrenal medullary zones and the iodate positive zones of the adrenal medulla on optical squared (mm) graph paper. The area thus traced was measured directly by counting the squares of the graph they covered. Then Camera Lucida drawings were made of the mm scale of the stage micrometer on the same graph paper. Then by dividing the areas drawn on the graph paper by the magnification, the actual area was determined.

In order to study the area, 10 µm serial sections of the adrenal glands were made and the sections containing medulla and cortex together were selected for measurement; in this manner every sixth sections was taken for area determination and thus six sections altogether were taken from each gland. The mean values of those six sections were considered to be representative of one gland.

RESULTS

The chromaffin reaction revealed a marked depletion of total catecholamines from the adrenal medulla following acute (Fig. 2) and chronic (Fig. 3) Δ⁹-THC treatment.

Depletion of total catecholamines from the medullary tissue was associated with a marked release of noradrenaline following acute Δ⁹-THC treatment. Both iodate reaction (Table 1) and glutaraldehyde silver sequence (Fig. 5) exhibited a similar type of change following acute Δ⁹-THC administration. It was of interest to note that the change in the noradrenaline containing area was negligible in the chronic treatment (Fig. 6, Table 1) although the total catecholamines showed noticeable depletion (Fig. 3).

The changes in the histometric measurement of adrenal medullary areas during different phases of Δ⁹-THC treatment are shown in Table 1 which
Table 1.  
Effect of $\Delta^9$-THC administration on the adrenal medullary and noradrenaline containing areas.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Acute $\Delta^9$-THC treatment</th>
<th>Chronic $\Delta^9$-THC treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal medullary areas ($\mu m^2$)</td>
<td>Mean $\pm$ 33.16</td>
<td>$\pm$ 28.78</td>
<td>$\pm$ 19.78</td>
</tr>
<tr>
<td></td>
<td>$t$ = 0.138**</td>
<td>$t$ = 5.85***</td>
<td></td>
</tr>
<tr>
<td>Noradrenaline containing iodate areas</td>
<td>Mean $\pm$ 2.17</td>
<td>$\pm$ 0.19</td>
<td>$\pm$ 1.67</td>
</tr>
<tr>
<td></td>
<td>$t$ = 5.479***</td>
<td>$t$ = 0.015**</td>
<td></td>
</tr>
</tbody>
</table>

* Each value represents the mean area of 6 glands, $t$-value compares in every case with control.
** Not significant.
*** Highly significant.

Indicate a significant hypertrophy of the chromaffin tissue following chronic administration.

It is evident from the optical sections of control adrenal medulla that calcium occurs in traces forming a network around the nuclei of chromaffin cells. Following acute $\Delta^9$-THC treatment, an increase in calcium mesh formation with an increased staining intensity was noted. Chronic $\Delta^9$-THC treatment produced a more marked increase in adrenal medullary calcium distribution. The entry of excess calcium within the chromaffin cells may be related to the release of catecholamines and this will be discussed later.

Figs. 1-6.
1. Chromaffin reaction showing normal distribution of the catecholamines in the adrenal medulla. $\times$ 96.
2. Chromaffin reaction showing marked depletion of total catecholamines from adrenal medulla in acute $\Delta^9$-THC treated animals. $\times$ 96.
3. Chromaffin reaction showing relatively less depletion of total catecholamines from adrenal medulla following chronic $\Delta^9$-THC treatment. Compare with Fig. 2. $\times$ 96.
4. Glutaraldehyde – K$_2$Cr$_2$O$_7$ silver stained adrenal medulla showing normal distribution of noradrenaline containing cells. $\times$ 192.
5. Glutaraldehyde – K$_2$Cr$_2$O$_7$ silver stained adrenal medulla showing marked depletion of noradrenaline following acute $\Delta^9$-THC treatment. $\times$ 192.
6. Glutaraldehyde – K$_2$Cr$_2$O$_7$ silver stained adrenal medulla showing no significant change in noradrenaline containing cells after chronic $\Delta^9$-THC treatment. $\times$ 192.
Incubation in Karnovsky and Roots medium enabled a contrast localization of both AChE and BChE activities in the rat adrenal medullary tissue. Sharp localization of AChE was noted in the control sections, along the splanchnic nerve fibres of the adrenal medulla (Fig. 7), while BChE showed a more general distribution in the chromaffin mass. Acute $\Delta^9$-THC treatment caused a moderate increase in AChE (Fig. 8), which was, however, more pronounced in the adrenal medulla of chronic $\Delta^9$-THC treated group (Fig. 9). The activity of BChE also showed a similar increase following acute and chronic $\Delta^9$-THC treatment. ATPase showed moderate activity in the adrenal medulla of the control animals (Fig. 10). $\Delta^9$-THC in either conditions of treatment caused enhanced ATPase activity. The alteration in ATPase activity after acute and chronic $\Delta^9$-THC treatment closely followed the pattern of AChE and BChE activity in the identical conditions of treatments (Figs. 11 and 12). The intensity of the enzyme activities observed histochemically in the adrenal medulla was similar among the animals of the same group.

DISCUSSION

Acute $\Delta^9$-THC administration

The present histochemical observations show a marked depletion of total catecholamines from the adrenal medullary tissues after acute $\Delta^9$-THC treatment. This finding corroborates with the earlier biochemical report (Welch et al. 1971) which suggests a rapid depletion of total adrenal medullary catecholamines in $\Delta^9$-THC treated mice. From the results of iodate reaction and glutaraldehyde-silver procedure, it is evident that the depletion of total catecholamines was associated with the noradrenaline liberation following acute $\Delta^9$-THC treatment. In addition an enhanced ATPase activity with the catecholamine release was noted after acute $\Delta^9$-THC treatment. Although the role of ATPase in the release of ATP bound catecholamines from the granules

Fig. 7–12.

7. Adrenal medulla showing normal AChE activity. The reaction products are visible as dark deposits along the splanchnic nerve. $\times$ 96.
8. Adrenal medulla showing increased AChE activity following acute $\Delta^9$-THC treatment. $\times$ 96.
9. Adrenal medulla showing marked increase of AChE treatment. Compare with Fig. 8. $\times$ 96.
10. Adrenal medulla showing normal ATPase activity. $\times$ 96.
11. Adrenal medulla showing increased ATPase activity following acute $\Delta^9$-THC treatment. $\times$ 96.
12. Adrenal medulla showing marked increase of ATPase activity following chronic $\Delta^9$-THC treatment. Compare with Fig. 11. $\times$ 96.
remain conjectural, reports are available which suggest an involvement of ATPase in the process (Banks 1965; Douglas et al. 1965).

It has long been known that ACh released by the splanchnic nerve acts as the immediate physiological transmitter for the catechoamine release (Douglas & Rubin 1961), while histochemical demonstration of AChE provides the information about the extent of cholinergic involvement (Koelle 1946). In our present study, acute $\Delta^9$-THC administration was found to be associated with a marked elevation of AChE and BChE activities in the medullary region. It is known that AChE causes a rapid hydrolysis of ACh following its production (Koelle 1946) and it has been suggested that BChE acts as a barrier against the unhydrolysed ACh (Abraham et al. 1957; Shapiro 1958). Hence an increase in both the enzyme activities after $\Delta^9$-THC treatment may account for the excess catecholamine liberation as has already been reported in this study.

That ACh exerts its stimulatory effect on the chromaffin cells by increasing the permeability of the plasma membrane to Ca$^{++}$, has been claimed by several investigators (Douglas & Poisner 1961; Douglas & Rubin 1961). Hence the present finding of an increased Ca$^{++}$ mesh formation in the medullary tissue may be a secondary effect of $\Delta^9$-THC induced ACh hyperactivity and may account for the enhanced liberation of endogenous amines by the Ca$^{++}$ dependent stimulation secretion coupling mechanism as suggested by Douglas & Poisner (1961, 1962) and Douglas (1966).

The single dose of $\Delta^9$-THC may act as a stressor the action of which is probably mediated through the sympathetic splanchnic nerve and thus may cause an enhanced liberation of catecholamines. Other psychopharmacological agents such as reserpine, morphine (Ray et al. 1968) and LSD (Ganong et al. 1961) are known to alter adrenomedullary secretions, although the mechanism by which they affect the adrenomedullary function may be different in each case.

**Chronic $\Delta^9$-THC treatment**

The main change in the adrenal medulla after chronic $\Delta^9$-THC treatment is a significant increase in the area of the medullary tissues (Table 1). The hypertrophy of the chromaffin cells was not associated with any significant change in either iodate positive areas (Table 1) or glutaraldehyde silver treated, noradrenaline containing cell distribution, while total catecholamines seemed to be depleted. These results may indicate an increased preferential release of adrenaline. It has already been reported (Barry et al. 1971; Dewey et al. 1970) that $\Delta^9$-THC causes adrenocortical hyperactivity which may account for the increased induction of phenyl ethanolamine-N-methyl transferase (Wurtman & Axelrod 1965, 1966) the enzyme responsible for the conversion of noradrenaline to adrenaline (Theonen et al. 1969). There are reports that an increased glucocorticoid level in the blood can not only affect the secretory
pattern but also influence the size of medullary cells and their protein constituents (Lempinen 1964; Mikulaskova-Rochova & Linet 1967). Hence $\Delta^9$-THC induced increased adrenocortical activity can again account for the observed hypertrophy of adrenal medulla.

In view of the fact that the adrenomedullary hypertrophy is not associated with any significant change in noradrenaline containing cells (Table 1), as has been observed in the present study, it may be concluded that the adrenaline containing cells may indirectly account for the hypertrophied mass of tissues. There are physiological indications that the secretion of noradrenaline and adrenaline is elicited differentially from the medullary tissues although the anatomical basis is not clear (De Robertis 1964). Several examples of such differential secretions have been described following chemical stimuli (Duner 1953, 1954). There are reports about the preferential release of adrenaline from the adrenal medulla after stimulation of the hypothalamus (Folkow & Euler 1954; Redgate & Gallhorn 1953) and amygdala regions of the brain (Gunne & Reis 1963; Reis & Gunne 1965). A selective accumulation of $\Delta^9$-THC or its metabolites in the amygdala region during maximal behavioural activity has also been reported (McIsaac et al. 1971). Hence the present report of increased adrenaline formation may be explained on the basis of differential CNS stimulation, brought about by $\Delta^9$-THC. It may be mentioned in this connection that prolonged hypothalamic stimulation may also lead to an increased tyrosine hydroxylase and PNMT activities (Reis et al. 1970) which are the rate limiting enzymes in the synthesis of noradrenaline and adrenaline respectively. In view of the well known interrelationship between the emotional state and the secretory activity of the adrenal medulla (Elmadjian et al. 1958) and characteristic patterns of adrenaline secretion for specific psychotic stress conditions (Bergman 1959), it seems likely, that the observed changes in the adrenal medulla brought by $\Delta^9$-THC may in someway be related to its well known psychomimetic property.

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