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EFFECT OF SINGLE AND REPEATED ADMINISTRATION OF △ 9-TETRAHYDROCANNABINOL ON 5-HYDROXYTRYPTAMINE, NORADRENALINE, DOPAMINE AND TRYPTOPHAN LEVELS IN THE BRAIN OF WISTAR RATS

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Single or repeated administration of △ 9-tetrahydrocannabinol (30 mg/kg i. p.) increased 5-hydroxytryptamine levels in rats' brain, but did not affect dopamine or noradrenaline levels. Brain and blood tryptophan levels were raised only after a single-dose regimen. The primary effect of △ 9-tetrahydrocannabinol may be a mobilization of blood tryptophan by displacement from its albumin-bound depots, due to a high affinity of the drug for plasma albumin.

Several studies concerned with the effects of △ 9-tetrahydrocannabinol (THC) on brain amines yielded contradictory results (1—4). A clarification of this issue seemed desirable in view of the recent suggestion that THC may be advantageously used as a therapeutic agent (5,6). We have, therefore, reexamined the effect of THC on three brain amines: dopamine (DA), noradrenaline (NA), and 5-hydroxytryptamine (5-HT), as well as on the latter's indirect precursor, tryptophan (TR). We followed their levels 1, 2, 3, and 4 hours after administration of a single dose of THC, and 2 hours after the last of a series of equally sized doses administered at 24-hour intervals for 5 or 10 days. As the brain levels of TR were affected by single doses of THC, we also followed blood TR under the same regimen to find out whether the change in brain TR can be correlated with a similar change in the circulation.

MATERIALS AND METHODS

THC was isolated (7) from a mixture of total cannabinoids extracted (8) from Macedonian marihuana, then purified (9) to satisfactory elemental analysis, IR data, and thin-layer chromatographic homogeneity. An injectable preparation containing 120 mg of drug per ml was obtained by dissolution of pure THC in propylene glycol (10). All solvents, thin-layer chromatographic supplies, and reagents used in these procedures were highest-grade purity products from E. Merck, Darmstadt, W. Germany.

Male Wistar rats weighing 150—200 g randomly assigned to experimental and control groups (N = 8 or 10) were kept under normal laboratory conditions until killed. The experimental animals received 30 mg/kg of THC intraperitoneally, once or at 24-hour intervals for 5 or 10 days. The controls received corresponding volumes (0.25 ml per kg) of pure propylene glycol so that possible action of this solvent could be detected. One group of controls was treated with 0.25 ml/kg of saline. Amine extracts were obtained (11) and used for fluorimetric determinations (Aminco Bowman Spectrofluorometer, American Instruments Company, Silver Springs, Maryland, U. S. A.) of DA (12), NA (13) and 5-HT (14) as well as TR (15). Free blood TR was determined according to *Wapnir* and *Stevenson* (16). The statistical significance of differences in mean values was assessed by Student's t-test.

RESULTS AND DISCUSSION

Table 1 shows that both single and repeated THC treatments produced statistically significant changes in brain concentrations only for 5-HT and TR.

After a single dose of THC the 5-HT concentration increased up till 4 hours after injection, at which time it reached about 275 per cent of the control value. Repeated administration of THC for 5 or 10 days produced somewhat smaller changes in brain 5-HT. Brain TR levels rose moderately after a single dose of THC, reaching 140 per cent of the control value in two hours while no difference between experimental and control values could be observed at the end of a 5-day regimen. The changes in free blood TR produced by THC were similar to those in the brain (Table 2). A single dose of THC caused a moderate increase initially (up to 160 per cent of the control value within two hours), but subsequently the TR level tended to decrease, and after another two hours it was reduced to 143 per cent of the control value. At the end of 5-day and 10-day treatments free blood TR levels did not significantly differ from the corresponding control values. We consider our results of brain amine levels to be qualitatively in accord with earlier

observations of *Sofia* and co-workers (3) and *Maitre* (4), but contrary to those reported by *Gallager* and co-workers (2). Our findings agree with the suggestion that elevated blood and brain TR levels enhance the biosynthesis of 5-HT (17,18). The primary effect of THC may, in fact, be a mobilization of blood TR by displacement from its albumin-

Table 1

Biogenic amines and tryptophan levels (μg/g S. E. M.) in rat's brain after single and repeated administration of △⁹-tetrahydrocannabinol in propylene glycol

Treatment		Dopamine	Noradrenaline	5-hydroxy-tryptamine	Tryptophan
PG	Saline	0.49±0.04	0.38±0.02	0.30±0.01	2.28±0.23
	2 hours	0.52±0.02	0.37±0.02	0.30±0.01	2.20±0.33
	5 days	0.55±0.03	0.46±0.06	0.35±0.04	2.51±0.30
	10 days	0.53±0.05	0.45±0.05	0.39±0.04	—
THC	1 hour	0.53±0.04	0.41±0.02	0.41±0.06	—
	2 hours	0.48±0.02	0.43±0.02	0.60±0.05**	3.29±0.35*
	3 hours	0.55±0.07	0.47±0.03	0.79±0.09	—
	4 hours	0.57±0.07	0.49±0.04	0.83±0.05**	—
	5 days	0.63±0.03	0.43±0.02	0.71±0.04**	2.25±0.20
	10 days	0.64±0.04	0.38±0.02	0.66±0.04**	—

PG = propylene glycol; THC = △⁹-tetrahydrocannabinol
* $p \leq 0.05$
** $p \leq 0.01$

Table 2

Free blood tryptophan (μ g/ X ± S. E. M) in rats (N = 8) after single and repeated administration of △⁹-tetrahydrocannabinol in propylene glycole (THC, 30 mg/kg)

Treatment		Tryptophan
PG	Saline	1.27±0.13
	2 hours	1.38±0.12
	4 hours	1.28±0.16
	5 days	1.60±0.15
	10 days	1.57±0.12
THC	1 hour	1.74±0.22
	2 hours	2.24±0.25* X
	3 hours	2.12±0.21* X
	4 hours	2.05±0.19* X
	5 days	1.44±0.23
	10 days	1.47±0.24

PG = propylene glycol; THC = △⁹-tetrahydrocannabinol
* $p < 0.05$

-bound store (19), due to a high affinity of the drug for plasma albumin (20, 21). Once released in free form, TR is driven across the blood--brain barrier and its concentration in the brain rises. The metabolism of brain TR is thereby accelerated, which results in an increase of brain 5-HT. Repeated THC administration during five days and longer probably depletes the albumin-bound TR store so that no further increase in free blood TR, and therefore no increase in brain TR, can occur thereafter and the latter is soon restored to normal. However, brain 5-HT remained at a level significantly above that in controls when brain TR was back at normal, which may indicate that THC not only enhances the biosynthesis but also reduces the degradation of 5-HT in the brain. *Sofia* and co-workers (10) consider that a decrease in brain 5-HT degradation may be caused by accumulation of THC in this organ. In our experiments with repeated administration accumulation was likely to have taken place as the drug is known to be protractedly retained by lipoproteinic constituents of the nervous tissue (22). The assumption that 5-HT degradation is reduced in the presence of THC is further supported by the observation of a markedly reduced oxygen consumption which occurs even after single small doses of THC (23) and is likely to retard essential catabolic processes. It may be taken that this THC effect must have offset any activation of monoamine oxidase the drug may have brought about (24). As a matter of fact, monoamine oxidase could have been hardly activated at all in our experiments, as indicated by the failure of the THC treatment to depress the levels of brain DA and NA.

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Sažetak

UČINAK JEDNOKRATNE I VIŠEKRATNE APLIKACIJE △⁹-TETRAHIDROKANABINOLA NA NIVO 5-HIDROKSITRIPTAMINA, NORADRENALINA, DOPAMINA I TRIPTOFANA U MOZGU ŠTAKORA

△⁹-tetrahidrokanabinol, apliciran jednokratno i višekratno (30 mg/kg, ip.) povećava nivo moždanog 5-hidroksitriptamina u štakora, ali ne i nivoe dopamina i noradrenalina. Nivo triptofana u mozgu i krvi povećava se samo nakon jednokratnog davanja. Primarni efekat △⁹-tetrahidrokanabinola može da bude mobilizacija krvnog triptofana vezanog za albumin, što je posljedica velikog afiniteta △⁹-tetrahidrokanabinola prema albuminu plazme.

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