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SHORT COMMUNICATIONS

Effect of Δ^9-tetrahydrocannabinol on monoamine oxidase activity of rat tissues *in vivo*

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Monoamine oxidase (MAO; monoamine:O_2 oxido-reductase (deaminating); EC 1.4.3.4) plays an important role in the metabolism of biogenic amines in neuronal tissue [1, 2], whereas in non-neuronal tissues, the enzyme has some protective influence from the harmful effects of circulating monoamines [3]. Several recent reports have attempted to correlate the behavioral effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) with changes in the levels of serotonin or its metabolites [4–7] and norepinephrine metabolism [8–11]. It is suggested that Δ^9-THC influences all the steps involved in the maintenance of biogenic amine levels in brain and other tissues [5, 12]. In the present study the effects of both acute and chronic administration of Δ^9-THC on MAO activity in rat blood platelets and in mitochondria from whole brain, hypothalamus and heart were studied.

Δ^9-THC, suspended in saline–tween 80, was injected intraperitoneally into male albino rats weighing 100–130 g at a dose of 50 mg/kg or 10 mg/kg. The rats were sacrificed after 6 hr. To study the long-term effect of Δ^9-THC, the treatment was continued for 21 days and the rats were sacrificed 6 hr after the last injection. Control rats received an equivalent volume of the saline–tween 80 vehicle. The rats were killed by cervical dislocation and tissue samples were collected in ice-cold conditions. The mitochondrial fraction from brain, hypothalamus, liver, kidney and heart was prepared according to the sucrose density gradient method of Gray and Whittaker [15]. The blood platelets were isolated from heparinized blood by the method of Collins and Sandler [16]. The platelets were then suspended in 0·1 M phosphate buffer, pH = 7·0.

MAO activity was estimated by the method of Green *et al.* [13], as modified by Guha [14]. Protein was estimated by the method of Gornall *et al.* [17].

Table 1 shows that the intraperitoneal administration of pure Δ^9-THC, both at low (10 mg/kg) and high (50 mg/kg) doses, increased the activity of MAO in blood platelets and in whole brain, hypothalamus and heart mitochondria but had no effect on MAO activity in liver and kidney mitochondria. A greater effect was produced by the higher dose of Δ^9-THC. MAO activity in hypothalamic mitochondria was stimulated more than that in other tissues (100–186%) by both low and high doses of Δ^9-THC. After treatment with Δ^9-THC for 21 days (Table 2) the mitochondrial MAO activities of whole brain and hypothalamus were markedly increased, whereas in liver, kidney, heart and blood platelets, the stimulatory effects were not so great.

In view of the fact that MAO is associated with the membrane components of mitochondria and because of the high lipid solubility of Δ^9-THC, it is possible that the increased MAO activity is due to the interaction of Δ^9-THC with the mitochondrial membrane components, as

Table 1. Effect of acute administration of Δ^9-THC on mitochondrial MAO activity of rat tissues

Sources	MAO activity (Δ O.D. 420 mμ/100 mg protein/hr) Control (saline–tween 80)	Δ^9-THC 10 mg/kg	Δ^9-THC 50 mg/kg
Platelets	5·85 ± 0·09	8·76 ± 0·16	13·21 ± 0·21
Whole brain	32·16 ± 0·98	38·39 ± 1·07	47·46 ± 1·20
Hypothalamus	173·90 ± 5·01	347·21 ± 6·31	491·47 ± 8·79
Heart	11·80 ± 0·44	17·78 ± 0·86	21·60 ± 0·27
Liver	241·64 ± 4·56	248·69 ± 5·56	249·61 ± 4·01
Kidney	126·88 ± 2·62	130·88 ± 3·12	131·05 ± 4·37

Results expressed as mean ± S.E.M. of four determinations.
MAO activity was measured 6 hr after Δ^9-THC administration (i.p.).

Table 2. Effect of chronic administration of Δ^9-THC on mitochondrial MAO activity

Sources	MAO activity (Δ O.D. 420 mμ/100 mg protein/hr) Control (saline–tween 80)	Δ^9-THC (10 mg/kg/day)
Platelets	5·56 ± 0·14	7·04 ± 0·19
Whole brain	32·16 ± 1·06	48·36 ± 1·71
Hypothalamus	173·90 ± 4·81	334·84 ± 6·38
Heart	16·35 ± 0·23	14·40 ± 0·34
Liver	241·64 ± 7·56	264·64 ± 8·92
Kidney	126·88 ± 5·62	128·12 ± 4·62

Results expressed as mean ± S.E.M. of four determinations. MAO activity was determined 6 hr after the last injection (i.p.) of Δ^9-THC. The treatment was continued for 21 consecutive days.

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a result of which there is increased accessibility and/or permeability of the mitochondrial membrane to the corresponding amine substrate. Bino *et al.* [18] have recently reported that Δ^9-THC has an effect on the outer membrane and cristae of isolated rat liver mitochondria.

The most interesting result of this study is the striking increase in the activity of hypothalamic MAO induced by both acute and chronic administration of Δ^9-THC. Since the hypothalamus is rich in monoaminergic neurons, the present findings, suggest that the central monoamine neurons are an important site of action of Δ^9-THC. The neuropharmacological implications of the present study in relation to the role of biogenic amines in Δ^9-THC-induced changes in brain function need further investigation.

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Modification of procaine metabolism in rat liver after administration of phenobarbital or ethyl *p*-nitrophenyl phenylphosphonothioate

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Our previous studies have shown that a liver microsomal arylamidase is responsible for the hydrolysis of several drugs possessing an amido bond such as isocarboxazid (ISOC), a monoamine oxidase (MAO) inhibitor [1, 2]. Recently, procaine (PROC) was also found to be readily hydrolyzed by this enzyme *in vitro*.* The arylamidase was known to be either induced by phenobarbital (PB) [3] or markedly inhibited by organophosphorus insecticides at doses lower than those which inhibited serum cholinesterase in our laboratory [4, 5].

The present studies were therefore undertaken to determine whether PROC metabolism *in vivo* is modified by

* K. Moroi and T. Satoh, manuscript in preparation.

Table 1. Effect of pretreatment with phenobarbital or ethyl *p*-nitrophenyl phenylphosphonothioate (EPN) on procaine metabolism in rats*

Treatment	Isocarboxazid†		Procaine†	
	Enzyme activity	(%)	Enzyme activity	(%)
Control	2·23 ± 0·19 (19)	100·0	2·74 ± 0·13 (12)	100·0
EPN (1 mg/kg, p.o.)				
2 hr	0·14 ± 0·07 (5)	5·7	0 (5)	0
4 hr	0·23 ± 0·02 (7)	9·3	0·68 ± 0·07 (8)	27·4
6 hr	0·46 ± 0·08 (7)	18·6	0·57 ± 0·01 (6)	22·9
Phenobarbital (80 mg/kg/day, 4 days)	5·08 ± 0·23 (12)	204·8	7·41 ± 0·72 (8)	298·8

* Figures in parentheses indicate number of animals used. Enzyme activity represents mean ± S.E.M. and values are expressed in μmoles product/g liver wet wt/30 min.

† Isocarboxazid and procaine were used as substrates.