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Further studies on brain concentrations of amphetamine and its metabolites in strains of mice showing different sensitivity to pharmacological effects of amphetamine

The mechanism by which amphetamine increases the body temperature remains in doubt.

Peripheral (McCullough, Milberg & Robinson, 1970; Jellinek, 1971; Gessa, Clay & Brodie, 1969) central (Matsumoto & Griffin, 1971; Hill & Horita, 1970) and both central and peripheral (Weis, 1973) mechanisms have been suggested because of the known interactions between amphetamine and the catecholaminergic systems.

We have examined the reasons for the insensitivity shown by C_3H mice towards amphetamine-induced hyperthermia.

Charles River CD_1 and C_3H mice (20–25 g) were housed in plastic cages, 6 animals per cage (25 × 25 × 15 cm) at 23° with a relative humidity (60%). All animals whether untreated or pretreated with reserpine (2·5 mg kg i.p., 16 h earlier) received an intraperitoneal injection of (+)-amphetamine sulphate at various doses. Body temperature was recorded before and every 30 min after amphetamine treatment.

Blood was collected following decapitation and the plasma was used for the determination of FFA according to Dole (1956) with minor modifications.

Brains were removed and immediately dissected on dry ice; whole brains and striatal areas were stored at −20° until analysis. Amphetamine and its hydroxylated metabolites (*p*-hydroxyamphetamine and *p*-hydroxynorephedrine) were measured according to Ånggård, Gunne & Niklasson (1970) and Belvedere, Caccia & others (1973) respectively. Homovanillic acid (HVA) was determined in the striatum using the procedure of Korf, Ottema & Van der Veen (1971).

From the results in Table 1, it can be seen that (+)-amphetamine sulphate (7·5 mg kg^{-1}, i.p.) elicits a hyperthermic effect in CD_1 mice, while not significantly increasing the body temperature in C_3H mice. Also, the concentration of homovanillic acid (HVA), the major metabolite of dopamine, in the striatum is increased in CD_1 mice but is unaffected in the C_3H strain. However, the concentrations of amphetamine in whole brain as well as in the striatum are similar for the two strains. Moreover no differences could be observed in concentrations of the *p*-hydroxylated metabolites of amphetamine. These differences in sensitivity do not apply to all the effects of amphetamine on the two strains of mice. For instance amphetamine elicits a similar dose-dependent increase of body temperature in both strains if the animals are pretreated with reserpine (2·5 mg kg^{-1}, i.p. 16 h earlier). Similarly the two strains respond with a comparable increase of plasma free fatty acids (FFA) to (+)-amphetamine sulphate (5 mg kg^{-1}, i.p.).

The data reported here confirm previous findings that C_3H mice are insensitive to the hyperthermic effect of amphetamine (Dolfini, Garattini & Valzelli, 1969a, b; Caccia, Cecchetti & others, 1973; Jori & Garattini, 1973). Concentrations of amphetamine in the whole brain and in the striatum, a site where amphetamine interacts with dopamine stores, have been found comparable for the sensitive (CD_1) and the insensitive (C_3H) strain of mice. Also *p*-hydroxyamphetamine and *p*-hydroxy-

Table 1. *Effect of (+)-amphetamine sulphate on body temperature and striatum homovanillic acid (HVA) and brain levels of amphetamine and its metabolites p-hydroxyamphetamine (pOH-A) and p-hydroxynorephedrine (pOHNE)*

Strain	Time	Body temperature $\Delta°$ ± s.e.	Amphetamine Brain in toto ng g^{-1} ± s.e.	Amphetamine Striatum ng g^{-1} ± s.e.	pOH-A Brainstem ng g^{-1} ± s.e.	pOHNE Brainstem ng g^{-1} ± s.e.	HVA Striatum ng g^{-1} ± s.e.
CD_1	30	+2·7 ± 0·1	5580 ± 250	4710 ± 610			
	60	+0·6 ± 0·2	2650 ± 190	2840 ± 340	15 ± 6·9	24 ± 3·8	507 ± 30
	120	−1·3 ± 0·2	—	1210 ± 60	14 ± 3·0	27 ± 3·4	377 ± 26
	180		—	—	8 ± 0	37 ± 4·9	
	300		—	70 ± 6	—	—	
C_3H	30	+0·2 ± 0·2*	5240 ± 310	5090 ± 720			
	60	−0·5 ± 0·1*	3200 ± 210	3300 ± 240	20 ± 1·2	24 ± 2·7	329 ± 16*
	120	−0·6 ± 0·1	—	1480 ± 210	15 ± 1·0	27 ± 2·1	264 ± 19*
	180		—	—	9 ± 0·5	40 ± 6·4	
	300		—	70 ± 1	—	—	

* $P < 0{\cdot}01$ versus the CD_1 strain.
The concentration of HVA in untreated mice was 252 ± 15 ng g^{-1} for CD_1 and 279 ± 20 ng g^{-1} for C_3H strain.

norephedrine, measured in the brainstem where they preferentially accumulate, as previously demonstrated for rats (Cattabeni, Racagni & Groppetti, 1973; Jori & Caccia, 1974), are present at low but comparable concentrations in both strains, thus confirming that *p*-hydroxylation represents a minor pathway in the metabolism of amphetamine in mice (Smith & Dring, 1970). That a preferential hydroxylation of amphetamine in C_3H mice may be the cause for the strain's insensitivity toward amphetamine hyperthermia is also excluded even though the accumulation of *p*-hydroxynorephedrine has been suggested as being responsible for the disappearance of the hyperthermic effect during a repeated amphetamine treatment (Groppetti & Costa, 1969; Brodie, Cho & Gessa, 1970; Lewander, 1971). It has been suggested (Gessa & others, 1969) that mobilization of FFA could be a major event in determining the hyperthermic response of amphetamine. Our findings do not support this hypothesis because amphetamine increased plasma FFA to a similar extent in both strains of mice, the sensitive and the insensitive to amphetamine hyperthermia. Other evidence is in favour of a dissociation between the elevation of plasma FFA and the hyperthermia induced by amphetamine. In fact Fuller, Shaw & Molloy (1972) have shown that structural modifications of the amphetamine molecule gives compounds with high lipolytic activity but without hyperthermic effect and Bizzi, Bonaccorsi & others (1970) blocked the increase of plasma FFA induced by amphetamine with lipolysis inhibitors without affecting the hyperthermia. Also, Matsumoto & Shaw (1971) reported that desipramine reduces the effect of amphetamine on plasma FFA but not on the increase of body temperature.

Our findings also show that amphetamine increases the concentration of striatum HVA only in the strain of mice sensitive to its hyperthermic effect. Therefore, according to the dopaminergic hypothesis (Hill & Horita, 1971; Matsumoto & Griffin, 1971), C_3H mice could be insensitive to the hyperthermia as a consequence of the lack of activity of amphetamine on dopamine metabolism. Dopamine turnover in the striatum of C_3H mice (Caccia & others, 1973) is lower than in sensitive mice. Also the hyperthermia in C_3H mice when amphetamine is given after a large dose of reserpine may be consistent with the dopaminergic hypothesis, since after reserpine the synthesis of dopamine is not impaired and the amine cannot be stored in the

vescicles (Rutledge & Weiner, 1967) but it is more available for the receptor sites (Roth & Stone, 1968), particularly because the presynaptic uptake is blocked by amphetamine.

In conclusion, our findings are consistent with the hypothesis that the hyperthermic effect of amphetamine in mice is mediated by an interaction of this drug with the dopaminergic system.

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