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Interaction of (+)-amphetamine with cerebral dopaminergic neurones in two strains of mice, that show different temperature responses to this drug

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Summary

1. (+)-Amphetamine sulphate elicits a dose-dependent hyperthermia in NMRI mice but it does not significantly increase the body temperature of C_3H mice.

2. When low doses of (+)-amphetamine are given, the body temperature of C_3H mice decreases.

3. (+)-Amphetamine decreases the noradrenaline concentration in the brain-stem and increases the homovanillic acid concentration (HVA) in the striatum of NMRI mice, but only slightly reduces the noradrenaline concentration and does not change the HVA concentration in the brains of C_3H mice.

4. The two strains appear to show a difference in the metabolism of dopamine in the striatum. The rates at which dopamine disappears from the tissue after blocking catecholamine synthesis with α-methyltyrosine and the rates at which HVA accumulates after blocking the active transport of this metabolite out of the brain with probenecid suggest that the turnover of dopamine is lower in C_3H mice than in NMRI mice.

Introduction

The symptoms induced by (+)-amphetamine in mice are strain-dependent (Weaver & Kerley, 1962; Brown, 1965). It has been reported that C_3H mice are considerably less sensitive than other strains to the hyperthermic and lethal effects of (+)-amphetamine (Dolfini, Garattini & Valzelli, 1969a; 1969b). The reduction in the concentration of noradrenaline in the brain produced by this drug is less pronounced in C_3H mice than in an albino strain (Dolfini, Ramirez del Angel, Garattini & Valzelli, 1970).

Pharmacogenetic experiments, conducted at this Institute, and involving the crossmating of C_3H (insensitive to (+)-amphetamine) and NMRI albino mice (sensitive to amphetamine) suggest that the hyperthermic response to amphetamine is a polygenically inherited trait (Jori, Price-Evans, unpublished results).

Furthermore, (+)-amphetamine interacts with cerebral dopaminergic neurones by releasing dopamine (Carlsson, 1970; Glowinski, 1970), blocking the neuronal uptake of dopamine (Fuxe & Ungerstedt, 1970) and increasing the turnover of this transmitter in the striatum (Javoy, Thierry, Kety & Glowinski, 1968; Javoy, Hamon & Glowinski, 1970; Costa & Groppetti, 1970). As additional evidence of this interaction, it was also reported that (+)-amphetamine increases the concentration of the major metabolite of dopamine, homovanillic acid (HVA), in the

striatum areas of mice and rats, in proportion to its hyperthermic effect (Jori & Bernardi, 1969 ; Jori & Bernardi, 1972). The suggestion of a relation between the increase in body temperature and dopamine is reinforced by the finding that a specific blockade of dopamine receptors in the brain antagonizes the amphetamine-induced hyperthermia in rats (Matsumoto & Griffin, 1971) and in rabbits (Hill & Horita, 1971). The purpose of the present study is to investigate whether the absence of the hyperthermic response to amphetamine in C_3H mice might be correlated with a different reactivity of central dopaminergic neurones.

Methods

Male and female mice of C_3H (Radiobiological Institute, T.N.O., Rjisvjik, Netherlands) and NMR, (Selvi, Milan, Italy) strains, weighing 25 ± 5 g, were used. Animals were kept in Makrolon cages ($25 \times 25 \times 15$ cm), six in each cage, at a room temperature of 23° C and with a relative humidity of 60 per cent.

(+)-Amphetamine, sulphate dissolved in 0·9% w/v sodium chloride solution, was injected intraperitoneally at doses of 3·75 ; 7·5 and 15 mg/kg (expressed as salt).

Body temperature was measured by inserting a thermistor in the rectal cavity to a depth of about 1·5 cm.

In the experiments with probenecid, the drug was injected at a dose of 200 mg/kg, i.p. at different times before killing (see Figure 1). α-Methyltyrosine and α-methyltyrosine, methyl ester, (200 mg/kg, i.p.) was given 1 or 2 h before killing.

The rate of synthesis and the turnover time of dopamine in the striatum area of mice were estimated according to Brodie, Costa, Dlabac, Neff & Smookler (1966).

HVA was determined on 3 pooled striata by the method of Korf, Ottema & Van der Veen (1971) ; noradrenaline and dopamine were measured in the brainstem by the method of Shore & Olin (1958) ; 5-hydroxyindolylacetic acid (5HIAA) and 5-hydroxytryptamine, according to Giacalone & Valzelli (1969).

Statistical evaluation of the data consisted of a factorial analysis of variance (2×2) for the hyperthermic effect of 7·5 mg/kg of amphetamine, reported in Table 1 ; the comparisons of the averages reported in all the other Tables were made with Student's *t* test.

Results

(+)-Amphetamine elicits a dose-dependent hyperthermia in male and female NMRI mice, while it does not significantly increase body temperature in C_3H mice even at the highest tested dose (Table 1). The administration of 3·75 and 7·5 mg/kg in female, and 3·75 mg/kg in male C_3H mice produces a hypothermic effect.

An analysis of variance for the data obtained 30 min after giving 7·5 mg/kg, shows no sex difference for hyperthermia in NMRI mice, but a significant difference between sexes for the body temperature changes in C_3H mice, the females being more sensitive than the males to the hypothermic effect of amphetamine.

The results given in Table 2 show that amphetamine (7·5 mg/kg) produced hyperthermia in NMRI mice at three different environmental temperatures. C_3H mice showed a weak hyperthermia only when the environmental temperature was

TABLE 1. *Effect of different doses of (+)-amphetamine sulphate on body temperature of female and male NMRI and C_3H mice*

Strain	Sex	Treatment	Dose mg/kg i.p.	Body temperature change (°C±S.E.) 30 min	60 min	90 min	120 min
NMRI	♂	Amphetamine	3·75	+0·6±0·2	−0·08±0·3	−0·01±0·2	−0·25±0·2
			7·5	+2·3±0·2	+2·8±0·2	+2·1±0·3	+1·1±0·3
NMRI	♀	„	3·75	+1·0±0·1	+0·5±0·2	+0·1±0·2	—1·1±0·3
			7·5	+2·5±0·1	+1·8±0·2	+0·2±0·2	+0·1±0·4
			15·0	+3·4±0·2	+2·7±0·2	+0·5±0·5	−1·5±0·5
C_3H	♂	„	3·75	−0·6±0·2	−0·08±0·3	+0·6±0·2	+0·5±0·1
			7·5	+0·2±0·1	+0·4±0·2	+0·1±0·2	+0·2±0·2
C_3H	♀	„	3·75	−1·7±0·1	−1·1±0·2	−0·2±0·2	+0·2±0·1
			7·5	−0·7±0·1	−1·0±0·2	−1·1±0·3	−1·3±0·3
			15·0	+0·2±0·1	+0·7±0·1	+0·6±0·1	+0·4±0·1

Eighteen females and 18 males of each strain received different doses of (+)-amphetamine sulphate. The interval between the treatments in the same group was one week.

TABLE 2. *Effect of (+)-amphetamine sulphate on body temperature of male NMRI and C_3H mice at different environmental temperatures*

No. of mice	Strain	Treatment	Room temperature °C	Body temperature °C±S.E.	Body temperature changes (°C±S.E.) after treatment 30 min	60 min
12	NMRI	Control	15	31·7±0·1	+0·3±0·08	+0·2±0·1
12		Amphetamine		31·7±0·2	+1·8±0·06	+1·1±0·1
18		Control	22	35·9±0·05	+0·01±0·07	—
18		Amphetamine		35·4±0·11	+2·3±0·2	+2·8±0·2
12		Control	31	36·2±0·1	−0·1±0·04	−0·4±0·1
12		Amphetamine		36·0±0·1	+3·9±0·1	+2·0±0·2
12	C_3H	Control	15	30·8±0·2	+0·3±0·06	+0·5±0·09
12		Amphetamine		31·3±0·3	+0·3±0·1	+0·5±0·08
18		Control	22	36·1±0·2	−0·5±0·1	−0·9±0·2
18		Amphetamine		35·0±0·1	+0·2±0·1	+0·4±0·2
12		Control	31	35·8±0·1	−0·2±0·05	−0·4±0·09
12		Amphetamine		35·6±0·1	+1·1±0·07	+1·0±0·06

Male mice were placed at the indicated room temperatures 16 h before the beginning of the experiment. (+)-Amphetamine sulphate was injected i.p. in a dose of 7·5 mg/kg. Controls received 0·9% w/v NaCl solution i.p. (1 ml/100 g).

TABLE 3. *Effect of (+)-amphetamine on the noradrenaline, homovanillic acid, 5-hydroxytryptamine (5-HT) and 5-hydroxyindolylacetic acid (5HIAA) concentrations in NMRI and C_3H mice*

Strain	Sex	(+)-Amphetamine sulphate 7·5 mg/kg, i.p.	5-HT ng/g brain ±S.E.	5HIAA ng/g brain ±S.E.	Noradrenaline ng/g brainstem±S.E.	HVA ng/g striatum±S.E.
NMRI	♂	Control	—	240±40	560±40	224±4
		Treated	—	260±10	310±10*	619±55*
	♀	Control	480±10	—	540±20	240±15
		Treated	510±10	—	390±30*	464±21*
C_3H	♂	Control	—	280±10	540±30	265±20
		Treated	—	250±20	430±10*	234±10
	♀	Control	490±10	—	570±20	279±15
		Treated	520±20	—	510±30	265±23

Each figure is the average of at least 4 determinations for HVA and 8 determinations for noradrenaline, 5-hydroxytryptamine (5HT) and 5-hydroxyindolylacetic acid (5-HIAA). Mice were killed for the determinations of noradrenaline or HVA 1 h and for 5HT or 5HIAA 2 h after the administration of (+)-amphetamine sulphate.

* $P<0·01$ versus untreated mice; (Student's t test).

31° C but the increase was very small in comparison to the body temperature changes produced under similar experimental conditions in NMRI mice.

The effect of (+)-amphetamine on the noradrenaline and HVA concentrations in brain is reported in Table 3. (+)-Amphetamine (7·5 mg/kg, i.p.) significantly decreased the noradrenaline concentration in the brainstem, while it increased the HVA concentration in the striatum of female and male NMRI mice. In C_3H mice, (+)-amphetamine did not change the central HVA concentration and slightly decreased the brainstem noradrenaline concentration, (the difference between treated and control mice reached a statistically significant value only in males).

When amphetamine was given to animals of the two strains kept at different environmental temperatures an increase in the concentration of cerebral HVA was seen only in the NMRI mice. Amphetamine produced no effect on the concentration of 5-hydrovytryptamine or 5-hydroxyindolylacetic acid (5HIAA) in the brain of either strain (Table 3).

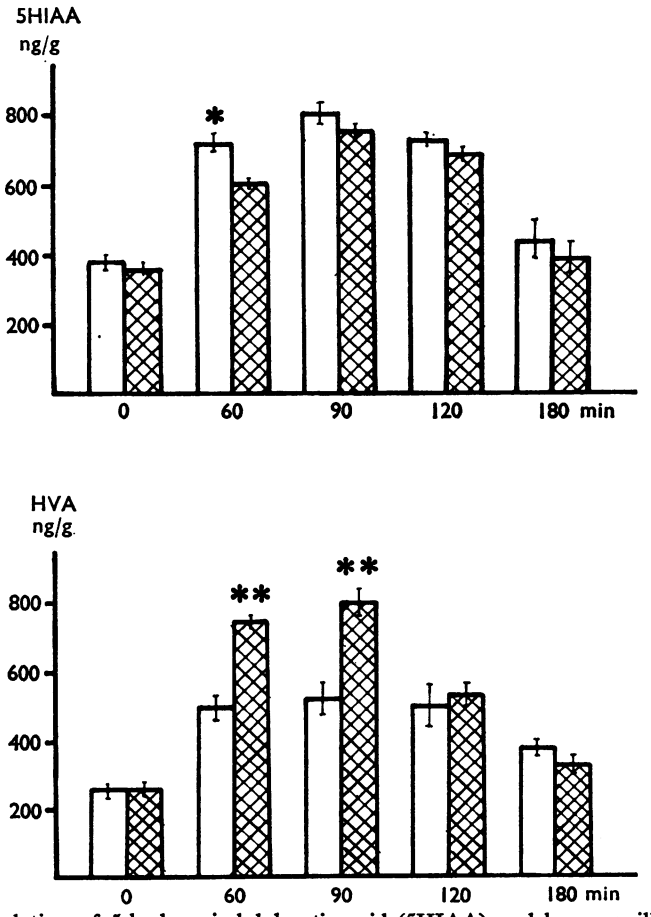

FIG. 1. Accumulation of 5-hydroxyindolylacetic acid (5HIAA) and homovanillic acid (HVA) in brain after probenecid administration in NHRI and C_3H mice. Probenecid was given in a dose of 200 mg/kg, i.p. 5HIAA was measured in brainstem and HVA in three pooled striata for each determination at different times after probenecid treatment. Each column (open for C_3H and shaded for NMRI) represents the average of at least 5 determinations. The vertical bars represent the standard error of the mean. $^{**}P<0{\cdot}01$; $^{*}P<0{\cdot}05$ differences between NMRI and C_3H values at the same times, corrected for the values at 0 time.

TABLE 4. *Turnover rates and turnover times of striatum dopamine in NMRI and C_3H mice*

No. of mice	Strain	Steady-state level μg/g±S.E.	Rate constant of amine loss K (h^{-1})±S.E.	Turnover time h	Turnover rate (μg/g)/h
26	NMRI	4·26±0·20	0·188±0·021	5·32	0·800
30	C_3H	4·28±0·16	0·136±0·017*	7·35	0·582

Mice were given α-methyltyrosine (200 mg/kg) and α-methyltyrosine methyl ester (300 mg/kg) together intraperitoneally 1, 2 or 3 h before killing. When mice were killed 3 h after the above treatment, a second administration of α-methyltyrosine methyl ester (200 mg/kg i.p.) was given 1 h before. K represents the slope ×2·3 of the curve obtained by plotting the $\log_{10}$ concentrations at 0, 1, 2 and 3 h after the first α-methyltyrosine treatment. Turnover rate is the product of the steady-state concentration and the rate constant of the decline in dopamine concentration (K). Turnover time is 1/K; * $P<0·05$.

The results obtained when mice were treated with probenecid (200 mg/kg i.p.), to block the active transport of the acid metabolites of dopamine and 5-hydroxytryptamine, are shown in Figure 1. The HVA concentrations in the striatum increased to a greater extent in NMRI than in C_3H. Conversely, 5HIAA accumulated more rapidly in C_3H than in NMRI mice.

Dopamine turnover, obtained by plotting the curves of its disappearance after synthesis had been blocked is shown in Table 4. Dopamine concentration declined exponentially at a rate constant which was lower for C_3H than for NMRI mice (Table 4).

Discussion

The results reported here confirm previous studies (Dolfini, *et al.*, 1969a, 1969b), showing that (+)-amphetamine does not elicit a hyperthermic effect in C_3H mice. In C_3H mice, a hypothermic effect appears at low doses of amphetamine, with a clear statistical difference between males and females. A sex difference in body temperature changes that occur in response to handling and in regard to amphetamine toxicity in C_3H mice was also reported by Brown & Julian (1968). It should be noted that a hypothermic effect was also observed by other authors in Swiss mice (McCullough, Milberg & Robinson, 1970) and in rats (Jellinek, 1971) after intraperitoneal doses of amphetamine (1 mg/kg) or after an intracerebral injection of few micrograms of this compound. These authors suggested that, at low doses, amphetamine can produce hypothermia through a direct action on the anterior hypothalamus, while, at high doses, the hyperthermia would depend on peripheral mechanisms involving a release of free fatty acids (Gessa, Clay & Brodie, 1969). Alternatively, it was suggested that both effects are mediated by biogenic amines released centrally but that the pattern of the amines released by small doses would differ from that produced by larger doses. However, an argument against the peripheral origin of hyperthermia is presented by Hill & Horita (1971) and Matsumoto & Griffin (1971), who showed that in rabbits and rats, pimozide, a drug that blocks the central actions of dopamine (Andén, Butcher, Corrodi, Fuxe & Ungerstedt, 1970), also antagonizes the amphetamine-induced hyperthermia. Furthermore, the effect of amphetamine on plasma free fatty acid concentrations was demonstrated not to be essential for the development of the hyperthermic response (Matsumoto & Shaw, 1971 ; Bizzi, Bonaccorsi, Jespersen, Jori & Garattini, 1970).

It is of interest to note that, in C_3H mice insensitive to amphetamine-induced hyperthermia, but more sensitive than the NMRI strain to the hypothermic effect, there is no effect on the HVA concentration in brain structures which contain dopamine. Also, the noradrenaline stores are less affected by amphetamine in C_3H than in albino mice, as previously reported by Dolfini *et al.* (1969a, b ; 1970).

These results do not depend on the rate of amphetamine metabolism, as similar concentrations of this compound were found in the brain of the two strains of mice (Dolfini *et al.*, 1969a). Neither do they result from a reduced general responsiveness to amphetamine since the drug elicits the same anorexic effect and it increases free fatty acids in a similar manner in both C_3H and NMRI mice (unpublished results).

The two strains of mice also show basic differences as far as catecholamine metabolism is concerned. In fact, the turnover rate of striatal dopamine is lower in C_3H than in NMRI mice, which is in agreement with the fact that probenecid increases the striatal HVA concentration less in the former than in the latter strain. Conversely, the increase of brain 5HIAA after administration of probenecid was higher for C_3H than for NMRI mice. As it has been postulated that biogenic amines may have a modulatory role in temperature regulation (Feldberg & Myers, 1963), different kinetic properties of catecholamine and 5-hydroxytryptamine synthesis could account for the different temperature responses to amphetamine in different strains of mice.

However, the fact that amphetamine does not affect the catecholamines in C_3H mice does not necessarily imply that the hyperthermia induced by this drug depends only on mechanisms involving central adrenergic neurones. Other systems, such as endocrine regulation, could be partially responsible for this genetic trait. Chai (1958) has shown that thyroid activity may be different in various strains of mice. A relative lack of thyroid function may explain the lack of hyperthermic activity of amphetamine in C_3H mice (Dolfini, *et al.*, 1970 ; Brown & Julian, 1968).

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