On the mechanism of the hyperthermia induced by amphetamine in the rat

One of the sympathomimetic effects of amphetamine in the rat is the production of a dose-dependent hyperthermia, lasting for 3–4 h, which is of uncertain origin. Amphetamine is an indirectly acting drug, releasing noradrenaline, dopamine and 5-hydroxytryptamine (5-HT) from nerve terminals, but it is not clear which of these transmitters is responsible for the hyperthermia, or whether this is a central or peripheral effect of the drug (Gessa, Clay & Brodie, 1968; Weis, 1973). When amphetamine is administered repeatedly, considerable tolerance develops to the hyperthermic effect (Lewander, 1971) and this has been attributed to the accumulation of *p*-hydroxynorephedrine, the suggested false transmitter metabolite of amphetamine, at noradrenergic nerve endings (Brodie, Cho & Gessa, 1970). However, when *p*-hydroxynorephedrine and drugs metabolized to it are given to rats, they confer protection against the hyperthermic effect of amphetamine only in male rats, these pretreatments being ineffective in females, (Sever, Caldwell & Williams, 1974). In view of this finding, the mechanism of amphetamine-induced hyperthermia in both male and female rats has been re-examined, to determine whether sex differences in this underlie the observed sex difference in *p*-hydroxynorephedrine protection. Rats were given a variety of pretreatments designed to modify the levels of the individual neurotransmitter amines in the peripheral and central nervous system, and then the hyperthermic effect of amphetamine was examined in these animals.

Male and female Wistar albino rats, 200–300 g, were housed at 22±2° in groups of five with free access to food (Oxoid No. 41B pellets) and water. Body temperatures were measured using a YSI No. 46 telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio, U.S.A.) equipped with a No. 402 probe (tip diam. 0·3 mm), which was placed in the colon 5 cm from the anus. The experimental design is indicated in Table 1. The animals were pretreated as shown with equivalent volumes of saline, and then challenged either with (+)-amphetamine (5 mg kg^{-1}, i.p.) or an equivalent volume of saline. Body temperatures were recorded immediately before and at 0·5, 1, 2 and 4 h after the challenging injection. The results are expressed as the mean maximal temperature rise after amphetamine and were statistically analysed by the Student *t*-test for unpaired data.

The pretreatments used (See Table 1) were chosen to affect noradrenaline, dopamine and 5-HT as follows. The ongoing biosynthesis of catecholamines was inhibited by pretreatment with α-methyl-*p*-tyrosine methyl ester hydrochloride (Sigma Chemical Co.) (see Table 1) as described by Buethin, Miya & others (1972), to give maximal depletion of noradrenaline and dopamine. Peripheral sympathectomy was achieved by a single intravenous injection of 6-hydroxydopamine hydrobromide (Aldrich Chemical Co.) (Smookler & Clark, 1972), while selective depletion of dopamine in the periphery was achieved by giving desipramine (Pertofran, Geigy) before 6-hydroxydopamine (Breese, Cooper & Smith, 1973). The passage of impulses through the peripheral autonomic nervous system was prevented by the ganglion blocker, tetraethylammonium bromide (Acheson & Pereira, 1948). Depletion of central 5-HT stores was achieved by the prior administration of *p*-chloroamphetamine (gift of Dr. R. E. McMahon, Eli Lilly Research Laboratories, Indianapolis, Ind. U.S.A.) which causes a transient lowering of brain noradrenaline levels but a very long lasting depletion of 5-HT (Miller, Cox & others, 1970) so that at 3 days after dosing, 5-HT is the only amine depleted.

The results are shown in Table 1 and it is clear that only those pretreatments which deplete noradrenaline reduce the amphetamine hyperthermia. Ganglion blockade

Table 1. *Influence of various pretreatments designed to impair nerve function on amphetamine-induced hyperthermia.* Values are mean maximum temperature rise after challenge in each group, ±s.e. n is given in parentheses.

Pretreatment	Effect of pretreatment	Mean maximum temperature rise in: Female challenged with Amphetamine	Female challenged with Saline	Male challenged with Amphetamine	Male challenged with Saline
α-Methyl-*p*-tyrosine methyl ester (150 mg kg^{-1}, i.p. 2 × daily for 3 days; last dose 12 h before challenge)	Inhibits catecholamine biosynthesis	0·1 ± 0·1 (8)*	0·1 ± 0·1 (5)	0·1 ± 0·1 (5)*	0·1 ± 0·2 (5)
Saline as above		2.0 ± 0·3 (6)	—	2·1 ± 0·1 (10)	—
6-Hydroxydopamine (100 mg kg^{-1}, i.v. 48 h before challenge)	Depletes peripheral catecholamines	0·8 ± 0·3 (7)*+	0·2 ± 0·1 (6)	0·5 ± 0·2 (5)*+	0·0 ± 0·0 (5)
Saline as above		2·2 ± 0·2 (10)	—	2·0 ± 0·3 (7)	—
Desmethylimipramine (15 mg kg^{-1}, i.p.) followed 30 min later by 6-hydroxydopamine (100 mg kg^{-1}, i.v.); challenge given 48 h later	Depletes peripheral dopamine	1·9 ± 0·2 (5)+	0·4 ± 0·2 (5)	2·4 ± 0·7 (4)+	0·4 ± 0·1 (5)
Saline as above		2·4 ± 0·3 (10)	—	2·1 ± 0·2 (8)	—
p-Chloroamphetamine (3 mg kg^{-1}, i.p.) 72 h before challenge	Depletes central 5-HT	2·4 ± 0·3 (5)+	0·3 ± 0·2 (5)	2·1 ± 0·2 (5)+	0·3 ± 0·1 (6)
Saline as above		2·3 ± 0·1 (6)	—	2·0 ± 0·1 (8)	—
Tetraethylammonium bromide (30 mg kg^{-1}, i.p.) 30 min before challenge	Ganglion blocker	2·7 ± 0·1 (6)+	0·6 ± 0·1 (3)	2·4 ± 0·5 (6)+	0·4 ± 0·1 (5)
Saline as above		2·4 ± 0·2 (6)	—	2·1 ± 0·1 (9)	—

* $P<0·01$ compared with saline pretreated amphetamine challenge control. + $P<0·01$ compared with similarly pretreated saline challenge control.

was ineffective suggesting that transmission through the autonomic nervous system is not involved in amphetamine hyperthermia. Similarly, the lack of effect seen with *p*-chloroamphetamine would appear to indicate that 5-HT in the cns is not involved in the hyperthermic response to amphetamine. None of these pretreatments affected the baseline temperatures of test and control groups immediately before challenge.

However, both inhibition of catecholamine synthesis and peripheral sympathectomy abolish the hyperthermic response, without affecting the basal temperature of the rats. When the peripheral sympathectomy was restricted to dopaminergic neurons, the hyperthermia was unaffected. This would appear to suggest that intact peripheral noradrenergic neurons are a prerequisite for the hyperthermic action of amphetamine. No sex differences were observed in this study.

It thus appears that the mechanism of the hyperthermia induced by amphetamine in the rat is different from other species, in that it is apparently peripheral while in the mouse and rabbit it appears to be a central effect. In the mouse, peripheral sympathectomy with 6-hydroxydopamine does not influence amphetamine hyperthermia (Wolf & Bunce, 1973) whereas in the rabbit evidence for a central mechanism for the hyperthermia has been presented by Hill & Horita (1971). The conclusion reached here for a peripheral mechanism in the rat is supported by the work of Weis (1973) who showed that amphetamine retained its hyperthermic effect in the decapitated rat. The finding that there is no sex difference in the mechanism of the hyperthermia induced in the rat by amphetamine indicates that other causes must be sought for the sex difference in *p*-hydroxynorephedrine protection found by Sever & others (1974).

We thank Prof. R. T. Williams, F.R.S. for his interest in this work. P.S.S. thanks the M.R.C. for a Junior Research Fellowship.

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July 3, 1974

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Evidence against the involvement of false neurotransmitters in tolerance to amphetamine-induced hyperthermia in the rat

When amphetamine is administered repeatedly to rats and guinea-pigs, considerable tolerance develops to its hyperthermic and anorectic effects (Lewander, 1971; Sever & Caldwell, 1974). Drug tolerance in general can be due to changes either in drug disposition occurring on chronic administration, or in the sensitivity of the target organ to the drug, but for tolerance to amphetamine, another type of mechanism has been proposed, that is, that a metabolite of amphetamine acts as an antagonist to it. This metabolite is *p*-hydroxynorephedrine, a false noradrenergic neurotransmitter which can be stored, released and taken up by the nerve ending in the same way as noradrenaline (Brodie, Cho, & Gessa 1970). Amphetamine acts by releasing noradrenaline from nerve endings, but on prolonged administration, *p*-hydroxynorephedrine accumulates in the place of noradrenaline and it is the presence of this much less active noradrenergic agonist which gives rise to tolerance.

The hyperthermic effect of amphetamine in the rat is evoked through intact peripheral noradrenergic neurons (Caldwell, Sever & Trelinski, 1974), and tolerance to this effect has been attributed to the false transmitter mechanism outlined above. The validity of this hypothesis can be tested by a study of the ability of metabolic precursors other than amphetamine of the false transmitter to protect against amphetamine-induced hyperthermia. Work on the metabolism of a range of amphetamine analogues (see Williams, Caldwell & Dring, 1974) has shown that both *p*-hydroxyamphetamine and norephedrine give rise to *p*-hydroxynorephedrine (see Table 1).

The results reported here show that these other precursors of the false transmitter protect only male rats and not female rats against amphetamine-induced hyperthermia. Lewander, (1971) has already reported that *p*-hydroxyamphetamine protects rats against amphetamine-induced hyperthermia. We have therefore