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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

Table 1. *Metabolism formation of* p-*hydroxynorephedrine from amphetamine analogues in male and female Wistar albino rats*

Compound	Sex	% of ^{14}C dose in 0–24 h urine	% of ^{14}C dose as -hydroxynorephedrine
p-Hydroxyamphetamine	M	92	2[a]
	F	84	3[b]
Norephedrine	M	84	27[a]
	F	92	28[c]

a. J. Caldwell & P. S. Sever, unpublished observations.
b. Sever, Dring & Williams, 1973.
c. Sinsheimer, Dring & Williams, 1973.

repeated Lewander's work exactly and found the above sex difference using his methods and our own.

p-Hydroxyamphetamine (Paredrine) hydrobromide was the gift of Smith, Kline & French Laboratories, Philadelphia, Pa, U.S.A. (+)-Amphetamine sulphate (Koch-Light Laboratories, Colnbrook, U.K.), (±)-amphetamine sulphate (Halewood Chemicals, Staines, U.K.), norephedrine hydrochloride (Sigma Chemical Co., Kingston-upon-Thames, U.K.) and *p*-hydroxynorephedrine hydrochloride (R. N. Emanuel Ltd., Wembley, U.K.) were purchased.

Male and female Wistar albino and Sprague-Dawley rats, 200-300 g, were housed at 22 ± 2° in groups of five in polythene cages (45 × 22 × 15 cm) with free access to food (Oxoid No. 41b pellets) and water. Body temperature was measured with a Yellow Springs Instrument No. 46 tele-thermometer (Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio, U.S.A.) equipped with a no. 402 probe (tip diam. 0·3 mm), which was inserted in the colon 5 cm from the anus.

Male and female Wistar albino rats were injected intraperitoneally with either *p*-hydroxyamphetamine (40 mg kg^{-1}), norephedrine (15 mg kg^{-1}), *p*-hydroxynorephedrine (5 mg kg^{-1}) or equivalent volumes of isotonic saline solution. They were challenged 20 h with an injection of (+)-amphetamine (5 mg kg^{-1}, i.p.) or an equivalent volume (2 ml kg^{-1}) of saline. Body temperature was measured immediately before and at 0·5, 1, 2 and 4 h after the challenging injection. In another series of experiments, designed to reproduce the work of Lewander (1971), male and female Wistar albino and Sprague-Dawley rats were injected intraperitoneally with either *p*-hydroxyamphetamine (40 mg kg^{-1}) or an equivalent volume of saline (2 mg kg^{-1}). They were challenged, 20 h later, with either (±)-amphetamine (20 mg kg^{-1}, i.p.) or an equivalent volume of saline (2 ml kg^{-1}, i.p.) and the body temperature measured as before. All the experiments were begun between 8.30 and 9.30 a.m. to minimize any

Table 2. *Effect of p-hydroxynorephedrine and its precursors on amphetamine-induced hyperthermia in the Wistar albino rat.* Figures represent the mean maximum temperature rise after (+)-amphetamine (5 mg kg^{-1}) ± s.e. for each group, as described in the text. n for each group is given in parentheses.

Pretreatment	Male	Female	P
p-Hydroxyamphetamine	0·7 ± 0·1 (5)**	1·8 ± 0·2 (6)	<0·001
Norephedrine	1·2 ± 0·2 (10)*	1·8 ± 0·2 (10)	<0·025
p-Hydroxynorephedrine	0·4 ± 0·1 (12)**	1·9 ± 0·2 (9)	<0·001
Saline	2·0 ± 0·1 (10)	1·8 ± 0·2 (10)	n.s.

** $P<0·001$.
* $P<0·01$ compared with saline controls.
n.s. not significant.

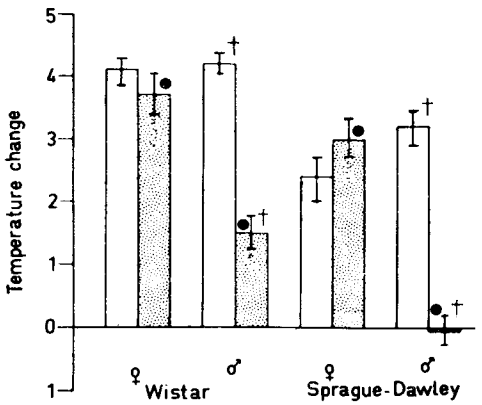

FIG. 1. Effect of *p*-hydroxyamphetamine pretreatment on amphetamine induced hyperthermia. Rats were pretreated with *p*-hydroxyamphetamine (40 mg kg^{-1}, i.p.; shaded columns) or saline (open columns) 20 h before amphetamine (20 mg kg^{-1}) as described in the text. Columns represent the mean rise in rectal temperature in °C (±s.e.) in 1 h after amphetamine, measured as stated in the text. n = 5 or 6.

† $P<0{\cdot}01$ compared with saline pretreated control.

● $P<0{\cdot}01$ compared with *p*-hydroxyamphetamine pretreated animals of opposite sex.

diurnal variation which might occur. Statistical analysis was performed with the Student *t*-test, for unpaired data.

The results for the pretreatments with *p*-hydroxyamphetamine, norephedrine and *p*-hydroxynorephedrine in male and female Wistar rats are shown in Table 2, from which it is clear that these three drugs only exert a protecting effect in the males, since the temperature rise after the amphetamine challenge in drug-pretreated females is the same as in saline controls. The reduction in the hyperthermic response is statistically significant in the males for all three agents. Control animals of both sexes (saline-saline and drug pretreatment-saline) did not show any significant temperature change on challenge and, furthermore, the drugs used for pretreatment had no effect on the baseline temperatures of the rats in any experiment.

In Fig. 1 the results obtained after the pretreatment of male and female Wistar and Sprague Dawley rats with *p*-hydroxyamphetamine following the method of Lewander (1971) are presented. The mean maximum rise in temperature after amphetamine is considerably reduced in *p*-hydroxyamphetamine-pretreated male rats, but this is not so in females. In both strains there is a statistically significant difference between the temperature rise in *p*-hydroxyamphetamine-pretreated male rats and in saline controls, but there is no significant difference between the two values in female rats similarly pretreated. Moreover, the maximum temperature rise after amphetamine in *p*-hydroxyamphetamine-pretreated male rats is significantly less than in pretreated females.

In the experiment illustrated in Fig. 1, there appears to be a strain difference, since male Wistar rats show a small temperature rise when amphetamine is given after *p*-hydroxyamphetamine whereas in male Sprague-Dawley rats the hyperthermia seems to be totally abolished by this pretreatment. The sex difference, however, is very clear-cut and any strain difference which may occur is secondary to this. When control animals of both sexes and strains were injected with saline after either saline or *p*-hydroxyamphetamine pretreatment, as described, the maximum temperature rise after challenge was ± 0·3°, which was not significant in any case examined.

The data do not appear to be in agreement with the concept of a false transmitter being involved in tolerance to amphetamine hyperthermia. Both male (Lewander,

1971) and female (Harrison, Ambrus & Ambrus, 1952; Sever & Caldwell, 1974) rats become tolerant to the hyperthermic action of amphetamine on prolonged dosage, so that there are apparently no sex differences in the tolerance mechanisms. Furthermore, there is no significant difference between the hyperthermic response to amphetamine in the two sexes, as shown in this work. Table 1 shows that both male and female rats convert the pretreating drugs in part to *p*-hydroxynorephedrine and this suggests that the production of this metabolite is not related to the tolerance to amphetamine hyperthermia seen in the rat since the sex difference in protection is not paralleled by a deficiency in the tolerance mechanism.

On the data available at present, a plausible explanation of the sex difference observed here is not possible. There may be sex differences in the pharmacokinetics and metabolism of the pretreating drugs, as has been shown with *p*-hydroxyamphetamine, whose metabolism is more extensive in the male than the female (P. S. Sever, J. Caldwell & L. G. Dring unpublished data).

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The influence of atrial temperature on the *in vitro* selectivity of isoprenaline for atria and of orciprenaline for trachea

Many studies on selective β-adrenoceptor agonists utilize isoprenaline as a reference drug and its potency is given an arbitrary value, for example of 100, on the various tissues. In previous *in vitro* studies from this laboratory isoprenaline has been found to be selective for atria, i.e. its potency on guinea-pig atria was significantly greater than that on tracheal chains (O'Donnell, 1970, 1972; O'Donnell & Wanstall, 1974). The experimental conditions for these two preparations differed in one respect, namely the atrial preparations were maintained at 30° whereas the tracheal preparations were at 37°. Other workers have previously described an increased sensitivity of isolated heart preparations to sympathomimetic amines if the temperature was decreased (Trendelenburg, 1968; Oppermann, Ryan & Haavik, 1969). The aims of the present study were to establish whether a similar temperature-dependent supersensitivity could account for the apparent selectivity for atria of isoprenaline noted above and to examine retrospectively whether the lower atrial temperature might also have influenced the selctivity for trachea observed for compounds related to isoprenaline and orciprenaline (O'Donnell & Wanstall, 1974). Thus isoprenaline and