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Effects of a dopamine β-hydroxylase inhibitor on amphetamine-induced hyperactivity in rats

The relative role of dopaminergic and noradrenergic systems of the brain in the locomotor activity response to (+)-amphetamine is still debated. Stein & Wise (1969) and Taylor & Snyder (1971) suggested that noradrenergic systems are responsible for both spontaneous and drug-induced locomotor activity. Others have indicated that dopaminergic systems alone may be responsible for the motor stimulant action of (+)-amphetamine (Costa, Groppetti & Naimzada, 1972; Thornburg, 1973; Hollister, Breese & Cooper, 1974). However, that both dopaminergic and noradrenergic mechanisms are essential to motility stimulation by amphetamine is widely supported (e.g., Svensson, 1970; Andén, Corrodi & others, 1970; Maj, Sowinska & others, 1972; Rolinski & Scheel-Krüger, 1973).

Hollister & others (1974) emphasized the importance of dopaminergic function to the amphetamine response in part because of their finding that a dopamine β-hydroxylase inhibitor, 1-phenyl-3-(2-thiazolyl)-2-thiourea (U-14, 624), did not antagonize the motor stimulant effects of (+)-amphetamine. However, this was in contrast to our preliminary findings with U-14,624 (Khalsa & Davis, 1974) which we have now extended in an effort to resolve the differences with the data of Hollister & others (1974).

Male albino rats (Holtzman Co., Madison, Wisc.), 350–400 g, were randomly assigned to treatment groups and caged in groups of six in a sound-attenuated room with a 14 h/10 h light-dark cycle and a temperature of 23 ± 1°. Food and water were freely available; 7–10 days were allowed for acclimatizion and 12 h for the rats to adjust to the photocell actometers (Pickens & Crowder, 1967). (+)-Amphetamine sulphate (SKF) was dissolved in 0·9% saline solution, and U-14,624 (Aldrich Chemical Co.) was suspended by first triturating it with 1% Tween 80 in saline and then diluting with saline. All injections were given intraperitoneally in a constant volume of 2 ml kg^{-1}.

Groups of 4 rats each were injected with saline or (+)-amphetamine 6 h after a single dose of either saline or 25 or 50 mg kg^{-1} of U-14, 624. These doses were validated

Table 1. *Locomotor activity effect of* (+)*-amphetamine sulphate* (1 mg kg^{-1}) *in rats and the antagonistic action of U-14,624* (25 or 50 mg kg^{-1}). Both drugs were given intraperitoneally at an interval of 6 h; n=4 for all groups.

Treatments	Activity counts 2 h mean ± s.e.
Saline + saline	121·0±24·7
Saline + amphetamine	995·5±144·9
U-14,624 (25 mg kg^{-1}) + amphetamine	421·8±146·7*
U-14,624 (50 mg kg^{-1}) + amphetamine	403·2±113·3*

* Significantly less than saline + amphetamine ($P<0·05$) by a 2-tailed Student's *t*-test.

by our earlier unpublished finding that whole brain noradrenaline was lowered after 4 h by 54 and 70%, respectively, while the dopamine concentration was not altered. These data agreed well with reports by Johnson, Boukma & Kim (1970) and von Voightlander & Moore (1970). Activity recording for 2 h began immediately after the second dose.

The results (Table 1) indicated that hyperactivity induced in rats by 1 mg kg^{-1} of (+)-amphetamine, was significantly reduced (2-tailed *t*-test, $P < 0.05$) by a single dose of 25 or 50 mg kg^{-1} of U-14,624. These results disagreed with those of Hollister & others (1974) who used a single intraperitoneal dose of 75 mg kg^{-1} of U-14,624 suspended in 0·5% carboxymethylcellulose which failed to reduce hyperactivity in rats after 2 mg kg^{-1} of amphetamine. But Hollister & others (1974) injected (+)-amphetamine only 1 h after the U-14,624 pretreatment, while with our rats the injection was 6 h later. Also, upon trying a 1% methylcellulose vehicle used by von Voightlander & Moore (1970), we found it nearly impossible to achieve a uniform suspension of U-14,624 to deliver an accurate and consistent dose. Furthermore, small pockets of unabsorbed drug were found in the peritoneal cavity at 48 h after U-14,624 in the methylcellulose vehicle, indicating a delayed and incomplete absorption of the drug from the site of injection. This did not occur when the drug was administered using 1% Tween 80. Therefore, we attempted to replicate part of the results of Hollister & others (1974) and to determine whether the different pretreatment interval and/or injection vehicle was responsible for our finding of antagonism toward amphetamine by U-14,624. Rats were injected intraperitoneally with either saline or U-14,624 in Tween 80/saline (75 mg kg^{-1}) 1 or 6 h before a single dose of either saline or (+)-amphetamine (2 mg kg^{-1}). Locomotor activity again was recorded immediately thereafter for 2 h. In addition, we determined the intraperitoneal LD50 of U-14,624. Groups of about 10 rats each were treated intraperitoneally with 25, 50, 75, 100 or 150 mg kg^{-1} of U-14,624 in Tween 80/saline. Mortality 48 h after the drug was recorded, and LD50 calculations were according to Litchfield & Wilcoxon (1949).

Results of our second activity study (Table 2) show that increased motility following the higher dose of (+)-amphetamine also was reduced significantly ($P < 0.05$; 1-tailed *t*-test used on basis of first results) by U-14,624 if the interval between amphetamine and U-14,624 was 6 h, but not if the interval was 1 h. This suggests that the period of pretreatment was the main factor responsible for the differing results described above. Further evidence for this inference is the fact that brain noradrenaline was

Table 2. *Locomotor activity effect of* (+)*-amphetamine sulphate* (2 mg kg^{-1}) *in rats pretreated with a single dose of U-14,624* (75 mg kg^{-1}). All injections were given intraperitoneally. Numbers in parentheses indicate the size of each group.

Treatments	Activity counts, 2 h mean ± s.e. at interval of pretreatment of	
	1 h	6 h
Saline + saline	44·8 ± 16·8(6)	53·3 ± 9·7(8)
U-14,624 + saline	57·0 ± 13·6(6)	37·0 ± 6·7(8)
Saline + amphetamine	1105·5 ± 163·6(6)	1056·0 ± 121·0(14)
U-14,624 + amphetamine	997·8 ± 108·7(6)	751·0 ± 99·0*(14)

* U-14,624 + amphetamine significantly different from saline + amphetamine (1-tailed Student's *t*-test, $P < 0.05$) at 6 h but not 1 h interval. All amphetamine-treated groups differed ($P < 0.01$) from the corresponding control groups pretreated with saline or U-14,624.

lowered only 35% at the 1 h interval after U-14,624 (75 mg kg^{-1}) observed by Hollister & others (1974), whereas we found a 54% depletion at a 6 h interval after U-14,624 (25 mg kg^{-1}).

The 75 mg kg^{-1} dose of U-14,624 used by Hollister & others (1974), who reported no deaths, was in our hands a lethal dose 24–48 h after injection, the intraperitoneal LD50 being 76 mg kg^{-1} (95% confidence limits of 61 and 96 mg kg^{-1}). There was no mortality at 25 mg kg^{-1} and only 17% at 50 mg kg^{-1}. The LD50 data might suggest that the reduction of amphetamine-induced activity after 75 mg kg^{-1} of U-14,624 could have resulted from some toxic action of U-14,624, rather than from inhibition of dopamine β-hydroxylase. While this alternative explanation cannot be excluded by the present data, it is weakened by the finding that amphetamine-induced motility was antagonized as well or even more effectively by 25 or 50 mg kg^{-1} of U-14,624, doses which caused little or no lethality.

These data are consistent with the view that noradrenergic systems are essential to motility stimulation of amphetamine, either independently of dopaminergic systems or, as seems more likely, together with dopaminergic function. The fact that U-14,624 achieved only a partial inhibition of activity after amphetamine agrees with experiments employing other inhibitors of dopamine β-hydroxylase, diethyldithiocarbamate (Pfeifer, Galambos & Gyorgy, 1966; Randrup & Scheel-Krüger, 1966; Mayer & Eybl, 1971), disulfiram (Maj & Przegalinski, 1967) and FLA-63 (Svensson, 1970; Rolinski & Scheel-Kruger, 1973). Considering the almost complete blocking of amphetamine-induced stimulation by α-methyl-*p*-tyrosine (Rolinski & Scheel-Krüger, 1973), it appears that dopamine may play a more significant role than noradrenaline in the mediation of locomotor excitation induced by amphetamine.

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