Inability of an inhibitor of amine uptake (Lilly 110140) to block depletion of brain 5-hydroxytryptamine by L-dopa

The depletion of brain 5-hydroxytryptamine (5-HT) by amine drugs such as *p*-chloroamphetamine and α-ethyl-3-hydroxy-4-methyl-phenethylamine (H75/12) is blocked by amine uptake inhibitors (Carlsson, Corrodi & others, 1969; Meek, Fuxe & Carlsson, 1971). Those depleting drugs apparently require the uptake pump for entry into the neuron; pump-blocking agents prevent the entry and hence the effects of the depleting drugs. We have described a 5-HT uptake inhibitor that is a highly potent antagonist of 5-HT depletion by *p*-chloroamphetamine (Fuller & Perry, 1974; Fuller, Perry & Molloy, 1975). That inhibitor is 3-(*p*-trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine hydrochloride (Lilly 110140). To provide evidence that the antagonism by 110140 of *p*-chloroamphetamine-induced depletion of 5-HT depended on the ability of 110140 to block *p*-chloroamphetamine uptake and not, for example, on interference with 5-HT release, we showed that 110140 did not block the depletion of 5-HT by reserpine. Reserpine apparently does not require active transport by the amine uptake pump. The intraneuronal actions of reserpine (as well as the mechanism of entry into the 5-HT neuron) may, however, be quite different from those of simple arylalkylamines like *p*-chloroamphetamine or H75/12. As further proof that 110140 does not interfere with the process of 5-HT release, we wanted to study an arylalkylamine agent that displaced 5-HT from intraneuronal storage sites but that did not require the amine uptake pump on the outer membrane for entry into the neuron. L-Dopa was chosen; presumably it enters the neuron as the amino acid and is then decarboxylated to dopamine, which displaces 5-HT. In this event, failure of 110140 to block the depletion of 5-HT by L-dopa would constitute evidence that 110140 does not interfere with 5-HT release by an arylalkylamine. On the other hand, 110140 should block the effects of L-dopa if the L-dopa were decarboxylated extraneuronally to dopamine which was then actively transported into the 5-HT neuron, or if 110140 interfered with the intraneuronal release of 5-HT. Experiments were done both in rats and in mice to determine if 110140 altered 5-HT release following L-dopa administration.

For these experiments, male Wistar-derived albino rats, about 150 g, or male Cox standard mice, about 20 g, were injected intraperitoneally with Lilly 110140 as an aqueous solution of the hydrochloride salt. One hour later, L-dopa was injected (i.p.) as an aqueous suspension. Animals were decapitated 30 min after L-dopa administration, and whole brains were quickly removed and frozen on dry ice. Spectrofluorometric analyses for 5-HT and 5-HIAA were made by condensation with *o*-phthalaldehyde (Miller, Cox, & others, 1970; Fuller, Perry & others, 1974a). All groups contained 5 animals, and statistical comparisons between groups were made by Student's *t*-test.

Table 1 shows the effects of L-dopa on brain 5-HT and 5-HIAA concentrations in control mice and in mice pretreated with 110140 at a dose 10 times that previously found to inhibit 5-HT depletion by *p*-chloroamphetamine (Fuller, Perry & others, 1974b). L-Dopa reduced 5-HT concentrations by about half both in control mice and in 110140-treated mice. Treatment with 110140 alone produced a slight but statistically significant decrease in 5-HIAA concentrations, as we have reported earlier (Fuller, Perry & Molloy, 1974); this decrease is due to a compensatory decline in 5-HT turnover. L-Dopa elevated 5-HIAA concentrations both in control mice and in 110140-pretreated mice. The ratio 5-HIAA:5-HT was markedly increased by L-dopa, and the magnitude of this effect was at least as great in 110140-pretreated mice as in controls.

Table 1. *5-HT release by* L-*dopa in control and 110140-pretreated mice and rats.*

Dose of L-dopa (mg kg^{-1})	Brain 5-hydroxyindole concentration (μg g^{-1}) 5-HT	5-HIAA	Ratio 5-HIAA 5-HT
Control	Mice		
0	0·81±0·02	0·60±0·02	0·73±0·02
400	0·43±0·06	0·70±0·03	1·68±0·13
	($P<0·001$)	($P<0·025$)	($P<0·001$)
110140-pretreated (20 mg kg^{-1})†			
0	0·84±0·01	0·49±0·01*	0·60±0·02*
400	0·36±0·01	0·65±0·02	1·80±0·01
	($P<0·001$)	($P<0·001$)	($P<0·001$)
Control	Rats		
0	0·64±0·02	0·42±0·01	0·65±0·01
400	0·51±0·02	0·55±0·02	1·07±0·04
	($P<0·005$)	($P<0·001$)	($P<0·001$)
110140-pretreated (10 mg kg^{-1})†			
0	0·61±0·02	0·35±0·01*	0·57±0·01*
400	0·53±0·03	0·50±0·05	0·86±0·14
	($P<0·025$)	($P<0·05$)	($P<0·025$)

* Significantly different from corresponding group not pretreated with 110140 ($P<0·005$).
† Both these doses block uptake into 5-HT neurons.

The effects of the same dose of L-dopa in rats were less pronounced than in mice (Table 1), but again it was as effective in the 110140-pretreated group as in controls. 5-HT was significantly lowered in both groups, and 5-HIAA was significantly increased by L-dopa in both groups. The ratio 5-HIAA:5-HT was increased to the same extent by L-dopa in control and in 110140-pretreated rats.

The fact that 5-HIAA is elevated while 5-HT is lowered by L-dopa implies that the effect of L-dopa is to release 5-HT rather than to inhibit its synthesis. The finding that the effects of L-dopa are not blocked by an inhibitor of the amine uptake system on the neuronal membrane indicates that L-dopa is transported into the 5-HT neuron as the amino acid and is decarboxylated intraneuronally to dopamine, which displaces 5-HT from storage vesicles. In contrast to the lack of effect of 110140 on 5-HT depletion by L-dopa, we have found that 110140 completely antagonizes the depletion of 5-HT by *p*-chloroamphetamine, *p*-bromoamphetamine, 6-chloro-2-aminotetralin, β,β-difluoro-*p*-chloroamphetamine, and H75/12. All of these amines appear to require active transport into neurons via the membrane pump responsible for 5-HT reuptake, and the explanation for the blockade of their effects by 110140 is that their entry into the neuron is prevented. The failure of 110140 to affect 5-HT depletion by L-dopa suggests that 110140 does not interfere with the intraneuronal release of 5-HT.

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REFERENCES

CARLSSON, A., CORRODI, H., FUXE, K. & HOKFELT, T. (1969). *Eur. J. Pharmac.*, **5**, 357–366.
FULLER, R. W. & PERRY, K. W. (1974). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **33**, 255.
FULLER, R. W., PERRY, K. W., BAKER, J. C., PARLI, C. J., LEE, N., DAY, W. A. & MOLLOY, B. B. (1974a). *Biochem. Pharmac.*, **23**, 3267–3272.
FULLER, R. W., PERRY, K. W. & MOLLOY, B. B. (1974). *Life Sci.*, **15**, 1161–1171.

FULLER, R. W., PERRY, K. W. & MOLLOY, B. B. (1975). *J. Pharmac. exp. Ther.*, in the press.
FULLER, R. W., PERRY, K. W., SNODDY, H. D. & MOLLOY, B. B. (1974b). *Eur. J. Pharmac.*, **28**, 233–236.
MEEK, J. L., FUXE, K. & CARLSSON, A. (1971). *Biochem. Pharmac.*, **20**, 707–709.
MILLER, F. P., COX, R. H., Jr., SNODGRASS, W. R. & MAICKEL, R. P. (1970). *Ibid.*, **19**, 435–442

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.