Effects of the dopamine-β-hydroxylase inhibitor FLA 63 on the kinetics of elimination of amphetamine in the rat

Disulfiram and its metabolite diethyldithiocarbamate (DDC) have both been shown to inhibit the *p*-hydroxylation of amphetamine in rats (Creaven, Barbee & Roach, 1970; Jonsson & Lewander, 1973). In the present paper an interaction between FLA 63 (bis-1-methyl-4-homopiperazinylthiocarbonyl)disulphide, which is chemically related to disulfiram, and amphetamine in rats is reported. FLA 63, like disulfiram and DDC, is a dopamine-β-hydroxylase inhibitor (Corrodi & Florwall, 1970).

Male Sprague-Dawley rats, 180–250 g, were used. Water and food were freely available, FLA 63, was dissolved in 2M HCl, neutralized, diluted with saline and injected in a dose of 50 mg kg^{-1} or 100 mg kg^{-1} (s.c.) 1 h before the administration of amphetamine. An Animex apparatus (Farad Electronics AB, Sweden) was used to measure motor activity. (+)-[^{3}H]Amphetamine (3·0 mCi mM^{-1}, 4 mg kg^{-1}) was injected intraperitoneally at 0·25, 0·5, 1, 2 and 4 h before the animals were decapitated and the brain and plasma concentrations of amphetamine were determined after extraction into toluene at pH 12 followed by counting in a liquid scintillation spectrometer. The pattern of urinary metabolites was determined after injection of (+)-[^{14}C]amphetamine sulphate (CIS, France) (5 mg kg^{-1}, i.p. 0·55 mCi mM^{-1}) into rats housed individually in metabolic cages. Urine was collected for 24 h. [^{14}C]Metabolites of amphetamine in the urine were separated by paper chromatography and quantified by liquid scintillation counting according to Ellison, Gutzait & van Loon (1966) as modified by Lewander (1968). Student's *t*-test was used for all statistical evaluations.

FLA 63 administered 1 h before amphetamine significantly prolonged the amphetamine-induced increase in motor activity by approximately 2·5 h (Fig. 1).

Pretreatment with FLA 63 significantly increased the concentration of brain amphetamine at 2 and 4 h after amphetamine (Fig. 2), when the rats were pretreated with 100 mg kg^{-1} of FLA 63. The effect of FLA 63 seemed to be dose dependent at this time interval after the injection of amphetamine. In Table 1 the distribution of amphetamine in plasma, lung, heart, liver and kidney is shown. Of all the tissues, the increase in the amphetamine concentration after pretreatment with 100 mg kg^{-1} of FLA 63 was most pronounced in plasma. The duration of the amphetamine-induced increase in motor activity followed roughly the concentration of amphetamine in brain and plasma.

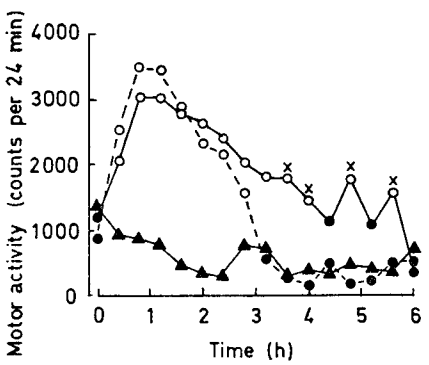

FIG. 1. Motor activity in rats after injection of (+)-amphetamine (A), 4 mg kg^{-1}, i.p. (○—●). FLA 63 50 mg kg^{-1}, s.c. + saline (▲—▲) and FLA 63 + (+)-amphetamine (○—●). (+)-Amphetamine was given 1 h after FLA 63. Open circles indicate statistically significant ($P<0·05$ or less) differences from FLA 63 + saline treated animals. The symbol "x" indicates statistically significant ($P<0·05$ or less) differences between amphetamine and FLA 63 + amphetamine treated rats. Each point represents the mean of 3–5 observations.

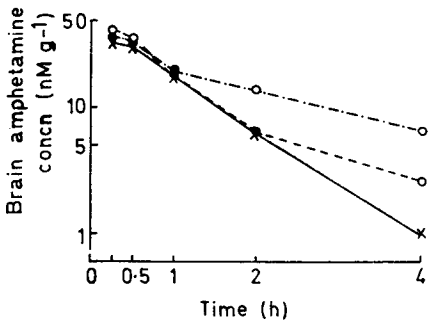

FIG. 2. Effect of pretreatment with FLA 63 on the disappearance of amphetamine from the rat brain. (+)-[^{3}H]Amphetamine sulphate, 4 mg kg^{-1} was given i.p. at time 0, 1 h after FLA 63. (—·—·—) FLA 63, 100 mg kg^{-1} + amphetamine; (— — —) FLA 63, 50 mg kg^{-1} + amphetamine, (× — ×) control, amphetamine only. Each point represents the mean concentration of amphetamine in 4 rats. Open circles indicate statistically significant ($P<0.05$ or less) differences from the controls.

Although the previous results with DDC suggested an inhibition of the oxidation of amphetamine, no influence of FLA 63 on the urinary metabolite pattern was found. Thus, the urinary data did not provide evidence for an inhibition of the *p*-hydroxylation of amphetamine by FLA 63.

Both disulfiram (Creaven & others, 1970) and DDC (Jonsson & Lewander, 1973) have a marked effect on the pattern of urinary metabolites of amphetamine, which is suggestive of an inhibition of the *p*-hydroxylation of amphetamine. This probably explains why DDC (400 mg kg^{-1} i.p.) caused a tenfold increase in the brain and plasma concentrations of amphetamine during 0·5–12 h after the injection of amphetamine (Jonsson & Lewander, 1973; Lal, Sourkes & Missala, 1974). The present experiments do not give conclusive evidence with respect to the mechanism for the

Table 1. *Tissue concentrations of amphetamine* (nM g^{-1}) *after pretreatment with FLA* 63. (+)-Amphetamine [^{3}H]sulphate 4 mg kg^{-1} was administered i.p. 1 h after FLA 63 or saline respectively. Each experiment represents the mean concentration of amphetamine in 3 or 4 rats.

		Amphetamine concentration (nM g^{-1}, mean ± s.e. n = 4) after: 15 min	30 min	1 h	2 h	4 h
Plasma	Controls	2·7 ± 0·1	2·2 ± 0·07	1·5 ± 0·1	0·6 ± 0·09	0·18 ± 0·01
	FLA 63 50 mg kg^{-1}	2·3 ± 0·4	2·8 ± 0·08†	1·7 ± 0·1	0·7 ± 0·08	0·40 ± 0·08* (3)
	FLA 63 100 mg kg^{-1}	4·2 ± 0·2*	3·2 ± 0·1*	2·0 ± 0·09‡(3)	1·4 ± 0·1†	0·43 ± 0·15(3)
Lung	Controls	62·4 ± 6·3	41·8 ± 1·8	25·1 ± 4·4	13·5 ± 1·5	2·7 ± 0·4
	FLA 63 50 mg kg^{-1}	78·7 ± 9·4	61·2 ± 3·3†	29·1 ± 2·7	16·5 ± 1·2	8·1 ± 1·3†(3)
	FLA 63 100 mg kg^{-1}	91·4 ± 14·2	45·3 ± 5·6	15·1 ± 2·7(3)	24·7 ± 1·9†	10·1 ± 3·1 (3)
Heart	Controls	15·5 ± 0·7	10·1 ± 0·4	6·6 ± 0·3	2·9 ± 0·4	0·5 ± 0·05
	FLA 63 50 mg kg^{-1}	17·0 ± 1·8	14·5 ± 1·2*	6·4 ± 0·7	3·8 ± 0·3	1·8 ± 0·2† (3)
	FLA 63 100 mg kg^{-1}	20·2 ± 1·4‡	11·4 ± 0·5	7·7 ± 0·4 (3)	5·3 ± 0·6‡	2·0 ± 0·6‡ (3)
Liver	Controls	44·7 ± 1·2	25·2 ± 0·5	19·2 ± 1·2	13·6 ± 1·4	4·2 ± 0·3
	FLA 63 50 mg kg^{-1}	48·7 ± 6·5	38·1 ± 2·8†	19·8 ± 1·1	13·1 ± 0·8	6·6 ± 0·4† (3)
	FLA 63 100 mg kg^{-1}	71·1 ± 9·9‡	36·9 ± 0·8‡	23·0 ± 2·4 (3)	21·9 ± 3·0‡	7·9 ± 1·2‡ (3)
Kidney	Controls	89·6 ± 2·2	66·1 ± 3·1	47·1 ± 3·5	26·0 ± 2·7	5·5 ± 0·3
	FLA 63 50 mg kg^{-1}	94·2 ± 7·5	81·5 ± 1·9†	36·6 ± 1·3‡	21·8 ± 0·5	12·2 ± 2·8 (3)
	FLA 63 100 mg kg^{-1}	90·6 ± 5·4	67·9 ± 7·6	43·9 ± 2·7 (3)	33·4 ± 1·5	12·4 ± 4·2 (3)

Differences from controls: * $P<0.001$; † $P<0.01$; ‡ $P<0.05$.

relatively small but clear effect of FLA 63 on tissue amphetamine concentrations. This is due to the fact that the maximal dose of FLA 63, which could be administered without a high mortality, was only 1/4–1/8 of that of the other disulphide derivatives. In addition, the 24 h interval chosen for collection of urine might have been too long for a transient inhibition of *p*-hydroxylation to be observed. All rats receiving FLA 63 had haematuria or possibly haemoglobinuria and the volume of urine produced was smaller than for the amphetamine controls. Further studies may reveal if this toxic side effect of FLA 63, which might interfere with the renal elimination of amphetamine, or some yet undefined interaction with the metabolism of amphetamine, causes the increased tissue concentration of amphetamine.

The present results demonstrate that FLA 63 pretreatment interferes with the kinetics of elimination of amphetamine, which is associated with a prolongation of one of its pharmacological effects. In the recent past, FLA 63 has been used as a potent dopamine-β-hydroxylase inhibitor in the elucidation of the mechanism of action of amphetamine in rats (Corrodi, Fuxe, & others, 1970; Ungerstedt, 1971) and mice (Svensson, 1970). In these studies, pretreatment with FLA 63 (25–50 mg kg^{-1} i.v. or i.p.) either potentiated or changed qualitatively the amphetamine induced effects. In view of the present findings, there is reason to reconsider the conclusions from such experiments, if based solely on the consequences of the dopamine-β-hydroxylase inhibitory effects of FLA 63.

The authors are indebted to Miss Sonja Ahlén and Mrs Christina Johansson for skilful technical assistance. The study has been supported by the Swedish Medical Research Council (Project No. B73–04X–1017–09 and B74–04P–4288–01A) and the Tri-Centennial Fund of the Bank of Sweden (Project No. 150).

Psychiatric Research Center,
University of Uppsala,
Ulleråker Hospital,
S-750 17 Uppsala,
Sweden.

JOHN JONSSON
TOMMY LEWANDER

July 8, 1974

REFERENCES

CORRODI, H. & FLORWALL, L. (1970). *Acta pharm. suec.*, **7**, 7–22.
CORRODI, H., FUXE, K., LJUNGDAHL. Å. & ÖGREN, S.-O. (1970). *Brain Research*, **24**, 451–470.
CREAVEN, P. J., BARBEE, T. & ROACH, M. K. (1970). *J. Pharm. Pharmac.* **22**, 828–831.
ELLISON, T., GUTZAIT, L. & VAN LOON, E. J. (1966). *J. Pharmac. exp. Ther.* **152**, 383–387.
JONSSON, J. & Lewander, T. (1973). *J. Pharm. Pharmac.*, **25**, 589–591.
LAL, S., SOURKES, T. L. & MISSALA, K. (1974). *Archs int. Pharmacodyn. Thér.*, **207**, 122–130.
LEWANDER, T. (1968). *Psychopharmac.*, **13**, 394–407.
SVENSSON, T. H. (1970). *Eur. J. Pharmac.*, **12**, 161–166.
UNGERSTEDT, U. (1971). *Acta physiol. scand., suppl.*, **367**, 49–68.