Antagonism by methylphenidate of the stereotyped behaviour produced by (+)-amphetamine in reserpinized rats

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Although (+)-amphetamine and methylphenidate produce similar central stimulation in rats characterized by increased locomotor activity and stereotyped behaviour, they differ in their sensitivity to reserpine and α-methyl-*p*-tyrosine (Scheel-Krüger, 1971). Whereas reserpine, antagonizes the effect of methylphenidate but not that of (+)-amphetamine, α-methyltyrosine antagonizes the effect of (+)-amphetamine but not that of methylphenidate. Thus both compounds appear to produce stimulation by an indirect action but with different mechanisms. There is evidence indicating that the stereotypies are produced by the increased concentration of dopamine at striatal receptors (Scheel-Krüger, 1971). (+)-Amphetamine seems to release dopamine from extravesicular sites (Carlsson, Fuxe & others, 1966; Farnebo, 1971) whereas the effect of methylphenidate is dependent on an intact vesicular store of dopamine (Scheel-Krüger, 1971).

In recent studies on the inhibition of the [^{3}H]dopamine accumulation in rat striatal homogenates it was observed that reserpine markedly potentiated the inhibitory action of (+)-amphetamine (Ross, 1977a). The inhibitory effect of methylphenidate was, on the other hand, not changed by reserpine (Ross, 1977b). These observations indicate that (+)-amphetamine is more potent in releasing dopamine than in inhibiting the dopamine uptake whereas methylphenidate is most potent as an inhibitor of the dopamine uptake. The stimulatory effect of methylphenidate was therefore proposed to be due to inhibition of the re-uptake of dopamine released by nerve activity (Ross, 1977b). Since the release of transmitter amines by indirectly acting agents is antagonized by inhibitors of the amine uptake (Trendelenburg, 1972), it is possible to test this hypothesis experimentally. If methylphenidate acts by inhibiting the dopamine uptake it should antagonize the stereotypied behaviour produced by (+)-amphetamine in reserpinized rats, in which methylphenidate itself has low activity, but not influence the stereotypies by apomorphine which acts directly on the dopamine receptors (Ernst, 1965). In the experiments reported here results were obtained which support this hypothesis.

Male Sprague-Dawley rats (160–200 g) were injected with reserpine (5 mg kg^{-1}) 4 h before the injection of methylphenidate hydrochloride. (+)-Amphetamine sulphate (5 mg kg^{-1}, i.p.) or apomorphine hydrochloride (1 mg kg^{-1}, s.c.) was injected after a further 0·5 h. The stereotypied behaviour was scored according to Costall, Naylor & Olley (1972) and observed during 1 h after the last injection. The observer did not know the injection schedules.

Methylphenidate itself caused a brief (about 15 min) stimulation of the locomotor activity with no or slight stereotypied behaviour. At the time of the injection of (+)-amphetamine or apomorphine the methylphenidate-treated rats behaved almost as the reserpine controls. (+)-Amphetamine and apomorphine caused pronounced stereotypied behaviour at the doses given (Table 1). Methylphenidate antagonized the effect of (+)-amphetamine in a dose related manner. The increase of the locomotor activity was also abolished at the two higher doses of methylphenidate. The stereotypies by apomorphine were not antagonized by methylphenidate.

The results obtained indicate that methylphenidate antagonized the effect of (+)-amphetamine at a presynaptic site. Since amphetamine causes stereotypies probably by releasing dopamine from extravesicular sites in the nerve terminals (Carlsson & others, 1966; Farnebo, 1971), a plausible explanation of the antagonism is that methylphenidate inhibits the dopamine release. This effect can be achieved by inhibition of the membrane dopamine carrier either by inhibiting a proposed active uptake of (+)-amphetamine into the nerve terminals (Trendelenburg, 1972) or by inhibiting the outward transport of dopamine released by amphetamine from extravesicular binding sites (Paton, 1974; Ross, 1976).

Several other observations support the hypothesis that the stereotypies produced by methylphenidate are due to inhibition of the uptake of dopamine. It is a rather potent inhibitor of the dopamine accumulation in a rat striatal homogenate *in vitro* (Ferris, Tang & Maxwell, 1972; Ross, 1977b). After administration *in vivo* at the same doses as those employed in the present study methylphenidate reduces the accumulation of

Table 1. *Effect of methylphenidate on the stereotypies produced by (+)-amphetamine or apomorphine in reserpinized rats.* Reserpine (5 mg kg^{-1}, i.p.) was injected 4 h before methylphenidate hydrochloride and the latter compound 0·5 h before (+)-amphetamine sulphate (5 mg kg^{-1}, i.p.) or apomorphine hydrochloride (1 mg kg^{-1}, s.c.). The stereotypies were observed during 1 h and scored every 15 min. The values are the mean score at maximal response (0·5 h) and the number of rats with stereotypied behaviour (response).

	Stereotypies				
	(+)-Amphetamine			Apomorphine	
Dose mg kg^{-1}, i.p.	n	Mean score ± s.e.m.	Response n	Mean score ± s.e.m.	Response n
0	18	2·33 ± 0·24	16	2·39 ± 0·20	18
10	12	1·83 ± 0·34	9	2·25 ± 0·25	12
20	12	0·33 ± 0·19*	4	2·33 ± 0·14	12
40	12	0·08 ± 0·08*	1	2·83 ± 0·11	12

* $P < 0.001$ (Student's *t*-test) compared with control.

[^{3}H]dopamine in striatal slices of mice (Ross & Renyi, 1975) and rats (ED50 = 67 mg kg^{-1}, i.p. 0·5 h after the injection, unpublished observation). Furthermore, methylphenidate produces stereotypies in normal (non-reserpinized) rats at the same doses (20 mg kg^{-1}, i.p. and more; cf. Scheel-Krüger, 1971) as those antagonizing the stereotypies produced by (+)-amphetamine in reserpinized rats. Other findings are also in accordance with the hypothesis that methylphenidate is an inhibitor of the dopamine uptake *in vivo*. Thus, the antagonism by reserpine of the stimulatory effect of methylphenidate is explained by the failure of the impulse propagated release of dopamine. Shore (1976) observed that methylphenidate + haloperidol lowers the striatal dopamine in α-methyltyrosine treated rats, which can be explained by the increased impulse flow combined with the inhibition of the re-uptake of dopamine. The stimulants of the methylphenidate group cause less tolerance than those of the amphetamine group (Biel, 1970), which is understood by the proposed difference in the mode of action between these goups of stimulants.

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Effects of reserpine, *para*-chlorophenylalanine, 5,6-dihydroxytryptamine and fludiazepam on the head twitches induced by 5-hydroxytryptamine or 5-methoxytryptamine in mice

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Reserpine decreases the concentration of brain catecholamines in rodents. Many authors have reported that behavioural activities of noradrenaline (intraventricularly) or apomorphine, a dopamine receptor agonist, are enhanced after treatment with reserpine (Rotrosen, Angrist & others, 1972; Geyer & Segal, 1973; Symes, Lal & others, 1977). These observations have been taken as evidence that pretreatment with reserpine results in increased catecholamine receptor activity. Moreover, chronic depletion of catecholamines following inhibition of their synthesis and destruction of nerve terminals produced an increase in catecholamine receptor activity (Dominic & Moore, 1969; Thornburg & Moore, 1973). On the other hand, destruction of central 5-HT nerve terminals with intraventricular 5,6-dihydroxytryptamine (5,6-DHT) produces supersensitivity to 5-HT precursors and agonists (Nygren, Fuxe & others, 1974; Nakamura & Fukushima, 1978). Chronic inhibition of 5-HT synthesis with *p*-chlorophenylalanine (*p*CPA) also produces supersensitivity to 5-HT agonists (Przegalinski, Zebrowska & others, 1976). Trulson, Eubanks & Jacobs (1976), however, reported that supersensitivity to 5-HT precursors and agonists occurs following destruction of central 5-HT nerve terminals with 5,7-dihydroxytryptamine (5,7-DHT), but no supersensitivity occurs following chronic 5-HT depletion with *p*CPA.

It has been shown that head twitches are due to increased activity of 5-HT neuron systems (Corne, Pickering & Warner, 1963; Nakamura & Fukushima, 1976; Nakamura, Fukushima & Kitagawa, 1976; Nakamura & Fukushima, 1977, 1978). Moreover, head twitches have been used to study 5-HT neuron activity. There are several drugs that induce head twitches in mice; 5-HT (intracerebrally; i.c.) and 5-hydroxytryptophan by increasing the free concentration of 5-HT

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