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The effects of blocking catecholamine uptake on amphetamine-induced circling behaviour in mice with unilateral destruction of striatal dopaminergic nerve terminals

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A number of tricyclic compounds used clinically as antidepressant agents are believed to function by blocking the uptake of released central neurotransmitters into their respective presynaptic nerve terminals. Thus, since re-uptake is an important mechanism for the inactivation of released substance, more transmitter will be available in the synaptic cleft for receptor interaction. Tricyclic antidepressants are used commonly in patients with Parkinson's disease, and have been shown to produce additional modest improvement in motor function (Strang, 1965). This may be due to their inherent antiacetylcholine properties, or, alternatively, to their capacity to block re-uptake of released dopamine in surviving nigrostriatal terminals. To investigate the latter possibility we have studied the effect of blocking the uptake of the catecholamines noradrenaline and dopamine on the dopamine-dependent circling behaviour in mice with unilateral destruction of one nigrostriatal dopamine pathway. In such animals the directly acting dopamine agonist, apomorphine, causes turning towards the intact side, due, it is suggested, to the preferential stimulation of supersensitive denervated striatal dopamine receptors, while indirectly acting dopamine agonists, such as amphetamine, cause circling towards the lesioned side, due to release of endogenous dopamine from the intact nigrostriatal terminals (Ungerstedt, 1971).

Unilateral destruction of nigrostriatal dopamine nerve terminals in mice was achieved by the free-hand injection of 16 μg 6-hydroxydopamine in 4 μl chilled saline containing 0·2 mg ml^{-1} ascorbic acid into the right striatum as previously described (von Voigtlander & Moore, 1973; Pycock, Tarsy & Marsden, 1975). Ten days later those animals showing tight ipsiversive circling to amphetamine (5 mg kg^{-1}, i.p.) and strong contraversive circling to apomorphine (2 mg kg^{-1}, i.p.) were selected for the following series of experiments to test the effect of pretreatment of catecholamine uptake blocking agents on amphetamine-induced circling behaviour. A complete Latin square design was used to randomize the distribution of nomifensine, amantadine, desipramine, amitriptyline and benztropine to groups of 10 mice, including a control series of saline-injected animals. At the end of 30 min, when the effects were maximal, the mice were observed for any circling behaviour or postural asymmetries produced by the blocking drugs. After such assessments the mice were injected with amphetamine (1·5 mg kg^{-1}, i.p.), a dose that causes submaximal rates of turning (Pycock & others, 1975). Thirty min after amphetamine administration, the number of full circles completed by each animal was counted and compared with the rate of circling in the control saline-treated groups. Mice were tested in this way to various doses of blocking agent on alternate days so that at least 10 observations for each dose of drug was obtained.

Of the five uptake inhibitors tested in this mouse model only nomifensine and benztropine produced circling behaviour. Nomifensine, in the dose range 5–40 mg kg^{-1}, caused a mild ipsilateral postural asymmetry and ipsiversive rotational behaviour in a dose graded response (Fig. 1A) confirming the observation of Costall, Kelly & Naylor (1975). Benztropine in the dose range 1·5–50 mg kg^{-1}, similarly caused some ipsilateral body posturing together with circling towards

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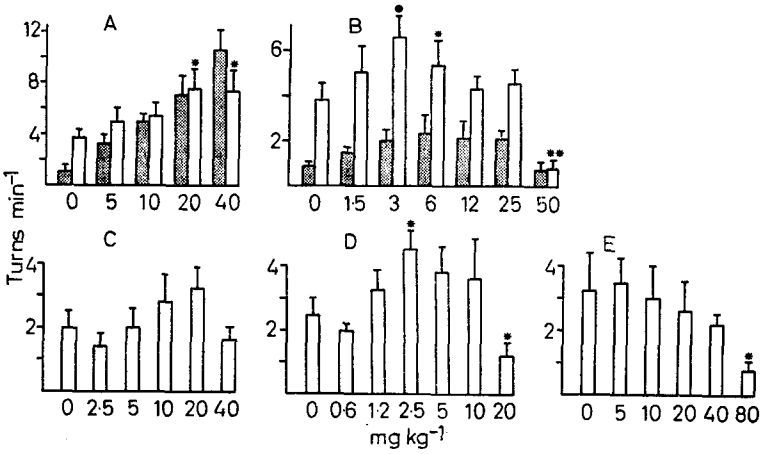

FIG. 1. (A—E) Dose response histograms for the intensity of circling to amphetamine (1·5 mg kg^{-1}, i.p.) in mice pretreated with A, nomifensine, B, benztropine, C, desipramine, D, amitriptyline and E, amantadine. O denotes animals pretreated with saline alone (see text for time course of pretreatments). In A, the shaded columns represent response to nomifensine administered alone, open columns represent amphetamine response after nomifensine pretreatment. In B the shaded columns represent response to benztropine alone, open columns represent amphetamine response after benztropine pretreatment. Means for 10 observations at each dose level are shown; vertical bars show s.e.m. * denotes statistical significance at the level $P<0·05$, ** denotes statistical significance at the level $P<0·001$.

the lesioned side, although much less marked than that seen after nomifensine (Fig. 1B). The highest dose of benztropine used caused sedation and no circling behaviour. Desipramine (2·5–40 mg kg^{-1}), amitriptyline (0·6–20 mg kg^{-1}), or amantadine (5–80 mg kg^{-1}) did not cause any marked body asymmetry or circling behaviour when administered alone (Fig. 1C, D, E).

The effect of these compounds on circling induced by 1·5 mg kg^{-1} amphetamine in these mice is shown in Fig. 1. This dose of amphetamine caused mice to turn ipsiversively at a submaximal rate of 2–4 turns min^{-1}; maximal rates of about 12 ± 2 turns min^{-1} being achieved with 8 mg kg^{-1} amphetamine (Pycock & others, 1975). Nomifensine increased the amphetamine response at the higher doses (20 and 40 mg kg^{-1}, $P < 0·05$) but circling rates were no different from those produced by the same doses of nomifensine given alone. Benztropine potentiated amphetamine-induced circling behaviour at 3 and 6 mg kg^{-1} ($P < 0·05$), and turning rates were greater than those obtained with benztropine alone at all except the highest dose. The highest dose of benztropine (50 mg kg^{-1}) inhibited amphetamine-induced circling ($P < 0·001$). Amitriptyline and desipramine had little effect on amphetamine-induced circling in the doses used: only amitriptyline in a dose of 2·5 mg kg^{-1} caused any significant potentiation, and the highest dose (20 mg kg^{-1}) caused sedation and depression of amphetamine circling. The effect of amantadine was to depress amphetamine-induced rotation which became statistically significant at the highest dose used (80 mg kg^{-1}, $P < 0·05$) (Fig. 1E).

Of the compounds examined, only nomifensine (Hoffmann, 1973; Hunt, Kannengiesser & Raynaud, 1974) and the antiacetylcholine compound benztropine (Coyle & Snyder, 1969) have been reported as potent inhibitors of dopamine uptake. The tricyclic antidepressants, desipramine and amitriptyline, block mainly the uptake of noradrenaline into noradrenergic neurons (Carlsson, Corrodi & others, 1969; Horn, Coyle & Snyder, 1971). Amantadine, however, is reported as causing the release of both noradrenaline and dopamine (von Voigtlander & Moore, 1971; Farnebo, Fuxe & others, 1971), while amphetamine probably releases all the central monoamine neurotransmitters (Fuxe & Ungerstedt, 1970).

Both dopamine uptake inhibitors, nomifensine and benztropine, elicited circling towards the side of the lesion, presumably by making more dopamine available at the synapse in the intact striatum. It is interesting to note that amphetamine did not add to the intensity of circling behaviour produced by nomifensine. As the latter drug was still active in control animals at this time, it may be supposed that nomifensine may block the uptake of amphetamine into dopaminergic nerve terminals and thus inhibit the subsequent release of endogenous dopamine. Benztropine, on the other hand, was synergistic with amphetamine, but this and its capacity to produce circling behaviour may be partly explicable in terms of its antimuscarinic actions, rather than being due to its capacity to block dopamine reuptake, for scopolamine, an antiacetylcholine drug that also induces circling in lesioned rodents (Pycock, Milson, Tarsy & Marsden, unpublished observations)

exerts little reuptake inhibition (Coyle & Snyder, 1969). In addition, benztropine is capable of releasing dopamine from striatal terminals (Orlansky & Heikkila, 1974).

In our hands amantadine, an antiviral agent mysteriously beneficial in Parkinson's disease, induced no circling behaviour, although the drug has been reported as eliciting ipsilaterial turning activity in rodents with unilateral lesions of the nigrostriatal dopaminergic pathway (Farnebo & others, 1971; Strömberg & Svensson, 1971; von Voigtlander & Moore, 1973). Initially amantadine was shown to be only a very weak inhibitor of dopamine uptake in the striatum (Heikkila & Cohen, 1972) although it had been suggested that it may release catecholamines from presynaptic nerve terminals (Farnebo & others, 1971). More recent work, however, has concluded that amantadine may exert partial agonist action at dopamine receptors (Stone & Bailey, 1975) which could, at least in part, explain its depression of amphetamine-induced circling activity in our model system.

The failure of the tricyclic antidepressant compounds, desipramine or amitriptyline, to greatly influence amphetamine-induced circling behaviour suggests that noradrenaline is not a crucial factor in this system. The modest antiparkinsonian action of such compounds are, thus, more likely to be due to their anti-acetylcholine properties, or to their general effects on mood which influences performance in Parkinson's disease. Only those agents acting directly upon the dopamine system (viz. nomifensine and benztropine) were noted as having consistently significant effects. In a similar study it was noted that blockade of the uptake of 5-hydroxytryptamine into central neurons also had no significant effect on drug-induced circling behaviour (Milson & Pycock, 1976). However, perhaps it should be noted that unilateral lesions of either the ascending noradrenaline (Pycock, Donaldson & Marsden, 1975) or 5-hydroxytryptamine (Costall, Naylor & others, 1976) neurons of the brain, without subsequent damage to the dopamine system, do produce animals that circle in response to drugs.

Perhaps the results of this study should be interpreted with some caution. The indirectly-acting dopamine agonist amphetamine must function by firstly being taken up into the neuron from which it then releases endogenous dopamine. If these blocking agents studied also interfere with the uptake of amphetamine, as may be suggested by the nomifensine results, then there will be obvious difficulties in interpreting the data. Similarly it has been noted that tricyclic compounds inhibit the rate at which amphetamine is metabolized (Lewander, 1969).

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