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THE INTERACTION OF NEUROLEPTIC AND MUSCARINIC AGENTS WITH CENTRAL DOPAMINERGIC SYSTEMS

P.H. KELLY

Department of Experimental Psychology, Downing Street, Cambridge

R.J. MILLER

M.R.C. Neurochemical Pharmacology Unit, Department of Pharmacology, Medical School, Hills Road, Cambridge CB2 2QD

1 The effect of muscarinic and neuroleptic agents on the turning behaviour induced by methamphetamine and apomorphine in rats with unilateral lesions of the substantia nigra induced by 6-hydroxydopamine has been examined.

2 Turning towards the side of the lesion induced by (+)-methamphetamine (5 mg/kg) was inhibited by α-flupenthixol (1 mg/kg) and α-clopenthixol (8 mg/kg) but not by high doses of their β-isomers.

3 Turning was inhibited by chlorpromazine (4 mg/kg) and pimozide (0.2 mg/kg). Thioridazine and clozapine (16 mg/kg) were ineffective. Turning in the same direction produced by scopolamine (10 mg/kg) was also inhibited by α-flupenthixol (1 mg/kg) and pimozide (0.25 mg/kg).

4 Turning produced by methamphetamine (5 mg/kg) was inhibited by oxotremorine (0.75 mg/kg) even in the presence of methylatropine (5 mg/kg).

5 Turning away from the side of the lesion induced by apomorphine (0.1 mg/kg) was inhibited by oxotremorine (0.75 mg/kg) but not by thioridazine or clozapine (16 mg/kg).

6 These results are discussed with regard to the mode of action of neuroleptic drugs in producing anti-psychotic effects and drug-induced Parkinsonism.

Introduction

Apart from their therapeutic antipsychotic action neuroleptic drugs may produce unwanted side-effects. One commonly observed side-effect is the occurrence of drug-induced parkinsonian symptoms (Klawans, 1973). Both the anti-psychotic action and the extrapyramidal parkinsonian side-effects of the neuroleptic drugs generally correlate well with their potency in blocking dopamine receptors as revealed by *in vitro* (Clement-Cormier, Kebabian, Petzold & Greengard, 1974; Horn, Cuello & Miller, 1974; Miller, Horn & Iversen, 1974) and *in vivo* (Andén, Butcher, Corrodi, Fuxe & Ungerstedt, 1970) models. However, it has been reported that some neuroleptics, notably thioridazine and clozapine, produce only minimal parkinsonian symptoms (Cole & Clyde, 1961; Burki, Ruch, Asper, Baggiolini & Stille, 1973) although they are potent blockers of dopamine effects *in vitro* (Miller *et al.*, 1974).

Since drug-induced extrapyramidal effects can be alleviated by antimuscarinic drugs (Shintami & Yamamura, 1973) it is possible that the recently demonstrated antimuscarinic action of clozapine and thioridazine (Miller & Hiley, 1974; Snyder, Greenberg & Yamamura, 1974) might account for their production of only minimal parkinsonian side-effects. Parkinsonism is related to deficient striatal dopaminergic function (Hornykiewicz, 1973) and drug-induced Parkinsonism may therefore result from blockade of striatal dopamine receptors. A possible basis for the alleviation of parkinsonian symptoms by antimuscarinic agents is the observed antagonistic effects of muscarinic agents and drugs acting on dopamine receptors in the striatum (Costall, Naylor & Olley, 1972).

In the present investigation we have used the rotation of rats with unilateral degeneration of the nigrostriatal pathway (Ungerstedt & Arbuthnott, 1970; Ungerstedt, 1971) as a quantitative *in vivo*

model of striatal dopaminergic activity to study the action of cholinomimetic agents and neuroleptics on striatal dopaminergic mechanisms.

Methods

Male albino Sprague-Dawley rats (250-300 g at the time of surgery) were given food and water *ad libitum* during the period of use.

Lesions

Animals were anaesthetized with Equithesin (Jensen-Salsbery Labs.), 2.5 ml/kg. They were then positioned in a stereotaxic apparatus and injected unilaterally with 6-hydroxydopamine through a 30 gauge stainless steel cannula aimed at the substantia nigra. The co-ordinates of the cannula tips were 2.8, 2.0, 8.0 according to the atlas of Pellegrino & Cushman (1967). 6-Hydroxydopamine hydrobromide was freshly dissolved (2 mg of base per ml) in cold 0.9% w/v NaCl solution (saline) containing 1 mg/ml of ascorbic acid; 4 μl of this solution was injected through the cannula at a rate of 1 μl/minute.

Turning behaviour

Drugs were made up in saline. The doses were calculated as the forms given below. Pimozide was first dissolved in a drop of glacial acetic acid and then diluted. Clozapine was dissolved with an equal weight of tartaric acid. Injection of vehicle solutions of these drugs into rats did not affect turning behaviour. All drugs were given intraperitoneally, except for apomorphine which was given subcutaneously. Fourteen days after the injection of 6-hydroxydopamine, the animals were screened for ability to turn with apomorphine (0.5 mg/kg) and (+)-methamphetamine (5 mg/kg). Only animals which responded with at least ten turns per minute were retained. Animals used in experiments with methamphetamine-induced turning were screened at least 10 days after operation, and those on apomorphine turning after at least three weeks. After initial selection, the animals were separated into groups of 4 or 5 animals and used in experiments with various drugs, at intervals of at least one week. The control responses of the various groups to methamphetamine and apomorphine during the period of the experiments did not change significantly. The experimental procedure was as follows. Animals were injected with the test drugs or vehicles 3 h before the experiments with the exception of oxotremorine which was given 5 min after the amphetamine or apomorphine. At the time of the experiment animals were injected with amphetamine or apomorphine and then placed in individual cages. All animals in a group received the same drug treatments. At 30 min ,intervals animals were placed in rotometer bowls for 5 minutes. The number of turns per min was measured on the fifth min by direct observation. The rotometers consisted of white perspex translucent bowls supported on metal rings. The bowls measured 30 cm in diameter and 26 cm in height.

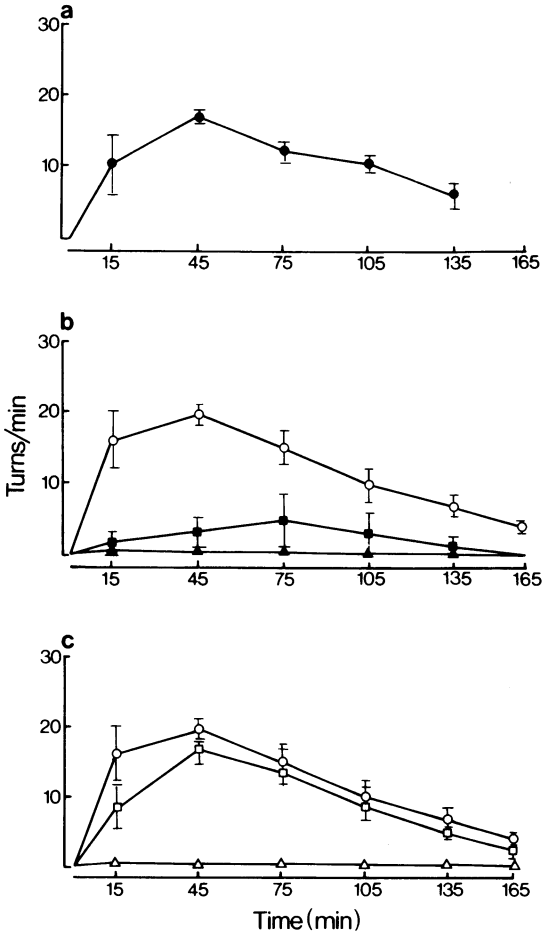

Figure 1 Effect of different isomers of thioxanthene neuroleptics on turning behaviour induced by methamphetamine (5 mg/kg). (a) Turning after 10 mg/kg β-flupenthixol (●). (b) Turning after 0.2 mg/kg (■) or 1.0 mg/kg (▲) α-flupenthixol and saline control (○). (c) Turning after 8 mg/kg α- (△) or β-clopenthixol (□) and saline control (○). Results are means from four rats. Vertical bars show s.e. mean.

Drugs

The following drugs were used: (+)-methamphetamine hydrochloride, 6-hydroxydopamine

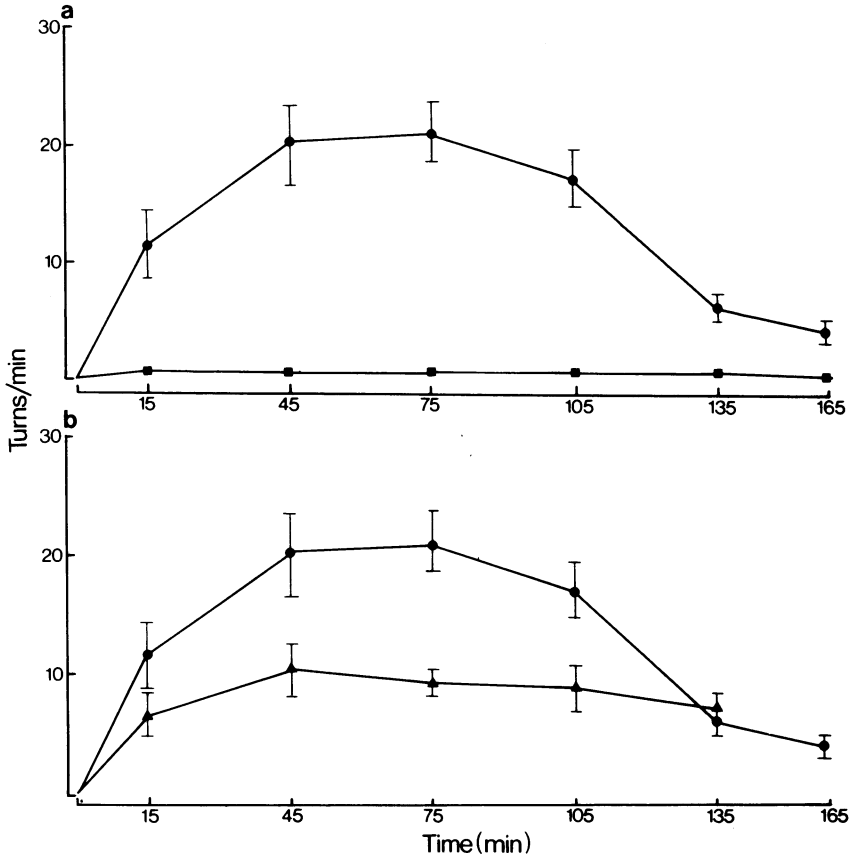

Figure 2 Effect of pimozide and chlorpromazine on methamphetamine- (5 mg/kg) induced turning behaviour. (a) Turning after 0.25 mg/kg pimozide (■) or saline (●). (b) Turning after 4 mg/kg chlorpromazine (▲) or saline (●). Results are means from four rats. Vertical bars show s.e. mean.

hydrobromide, (Sigma); thioridazine hydrochloride (Sandoz); clozapine (base) (Sandoz/Wander); scopolamine hydrochloride (BDH); apomorphine hydrochloride (MacFarlane Smith); oxotremorine sesquifumarate (Aldrich); pimozide (base) (Janssen); α- and β-flupenthixol and α- and β-clopenthixol dihydrochloride (Lundbeck).

Results

Blockade of methamphetamine-induced turning by cis- and trans-thioxanthene isomers

In accordance with previous reports (Ungerstedt & Arbuthnott, 1970) intraperitoneal injection of methamphetamine in lesioned animals produced turning towards the side of the lesion in a dose-dependent fashion. The thioxanthene neuroleptics flupenthixol and clopenthixol exhibit geometric isomerism, existing as *cis* (α) or *trans* (β) isomers. Previously we have shown that only the α-isomers are effective antagonists of the dopamine-stimulated adenylate cyclase in homogenates of rat striatum. Figure 1 shows the effects of the different isomers on turning produced by 5 mg/kg of methamphetamine. For both neuroleptics the β-isomers were ineffective whereas the α-isomers were effective in blocking such turning. α-Flupenthixol was more potent than α-clopenthixol. This again parallels the activity of the drugs on the dopamine-sensitive adenylate cyclase.

Effects of other neuroleptics

Pimozide (Figure 2) completely abolished turning behaviour at low doses. At 4 mg/kg chlorpromazine partially inhibited turning (Figure 2). This result is similar to that reported by Crow & Gillbe (1973). These authors also showed that

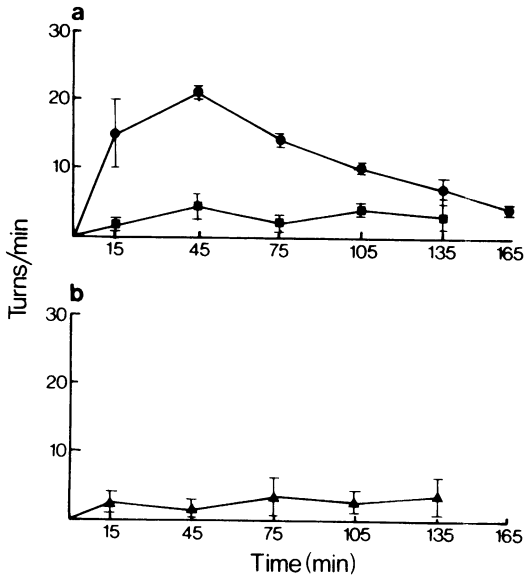

Figure 3 Effect of oxotremorine on turning behaviour induced by methamphetamine (5 mg/kg). (a) Turning after 0.75 mg/kg oxotremorine (■) given 5 min after the amphetamine and saline control (●). (b) Effect of 0.75 mg/kg oxotremorine and 5 mg/kg methylatropine (▲) given 5 min after the amphetamine. Results are means from four rats. Vertical bars show s.e. mean.

higher doses of chlorpromazine completely blocked turning. In contrast the two neuroleptics clozapine and thioridazine had no effect on turning behaviour even at very high doses (16 mg/kg). Similar observations with thioridazine at lower doses have been reported previously (Crow & Gillbe, 1973).

Effect of muscarinic agents

When 0.75 mg/kg oxotremorine was administered just after methamphetamine (5 mg/kg) the resulting turning behaviour was considerably reduced. In order to see whether this blockade of turning was due to central or peripheral effects of oxotremorine, some animals were also given 5 mg/kg of methylatropine, to block peripheral muscarinic receptors. In these animals oxotremorine (0.75 mg/kg) still produced a considerable decrease in methamphetamine-induced turning behaviour (Figure 3). In confirmation of previous reports (Ungerstedt, Avemo, Avemo, Ljungberg & Ranje, 1973), the antimuscarinic drug scopolamine (10 mg/kg) produced turning behaviour in the same direction as methamphetamine. However, scopolamine was considerably less effective than methamphetamine. Scopolamine-induced turning could be inhibited by α-flupenthixol and pimozide (Figure 4).

Effect of drugs on apomorphine-induced turning

When animals were given apomorphine, a directly acting dopamine receptor agonist, the animals turned away from the side of the lesion in a dose-dependent fashion. This phenomenon has been attributed to the action of apomorphine on supersensitive receptors on the lesioned side (Ungerstedt, 1971). Turning could be elicited by very low doses of apomorphine, down to 0.01 mg/kg. With doses of 0.05 mg/kg or higher the maximal number of turns per minute did not increase but the duration of turning did. It was found that oxotremorine (0.75 mg/kg) reduced apomorphine-induced turning (Figure 5). It was

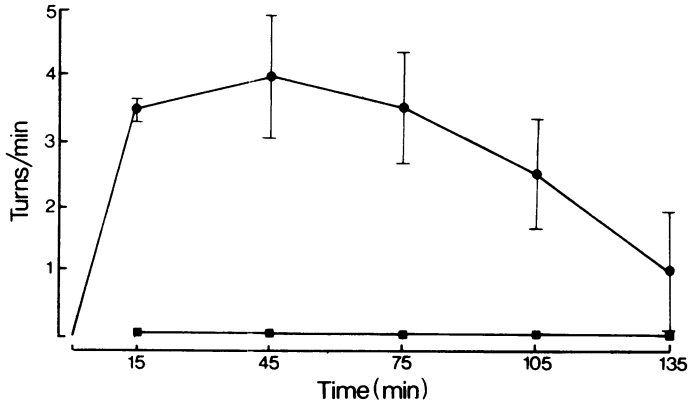

Figure 4 Turning towards the side of the lesion produced by scopolamine (10 mg/kg) (●). The turning was blocked by pimozide (0.25 mg/kg) (■) or α-flupenthixol (1.0 mg/kg) (■). Results are means from four rats. Vertical bars show s.e. mean.

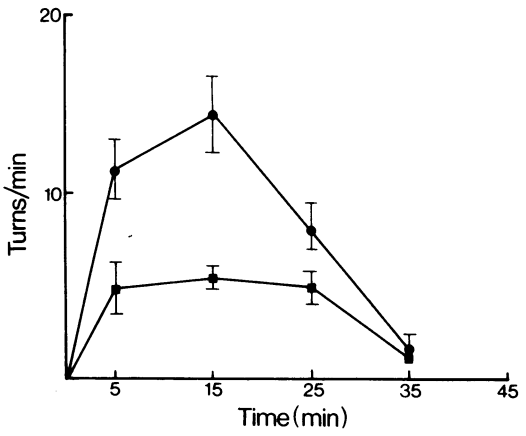

Figure 5 Effect of oxotremorine 0.75 mg/kg (■) or saline (●) given 5 min after apomorphine on turning behaviour produced by 0.1 mg/kg apomorphine. Results are means from four rats. Vertical bars show s.e. mean.

also found that clozapine and thioridazine (16 mg/kg) were ineffective in inhibiting apomorphine-induced turning (Figure 6).

Discussion

In the present investigation we have confirmed that some neuroleptic drugs are able to block the methamphetamine-induced rotation of rats with unilateral degeneration of the nigrostriatal pathway. The potency of chlorpromazine and the α- and β-isomers of flupenthixol and clopenthixol as amphetamine antagonists in this test correlated well with the dopamine blocking potencies of these drugs previously measured in an *in vitro* biochemical system (Miller *et al.*, 1974). However, the neuroleptics thioridazine and clozapine did not block the turning provoked by methamphetamine or apomorphine even in high doses. On the basis of their original observation that thioridazine did not block methamphetamine-induced turning Crow & Gillbe (1973) suggested that thioridazine does not block dopamine receptors. However, both clozapine and thioridazine are potent blockers of the stimulation by dopamine of adenylate cyclase in homogenates of the rat striatum (Miller *et al.*, 1974), an *in vitro* system which has proved a useful model of the dopamine receptor.

An alternative explanation of the failure of thioridazine and also of clozapine to block methamphetamine-induced rotation is that their antimuscarinic action (Miller & Hiley, 1974; Snyder *et al.*, 1974) may alleviate the anti-

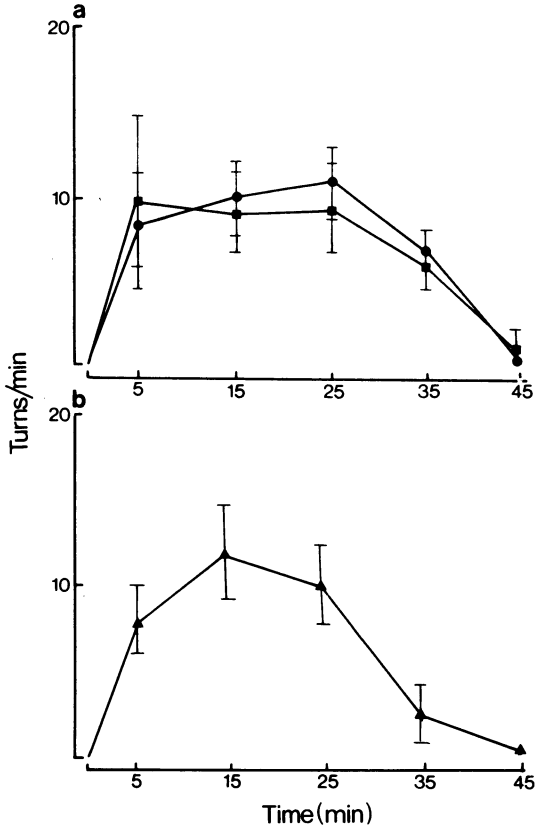

Figure 6 Effect of clozapine and thioridazine on turning induced by apomorphine (0.1 mg/kg). (a) Effect of thioridazine (16 mg/kg) (■) and saline control (●). (b) Effect of clozapine (16 mg/kg) (▲). Results are means from four rats. Vertical bars show s.e. mean.

dopaminergic effects of this drug in the striatum. We have demonstrated that central cholinergic stimulation produced by oxotremorine can block methamphetamine-induced turning and conversely that the antimuscarinic agent scopolamine produces turning in the same direction as methamphetamine.

The neuroanatomical basis of these cholinergic-dopaminergic interactions could involve both the substantia nigra and the striatum. Local application of cholinomimetic agents to the substantia nigra decreases the turnover of dopamine in nigrostriatal dopaminergic neurones, while antimuscarinic drugs have the opposite effect (Javoy, Agid, Bouvet & Glowinski, 1974), suggesting that cholinergic synapses are involved in an inhibitory effect on nigrostriatal neurones. The turning produced by the anti-acetylcholine agent,

scopolamine, may involve activity in the intact nigrostriatal dopaminergic neurones, since this turning could be blocked by dopamine receptor blocking drugs such as pimozide and α-flupenthixol.

A further site of cholinergic-dopaminergic interaction is probably the striatum itself. In the present investigation we have observed that the turning produced by the dopamine agonist apomorphine is inhibited by the cholinomimetic agent oxotremorine. Since apomorphine-induced turning is thought to result from the direct stimulation of the supersensitive denervated striatal dopamine receptors (Ungerstedt, 1971), the effect of oxotremorine on apomorphine-induced turning does not depend on an action on nigrostriatal dopaminergic neurones. One mechanism which may be important in the effect of oxotremorine on apomorphine-induced turning is an increase of striatal cholinergic activity. This would tend to antagonize dopaminergic activity since there is evidence that dopamine inhibits cholinergic interneurones in the striatum. For example, blockade of dopaminergic transmission by neuroleptics increases the release of acetylcholine from the caudate nucleus (Stadler, Lloyd, Gadea-Ciria & Bartholini, 1973) and reduces striatal levels of acetylcholine (Sethy & Van Woert, 1974; Agid, Guyenet, Javoy, Beaujouian & Glowinski, 1974). Treatment with dopamine agonists such as apomorphine and L-DOPA increases striatal levels of acetylcholine (Sethy & Van Woert, 1974).

In conclusion, using the rotation model we have demonstrated an inhibitory interaction between cholinergic activity and dopaminergic activity in the extrapyramidal system. Evidence has been discussed that this interaction can occur both in the substantia nigra and in the striatum itself. These interactions may explain why neuroleptic drugs such as thioridazine and clozapine which possess both antidopaminergic and antimuscarinic actions do not block the turning behaviour produced by dopamine release (methamphetamine) or by direct stimulation of dopamine receptors (apomorphine). The antimuscarinic actions of these drugs may also be responsible at least in part, for their lack of production of extrapyramidal symptoms when used clinically. Their therapeutic antipsychotic activity may therefore result from blockade of dopamine receptors other than those in the striatum.

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