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Interaction of atropine-like drugs with dopamine-containing neurones in rat brain

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Summary

1. A variety of atropine-like drugs effective in the treatment of drug-induced extrapyramidal syndromes have been investigated with regard to their interaction with dopamine-containing neurones in rat brain.

2. Under some conditions benztropine, trihexyphenidyl, atropine and ethopropazine significantly antagonized the chlorpromazine-induced increase in subcortical concentrations of homovanillic acid.

3. Most of the atropine-like drugs investigated also decreased the turnover of dopamine in the subcortex as measured by following the disappearance of dopamine after administration of α-methyl-*p*-tyrosine.

4. These findings are suggestive that an imbalance between a dopaminergic and cholinergic system might be closely linked to the pathogenesis of extrapyramidal movement disorders.

Introduction

An increased synthesis and turnover of dopamine is routinely observed in animal brain after the administration of various antipsychotc drugs which produce extrapyramidal symptoms in man. Thus, one common feature of all the neuroleptic drugs which have extrapyramidal side effects is their ability to produce large increases in the homovanillic acid content of mammalian brain. However, the ability of certain antipsychotic drugs to produce extrapyramidal disorders is thought not to be due to their ability to stimulate dopamine synthesis and turnover directly, but rather related to the ability of these agents to block dopamine receptors (Anden, Carlsson & Haggendal, 1969). It is currently believed that this blockade of dopamine receptors ultimately leads to a feedback activation of dopaminergic neurones and the resultant increase in dopamine synthesis and turnover.

It has been known for some time that several drugs which are acetylcholine antagonists are quite useful clinically in the treatment of extrapyramidal reactions produced by these antipsychotic compounds. These observations suggest that centrally acting atropine-like drugs may interact in some way with dopamine containing neurones in the central nervous system and thus alter the activity of these neuroleptic drugs. To investigate this possibility the following experiments were performed.

Methods

In one series of experiments, 4 h after injection with chlorpromazine (10 mg/kg, i.p.) and 2 h before being sacrificed, male Sprague-Dawley rats (200–250 g) obtained from Charles River, Inc. were injected intraperitoneally with the following atropine-like compounds: benztropine mesylate, 10, 25, or 50 mg/kg; procyclidine hydrochloride, 25 or 100 mg/kg; benzhexol hydrochloride, 25 and 50 mg/kg; ethopropazine hydrochloride, 25 mg/kg; atropine sulphate, 25 mg/kg; or atropine methonitrate, 50 mg/kg. Doses were calculated on the basis of the salt form of each compound. Controls (animals that received only chlorpromazine and animals that received only saline) were included with each dosage of every atropine-like drug tested. Administration of atropine-like drugs alone did not significantly affect subcortical homovanillic acid concentrations nor dopamine concentrations in any uniform way. The first group of experiments were performed at room temperature, although several parallel experiments conducted at 32° C demonstrated comparable effects. In a second group of determinations, 15 min before intraperitoneal injection with chlorpromazine (10 mg/kg) and 105 min before being sacrificed, rats were injected intraperitoneally with benztropine, 25 or 50 mg/kg; ethopropazine, 25 mg/kg; benzhexol, 50 mg/kg; atropine, 25 mg/kg; or orphenadrine, 25 mg/kg. Control animals were also used as mentioned above. The second group of experiments were performed at 32° C to avoid any undue hypothermia as a result of the combined drug treatment.

After sacrifice, in the first two groups of experiments, the subcortex (including midbrain, basal ganglia and diencephalon) was dissected out and rapidly frozen on dry ice before the measurement of homovanillic acid. In a third group of experiments α-methylparatyrosine (250 mg/kg) methyl ester was injected 10 min after intraperitoneal administration of the following compounds (25 mg/kg); benzhexol, orphenadrine, procyclidine, benztropine, or atropine. Two hours after α-methylparatyrosine the animals were sacrificed and the subcortex was quickly excised and frozen on dry ice. These samples were analysed fluorimetrically for dopamine. Control animals which received only α-methylparatyrosine were included in experiments with each atropine-like compound. Dopamine and homovanillic acid were measured fluorimetrically essentially by the methods described by Roth & Surh

TABLE 1. *Antagonism at 4 h by atropine-like drugs of the phenothiazine-induced increase in homovanillic acid in the rat subcortex*

Treatment	Dose (mg/kg)	No. determinations	Homovanillic acid (ng/g±S.E.M.)	*P*
Saline controls*		44	126± 7	—
Chlorpromazine	10*	63	523±16	—
Benztropine	10†	6	290±35	<0·02
	25	6	232±57	<0·02
	50	3	165± 4	<0·005
Procyclidine	25	3	398±46	N.S.
Benzhexol	25	6	458±38	N.S.
	50	5	351±24	<0·01
Ethopropazine	25	10	218±29	<0·001
Atropine	25	7	397±42	<0·02
Atropine methyonitrate	50	6	611±50	N.S.

* For each experiment some animals received saline or chlorpromazine only and these values are pooled in the table. † Each atropine-like drug was administered 4 h after chlorpromazine (10 mg/kg) and the animals were sacrificed 2 h later. *t*-tests (two tailed) compared animals receiving chlorpromazine plus an atropine-like drug with chlorpromazine controls from the same experiments. These experiments were performed at room temperature.

(1970) after isolation from tissue extracts by alumina column chromatography. All drugs were administered in the indicated salt form.

Results

In experiments where atropine-like compounds were administered 4 h after chlorpromazine (Table 1) benztropine (each dose), benzhexol (50 mg/kg only), ethopropazine (25 mg/kg), and atropine (25 mg/kg) significantly blocked the chlorpromazine induced increase in subcortical homovanillic acid. Benztropine appeared to be the most effective compound in this respect since it blocked the chlorpromazine-induced rise in homovanillic acid at a dose of only 10 mg/kg. Atropine methonitrate (50 mg/kg) was ineffective. In the second group of experiments, only benztropine and atropine were effective in blocking the homovanillic acid increase (Table 2). In the third series of experiments each compound except atropine significantly slowed the utilization of dopamine after inhibition with α-methylparatyrosine (Table 3).

Discussion

An increase in homovanillic acid, one of the major metabolites of dopamine, accompanies the administration of certain antipsychotic compounds to animals (Anden, Roos & Werdinius, 1964; Laverty & Sharman, 1965; Sharman, 1966; Juorio, Sharman & Trajkov, 1966; O'Keefe, Sharman & Vogt, 1970). A similar increase in homovanillic acid has been reported in the cerebrospinal fluid of psychiatric patients who have received large doses of antipsychotic drugs (Persson & Roos,

TABLE 2. *Antagonism at 90 min by atropine-like drugs of the phenothiazine-induced increase in homovanillic acid in the rat subcortex*

Treatment	Dose (mg/kg)	No. determinations	Homovanillic acid (ng/g±S.E.M.)	*P*
Saline controls*		5	178±17	—
Chlorpromazine	10	18	614±24	—
Benztropine	25†	4	296±29	<0·001
	50	4	322±26	<0·001
Ethopropazine	25	4	716±42	N.S.
Benzhexol	50	3	515±45	N.S.
Atropine	25	4	421±60	<0·02
	50	4	253±32	<0·001
Orphenadrine	25	3	474±43	N.S.

* For each experiment some animals received saline or chlorpromazine only and these values are pooled in the table. † Each atropine-like drug was administered 15 min before chlorpromazine (10 mg/kg dose), and 105 min before being sacrificed. *t*-tests (two-tailed) compared animals receiving chlorpromazine plus an atropine-like drug with chlorpromazine controls from the same experiment. These experiments were performed at 32° C.

TABLE 3. *Effect of atropine-like drugs upon dopamine concentrations in the rat subcortex after α-methylparatyrosine*

Treatment	Dose (mg/kg)	No. determinations	Dopamine (% control)*	*P*
Benzhexol	25	4	126	<0·05
Orphenadrine	25	4	160	<0·05
Procyclidine	25	4	138	<0·05
Benztropine	25	4	138	<0·001
Atropine	25	4	109	N.S.

* Actual control value for dopamine ± S.D. 2 h after α-methylparatyrosine methyl ester (250 mg/kg) was 560±151 (twenty-four determinations).

1968, 1969). This effect has been ascribed to an accelerated turnover of dopamine (Sharman, 1967) through the mechanism of increased synthesis, possibly initiated by a compensatory activation of a dopamine-containing neuronal system following blockade of dopamine receptors by the antipsychotic compounds in question (Nyback & Sedvall, 1969). Clinically, the use of effective antipsychotic medication is usually accompanied by a variety of extrapyramidal symptoms which are usually relieved by atropine-like drugs. Although there is some species variation, the ability of an antipsychotic drug to increase dopamine turnover in animals correlates with its tendency to produce extrapyramidal symptoms in man (Laverty & Sharman, 1965).

O'Keefe *et al.* (1970) reported that atropine (25 mg/kg) attenuated the increase in homovanillic acid produced by chlorpromazine (10 mg/kg) or haloperidol (1 mg/kg). They suggested that atropine may exert its effects by blocking the action of acetylcholine at sites where acetylcholine normally activates a dopaminergic pathway, perhaps in the substantia nigra. The results of the present experiments indicate that several atropine-like compounds which are highly effective clinically in preventing or treating drug-induced Parkinsonism can antagonize the increase in homovanillic acid produced by antipsychotic compounds. In our experiments benztropine appeared to be the most potent compound in this regard. Atropine methonitrate (a quaternary atropine derivative which does not get into the central nervous system) was ineffective, suggesting that the atropine-like blockade of dopamine turnover is primarily an effect related to an action of atropine directly on the central nervous system and not related to its peripheral activity as an acetylcholine antagonist. Two compounds (benzhexol and ethopropazine) were effective only in the 4 h experiments and the reasons for these differential results are not clear. In the case of ethopropazine it is possible that this phenothiazine is not exerting its effect by an atropine-like mechanism (Doshoy, Constable & Agate, 1956) but rather is competing with chlorpromazine for the dopamine receptor more effectively at a time (4 h) when the chlorpromazine effect is diminishing. In any event our results appear to be consistent with the suggestion of Arnfred & Randrup (1968), O'Keefe *et al.* (1970), that a cholinergic mechanism exerts an inhibitory effect upon dopaminergic neurones in the striatum. Evidence at the clinical level has also implicated an interaction between cholinergic and dopaminergic systems in the central nervous system. Thus Weintraub & Van Woert (1971) have shown that the exacerbation of Parkinsonian symptoms produced by physostigmine is greatly reduced by L-dopa therapy. It is perhaps noteworthy that the effect of the atropine-like drugs reported here upon dopamine metabolism is not as potent as that produced by γ-hydroxybutyrate, a compound which appears to block dopamine release (Roth, 1971).

Recently, Coyle & Snyder (1969) showed that many drugs useful in the treatment of Parkinsonism were potent inhibitors of dopamine uptake into striatal synaptosomes. Benztropine was the most potent compound in their experiments. Our finding of decreased dopamine turnover with atropine-like drugs after chlorpromazine may be consistent with their hypothesis that atropine-like anti-Parkinson drugs increase the availabilty of dopamine at the receptor. This increased availability of dopamine at dopamine receptive neurones could lead to a negative feedback on dopamine neurones suppressing their activity and decreasing dopamine turnover. However, it is also conceivable that these compounds both affect dopamine reuptake and block some cholinergic activation of dopamine synthesis.

A recent report (Anden & Bedard, 1971), using several other atropine-like drugs, confirms our independent observations demonstrating that these drugs decrease dopamine turnover.

The interpretation of atropine-like drug action in relationship to antipsychotic drugs is complicated by the fact that large doses of chlorpromazine also appear to have some cholinomimetric activity (Maickel, 1968). However, despite the complexity of action of these drugs it appears that antipsychotic drugs can set in motion a series of behavioural and chemical events which can in part be blocked by atropine-like drugs (Hanson, Stone & Witoslawski, 1970). The present demonstration that the increase in dopamine turnover produced by antipsychotic drugs can be blocked by some clinically effective atropine-like compounds is further evidence that a dopaminergic-cholinergic imbalance is closely linked in the pathogenesis of extrapyramidal movement disorders.

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