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Oxotremorine- and atropine-induced changes of dopamine metabolism in the rat striatum

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The effects of various doses of oxotremorine and of atropine (30 min, i.p.) on the metabolism of dopamine were examined in the striatum of the rat. Changes in striatal dopamine metabolism were estimated by following either the accumulation of [^{3}H] dopamine 15 min after intravenous injection of [^{3}H] tyrosine or the accumulation of dopa (taken as an index of dopamine synthesis) in animals pretreated with a dopa decarboxylase inhibitor Ro4-4602. Oxotremorine, 0·1 mg kg^{-1}, and atropine, 1 mg kg^{-1}, did not affect dopamine metabolism. Oxotremorine, 0·5 and 1·5 mg kg^{-1}, did not modify dopamine concentrations but increased the accumulation of [^{3}H]dopamine. The drug enhanced dopa formation in animals pretreated with Ro4-4602. Atropine, 5 and 20 mg kg^{-1}, increased the accumulation of [^{3}H]dopamine but did not affect dopamine concentrations. The accumulation of dopa was not modified there being no difference from the saline value in animals pretreated with the dopa decarboxylase inhibitor. Thus at high doses oxotremorine stimulated dopamine metabolism and atropine reduced dopamine utilization.

The extrapyramidal disorders seen in patients treated with neuroleptic drugs which block dopaminergic transmission are relieved by antiacetylcholine drugs. On the other hand, centrally acting acetylcholine-like compounds may induce catalepsy (Zetler, 1968; Costall & Olley, 1971). Cholinergic and neuroleptic-induced catalepsy can be blocked by antiacetylcholine drugs (Costall & Olley, 1971; Corrodi, Fuxe & Lidbrink, 1972). Furthermore, high doses of atropine slightly reduce dopamine utilization in the brain (Andén & Bedard, 1971; Bartholini & Pletscher, 1971; Corrodi & others, 1972). The increase in central dopamine turnover induced by neuroleptics can be prevented by antiacetylcholine agents (O'Keefe, Sharman & Vogt, 1970; Andén, 1972; Corrodi & others, 1972). Similarly, the activation of brain dopamine turnover by the acetylcholine-like agent, oxotremorine, may be blocked by atropine (Corrodi, Fuxe & others, 1967). We have further investigated the action of atropine and of oxotremorine on striatal dopamine metabolism by analysing the effects of systemic injections of various doses of these agents in the striatum (putamen + caudate nucleus) of the rat.

METHODS

Physiological procedure

Male Charles River rats (250 g) were treated for 30 min with oxotremorine fumarate, atropine sulphate or saline injected intraperitoneally. Drug doses (Table 1) are expressed as bases. Fifteen min after injection a pulse injection of purified (Javoy, Agid & others, 1974a) L-[3, 5-^{3}H] tyrosine (49 Ci mmol^{-1}, CEA, France) was made intravenously and the animals were decapitated 15 min later and brains quickly

removed. Striata were dissected and analysed for their content in endogenous and labelled dopamine and tyrosine.

In other experiments, rats were injected with the inhibitor of dopa decarboxylase, Ro4-4602 [N^1-(DL-seryl)-N^2-(2, 3, 4-trihydroxybenzyl) hydrazine] (800 mg kg^{-1}, i.p.) administered simultaneously with oxotremorine, atropine or saline for 30 min and then dopa was assayed in the striatum.

Biochemical procedure

The striatum was homogenized in 0·4 N perchloric acid. Tissue homogenates were adjusted to pH 6·1. Dopamine and [^{3}H] dopamine were separated by ion exchange chromatography on Amberlite CG-50 and adsorption chromatography on alumina (Javoy, Agid & others, 1972). Dopamine was estimated on an aliquot of the Amberlite eluate by the spectrofluorimetric technique of Laverty & Taylor (1968). The effluents of the Amberlite column, containing tyrosine, [^{3}H] tyrosine and dopa were passed through an alumina column (Javoy & others, 1974a). The hydrochloric acid eluate from the alumina column was adjusted to pH 2 and passed through a Dowex 50 W X4 column buffered at pH 6·5. Dopa was eluted from the column by a 0·2 M KH_2PO_4 K_2HPO_4 buffer at pH 5·4 and estimated spectrofluorimetrically (Laverty & Taylor, 1968). The effluent of the alumina column was adjusted to pH 2 and passed through a Dowex 50 W X4 (H^+) column. Tyrosine and [^{3}H]tyrosine were eluted in N perchloric acid. Tyrosine was estimated spectrofluorimetrically (Udenfriend, 1962).

The radioactivity ([^{3}H]dopamine and [^{3}H]tyrosine) was estimated on aliquots of the respective collected fractions by liquid scintillation. All data were corrected for respective recoveries. Absolute experimental values were statistically evaluated with Student's *t*-test. Differences were accepted as significant for $P < 0{\cdot}05$.

RESULTS

Effect of various doses of oxotremorine or atropine on dopamine concentrations and on the accumulation of newly formed [^{3}H]dopamine in the striatum

No modification of dopamine concentrations were found after oxotremorine and atropine treatments (Table 1). At the lowest doses oxotremorine (0·1 mg kg^{-1}) and atropine (1 mg kg^{-1}) did not affect the initial accumulation of [^{3}H]dopamine newly formed from [^{3}H]tyrosine (Table 1). At higher doses both oxotremorine (0·5 and 1·5 mg kg^{-1}) and atropine (5 and 20 mg kg^{-1}) increased the concentrations of [^{3}H] dopamine compared to those of saline-treated rats.

Oxotremorine 0·1 and 0·5 mg kg^{-1} did not affect tyrosine and [^{3}H]tyrosine concentrations, but at 1·5 mg kg^{-1} enhanced the accumulation of [^{3}H]tyrosine and the tyrosine content (Table 1). However, tyrosine specific activity was never significantly affected by the drug.

Atropine significantly decreased tyrosine concentrations as well as the accumulation of [^{3}H]tyrosine after 1 mg kg^{-1} and the effect was more striking after 5 and 20 mg kg^{-1} of atropine. Yet tyrosine specific activity remained unchanged (Table 1).

Effect of various doses of oxotremorine and atropine on the initial accumulation of dopa in the striatum of Ro4-4602 treated animals

When animals were treated with Ro4-4602, administered simultaneously with oxotremorine, atropine or saline, the accumulation of dopa was used as an index of the rate of tyrosine hydroxylation which is a limiting step of dopamine synthesis (Table 2).

Table 1. *Effect of oxotremorine and atropine on dopamine metabolism in the rat striatum.* Animals were treated with drug or saline for 30 min (i.p.), [^{3}H]tyrosine (285 μCi, i.v. in oxotremorine-treated animals and 220 μCi in atropine-treated rats) was injected 15 min before decapitation. Results are the mean $\pm$ s.e.m. of data obtained with groups of 7 to 10 rats. * Represents significant statistical differences for all P values $\leqslant 0{\cdot}05$ when compared to saline treated animals. (Tyrosine S.A. Tyrosine Specific Activity).

	Oxotremorine (mg kg^{-1})				Atropine (mg kg^{-1})			
	0	0·1	0·5	1·5	0	1	5	20
Dopamine μg g^{-1}	8·7 ± 0·4	9·1 ± 0·3	8·3 ± 0·2	9·1 ± 0·3	8·6 ± 0·2	8·6 ± 0·3	8·7 ± 0·2	9·1 ± 0·6
[^{3}H]Dopamine nCi g^{-1}	25·6 ± 1·6	24·7 ± 1·8	30·5*± 1·2	36·9*± 1·9	16·8 ± 0·7	16·9 ± 0·5	23·1*± 1·4	22·2*± 1·2
Tyrosine μg g^{-1}	17·8 ± 0·7	16·4 ± 0·6	17·9 ± 0·6	18·5 ± 0·4	19·1 ± 0·8	17·0 ± 0·8	13·9*± 0·5	14·5*± 0·7
[^{3}H]-Tyrosine nCi g^{-1}	314 ± 20	278 ± 24	341 ± 26	406*± 31	245 ± 15	199*± 4	161*± 10	191*± 6
Tyrosine S.A. nCi μg^{-1}	18·7 ± 1·1	17·9 ± 1·2	19·0 ± 1·1	21·9 ± 1·6	12·8 ± 0·5	12·0 ± 0·8	11·7 ± 0·5	13·2 ± 0·6

Oxotremorine 0·1 mg kg^{-1}, did not affect the concentration of dopa compared to corresponding controls but at 0·5 or 1·5 mg kg^{-1} significantly enhanced the accumulation of dopa. On the other hand atropine 1·5 or 20 mg kg^{-1} did not modify the accumulation of dopa there being no significant differences from the control value.

Ro4-4602 increased the concentrations of tyrosine (Table 2) compared to those of saline-treated rats (Table 1) as previously reported (Carlsson, Davis & others, 1972; Javoy, Agid & others, 1974b). However no differences in tyrosine concentrations were seen between oxotremorine, atropine or saline-treated animals simultaneously injected with the dopa decarboxylase inhibitor (Table 2). Thus the depressant effect of atropine on the endogenous tyrosine content (Table 1) was masked by the simultaneous injection of Ro4-4602.

DISCUSSION

No changes in dopamine metabolism could be detected in the striatum, shortly after a systemic injection of low doses of oxotremorine or atropine. Both drugs adminis-

Table 2. *Effect of oxotremorine or atropine on the accumulation of dopa (dihydroxyphenylalanine) in striatum of rats pretreated with Ro4-4602.* All animals were treated with a dopa decarboxylase inhibitor Ro-4602 (800 mg kg^{-1}, i.p.) simultaneously to saline, oxotremorine or atropine. Thirty min later dopa and tyrosine was estimated in the striatum. Each value represents the mean $\pm$ s.e.m. of data obtained on 6 to 8 animals. *Represents significant statistical differences $P < 0{\cdot}05$ when compared to saline treated animals.

	Saline	Oxotremorine (mg kg^{-1})		
		0·1	0·5	1·5
Dopa (ng g^{-1})	386 ± 34	411 ± 25	489* ± 25	476* ± 33
Tyrosine (μg g^{-1})	20·6 ± 0·8	18·8 ± 0·6	18·4 ± 0·5	20·2 ± 0·9
	Saline	Atropine (mg kg^{-1})		
		1	5	20
Dopa (ng g^{-1})	390 ± 23	395 ± 26	372 ± 22	427 ± 27
Tyrosine (μg g^{-1})	21·5 ± 0·9	23·2 ± 1·7	19·6 ± 0·7	21·5 ± 0·7

tered in higher doses affected dopamine metabolism since they significantly increased the initial accumulation of newly-formed [^{3}H]dopamine. The differences in the [^{3}H] dopamine accumulation are very likely not due to changes in tyrosine and [^{3}H]tyrosine since tyrosine specific activity was never statistically different from that of controls after oxotremorine as well as after atropine pretreatment.

Oxotremorine at 0·5 and 1·5 mg kg^{-1} stimulated dopamine synthesis, as indicated by the greater accumulation of dopa in animals pretreated with the dopa-decarboxylase inhibitor. Moreover, the unchanged dopamine concentrations (in agreement with Corrodi & others, 1967) associated with the increased accumulation of [^{3}H]dopamine (Table 1) observed in striatum of rat treated only with oxotremorine may indicate that the drug activated dopamine synthesis and utilization simultaneously. This is in agreement with previous histochemical (Corrodi & others, 1967) and biochemical (Corrodi & others, 1967; Perez-Cruet, Gessa & others, 1971; Handbrich & Reid, 1972; Andén, 1974) observations indicating an activation of striatal dopamine neurons by acetylcholine-like drugs.

Atropine at 5 and 20 mg kg^{-1} did not affect dopamine synthesis since the accumulation of dopa was not affected in animals pretreated with Ro4-4602. Atropine alone, did not affect dopamine concentrations (in accordance with O'Keefe & others, 1970; Perez-Cruet & others, 1971; Bartholini & Pletscher, 1971) but markedly increased the accumulation of newly formed [^{3}H]dopamine (Table 1) suggesting that the drug reduced the utilization of the newly synthesized amine. Indeed, antiacetylcholine drugs reduce the α-methyltyrosine-induced rate of disappearance of brain dopamine (Andén & Bedard, 1971). Furthermore, large doses of atropine reduce the accumulation of striatal homovanillic acid in normal (Bartholini & Pletscher, 1971) or in neuroleptic-treated rats (Andén, 1972). The absence of detectable modifications of the dopamine synthesis or in concentrations of dopamine which could be expected as a feedback response to the modification of the amine utilization (Agid, Javoy & Glowinski, 1974; Javoy & others, 1974a) may be explained by the weakness of the atropine effect on dopamine neurons. Thus, the changes in dopamine metabolism induced by oxotremorine and atropine suggest that the functional activity of the dopamine nigrostriatal pathway is partly but weakly under the influence of cholinergic neurons.

The present data combined with results previously reported (Javoy & others, 1974a) add further support to the concept of a control of the dopamine nigrostriatal neurons by at least two cholinergic neuronal systems: one acting at the level of the substantia nigra the other at the level of the striatum. Indeed, intranigral application of the acetylcholine-like agonist carbachol or of atropine resulted respectively in a reduction and an activation of striatal dopamine neurons (Javoy & others, 1974a). According to electrophysiological studies, these effects are possibly mediated by cholinergic receptors localized on nigral inhibitory interneurons connected to the dopamine neurons (Aghajanian & Bunney, 1973). In contrast, atropine when directly infused in the striatum has been shown to inhibit dopamine synthesis (Javoy & Agid, unpublished observations). Thus, changes in striatal dopamine metabolism following the systemic administration of oxotremorine or atropine (present report) may correspond to the summation of opposite effects induced by the modification of cholinergic transmission occurring at the striatal and the nigral levels. At low systemic (clinical) doses of atropine the respective actions of the drug at the nigral and striatal levels could counteract each other and explain the failure to detect any modification of dopamine metabolism. The reduction of dopamine utilization induced by high doses of atropine

suggests that in these conditions the influence of the drug at the level of the striatum predominates.

Antiacetylcholine drugs are commonly used in the treatment of Parkinson's disease, a disorder known to be mainly related to a striatal dopamine deficiency (Bernheimer & Hornykiewicz, 1965). The clinical efficiency of atropine-like compounds is unexpected from biochemical results since these drugs, when administered at high doses have been shown to decrease dopamine turn-over. In fact, at the low doses used in patients, it is possible that antiacetylcholine drugs do not change striatal dopamine metabolism as a result of opposite actions on dopamine nigral cell bodies and striatal terminals. In Parkinsonism hyperactivity of striatal cholinergic neurons has been put forward as a consequence of impairment of dopaminergic transmission (Stadler, Lloyd, & others, 1973; Bartholini, Stadler & Lloyd, 1973; Agid, Guyenet, & others, 1975). Therefore it is likely that the therapeutic effects of antiacetylcholine compounds are mainly related to direct reduction of the increased activity of striatal cholinergic neurons.

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