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Effect of some antiparkinsonian drugs on catecholamine neurons

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The inhibitory effect of some antiparkinsonian drugs on the accumulation of catecholamines into rat dopamine and noradrenaline neurons was studied with isotope and histochemical fluorescence methods *in vivo* and *in vitro*. Benztropine was the most potent drug hitherto tested to inhibit accumulation of catecholamines into dopamine neurons and was effective down to a dose of 10 mg/kg. Also ethybenztropine, brompheniramine, diphenylpyraline, chlorpheniramine and methixene, in doses of 50 mg/kg, inhibited accumulation of catecholamines. Atropine, scopolamine, benzhexol, diphenhydramine and many other antiparkinsonian agents were ineffective.

The dopamine content in the neostriatum and the substantia nigra is reduced in Parkinson's disease due to degeneration of the nigro-neostriatal dopamine neuron system (see Gillingham & Donaldson, 1969). This leads to a decreased neostriatal dopamine neuro-transmission which probably results in an imbalance between α- and γ-motoneuron activity (Steg, 1969) and in the symptoms rigidity and akinesia found in Parkinsonism. It is conceivable that the decreased dopamine neurotransmission could partially be counteracted by inhibition of re-uptake of released dopamine into the dopamine nerve terminals. Therefore, in the present experiments some antiparkinsonian drugs of the anticholinergic and antihistamine type (see Yahr & Duvoisin, 1968) were tested for their ability to inhibit dopamine uptake in central dopamine nerve terminals *in vivo* and *in vitro* using isotope and histochemical fluorescence methods. The available evidence suggest that such a site of action may exist only for certain antiparkinsonian agents.

EXPERIMENTAL

Isotope methods

Sprague-Dawley rats, 180–200 g, were killed and neostriatal slices with a thickness of about 0·5 mm and a diameter of 3 mm were prepared. The slices were preincubated for 15 min in a Krebs–Ringer bicarbonate medium, pH 7·4, containing the drug to be tested. Tritiated dopamine (^{3}H-DA) was then added to the incubation medium (final concentration of ^{3}H-DA: 2×10^{-8}M; specific activity 8 Ci/mmol). The slices were incubated for another 30 min, and then rinsed in ^{3}H-DA-free buffer for 10 min, after which they were dissolved in Soluene and the radioactivity determined by liquid scintillation counting and the activity/slice used for calculations of uptake inhibition. This was possible because early experiments had shown that ^{3}H-uptake/slice gave reproducible results. Variation in thickness of the slice, which changes the weight, did not affect the uptake of tritiated noradrenaline (^{3}H-NA) proportionally. This is probably due to the poor penetration of the catecholamines into the slices (Hamberger, 1967). The effect of the same drugs on

the uptake of [^{3}H]noradrenaline (^{3}H-NA) (final concentration of ^{3}H-NA: 10^{-7}M; specific activity 10 Ci/mmol) in the isolated rat iris was determined analogously (Jonsson, Hamberger & others, 1969). In other experiments drugs were injected intraperitoneally in a dose of 50 mg/kg 1 h before the animals were killed. After the animals had been killed we measured the uptake of ^{3}H-DA or ^{3}H-NA in slices of neostriatum or in the isolated iris respectively as described above.

Fluorescence histochemistry

Studies were made on the dopamine nerve terminals in the median eminence which lie outside the blood brain barrier (see Fuxe, Hamberger & Malmfors,1967). The rats were pretreated with reserpine (5 mg/kg, i.p. 18–24 h before killing) to deplete the endogenous catecholamine stores and with the α-adrenergic receptor blocking agent, azapetine phosphate (20 mg/kg, i.p., 20 min before the amine injection; Ilidar, Roche) to protect the rat from the peripheral actions of α-methyl-noradrenaline (α-methyl-NA) (0·5 mg/kg) given intravenously 30 min before killing. 30 min before the α-methyl-NA injection, the drugs mentioned above were injected intraperitoneally in doses of 25–50 mg/kg. The effects of benztropine and ethybenztropine were also examined in doses of 5 and 10 mg/kg. At least 5 and at most 10 rats were used in each single experiment with the different compounds. The rats were killed by decapitation and the hypothalamus was taken for the histochemical fluorescence analysis of catecholamines (Falck, Hillarp & others, 1962; Corrodi & Jonsson, 1967).

Drugs. The following drugs were tested: (belladonna alkaloids) atropine sulphate, scopolamine hydrobromide; (synthetic tropane derivatives) benztropine mesylate (Cogentin, MSD), ethybenztropine (Ponalide, Sandoz); (piperidyl compounds) benzhexol hydrochloride (Pargitan, Kabi); procyclidine hydrochloride (Kemadrin, B.W. & Co.); (antihistamines) diphenhydramine hydrochloride (Desentol, LEO), orphenadrine hydrochloride (Disipal, Brocades), chlorpheniramine maleate (Allergisan, Pharmacia), brompheniramine maleate, diphenylpyraline hydrochloride (Histyn, Hässle), diethylaminoethylbenzhydryl ether hydrochloride (Rigidyl, Dumex); (phenothiazine derivatives) profenamine hydrochloride (Lysivane, Rhodia); (a derivative of thioxantene) methixene hydrochloride (Tremoquil, Tika); desipramine hydrochloride (Pertofran, Geigy). We are grateful to the drug companies for the supply of their respective compounds. The doses given refer to the form shown.

RESULTS

Isotope measurements

Benztropine was the most potent drug tested in inhibiting the uptake of ^{3}H-DA in neostriatal slices (Table 1). Several other drugs with anticholinergic or antihistamine properties were also able to inhibit ^{3}H-DA uptake. However, both atropine and scopolamine were without effect. Benztropine, brompheniramine and methixene inhibited the uptake of ^{3}H-NA in the isolated iris. The inhibitory effects of these drugs on the uptake of ^{3}H-NA by the iris are, however, far weaker than those of the tricyclic antidepressants such as desipramine (see Hamberger, 1967). This drug is more than a 1000 times more potent on noradrenaline nerve terminals than on dopamine nerve terminals.

Table 1. *Effect of certain antiparkinsonian drugs on the uptake of* ^{3}H-DA *and* ^{3}H-NA *in neostriatal slices and isolated irides respectively of untreated rats*

Drug*	Drug *in vitro*		Drug *in vivo*	
	Neostriatum	Iris	Neostriatum	Iris
Atropine	88† ± 6·9 (6)	130 ± 14 (4)	96 ± 2·2 (12)	82 ± 7·6 (8)
Scopolamine	93 ± 3·2 (6)	120 ± 13 (4)	76 ± 5·9 (6)	111 ± 5·9 (4)
Benztropine	33 ± 2·0 (12)	17 ± 0·6 (8)	64 ± 5·1 (11)	63 ± 2·6 (8)
Ethybenztropine ..	41 ±1·0 (6)	35 ± 2·1 (4)	83 ± 6·9 (6)	115 ± 2·9 (4)
Procyclidine	80 ± 2·0 (6)	104 ± 14·1 (4)	76 ± 4·9 (6)	78 ± 4·6 (4)
Diphenhydramine ..	87 ± 5·2 (5)	46 ± 6·3 (4)	92 ± 2·2 (6)	77 ± 6·0 (3)
Brompheniramine ..	55 ± 4·0 (6)	32 (2)	69 ± 4·8 (6)	43 ± 5·2 (3)
Diphenylpyraline ..	59 ± 1·1 (6)	32 (2)	78 ± 2·8 (12)	84 ± 8·5 (4)
Methixene	48 ± 5·6 (12)	34 ± 1·5 (8)	73 ± 4·4 (12)	58 ± 7·0 (8)
Cocaine	53 ± 1·4 (6)	17 ± 1·1 (4)		
Desipramine	86 ± 4·6 (6)	‡	85 ± 2·8 (9)	6·1 ± 1·1 (8)

* The drugs were either added *in vitro* in a concentration of 10^{-5}M or the rats were pretreated with the drug in a dose of 50 mg/kg i.p. 1 h except for ethybenztropine, where 25 mg/kg 1 h was given.

† The values are expressed as % of control radioactivity without drug and are the means ± s.e. ( ) Number of experiments. In each experimental run at least 6 neostriatal and 4 iris controls were included.

‡ The concentration of desipramine giving a 50% reduction in the noradrenaline uptake in the isolated rat iris is 6×10^{-9}M.

Fluorescence microscopy

Benztropine in doses of 25–50 mg/kg caused a strong blockade of the accumulation of α-methyl-NA in the dopamine nerve terminals of the median eminence (Table 2). A clear effect was observed in a dose of 10 but not of 5 mg/kg. Ethybenztropine reduced the amine accumulation to a moderate degree in a dose of 25 mg/kg. In doses of 50 mg/kg diphenylpyraline, brompheniramine, chlorpheniramine and methixene also had moderate blocking actions. Diphenylpyraline and brompheniramine were studied also at a dose of 25 mg/kg and blocking effects were still observed. The belladonna alkaloids, the piperidyl compounds, and profenamine were without effects. Furthermore, the other antihistamine compounds tested had no clear-cut blocking actions.

DISCUSSION

It is obvious from the present results that most of the antiparkinsonian agents used clinically today do not owe their therapeutic effects to blockade of dopamine uptake in the central dopamine neurons. Thus, atropine, scopolamine, benzhexol and procyclidine probably act by way of their anticholinergic activities which are clearly apparent with regard to behaviour in doses below those used in the present study (Carlton, 1961, 1963; Arnfred & Randrup, 1968; Scheel-Krüger, 1970). However, the diphenylester of tropanol, benztropine, was a potent blocker of dopamine uptake in doses of 25–50 mg/kg both in the nigro-neostriatal dopamine neurons and the tubero-infundibular dopamine neurons. This drug is known to be a potent synthetic anticholinergic agent. However, obviously, as shown in the present paper, at higher doses an additional site of action exists, i.e. blockade of dopamine uptake, which also may be of functional importance (Fuxe, Goldstein & Ljungdahl, 1970). Thus, it seems reasonable that in combination with dopa treatment in parkinsonian patients (Cotzias, van Woert & Schiffer, 1967; Cotzias, Papavasiliou

Table 2. *Effect of some antiparkinsonian agents on the accumulation of α-methyl-noradrenaline in dopamine nerve terminals of the rat median eminence*

Drug	mg/kg	Fluorescence intensity	Effect on accumulation of α-methyl-NA
No drug		1+ (4)—2+ (8)	
Atropine	50	1+ (1)—2+ (4)	
Atropine	25	2+ (3)	
Scopolamine	50	2+ (4)	
Benztropine	50	½+ (4)—1+ (1)	diminished
Benztropine	25	½+ (3)—1+ (1)	diminished
Benztropine	10	1+ (4)—2+ (1)	diminished
Benztropine	5	1+ (2)—2+ (5)	
Ethybenztropine	25	1+ (4)—2+ (2)	diminished
Benzhexol	50	2+ (4)	
Procyclidine	50	1+ (2)—2+ (4)	
Diethylaminbenzhydrylether ..	50	1+ (2)—2+ (6)	
Diphenhydramine	50	1+ (1)—2+ (4)	
Chlorpheniramine	50	1+ (4)	diminished
Chlorpheniramine	25	1+ (3)—2+ (3)	
Brompheniramine	50	1+ (4)	diminished
Brompheniramine	25	1+ (4)	diminished
Brompheniramine	10	1+ (1)—2+ (4)	
Diphenylpyraline	50	1+ (4)	diminished
Diphenylpyraline	25	1+ (4)—2+ (1)	diminished
Diphenylpyraline	10	2+ (3)	
Orphenadrine	50	1+ (3)—2+ (4)	
Profenamine	50	2+ (3)	
Methixine	50	1+ (4)—2+ (2)	diminished

All rats were treated with reserpine (5 mg/kg, i.p., 18–24 h), azapetine (20 mg/kg, i.p., 50 min) and α-methyl-NA (0·5 mg/kg, i.v., 30 min). The drugs to be tested were injected i.p. 30 min before the α-methyl-NA injection. A semiquantitative estimation of fluorescence intensity has been made: moderate = 2+; weak = 1+; very week = 1/2+. When the fluorescence intensity of an individual brain preparation could not be definitely determined as belonging to one intensity grade or the next, this was indicated by adding one half to the lower grade. The range in the experiments are given. Number of rats within parentheses.

& Gellene, 1969; Yahr, Duvoisin & others, 1969) this action could be of some therapeutic importance. In agreement with our results it has recently been found that benztropine blocks dopamine uptake in synaptosomes (Coyle & Snyder, 1969).

In view of the present results it may be that a compound with an antihistamine-like ring-structure in combination with a side-chain containing a ring nitrogen atom could be a potential blocker of the dopamine uptake mechanism. This could explain why diphenylpyraline was effective whereas many other antihistamine compounds studied (diphenhydramine, orphenadrine, etc.) were relatively ineffective since they all had dimethylamine alkyl side-chains. In agreement with this suggestion, methixene, which was effective in blocking the dopamine uptake, is a thioxanthene derivative having a piperidyl side-chain. One exception, however, is brom- and chlorpheniramine which had a clearcut blocking activity in the high dose studied. This is probably due to the halogen atom in the ring.

The above results suggest that antiparkinsonian drugs of the antihistamine type available at present either exert their therapeutic effects via their anticholinergic effects or via some other unknown action. The fact that Coyle & Snyder (1969) obtained relatively good blocking effects with diphenhydramine, orphenadrine and benzhexol *in vitro* on dopamine uptake in synaptosomes from the neostriatum may not be incompatible with the present results, since the synaptosomes may be more susceptible to the blocking action of drugs than when left undetached in the tissue.

As seen from the results benztropine is not a selective blocker of dopamine uptake, since there is about the same degree of inhibition of noradrenaline uptake in the rat iris as of DA uptake in the neostriatum. However, the difference towards desipramine should be pointed out. As seen methixene is similar to benztropine in this respect. It may be mentioned that Carlsson & Lindqvist (1969) have found that many drugs belonging to the antihistamine group are blockers of the amine membrane pump in the central noradrenaline and 5-hydroxytryptamine neurons.

In conclusion, the results obtained with benztropine, ethybenztropine, diphenylpyraline, brompheniramine, chlorpheniramine and methixene suggest that a new type of antiparkinsonian drug related to the antihistamine compounds may be developed with its main action being a blockade of the membrane pump in the central dopamine neurons.

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