J. Pharm. Pharmac., 1972, **24**, 875–879 *Received March* 17, 1972

Effects of amantadine on uptake and release of dopamine by a particulate fraction of rat basal ganglia

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Amantadine, *in vitro*, produced dose-dependent blockade of dopamine uptake into a synaptosome-rich particulate fraction of an homogenate of the basal ganglia of rats. A concentration of $3{\cdot}6 \times 10^{-6}$M was required to inhibit uptake by 50%. Diethazine was less potent in blocking dopamine uptake, the equiactive concentration being $8{\cdot}0 \times 10^{-6}$M. Amantadine and (+)-amphetamine in concentrations in excess of 10^{-5}M caused the release of small quantities of added dopamine from the particulate fraction.

The findings of Vernier, Harmon & others (1969) and of Heimans, Rand & Fennessy (1972) suggest that amantadine potentiates the peripheral pharmacological effects of dopamine by blocking uptake of amines in a variety of adrenergically innervated preparations. We have investigated the effect of amantadine on dopamine uptake and release by the particulate fraction of an homogenate obtained from basal ganglia, the presumed site of pathology in Parkinson's disease (Hornykiewicz, 1963).

METHODS

Preparation of homogenate from rat basal ganglia

The preparation was modified from that described by Horn, Coyle & Snyder (1971). Rats, 200–300 g, were stunned and decapitated and the basal ganglia, consisting of corpus striatum, caudate and putamen were rapidly dissected and removed to ice, according to Glowinski & Iverson (1966). The tissue was weighed (100–125 mg) and homogenized in 4 ml of 0·25M sucrose at 0° before centrifuging for 10 min at 100 *g* in an M.S.E. refrigerated centrifuge. The supernatant fluid was then carefully decanted from the pellet of tissue debris which was discarded. The resulting fluid containing particulate fraction was gently stirred to produce a homogeneous suspension.

Dopamine uptake by the synaptosome-containing homogenate

An aliquot of the homogenate (0·1 ml) was transferred to a 20 ml vial containing 3·9 ml of modified Krebs-Henseleit solution of the following composition: NaCl, 118 mM; KCl, 4·7 mM; $NaHCO_3$, 25 mM; $MgSO_4$, 0·45 mM; KH_2PO_4, 1·03 mM; $CaCl_2$, 1·25 mM; D-(+)-glucose, 11·1 mM; together with nialamide $1{\cdot}25 \times 10^{-5}$M and ascorbic acid, 0·2 mg/ml. Disodium EDTA, 0·1 mg/ml, and various concentrations of amantadine or diethazine were added to this solution. The solution was kept at 37° and continuously aerated with a stream of 5% carbon dioxide in oxygen. The preparation was incubated for 5 min, 5 to 40 μl of 30 mM dopamine (side-chain 1,2-^{3}H; specific activity 1·3 Ci/mmol) was added and incubation continued for 5 min.

The particulate material, containing synaptosomes, was then separated from the liquid by millipore filtration using a single filter of 0·22 μm pore size.

The filters were washed with 10 ml of 0·9% NaCl and the residue dissolved in 1 ml of acetone in a counting vial. One ml of the filtrate was also assayed to determine extra-particulate dopamine. Nine ml of scintillation fluid (Instagel, Packard) was added to each vial and any precipitated material was re-suspended by subjecting the contents to ultrasonic vibration. After this treatment, vials remained free of precipitate for the remainder of the experiment. Vials were assayed for tritium in a Packard Tricarb 3380 liquid scintillation counter and dopamine uptake in nmol/min per g equivalent of basal ganglia tissue was calculated. Corrections were made for non-specific binding of tritiated dopamine to the millipore filter by subtracting uptake values obtained by using a particle-free supernatant obtained by centrifuging samples of homogenate at 48 000 *g*.

Since uptake by the particulate fraction was linear during the incubation period, this value was equivalent to the initial velocity of uptake.

Release of dopamine from the particulate fraction

The homogenate was prepared as described and tritiated dopamine was incorporated into the particulate material. Two ml of the preparation was incubated at 37° for 20 min in 19 ml of the modified Krebs-Henseleit solution, the incubation solution being made 1 mM with respect to [^{3}H]dopamine. At the end of the incubation, the material was centrifuged at 9000 *g* to pack the particulate fraction; the supernatant solution was discarded. The pellet was washed twice in fresh modified Krebs-Henseleit solution, re-suspended and centrifuged at 9000 *g*. After the final centrifugation, the pellet was re-suspended in 10 ml of modified Krebs-Henseleit solution. A 0·4 ml aliquot of this suspension of particulate matter was preincubated for 5 min with 3·2 ml of modified Krebs-Henseleit solution before the addition of 0·2 ml of amantadine or amphetamine solution, sufficient to bring the drug concentration to 10^{-7} to 10^{-4}M in the final mixture, and incubation was continued for a further 15 min. Drug-free controls were run in parallel. At the end of the incubation period, the particulate material was separated from the solution containing released dopamine by millipore filtration as described above. Filters and filtrate were assayed for tritium as before and corrected for non-specific binding. Release was calculated as pmol of dopamine released per min per g equivalent of basal ganglia tissue.

Drugs

Drugs used were amantadine hydrochloride (Geigy, Australia), (+)-amphetamine sulphate, diethazine hydrochloride (May & Baker) and nialamide (Pfizer).

RESULTS

Effect of drugs on dopamine uptake

The K_m value for the uptake of dopamine by particulate fraction was 0·2 μM.

Both amantadine and diethazine diminished the uptake of dopamine by the synaptosome-containing particulate preparation (Table 1). When the results were displayed as a double reciprocal plot (Lineweaver & Burk, 1934), it was evident that both amantadine and diethazine produced non-competitive blockade of dopamine uptake (Fig. 1). The calculated K_i values (drug concentration causing 50% uptake

Table 1. *Inhibition of dopamine uptake by a particulate fraction of rat basal ganglia.*

Blocking drug	Dopamine uptake (nmol/min per g equivalent tissue)	% of control
—	0·132 ± 0·007	100 ± 5
Amantadine, 1 μM	0·112 ± 0·005	86·5 ± 4·5*
Amantadine, 5 μM	0·0498 ± 0·0015	35·3 ± 3·0†
Diethazine, 5 μM	0·0827 ± 0·0029	57·5 ± 3·5†

Each entry is the mean and standard error of eight separate determinations.
* Significantly different from control value $P < 0.05$ (*t*-test).
† Significantly different from control value $P < 0.01$ (*t*-test).

block) were 8·0 ± 1 × 10^{-6}M for diethazine and 3·8 ± 0·5 × 10^{-6}M for amantadine (means ± standard errors of eight experiments).

Effect of drugs on dopamine release

Both amantadine and (+)-amphetamine caused release of added dopamine from the particulate preparation (Fig. 2). The magnitude of release was very low, and required about ten times the concentration of amantadine that was effective in blocking dopamine uptake. (+)-Amphetamine was more potent than amantadine, in equivalent concentrations, in causing dopamine release.

DISCUSSION

Amantadine, in concentrations in excess of 10^{-6}M (0·15 μg/ml), produced detectable blockade of dopamine uptake by a particulate preparation of basal ganglia containing synaptosomes. This finding provides direct evidence for the suggestion made by Heimans & others (1972). The effective concentration is likely to be reached in the tissues of patients taking clinically effective doses of amantadine, assuming that

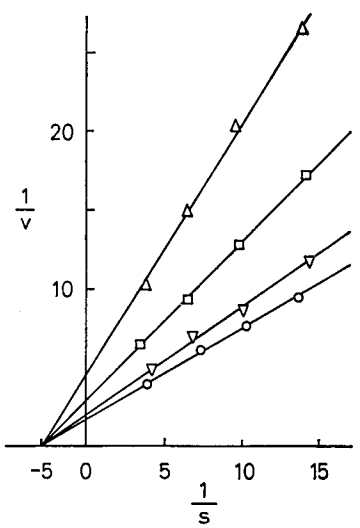

FIG. 1. Double reciprocal plot of dopamine uptake kinetics by particulate material from rat basal ganglia. ○ drug free controls; ▽, amantadine, 1 μM; △, amantadine, 5 μM; □, diethazine, 5 μM. The units of initial velocity (v) are nmol dopamine/min per g equivalent of tissue. Dopamine concentration (s) in μM.

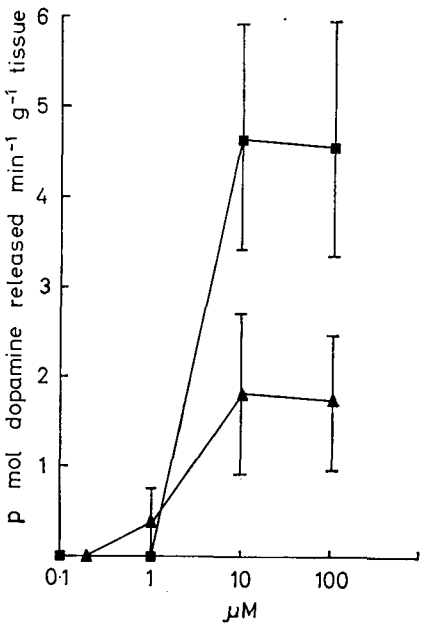

FIG. 2. Release of dopamine from a particulate fraction of basal ganglia. ■, (+)-amphetamine; ▲, amantadine. The vertical axis depicts dopamine release in pmol/min per g equivalent of tissue. The horizontal axis depicts drug concentration (μM).

the usual dose of 200 to 400 mg is distributed evenly in body water. It has been reported previously that amantadine inhibited dopamine uptake by rat brain preparations, but effective concentrations were in excess of 10^{-4}M. Fletcher & Redfern (1970) used whole brain homogenates which took up dopamine through a dialysis membrane. Baldessarini, Lipinski & Chace (1972) used basal ganglia homogenates and uptake of dopamine was determined after chilling the mixture to terminate uptake and depositing the particulate material by high speed centrifugation. It appears that the method of millipore filtration is more satisfactory for determining the inhibitory effect of amantadine on dopamine uptake.

Amantadine was approximately twice as effective as diethazine in blocking dopamine uptake, the K_i values being $3{\cdot}8 \times 10^{-6}$M and $8{\cdot}0 \times 10^{-6}$M respectively. The value for diethazine agrees closely with that obtained by Coyle & Snyder (1969).

The K_m value for uptake of dopamine by the particulate fraction, using millipore filtration was identical with that obtained with a similar preparation by Coyle & Snyder (1969) using ultracentrifugation to separate the particulate matter, which they showed to consist predominantly of synaptosomes.

It has been stated that amantadine causes release of dopamine (Offermeier, 1971), the evidence for this statement being that amantadine released noradrenaline from the mouse heart (Stromberg, Svensson & Waldeck, 1970) and that synthesis of dopamine in striatal slices of rats was enhanced by pretreatment with 40 mg/kg amantadine and there was enhanced accumulation of dopamine in extracellular spaces (Scatton, Cheramy & others, 1970). Furthermore, von Voigtlander & Moore (1971) reported an enhanced concentration of dopamine in ventricular perfusates after intraventricular administration of 100 μg/ml of amantadine. All these findings, however, are equally

explicable in terms of inhibition of dopamine uptake by amantadine, an effect which would lead to enhanced extracellular accumulation of spontaneously released dopamine.

Amantadine does appear to release dopamine from stores within particulate matter, presumably the synaptosomes, but this effect is small in magnitude and is evident only with concentrations higher than those likely to be attained in the tissues of patients taking clinically effective doses of the drug. Furthermore, the possibility that this effect is secondary to blockade of uptake cannot be ruled out.

In conclusion, we suggest that the beneficial effect of amantadine in the treatment of Parkinson's disease may be the result of accumulation of dopamine at synaptic clefts of dopaminergic neurons in the basal ganglia following blockade of re-uptake of dopamine into the presynaptic neurons by amantadine.

Acknowledgements

We are grateful to Geigy Australia Pty. Ltd. for providing the amantadine hydrochloride used in these investigations. The expenses of the research were defrayed from a grant by the National Health and Medical Research Council to M.J.R. R.L.H.H. was in receipt of an Australian Commonwealth Research Grant.

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