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EFFECTS OF AMANTADINE HYDROCHLORIDE ON CATECHOLAMINE METABOLISM IN THE BRAIN OF THE RAT*

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Abstract—We have examined the effects of the cyclic amine, amantadine, on the metabolism of catecholamines in the rat. Large doses of the drug had minimal effects on cardiac norepinephrine stores, but produced small decreases in concentrations of norepinephrine in the brain, while having less effect on dopamine and no effect on serotonin. The drug altered the metabolism of previously intrathecally administered [^{3}H]norepinephrine: there were small decreases of [^{3}H]norepinephrine, but clear decreases of [^{3}H]deaminated catechol metabolites and marked increases of [^{3}H]normetanephrine for 1–6 hr, at times paralleling decreases in endogenous norepinephrine levels. Later, smaller increases of [^{3}H]deaminated-*O*-methylated products appeared. These metabolic changes were not due to inhibition of monoamine oxidase by this drug, as amantadine had no effect on the deamination of [^{14}C]serotonin or [^{3}H]norepinephrine even at high concentrations *in vitro* or doses *in vivo*. Amantadine decreased the retention by isolated nerve endings *in vitro* of previously intrathecally injected [^{3}H]norepinephrine by a very small amount and competitively inhibited the initial uptake of [^{3}H]*d,l*-norepinephrine by nerve endings of whole brain, with a K_i of approximately $1{\cdot}5 \times 10^{-5}$ M. This inhibition of uptake was somewhat greater in the cerebral cortex than in the corpus striatum. There was much less inhibition of the uptake of [^{3}H]dopamine in both regions. Thus, part of the action of this drug in Parkinson's disease may be due to inhibition of uptake and retention of catecholamines at central nerve terminals. However, since the doses required to produce changes *in vivo* were much higher than those used clinically, these findings may reflect non-specific or toxic effects of the drug.

AMANTADINE hydrochloride (1-adamantanamine), the salt of a symmetrical 10-carbon polycyclic primary amine, has been used as an antiviral agent.[1] More recently, it has been found useful in the management of Parkinson's disease[2,3] and it may potentiate both clinical[2] and animal motor responses[4] to L-dopa. It was also noted that this amine lacks significant anticholinergic activity but it potentiated the pressor response to injected dopamine in the dog, leading to the suggestion that amantadine may act by interfering with catecholamine retention in peripheral and central nerve endings.[5] It has also been found that amantadine can interfere with the uptake of dopamine and norepinephrine by rat brain homogenates[6] and increase the turnover of dopamine in the corpus striatum of the rat brain *in vitro*.[7] We have reported recently that amantadine and a variety of aromatic or non-aromatic primary amines can interfere

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with the retention of [^{3}H]norepinephrine by homogenates of rat brain *in vitro*.[8] In order to investigate further, possible effects of this unusual compound on the metabolism of biogenic amines in the mammalian central nervous system, the following studies were undertaken.

MATERIALS AND METHODS

Animals. Young adult (190–210 g) male albino rats (Charles River Co., Wilmington, Mass.) were maintained *ad lib.* on Charles River Laboratory Chow and water.

Chemicals. The following radiochemicals and drugs were used: *d,l*-norepinephrine [7-^{3}H] (8–13 c/m-mole), serotonin [2-^{14}C] binoxalate (15 c/mole), dopamine [2-^{3}H] (8 c/m-mole) (New England Nuclear Corp., Boston, Mass.), *l*-norepinephrine [7-^{3}H] (2–3 c/m-mole, Amersham/Searle Corp., Arlington Heights, Ill.), amantadine-HCl (Symmetrel, donated by the DuPont Co., Wilmington, Del.), cocaine-HCl (Merck & Co., Rahway, N.J.), desmethylimipramine-HCl (DMI) (donated by Lakeside Labs., Milwaukee, Wis.), pargyline-HCl (donated by Abbott Labs., No. Chicago, Ill.), and tyramine-HCl (CalBiochem Co., Los Angeles, Cal.). Authentic standards of amines and their metabolites were obtained in the highest available purity from CalBiochem Co.

Tissue preparation. In studies of endogenous amine concentrations, rats were stunned and decapitated at selected times after the intraperitoneal injection of amantadine-HCl in 0·9% (w/v) saline, or saline alone. Brains and hearts were quickly removed, iced, weighed, homogenized in 5 vol. of ice-cold 0·4 N perchloric acid, and centrifuged at 17,000 *g* for 15 min. In studies of the metabolism of [^{3}H]norepinephrine (NE) in the brain, rats were given 12 μc (1·5 nmole) *d,l*-[^{3}H]NE in artificial CSF intracisternally[9] under light ether anaesthesia. After 5 or 10 min to permit uptake of [^{3}H]NE to occur, 100 mg/kg doses of amantadine-HCl (or an equal volume of saline) were injected intraperitoneally. Animals were sacrificed at specific times after intracisternal injection, up to 24 hr in three separate experiments, with overlapping time points. The brains (after removing the cerebellum) were extracted into perchloric acid as described above. A "zero-time" point was included in each experiment to monitor the amount of [^{3}H]NE and [^{3}H]metabolites present at the time of injection of amantadine.

Isolation and assay of amines and metabolites. Endogenous and tritiated catecholamines were isolated by alumina column chromatography,[10] and eluted with 0·2 N acetic acid (HAc). Fluorimetric methods were used to estimate endogenous NE[11] and dopamine[12] in the eluates. Portions of the perchloric acid tissue extracts were used for the estimation of endogenous serotonin.[13] The recovery of endogenous amines was estimated by the addition of authentic amines to tissue homogenates. Recoveries were approximately 80–85 per cent for all three endogenous amines and were used to correct the estimated tissue concentrations.

Tritiated metabolites of [^{3}H]NE were separated by alumina column chromatography to isolate catechols,[10] followed by Dowex-50 W × 8 (200–400 mesh) column chromatography to separate amines from deaminated products.[14] The radioactivity in samples of the various fractions was estimated by liquid scintillation spectrometry.[8] The radioactive compounds measured included [^{3}H]NE.[10] Furthermore, [^{3}H]normetanephrine (NMN), [^{3}H]deaminated catechols [3,4-dihydroxymandelate (DHMA) and 3,4-dihydroxyphenylglycol (DHPG)]; total [^{3}H]deaminated-*O*-methylated metabolites [3-methoxy-4-hydroxyphenylglycol (MHPG) and its sulfate conjugate and 3-methoxy-4-hydroxymandelate (VMA)] vs. free (ethyl acetate extractable at acid pH) [^{3}H]de-

aminated-*O*-methylated products were assayed by the methods of Kopin *et al.*[14] as modified by Schanberg *et al.*[15] The recovery of [^{3}H]NMN added to tissue extracts of normal or amantadine-treated rats was 80–85 per cent. The recovery of authentic NMN, VMA and DHMA added to perchloric acid preparations of tissues of normal and amantadine-treated rats, carried through the chromatographic separation procedures and estimated by native fluorescence, was greater than 90 per cent. The recovery of [^{3}H]NE radioactivity and of authentic un-labeled NE, measured by fluorescence, was also found to be over 90 per cent if the material removed from the alumina columns by 2 N HCl (less the DHMA and DHPG extracted by ethyl acetate) was added to that removed by elution with 0·2 N HAc,[16] and the recovery of NE was found not to be altered in amantadine-treated tissues. Although estimations of metabolites were not corrected for recoveries, the over-all accountability of the total tritium in brain homogenates as the sum of the various fractions of [^{3}H]NE and metabolites was about 85–90 per cent.

Assay of monoamine oxidase (MAO). Crude mitochondrial-synaptosomal ("P_2", or 17,000 *g* pellet) fractions of whole rat brain were prepared,[17] taken up in 1·15% (w/v) KCl, and incubated with [^{14}C]serotonin or [^{3}H]NE which were separated by Dowex-50 column chromatography from labeled deaminated metabolites, the radioactivity of which appeared in the effluent and was estimated by liquid scintillation spectrometry.[18] The reactions were linear with incubation time and amount of tissue.

Uptake and efflux of [^{3}H]amines. Either [^{3}H]NE or [^{3}H]dopamine (DA) was incubated at various concentrations, with or without amantadine-HCl added to a Krebs-bicarbonate buffered physiological medium maintained at pH 7·2. The methods of tissue preparation, incubation, extraction and counting have been described in more detail elsewhere.[8] Tissue (approximately 10 mg of protein, as determined by the method of Lowry *et al.*[19] in 10-ml incubation volume) was added as a homogenate of brain tissue separated from debris and nuclei (1000 *g* supernatant) after preparation with isotonic sucrose in a glass homogenizer with a Teflon pestle modified to provide 0·25 mm clearance to preserve synaptosomal integrity which was checked by electron microscopy. Whole brain or separate preparations of cerebral cortex and the corpus striatum, dissected as previously described,[20] were used. Incubation was done at 37° under air in a Dubnoff-type shaker for 5 min, during a period of initial uptake found to be linear with time. The tubes were rapidly chilled and pellets were preparad by centrifugation at 48,000 *g*, and extracted in 0·4 N perchloric acid. Total ^{3}H radioactivity was estimated by liquid scintillation spectrometry. Estimates of tissue-bound radioactivity after incubation at 4° were used as "blanks". When kinetic data were plotted, lines of "best fit" were determined by the method of least squares analysis.

Findings concerning the uptake of amines by homogenates and their pellets were compared with experiments in which partially isolated nerve endings were used. Thus, a crude synaptosomal (and mitochondrial) fraction[17] was obtained by centrifuging the 1000 *g* supernatant material at 17,000 *g*, and was taken up in the incubation medium, and otherwise treated as described above. Alternatively, synaptosomes were separated from suspensions of crude homogenates by a rapid ultrafiltration technique described elsewhere,[18] and the ^{3}H in tissue entrapped by 0·8 μ pore Millipore filters was counted.

In order to evaluate the effects of drugs on the retention of catecholamines by brain tissues, [^{3}H]NE (10 μc) was given to normal rats by intracisternal injection. In 30

min the brains were removed, homogenized in sucrose, and the 1000 *g* supernatant material was incubated for another 30 min as described for the uptake experiments, with or without drugs added to the medium. Again, a 48,000 *g* pellet was prepared, extracted and counted.

RESULTS

Concentrations of endogenous amines. When rats were given doses of amantadine-HCl of 25 mg/kg, i.p., at 5·5 and 0·5 hr before sacrifice, there was no change in cardiac NE concentration and only a small decrease in brain NE (10%), which was not statistically significant. When a single dose of 100 mg/kg, i.p., was given, there was an insignificant decrease (10%) of cardiac NE levels 0·5 and 3 hr later (Table 1). The same dose of amantadine-HCl decreased whole brain NE concentrations by about 20 per cent for 1–3 hr (Table 2). Similarly, brain dopamine concentration underwent a significant but small decrease of 14 per cent, 3 hr after a 100 mg/kg, i.p., dose, while brain serotonin concentration remained unchanged (Table 3).

TABLE 1. NOREPINEPHRINE CONCENTRATION IN HEARTS OF RATS AFTER AMANTADINE-HCl OR SALINE INJECTION*

Time after injection (hr)	Control	Amantadine
0·5	759·0 ± 61·2 (12) (100·0%)	681·1 ± 41·1 (12) (89·5%)
3·0	709·2 ± 38·9 (5) (100·0%)	646·1 ± 24·6 (5) (91·0%)

* Rats were given a 100 mg/kg, i.p., dose of amantadine-HCl in saline, or saline alone and sacrificed at 30 min or 3 hr. Data are given as ng NE/g wet tissue ± S.E. and as per cent of control. (N) = number of animals per group. The changes are not significant statistically.

Metabolism of intracisternally injected [^{3}H]NE. Following time to permit the uptake of intracisternally injected [^{3}H]NE, amantadine-HCl was given intraperitoneally. While total radioactivity in brain was not altered throughout the 24 hr after drug injection, changes in the radioactive metabolite concentrations were noted (Fig. 1). There was an insignificant (7 per cent) decrease in [^{3}H]NE concentration at 1–3 hr after intracisternal injection, while deaminated catechol metabolites (mainly [^{3}H]DHPG and [^{3}H]DHMA) decreased by as much as 30–35 per cent at the same times. In contrast, methylated metabolites increased markedly, particularly [^{3}H]NMN which was increased from 0·5 to 12 hr, to as much as 238 per cent of control at 3 hr. The free (mostly [^{3}H]VMA) and conjugated (mostly [^{3}H]MHPG sulfate) *O*-methylated-deaminated products increased to a much lesser extent and more slowly than [^{3}H] NMN.

Effect of amantadine-HCl on MAO activity in the brain. In view of the marked increase in *O*-methylated products and decrease of deaminated catechols in the brain, MAO activity in a crude mitochondrial-synaptosomal (P_2) fraction of the brain was

TABLE 2. NOREPINEPHRINE CONCENTRATION IN WHOLE BRAIN OF RATS AFTER AMANTADINE-HCl OR SALINE INJECTION*

Time after injection (hr)	% of Control ± S.E. (N)
0·5	90·2 ± 3·4 (14)†
1·0	79·6 ± 10·2 (8)†
1·5	80·4 ± 4·1 (8)†
3	74·9 ± 10·0 (8)†
6	98·6 ± 1·7 (8)
12	104·4 ± 7·4 (9)
24	95·3 ± 4·1 (8)

* Rats were given a dose of 100 mg/kg, i.p., of the drug in saline or saline alone, and sacrificed at specified times. Data were calculated as ng NE/g wet brain tissue and are presented as per cent of control concentrations ± S.E. (N).
† P at least < 0·05 for significance of difference from control by Student's *t*-test. The mean control NE (± S.E.) = 441·5 ± 6·2 ng/g.

TABLE 3. AMINE CONCENTRATIONS IN BRAINS OF RATS AFTER AMANTADINE-HCl OR SALINE INJECTION*

Time after injection (hr)	Dopamine		Serotonin	
	Control	Amantadine	Control	Amantadine
0·5	599·8 ± 56·5 (6) (100·0%)	606·8 ± 35·9 (6) (101·2%)	612·1 ± 31·4 (6) (100·0 %)	581·2 ± 34·5 (6) (95·0%)
3·0	602·6 ± 29·7 (19) (100·0%)	518·4 ± 24·2 (18) (86·0%)†	402·9 ± 14·4 (10) (100·0%)	403·8 ± 20·2 (8) (100·4%)

* Rats were given a 100 mg/kg, i.p., dose of amantadine-HCl in saline, or saline alone and sacrificed at 30 min or 3 hr. Data are given as ng/g wet whole brain ± S.E. (N) and as % of control.
† P value from Student's *t*-test < 0·05.

estimated. There were no inhibitory effects of amantadine-HCl upon the deamination of [^{14}C]serotonin *in vitro* over a wide range of drug concentrations up to 10^{-3} M, although pargyline-HCl produced almost complete inhibition of MAO activity at 10^{-5} M in the same experiment. Similarly when amantadine-HCl (100 mg/kg, i.p.) or saline alone was given *in vivo* 3 hr before rats were killed, there were no differences in the ability of P_2 preparations of brain to deaminate [^{3}H]NE.

Effect of amantadine-HCl on the efflux of [^{3}H]*NE.* The ability of homogenates of the brain to retain previously intracisternally administered l-[^{3}H]NE in the presence of amantadine *in vitro* was examined. Both total ^{3}H radioactivity and [^{3}H]NE isolated by alumina column chromatography retained by tissue were measured. Amantadine led to small decreases in [^{3}H]NE retention (less than 10 per cent) at lower concentrations

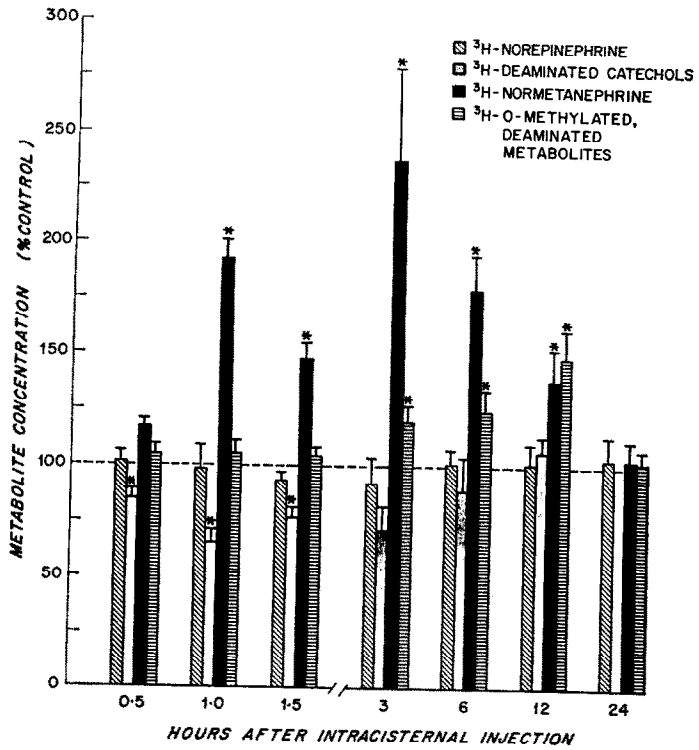

FIG. 1. Radioactive metabolites of *d,l*-[^{3}H]NE-injected intracisternally. Rats were given 100 mg/kg, i.p., of amantadine-HCl in saline or saline alone after 5 or 10 min to permit ^{3}H-NE uptake. Brains (less cerebellum) were extracted and metabolites were separated as described in Materials and Methods. Data were calculated as concentrations (ng ^{3}H/g wet brain) and presented as per cent of control ± S.E. (vertical lines) for: [^{3}H]NE, [^{3}H]deaminated catechols, [^{3}H]NMN, and total [^{3}H]deaminated-*O*-methylated products (VMA and MHPG). Asterisk indicates P at least < 0·05 by Student's *t*-test.

(10^{-6} M or less) and somewhat greater decreases as drug concentration was increased, to a maximum of 45 per cent decrease in retention at 10^{-3} M. The effects of the drug on tissue retention of [^{3}H]NE radioactivity were about 10 per cent greater than the effects on total ^{3}H. The effects of amantadine on the retention of previously bound *d,l*-^{3}H-NE were compared with those of other drugs known to be potent inhibitors of catecholamine uptake (DMI and cocaine) or capable of displacing catecholamines from intraneuronal storage sites (tyramine).[21] Amantadine, tyramine and cocaine produced similar effects and DMI produced a much greater decrease in retention of labeled material (Table 4).

Effect of amantadine-HCl on [^{3}H]catecholamine uptake. When the initial (5 min) uptake of [^{3}H]NE by homogenates of brains of normal rats was studied *in vitro*, it was found that the drug had an inhibitory effect. When the data were analyzed by the double reciprocal plot technique of Lineweaver and Burk[22] (Fig. 2), by analogy to the analysis of the kinetics of enzymatic reactions, the normal affinity constant (K_m) of *d,l*-[^{3}H]NE was approximately $4{\cdot}5 \times 10^{-7}$ M, a value similar to those previously reported.[23] In the presence of amantadine-HCl, the line describing [^{3}H]NE uptake

TABLE 4. EFFECT OF DRUGS ON TISSUE RETENTION OF [^{3}H]NE*

Drugs (5×10^{-4} M)	% Control [^{3}H]retention ± S.E.
Control	100·0 ± 1·4
Amantadine	70·0 ± 1·6†
Tyramine	61·9 ± 1·3†
Cocaine	76·1 ± 2·0†
DMI	28·2 ± 0·4†

* Rats were given 10 μc of *d,l*-[^{3}H]NE intracisternally 30 min before sacrifice. Sucrose homogenates of the brains were incubated at 37° for 30 min in Krebs-bicarbonate medium at pH 7·2, containing the drug. Total residual ^{3}H in 48,000 *g* pellets was measured. Data are nc ^{3}H per mg of tissue protein incubated, expressed as mean per cent of control ± S.E. for five determinations.
† $P < 0{\cdot}001$, calculated by Student's *t*-test.

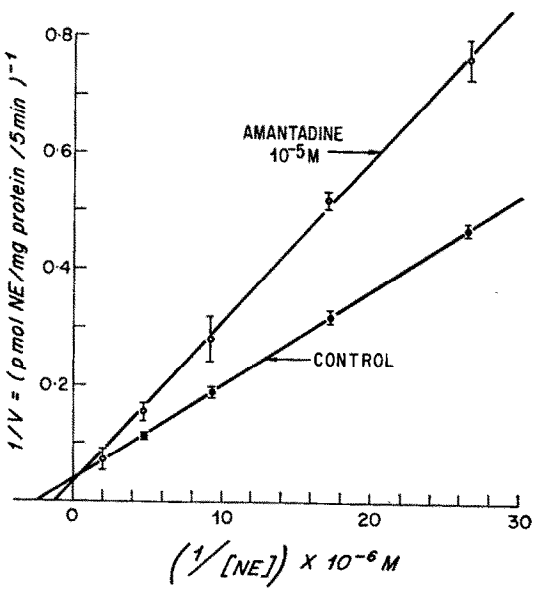

FIG. 2. Graphic analysis of the inhibition of *d,l*-[^{3}H]NE initial uptake into homogenates of brain by amantadine-HCl. The points are means of four determinations ± S.E. (vertical lines), with initial uptake velocity (V) expressed as pico moles of NE accumulated per milligram of tissue protein, recovered as pellets prepared at 48,000 *g* after 5 min of incubation at 37°. Data are analyzed by the method of Lineweaver and Burk,[22] with amantadine-HCl present at 0 and 1×10^{-5} M, and [^{3}H]NE, present as labeled and un-labeled *d,l*-NE at concentrations from 4×10^{-8} to 5×10^{-7} M. Points were fit to a "best line" by the method of least squares analysis.

appeared to rotate about a constant Y-intercept (V_{max}), suggesting competitive inhibition of uptake (Fig. 2). Analysis of the change of slope[22] provided an estimate of the inhibitor affinity constant (K_i) for amantadine of $1{\cdot}3 \times 10^{-5}$ M. When the data were analyzed by the method of Dixon,[24] a K_i of approximately 2×10^{-5} M for amantadine-HCl was found (Fig. 3). This apparently competitive inhibitory action of the drug upon the uptake of [^{3}H]NE by relatively crude homogenates of whole brain was confirmed by repeating the experiments several times with partially purified synaptosomes, using P_2 (mitochondrial-synaptosomal) pellets, or the selective removal of nerve endings from suspensions of crude homogenates by ultrafiltration.[18] With the latter method, K_i values of $1–2 \times 10^{-5}$ M for amantadine-HCl were found.

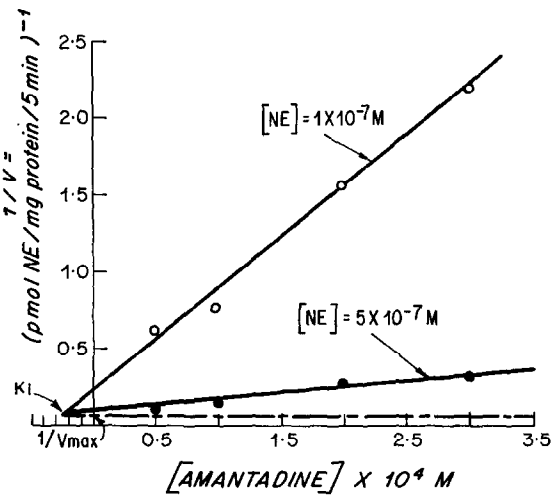

FIG. 3. Graphic analysis of the inhibition of *d,l*-[^{3}H]NE uptake into homogenates of brain by amantadine-HCl. The points are means of four determinations of initial uptake velocity (V) obtained by methods described for Fig. 2. Data were analyzed by the method of Dixon[24] to estimate K_i. ($2{\cdot}0 \times 10^{-5}$ M) of amantadine. The drug was present at concentrations from 5×10^{-5} to $2{\cdot}5 \times 10^{-4}$ M and NE was present at 1 and 5×10^{-7} M. V_{max} was determined separately as in Fig. 2.

When the effects of this drug on the uptake of [^{3}H]NE and [^{3}H]DA in specific regions of the brain were compared, it was found that amantadine-HCl had its greatest effects against the uptake of [^{3}H]NE by homogenates of cerebral cortex (Table 5). The inhibitory effects against the uptake of either of the catecholamines were consistently about 10–20 per cent smaller with striatal tissues than with cortical tissues. The inhibition by amantadine-HCl of [^{3}H]DA uptake was much less than for [^{3}H]NE. As previously observed,[23] the uptake of both catecholamines was much greater in untreated striatal tissues than in cortical preparations, and the uptake of [^{3}H]DA was much greater than that of [^{3}H]NE in both tissues (Table 5).

DISCUSSION

The present findings suggest that the non-aromatic cyclic amine, amantadine, may have effects upon the uptake, retention and storage of catecholamines in the central

TABLE 5. EFFECT OF AMANTADINE-HCl ON THE UPTAKE OF [^{3}H]CATECHOLAMINES BY BRAIN TISSUES *in vitro**

Amantadine M	[^{3}H]norepinephrine		[^{3}H]dopamine	
	Cortex	Striatum	Cortex	Striatum
0	341·2 ± 25·2 (100·0%)	2080·6 ± 95·4 (100·0%)	549·4 ± 26·4 (100·0%)	4119·4 ± 56·4 (100·0%)
10^{-5}	202·8 ± 22·2 (59·4%)†	1849·0 ± 45·8 (74·5%)†	436·6 ± 50·1 (79·5%)	4135·4 ± 20·8 (100·4%)
10^{-4}	63·7 ± 14·8 (18·7%)†	689·7 ± 20·8 (28·2%)†	280·6 ± 26·0 (51·1%)†	2842·6 ± 17·2 (69·0%)†

* Homogenates of regions of brain were exposed to [^{3}H]NE or [^{3}H]DA, and total ^{3}H accumulation in 48,000 *g* pellets after 5 min incubation was determined. Data are nc ^{3}H/g wet tissue/5 min for three determinations ± S.E., and as per cent of control.
† P at least < 0·01 by Student's *t*-test.

nervous system. The drug appears to produce only very small decreases of cardiac norepinephrine in the rat (Table 1) and mouse,[25] but decreased CNS norepinephrine stores by 20–25 per cent for at least 3 hr (Table 2), while it had less effect on dopamine stores and no effect on serotonin levels in the brain, following a single large intraperitoneal injection (Table 3). Clear effects of this drug on the central metabolism of intrathecally administered [^{3}H]NE were observed. There were no changes in total radioactivity and there were only small decreases in [^{3}H]NE concentrations after a large dose of amantadine. Large increases in [^{3}H]NMN were found (more than double the control values at 3 hr) (Fig. 1), and they appeared to correspond in time to the relatively small decreases of endogenous catecholamines (Tables 2 and 3), and of ^{3}H-deaminated catechol metabolites (Fig. 1) observed. The ^{3}H-deaminated-*O*-methylated metabolites underwent a later increase (maximal at 12 hr) (Fig. 1), and may represent the later deamination of the more rapidly formed [^{3}H]NMN. It has been suggested that extra-neuronal *O*-methylation, which converts NE to NMN, is a relatively sensitive measure of release or displacement of NE to an extraneuronal (including post-synaptic) location.[26–28] Thus, the present findings of altered [^{3}H]NE metabolism are consistent with an initial decrease of storage or of re-uptake of bound [^{3}H]NE, leading to release and extraneuronal *O*-methylation which persists for at least 3 hr and perhaps as long as 6–12 hr, followed by secondary deamination. This time course of action is consistent with the reported half-life of amantadine of approximately 10–12 hr, with removal mainly by urinary excretion of the unaltered compound.[29] The attractive alternative possibility, that [^{3}H]NE metabolism is shifted away from deamination and toward *O*-methylation by the inhibition of MAO by amantadine, does not appear to be tenable since the drug did not decrease the deamination of [^{14}C]serotonin or of [^{3}H]NE by nerve endings and mitochondria, even at high doses *in vivo* or high concentrations *in vitro* of the drug, nor did it inhibit the deamination of tyramine by rat liver homogenates even at 6×10^{-3} M.[25]

A partial explanation of these metabolic actions of amantadine may be reflected in its apparent ability to block competitively the initial uptake (presumably at the neuronal cell membrane) of [^{3}H]NE by central nerve endings (Figs. 2 and 3, and cf.

Fletcher *et al.*[6]). Other drugs, such as the tricyclic antidepressants, including DMI, are known to have similar effects on the increased production of methylated metabolites of [^{3}H]NE in the brain, and they are potent inhibitors of catecholamine uptake.[26,27] However, the inhibitors of uptake usually do not decrease endogenous central stores of catecholamines.[26,27] In this action of amantadine, it appears to resemble amphetamine[26] or the indirectly-acting sympathomimetic aromatic amines, including tyramine (Table 4), which appear to interfere with catecholamine retention in peripheral[21] and central[8] nerve-ending storage sites. Evidence that amantadine may interfere with the retention of bound [^{3}H]NE *in vitro* was presented (Table 4). Thus, effects at intraneuronal storage sites and effects on the reuptake of catecholamines may occur with amantadine, although inhibition of reuptake, itself, could contribute to decreased retention of catecholamines, since other inhibitors of uptake, DMI and cocaine, also hastened the spontaneous efflux of bound [^{3}H]NE *in vitro* (Table 4).

Other evidence that the uptake of catecholamines may be decreased has been presented in a study *in vivo* of uptake and retention of intravenously administered [^{3}H]NE by the mouse heart.[25] Furthermore, amantadine enhanced the blood pressure elevation response to intravenously administered NE in the dog, but decreased the pressor response to an indirectly acting amine, phenethylamine.[25] These findings are consistent with an inhibition of the uptake of NE and other aromatic amines at sympathetic nerve endings. Amantadine itself is reported to have little effect on blood pressure in low doses, and can even decrease it at high doses,[25] and thus does not appear to be an effective directly or indirectly acting amine in the periphery. It has also been noted that the ED_{50} for amantadine in producing signs of central excitation, such as spontaneous motor activity or reversal of tetrabenazine-induced sedation, is 10–40 times that of *d*-amphetamine and 6–10 times that of imipramine.[4,25] It should also be noted that amantadine can enhance the central excitatory effects of L-dopa in mice.[4] It has been suggested[2] that amantadine may have additive therapeutic effects for Parkinsonian patients when given with L-dopa, although this point is not clear.[3] The latter effects with L-dopa might be produced by the interference by amantadine of catecholamine reuptake at central catecholamine nerve endings, thus potentiating the postsynaptic effects of new catecholamine molecules synthesized directly from their precursor. It is also of interest that pre-treatment with amantadine has been shown to increase the rate of formation of dopamine from labeled tyrosine, and the rate of exit of newly synthesized dopamine in striatal tissue of the rat *in vitro*,[7] possibly by decreasing the reuptake and retention of the catecholamine.

In comparison with the amphetamines and the tricyclic antidepressants,[30,31] and in view of the rather limited efficacy of amantadine in the treatment of Parksonism, the drug appears to be relatively weakly active at central catecholamine-containing neurons, particularly at striatal nerve terminals presumed to be dopaminergic. Our present findings of a relatively high K_i for the inhibition of uptake of [^{3}H]NE by amantadine (probably not less than 10^{-5} M), even weaker antagonism of [^{3}H]DA uptake, and a limited effectiveness in enhancing the spontaneous efflux of bound [^{3}H]NE are all consistent with that view. Other drugs, ordinarily considered to be anticholinergic or antihistaminic, which are used in treating Parkinson's disease, are also known to be active in inhibiting catecholamine uptake.[30,31] These compounds include benztropine, trihexyphenidyl and diphenhydramine. Their potencies in inhibiting the uptake of [^{3}H]NE by hypothalamic homogenates are similar to that

of amantadine (K_i of 0·4–4·7 $\times$ 10^{-5} M).[30] However, in contrast to amantadine, they are quite active inhibitors of uptake of [^{3}H]DA by striatal homogenates (K_i of 0·2–7·0 $\times$ 10^{-6} M).

In conclusion, it would appear from the present and other available findings that the cyclic amine, amantadine, while of limited potency, may act on catecholamine containing nerve endings by interfering with neuronal uptake in the CNS and in the peripheral sympathetic nervous system. The apparent interference by amantadine with intraneuronal retention of these putative neurotransmitters in the brain may also be mediated by the inhibition of reuptake. The drug does not appear to inhibit or to be a substrate for monoamine oxidase. Since the effects observed *in vitro* and *in vivo* required large doses of the drug, it is possible that these effects may not occur in patients given therapeutic doses of amantadine-HCl of the order of 5 mg/kg a day orally, or less.

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REFERENCES

1. W. Davies, R. Grunert, R. Haff, J. McGahen, E. Neumayer, M. Paulshock, J. Watts, T. Wood, E. Hermann and C. Hoffmann, *Science, N.Y.* **144,** 862 (1964).
2. R. Schwab, A. England, D. Poskanzer and R. Young, *J. Am. med. Ass.* **208,** 1168 (1969).
3. J. Parkes, K. Zilkha, D. Calver and R. Knill-Jones, *Lancet* **I,** 259 (1970).
4. T. H. Svensson and U. Strömberg, *J. Pharm. Pharmac.* **22,** 639 (1970).
5. R. P. Grelak, R. Clark, J. Stump and V. Vernier, *Science, N.Y.* **169,** 203 (1970).
6. E. A. Fletcher and P. H. Redfern, *J. Pharm. Pharmac.* **22,** 957 (1970).
7. B. Scatton, A. Chermany, M. Besson and J. Glowinski, *Eur. J. Pharmac.* **13,** 131 (1970).
8. R. J. Baldessarini, *Biochem. Pharmac.* **20,** 1769 (1971).
9. S. Schanberg, J. Schildkraut and I. Kopin, *J. Pharmac. exp. Ther.* **157,** 311 (1967).
10. L. Whitby, J. Axelrod and H. Weil-Malherbe, *J. Pharmac. exp. Ther.* **132,** 193 (1961).
11. A. Anton and D. Sayre, *J. Pharmac. exp. Ther.* **138,** 360 (1962).
12. A. Carlsson and B. Waldeck, *Acta physiol. scand.* **44,** 293 (1958).
13. S. Snyder, J. Axelrod and M. Zweig, *Biochem. Pharmac.* **14,** 831 (1965).
14. I. J. Kopin, J. Axelrod and E. Gordon, *J. biol. Chem.* **193,** 265 (1961).
15. S. Schanberg, J. Schildkraut, G. Breese and I. Kopin, *Biochem. Pharmac.* **17,** 247 (1968).
16. J. J. Schildkraut, *Am. J. Psychiat.* **126,** 925 (1970).
17. V. P. Whittaker, *Prog. Biophys. molec. Biol.* **15,** 39 (1965).
18. R. J. Baldessarini and M. Vogt, *J. Neurochem.* **18,** 951 (1971).
19. O. Lowry, N. Rosebrough, A. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
20. J. Glowinski and L. Iversen, *J. Neurochem.* **13,** 655 (1966).
21. L. Iversen, in *The Uptake and Storage of Noradrenaline in Sympathetic Nerves*, p. 151. The University Press, Cambridge (1967).
22. H. H. Lineweaver and D. Burk, *J. Am. chem. Soc.* **56,** 658 (1934).
23. J. T. Coyle and S. S. Snyder, *J. Pharmac. exp. Ther.* **170,** 221 (1969).
24. M. Dixon, *Biochem. J.* **55,** 170 (1953).
25. V. Vernier, J. Harmon, J. Stump, T. Lynes, J. Marvel and D. Smith, *Toxic. appl. Pharmac.* **15,** 642 (1969).
26. J. Glowinski, J. Axelrod and L. Iversen, *J. Pharmac. exp. Ther.* **153,** 30 (1966).
27. J. Schildkraut, S. Schanberg, G. Breese and I. Kopin, *Am. J. Psychiat.* **124,** 660 (1967).
28. N. Weiner, *A. Rev. Pharmac.* **10,** 273 (1970).
29. W. Bleidner, J. Harmon, W. Hewes, T. Lynes and E. Hermann, *J. Pharmac. exp. Ther.* **150,** 484 (1965).
30. J. T. Coyle and S. H. Snyder, *Science, N.Y.* **166,** 899 (1969).
31. S. H. Snyder, *Biol. Psychiat.* **2,** 367 (1970).