The enzyme activity decreased in the mixture of enzyme and benzydamine, but it recovered by gel filtration. This shows that the benzydamine was removed and that the inhibition by benzydamine was reversible.

The kinetics of the inhibition by benzydamine were of non-competitive type with respect to fructose 6-phosphate and glutamine, respectively (Figs. 2 and 3). K_m values were 1·2 mM for fructose 6-phosphate and 1·0 mM for glutamine. Kornfeld[11] reported that K_m value for fructose 6-phosphate was 0·22 mM, and the higher value obtained in this study may be due to the amount of phosphoglucose isomerase (D-glucose 6-phosphate ketol-isomerase EC 5.3.1.9) in the crude enzyme as reported by Kornfeld *et al.*[12]

Although there are many testing methods of nonsteroid anti-inflammatory agents,[1,13] the present method can be used as one of the screenings of the agents for chronic inflammation such as rheumatic arthritis. This method is suitable for routine test because the enzyme of $(NH_4)_2SO_4$ fraction can be stored at − 20° at least 4 weeks without loss of activity, and even after 3 months it still retains 80 per cent of original activity.

Acknowledgement—We are indebted to Angelini Francesco, Boots Pure Drug, Takeda and Bristol for generous gifts of the samples used in this study.

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Biochemical Pharmacology, Vol. 21, pp. 1993–1995. Pergamon Press, 1972. Printed in Great Britain.

Amantadine and catecholamine uptake

(*Received* 7 *November* 1971; *accepted* 7 *February* 1972)

SEVERAL recent developments have created interest in the interaction of amantadine with biogenic amines, dopamine in particular. Amantadine has been shown to be effective in relieving the symptoms of Parkinsonism,[1,2] as has L-dopa,[3,4] the precursor of dopamine. Anti-Parkinson activity has been shown to be correlated for some drugs with the blockade of dopamine uptake in the striatum.[5] These findings have led several investigators to suggest that amantadine may act by blocking the uptake of dopamine.[6]

Some workers have examined the inhibition of amine uptake by amantadine with weak[7] or negative results,[8,9] but others have suggested that their results could be explained in this manner.[10,11] Numerous variables such as amine, brain region, preparation, etc. have not been adequately studied. We have examined the effects of amantadine on the uptake of norepinephrine and dopamine by synaptosomes from the striatum and the hypothalamus of normal and reserpinized rats. In this way, we hoped to obtain definitive information on the potential involvement of uptake inhibition in the mechanism of action of amantadine in Parkinsonism. The effects of amantadine on serotonin uptake were also determined.

DL-noradrenaline-7-^{14}C acetate, dopamine-1-^{14}C-HBr, and 5-hydroxytryptamine-2-^{14}C binoxalate were obtained from New England Nuclear Corp. and our methods were similar to those of Coyle and Snyder.[5] Female Holtzman rats were decapitated, and the brain was dissected according to Glowinski and Iversen.[12] The tissues were homogenized in 20 vol. of ice-cold 0·25 M sucrose and centrifuged at 1000 *g* for 10 min. Aliquots of this supernatant (0·2 ml) were combined with 3·6 ml of modified Krebs–Henseleit buffer at pH 7·0 which contained 1×10^{-7} M dopamine, norepinephrine or serotonin. When the uptake of norepinephrine by hypothalamic synaptosomes was examined, the concentration of the amine (racemic mixture) was raised to 2×10^{-7} M to offset the stereospecific nature of this system.[13] Finally amantadine was added (0·2 ml), and the mixture incubated at 37° under 95% O_2–5% CO_2 for 10 min, cooled in an ice-bath, and centrifuged at 0–4° (44,000 *g* for 30 min). Aliquots of the supernatant and an ethanol extract of the pellet were assayed for ^{14}C and the uptake determined by calculating a ratio (R) of counts released from the pellet (counts per minute per gram) to counts present in the supernatant (counts per minute per milliliter). Reserpinized rats were prepared by injecting reserpine (5 mg/kg, i.p.) 16–18 hr before sacrifice.

For striatal or forebrain synaptosomes, inhibition was calculated directly from the R values. For hypothalamic synaptosomes, desipramine at 1×10^{-4} M was used as a positive control and defined as 100 per cent inhibition.[14]

Amantadine inhibits the uptake of norepinephrine by both striatal and hypothalamic synaptosomes (Table 1), but is about twice as potent against the hypothalamic or "natural" system. Reserpinization has little effect on either the absolute amount of uptake observed or the extent of inhibition by amantadine. The only inhibition of dopamine uptake seen was with hypothalamic synaptosomes from non-reserpinized rats, which amounted to about 20 per cent. Reserpinization did not affect uptake or inhibition of the striatal system but seemed to reduce both uptake and inhibition of the hypothalamic system. Serotonin is taken up by forebrain synaptosomes in a time-dependent manner. R values were

TABLE 1. INHIBITION OF CATECHOLAMINE UPTAKE BY AMANTADINE*

		Amantadine							
		10^{-4} M		10^{-5} M		10^{-6} M		10^{-7} M	
	Control R	R	%I	R	%I	R	%I	R	%I
Norepinephrine									
Striatum									
Normal	46·9	32·7	30	47·6	0	50·1	0		
Reserpinized	51·9	31·4	39	48·8	2	46·3	7		
Hypothalamus									
Normal	2·4	0·5	79	1·5	37	2·5	0	2·5	0
Reserpinized	3·0	1·0	67	2·3	23	2·3	23	2·9	3
Dopamine									
Striatum									
Normal	99·6	109·0	0	97·5	2	102·7	0		
Reserpinized	112·2	107·2	4	113·9	0	120·1	0		
Hypothalamus									
Normal	6·4	5·1	20	5·0	22	5·8	9	6·6	0
Reserpinized	3·7	4·2	0	4·0	0	4·0	0	4·1	0

* Each value is the average of at least three separate determinations.

9·0 at 5 min, 16·9 at 15 min, and 20·0 at 30 min. Amantadine at 1×10^{-4} M inhibited this uptake by 13, 19 and 23 per cent respectively.

The emphasis of this study was on the comparison of values obtained from different systems with similar experimental procedures. The actual numbers can be manipulated by varying certain parameters such as order of addition, preincubation, incubation time, etc. In fact, the extent of inhibition can be increased by preincubating the synaptosomes with amantadine for various times. But since this is true for all preparations, the comparative picture remains the same.

The complete lack of effect of 1×10^{-4} M amantadine on dopamine uptake by striatal synaptosomes argues strongly against this being involved in the mechanism of the anti-Parkinson activity of this drug. The inhibition of dopamine uptake by hypothalamic synaptosomes probably reflects an effect on exchange of dopamine with endogenous norepinephrine, since both the uptake and the inhibition are drastically reduced by reserpine pretreatment.

Amantadine does inhibit the uptake of norepinephrine, particularly into hypothalamic synaptosomes, and the lack of effect of reserpine pretreatment suggests that this is a direct effect on the membrane process itself. Whether or not this effect has any physiological significance will most likely depend on the dose of amantadine and may assert itself at high levels. Its effects in the normal amantadine dose range of 2–3 mg/kg are almost certainly minimal unless Parkinsonian patients exhibit a greatly increased sensitivity.

Recently, effects of amantadine on the synthesis and/or release of dopamine have been proposed with some supporting evidence.[11,15] The validity of these proposals will have to be determined by further experimental work, but the hypothesis that uptake inhibition is involved seems very unlikely in view of the results reported here.

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Biochemical Pharmacology, Vol. 21, pp. 1995–1998. Pergamon Press, 1972. Printed in Great Britain.

Multiple forms of monoamine oxidase. Comparison of *in vitro* and *in vivo* inhibition patterns

(*Received* 21 *January* 1972; *accepted* 7 *March* 1972)

MITOCHONDRIAL monoamine oxidase (MAO; EC 1.4.3.4) from various animal species and tissues has recently been purified and shown to occur in several molecular forms.[1–6] Although these *in vitro*