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Some actions of amantadine on peripheral tissues

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Amantadine has been tested on several peripheral preparations. It depressed responses of the chick isolated oesophagus to nicotine and preganglionic parasympathetic nerve stimulation without depressing responses to acetylcholine. It did not affect the amplitude or duration of action potentials in toad sciatic nerve. It enhanced the effects of noradrenaline and dopamine while reducing the effect of tyramine on the coaxially stimulated guinea-pig ileum, isolated central artery of the rabbit ear and spontaneously beating guinea-pig isolated atria preparations. Amantadine enhanced the depressor effect of dopamine in the rabbit.

Since the chance discovery by Schwab, England & others (1969) of the alleviation of symptoms of Parkinson's disease (paralysis agitans) by amantadine hydrochloride, its efficacy in that disease has been confirmed by Parkes, Calver & others (1970) and by Boman & Porras (1970).

We have examined possible mechanisms of action of amantadine by investigating its effects on cholinergic and catecholaminergic function in several peripheral tissues.

METHODS

Chick oesophagus preparation. The preparation was set up as described by Bowman & Everett (1964). The nerve was stimulated with electrodes as described by Burn & Rand (1960).

Toad sciatic nerve preparation. The sciatic nerve from a freshly killed toad was placed in a Perspex three-compartment bath at 20°. The first and third compartment contained bipolar platinum stimulating and recording electrodes in paraffin oil. They were separated from the centre compartment, which contained Ringer solution, by thin (0·1 mm) rubber diaphrams. The nerve was threaded through perforations in the diaphragms and stimulated with supramaximal pulses of 10 μs duration every 10 s. The action potentials were recorded. Amantadine in Ringer solution was added to the centre compartment.

Coaxially stimulated guinea-pig ileum preparation. The preparation was set up as described by Paton (1957).

Rabbit ear artery preparation. Most experiments were made with the preparations described by de la Lande & Rand (1965). Some arteries were doubly cannulated as described by de la Lande, Connell & Waterson (1966).

Guinea-pig atria preparations. Spontaneously beating atria from a freshly killed guinea-pig were cleared of ventricular tissue and set up at 30° in McEwen (1956) solution aerated with 5% carbon dioxide in oxygen. The isometric contractions were recorded.

Rabbit blood pressure. Rabbits of either sex, 2 to 2·5 kg, were anaesthetized with urethane, 1·5 g/kg. Injections of drugs were made into the left brachial vein and femoral arterial blood pressure was recorded.

Drugs. These were: acetylcholine chloride (Hopkin & Williams); amantadine hydrochloride (Geigy, Australia); dopamine hydrochloride (Sigma); isoprenaline sulphate (Winthrop, Australia); nicotine hydrogen tartrate (BDH); noradrenaline (Winthrop, Australia); propranolol hydrochloride (ICI, Australia); suxamethonium chloride (May & Baker) and tyramine hydrochloride (Koch-Light). All drug concentrations are expressed in terms of the base.

RESULTS

Chick oesophagus preparation. Amantadine had no effect on responses to acetylcholine in concentrations up to 100 μg/ml, but depressed responses to nicotine and stimulation of the preganglionic parasympathetic nerves (Fig. 1). After washing out

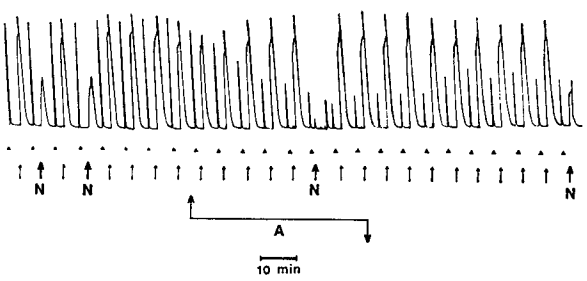

FIG. 1. Responses of the chick isolated oesophagus preparation to supramaximal voltage nerve stimulation (▲) with 1 ms pulses at 20 Hz for 10 s, nicotine 10 μg/ml (N) and acetylcholine, 5 μg/ml (↑). Nicotine and acetylcholine were left in contact for 1 min. The recording was turned off during the periods of washout. Amantadine (A), 100 μg/ml, was present in the bath fluid during the period indicated by the horizontal line with arrows.

the amantadine, its effects slowly wore off. The degree of depression of responses to nerve stimulation was concentration-dependent in the range of 1 to 100 μg/ml.

Toad sciatic nerve preparation. The size and duration of the recorded action potentials were unimpaired after allowing amantadine in concentrations of 0·01, 0·1, 1 and 10 mg/ml to remain in contact with the nerve for 10 min.

Coaxially stimulated guinea-pig ileum preparation. Amantadine, 0·1 to 100 μg/ml did not affect the height of the twiches elicited by supramaximal coaxial stimulation of the guinea-pig ileum. The twitches were reduced by noradrenaline, dopamine and tyramine and these effects were modified by amantadine (Fig. 2). In all the prepara-

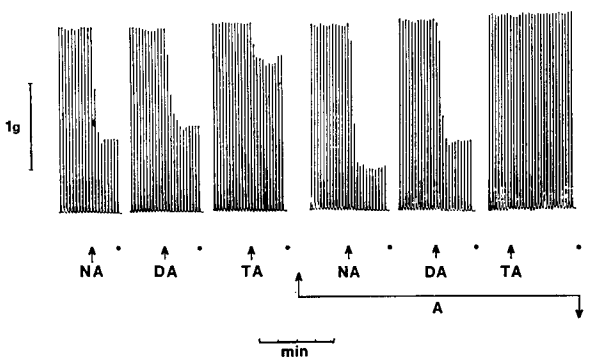

FIG. 2. Responses of the coaxially stimulated guinea-pig ileum preparation to 10 ng/ml of noradrenaline (NA), 1 μg/ml of dopamine (DA) and 2·5 μg/ml of tyramine (TA). Amantadine, 10 μg/ml, was present in the McEwen solution bathing the preparation during the period indicated by the horizontal bar.

tions examined, amantadine (10 μg/ml) further reduced the reductions in twitch amplitude produced by noradrenaline and dopamine and abolished the response to tyramine. The dose response curves for depression of the twitches by noradrenaline and dopamine were shifted parallel and to the left by amantadine. These effects of amantadine were readily reversible on washing the preparation.

Isolated central artery of the rabbit ear. Amantadine, perfused in concentrations of up to 10 μg/ml, had no effect on the perfusion pressure. Concentrations ranging from 0·1 to 10 μg/ml caused graded increases in the responses of the preparation to sympathetic nerve stimulation, noradrenaline and dopamine. The log frequency response curve produced by stimulation, noradrenaline or dopamine was shifted parallel and to the left by amantadine. Tyramine caused a biphasic response, consisting of an initial rapid increase in perfusion pressure followed by a larger and more prolonged increase; amantadine enhanced the first phase but depressed the second phase (Fig. 3). The effects were reversed on washing the preparation.

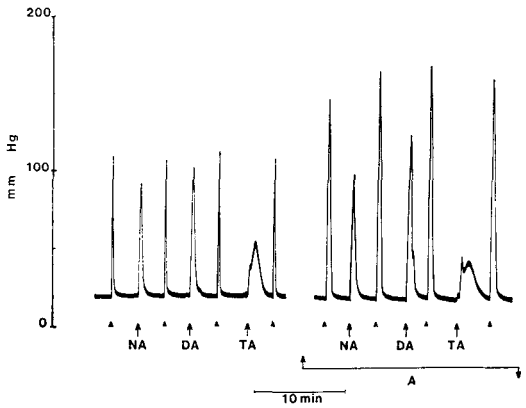

FIG. 3. Responses of the isolated central artery of the rabbit ear to sympathetic nerve stimulation with supra-maximal pulses of 1 ms duration at 10 Hz for 10 s (▲) and intraluminal injections of noradrenaline, 15 ng (NA), dopamine 6 μg (DA) and tyramine, 60 μg (TA). During the period represented in the second panel, 10 μg/ml amantadine (A) was present in the McEwen solution perfusing the preparation.

The observations suggest that amantadine may be blocking the uptake of amines. Since de la Lande & others (1966) have demonstrated that drugs having such an action produce a greater modification of responses to extraluminally than intraluminally applied amines, the effects of amantadine were examined on doubly cannulated preparations. In these, amantadine was applied extraluminally and produced a much greater enhancement of responses to extraluminal than to intraluminal noradrenaline. Responses to intraluminal dopamine were enhanced, whereas those of extraluminal dopamine were slightly decreased. The responses to tyramine applied extra- or intraluminally were decreased, those to extraluminal application being decreased most (Fig. 4).

Guinea-pig atria preparations. The positive inotropic and chronotropic responses of the preparation to noradrenaline (0·01 μg/ml) and dopamine (1 μg/ml) were increased by 20 μg/ml of amantadine to 120 and 115% of the pre-amantadine responses respectively, the response to tyramine (1 μg/ml) was diminished to about 70% of the pre-amantadine response, and responses to acetylcholine were not affected

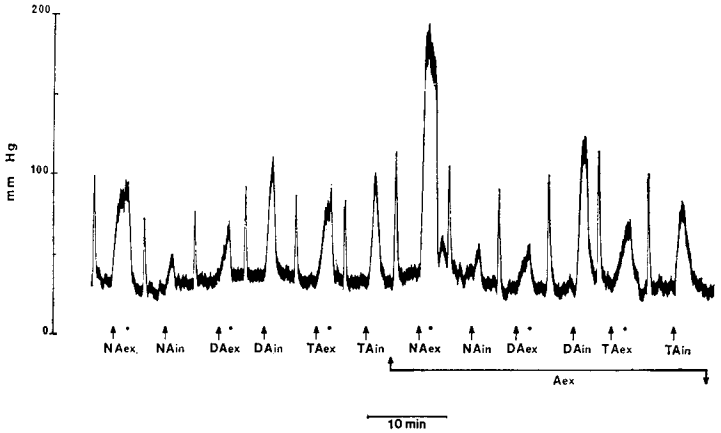

FIG. 4. Responses of the doubly cannulated rabbit ear artery to extraluminally applied noradrenaline, 100 ng/ml (NAex), intraluminally applied noradrenaline, 15 ng (NAin), extraluminally applied dopamine, 500 ng/ml (DAex), intraluminally applied dopamine, 6 μg (DAin), extraluminally applied tyramine, 500 μg/ml (TAex) and intraluminally applied tyramine, 60 μg (TAin). Unmarked responses are to sympathetic stimulation at 10 Hz for 10 s. During the period indicated, 10 μg/ml of amantadine (Aex) was present in the McEwen solution bathing the extraluminal surface. At the dots, the extraluminal bath fluid was changed.

(Table 1). Amantadine, 20 μg/ml alone, had no effect on the preparation. The responses to noradrenaline, dopamine and tyramine returned to control levels after washing the amantadine from the preparation.

Rabbit blood pressure. Neither the depressor response to isoprenaline nor the pressor response to noradrenaline given intravenously were significantly affected by amantadine, but the configuration of response to noradrenaline was changed in that a depressor component appeared after administration of amantadine. The depressor responses to dopamine were increased and prolonged by amantadine but the pressor components of responses to high doses of dopamine was not greatly affected. In other experiments, after β-adrenoceptor blockade with 0·5 mg/kg of propranolol, the enhancement of the depressor effects of dopamine by amantadine was still obtained.

Table 1. *Effect of amantadine (20 μg/ml) on chronotropic and inotropic responses of isolated spontaneously beating atria of the guinea-pig to noradrenaline, dopamine, tyramine and acetylcholine.*
Mean values $\pm$ s.e., number of experiments in brackets.

	Before amantadine		In presence of amantadine	
	Inotropic response (mg systolic tension)	Chronotropic response (beats/min)	Inotropic response	Chronotropic response
Noradrenaline, 0·01 μg/ml	280 $\pm$ 10(6)	22·5 $\pm$ 1·5(6)	330 $\pm$ 10*	30 $\pm$ 2*
Dopamine, 1 μg/ml	290 $\pm$ 10(6)	23 $\pm$ 1·5(6)	315 $\pm$ 10*	26 $\pm$ 2*
Tyramine, 1 μg/ml	250 $\pm$ 10(5)	23 $\pm$ 2 (5)	160 $\pm$ 10*	7 $\pm$ 1*
Acetylcholine, 0·01 μg/ml	—100 $\pm$ 8(4)	—12 $\pm$ 1 (4)	—95 $\pm$ 8	—10 $\pm$ 1

* Significantly different from control values, $P < 0·01$.

DISCUSSION

Cholinergic preparations. Amantadine, even in high concentrations, did not possess atropine-like activity in chick oesophagus, guinea-pig ileum or guinea-pig atria. Its effect in decreasing the responses to preganglionic nerve stimulation and nicotine on the chick oesophagus preparation could be explained by (i) a local anaesthetic action, (ii) reduction of acetylcholine output from the postganglionic nerve endings, or (iii) blockade of nicotinic receptors of the parasympathetic ganglion cells. The first explanation appears to be negated by the lack of effect of high concentrations of amantadine on conduction of action potentials in the toad sciatic nerve and the persistence of nerve mediated effects in other preparations. The second explanation may also be negated by the lack of effect of amantadine alone on the height of twitches elicited by coaxial stimulation of the guinea-pig ileum. It is suggested therefore, that amantadine acts as an antagonist at the nicotinic receptors in the parasympathetic ganglia of the chick oesophagus preparation.

Adrenergic preparations. The effects of amantadine in potentiating the actions of noradrenaline and dopamine and decreasing the actions of tyramine in the isolated ear artery of the rabbit, the coaxially stimulated guinea-pig isolated ileum and the isolated atria of the guinea-pig could be explained by blockade of uptake of amines into the adrenergic neuro-transmitter apparatus. The potentiation of the actions of dopamine was generally less than of the corresponding actions of noradrenaline presumably because dopamine, as well as having a direct effect, also exhibits a weak indirect effect (Bejrablaya, Burn & Walker, 1958). The findings with extraluminally applied dopamine in the doubly cannulated rabbit ear artery indicate that it had a predominantly indirect action in this preparation. Blockade by amantadine of amine uptake was clearly demonstrated by its effect on the two phases of the response to tyramine in the rabbit ear artery. This biphasic response has been explained as an initial direct action of tyramine on receptors followed by a more prolonged indirect action (Farmer, 1966): the first phase was enhanced by amantadine, the second was reduced. There is other evidence that amantadine blocks uptake of catecholamines into peripheral adrenergic neurons. Thus Vernier, Harmon & others (1969) found that amantadine potentiated pressor responses to noradrenaline in dogs pretreated with a ganglion blocking drug while the pressor response to phenethylamine was diminished; and Offermeier (1971) has found that amantadine potentiated the actions of noradrenaline on the rat vas deferens and guinea-pig tracheal chain.

Amantadine caused a striking potentiation of the depressor action of dopamine on the rabbit blood pressure. This depressor response is not due to an action of dopamine on β-adrenoceptors (Holtz, Stock & Westerman, 1963; Vanov, 1963), and its potentiation by amantadine still occurred after β-adrenoceptor blockade. Suggested explanations for the depressor action of dopamine include (i) formation of a depressor metabolite (Holtz & others, 1963) and (ii) competition with endogenously acting noradrenaline by dopamine acting as a partial agonist (Burn & Rand, 1958). In either event, the effect of amantadine in enhancing the response to dopamine could be ascribed to blockade of dopamine uptake.

Recently, a number of atropinic drugs useful in Parkinson's disease have been found to possess effects on the uptake of dopamine by basal ganglia synaptosomes (Coyle & Snyder, 1969). Since central dopaminergic mechanisms are known to be impaired in Parkinson's disease (Hornykiewicz, 1966), the inhibition of uptake of

dopamine would be expected to ameliorate the symptoms of the disease. There is evidence consonant with the suggestion that amantadine blocks the uptake of dopamine and other amines in the periphery and it is likely therefore that amantadine could block the uptake of dopamine centrally. Furthermore, it has been shown that amantadine blocks the uptake of dopamine by synaptosomes prepared from rat basal ganglia (Heimans, Rand & Fennessy, 1972).

It is suggested that the beneficial effect of amantadine in Parkinson's disease may be due to enhancement of a dopaminergic mechanism resulting from a blockade of dopamine re-uptake within the basal ganglia.

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