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On the mode of action of amantadine

Amantadine has been shown to have some therapeutic effect in parkinsonism, initially by Schwab, England & others (1969). In animal experiments it has been found to cause moderate central stimulation, reversal of tetrabenazine-induced sedation, a slight block of the noradrenaline uptake in the heart and to be ineffective in decreasing monoamine oxidase activity *in vitro* (Vernier, Harmon & others, 1969) An anti-cataleptic effect is reported (Simon, Malatray & Boissier, 1970; Zetler, 1970) and the drug potentiates L-dopa-induced effects in mice (Svensson & Strömberg, 1970). We have now examined some functional and biochemical aspects of the effect of amantadine on central and peripheral catecholamine neurons, and compared its mode of action with (+)-amphetamine.

We have confirmed the stimulant action of amantadine on the motor activity of mice. To investigate the effect of catecholamine depletion on this effect, amantadine HCl (150 mg/kg) was injected to female mice (strain NMRI, about 20 g) pretreated with reserpine (10 mg/kg) 5 h before. The animals were put into a test cage 90 min later, 10 min after which the motility was measured for the next 30 min by means of an Animex activity meter (Svensson & Thieme, 1969). Some mice received α-methyl-tyrosine methylester (H 44/68), an inhibitor of tyrosine hydroxylase (200 mg/kg) 15 min before amantadine, and some L-dopa (25 mg/kg) 85 min after amantadine. All injections were given intraperitoneally. Controls were given either reserpine and H 44/68 or these drugs together with L-dopa (Fig. 1). Statistical evaluation showed that amantadine caused an increased motor activity in the reserpine-pretreated mice ($P < 0.005$), which was inhibited by H44/68 ($P < 0.01$). A small dose of L-dopa, causing no motor stimulation by itself, restored the amantadine effect ($P < 0.05$). The general picture strongly resembles that of (+)-amphetamine (*c.f.* Hanson, 1966, 1967) and indicates, that amantadine requires small amounts of catecholamines for its motor stimulant effect.

For assay, amantadine HCl or (+)-amphetamine sulphate were injected in mice in various doses 105 and 45 min, respectively, before death. All mice were pretreated with reserpine (10 mg/kg) 22 h and nialamide (100 mg/kg) 4 h before death. L-Dopa (25 mg/kg) was injected subcutaneously 30 min before death. All other drugs were given intraperitoneally. Noradrenaline was determined according to Bertler, Carlsson & Rosengren (1958); dopamine according to Carlsson & Waldeck (1958, as modified by Carlsson & Lindqvist, 1962a); normetanephrine according to Carlsson & Lindqvist (1962b); methoxytyramine according to Carlsson & Waldeck (1964). Amantadine, 50 or 100 mg/kg, or (+)-amphetamine, 0·5 or 1·5 mg/kg, caused a decrease in the noradrenaline accumulation in the brain after L-dopa ($P < 0.001$). In the heart, amantadine, 50 mg/kg, caused a decrease in noradrenaline accumulation ($P < 0.025$) and so did (+)-amphetamine, 2·5 and 1·5 mg/kg, ($P < 0.001$ and < 0.01, respectively) (Table 1). No decrease of the dopamine accumulation in the brain or in the heart was found after amantadine or (+)-amphetamine but it was enhanced in the brain after 25 mg/kg amantadine ($P < 0.025$) and in the heart after 50 mg/kg ($P < 0.005$). A decrease in

the dopamine: methoxytyramine ratio with increasing doses of amantadine was also found. This could be interpreted in terms of a net release of dopamine into the extraneuronal space. The normetanephrine analyses showed amounts too small to be measured by the technique used.

The effect of amantadine on brain levels of noradrenaline, dopamine, normetanephrine and methoxytyramine after nialamide pretreatment was also examined.

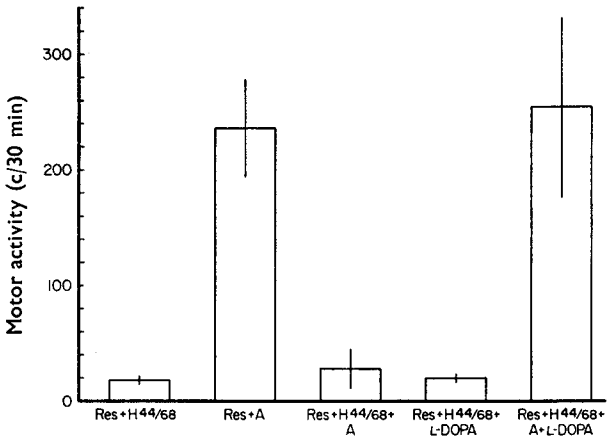

FIG. 1. Effect of amantadine on motor activity in mice after catecholamine depletion. Drugs were given intraperitoneally, using the following doses and time intervals before the start of the measurement: Reserpine (Res) 10 mg/kg 6 h 40 min, H44/68 200 mg/kg 1 h 55 min, amantadine HCl (A) 150 mg/kg 1 h 40 min and L-dopa 25 mg/kg 15 min. Each value represents the mean of activity of 4–6 groups, consisting of three mice, ± s.e.

Table 1. *Effect of amantadine and* (+)*-amphetamine on* L*-dopa induced accumulation of noradrenaline* (NA), *dopamine* (DA), *and methoxytyramine* (MT) *in mice pretreated with reserpine and nialamide.* The drugs were given i.p. in the following doses and time intervals before death: reserpine, 10 mg/kg—22 h, nialamide, 100 mg/kg—4 h, amantadine HCl—105 min, (+)-amphetamine sulphate—45 min. L-Dopa, 25 mg/kg s.c., was given 30 min before death. Shown are the means ±s.e. in μg/g of (n) determinations.

Drug, mg/kg	Heart		Brain		
	NA	DA	NA	DA	MT
Amantidine					
100	0·04 (2) ±0·01	0·52 (2) ±0·12	0·05 (2) ±0·01	1·41 (2) ±0·07	
50	0·04 (6) ±0·01	0·82 (6) ±0·10	0·07 (8) ±0·01	1·65 (8) ±0·06	3·03 (4) ±0·38
25	0·05 (2) ±0·01	0·64 (2) ±0·06	0·11 (2) ±0·02	2·20 (2) ±0·00	
0	0·07 (8) ±0·01	0·50 (7) ±0·05	0·10 (10) ±0·01	1·72 (10) ±0·10	2·24 (6) ±0·24
(+)-Amphetamine					
2·5	0·01 (3) ±0·00	0·38 (2) ±0·06	0·05 (3) ±0·01	1·42 (3) ±0·02	
1·5	0·02 (2) ±0·01	0·34 (2) ±0·10	0·06 (4) ±0·01	1·54 (4) ±0·08	2·82 (2) ±0·03
0·34	0·09 (2) ±0·00	0·66 (2) ±0·02	0·10 (2) ±0·01	1·74 (2) ±0·20	

Table 2. *Effect of amantadine on the levels of noradrenaline* (NA), *dopamine* (DA), *normetanephrine* (NM), *and methoxytyramine* (MT) *in nialamide pretreated mice.* Nialamide 100 mg/kg and amantadine HCl (100 mg/kg) were given i.p. 4 and 2 h, respectively, before death. Each value represents the mean of 6 determinations ± s.e. The increase in NM and MT caused by amantadine is statistically significant ($P < 0.001$).

Treatment	NA μg/g	DA μg/g	NM μg/g	MT μg/g
Nialamide + amantadine	0·43 ± 0·02	1·15 ± 0·06	0·25 ± 0·02	0·42 ± 0·02
Nialamide	0·47 ± 0·03	1·17 ± 0·05	0·07 ± 0·01	0·26 ± 0·02

Nialamide (100 mg/kg, l.p.) and amantadine HCl (100 mg/kg, l.p.) were given 4 and 2 h respectively, before death. Amantadine caused an increase in both normetanephrine and methoxytyramine ($P < 0.001$), but no change in the catecholamine levels (Table 2). The data in Tables 1 and 2 are similar to those found after (+) amphetamine treatment (Carlsson, Fuxe & others, 1966) and indicate an increased release of catecholamines into the extraneuronal space (*c.f.* Carlsson, 1970) after amantadine.

Finally, reserpine (10 mg/kg) and nialamide (10 mg/kg) were injected intraperitoneally in mice 6 and 2 h, respectively, before ^{3}H-NA, 0·5 μg/kg, intravenously. Fifteen min later, amantadine HCl in various doses was given intravenously and the mice were killed after another 15 min. ^{3}H-NA was measured in the heart (Carlsson & Waldeck, 1963). Amantadine released ^{3}H-NA taken up by the heart through a reserpine-resistant mechanism (^{3}H-NA ng/g ± s.e.; 0·32 ± 0·05 n = 5; 0·20 ± 0·06 n = 3; 0·09 ± 0·00 n = 4; 0·13 ± 0·01 n = 5 for amantidine 0, 2·5, 10, 25 mg/kg respectively, $P < 0.001$ at the 10 mg dose and <0·005 at the 25 mg dose compared to control value), an effect shared by (+)-amphetamine (Carlsson & Waldeck, 1966). This specific release is not due to a block of the uptake mechanism at the level of the cell membrane (the so-called membrane pump), since a potent inhibitor of this mechanism, protriptyline, is ineffective in this respect (Carlsson & Waldeck, 1966).

We thus have to consider the possibility that the activity of amantadine against parkinsonism is brought about by an amphetamine-like mechanism, i.e. by release of catecholamines from an extragranular, though intraneuronal pool, apparently dependent on the rate of catecholamine synthesis. As is well known, amphetamine belongs to the traditional armamentarium of drugs used in parkinsonism. An important difference between the two drugs may be a higher selectivity of amantadine for *central* catecholamine neurons for amine release. As indicated above, the actions of amantadine described here cannot be entirely explained by inhibition of the membrane pump. Nevertheless the evidence does not exclude the possibility that amantadine has this action in addition to its amine-releasing properties.

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The effect of prenylamine on adrenaline-induced hypercholesterolemia in mice

The administration of adrenaline causes a rise in serum cholesterol level in many animal species. The hypercholesterolemia is preceded by an elevation of free fatty acid levels in rats (Shafrir, Sussman & Steinberg, 1960; Shafrir & Steinberg, 1960), dogs (Kaplan, Stafford & Gant, 1957; Shafrir, Sussman & Steinberg, 1959), and rabbits (Dury, 1957), and it seems that the rise in serum cholesterol is secondary to the rise in blood free fatty acids (Gidez, Roheim & Eder, 1962; Nestel & Steinberg, 1963; Steinberg, 1963). We now report the effect of adrenaline on cholesterol serum levels in mice, and the modulation of this effect by prenylamine and other adrenergic blocking drugs known to inhibit the free fatty acid response to adrenaline.

Swiss-Webster (ICR) albino male mice (Harlan Industries, Cumberland, Indiana) 25–28 g were housed in groups of five at 25 $\pm$ 3° with freely available food and water. All injections were subcutaneous at varying sites in the dorsal region. The drugs were dissolved or suspended in sesame oil in a volume of 10 ml/kg weight. The doses as base of drug were: (−)-adrenaline, 1 mg/kg; prenylamine, 5, 10, 25 and 50 mg/kg; propranolol, 10 mg/kg; phenoxybenzamine hydrochloride, 1 mg/kg; and phentolamine hydrochloride, 10 mg/kg. The schedule for drug administration was: blocking drug at 5 30 am on days 1, 2 and 3 and again 12 h later on days 1 and 2. Adrenaline was given at 6 am on days 2 and 3 and 12 h later on day 2. Analysis was at 6 pm on day 3. In experiments where either adrenaline or prenylamine (25 or 50 mg/kg) was given alone, in injection of 10 ml/kg of sesame oil replaced the blocking drug; thus eight injections were always made in the three-day period. The controls similarly had only sesame oil. A group of older male mice, 32–36 g was also included.

Blood was obtained from the mice by decapitation, and serum cholesterol measured colorimetrically (Watson, 1960).

No difference was found between control serum levels of cholesterol of either weight groups of mice (Table 1). Whereas adrenaline administration caused no significant change in serum cholesterol level in the older mice (32–36 g), there was a statistically significant ($P < 0{\cdot}001$) elevation in mean cholesterol value above control