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O EKSPERIMENTALNOM TREMORU

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B. COX, E. M. R. CRITCHLEY, H. SCHNIEDEN AND C. WILLIAMS

SOME PHARMACOLOGICAL ACTIONS OF AMANTADINE HYDROCHLORIDE AND ITS EFFECTS ON EXPERIMENTAL AND CLINICAL TREMOR

INTRODUCTION

Amantadine hydrochloride was first introduced as an antiviral agent effective against A_2 influenza (8, 9). In subsequent use it was found to have a beneficial effect in Parkinson's disease (11, 15). The work to be reported is a general examination of the pharmacology of amantadine and also its application in the treatment of essential tremor in man.

METHODS

(i) Isolated guinea-pig ileum preparation.

Segments of ileum 2.5 cm in length were suspended in Krebs solution maintained at 37 ± 1°C bubbled with a mixture of 95% O_2 and 5% CO_2. Tension changes were recorded isometrically. The initial tension applied to the ileum was lg.

(ii) Isolated rat and rabbit preparations.

Isolated rat uteri were set up as described by Gaddum and Lembeck (5), except that isometric rather than isotonic recordings were made.

Spiral strips of rabbit aorta were set up as described by Furchgott and Bhadrakom (4) except that isometric recording techniques were used.

Isolated perfused rat and rabbit hearts were set up for force and rate recordings (1).

(iii) Effect of amantadine on drug induced tremor in mice.

The dose of tremorgenic agent producing tremor in 50% of a group of mice treated with the drug was determined by observation using a »double-blind« technique. The results were calculated according to the method of Litchfield and Wilcoxon (10).

(iv) Clinical Trials.

Patients diagnosed as essential tremor were asked if they would participate in a clinical trial. The diagnosis was based on criteria for this disease laid down by Critchley, 1949 (2).

The patients' ages ranged from 43—67 years and patients of both sexes were included in the trial. Double blind conditions were observed and clinical assessments made whilst the patients were on placebo or on drug. The clinician ranked improvement or deterioration on a ranking scale ranging from — 3 to + 3. — 3 represented marked deterioration whilst + 3 represented marked improvement.

A number of tests were carried out. The patient was presented with 6 wooden blocks (each side of the cube 2.5 cm) and asked to arrange 3 blocks on the bottom row, 2 blocks in the middle row and one on top. The time taken to perform this exercise was noted. The time taken for the patient to fold a piece of paper and place it in an envelope was noted as well as the time taken to open and close a safety pin. The patient was asked to trace round the outline of a 3 cm diameter circle. The time taken was noted. In addition a score of 1 to 3 was given for closeness to the outline, 1 being very close, 2 following the outline moderately well and 3 marked excursions from the outline. The time taken to perform this exercise was multiplied by the 'closeness' factor.

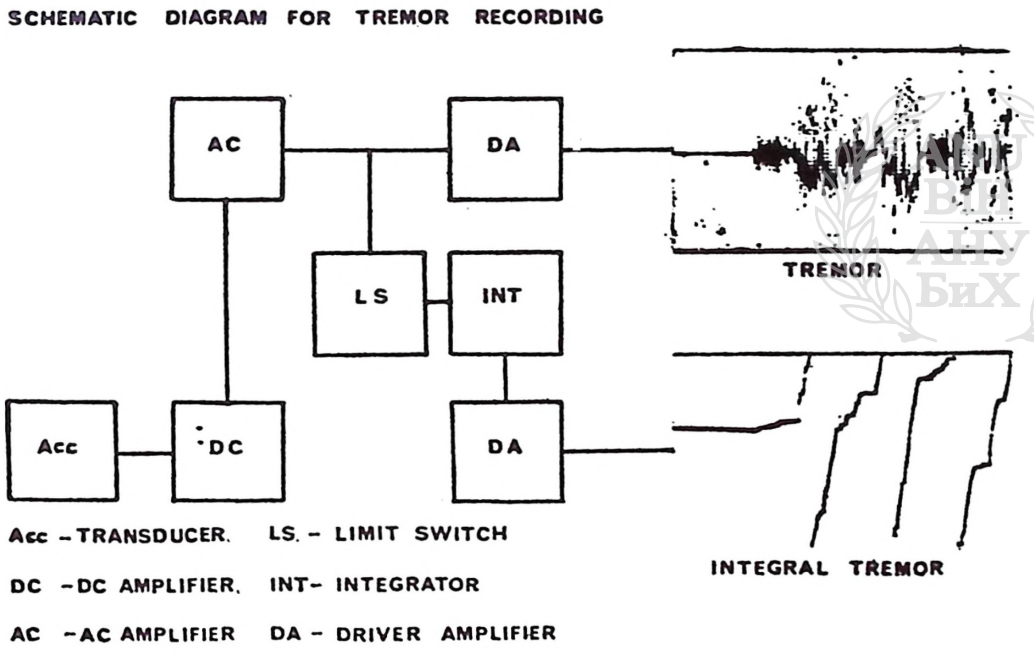

Fig. 1

Schematic diagram for tremor recording.

ACC *Grass accelerometer SPA 1*
DC *DC amplifier Devices DC2D*
AC *AC amplifier Devices AC 1*
DA *Driver amplifiers DC5 (M2 Devices Recorder)*
LS *Limit Switch*
INT *Intergrator* } *Devices 3020*

A recording of tremor and integral of tremor is shown. The integrator pen is automatically zeroed on reaching full deflection.

Similarly the time taken to print a phrase was noted and a ranking score of 1 — 3 (1 very neat) was given. The time taken was multiplied by the 'neatness' factor. The patients was also instructed to remove a match from a box of matches and strike it and time taken was noted.

In addition tremor was estimated using a Grass Parkinsonian transducer, the output of which was fed through amplifiers so that tremor trace and integral of tremor could be obtained (fig. 1). All patients were allowed a pre-trial run on the tests described above and a pre-trial tremor recording was taken so that they could get acclimatised to the procedure. The results were analysed using non-parametric statistics (6).

DRUGS USED

We would like to thank Dr. A. W. Galbraith, Geigy Pharmaceuticals Ltd., Macclesfield, Cheshire, England for kindly supplying amantadine hydrochloride, and Dr. R. W. Brimblecombe, Ministry of Defence Chemical Defence Establishment, Porton Down, Wiltshire, England for generously donating some oxotremorine pure substance. Other drugs used were: Acetylcholine (Acecoline, Lematte et Boinot, Paris), bradykinin, Sandoz, Ltd., carbachol chloride and histamine phosphate (B. D. H. Ltd.,), dopamine hydrochloride, harmine hydrochloride, homovanillic acid, 5-hydroxyindole-3-acetic acid, 5-hydroxytryptamine, noradrenaline bitartrate (Koch-Light Ltd.), propranolol hydrochloride (I. C. I.) and tremorine (Sigma Chemicals Ltd.)

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RESULTS

(i) Isolated guinea-pig ileum preparation.

The effect of Amantadine hydrochloride on guinea-pig ileum is shown in Table 1. Amantadine in concentrations of 5 μg/ml or less

Table 1

INTERACTION BETWEEN AMANTADINE AND SOME AGONISTS ON THE ISOLATED GUINEA-PIG ILEUM PREPARATION

Agonist	Amantadine μg/ml				
	5	10	40	160	640
None	0	+	++	+	—
Acetylcholine	0	+	++	0	—
Bradykinin	0	++	+	0	—
5-HT	+	++	+	0	—
Histamine	+	+	++	0	—

0 = no effect.
+ = potentiation or increased activity.
— = antagonism or decreased activity.
Minimum number of observations for each concentration = 5.

was without effect on the resting tension. A concentration of 10 μg/ml induced small rhythmic tension changes in some segments. All segments tested showed this response at 40 μg/ml, and the effect persisted up to a concentration of 160 μg/ml. At higher concentrations the ileum became quiescent. Table 1 also shows the effect of amantadine on the response of the ileum to various agonists. A dose response curve was established for acetylcholine and repeated in the presence of various concentrations of amantadine, five separate experiments being made at each concentration. With 5 μg/ml amantadine hydrochloride no change occurred in the dose response curves. At 10 and 40 μg/ml all the dose response curves were shifted to the left indicating potentiation, no leftward shift was apparent at 160 μg/ml. The ileum did not respond to acetylcholine added in the presence of amantadine 640 μg/ml. Segments of ileum were also tested with ED 70 doses of bradykinin, 5-hydroxytryptamine and histamine (table 1) similar results to those with acetylcholine were obtained.

(ii) Isolated rat and rabbit preparations.

Table 2 shows the effect of amantadine alone on some isolated tissue preparations and the effect of amantadine on responses of these tissues to dopamine and noradrenaline.

Table 2

EFFECT OF AMANTADINE ON SOME ISOLATED RAT AND RABBIT TISSUES

	Minimum effective concentration+ of Amantidine (g/ml)		
	Alone	With Dopamine	With Noradrenaline
Rat Uterus	0, 4×10^{-5}	+, 1×10^{-5}	+, 4×10^{-5}
Rabbit Aorta	0, 1×10^{-3}	0, 1×10^{-4}	0, 1×10^{-4}
Rabbit Heart	+, 2×10^{-7}*	0, 1×10^{-5}	0, 1×10^{-5}
Rat Heart	+, 2×10^{-7}*	0, 1×10^{-5}	0, 1×10^{-5}

0 = no effect.

+ = potentiation or increased activity.

All other figures represent concentration of amantadine hydrochloride in Krebs, except at* where figures represent dose injected in a volume of 0.2 ml. For significance of changes see text. + Minimum effective concentration when amantadine active, when inactive figures represent maximum concentration used.

Amantadine alone was without effect on the uterus in concentrations up to 4×10^{-5} g. Concentrations higher than this reduced the response of the uterus to carbachol. Dopamine was used as an inhibitor of the carbachol spasm and its inhibitory effect measured in the presence and absence of amantadine slight potentiation of dopamine was observed with amantadine 10 μg/ml, which became significant ($p < 0.05$ Mann-Witney 'U' test) (6) when the concentration was increased to 4×10^{-5}

g/ml. When noradrenaline was used as the inhibitor of the carbachol spasm, slight non-significant potentiation was observed only at 4×10^{-5} g/ml amantadine.

Amantadine in concentrations as high as 10^{-3} g/ml was without effect on the resting tension of spiral strips of rabbit aorta. Amantadine hydrochloride 10^{-4} g/ml in the Krebs solution was without effect on cumulative log dose-tension response curves obtained with either dopamine or noradrenaline.

The effect of amantadine on isolated heart preparations is also shown in Table 2. Some hearts (rat, 3 of 13 tested, rabbit, 1 of 6 tested) showed increased force of contraction but no change in rate of beating with a dose of 2×10^{-7} g amantadine hydrochloride. The increased force response was blocked by propranolol. The remaining hearts tested did not respond to amantadine until doses of 2×10^{-4} g were used, when there was usually a decrease in heart rate. Amantadine (1×10^{-5} g/ml) was added to the perfusing Krebs solution and was without effect on either dopamine or noradrenaline induced rate and force changes (6 experiments).

Table 3

EFFECT OF AMANTADINE ON DRUG INDUCED TREMOR IN MICE

TREMOR DRUG	PRETREATMENT	
	SALINE 0.9%	AMANTADINE 100 mg/kg
TREMORINE TD 50	7.2 mg/kg	4.2 mg/kg
Confidence limits	5,3—9,7	3,0—5,9
Number of mice	60	59
OXOTREMORINE TD 50	0.18 mg/kg	0.04 mg/kg
Confidence limits	0,15—0,22	0,025—0,06
Number of mice	110	60
HARMINE TD 50	5.8 mg/kg	1.8 mg/kg
Confidence limits	3,9—8,7	1,1—2,9
Number of mice	40	40

TD 50 = dose of tremor-drug producing tremor in 50% of mice treated calculated according to Litchfield & Wilcoxon (1949) (10).

(iii) Effect of amantadine on drug-induced tremor in mice.

Table 3 shows the effect of amantadine hydrochloride 100 mg/kg on drug induced tremor in mice. The dose of tremorgenic agent producing tremor in 50% of the mice treated (TD 50) was determined (10). The TD 50 for tremorine, oxotremorine and harmine was reduced by pretreatment of the mice with amantadine. In each case the potentiation was significant at the 95% confidence limits.

A preliminary trial was carried out in which nine patients received 100 mg daily of amantadine hydrochloride for 1 week followed by 200 mg daily for 5 weeks. 8 other patients received placebo over the

same period. Clinically no significant improvement occurred as compared to placebo. 3 patients in each group showed some evidence of improvement over their base line performance. There was no significant improvement in recorded tremor.

A double blind crossover trial using higher doses of amantadine hydrochloride for a similar period was then conducted. Ten patients received one capsule daily during the first week of drug (100 mg) or placebo, 2 capsules daily the second week and 3 capsules daily on subsequent weeks. On comparing results at the end of the placebo period with the end of the amantadine period, 5 patients clinically scored better on drug than placebo and 2 scored better on placebo than drug. 3 others had equal scores. The scores on drug were higher than on placebo and were significant ($p < 0.05$ signed Rank test one tail) (6).

The result of the tests are shown in table 4. It will be seen that the patients ability to fold paper improved but their ability to remove

Table 4

TEST	TREND IN FAVOUR OF	SIGNIFICANCE*
BLOCKS	DRUG	$p > 0.05$
FOLDING PAPER	DRUG	$p < 0.01$
SAFETY PIN	DRUG	$p > 0.05$
CIRCLE	PLACEBO	$p > 0.05$
SENTENCE	DRUG	$p > 0.05$
TREMOR	DRUG	$p < 0.05$
MATCH	PLACEBO	$p < 0.05$

* Wilcoxon signed Rank Test. (Two tailed test).

and strike matches did not. A significant diminution in tremor occurred (fig. 2). The trends were mainly in favour of the drug and not of the placebo.

Among the patients'; reports of improvement, one patient reported he could now shawe with a safety razor and another that she could dress herself.

Side effects were noted by a number of patients. Sleep problems were the commonest complaints on the drug. These included dreaming nightmares, early wakening and difficulty in getting to sleep (three patients). One patient complained of dizziness and one had palpitations. No serious side effects were seen. On placebo, one patient complained of dizziness, two had sleep problems, difficulty in getting to sleep or dreaming and two complained of chest pain.

Some determinations of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5 HIAA) were made from samples of cerebrospinal fluid (C. S. F.) taken from patients with Parkinson's disease (3). Wide variation was found from patient to patient. No significant difference could be detected between samples taken during treatment with amantadine when compared with samples taken whilst the patients were receiving L-dopa.

The minimum number of samples estimated was 8. In a number of patients 2 samples were taken from the same person once during amantadine therapy and once during L-dopa medication. Nine paired samples were examined for 5 HIAA content. Five patients had higher 5 HIAA/CSF levels during the amantadine period and four had higher CSF levels

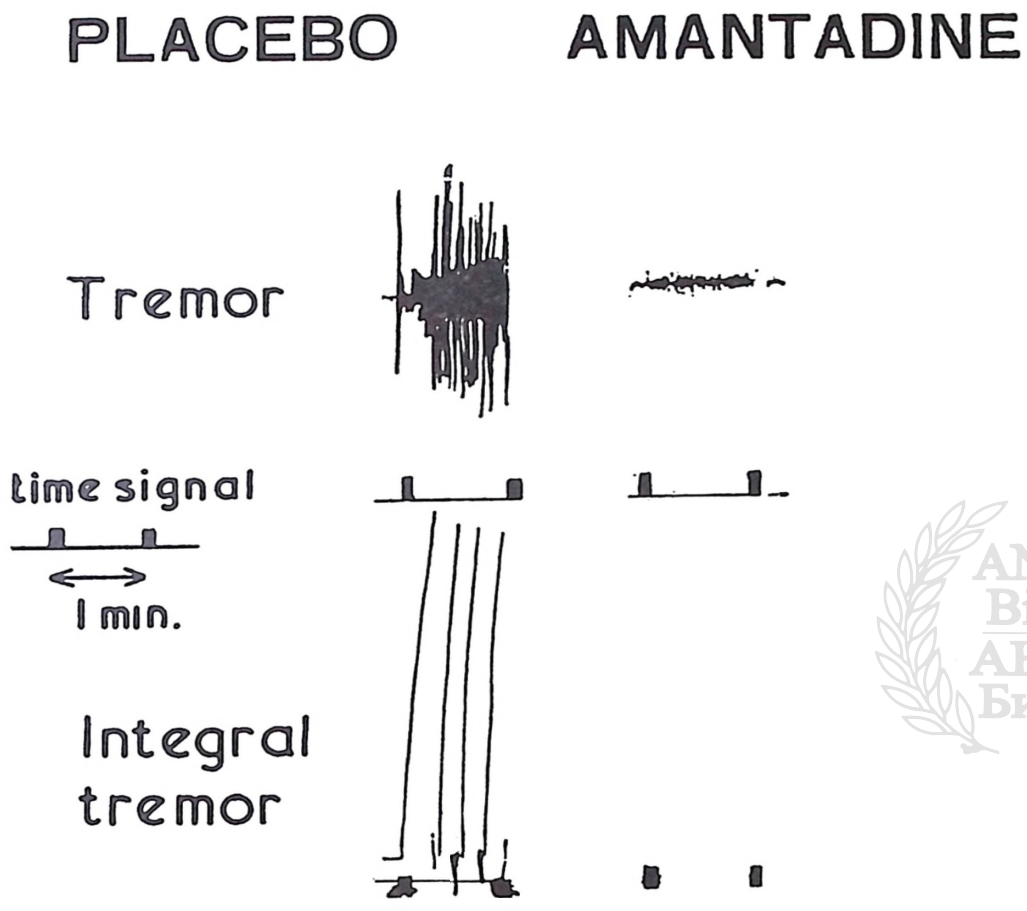

Fig. 2

Tremor recording. Patient on 300 mg amantadine daily or placebo. Recording taken at end of the placebo period or period on amantadine. Tremor was recorded for 60 seconds.

during L-dopa therapy. Eight paired samples were also obtained for HVA. In this case 5 patients had higher CSF levels during amantadine therapy, two patients had higher CSF levels during L-dopa treatment. No HVA could be detected in either sample taken from the remaining patient. When examined statistically using the Wilcoxon matched pairs signed rank test (6), the differences in HVA content of CSF just failed to achieve statistical significance ($p < 0.05$). The difference in 5 HIAA content of CSF also failed to achieve the accepted level for statistical significance. Both tests being »one-tailed tests«.

DISCUSSION

Experiments on isolated tissues indicated that amantadine had very little activity on its own. Also its interaction with various agonists on the guinea-pig ileum was non-specific, causing potentiation at low concentrations and antagonism at high concentrations. A non-specific blockade of histamine and acetylcholine on guinea-pig ileum has been reported previously (7). Of all the tissues tested only on the rat uterus did amantadine show a significant potentiation of a dopamine effect. There was also a slight potentiation of noradrenaline on this tissue.

Amantadine was found to potentiate drug-induced tremor in mice. Since it has been shown to alleviate the Parkinsonian condition in man (11, 15), these findings must throw more doubt on the usefulness of tremorgenic drugs as models of Parkinson's disease.

On the objective tests in patients, one would have expected that an improvement on the folding paper test which is a test of manual dexterity would have been accompanied by a similar improvement on the match test which again tests dexterity in handling objects. However it should be noted that patients on placebo took a mean time of 6.6 sec to complete this test whilst the mean time for the folding paper test was 16.2 sec. The reaction time of the observer using the stopwatch would therefore be of greater importance in the first instance than in the second and this factor of observer error may therefore have been of importance in producing the result obtained in the match test.

There have been a number of clinical trials of amantadine in Parkinsonism and Parkinsonian tremor has been reported to be improved by the drug (13, 15). Schwab, England, Poskanzer and Young (15) state the drug is of no benefit in familial tremor but do not give details. Partera, Varala de Seijas, Mara, Ramírez Echevarría and Amaya Pembo (14) treated 9 patients with senile tremor with 300 mg per day of amantadine for 25 days. They observed no improvement. Though the dose was the same as in the present series the duration of treatment was less. Parkes, Knill-Jones and Clements (12) report that the familial tremor of 2 female patients was exacerbated by amantadine 300 mg daily. The duration of treatment is not noted.

A significant change in response is not necessarily of therapeutic importance as it is the degree of improvement that matters. In the present series clinically in some patients improvement occurred and this was borne out by the patients subjective impressions. On the basis of this small trial it would appear that amantadine is of some benefit in this disease though if one takes into account side effects the benefits maybe only marginal.

In Parkinsonian patients it was not possible to detect any significant change in the concentrations of HVA and 5 HIAA in CSF samples taken during amantadine therapy when compared with patients on L-dopa. This was probably due to the wide individual variations in the CSF content of these substances. When the results for patients giving two CSF samples were extracted from all the results obtained, then a

slightly different pattern emerged. For the majority of patients (5 out of 8) there were lower concentrations of HVA in the CSF samples taken during the L-dopa period than the concentrations found whilst the same patients were receiving amantadine. One possible explanation for this finding is that amantadine is increasing the release and conversion of dopamine to its acid metabolite. A decrease in the concentrations of 5 HIAA in CSF of Parkinsonian patients receiving L-dopa has been reported previously (16). In our trial though not significant the mean 5 HIAA concentration was lower for CSF taken during L-dopa therapy than the concentration during amantadine therapy.

SUMMARY

Amantadine hydrochloride was tested for its effect on isolated guinea-pig ileum, rat uterus, rabbit aortic strip and rat or rabbit perfused heart. Amantadine on its own produced small rhythmic tension changes in the guinea-pig ileum in concentrations of 40 μg/ml. It was without effect on rat uterus and rabbit aorta, but had a variable action on perfused hearts. Some hearts showed an increased force of contraction at a dose of 2×10^{-7} g/ml but the majority failed to respond in this way even at 2×10^{-4} g/ml. Its interaction with various agonists on the guinea-pig ileum suggests it is a non-specific potentiating agent at low doses (40 μg/ml) and a non-specific inhibitor at high doses (640 μg/ml). Amantadine did not potentiate either dopamine or noradrenaline on rabbit aorta and rat or rabbit heart. It did however increase the ability of dopamine and noradrenaline to inhibit carbachol spasms of rat uterus. Amantadine potentiated the tremorgenic actions of tremorine, oxotremorine and harmine.

A double blind crossover trial of 10 patients with essential tremor was carried out using amantadine hydrochloride and placebo. 300 mg of amantadine hydrochloride produced some slight improvement and this was borne out by tremor recordings. The drug produced some side effects as did the placebo.

No significant difference could be detected in the HVA and 5 HIAA content of CSF taken from Parkinsonian patients receiving either L-dopa or amantadine.

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NEKA FARMAKOLOŠKA DJELOVANJA AMANTADINA I NJEGOVI EFEKTI NA TREMOR

KRATAK SADRŽAJ

Rad se odnosi na istraživanja raznih aspekata farmakologije amantadina. Inicijalni eksperimenti odnosili su se na upotrebu izoliranih tkiva i također na neke in vivo eksperimente sa mišem. Daljni eksperimenti odnose se na evaluaciju terapeutske efikasnosti amantadina na izvjesna čovjekova tremor-stanja.

Determiniran je efekat amantadina na izvjestan broj izoliranih preparata tkiva (npr. ileum zamorčeta, uterus štakora, aorte zeca i izolirano perfuzirano srce). Ista ova izolirana tkiva upotrijebljena su za ispitivanje interakcije između amantadina i nekih transmiterskih supstanci. Ispitivano je i djejstvo amantadina na tremor miša izazvan pomoću farmaka.

Vršena su klinička ispitivanja djelovanja amantadina na pacijente sa esencijalnim tremorom. Tremor je praćen objektivima i pacijenti su učestvovali u izvjesnom broju testova čiji je cilj bio da se pokaže da li lijek izaziva bilo kakvo poboljšanje na sposobnost izvođenja finih pokreta. Prikupljeni su CSF-uzorci od nekoliko pacijenata i koncentracije homovanilične kiseline i 5-hydroxyindoloctene kiseline mjerene za vrijeme dok su pacijenti uzimali ili placebo ili aktivni lijek.

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DISCUSSION

Jurna: Did I understand you correctly when you said that the sensitivity of aortic strips towards NA or DA was not increased by amantadine?

Cox: The action of amantadine was determined on the aortic strip preparation in the following way. Cumulative dose response curves were obtained to noradrenaline and dopamine, and then repeated in the presence of amantadine concentrations up to those shown on the slide. Amantadine failed to potentiate either noradrenaline or dopamine.