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# Amantadine Decreases *d*-Amphetamine Stimulation and Increases *d*-Amphetamine Anorexia in Mice (39228)

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(Introduced by A. A. Rubin)

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Amantadine hydrochloride (1-adamantanamine hydrochloride, Symmetrel) is an antiviral agent used against $A_2$ (Asian) influenza in animals (1) and in man (2). Amantadine also has been used in the treatment of parkinsonism (3), the available evidence from animal experiments pointing to an interaction with dopamine and perhaps other catecholamines within the brain as its major mode of action (4).

The present study in mice confirms and reveals new actions for amantadine; it decreased the CNS stimulant side-effects of *d*-amphetamine, and simultaneously it increased *d*-amphetamine-induced anorexia. The unexpected blockade by amantadine of the stimulant (and also the lethal) effect of *d*-amphetamine was reported by Menon, Clark, and Fleming (5). This finding is of particular interest since amantadine itself has been reported to cause weak, atypical stimulant effects at high doses (6).

Using the test method of Clark (7), female mice (Carworth Farms, $CF_1S$) were food-deprived for 26 to 29.5 hr and orally given coded doses of either amantadine HCl (1, 2.5, 5, or 25 mg/kg) or water, followed 30 min later by a second, coded oral dose of either *d*-amphetamine $SO_4$ (0.0625 to 16 mg/kg, 2X increment between dose levels) or water. Thirty minutes after the second dose the mice were put into individual feeding chambers. Each chamber contained a brass bar with 10 small depressions; each depression was about half-filled with a drop (0.05 ml) of a 50% aqueous solution of sweetened, condensed milk. The mice remained in the feeding chamber for 45 min, at which time a count was made of the number of milk drops consumed. The mice were observed for signs of CNS stimulation (increased spontaneous motor activity) just before they were put into the feeding chambers (when control mice are asleep), and were observed for signs of CNS depression (decreased spontaneous motor activity) after 5 min in the feeding chambers (when control mice are quite active). The observations were made blind; that is, the observer was unaware of the treatment condition. Forty to fifty mice were tested at a time including 10 or 20 controls. Four-hundred-ninety mice were distributed among the various drug-treated conditions; there were 290 controls. Drugs were dissolved in distilled water and were given orally on a milligram base weight per kilogram of body weight basis at a constant volume of 10 ml/kg. Control mice received two doses of water at 10 ml/kg.

The CNS stimulation data were analyzed by plotting, on a log-probit grid, the percentage of mice that showed signs of increased spontaneous motor activity as a function of the dose level of *d*-amphetamine administered alone and in combination with each of four preceding dose levels of amantadine (1, 2.5, 5, and 25 mg/kg). The five dose–response lines were fitted visually. The methods of Litchfield and Wilcoxon (8) were used to obtain the 95% confidence limits around the doses that caused stimulation in 50% of the mice (ED50) and to test for potency differences among the ED50 values. The anorexia data were analyzed by plotting mean milk intake values on a semilog grid as a function of the dose level of *d*-amphetamine administered alone and in combination with each of three preceding dose levels of amantadine (1, 2.5, and 5 mg/kg). Each of the four dose–response lines was derived by the method of least squares. The methods of Bliss (9) were used to obtain the 95% confidence limits around the

TABLE I. AMANTADINE DECREASED *d*-AMPHETAMINE STIMULATION AND INCREASED *d*-AMPHETAMINE ANOREXIA IN MICE.

| | *d*-Amphetamine | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Stimulation (S) | | | | Anorexia (A)[b] | | | | |
| Amantadine[a] oral dose in mg/kg | Oral ED50 in mg/kg (95% C.L.) | Slope (95% C.L.) | *n* (Number of doses) | Potency relative to *d*-amphetamine alone (95% C.L.) | Oral ED50 in mg/kg (95% C.L.) | Slope (95% C.L.) | *n* (Number of doses) | Potency relative to *d*-amphetamine alone (95% C.L.) | S/A |
| 0.0 (*d*-Amphetamine alone) | 2.1 (1.8, 2.5) | 1.6 (1.4, 1.9) | 60 (3) | – | 0.74 (0.45, 1.21) | −5.6 (−3.3, −7.9) | 40 (4) | – | 2.8 |
| 1.0 | 2.2 (1.7, 2.8) | 1.5 (1.2, 1.9) | 30 (3) | 0.95 (0.70, 1.28) | 0.65 (0.25, 1.68) | −4.5 (−1.8, −7.1) | 40 (4) | 1.14 (0.63, 2.08) | 3.4 |
| 2.5 | 2.7[c, e] (2.2, 3.2) | 1.8 (1.5, 2.2) | 90 (3) | 0.78 (0.61, 0.99) | 0.37[d, f] (0.10, 1.40) | −5.3 (−0.8, −9.9) | 30 (3) | 2.00 (1.09, 4.17) | 7.3 |
| 5.0 | 3.4[c, e] (2.4, 4.8) | 1.9 (1.4, 2.5) | 30 (3) | 0.62 (0.43, 0.89) | 0.39[d, f] (0.29, 0.53) | −6.0 (−3.9, −8.1) | 130 (3) | 1.90 (1.28, 2.86) | 8.7 |
| 25.0 | 3.6[c, e] (2.9, 4.4) | 1.6 (1.3, 1.8) | 40 (3) | 0.58 (0.45, 0.76) | [g] | – | – | – | – |

[a] For amantadine alone at 1, 2.5, 5, and 25 mg/kg, the mean milk intake values were 5.5 ($n$ = 20), 5.4 ($n$ = 30), 5.9 ($n$ = 80), and 1.9 ($n$ = 20) drops, respectively. Only the amantadine value at 25 mg/kg differed significantly from control by 2-tailed Student's $t$ test. None of the amantadine-alone-treated mice showed signs of either stimulation or depression at any dose.

[b] The mean milk intake for control mice ($n$ = 290) was 5.6 drops. None of the control animals showed signs of stimulation; 2.4% were depressed.

[c] Significantly different from *d*-amphetamine alone at the 0.05 level or beyond (8).

[d] Significantly different from *d*-amphetamine alone at the 0.05 level or beyond (9).

[e, f] Values not significantly different from one another.

[g] Data not interpretable since this dose of amantadine alone caused significant anorexia.

doses that reduced milk intake to 50% of control (ED50), and to test for potency differences among the ED50 values.

The results are summarized in Table I, which shows (i) oral ED50 values and confidence limits (C.L.) for CNS stimulation and anorexia for *d*-amphetamine alone and in combination with each of three or four preceding dose levels of amantadine, (ii) the slope value and confidence limits for each dose–response line; (iii) the number of mice and the number of doses of *d*-amphetamine on which each line was based, (iv) the potency and confidence limits of each line relative to that of *d*-amphetamine alone, and (v) the ratio (S/A) of the stimulant ED50 (S) to the anorexia ED50 (A). *d*-Amphetamine alone stimulated 50% of the mice at 2.1 mg/kg and caused a 50% reduction in milk intake at 0.74 mg/kg. Amantadine at 1 mg/kg had no significant effect on the *d*-amphetamine ED50 values for stimulation or anorexia. Amantadine at 2.5 and at 5 mg/kg significantly raised the *d*-amphetamine stimulation ED50 values to 2.7 and 3.4 mg/kg, respectively, and at the same time it significantly lowered the *d*-amphetamine anorexia ED50 values to 0.37 and 0.39 mg/kg, respectively. Amantadine at 25 mg/kg raised the *d*-amphetamine stimulant ED50 to 3.6 mg/kg. The ratio of the stimulant to the anorexic ED50 (S/A) which is one measure of the "selectivity" of an anti-appetite drug, increased from 2.8 for *d*-amphetamine alone to 8.7 for *d*-amphetamine in combination with 5 mg/kg of amantadine. Amantadine alone, at 1, 2.5, and 5 mg/kg, had no effect on either spontaneous motor activity or on milk intake. The 25 mg/kg dose of amantadine alone caused significant anorexia but had no stimulant or depressant effect. This is consistent with previous reports of lack of CNS activity of amantadine in mice and other animals at oral doses below 30 mg/kg (6). Thus in mice, amantadine at "no effect" dose levels, which are within the clinical range (2 to 3 mg/kg), significantly decreased *d*-amphetamine stimulation and simultaneously increased *d*-amphetamine anorexia. The present data suggest that amantadine could serve as a useful laboratory tool in furthering our understanding of the pharmacology of the CNS system or systems which subserve stimula-

tion and appetite, since it appears to differentially affect the actions of *d*-amphetamine on these two aspects of behavior.

*Summary.* Amantadine hydrochloride (Symmetrel), an antiviral, antiparkinson agent that is most frequently used clinically at oral doses of 2 to 3 mg/kg, significantly decreased *d*-amphetamine-induced CNS stimulation (motor activity) and simultaneously increased *d*-amphetamine-induced anorexia (milk intake) in mice. Amantadine did this at oral doses of 2.5 and 5 mg/kg, which alone had no effect on either motor activity or milk intake.

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1. Davies, W. L., Grunert, R. R., Haff, R. F., McGahen, J. W., Neumayer, E. M., Paulshock, M., Watts, J. C., Wood, T. R., Hermann, E. C., and Hoffmann, C. E., Science **144,** 862 (1964); McGahen, J. W., and Hoffmann, C. E., Proc. Soc. Exp. Biol. Med. **129,** 678 (1968).
2. Jackson, G. G., Muldoon, R. L., Akers, L. W., "Antimicrobial Agents and Chemotherapy" (J. D. Sylvestor, ed.) p. 703. American Society for Microbiology, Ann Arbor, Michigan (1964); Wingfield, W. L., Pollack, D., Grunert, R. R., N. Engl. J. Med. **281,** 579 (1969).
3. Schwab, R. S., England, A. C., Poskanzer, D. C., and Young, R. R., J. Amer. Med. Assoc. **208,** 1168 (1969); Parkes, J. D., Calver, D. M., Zilkha, K. T., Knill-Jones, R. P., Lancet, **1970-I,** 259 (1970); Weeth, J. B., Shealy, C. N., Mercier, D. A., Wis. Med. J. **68,** 325 (1969); Millac, P., Hason, I., Espir, M. L. E., and Slyfield, D. G., Lancet **1970-I,** 464 (1970).
4. Vernier, V. G., "Annual Reports in Medicinal Chemistry, 1970," Chapter 5, p. 42. Academic Press, New York and London (1971).
5. Menon, M. K., Clark, W. G., and Fleming, R. M., Eur. J. Pharmacol. **21,** 311 (1973).
6. Vernier, V. G., Harmon, J. B., Stump, J. M., Lynes, T. E., Marvel, J. P., and Smith, D. H., Toxicol. Appl. Pharmacol. **15,** 642 (1969).
7. Clark, R., Toxicol. Appl. Pharmacol. **15,** 212 (1969).
8. Litchfield, J. T., Jr., and Wilcoxon, F., J. Pharm. Exp. Therap. **96,** 99 (1949).
9. Bliss, C. I., "The Statistics of Bioassay." Academic Press, New York (1952).

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