the serotonin and tryptophan hydroxylase is located in cell bodies, the activity of this enzyme does not control the amine concentrations. However, the data of Table 2 show that the enzyme controls the conversion index for serotonin.

This report suggests that tryptophan hydroxylase of brainstem can be decreased without changing the serotonin concentrations in this tissue. Perhaps in brainstem the enzyme is not directly related to serotonin content because of its cellular location. This area is rich in serotonergic cell bodies where tryptophan hydroxylase is synthesized. One could propose that at its sites of synthesis the concentrations of the enzyme exceed the amount required to control the steady state of serotonin. In conclusion, these experiments substantiate the working hypothesis that adrenal steroids regulate the biosynthesis of tryptophan hydroxylase and through this mechanism they also control the turnover rate of brain serotonin.

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Amantadine-Dopamine Interaction: Possible Mode of Action in Parkinsonism

Abstract. *Intravenous doses of amantadine hydrochloride, an antiviral drug, as small as 0.08 milligram per kilogram may release dopamine and other catecholamines from neuronal storage sites in dogs primed with dopamine. This release may account for the reported efficacy of amantadine hydrochloride in the treatment of human parkinsonism.*

Amantadine hydrochloride (1-adamantanamine hydrochloride, Symmetrel) is an antiviral agent effective against A2 (Asian) influenza in animals (*1*) and in man (*2*). Although it has no anticholinergic activity in tests on animals, amantadine is reported to be effective in the treatment of human parkinsonism (*3*), Schwab's initial observation having been confirmed in a controlled trial by Parkes.

We now describe experiments which may relate observations in animals to the mechanism of action of amantadine in human parkinsonism. Because extrapyramidal function in the basal ganglia, which is disordered in Parkinson's disease, consists of both a cholinergic facilitatory component and an adrenergic inhibitory component (dopaminergic), we studied the interaction of amantadine with these transmitters.

We could not demonstrate a selective anticholinergic effect of amantadine in any of several animal tests. Thus, (i) amantadine was only 1/209,000 as potent as atropine in antagonizing contractions of the guinea pig ileum induced by acetylcholine, and its block of contraction was nonselective. The *p*A2 values [the negative logarithm of the concentration of antagonist that doubles the ED_{50} of agonist (*4*)] were 3.23 ($6.0 \times 10^{-4}M$) and 3.13 ($7.4 \times 10^{-4}M$) for amantadine HCl against acetylcholine and histamine, respectively; and 8.55 ($2.8 \times 10^{-9}M$) and 5.48 ($3.3 \times 10^{-6}M$) for atropine sulfate against acetylcholine and histamine respectively. (ii) Amantadine, at sublethal intravenous doses, did not significantly block the vasodepressor response to acetylcholine in dogs (*5*, table 9). In this test, atropine blocked the acetylcholine response by 50 percent at 0.005 mg/kg administered intravenously. (iii) Unlike atropine, but similar to levodopa, very high oral or intravenous doses of amantadine failed to antagonize tremors induced in mice by oxotremorine (*6*). Thus, it is unlikely that amantadine exerts its antiparkinson effect through an anticholinergic mechanism.

In contrast, our data do indicate that, in dogs primed with dopamine, amantadine may release dopamine and other catecholamines from neuronal storage sites at intravenous doses well below those used orally in the treatment of parkinson patients. Because a deficiency of dopamine, a major neurotransmitter in basal ganglia, is strongly implicated in the etiology of parkinsonism (*7*), we think that the interaction of amantadine with dopamine may be related to its reported clinical effectiveness.

Three groups of six mongrel dogs (5 to 12 kg) were anesthetized (sodium barbital, 200 mg/kg, and sodium pentobarbital, 15 mg/kg, administered intravenously), bilaterally vagotomized, and tracheotomized. Arterial blood pressure was measured with a Statham pressure

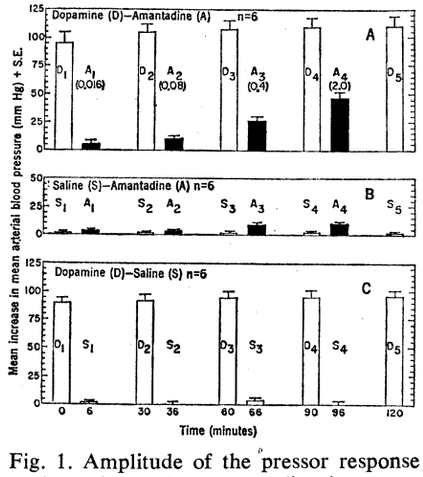

Fig. 1. Amplitude of the pressor response to dopamine and to amantadine in groups of six vagotomized, anesthetized dogs. (A) Each injection of dopamine HCl (0.1 mg/kg, base weight; intravenous) preceded each dose of amantadine HCl by 6 minutes. Amantadine doses (in parentheses) are cumulative milligrams of base weight per kilogram of body weight intravenous (B and C) The dogs were treated similarly to those in (A) except that saline (0.1 ml/kg intravenous replaced dopamine in (B) and saline replaced amantadine in (C). The mean pressor-response values and their standard errors are based on the peak increase in mean arterial pressure recorded during the 60-second period after each injection of dopamine, amantadine, and saline in each dog.

transducer through a cannula implanted in the femoral artery and recorded on a Grass polygraph. Drugs (dopamine hydrochloride and amantadine hydrochloride) were rapidly injected through a polyethylene cannula in the femoral vein in aqueous solutions (0.1 ml/kg). Body temperature was maintained at 37° ± 1°C.

One group of six dogs (Fig. 1A) received five injections of dopamine hydrochloride (0.1 mg/kg, base weight; intravenous) spaced 30 minutes apart. Each of the first four injections of dopamine was followed generally 6, but as much as 8, minutes later by a dose of amantadine hydrochloride [0.016 to 2.0 mg/kg, base weight (cumulative); intravenous]. Two control groups of six dogs each were treated similarly except that one group (Fig. 1B) received saline (0.1 ml/kg; intravenous) instead of dopamine, and the other group (Fig. 1C) received saline in place of amantadine.

Amantadine caused a dose-related pressor response in dogs that were given amantadine 6 minutes after an injection of dopamine (Fig. 1A). The amantadine pressor effect in these dogs primed with dopamine was significant ($P < .05$ by two-tailed t-test) at a dose of 0.08 mg/kg or more when compared with the corresponding response to amantadine in a group of saline control dogs (Fig. 1B). Priming with dopamine had an obvious effect on the pressor response to amantadine, but amantadine did not seem to affect the amplitude of the pressor response to dopamine given 24 minutes later. Although the pressor response to dopamine increased in amplitude over the five successive doses of dopamine (Fig. 1A), the change in amplitude over the five doses was not significantly different from that of the dogs receiving dopamine and saline (Fig. 1C). Thus, we have no convincing evidence that amantadine blocked the uptake of dopamine into peripheral nerve storage sites; at least not under the present experimental conditions. If amantadine had blocked dopamine uptake we should have seen a significant increase in the amplitude of the pressor response to dopamine as a consequence of prior treatment with amantadine. Amantadine blocks uptake of norepinephrine at relatively high intravenous doses (*5*).

In contrast to the pronounced pressor response to amantadine in the dogs primed with dopamine (Fig. 1A), the control dogs which received amantadine after saline (Fig. 1B) showed only a small transient pressor effect which was statistically significant only at the highest dose of amantadine [when compared with the corresponding pressor response to saline (Fig. 1C)]. The pressor response to repeated injections of dopamine increased somewhat, and an injection of saline after dopamine had no significant effect on blood pressure (Fig. 1C). Some dogs receiving saline and amantadine showed a biphasic blood pressure response (a rise followed by a fall in pressure) after the dose of 2.0 mg of amantadine per kilogram (Fig. 1B).

The data in Fig. 1 suggest that amantadine releases catecholamines from peripheral nerve storage sites. We think that amantadine may have the same action within the central nervous system. The slight vasopressor response to amantadine alone (Fig. 1B) was probably not due to a direct action upon receptors, since Vernier *et al.* (*5*) reported that a small positive inotropic effect of amantadine in dogs was abolished by prior treatment with reserpine and was restored by infusion with norepinephrine. The catecholamine-releasing action of amantadine was considerably enhanced in the present study by priming the dogs with dopamine shortly before each injection of amantadine (Fig. 1A). Priming with dopamine did not create but simply amplified an inherent pharmacological action of amantadine, thus making it more readily measurable. The lowest effective intravenous dose of amantadine in dogs primed with dopamine (0.08 mg/kg; intravenous) was well below the oral doses of amantadine used in the treatment of parkinson patients (2 to 3 mg/kg).

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Automated Continuous Culture of Mammalian Cells in Suspension

Abstract. *A system has been developed for the continuous culture of mammalian cells in suspension. The system maintains constant cell concentrations (monitored as the degree of light scattering) over a wide range of previously selected values by automatic additions of known amounts of medium and simultaneous withdrawals of equal volumes of cell suspension.*

We have devised an automated system for the continuous culture of mammalian cells and have used it to maintain a suspension culture of HeLa cells for several months. Our objective was the development of a small bench-top facility which could be used to maintain growing stock cultures of mammalian cells indefinitely in a laboratory not equipped with standard facilities for cell culture, and to grow cells in sufficiently large quantities for biochemical studies. Both criteria have been satisfied. The flexibility of the system allows broad adjustments in performance to accommodate differences in the behavior of various types of cultures; its capacity can be readily varied to suit the requirements of the investigator.

The system incorporates a Nephelostat, controlled by a photocell and originally designed for the continuous culture of microorganisms (Fig. 1) (*1*).