with diazomethane. An isomeric compound, *m/e* 322, present in lower yield, must also be a tetrachlorophenoxyphenol as it formed only a monomethyl ether with diazomethane. The absence of *m/e* 146 in the spectrum of the isomer leaves only the two alternative possibilities with an ether linkage meta to the free hydroxyl group. Two isomeric tetrachlorodihydroxybiphenyls (*m/e* 322) were also present in the reaction products. They formed dimethyl ethers (*m/e* 350) on treatment with ethereal diazomethane.

Studies of product composition were made at several intervals. Conversion to dimeric products was usually less than 5 percent, and no dibenzo-*p*-dioxins could be detected in the reaction mixture. In solution, by sunlight irradiation, the rate of photolysis of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and trichlorodibenzo-*p*-dioxins is relatively rapid (*13*). This probably holds for all tetrachlorodibenzo-*p*-dioxin isomers, so that the extremely rapid breakdown of any dioxin by light may account for our failure to detect dioxins as reaction products. Octachlorodibenzo-*p*-dioxin, in contrast, is broken down more slowly by light than are tetrachloro compounds, and may thus accumulate as a reaction product (*9, 13*). It seems unlikely, therefore, that in an aquatic environment dioxins will be formed from di- or trichlorophenol derivatives such as 2,4-D or 2,4,5-T. At the surfaces of soils or plants, dimerization of chlorophenols is probably limited by the ready availability of other molecular species for reaction with the radicals initially formed by photolysis.

The presence of both riboflavin and oxygen was necessary to obtain measurable yields of products in water. The reaction may proceed through the intermediacy of singlet oxygen which can function by abstraction of hydrogen from the phenol (*14*). Alternatively, riboflavin may participate in hydrogen abstraction reactions (*15*). Further experiments indicate that some reaction also occurs in methanol, but the addition of 2',7'-dichlorofluorescein as sensitizer does not change the quantity of dimer obtained. The former hypothesis, therefore, appears unlikely.

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Dopamine: Release from the Brain in vivo by Amantadine

Abstract. *After dopamine stores in the caudate nucleus of cats were labeled with [^3H]dopamine, the ventricular system was perfused with artificial cerebrospinal fluid. The addition of amantadine to the perfusing fluid caused a dose-related increase in the concentrations of [^3H]dopamine appearing in the perfusion effluent. Subthreshold concentrations of amantadine also enhanced the efflux of [^3H]dopamine induced by electrical stimulation of the caudate nucleus.*

Several clinical reports (*1*) have now confirmed the serendipitous discovery (*2*) that amantadine (*l*-adamantanamine hydrochloride, Symmetrel) ameliorates the symptoms of Parkinson's disease. Efforts are now under way to determine the mechanism of this action.

Reports that the brains of patients suffering from Parkinson's disease contained reduced amounts of dopamine (*3*) have stimulated research on the functional role of this amine in the central nervous system. This research has led to the identification of a dopaminergic nigrostriatal pathway (*4*) which probably has an inhibitory action on neurons in the basal ganglia (*5*). This basic research has culminated in the successful use of L-dopa in the treatment of Parkinson's disease (*6*).

It is reasonable, therefore, that attempts have been made to relate the pharmacological actions of amantadine to an interaction with dopaminergic neuronal systems. In studies in vitro with rat brain striatal slices amantadine increased the rate of dopamine synthesis and increased the release of this amine into the incubating medium (*7*). The pressor effects of amantadine in dogs are enhanced by prior treatment with dopamine, suggesting that amantadine releases dopamine from peripheral sympathetic nerve terminals (*8*). We now report that amantadine causes a selective and dose-related efflux of dopamine from brain structures that line the cerebroventricular system, most likely from the caudate nucleus. Furthermore, low doses of amantadine enhance the efflux of dopamine resulting from electrical stimulation of the caudate nucleus. These results suggest that the antiparkinsonian properties of amantadine may be related to the ability of this drug to increase the concentration of dopamine at postulated receptor sites in the caudate nucleus.

Cats weighing 2 to 3 kg were prepared for perfusion of the cerebroventricular system and for electrical stimulation of the caudate nucleus by modifications of described methods (*9*). The animals were anesthetized with methoxyflurane and placed in a stereotaxic apparatus. The spinal cord was sectioned at the level of the atlas and the animal was maintained on artificial respiration. All wound margins and pressure points were infiltrated with local anesthetic (hexylcaine). A screw-type stainless steel cannula was fixed in a lateral ventricle, and a polyethylene catheter was inserted through the cisterna, under the cerebellum and

into the cerebroaqueduct. Five microcuries of [^{3}H]dopamine (10.6 c/mmole) in a volume of 10 μl was injected into the lateral ventricle. Fifteen minutes later the lateral and third ventricles were perfused with artificial cerebrospinal fluid at a rate of 0.5 ml/min, with the lateral ventricular cannula as the inflow and the cerebroaqueduct as the outflow. In the inital experiments 2.5 μc of [^{14}C]urea (0.27 mc/mmole) in a volume of 20 μl was injected concurrently with the [^{3}H]dopamine. After a 2-hour washout period, when the efflux of total radioactivity had reached a stable level, 1-ml samples of perfusates were collected every 2 minutes. [^{3}H]Dopamine and its deaminated and *O*-methylated metabolites were separated by alumina adsorption and ion-exchange chromatography, and the fractions were subjected to liquid scintillation counting (*9*).

In some experiments the caudate nucleus was stimulated electrically with two electrodes (David Kopf NE-200) placed 5 mm apart; the cathode was at A 18.0, L 4.0, and H + 5, and the anode was at A 13.0, L 4.0, and H + 5 (*10*). The electrodes were inserted at a 24° angle to avoid puncturing the ventricle. Electrode placements were verified by gross examination of sections of the formalin-fixed brain.

When [^{3}H]catecholamines are injected into the lateral ventricle in the manner described, they are concentrated primarily in the ipsilateral caudate nucleus (*11*). Other investigators have demonstrated that intraventricularly administered catecholamines assume cellular and subcellular distribution patterns that are similar to those of endogenous amines (*12*). Accordingly, we assume that the injected [^{3}H]dopamine is distributed primarily to the dopaminergic nerve terminals in the caudate nucleus. This assumption is strengthened by the demonstration that destructive lesions of the nigrostriatal neuronal pathway results in degeneration of dopaminergic but not serotonergic nerve terminals, and in the reduced uptake of [^{3}H]dopamine by the caudate nucleus (*13*).

After 2 hours of washout, the radioactivity in the perfusates consisted of approximately 38 percent dopamine, 5 percent deaminated catechols, 40 percent *O*-methylated amines (presumably 3-methoxytyramine), 17 percent *O*-methylated deaminated metabolites, and less than 1 percent norepinephrine.

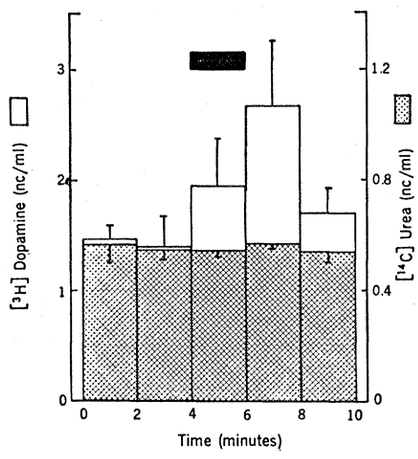

Fig. 1. Effects of amantadine hydrochloride on the concentrations of [^{3}H]dopamine and [^{14}C]urea in cerebroventricular perfusates. The heights of the open bars and the cross-hatched bars represent the mean concentration of [^{3}H]dopamine and [^{14}C]urea, respectively (vertical lines denote 1 standard error; $n = 4$). During the 2-minute period indicated by the solid horizontal bar, amantadine hydrochloride (100 μg/ml) was added to the artificial cerebrospinal fluid. The [^{3}H]dopamine concentrations during and immediately after amantadine perfusion are statistically greater ($P < .05$, Student's *t*-test) than the concentration before amantadine perfusion.

Addition of amantadine (100 μg/ml) to the cerebrospinal fluid during one 2-minute collection period significantly increased the efflux of [^{3}H]dopamine during this and the subsequent collection period (Fig. 1); the concentrations of dopamine metabolites were not consistently altered. The efflux of [^{14}C]urea, however, was not altered by amantadine, suggesting that there is some specificity in the amantadine-induced efflux of [^{3}H]dopamine. The magnitude of this latter effect was related to the concentration of amantadine perfused. In four experiments, the addition of 10 μg of the drug per milliliter had no effect, but amantadine at 30, 100, and 300 μg/ml caused a progressive and statistically significant increase in the efflux of [^{3}H]dopamine (1.92 $\pm$ 0.80, 2.37 $\pm$ 0.13, and 4.95 $\pm$ 0.81 nc/ml, respectively). These values represent the difference between the [^{3}H]dopamine concentration in the perfusate during the 4-minute period immediately after the start of the amantadine infusion and the concentration of [^{3}H]dopamine during the 4-minute period just before the drug was added.

Electrical stimulation of the caudate nucleus increases the efflux of [^{3}H]dopamine into the cerebrospinal fluid (*14*). The efflux of [^{3}H]dopamine did not change when the nucleus was stimulated for 2 minutes at a subthreshold intensity (1-msec pulses of 200 μa intensity at 50 hz). Nevertheless, when this same stimulus was presented in the presence of a subthreshold concentration of amantadine (10 μg/ml), the combination increased the efflux of [^{3}H]dopamine. That is, in four experiments the efflux of [^{3}H]dopamine that accompanied the combination of electrical stimulation and amantadine (1.95 $\pm$ 0.25 nc/ml) was substantially greater than the sum of the effluxes of this amine when the drug (0.10 $\pm$ 0.28 nc/ml) and electrical stimulation (0.07 $\pm$ 0.10 nc/ml) were presented singly.

Amantadine might increase the efflux of [^{3}H]dopamine from the brain by any one of a number of different mechanisms. Unfortunately, it is not possible with our technique to distinguish between an active release and a blockade of reuptake of amine at nerve terminals. The efflux of [^{3}H]dopamine that accompanies the administration of high concentrations of amantadine may be associated with the latter mechanism since similar concentrations block the transport of [^{3}H]dopamine into crude brain synaptosomes (*15, 16*). The same mechanism may also explain why amantadine potentiated the efflux of [^{3}H]dopamine induced by electrical stimulation.

Several other drugs that increase the efflux of catecholamines into cerebroventricular perfusates have central nervous stimulating properties (amphetamine, ephedrine, and methylphenidate) (*17*). In contrast to the actions of these obvious stimulant drugs, amantadine only slightly increases locomotor activity in rodents (*16, 18*). Lack of marked central stimulation would be a desired feature of any drug used in long-term treatment of Parkinsonism.

Amantadine, when administered in vivo, increases the extracellular concentration of dopamine and also enhances the release of this amine in response to electrical stimulation of dopaminergic nerve terminals in the caudate nucleus. These actions may be responsible for the ability of this drug to relieve some of the symptoms of Parkinson's disease.

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Differences in the Distribution of Catecholamine Varicosities in Cat and Rat Reticular Formation

Abstract. *The distribution of catecholamine varicosities within the brainstem reticular formation of the immature cat was determined by means of the formaldehyde-induced fluorescence technique. A continuous pattern of intense, green, medium-sized varicosities exists at nearly all brainstem levels. At most of these levels the varicosities appear within the boundaries of reticular formation nuclei. However, in rostral mesencephalon, some of the varicosities of the pattern lie in proximity to perikarya of the red nucleus. In addition, numerous varicosities in caudal medulla appear to extend from the pattern into nonreticular formation nuclei. A comparable pattern of reticular formation fluorescence is absent in the rat and this finding is believed to represent a true interspecies difference.*

Many data exist concerning the distribution of biogenic amine varicosities within the central nervous systems of certain rodents and carnivores (*1*). While these investigations have reported findings from a great number of rats, little or no definitive data were presented regarding findings from other mammalian species. These detailed studies appear to be representative of the rat, but the extent of correlation which may exist between these findings and other members of the class Mammalia is in question.

Biochemical determinations of relative whole brain concentrations of norepinephrine and dopamine indicate that marked species differences occur between rat and other mammals (*2*). These whole brain differences may reflect a species variability demonstrable with the aid of the fluorescence technique, thus affording a precise anatomic localization of these interspecies differences.

Twelve 6- to 9-week-old kittens and twelve 150- to 700-g rats of both sexes were used. Kittens of this age were chosen for three reasons. First, the cartilaginous nature of their calvaria facilitated rapid removal of the brain stem. Second, I found that adult cats, but not kittens, possess intense yellow-orange autofluorescent granules within perikarya of the majority of brainstem nuclei, and this autofluorescence tends to obscure photographic representations of catecholamine fluorescence. Finally, kittens of the age employed possess relatively stable and adultlike concentrations of brainstem norepinephrine (*3*).

Animals were anesthetized with Nembutal and decapitated. Each brainstem was rapidly removed, transected into five blocks, and prepared for fluorescence microscopy (*4*). Owing to the larger size of the kitten brain a standardization of the method was performed in order to produce an optimal formaldehyde-induced fluorescence (*5*). All animals were killed as close to 1:00 p.m. as possible in an attempt to minimize any effect that endogenous circadian norepinephrine rhythms might have upon the fluorescence microscopic picture (*6*).

The most marked dissimilarity was seen in the form of a continuous pattern of varicosities in the reticular formation of the kitten, but not the rat. In transverse sections it appeared as an oval-shaped pattern of large, bright green varicosities of a medium (3+) density (Fig. 1, A, C, E–I). The pattern was observed at all levels of the brainstem reticular formation with the exception of levels rostral to the red nucleus and caudal to the hypoglossal nucleus. It maintained a position ventrolateral to the medial longitudinal fasciculus at all observed levels. In rostral mesencephalon numerous varicosities were seen in association with the most dorsolateral perikarya of the red nucleus (Fig. 1, A, B, E). Its position in mesencephalon corresponded with the ventromedial portion of the nucleus subcuneiformis of Taber (*7*). In rostral pons it appeared within the central portion of the nucleus reticularis pontis oralis of Brodal (*8*) (Fig. 1F). At midpontine levels, the pattern appeared within the central portion of the nucleus reticularis pontis caudalis just dorsomedial to the trigeminal motor nucleus (Fig. 1, D, G). Within more caudal pons, at the level of the genu of the facial nerve, the constant pattern was seen within the central portion of the nucleus reticularis parvicellularis (Fig. 1C). Its position was dorsolateral to that of the previous level and remained unchanged caudally in pons, at levels of the facial nucleus, and in rostral medulla, at the level of the nucleus ambiguus (Fig. 1H).

In caudal medulla, the pattern was localized within the nucleus reticularis ventralis, just ventrolateral to the hypoglossal nucleus (Fig. 1I). At this level long "strings" of varicosities were seen that extended dorsomedially into the area of the nucleus intercalatus, dorsally toward the dorsal motor nucleus of the vagus and the medial solitary nucleus, ventrally into the remainder of the nucleus reticularis ventralis, and medially into the raphe. In addition, many of the "strings" appeared to traverse one another.

No portion of this constant pattern of reticular formation fluorescence was