percent each of BME amino acid and vitamin solutions (100×, from GIBCO), and 2 percent phytohemagglutinin (Burroughs Wellcome).
4. Dow Chemical ASA, lot No. 36-023-01, dissolved at 37°C in GIBCO-BME culture medium.
5. Nine healthy adults ranging in age from 21 to 48 years participated in the study. X-ray technicians, subjects who have had diagnostic x-ray procedures other than chest x-rays, subjects exposed to x-rays during the previous 6 months, individuals with upper respiratory tract infections or other recent viral diseases, individuals taking any medications chronically, pregnant females, subjects with a history of peptic ulcer or bleeding diathesis, and individuals allergic to aspirin were excluded from the study.
6. Bayer Aspirin, 300-mg tablets, lot No. D 8078.
7. P. S. Moorhead, P. C. Nowell, W. J. Mellman, D. M. Batipps, D. A. Hungerford, *Exp. Cell Res.* **20**, 613 (1960).
8. P. Trinder, *Biochem. J.* **57**, 301 (1954).
9. W. J. Mellman, in *Human Chromosome Methodology*, J. J. Yunis, Ed. (Academic Press, New York, 1965), p. 21.
10. R. G. Scherz, *Stain Tech.* **37**, 386 (1962).
11. T. H. Chang, P. S. Moorhead, J. G. Boué, S. A. Plotkin, J. M. Hoskins, *Proc. Soc. Exp. Biol. Med.* **122**, 236 (1966); M. M. Cohen, K. Hirschhorn, W. A. Frosch, *N. Eng. J. Med.* **277**, 1043 (1967).
12. Using tritiated thymidine pulse labeling, M. A. Bender and D. M. Prescott [*Exp. Cell Res.* **27**, 221 (1962)] have suggested that leukocytes may undergo as many as four divisions in culture.
13. W. L. Stevens, *J. Genet.* **43**, 301 (1942).
14. A. Goldstein and R. Warren, *Biochem. Pharmacol.* **11**, 166 (1962).
15. D. G. Trasler, *Lancet* **1965-I**, 606 (1965); H. J. Klein Obbink and L. M. Dalderup, *ibid.* **1964-I**, 565 (1964); ———, *ibid.* **1964-II**, 152 (1964).
16. L. F. Meisner, S. L. Inhorn, P. M. Nielson, paper presented at Somatic Cell Genetics, 8th Conference, Chatham, Mass., 7–10 October 1969.
17. We thank Marion L. Katz and Lanny Katz (Cytogenetics Group) for technical assistance; Marian Barakat and Connie Ashley (Special Treatment Unit) for collecting blood samples from volunteers; and the subjects who participated in the in vivo study.

17 February 1970; revised 6 April 1970

In vivo Conversion of ^{3}H-L-Tryptophan into ^{3}H-Serotonin in Brain Areas of Adrenalectomized Rats

Abstract. *Rats were adrenalectomized 10 days before we estimated in vivo the conversion index of ^{3}H-tryptophan into radioactive serotonin in brainstem and telediencephalon. We found that the conversion index in the brainstem of adrenalectomized rats is smaller than in the same area of sham-operated rats. Conversely, the conversion index in the telediencephalon was similar in the two groups of rats. The serotonin concentrations were unchanged by adrenalectomy, which suggests that in brainstem the decrease of tryptophan hydroxylase is reflected by the conversion index estimation and not by measurement of serotonin steady-state concentrations.*

Studies on the localization of serotonin (*1*) in brainstem have revealed that most of the serotonin and perhaps the tryptophan hydroxylase activity is associated with cell bodies, where the enzyme is probably synthesized. Corticosterone, the main component of the secretion of rat adrenal glands (*2*), stimulated de novo synthesis of tryptophan hydroxylase in the midbrain when injected into bilaterally adrenalectomized rats (*3*). In these animals the activity of the midbrain tryptophan hydroxylase was reduced by 75 percent when compared with that of sham-operated rats (*3*). Since tryptophan hydroxylase is the rate-limiting enzyme for serotonin biosynthesis (*4*), one might expect that adrenalectomy or injections of adrenal corticosteroids should change the concentrations of brain serotonin in opposite directions. This prediction is not supported by reports which show that adrenalectomy can decrease (*5*), increase (*6*), or leave unchanged (*7*) whole brain serotonin content, while cortisone injections can either increase (*8*) or fail to change (*9*) the brain concentrations of serotonin.

A discrepancy between turnover rate of serotonin and the steady-state concentrations of brain serotonin was reported following inhalation of 100 percent oxygen (*10*) during ether anesthesia (*10*) and after LSD injections (*11*). Therefore, we decided to test whether the reported reduction of tryptophan hydroxylase in the rat midbrain after bilateral adrenalectomy (*3*) changed the in vivo conversion rate of tryptophan (TP) into midbrain serotonin and decreased the serotonin concentrations in midbrain. We have compared the conversion indexes of TP into serotonin in brainstem and in telediencephalon of sham-operated and adrenalectomized rats receiving a pulse injection of ^{3}H-TP. This conversion index (CI) was calculated as proposed by Sedvall *et al.* (*12*) from the relationship

$$CI = \frac{\text{nc serotonin/g}}{\text{nc TP/nmole TP}} \quad (1)$$

where the conversion index has the dimension of nanomoles of serotonin. This formula neither corrects for the continuous efflux rate of the ^{3}H-serotonin from brain nor for the rapid changes of the TP specific activity at the initial times after labeling (*13*). Although the conversion index is not a measure of serotonin biosynthesis, a comparison of the conversion index in different experimental situations may be used to approximate whether or not the turnover rate of serotonin has changed.

Male, Sprague-Dawley rats (180 to 220 g) were bilaterally adrenalectomized or sham-operated by Zivic Miller, Pittsburgh, Pennsylvania. The animals were housed in large group cages upon delivery and maintained with free access to a NaCl solution (0.9 percent) and standard lab chow. A pulse intravenous injection of 500 μc of ^{3}H-L-tryptophan per kilogram (5 c/mmole, New England Nuclear Co.) in 10 ml of phosphate buffer per kilogram (7.4 *p*H, 0.2*M*) was made in the afternoon of the tenth postoperative day and the rats were decapitated either 20 or 40 minutes later. Sham-operated and adrenalectomized rats used as controls received only phosphate buffer. The brain was quickly removed and dissected into brainstem (pons, medulla, and midbrain) and telediencephalon. The latter included all structures anterior to a cut from the anterior part of the superior colliculus to the posterior edge of the mammillary bodies (*14*). These two brain areas were immediately frozen and they were kept frozen until assay of the specific activity of TP and serotonin (*15*). Blood was collected in heparinized tubes and immediately centrifuged to separate the plasma where we assayed TP (*15*) and corticosterone concentrations (*16*). Radioactive TP and serotonin were estimated by liquid scintillation spectrometry in 2.0-ml aliquots from the aqueous phases used to estimate fluorimetrically the amine and the amino acid concentrations.

The average concentration of corticosterone in plasma of sham-operated rats was 34.2 μg/100 ml and that of adrenalectomized rats was 2.7 μg/100 ml. Adrenalectomized rats with concentrations of plasma corticosterone greater than 10 μg/100 ml were discarded.

Table 1 lists the concentrations and specific activity of TP in plasma, brainstem, and telediencephalon of adrenalectomized and sham-operated rats 20 and 40 minutes after the injection of ^{3}H-TP. The concentration of TP of adrenalectomized rats was significantly greater than that of sham-operated rats only in the plasma of experiment 2 (Table 1).

Following adrenalectomy the decrease

Table 1. Concentrations and specific activity of tryptophan in plasma, brainstem, and telediencephalon of sham-operated and adrenalectomized rats injected with ^{3}H-L-tryptophan.

Experiment*	Condition	Plasma†		Brainstem†		Telediencephalon†	
		nmole/g	nc/nmole	nmole/g	nc/nmole	nmole/g	nc/nmole
1 (20)	ADX‡	116 ± 7.2 (9)	2.6 ± 0.12 (4)	23.60 ± 1.63 (9)§	2.0 ± 0.046 (5)	25.9 ± 1.96 (8)	2.0 ± 0.099 (4)
1 (20)	Sham operated	101 ± 4 (10)	2.4 ± 0.21 (5)	18.15 ± 0.89 (10)	2.3 ± 0.22 (5)	21.6 ± 0.98 (10)	2.0 ± 0.18 (5)
2 (40)	ADX‡	130 ± 10 (10)¶	.98 ± 0.04 (5)‖	18.90 ± 0.68 (10)	1.7 ± 0.26 (3)	16.19 ± 0.78	1.3 ± 0.12 (10)
2 (40)	Sham operated	89 ± 4 (10)	0.80 ± 0.038 (5)	21.00 ± 1.18 (10)	1.8 ± 0.28 (4)	17.3 ± 0.92	1.1 ± 0.12 (9)

*Minutes from the intravenous injection of ^{3}H-L-tryptophan in parentheses. †Values are means ± S.E. Numbers of rats in parentheses. ‡ADX = adrenalectomized 10 days before the experiment and kept with 0.15 mole of NaCl. §$P < .01$ when compared with sham-operated rats. ¶$P < .001$ when compared with sham-operated rats. ‖$P < .02$ when compared with sham-operated rats.

Table 2. Conversion of ^{3}H-tryptophan into serotonin in brainstem and telediencephalon of sham-operated and adrenalectomized rats.

Experiment*	Condition	Brainstem serotonin†			Telediencephalon serotonin†		
		nmole/g	nc/nmole	Conversion index‡	nmole/g	nc/nmole	Conversion index‡
1 (20)	ADX§	3.59 ± 0.16 (10)	1.1 ± 0.10 (3)¶	1.62 ± 0.28 (3)‖	1.96 ± 0.16 (10)	0.70 ± 0.17 (4)	1.44 ± 0.22 (4)
1 (20)	Sham-operated	3.23 ± 0.089 (10)	4.1 ± 0.79 (3)	4.68 ± 0.34 (3)	1.96 ± 0.33 (9)	1.0 ± 0.22 (4)	1.92 ± 0.61 (4)
2 (40)	ADX§	3.01 ± 0.13 (10)	1.4 ± 0.13 (4)	1.90 ± 0.18 (3)¶	1.31 ± 0.06 (15)	1.5 ± 0.18 (10)	1.01 ± 0.14 (9)
2 (40)	Sham-operated	3.38 ± 0.15 (10)	2.2 ± 0.55 (6)	3.65 ± 0.62 (6)	1.38 ± 0.14 (12)	2.0 ± 0.29 (6)	1.44 ± 0.56 (6)

*Minutes from the intravenous injection of ^{3}H-L-tryptophan in parentheses. †Values are means ± S.E. Numbers of rats in parentheses. ‡Expressed in nanomoles of newly formed serotonin remaining in the tissue sample, per gram, calculated for each animal according to Eq. 1. §ADX = adrenalectomized 10 days before the experiment and kept with 0.15 mole of NaCl. ¶$P < .01$ when compared with sham-operated rats. ‖$P < .001$ when compared with sham-operated rats.

of the plasma concentrations of cortical steroids may lower the activity of TP pyrrolase to a basal level and therefore lead to a higher plasma or brain concentration of TP. In plasma, a great proportion (70 to 80 percent) of TP (*17*) is highly bound to proteins, and this binding may also control the plasma concentrations of the free amino acid which presumably is the fraction available to the uptake of brain neurons.

The serotonin concentrations in brainstem and telediencephalon of sham-operated and adrenalectomized rats are comparable, which suggests that 10 days after adrenalectomy the adrenal steroids are not essential for the maintenance of steady-state concentrations of brain serotonin. However, in experiment 1 of Table 2 the serotonin specific activity of the brainstem was lower in adrenalectomized than in sham-operated rats by about 75 percent. However, a comparison of the actual turnover rate of serotonin after pulse injections of ^{3}H-TP could not be based on the serotonin specific activity measured only at one time. This view is supported by the curve describing the changes of serotonin specific activity after pulse injections of the ^{3}H-TP (*13*). When the turnover rate of brain serotonin is either increased or decreased all the characteristics of the curve change (*13*). On a semilogarithmic plot the slopes of the upward and downward phase are steeper than normal when the turnover rate is increased; the reverse is true when the turnover rate is decreased. Both changes determine a shift of the plateau: to the left when turnover is increased or to the right when turnover is decreased. Hence, if we compare points taken during the downward slope an increased turnover rate is characterized by a brain serotonin specific activity lower than that of controls, while the reverse is true when the serotonin turnover rate is decreased. The specific activity of serotonin in brainstem and telediencephalon reported in Table 2 is in agreement with the foregoing. It has also been reported (*13*) that in control rats the turnover rate of serotonin in brainstem is faster than in telediencephalon; accordingly the data reported in Table 2 show that the specific activity of serotonin in brainstem of sham-operated rats declines from 20 to 40 minutes after labeling; in contrast, that of telediencephalon is still increasing from 20 to 40 minutes. In addition, the data listed in Table 2 show that the specific activity of serotonin in brainstem of adrenalectomized rats but not that of sham-operated rats is similar at the two times studied; in contrast, the specific activity of serotonin in telediencephalon appears to increase both in sham-operated and adrenalectomized rats. This consideration would suggest that in adrenalectomized rats the serotonin turnover rate is decreased in the brainstem and is unaffected in telediencephalon. These indexes are listed in Table 2 and show that in the brainstem of adrenalectomized rats the conversion index is significantly lower than that of sham-operated rats. However, in telediencephalon, adrenalectomized and sham-operated rats have similar conversion indexes. A preliminary in vitro comparison of the tryptophan hydroxylase activity of telediencephalon of sham-operated and adrenalectomized rats (10 days before the experiment) revealed that the enzyme activity of the latter area is slightly reduced (*18*).

The data reported in Tables 1 and 2 suggest that the decrease of the conversion index in brainstem of adrenalectomized rats is neither related to a change in substrate concentration nor to a change in the serotonin concentration due to a defect of serotonin binding and consequent acceleration of metabolism. As an alternative, we suggest that adrenocortical secretion controls in vivo activity of tryptophan hydroxylase in brain, thus confirming and extending previous reports (*3*). These results indicate that in brainstem, where most of

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.

E. C. AZMITIA, JR.
Rockefeller University, New York
S. ALGERI, E. COSTA
Laboratory of Preclinical Pharmacology, National Institute of Mental Health, Saint Elizabeths Hospital, Washington, D.C. 20032

References

1. K. Fuxe, *Z. Zellforsch.* **61**, 710 (1964); A. Dahlström and K. Fuxe, *Acta Physiol. Scand. Suppl. 232* (1964).
2. I. E. Bush, *J. Endocrinol.* **9**, 95 (1953).
3. E. C. Azmitia, Jr., and B. S. McEwen, *Science* **166**, 1274 (1969).
4. H. Green and J. L. Sawyer, *Fed. Proc.* **24**, 604 (1965).
5. D. DeMaio, *Science* **129**, 1678 (1959).
6. T. R. Put and J. W. Meduski, *Neerlandia* **11**, 240 (1962); A. K. Pleifer, E. C. Vizi, E. Sartory, E. Galambos, *Experientia* **19**, 482 (1963).
7. S. Garattini, L. Lamesta, A. Mortari, V. Palma, R. Valzelli, *J. Pharm. Pharmacol.* **13**, 385 (1961).
8. D. DeMaio and C. Marbbrio, *Arch. Sci. Med.* **111**, 369 (1961).
9. C. T. McKennee, P. S. Timiras, W. B. Quay, *Neuroendocrinology* **1**, 251 (1968).
10. P. M. Diaz, S. H. Ngai, E. Costa, *Advan. Pharmacol.* **6B**, 75 (1968).
11. R. C. Lin, S. H. Ngai, E. Costa, *Science* **166**, 237 (1969).
12. G. C. Sedvall, V. K. Weise, I. J. Kopin, *J. Pharmacol. Exp. Ther.* **159**, 274 (1968).
13. N. H. Neff, A. Groppetti, P. F. Spano, E. Costa, *ibid.*, in press.
14. W. Zeman and J. R. Innes, Eds., *Craigies's Neuroanatomy of the Rat* (Academic Press, New York, rev. ed. 1963). This dissection was selected because histochemical studies have shown that most of the serotonin contained in brainstem is located in cell bodies, while the serotonin in telediencephalon is stored mainly in axons and nerve terminals (*1*).
15. E. Costa, P. F. Spano, A. Groppetti, S. Algeri, N. H. Neff, *Atti Accad. Med. Lombarda* **23**, 1096 (1968).
16. D. Glick, D. von Redlick, S. Levine, *Endocrinology* **74**, 656 (1964).
17. R. H. McNenamy, C. C. Lund, D. F. H. Wallach, *J. Clin. Invest.* **39**, 1675 (1960).
18. E. C. Azmitia, Jr., and B. S. McEwen, unpublished observation.

23 January 1970; revised 23 April 1970

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

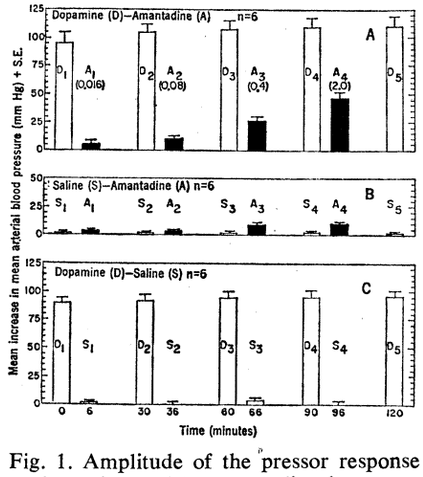

Fig. 1. Amplitude of the pressor response to dopamine and to amantadine in groups of six vagotomized, anesthetized dogs. (A) Each injection of dopamine HCl (0.1 mg/kg, base weight; intravenous) preceded each dose of amantadine HCl by 6 minutes. Amantadine doses (in parentheses) are cumulative milligrams 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.