mented with various nutritives. Any growth resulting from the addition of a particular material would imply that it contained essential nutrient or nutrients normally supplied to the flagellates by diplosomes or bipolar bodies. For each test, 2 ml of desired medium was inoculated with 2×10^6 flagellates, and growth, if any, was determined by hemocytometer count at late-log phase. Growth was considered positive only when flagellates continued to grow successfully for at least five transfers in the same medium. Each medium was tested at least twice in duplicate. Among various additives tested, only aqueous liver extract (1 : 20, Nutritional Biochemicals Corp.) at a concentration of 0.25 percent in Trager's medium (TM + L) stimulated the growth of cured strains (Table 1). A higher concentration (over 1 percent) of liver extract was inhibitory. Cured strains did not grow in Trager's medium enriched with fetal bovine serum, whole blood, or blood lysate of rabbit. However, the presence of these materials in TM + L seemed to improve or stabilize their growth in that thin pellicles were often formed and the cell count increased slightly (Table 1).

Although the growth factor in liver extract for the cured strains has not been characterized, its use with Trager's medium (which normally contains hemin) has made possible a comparison of the hemin requirement of normal and cured strains. The defined medium from which hemin was omitted (TM − H) was tested for growth of the normal strains and, supplemented with 0.25 percent liver extract (TM − H + L), for that of the cured strains (Table 1). The results showed clearly that normal strains of both species do not require hemin. On the other hand, strains free from endosymbiotes have a hemin requirement that is not supplied adequately by the liver extract. These symbiote-free strains thus resemble in their hemin requirement other species of trypanosomatids that normally do not have endosymbiotes, such as *C. fasciculata* and *Leishmania tarentolae* (*18*).

Our findings confirm that diplosomes of *B. culicis* and bipolar bodies of *C. oncopelti* are bacterial endosymbiotes. Furthermore, they indicate that these endosymbiotes supply their hosts with hemin and other essential nutrients.

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Cyclic Adenosine Monophosphate: Selective Increase in Caudate Nucleus after Administration of L-Dopa

Abstract. *Treatment with the dopamine precursor* L-*dopa produced a significant accumulation of adenosine 3′,5′-monophosphate (cyclic AMP) in the caudate nucleus of the rat. In contrast, there was no change in the amount of cyclic AMP in the cerebellum. Accumulation of cyclic AMP in the caudate nucleus after administration of* L-*dopa was prevented by prior treatment with the decarboxylase inhibitor RO 4-4602. These observations and those in other laboratories support the assumption that dopamine formed from* L-*dopa selectively activates striatal adenylate cyclase. The in vivo activation of adenylate cyclase after treatment with* L-*dopa may be a useful model for studying neurological and psychiatric disorders that are thought to involve the dopaminergic system of the brain.*

A dopamine-sensitive adenylate cyclase has been identified in the sympathetic ganglia (*1*), the retina (*2*), and the caudate nucleus (*3*). These in vitro studies and a variety of supporting evidence led to the hypothesis that this enzyme represents a "dopamine-receptor" and that adenosine 3′,5′-monophosphate (cyclic AMP) may be implicated in synaptic transmission in the central nervous system (*3*).

We report here that when L-dopa is

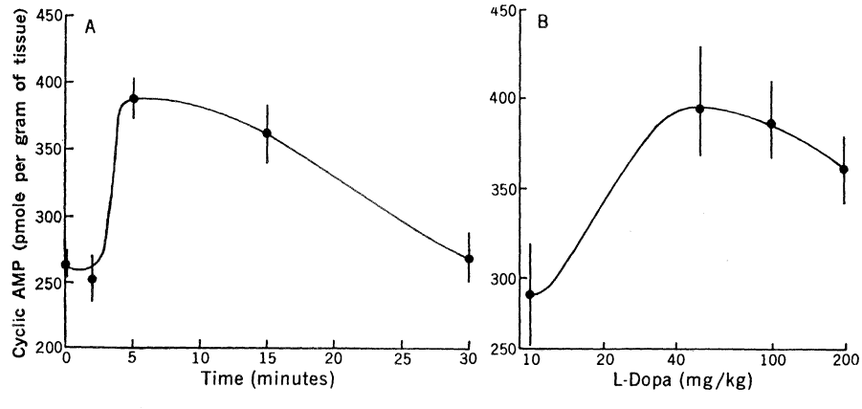

Fig. 1. (A) Concentration of cyclic AMP in the caudate nucleus of the rat after injection of L-dopa (100 mg/kg, intraperitoneally). Rats were killed in a microwave oven, and cyclic AMP was assayed by a protein-binding procedure (*7*). Data are means ± standard errors of mean (S.E.M.) for four to nine determinations. Cyclic AMP concentrations were significantly different ($P < .01$) from control values 5 and 15 minutes after L-dopa treatment. (B) Concentration of cyclic AMP in the caudate nucleus of the rat after various doses of L-dopa. Animals were killed 5 minutes after the injection; data are as in (A). Cyclic AMP concentrations were significantly different ($P < .01$) from control values at L-dopa doses of 50, 100, and 200 mg/kg.

given systemically, there is an increase in the concentration of cyclic AMP in the caudate nucleus. Presumably, L-dopa is converted by aromatic amino acid decarboxylase to dopamine, which in turn activates a dopamine-sensitive adenylate cyclase found in this region of the brain. In support of this hypothesis, we found that the accumulation of cyclic AMP in the caudate nucleus was prevented by treatment with the decarboxylase inhibitor *N*-(DL-seryl)-*N'*-(2,3,4-trihydroxybenzyl)-hydrazine (RO 4-4602) (*4*). In the cerebellum, where there are few dopamine-containing neurons and therefore few dopamine receptor sites, L-dopa treatment did not change the concentration of cyclic AMP even though dopamine is formed from L-dopa in the cerebellum (*5*).

L-Dopa, RO 4-4602, and solvent were administered intraperitoneally to male Sprague-Dawley rats of about 160 to 180 g. The RO 4-4602 was administered in water; L-dopa was titrated with hydrochloric acid until a clear solution was obtained and the solution was then diluted with water. Diluted solutions of acid had no effect on the concentration of cyclic AMP of brain. Animals were killed in a microwave oven (*6*), and the cerebellum and the caudate nuclei were rapidly removed and immediately frozen on Dry Ice. Cyclic AMP was assayed by a protein-binding method (*7*).

A highly significant rise in cyclic AMP concentration was observed in the caudate nucleus 5 minutes after injection of L-dopa (100 mg per kilogram of body weight), and the concentrations returned to normal in about 30 minutes (Fig. 1A). A dose-response curve for the effect of L-dopa on cyclic AMP in the caudate nucleus is shown in Fig. 1B. Accumulation of cyclic AMP was maximal after L-dopa doses of about 50 to 100 mg/kg; the response tended to diminish at doses of 200 mg/kg. Prior treatment with the decarboxylase inhibitor RO 4-4602 (500 mg/kg) prevented the accumulation of cyclic AMP in the caudate nucleus (Table 1). The cerebellum, in contrast to the caudate nucleus, showed no significant changes of cyclic AMP concentration after L-dopa treatment (Table 2).

The absence of effect of L-dopa treatment on cerebellar cyclic AMP content and the absence of an in vitro effect of dopamine on the adenylate cyclase of the cerebellum and other brain areas (*3*, *8*) suggests that the increase of cyclic AMP in the caudate nucleus is induced by activation of a dopamine-sensitive adenylate cyclase in this tissue (*3*), the dopamine being formed from the injected L-dopa. In support of this notion, blocking the formation of dopamine from L-dopa by administering RO 4-4602 (*4*) prevented the accumulation of cyclic AMP. Dopamine does not inhibit striatal phosphodiesterase (*3*), which might be an alternative explanation for the cyclic AMP accumulation. Also, the possibility that L-dopa itself might inhibit phosphodiesterase in vivo can be excluded in our study; after a high dose of RO 4-4602, L-dopa concentration rose sharply in the caudate nucleus (*4*), but there was no increase in cyclic AMP (Table 1).

Table 1. Prevention of L-dopa–induced accumulation of cyclic AMP in the caudate nucleus of the rat by prior treatment with RO 4-4602. All animals received two intraperitoneal injections 30 minutes apart. They were killed in a microwave oven 5 minutes after the second injection, and cyclic AMP was measured by a protein-binding assay (*7*). Treatments were as follows: group A, water followed by L-dopa solvent; group B, water followed by L-dopa (100 mg/kg); group C, RO 4-4602 (500 mg/kg) followed by L-dopa solvent; group D, RO 4-4602 (500 mg/kg) followed by L-dopa (100 mg/kg). Data are means ± standard error of the mean.

Group and treatment	Cyclic AMP (picomoles per gram of tissue) (*4*)
A. Solvent	262 ± 38
B. L-Dopa	448 ± 22*
C. RO 4-4602	253 ± 8
D. RO 4-4602 + L-dopa	237 ± 59

* $P < .01$ compared with solvent treatment.

Table 2. Effect of L-dopa on cyclic AMP concentrations of rat cerebellum. Rats were killed in a microwave oven, and cyclic AMP was measured by a protein-binding assay (*7*). Data are means ± standard error of the mean. None of the values differed significantly from those for solvent-treated animals; *N*, number of determinations.

Treatment	Cyclic AMP (picomoles per gram of tissue)	*N*
*Effect of L-dopa dose**		
Solvent	783 ± 74	6
L-Dopa (50 mg/kg)	780 ± 86	4
L-Dopa (100 mg/kg)	791 ± 189	4
L-Dopa (200 mg/kg)	828 ± 13	4
Effect of time after injection†		
Solvent	806 ± 79	9
5 minutes	791 ± 189	4
15 minutes	750 ± 195	4

* Animals were killed 5 minutes after intraperitoneal injection. † L-Dopa (100 mg/kg) was administered intraperitoneally.

Our studies, as well as those in vitro, suggest that the therapeutic effects of L-dopa in Parkinson's disease may be due to the activation of a dopamine-sensitive cyclase associated with the striatum. We and others (*9*) observed a decreased responsiveness of adenylate cyclase with increasing doses of agonist (Fig. 1B). Thus, there may be a dose of L-dopa that produces a maximal therapeutic effect in man, and larger doses may actually decrease the therapeutic response and at the same time expose the patient to central side effects.

Altered sensitivity of the dopamine receptor in the striatum has been implicated in the hyperkinesias associated with L-dopa treatment, in Huntington's disease, in drug-induced dyskinesias, and in manic-depressive psychosis (*10*). Activation of the dopamine-sensitive adenylate cyclase in the caudate nucleus after treatment with L-dopa may be a useful model for studying the pathophysiology and treatment of these disorders.

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