gine concentrations for more than 19 days as compared to only 10 days for those receiving the free enzyme. We believe that in vivo lysis of the asparaginase-loaded cells slowly released the entrapped enzyme and maintained a higher enzyme concentration for the longer interval.

In summary, resealed RBC ghosts show promise as a slow-release vehicle for enzyme therapy. A single injection of asparaginase-loaded RBC's results in prolonged asparaginase activity which may produce safer and more effective therapeutic results than the conventional regimen of multiple injections of the enzyme. Substantial protection against degradation of the therapeutic enzyme by endogenous proteolytic enzyme or by antibody-complexing appears probable. For those therapeutic enzymes that should be targeted at the reticuloendothelial system, immobilization in RBC's may offer the additional advantage of release directly into this system. Partial uptake of the labeled RBC's was detected by gamma counter scans over the spleens and livers of monkeys used in these experiments.

The effect of slow release of the enzyme into the reticuloendothelial system is not yet clear. Tumor located in the reticuloendothelial system might be destroyed more effectively. Because the enzyme is released slowly at a site distant from the bronchial smooth muscle, this technique might also prevent life-threatening allergic reactions.

We conclude that entrapment of enzyme in RBC's offers a promising new strategy for enzyme therapy. This approach appears particularly advantageous for treatment of the enzyme deficiency–related storage diseases and the asparagine-dependent cancers of the reticuloendothelial system.

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Dopamine-Sensitive Adenylate Cyclase Occurs in a Region of Substantia Nigra Containing Dopaminergic Dendrites

Abstract. *The zona reticulata, the subdivision of the substantia nigra containing dendrites of the dopaminergic nigro-neostriatal neurons, contains dopamine-sensitive adenylate cyclase activity. This nigral dopamine receptor is similar to the striatal dopamine receptor. These and previous data suggest a physiological role (or roles) for dopamine in the substantia nigra.*

Dopamine has been implicated as a neurotransmitter in the mammalian nervous system (*1*). Also, dysfunctions in dopamine metabolism in the brain may be involved in the etiology of parkinsonism (*1*) and schizophrenia (*2*). Biochemical, physiological, and pharmacological evidence suggests that a postsynaptic receptor for dopamine regulates the formation of adenosine 3′,5′-monophosphate (cyclic AMP) (*3*). Thus, a dopamine-sensitive adenylate cyclase has been demonstrated in caudate nucleus, nucleus accumbens, and olfactory tubercle, regions of the brain containing the axonal terminals of dopaminergic neurons (*4–6*). Furthermore, lesions that destroyed the dopaminergic nerve terminals within the caudate nucleus did not diminish this dopamine-sensitive enzyme activity, thereby suggesting that this enzyme is located predominantly on postsynaptic structures (*7*).

Electrophysiological evidence suggests that dopamine receptors might also occur on the dopaminergic neurons located within the substantia nigra (*8*). Because of the possibility that a presynaptic dopamine receptor might exist in this region, we initiated a search for the biochemical model of this receptor. We now report a dopamine-sensitive adenylate cyclase activity in homogenates of the zona reticulata of the substantia nigra of the rat brain.

The substantia nigra is divided into three parts: the zona compacta, which contains the cell bodies of the nigro-neostriatal dopaminergic neurons; the zona reticulata, which contains the dendrites of these dopaminergic neurons; and the pars lateralis (*9*). These three areas were separated by a microdissection technique and the adenylate cyclase activity was determined (*10*). A dopamine-sensitive adenylate cyclase activity occurred in homogenates of the zona reticulata (Table 1); in this region, dopamine caused a 74 percent increase in enzyme activity. In homogenates of either the zona compacta or the pars lateralis the effect of dopamine was less pronounced

Table 1. Regional distribution of dopamine-sensitive adenylate cyclase in rat substantia nigra. Data represent mean ± S.E.M. of adenylate cyclase activity, which was determined in homogenates of the three subdivisions of the substantia nigra as described (*10*). *N* represents the number of individual tissue homogenates in which the effect of dopamine was determined; for each separate tissue homogenate, enzyme activity was measured in three to six replicate samples of the homogenate, both in the absence and in the presence of dopamine. The statistical significance of the data was determined with a two-tailed *t*-test.

Region	*N*	Adenylate cyclase activity (cyclic AMP per mg protein per minute)		
		No addition	Dopamine (100 μM)	Percent stimulation by dopamine
Zona reticulata	16	45.7 ± 2.5	79.9 ± 3.7*	74
Zona compacta	10	52.8 ± 5.6	72.1 ± 8.7†	36
Pars lateralis	9	44.5 ± 3.5	51.0 ± 3.6†	15

*P = .001 versus no addition. †Not significantly different from no addition.

than in homogenates of zona reticulata.

The dopamine receptor that stimulates the nigral adenylate cyclase activity was further characterized (Fig. 1, A and B); we used homogenates of the zona reticulata, since this tissue gave the greatest response to dopamine. Both dopamine and *l*-norepinephrine could increase the adenylate cyclase activity in homogenates of the zona reticulata (Fig. 1A). However, dopamine was more potent than *l*-norepinephrine; concentrations producing half-maximal stimulation were approximately 10 μM for dopamine and 100 μM for *l*-norepinephrine, respectively. The synthetic catecholamine, *l*-isoproterenol, which stimulates β-adrenergic receptors, had no effect on the enzyme activity when tested at concentrations as high as 1000 μM. Maximally effective concentrations of the *N*-methyldopamine analog, epinine, caused slightly more than a doubling of enzyme activity (Fig. 1B); half-maximal stimulation of enzyme activity was achieved with a concentration of 8 μM epinine.

Since chlorpromazine is a competitive antagonist of the dopamine receptor in other brain regions (*6*), the potency and kinetic mechanism of action of this compound as an antagonist of the nigral dopamine receptor were examined. In the presence of low concentrations of chlorpromazine, higher concentrations of dopamine were required to stimulate the enzyme activity (Fig. 1C); however, the maximal stimulatory effect of dopamine was unaltered. These results are consistent with a kinetic competition between dopamine and chlorpromazine for the dopamine receptor; if we assume such a kinetic competition, the calculated affinity of chlorpromazine for the dopamine receptor is 67 nM (*6*).

The occurrence in the substantia nigra of adenylate cyclase activity that is sensitive to dopamine raises the possibility that a receptor for dopamine, similar to the well-characterized striatal dopamine receptor regulating adenylate cyclase, may occur within the substantia nigra. The comparison of the properties of the nigral and striatal dopamine receptors regulating adenylate cyclase activity does not distinguish between them. Thus, for both the nigral and striatal dopamine receptors, dopamine is a more potent agonist than is *l*-norepinephrine (*4*). Likewise, the affinity of chlorpromazine for these two dopamine receptors (67 nM in nigra; 66 nM in caudate) are approximately equal (*6*). Furthermore, the ineffectiveness of *l*-isoproterenol as an agonist, and the approximate equipotency of dopamine and epinine as agonists distinguish both the nigral and the striatal dopamine receptors from a β-adrenergic receptor (*11*).

Our results demonstrate the occurrence within the substantia nigra of a dopamine-sensitive adenylate cyclase. This enzyme activity may represent a receptor mechanism for dopamine within the substantia nigra; however, the precise cellular location of the nigral dopamine-sensitive adenylate cyclase is still unknown. It was necessary to determine whether the nigral dopamine-sensitive adenylate cyclase is associated with the dopaminergic nigro-neostriatal neurons. Thus, unilateral injections of 6-hydroxydopamine (8 μg in 4 μl) were made into the left substantia nigra of five rats. Approximately 3 months after the injection, the animals were killed, and adenylate cyclase activity was measured in homogenates of the zona reticulata of both the left and the right substantia nigra of each animal (*12*). There was no difference in either the basal or the dopamine-stimulated adenylate cyclase activities in homogenates of zona reticulata from the intact and injected sides of the brains;

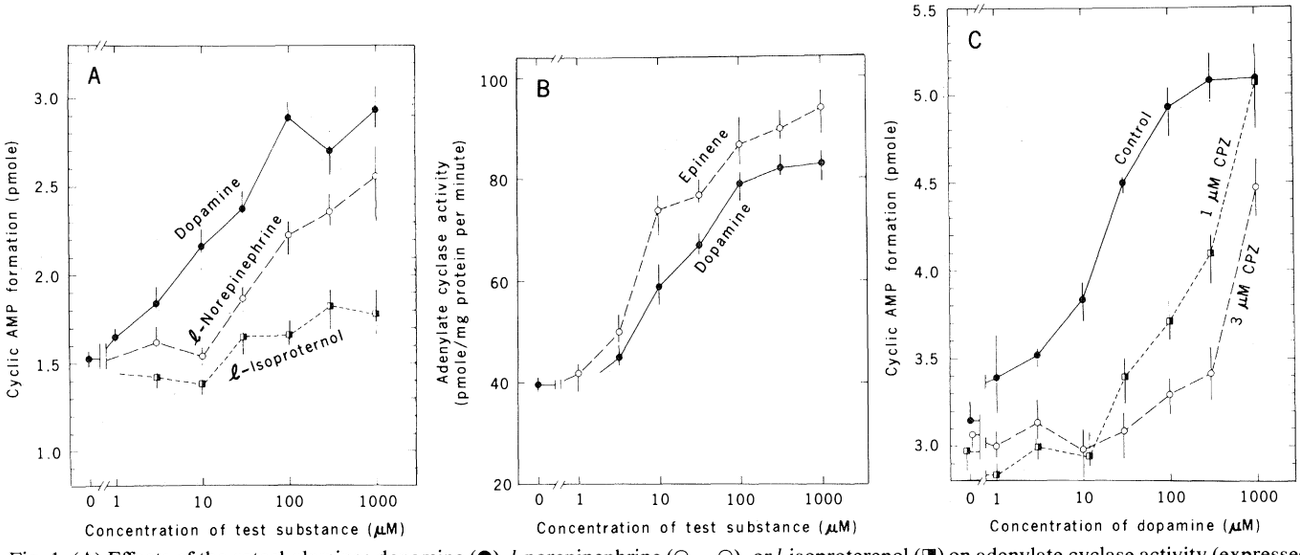

Fig. 1. (A) Effects of the catecholamines dopamine (●), *l*-norepinephrine (○----○), or *l*-isoproterenol (◨) on adenylate cyclase activity (expressed as picomoles of cyclic AMP formed per 5 minutes) in a homogenate of the zona reticulata of the substantia nigra. Enzyme activity was measured as described (*10*). The data for no test substance addition represent mean ± S.E.M. (N = 18) and the data for test substance additions represent mean and range (N = 3) for enzyme activity measured in replicate samples of the single homogenate. In this experiment, no cyclic AMP was detected when enzyme assay tubes were placed in boiling water for 2 minutes prior to the addition of ATP. (B) Effects of dopamine (●——●) or epinine (○----○) on adenylate cyclase (expressed as picomoles of cyclic AMP formed per milligram of protein per minute) in a homogenate of the zona reticulata of the substantia nigra. Enzyme activity was measured as described (*10*). The data for no test substance addition represent mean ± S.E.M. (N = 14) and the data for test substance additions represent mean and range (N = 3) for enzyme activity measured in replicate samples of a single homogenate. No cyclic AMP was measured when enzyme assay tubes were placed in boiling water for 2 minutes prior to the addition of ATP. (C) Effects of dopamine on adenylate cyclase activity (picomoles of cyclic AMP formed per 5 minutes) in a homogenate of the zona reticulata and pars lateralis of the substantia nigra either in the absence (●) or in the presence of 1 μM (◨) or 3 μM (○) chlorpromazine. Enzyme activity was measured as described (*10*). The data represent mean ± S.E.M. (N = 6) for no dopamine addition, and the data for dopamine additions represent mean and range (N = 3) for enzyme activity measured in replicate samples of a single homogenate. In this experiment, no cyclic AMP was detected when enzyme assay tubes were placed in boiling water for 2 minutes prior to the addition of ATP. The inclusion of the pars lateralis in the tissue homogenate used for this experiment may account for both the higher enzyme activity in the absence of added dopamine and the lesser fold stimulation of enzyme activity by dopamine in comparison with the experiment represented in (A).

furthermore, there was no change in the affinity of dopamine for the receptor regulating adenylate cyclase activity in these homogenates. Examination by the Falk-Hillarp technique of both substantia nigra from an additional rat which received a comparable injection of 6-hydroxydopamine demonstrated that the experimental treatment destroyed the dopaminergic nigro-neostriatal neurons on the injected side of the brain. These data demonstrate that the dopamine-sensitive adenylate cyclase in the zona reticulata of the substantia nigra can be separated from the dopaminergic nigro-neostriatal neurons and, therefore, suggest that this enzyme activity is not associated with these cells.

The presence in the substantia nigra of mechanisms (*13*) for both the uptake of dopamine and the potassium-stimulated, calcium-dependent release of dopamine, as well as the presence of dopamine receptors on both the dopaminergic neurons (*8*) and other anatomically undefined cells (our results reported here) raises the possibility that within this region of the brain dopamine may have a physiological role (or roles) similar to the roles of dopamine in the striatum, where the uptake, storage, and release of dopamine have been demonstrated and where both pre- and postsynaptic receptors for dopamine occur (*7, 13, 14*). Our results suggest that the substantia nigra might represent another site (in addition to the neostriatum) where either antipsychotic drugs or drugs used to treat Parkinson's disease could affect the regulation by dopamine of physiological activity in the extrapyramidal system (*15*).

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10. Samples of the tissue were isolated with a 300-μm (inside diameter) needle from frozen sections (300 μm) of rat brain; the zona compacta, zona reticulata, and pars lateralis of the substantia nigra are the subdivisions of the substantia nigra identified as regions 44, 45, and 46, respectively, by M. Palkovits, M. Brownstein and J. M. Saavedra [*Brain Res.* **80**, 237 (1974)]. Tissue was homogenized in a solution of 2 m*M* tris(hydroxymethyl)aminomethane maleate (tris-maleate) (*p*H 7.4) and 2 m*M* EGTA ([ethylenebis (oxyethylenenitrilo)]tetraacetate). Adenylate cyclase activity in homogenates was assayed in a mixture (final volume, 0.05 ml) containing (mmole/liter): tris-maleate, *p*H 7.4, 80; theophylline, 10; $MgSO_4$, 6.0; EGTA, 0.8; and adenosine triphosphate (ATP), 1.5; 0.01 ml of tissue homogenate containing approximately 10 μg of protein; the test compounds were added at the concentrations indicated. The assay of enzyme activity was initiated with the addition of ATP; all samples were incubated at 30°C for 5 minutes in a shaken water bath. The incubations were terminated by placing the tubes in a boiling water bath for 2 minutes. Under these assay conditions, the enzyme activity in all regions studied was proportionate to both the duration of the incubation at 30°C and the concentration of tissue homogenate. All salts were of reagent grade. Catecholamines were obtained from Sigma, Calbiochem, or Aldrich Chemical Company; chlorpromazine was supplied by Smith Kline & French. The cyclic AMP content of the entire sample was measured by the method of B. L. Brown, J. D. M. Albano, R. P. Ekins, A. M. Sgherzi, and W. Tampion [*Biochem. J.* **212**, 561 (1971)] with the use of 4 pmole (30 c/mmole) of cyclic AMP. Protein was determined by the method of O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall [*J. Biol. Chem.* **193**, 265 (1951)].
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Binding of C-Reactive Protein to Antigen-Induced but Not Mitogen-Induced T Lymphoblasts

Abstract. *The C-reactive protein (CRP), an acute phase reactant which binds selectively to T (thymus-derived) lymphocytes, was found to bind to lymphoblasts formed upon stimulation with antigens but not with mitogens. Binding of CRP thus serves as a marker for antigen-reactive (-reacted) as opposed to mitogen-reactive (-reacted) T cells, suggesting that these represent separate subpopulations, and supports the developing concept that CRP plays an important role in the regulation of responses critical to inflammation, host defense, and tissue repair.*

C-reactive protein (CRP) is a trace constituent of serum that was originally defined by its calcium-dependent precipitation with the C polysaccharide (CPS) of the pneumococcus (*1*). Its concentration in the blood, which increases during the acute phase of febrile illnesses and a variety of tissue-destructive or inflammatory processes, has long served as a clinical indicator of these states (*2*). It has been reported that CRP binds selectively to 30 to 40 percent of human peripheral blood lymphocytes, and that the lymphocytes to which the CRP binds are primarily T cells (*3*). This binding is associated with inhibition of the formation of T cell rosettes with sheep erythrocytes, the mixed lymphocyte reaction (MLR), and the generation of cytotoxic lymphocytes (*3, 4*). By contrast, proliferative responses to the T cell mitogens phytohemagglutinin (PHA) and concanavalin A (Con A) are unaltered (*3, 4*). Further, CRP by itself does not induce lymphocyte blastogenesis, is not cytotoxic for lymphocytes in culture, and does not bind to lymphocytes with complement receptors or surface immunoglobulin (*3*); it reacts with theta-bearing cells derived from mouse spleen but not from mouse thymus (*4*). Taken together, these findings suggested that CRP binds to and alters the function of a certain fraction, but not all, of T lymphocytes.

It seemed possible that the T cells binding CRP were cells at a particular stage in their reproductive cycle, cells that had been injured, or cells of a T cell subpopulation. To examine these possibilities, we studied the binding of CRP to human lymphoblasts obtained by stimulation with PHA, Con A, allogeneic cells, and purified protein derivative (PPD) of tuberculin.

For these experiments, the CRP substrate CPS was obtained as described (*5*) from a capsulated pneumococcal variant (Cs), lyophilized, and stored until use. Purified human CRP was obtained by affinity chromatography of ascites or pleural fluids on CPS covalently bound to Bio-Gel according to a method previously described (*6*); the CRP was then dialyzed against saline, sterilized with a Millipore filter, stored at 4°C, and used within 30 days. Lymphocytes were obtained by centrifugation of heparinized blood from normal human donors on Ficoll-Hypaque; they were washed three times in RPMI 1640 medium containing Penn-Strep (50 μg/ml) and 10 percent heat-inactivated fetal calf serum (International Scientific Industries), and resuspended to the appropriate concentration.