Localization of Nigral Dopamine-Sensitive Adenylate Cyclase on Neurons Originating from the Corpus Striatum

Abstract. *Nigral basal adenylate cyclase and dopamine-sensitive adenylate cyclase, glutamate decarboxylase, choline acetyltransferase, and tyrosine hydroxylase activities were measured in rats with hemitransections at various levels or with electrolytic lesions of the medial forebrain bundle or the crus cerebri. The loss of nigral dopamine-sensitive adenylate cyclase activity after the various brain lesions was correlated with the loss of nigral glutamic acid decarboxylase but not that of tyrosine hydroxylase; nigral choline acetyltransferase was unaffected in all cases. The data indicate that the nigral dopamine-sensitive adenylate cyclase activity may be localized on neurons afferent to the nigra, probably originating from the globus pallidus and possibly from the tail of the caudate. The results suggest that dopamine, released from nigral dendrites, may influence dopaminergic activity indirectly by modulating impulses transmitted to the nigrostriatal neurons through the crus cerebri.*

The substantia nigra (SN) contains the cell bodies (pars compacta) and the dendritic processes (pars reticulata) of the nigro-neostriatal dopaminergic neurons (*1*). Recent biochemical and pharmacological evidence indicates that dopamine (DA) released from the dendrites of the pars reticulata acts as a neurotransmitter in the nigra (*2*) and possibly mediates an intranigral mechanism regulating the activity of the dopaminergic nigro-neostriatal neurons (*3–5*). This hypothesis has been strengthened by the finding in the nigra of DA-sensitive adenylate cyclase, demonstrating the existence of DA receptors in this area (*6*).

To clarify the origin and localization of nigral DA-sensitive adenylate cyclase, we studied the effect of brain lesions on the adenylate cyclase activity and on enzymatic markers of specific nigral neurons. Our results strongly suggest that nigral DA-sensitive adenylate cyclase is located on neurons originating from the corpus striatum. The data suggest that DA, released from nigral dendrites, may influence dopaminergic activity indirectly by modulating impulses transmitted to the nigro-neostriatal neurons through the crus cerebri.

Male Charles River rats (200 to 250 g) were used in all the experiments. Hemitransections were performed as described by McGeer *et al.* (*7*), using the coordinates of König and Klippel (*8*). Electrocoagulations were made by unipolar tungsten electrodes, and the extent of the lesion was verified histologically and recorded as described by Costall and Naylor (*9*). Four days after the lesions the rats were killed and substantia nigra tissue was obtained as described in Table 1. Nigral adenylate cyclase activity was measured as described by Kebabian *et al.* (*10*); adenosine 3′,5′-monophosphate (cyclic AMP) was assayed by the protein kinase method of Kuo and Greengard (*11*). Dopamine-sensitive adenylate cyclase activity was always measured in the presence of 100 μM DA.

In addition to adenylate cyclase activity, we measured in the same homogenate of nigra the following enzymatic activities: glutamic acid decarboxylase (GAD) (*12*), a marker of neurons containing γ-aminobutyric acid (GABA); choline acetyltransferase (CAT) (*13*), a marker of cholinergic neurons; and tyrosine hydroxylase (TH) (*14*), a marker of the dopaminergic neurons ascending to the neostriatum. Protein was determined as described by Lowry *et al.* (*15*). The effect of the lesions on nigral enzymes was estimated by comparing the activities of nigral homogenates from the intact and from the lesioned side.

Figure 1 shows the levels at which hemitransections were performed and the extent and location of the electrocoagulations. As Table 1 shows, prepallidal hemitransections carried at the level of the anterior commissure (A7190 of König and Klippel) failed to affect both basal and DA-stimulated adenylate cyclase activity of nigral homogenates. In agreement with the results of McGeer *et al.* (*7*), these hemitransections failed to affect significantly nigral GAD or

Table 1. Effect of brain hemitransections and electrocoagulations on the activities of basal and DA-stimulated adenylate cyclase, glutamic acid decarboxylase (GAD), tyrosine hydroxylase (TH), and choline acetyltransferase (CAT) in homogenates of rat substantia nigra pars reticulata. Four days after the lesions, rats were killed and substantia nigra was punched out by stainless steel cannulas, using the anterior commissure as the landmark. Serial 400-μm coronal slices were made by cryostat. The wet weight of substantia nigra tissue was 2.6 ± 0.2 mg [mean ± standard error of the mean (S.E.M.) for 20 samples]. Enzyme activities and protein concentration were measured according to the methods cited in the text, with minor modifications. Stimulation of adenylate cyclase activity by DA was estimated in the presence of 100 μM DA. For adenylate cyclase assay, 50 μl of homogenate (1 : 40 by volume) was incubated with [8-^{14}C]adenosine triphosphate (~ 600,000 counts per minute; 60 mc/mmole; Amersham/Searle). After incubation for 3 minutes the following counting rates were obtained (counts per minute; mean ± S.E.M. for ten samples): blank, 36 ± 5; basal, 235 ± 25; and with DA present (+DA), 436 ± 40. Enzyme activities (nanomoles per hour per milligram of protein) in nigral homogenates from the intact side were as follows: TH, 11.5 ± 0.6; GAD, 985 ± 42; and CAT, 8.6 ± 0.7 (mean ± S.E.M. of ten determinations). Protein concentration was not significantly modified by any of the lesions. Cyclic AMP formation is given as picomoles per minute per milligram of protein; GAD, TH, and CAT are expressed as percentages of the values for the intact side. Results are expressed as the mean ± S.E.M. of at least seven determinations; *N* is the number of animals receiving lesions.

Area	*N*	Cyclic AMP formation				GAD	TH	CAT
		Intact side		Lesioned side				
		−DA	+DA	−DA	+DA			
				Hemitransections				
Prepallidal	32	201 ± 13	414 ± 23*	180 ± 9	398 ± 19*	80 ± 6.5	66 ± 3.8†	102 ± 7.2
Postpallidal	36	193 ± 8	390 ± 20*	77 ± 5†	83 ± 7‡	32 ± 2.5†	55 ± 3.0†	96 ± 6.3
Midhypothalamic	28	195 ± 9	386 ± 18*	75 ± 6†	80 ± 6‡	30 ± 2.2†	52 ± 3.5†	98 ± 7.5
				Electrocoagulations				
Globus pallidus	32	180 ± 12	380 ± 15*	107 ± 9†	135 ± 18‡	42 ± 3.5†	72 ± 4.5†	103 ± 6.8
Crus cerebri	32	179 ± 11	375 ± 20*	110 ± 10†	125 ± 15‡	37 ± 2.8†	88 ± 6.5	97 ± 6.5
Medial forebrain bundle	28	184 ± 13	373 ± 22*	165 ± 12	295 ± 30*	65 ± 7.2†	56 ± 3.8†	101 ± 8.5

*$P < .01$ compared to values obtained in the absence of DA (−DA). †$P < .01$ compared to corresponding values for the intact side. ‡Not significantly different from values obtained in the absence of DA.

CAT, but produced a 35 percent decrease of nigral TH, which is likely to be the result of a retrograde degeneration of the nigro-neostriatal dopaminergic neurons secondary to the interruption of the connections between the nigra and the head of the caudate (*16*). Postpallidal hemitransections (level A5660) produced a 60 percent decrease of basal adenylate cyclase activity and a complete loss of the ability of DA to stimulate nigral adenylate cyclase activity. Glutamic acid decarboxylase activity also decreased by 68 percent, while CAT was not affected. Tyrosine hydroxylase decreased by 45 percent. Midhypothalamic hemitransections (A4230) produced changes in nigral enzymes very similar to those obtained after postpallidal hemitransections, and in agreement with the results of McGeer *et al.* (*7*) and of Störm-Mathisen (*17*).

Large electrocoagulations of the globus pallidus (see Fig. 1) produced a large loss of nigral DA-sensitive adenylate cyclase and of GAD, a 40 percent decrease of basal adenylate cyclase activity, and a 30 percent decrease of TH, but no change in nigral CAT.

Electrocoagulations of the crus cerebri (see Fig. 1), where the striatonigral neurons are reported to course (*18*), resulted in a total loss of DA-stimulated adenylate cyclase activity and in a significant loss of basal adenylate cyclase activity, while GAD decreased by 65 percent and CAT remained unaffected. The lesions of the crus cerebri did not significantly affect nigral TH activity.

Electrocoagulations of the medial forebrain bundle (see Fig. 1), which decreased nigral TH by 45 percent, resulted in a slight loss of DA-stimulated adenylate cyclase activity and GAD, but not of basal adenylate cyclase activity or CAT.

The results obtained with the postpallidal hemitransections show that interruption of the connections between the complex "globus pallidus–postcommissural caudate" and the substantia nigra produces a rapid loss of both basal and DA-stimulated adenylate cyclase activity in the substantia nigra. Hemitransections at the level of the anterior commissure, which interrupt the connections between the head of the caudate and the substantia nigra, are without effect on nigral adenylate cyclase activity. On the other hand, lesions of the globus pallidus and of the crus cerebri, where most of the afferent nigral connections and in particular the pallidonigral and caudatonigral neurons are reported to run (*18*), completely and rather selectively abolish nigral DA-sensitive adenylate cyclase activity.

These results strongly suggest that all the DA-sensitive adenylate cyclase activity of the substantia nigra is localized on neurons connecting the complex globus pallidus–postcommissural caudate with the substantia nigra. The slight decrease of nigral DA-sensitive adenylate cyclase after electrocoagulations of the medial forebrain bundle can be explained as due to partial damage of the adjacent crus cerebri. The lack of correlation between the decrease of TH, the marker of dopaminergic neurons, and the loss of DA-sensitive adenylate cyclase activity agrees with localization on nondopaminergic neurons (*6*).

The rapidity of the loss of nigral adenylate cyclase activity after brain lesions favors its localization on neurons undergoing anterograde degeneration; that is, afferent to the nigra. This, in turn, is in agreement with the afferent nature of the nondopaminergic connections between the striatum and the substantia nigra (*18*).

The loss of nigral DA-sensitive adenylate cyclase produced by the lesions appears to be correlated with a loss of nigral GAD, a marker of GABA-containing terminals (*7, 17*). This correlation, while confirming the afferent nature of the connections containing the cyclase, might suggest its localization on GABA-containing neurons. On the other hand, one cannot dismiss the possibility that the DA-sensitive adenylate cyclase of the nigra is localized on striatal non-GABA-containing neurons running parallel to the GABA-containing ones. Indeed, there is now evidence for a substance P–containing tract descending from the striatum to the nigra, which might well contain the nigral DA-sensitive adenylate cyclase (*19*).

An interesting result of our experiments is that postpallidal hemitransections produce a large loss of basal adenylate cyclase activity. This does not appear to be due to nonspecific damage of the nigra, since nigral CAT remains unmodified. On the other hand, the decrease of DA-sensitive adenylate cyclase activity can be dissociated from the loss of basal activity, since discrete lesions of the crus cerebri produce complete loss of DA-sensitive cyclase activity and only a 30 percent decrease of basal activity. These results, while indicating that basal and DA-sensitive adenylate cyclase of the nigra originate from the striatum, suggest that they are associated with different neuronal systems.

Since DA-sensitive adenylate cyclase

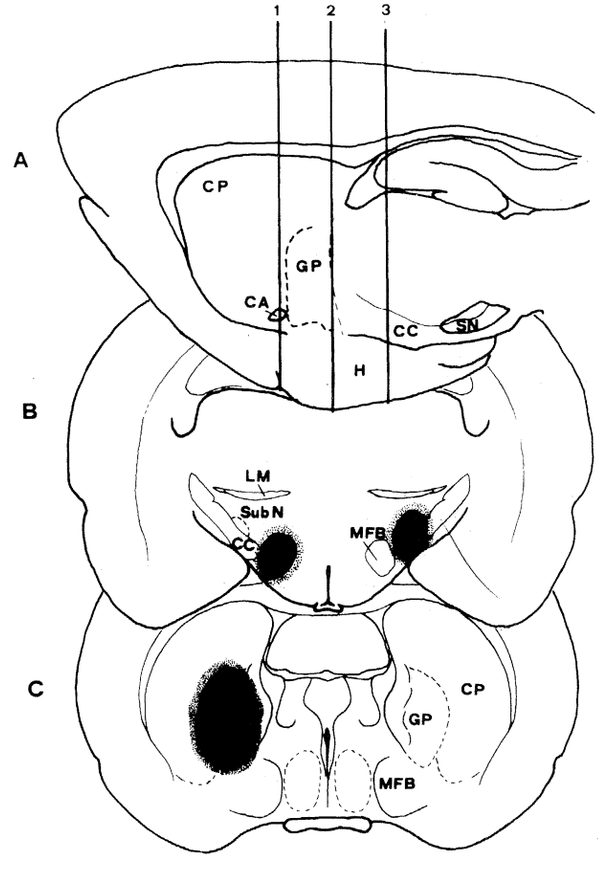

Fig. 1. Diagrammatic representation of the localization and extent of the brain lesions. (A) Level (*8*) of the hemitransections, which are numbered as follows: 1, prepallidal, A7190; 2, postpallidal, A5660; and 3, midhypothalamic, A4230. (B) Diagram (A3750) (*8*) of the electrocoagulative lesion produced in the crus cerebri and in the medial forebrain bundle as constructed from the histological data obtained from ten and eight rats, respectively. (C) Diagram (A6360) (*8*) of the electrocoagulative lesion of the globus pallidus as constructed from data obtained from nine rats. (Dark shading) Tissue damage common to all rats; (stippling) damage observed occasionally. Abbreviations: *CA*, anterior commissure; *CC*, crus cerebri; *CP*, caudatus putamen; *GP*, globus pallidus; *H*, hypothalamus; *LM*, lemniscus medialis; *MFB*, medial forebrain bundle; *SN*, substantia nigra; and *Sub N*, subthalamic nucleus.

is a marker of DA receptors (*20*), our results can be taken to indicate the presence in the nigra of DA receptors localized on connections afferent to it.

Based on our findings and on recent results indicating DA release within the substantia nigra (*2*), a new mechanism of regulation of the activity of dopaminergic neurons may be postulated: nigral DA, by activating nigral DA-sensitive adenylate cyclase, would modulate transmitter release from nigral afferent connections, thus influencing the activity of the DA neurons. In particular, nigral DA might inhibit the release onto DA neurons of an excitatory transmitter (such as substance P) (*17*) or stimulate that of an inhibitory one (such as GABA) (*7, 17*), thus mediating a depression of dopaminergic activity. This model would explain the stimulation of DA firing produced by neuroleptics as due to blockade of nigral DA-sensitive adenylate cyclase and the inhibition produced by amphetamine as due to activation of the cyclase by DA released within the nigra.

This interpretation appears to reconcile the findings of Groves *et al.* (*4*) and of Bunney and Aghajanian (*5*) on the inhibition of dopaminergic firing by amphetamine. If one assumes with Groves *et al.* that amphetamine acts by releasing DA onto nigral DA receptors located on DA neurons, it is not possible to explain why interruption of afferent nigral connections blocks amphetamine effects, as shown by Bunney and Aghajanian. Conversely, if one postulates with Bunney and Aghajanian that amphetamine inhibits its DA firing by releasing DA onto striatal postsynaptic DA receptors, then it is difficult to justify its effectiveness when infused within the substantia nigra, as shown by Groves *et al.* Our model provides a unitary interpretation of these results. However, our findings do not exclude the existence of DA receptors localized on the membrane of DA neurons (autoreceptors) (*21*). Stimulation of these receptors would explain the finding of Bunney and Aghajanian that inhibition of dopaminergic firing by apomorphine is not abolished by interruption of afferent nigral connections. The significance of autoreceptors for the physiological regulation of dopaminergic activity, however, remains to be established (*22*).

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Lens Cataract Formation and Reversible Alteration in Crystallin Synthesis in Cultured Lenses

Abstract. *Embryonic chick lenses developed cortical cataracts and altered their pattern of δ-crystallin synthesis within 3 hours, if cultured without their vitreous body or traumatized with their vitreous body attached. δ-Crystallin reverted to the normal pattern by 24 hours in the cataractous lenses. Thus, biochemical differences that are only observable during the initial stages of cataractogenesis can exist between opaque and normal lenses.*

An ocular lens opacity, or cataract, often occurs naturally with age (senile cataract), develops as a congenital anomaly of chromosomal, genetic, or viral origin, or arises as a consequence of diseases occurring later in life, of metabolic stress, of trauma, or of various forms of physical or chemical insults to the lens (*1*). Cataracts can affect vision seriously and lead to blindness. Lens opacities cannot generally be reversed, and thus the usual treatment for an advanced cataract is surgery. Although many histological, cytological, and biochemical aberrations are associated with opaque lenses, the mechanisms underlying cataract formation are not known. Two very important advances concerning this problem include the recognition that polyol accumulation leads to osmotic imbalance in sugar-induced cataracts (*2*) and the evidence that protein aggregation leads to light scattering in senile cataracts (*3*). To the best of our knowledge, there has never been a direct demonstration of an alteration in protein synthesis associated with cataract formation. We now report that the synthesis of the principal crystallin of the embryonic chick lens, δ-crystallin (*4*), is reversibly affected during the initiation of cataract formation in vitro.

Our ability to study protein synthesis during cataract formation became possible when we discovered that surgically excised embryonic chick lenses develop cortical cataracts within 3 hours of culture unless the vitreous body is not detached from the posterior lens capsule (Fig. 1). Vitreous-associated lenses remained clear when cultured for 48 hours. A similar protective action by the vitreous body toward cataract formation has been observed earlier in cultured rabbit lenses, where Chylack and Kinoshita pointed out that a disordered relation between the vitreous body and the lens may contribute to the development of a posterior subcapsular human senile cataract or affect the ability of the human lens to withstand metabolic stress (*5*). Those authors provided evidence that