evidence suggests that oxidative metabolism is only marginally involved, if at all, with Na-K transport.

Addition of KCl to the PSS stimulated Na-K transport and development of isometric force; both O_2 consumption and lactate production were substantially increased (Table 1). The steady-state isometric force developed under stimulation by KCl, however, was found to be relatively unaffected by ouabain. Therefore one can investigate the effects of ouabain on stimulated Na-K transport at nearly constant levels of contractility. As Fig. 1 shows, steady-state J_{O_2} under stimulation by KCl in the presence of ouabain was virtually identical to the J_{O_2} under such stimulation in the absence of ouabain. On the other hand, lactate production, even in the presence of KCl, was again found to be greatly inhibited by ouabain. Thus, whereas stimulation and inhibition of Na-K transport is accompanied by parallel changes in aerobic glycolysis in VSM, oxygen consumption bears little relation to Na-K transport even though it is strongly associated with increases in isometric force.

Since aerobic glycolysis was estimated from measurements of lactate in the bathing medium, these experiments cannot distinguish between a direct coupling of glycolysis to the energetics of Na-K transport or to an effect on lactate permeability. The latter mechanism is unlikely, however, since the reported values of vascular lactate content, and changes in content with stimulation (*13*), are small compared to the rates of lactate efflux. Preliminary experiments measuring the lactate content of porcine coronary vessels indicate that, under conditions in which KCl has been added or potassium has been substituted for sodium, the change in lactate content from basal levels can be appreciable; however, the changes parallel those measured in the bathing medium. Thus the effects reported here for aerobic glycolysis would be minimal estimates, suggesting that glycolysis and Na-K transport are very tightly coupled.

Complete understanding of the nature of the coupling between Na-K transport and glycolysis may come with further experimentation. However, the coupling of glycolysis to Na-K transport via membrane-bound glycolytic enzymes, as postulated for erythrocytes (*14*), would appear to be a plausible model. A distribution of mitochondria suitable to the energy demands of membrane transport processes may not be possible in the smooth muscle cell, given that contractile force is transmitted by attachments of the myofilaments to dense bodies on the plasma membrane. Thus aerobic glycolysis in VSM, which derives most of its adenosine triphosphate from oxidative phosphorylation, may have evolved to favor the mechanical efficiency of the cell.

Although the exact nature of the coupling of aerobic glycolysis to Na-K transport-related processes is unknown, our results suggest that the reported increase in lactate production associated with vascular myopathy (*5*) may more reflect changes in Na-K transport processes than a nonspecific degradation of metabolism.

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A Direct Role of Dopamine in the Rat Subthalamic Nucleus and an Adjacent Intrapeduncular Area

Abstract. *The subthalamic nucleus, a clinically important component of the extrapyramidal motor system, and a lateral area extending into the peduncle contain catecholamine terminals and dopamine receptors coupled to adenylate cyclase. In addition, dopamine agonists administered in vivo enhance glucose utilization in the region. Thus, neuronal function in this region is directly affected by dopamine and dopaminergic drugs.*

The subthalamic nucleus (STN), a relatively small nucleus of the extrapyramidal motor system, is known for its vulnerability to stroke damage in humans and the resultant involuntary limb-flinging movements called hemiballismus (*1*). Previous neuroanatomical studies in animals have suggested that catecholamines do not play a direct role in the function of the STN (*2*). In a study of rats injected with [^{14}C]deoxyglucose as a metabolic tracer, however, we found that a dopamine (DA) agonist, apomorphine, markedly increased glucose utilization in the STN (*3*), suggesting either a direct action of apomorphine on DA receptors in the STN or an indirect action via the striatum, where a high density of DA receptors exists (*4*). We present anatomical and biochemical evidence here that the STN of rats is a DA receptor area with DA afferents, and that in addition, and unexpectedly, there is a region of neuropil within the cerebral peduncle, just lateral to the STN, which is also a DA receptor area.

Anatomical studies of the STN region were carried out with eight male Sprague-Dawley rats (200 to 300 g). Three rats were perfused with a glutaraldehyde solution for light and electron microscopy (*5*), and five rats were perfused with glyoxylic acid for catecholamine fluorescence histochemistry (*6*). Examination of the STN region by light and electron microscopy revealed

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that many of the large cells of the STN extend ventrolaterally into the peduncle in bands (Fig. 1A). Deep into the peduncle there was a small number of isolated neurons and neuropil. Synaptic profiles were seen on neurons and in neuropil within the white matter of the peduncle, and many could be classified either as axosomatic or axodendritic (Fig. 1B). Some synaptic profiles were indeterminate, and some may have been axo-axonic.

The glyoxylic acid histochemical studies of the STN region revealed catecholamine-containing axons and varicosities in most parts of the STN (Fig. 1C) and in the adjacent intrapeduncular area. Many of these varicosities had the extremely delicate and fine-varicose appearance of known DA axons and terminal varicosities (*7*). Whereas ascending catecholamine axons traverse the STN at its most anterior and dorsal portions, a significant portion of the varicosities we observed had the appearance of a terminal plexus area, perhaps from axon collaterals. Although the number and intensity of these varicosities in the STN were low compared with those of the striatum, they were similar to the density and intensity seen in the substantia nigra reticulata studied with the same technique. Dopamine-containing cell bodies were not observed within the STN of the rat, although they have been described in the posterior STN of the cat (*8*).

For assessment of DA receptors in the STN and related areas, the DA-stimulated adenylate cyclase (AC) system was used. This system has served as a sensitive biochemical marker for study of DA receptors in other brain regions (*4*). In the present studies, tissue punches from a brain area from both halves of the brains of six to eight male Sprague-Dawley rats (200 to 300 g) were pooled. To confirm the anatomical location of the punch, the remainder of the slices were fixed, sectioned, and stained with Luxol blue (*9*). The pooled tissues were homogenized and assayed for AC activity (*10*).

Dopamine effectively stimulated AC activity in the STN punch (Fig. 2). Norepinephrine also stimulated activity, but a tenfold greater concentration was required for an equivalent effect. Isoproterenol, a β-adrenergic receptor agonist, showed little effect even at high concentrations ($10^{-4}M$). Apomorphine, on the other hand, was effective. The stimulation by DA was effectively inhibited by the potent DA receptor antagonists fluphenazine (Fig. 2) and haloperidol (85 percent inhibition of 10 μM DA by 10 μM haloperidol). Basal activity and the absolute amount of DA-stimulated activity in the STN were nearly equal to those in the substantia nigra, but were one-third to one-fourth of those in the striatum when dissected and assayed under the same conditions (Fig. 2). The percent stimulation produced by DA in the STN at maximally effective concentrations (113 ± 15 percent; $10^{-4}M$ DA) was as great as that generally observed in rat striatum (*4*). Also, the relative potency of the agents used was similar to that generally obtained in the striatum (*4*).

The cerebral peduncle adjacent to the STN was also examined for AC activity. We found DA-stimulated activity comparable in absolute amount and in percentage stimulation (112 ± 32 percent; $10^{-4}M$ DA) to that in the STN itself (Fig. 2). The activity in the peduncle was stimulated by apomorphine as well as by DA, and the stimulation by DA was blocked by fluphenazine. By contrast, another white matter area, the corpus callosum, did not contain DA-stimulated activity (Fig. 2). Activity in the peduncle was also stimulated by isoproterenol, and the stimulation by norepinephrine was somewhat greater than DA, which suggests the presence of another receptor that remains to be characterized, but which is probably β-adrenergic. The intrapeduncular area may represent a unique system for the study of DA receptors coupled to AC, because, in comparison with the ratio in the striatum, the ratio of the DA receptors to other receptors and neural elements may be high.

Finally, we examined the intrapeduncular area for changes in glucose utilization with a DA agonist. This study was carried out using the [^{14}C]deoxyglucose autoradiographic technique (*11*) in combination with a systemic injection of L-dopa (*12*) in unanesthetized rats. The DA agonist L-dopa caused a marked increase over controls in the [^{14}C]deoxyglucose density in the STN itself (Fig. 1D), as previously reported with apomorphine.

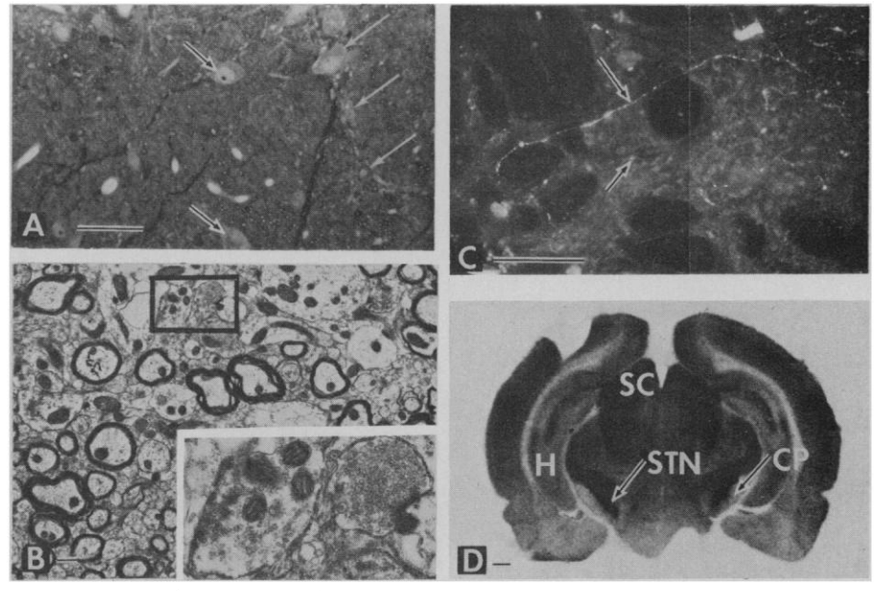

Fig. 1. (A) A toluidine blue section (1 μm thick) of the cerebral peduncle area just ventrolateral to the junction of STN proper and the peduncle. The field is occupied almost exclusively by myelinated fibers except for a few large isolated neurons (black arrows). White arrows point to a band of neurons and neuropil radiating ventrolaterally away from STN proper. (B) Electron micrograph of a neuropil area deep into the white matter of the cerebral peduncle. A small region of neuropil with axodendritic synapses (rectangle and inset) is seen amid the myelinated and unmyelinated axons. Inset is an enlargement of the rectangular area, where two typical synapses are visible. The vesicles in the synapse on the left in the inset form distinct clusters. (C) Fluorescence photomicrograph of catecholamine axons and varicosities in the anterior STN. The tissue was cut in a horizontal plane through the left side. A varicose axon with visible intervaricose segments (top arrow) crosses the anterior pole of STN from its medial border to its lateral region. In this anterior serrated region of STN, fibers of the peduncle form large bundles (round and oval dark areas). Other very fine fluorescent fibers and single varicosities are visible in STN proper, but they have a low intensity and are sparsely distributed. The relatively large number of varicosities in the upper right corner are outside (medial to) the STN. (D) [^{14}C]Deoxyglucose autoradiogram of a coronal brain section of a rat treated with L-dopa. The lens shape of the STN proper (left arrow) is well delineated on both sides by its high density, which has been enhanced over controls by the L-dopa treatment. In the cerebral peduncle just ventrolateral to the STN, fine lines of higher density than the surrounding region are visible. They are oriented in a ventrolateral direction (right arrow and left side). The peduncle area ventrolateral to the STN is also diffusely grayer than the area of the peduncle dorsolateral to the STN. Scale bars: (A) 100 μm, (B) 1 μm, (C) 100 μm, and (D) 500 μm; *STN*, subthalamic nucleus; *CP*, cerebral peduncle; *SC*, superior colliculus; *H*, hippocampus.

Also fine lines of increased density projected ventrolaterally into the peduncle from STN (Fig. 1D); a diffuse increase in density was also present in the peduncle adjacent to the anterior two-thirds of the STN. Reexamination of autoradiograms of rats treated with apomorphine showed the same effect. Thus, an in vivo functional measure of gray matter activity delineated the intrapeduncular region.

Although no other studies have suggested that STN is a DA-sensitive area, Versteeg *et al.* (*13*) reported that STN contained DA; the amounts of DA were similar to those found in the substantia nigra. As demonstrated in our experiments, the two regions also had similar DA-stimulated AC activity. Both regions also receive input from neurons containing γ-aminobutyric acid (GABA) (*14*), and in the case of the substantia nigra it has been proposed that the DA receptor is localized on the GABA (or substance P) terminal (*15*). The DA receptors in STN may be similarly located, thus exerting a presynaptic influence.

The question arises whether the neurons and neuropil of the intrapeduncular area represent an extension of STN or a distinct group of cells. The bands of increased density that are continuous with STN in the autoradiograms suggest that the intrapeduncular area is an extension of STN. Others (*16*) have noted that a few STN cells are embedded in the peduncle or that the most anterior STN in rats has a serrated appearance, with gray matter and dendrites extending for short distances into white matter. Our data expand this concept and increase its potential for functional importance. Alternatively, the peduncle area may be considered distinct from STN. In his atlas of the human brain, Riley (*17*) labeled an area distinct from STN and within the peduncle as nucleus pontes grisei pedunculares, and this may have an analogous area in the rat.

Damage to the intrapeduncular gray as well as to the STN may indeed be an important part of the pathology involved in hemiballismus. In rhesus with ballismus, the histological data show damage to both peduncle and STN (*18*). Also, antagonism of DA by haloperidol at receptor sites in the STN or peduncle may help to explain the clinical improvement produced by haloperidol in some patients with hemiballismus (*19*).

We have shown that dopaminergic receptor mechanisms are present in the subthalamic nucleus and in a lateral extension into the cerebral peduncle, that there is indeed a catecholaminergic substrate for the receptor activity, and that with systemic administration of L-dopa these receptor areas are metabolically active. Thus, dopamine is likely to influence output of the extrapyramidal system through an unexpected route.

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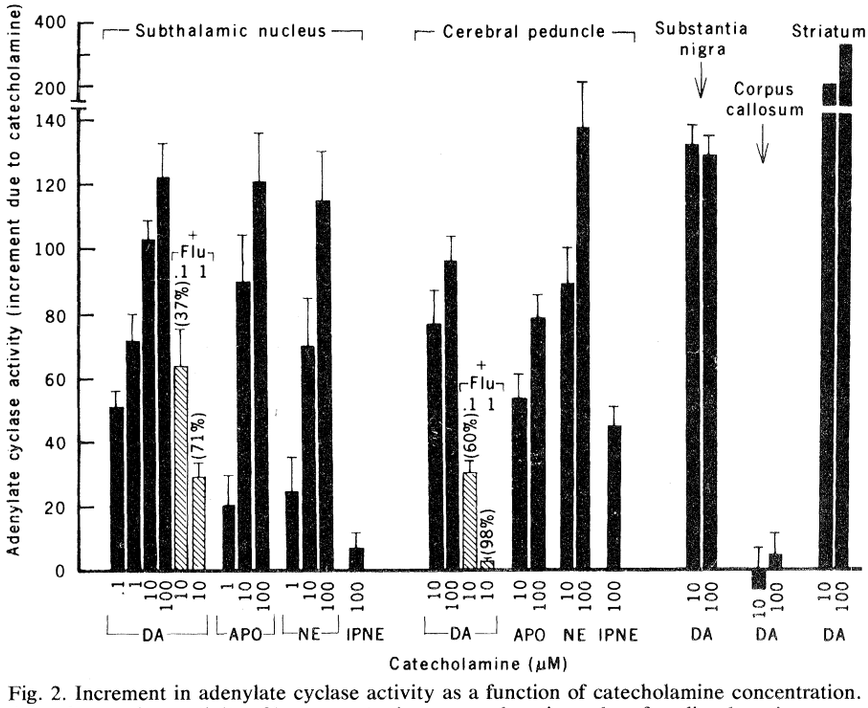

Fig. 2. Increment in adenylate cyclase activity as a function of catecholamine concentration. Adenylate cyclase activity of homogenates is expressed as picomoles of cyclic adenosine monophosphate formed per milligram of protein in 5 minutes. Values represent increments ± standard errors attributable to dopamine (*DA*), apomorphine (*APO*), norepinephrine (*NE*), or isoproterenol (*IPNE*) over the basal values. Average basal values were 121 ± 10 (N = 24), STN; 101 ± 7 (N = 24), adjacent cerebral peduncle; 68 ± 15 (N = 4), substantia nigra; 96 ± 11 (N = 4), corpus callosum; and 199 ± 20 (N = 4), striatum. All increments were significant (t-test, $P < .05$), except for those due to isoproterenol in the STN and DA in the corpus callosum. Increments due to 10 μM DA in the presence of the antagonist fluphenazine (*Flu*) at 0.1 and 1.0 μM are also shown (N = 4 to 7), with the percentage inhibitions due to fluphenazine given in parentheses.

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