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Role of excitatory amino acids in the regulation of dopamine synthesis and release in the neostriatum

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Summary. We have explored the role of excitatory amino acids in the increased dopamine (DA) release that occurs in the neostriatum during stress-induced behavioral activation. Studies were performed in awake, freely moving rats, using *in vivo* microdialysis. Extracellular DA was used as a measure of DA release; extracellular 3,4-dihydroxyphenylalanine (DOPA) after inhibition of DOPA decarboxylase provided a measure of apparent DA synthesis. Mild stress increased the synthesis and release of DA in striatum. DA synthesis and release also were enhanced by the intra-striatal infusion of N-methyl-D-aspartate (NMDA), an agonist at NMDA receptors, and kainic acid, an agonist at the DL-α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionate (AMPA)/kainate site. Stress-induced increase in DA *synthesis* was attenuated by co-infusion of 2-amino-5-phosphonovalerate (APV) or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), antagonists of NMDA and AMPA/kainate receptors, respectively. In contrast, intrastriatal APV, CNQX, or kynurenic acid (a non-selective ionotropic glutamate receptor antagonist) did not block the stress-induced increase in DA *release*. Stress-induced increase in DA release was, however, blocked by administration of tetrodotoxin along the nigrostriatal DA projection. It also was attenuated when APV was infused into substantia nigra. Thus, glutamate may act via ionotropic receptors within striatum to regulate DA synthesis, whereas glutamate may influence DA release via an action on receptors in substantia nigra. However, our method for monitoring DA synthesis lowers extracellular DA and this may permit the appearance of an intra-striatal glutamatergic influence by reducing a local inhibitory influence of DA. If so, under conditions of low extracellular DA glutamate may influence DA release, as well as DA synthesis, by an intrastriatal action. Such conditions might occur during prolonged severe stress and/or DA neuron degeneration. These results may have implications for the impact of glutamate antagonists on the ability of patients with Parkinson's disease to tolerate stress.

Keywords: Dopamine release – Dopamine synthesis – Excitatory amino acids – Microdialysis – Parkinson's disease – Substantia nigra

Introduction

The neostriatum receives two major inputs. One descends from the cerebral cortex and utilizes an excitatory amino acid (EAA), likely to be glutamate, as its transmitter. The other pathway ascends from substantia nigra and utilizes dopamine (DA). Electron microscopic and electrophysiological analyses indicate that both projections often synapse on the same striatal GABA neuron. In addition, neurochemical evidence has accumulated to suggest that EAAs can act within the striatum to increase the release of DA via a local influence. The earliest reports in support of this proposal demonstrated that the addition of glutamate or glutamate agonists to striatal slices resulted in the overflow of ^{3}H-DA formed from ^{3}H-tyrosine (Giorguieff et al., 1977). Comparable results were obtained in studies in which endogenous DA was monitored (Jhamandas and Marien, 1987; Lonart and Zigmond, 1991). Studies using push-pull cannulae or microdialysis provided analogous results in the intact animal (Cheramy et al., 1990; Carter et al., 1988). In addition to a local striatal site for a direct glutamate-DA interaction, an interaction might also occur within the substantia nigra. EAA projections terminate in substantia nigra as well as striatum, and application of glutamate agonists in substantia nigra also have been shown to increase DA release (Westerink, et al., 1992).

Thus, there are several ways in which EAAs might act to modulate the activity of DA neurons of the nigrostriatal projection. However, despite this extensive literature on glutamate-DA interactions, most such studies have been limited to an examination of *exogenous* EAA agonists. We, therefore, wished to explore the role of *endogenous* glutamate released under physiological conditions. The condition that we have examined is stress-induced behavioral activation, using mild electric shock applied to the tail or paws as our stressor. This stimulus causes an increase in the extracellular levels of both DA (Abercrombie et al., 1989) and glutamate (Keefe et al., 1993). We reasoned that if endogenous glutamate played a role in the stress-induced elevation in DA release, then it would be attenuated by appropriate EAA receptor antagonists applied to the striatum.

EAAs can act on a large number of receptors. In our studies, we have chosen to focus on a particular group of these receptors, those that are directly coupled to ion channels. It is possible to distinguish pharmacologically between two major classes of such ionotropic receptors, those activated by N-methyl-D-aspartate (NMDA) (NMDA receptors) and those activated by kainic acid (AMPA/kainate receptors). Thus, we chose to examine the impact of ionotropic glutamate receptor antagonists on the response of the nigrostriatal DA system to stress. We used 2-amino-5-phosphonovalerate (APV), an antagonist of NMDA receptors, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), an antagonist of kainate receptors, and kynurenic acid, a broad-spectrum antagonist of ionotropic glutamate receptors.

Influence of glutamate acting in the striatum

DA synthesis

Exposure of rats to 30 min of intermittent mild electric shocks produced significant behavioral arousal and increased motor activity. We used *in vivo* microdialysis to examine the effects of this stressor on striatal DA synthesis in awake, freely moving rats. An inhibitor of aromatic amino acid decarboxylase, NSD-1015 (100 μM), was infused via the microdialysis probe beginning 90 min before the onset of the stressor. Changes in extracellular DOPA then were monitored as an index of DA synthesis (Westerink et al., 1990). We observed that the stress-induced behavioral activation was associated with an apparent increase in DA synthesis. Infusion into striatum of either NMDA (100 μM) or kainic acid (10 μM) also increased apparent DA synthesis. Moreover, the infusion of glutamate antagonists into striatum attenuated the stress-induced increase in apparent DA synthesis in this region (Castro et al., 1996).

DA release

For an increase in DA synthesis to have a functional impact, it must be translated into an increase in DA release. Exposure to electric shocks increased extracellular DA as measured by microdialysis, as did the local infusion of NMDA or kainic acid (Keefe et al., 1992). However, the infusion of either APV or CNQX into striatum had no significant effect on the concentration of extracellular DA in striatum, either under basal conditions or during exposure to stress (Keefe et al., 1993).

Influence of glutamate in the substantia nigra

Glutamate projections innervate substantia nigra, as well as striatum, and EAA agonists infused in this region also increase DA release in striatum (Westerink et al., 1992). Moreover, tetrodotoxin (10 mM) infused along the nigrostriatal projection reduced extracellular DA to below the limits of detection of the assay and blocked the stress-induced increase in striatal extracellular DA (Keefe et al., 1992; 1993). Thus, we explored the influence of EAAs on NMDA receptors in substantia nigra. Whereas APV (0.1 mM) infused in substantia nigra did not alter basal extracellular DA, it abolished the stress-induced increase in extracellular DA (Castro and Zigmond, unpublished observations; see also Karreman et al., 1996; Taber and Fibiger, 1997). In contrast, we observed no effect of APV on the stress-induced increase in DA synthesis.

Role of striatal D2 receptors in the regulation of DA synthesis

Our results could be taken to suggest that EAAs influence DA synthesis and release at two separate sites (see also Kapatos and Zigmond, 1979). DA release might be affected by an action of EAA in substantia nigra, while DA synthesis might be modulated within striatum. However, there is another way in which to interpret the data. Stress normally is associated with an increase in extracellular

DA. Yet, our method for measuring DA synthesis involves inhibiting the conversion of DOPA to DA, and this inhibition causes a decrease in extracellular DA (Castro et al., 1996). The synthesis and release of DA, as well as the release of glutamate, appear to be under the inhibitory control of D2 receptors within striatum. Thus, a decrease in extracellular DA would reduce autoinhibition of DA synthesis while increasing the release of glutamate. This might permit the emergence of a glutamate influence on DA release.

We have begun to examine this hypothesis. First, we have measured the impact of a D2 agonist, quinpirole, on the stress-induced increase in DA synthesis. When quinpirole (100 μM) was infused locally into striatum, we observed no significant alteration in the basal rate of DOPA accumulation in that region. However, the D2 agonist completely blocked the stress-induced increase in DOPA accumulation (Castro et al., 1996). Second, we examined the impact of stress on DOPA accumulation 30 min after the onset of decarboxylase inhibition, rather than 90 min as before. (At 30 min, extracellular DA levels were still normal.) Again, stress failed to increase apparent DA synthesis. Finally, we again looked at the effects of stress beginning 30 min after the onset of decarboxylase inhibition but in the presence of the D2 antagonist eticlopride (50 nM). Under these conditions the stress-induced increase in apparent DA synthesis was restored. Collectively, these results suggest that the stress-induced increase in DA synthesis occurs only when D2 receptors on DA and/or glutamate terminals are not fully occupied. We have already shown that when stress increases DA synthesis it does so via EAAs acting within striatum. It now must be determined whether EAAs also are involved in modulating stress-induced increases in DA *release* under conditions of reduced DA availability.

Relationship to Parkinson's disease

Our data suggest that under normal conditions the regulation of DA release by EAAs occurs within substantia nigra. However, under conditions in which extracellular DA in striatum is reduced, a glutamate projection to this region may also stimulate DA activity (Castro et al., 1996) (Fig. 1). Parkinson's disease is characterized by the severe loss of DA neurons. The application of our model to this disorder predicts that the reduction in extracellular DA will trigger an increase in DA release due in part to glutamate acting within striatum. Although we have not fully tested this prediction, the 6-OHDA-induced destruction of DA terminals does lead to an increase in DA turnover in the remaining DA neurons (see review, Zigmond et al., 1993). Moreover, a recent report indicates that both behavioral and neurochemical compensations can be blocked by treatment with NMDA antagonists (Emmi et al., 1996).

Glutamate has been implicated in the neurotoxicity associated with Parkinson's disease and it has been suggested that treatment with glutamate antagonists might attenuate the neurodegenerative process. Likewise, glutamate antagonists have been used with some success in the treatment of the symptoms of parkinsonism. However, our results and those of others suggest that glutamate may also play a role in the compensatory events occurring

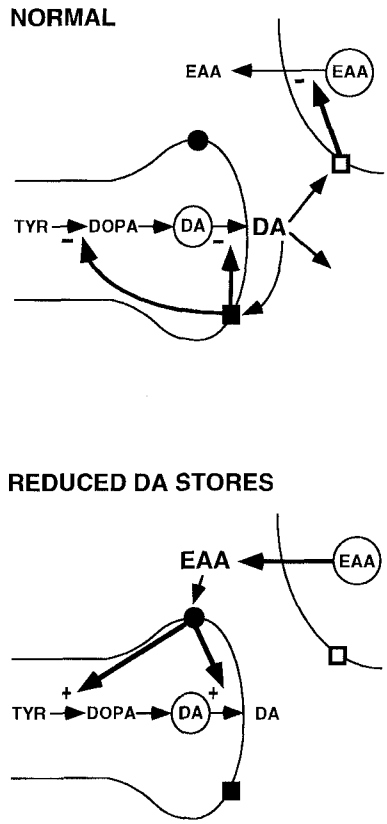

Fig. 1. A model of dual regulation of DA synthesis and release in striatum. *Upper panel*: Under normal conditions, both the synthesis of DA from tyrosine (TYR) and the release of DA are inhibited by extracellular DA acting on terminal autoreceptor (filled square). In addition, EAA release is under tonic inhibition by DA (open square). *Lower panel*: When extracellular DA levels are significantly reduced, the influence of DA on the autoreceptor is absent and EAA terminals, now also freed from inhibition by DA, can exert an excitatory influence on DA synthesis and release (filled circle), via either a direct or indirect feedback circuit. This would serve as an emergency regulatory loop utilized under conditions of reduced extracellular DA such as might occur during prolonged, severe stress and/or the extensive loss of dopaminergic input in Parkinson's disease (reprinted from Castro et al., 1996)

during the loss of DA neurons. If so, then glutamate antagonists must be used with caution in the treatment of Parkinson's disease, since in addition to their own salutatory actions, they may also reduce DA release within the striatum, at least during exposure to stress.

Acknowledgments

This review is based on our published findings (Keefe et al., 1992; 1993; Castro et al., 1996) and on results presented at the 26th meeting of the Society for Neuroscience, Washington DC, November 16–21, 1996. Space does not permit us to provide extensive references. However, most of these can be found in the papers noted above. The work was supported in part by USPHS grants MH18273, MH43947, NS19608, and MH00058.

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