Presynaptic Regulation of Dopamine Transmission in Schizophrenia

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A role for dopamine (DA) release in the hallucinations and other positive symptoms associated with schizophrenia has long been inferred from the antipsychotic response to D2 DA receptor antagonists and because the DA releaser amphetamine can be psychotogenic. Recent studies suggest that patients with schizophrenia, including those never exposed to antipsychotic drugs, maintain high presynaptic DA accumulation in the striatum. New laboratory approaches are elucidating mechanisms that control the level of presynaptic DA stores, thus contributing to fundamental understanding of the basic pathophysiologic mechanism in schizophrenia.

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Introduction

Approximately 1% of humanity develops schizophrenia during early adulthood, but the causes of this devastating disorder remain unknown. Over 20 susceptibility genes have been suggested to play a role,1,2 and environmental and/or epigenetic factors are also important, as evident from the approximately 50% discordance for schizophrenia between identical twins.3,4 Prenatal conditions, including malnutrition, maternal immune activation, and paternal age, may also provide susceptibility factors.2

Evidence of dopamine (DA) involvement in schizophrenia developed in tandem with the discovery of this neurotransmitter in the 1950s. Reserpine, from the plant Rauwolfia serpentina that has been used to treat insanity for centuries in India, was approved for antipsychotic use in the West in 1954 and was soon discovered to block the accumulation of DA and other catecholamines into adrenal chromaffin secretory vesicles.5 Carlsson6 speculated nearly 50 years ago that an imbalance in DA release might underlie psychosis.

Carlsson’s suggestion was buttressed when the clinical potencies of chlorpromazine, the first antipsychotic drug used in the West, and other “first-generation” antipsychotics in the 1950s and 1960s were found to correlate with their binding affinities for D2-type DA receptors.7,8 Clinical response to most antipsychotics requires a minimum of 50% occupancy of striatal D2 receptors.9,10 While effective “second-” and “third-generation” antipsychotic drugs have been developed,11 the relationship between antipsychotic potency and D2 receptor affinity has been sustained,12–14 and the recent clinical antipsychotic trials of intervention effectiveness (CATIE) trial detected little difference between an older typical first-generation antipsychotic, perphenazine, and newer generation drugs.15

Additional support for a role of DA in schizophrenia arrived from the effects of psychostimulant drugs, including reports of widespread psychosis in populations in which amphetamines were widely abused, such as post–World War II Japan and Sweden in the 1940s and 1950s.16,17 Amphetamines release massive amounts of DA from presynaptic terminals, far more than with synaptic activity, and do so through mechanisms independent of synaptic vesicle exocytosis.18 Repeated amphetamine or methamphetamine use by individuals without schizophrenia can lead to a state similar to schizophrenic psychosis, with a propensity for psychotic reaction to subsequent psychostimulant exposure decades after the initial use.19 Premorbid schizoid or schizotypal personality predisposes methamphetamine users to psychosis, and the greater the personality vulnerability, the longer the psychosis persists.20 Many patients with schizophrenia exhibit an emergence or worsening of psychotic symptoms after acute exposure to amphetamine at doses that do not induce psychosis in healthy subjects.21 The patients who exhibit a worsening of psychotic symptoms in response to psychostimulant challenge during acute episodes are more likely to relapse upon antipsychotic discontinuation.22 Thus, both chronic and...
Mechanisms underlying amphetamine sensitization, in which previous amphetamine exposure increases striatal DA release upon readministration, have been proposed to be relevant to psychosis. Amphetamine sensitization may be persistent; in humans, enhancement of amphetamine-evoked striatal DA efflux persists for at least 1 year following as few as 3 doses 48 hours apart. Patients with schizophrenia have been proposed to exhibit an “endogenous sensitization” in which repeated psychotic episodes show progressively higher elevations of presynaptic striatal DA, that contribute to the progressively poorer clinical response to antipsychotic medications after each psychotic episode.

Recent Evidence for Enhanced DA Accumulation

Amphetamine Displacement of D2 Receptor Occupancy

Much of the newer evidence for increased striatal DA levels in schizophrenia is from studies of D2 receptor occupancy in patients. DA receptors are found in nearly every class of neuron in the striatum. D2 autoreceptors are located on striatal DAergic axonal terminals, where they mediate negative feedback by inhibiting DA synthesis and release. On γ-aminobutyric acid–mediated (GABAergic) medium spiny neurons (MSNs), D2 receptors are highly expressed on neurons within the “indirect” striatopallidal pathway, particularly on dendritic spines proximal to the appositions or synapses formed by DA terminals. A presence of D2 receptors on the axons of some of the glutamatergic afferents from the cortex is supported by anatomical and electrophysiological studies. DA D1 and D2 receptors are also found on cholinergic and GABAergic interneurons of the striatum. Imaging findings of altered D2 receptor binding in striatum could reflect changes in any or all of these striatal D2 receptor populations. The displacement of a radiolabeled D2/D3 ligand (iodobenzamide [IBZM], raclopride, or fallypride) in response to amphetamine can be used as an index of amphetamine-induced DA efflux in the striatum. Studies using this approach have shown that amphetamine-induced DA efflux is abnormally high in patients at the onset of psychosis, prior to treatment with antipsychotic drugs. A meta-analysis of imaging studies comparing D2 receptor parameters in patients with schizophrenia and healthy controls also revealed a small (12%) but significant elevation of striatal DA receptors in untreated patients with schizophrenia (see table 1). It is unclear what effect, if any, that this receptor elevation has in regard to the symptomatology in schizophrenia. However, because amphetamine causes substantial DA release through stimulation-independent nonexocytic mechanisms, these results indicate that there are higher-than-normal presynaptic levels of DA in the striatum of schizophrenic patients.

L-3,4-Dihydroxyphenylalanine Accumulation Studies

Presynaptic DA accumulation can be estimated in an alternate manner independent of D2 receptor binding from the accumulation of [13F] or [11C]-labeled 3,4-dihydroxyphenylalanine (DOPA). L-DOPA is rapidly converted to DA and [18F]DOPA to [18F]fluorodopamine in the cytosol by aromatic acid decarboxylase (AADC), and as nearly all the cytosolic DA in DA neurons is accumulated into synaptic vesicles, this approach mostly estimates presynaptic vesicular DA stores. Six of 8 studies report increased accumulation of [18F]DOPA or [11C]DOPA in the striatum of patients with schizophrenia (table 1). Consistent with the studies of amphetamine-induced DA release in antipsychotic-naïve patients, 3 studies found increased striatal uptake of [18F]DOPA or [11C]DOPA in antipsychotic-naïve subjects with schizophrenia compared with healthy control subjects (table 1). These data are consistent with the studies of amphetamine-induced displacement of DA D2 receptor radioligands as both indicate that there are increased levels of striatal presynaptic DA stores in patients with schizophrenia. It has also been shown that there is nearly 2-fold increased [18F]fluorodopamine turnover in the brains of untreated patients with schizophrenia, which is the largest biochemical difference yet reported.

Interestingly, the 3 catatonic subjects tested in these studies possessed [18F]DOPA accumulation rates that were by far the lowest of any of the schizophrenic subjects and were also lower than controls, with [18F]DOPA accumulation rates comparable in magnitude to those measured in the putamen in Parkinson disease. This is consistent with a number of parallel features in the catatonic syndrome and neurological extrapyramidal disorders. While the [18F]DOPA findings suggest that the catatonic subtype of schizophrenia might be associated with abnormally low levels of DA, this is only based on studies in 3 subjects, and more patients need to be studied to substantiate this finding.

DA Depletion Studies

The amphetamine-D2 occupancy and L-DOPA imaging experiments indicate higher levels of presynaptic accumulation of DA in patients but do not indicate if there are higher levels of DA receptor occupancy in the patients. This question has been addressed by depleting DA with the tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine (a-MPT), for 2 days to “unmask” D2 receptors that would otherwise be occupied by endogenous DA and would be unavailable to the labeled D2 ligand. The difference in binding between a DA-depleted and -nondepleted patient should indicate the D2 receptors that were bound by DA.
Following DA depletion, $[^{123}\text{I}]$IBZM binding to D2 receptors was higher in patients with schizophrenia than in healthy controls. Within patients with schizophrenia, binding was higher for those experiencing an exacerbation of illness than for those who were not experiencing a psychotic episode. Assuming that the number and affinity of D2 receptors for DA are similar during and between psychotic episodes and that DA depletion does not alter D2 affinity (2 critical caveats), a higher fraction of striatal D2 receptors was apparently occupied by DA in patients with schizophrenia and the levels of DA correlated with the severity of psychosis. Higher synaptic DA levels in patients with schizophrenia were found to be predictive of a better therapeutic response to antipsychotic medications.

### Summary of Imaging Studies

The results with labeled L-DOPA and amphetamine displacement of D2 ligands independently indicate that more DA is present in presynaptic terminals in the striatum of many patients with schizophrenia, with the possible exception of those with the catatonic subtype. The $\alpha$-MPT studies further indicate that baseline levels of striatal DA in patients are higher. Importantly, increased striatal DA associated with the disease does not seem to be due to antipsychotic treatment.

The important related question of whether DA neuronal activity, ie, the firing rate, is higher in patients with schizophrenia at baseline or during acute psychotic exacerbations has not been resolved (see below), and this could fundamentally alter the interpretation of these studies. It may be that the relatively long time windows measured by these imaging techniques reflect turnover of DA, so that at any given time, presynaptic levels are normal, but that there is a greater opportunity during the measurements for L-DOPA accumulation or amphetamine displacement of vesicle stores from the terminals that are more actively accumulating DA.

It is important to note that in contrast to the evidence for increased striatal presynaptic DA in schizophrenia, the DAergic innervation of some cortical regions appears to be decreased, as observed with tyrosine hydroxylase immunocytochemistry. Interestingly, in patients with schizophrenia, decreases in prefrontal cortical activation during performance of cognitive tasks correlated with increased $[^{18}\text{F}]$DOPA signal in the striatum. As
discussed below, the phenomena of enhanced striatal DA and low cortical DA could be related.

As increased presynaptic DA accumulation in the striatum in schizophrenia occurs independently of antipsychotic administration, it is possible that labeled L-DOPA could be used as a biomarker to identify disease progression or at-risk individuals. During the prodromal period, individuals exhibit symptoms of depression or “negative symptoms” of schizophrenia and sometimes subthreshold levels of positive symptoms. A recent \(^{18}\)F]DOPA imaging study of prodromal patients compared controls, “at-risk mental state” (ARMS) prodromal patients, and new-onset psychotic patients (table 1). This study replicated prior results within new-onset psychoses, showing that the psychotic patients had elevated \(^{18}\)F]DOPA in relation to controls.

In addition, elevation of \(^{18}\)F]DOPA in the striatum (specifically the associative striatum) of 24 ARMS patients was significantly higher than controls. \(^{18}\)F]DOPA uptake was correlated with total prodromal symptom ratings and Positive and Negative Syndrome Scale score but was not correlated with depression or anxiety scores. Future imaging of these prodromal patients might provide insight concerning the “state- or trait-like” characteristics of DA accumulation in schizophrenia.

An example of a cortical alteration in DA that could cause downstream striatal effects involves catechol-O-methyltransferase (COMT), which metabolizes extracellular DA and regulates its availability in the cortex where DA transporter expression is quite low. There are 2 major allele types for this enzyme at amino acid 158.
When exposed to amphetamine, individuals without schizophrenia who exhibit the common Met/Met COMT genotype, which leads to slower DA metabolism than seen with the Val/Val form, 71 exhibit poorer performance on high task-load tests than do those with the Val/Val form. 73 A clinical analysis of the polymorphism in Japanese patients who take methamphetamine found that the Met allele is associated with patients who experienced methamphetamine psychosis and spontaneous relapse, suggesting that patients with 1 or 2 met alleles appear to be at increased risk of an adverse response to methamphetamine. 74

**Evidence From the Laboratory for Altered Striatal DA**

As discussed above, evidence in patients indicates that altered DA release in schizophrenia may be due to either intrinsic changes in the presynaptic terminal or changes in neuronal activity regulated by inputs from other neurons, particularly via altered cortical and subcortical or limbic regulation.

**Presynaptic Studies**

What underlies the increased presynaptic DA in patients with schizophrenia? Over the past decade, several new manipulations have been shown to alter presynaptic DA accumulation, including changes in DA synthesis, metabolism, and a variety of effects on quantal size (figure 1). One possibility is that there is a higher density of DA terminals in the striatum of patients with schizophrenia. This is however inconsistent with reports of a normal density of DAergic mesostriatal neuronal terminals in the striatum of patients, using tyrosine hydroxylase immunocytochemistry. 75–78

Another possibility, that patients have increased DA synthesis due to higher activity of tyrosine hydroxylase (generally the rate-limiting step for DA synthesis), does not explain the phenomenon because imaging studies show enhanced accumulation of exogenous L-DOPA, the tyrosine hydroxylase product. Enhanced DA uptake transport into neurons likewise does not appear to play a role, as postmortem studies consistently report unaltered dopamine transporter (DAT) levels in the striatum of patients with schizophrenia. 75–78 Four of 5 in vivo imaging studies report normal striatal DAT density in young and/or antipsychotic-naïve or -treated patients with schizophrenia. 27,55,79,80

There is likewise no evidence for enhanced expression of DA synaptic vesicle uptake transporters, although this has not been widely examined: One study finds normal vesicular monoamine transporter (VMAT) type 2 levels in patients using radiolabeled tetrabenazine as a ligand. 81 Vesicular transporter activity has however not been assessed directly in patients.

An increased packaging of DA within synaptic vesicles might be the culprit. Amperometric recordings indicate that within the DA presynaptic terminal of cultured ventral midbrain DA neurons, the vast majority of DA is stored within the synaptic vesicles, which can maintain a concentration of nearly a molar. 82 A new technique known as intracellular patch electrochemistry demonstrates that in contrast, the DA cell body cytosol normally contains <100 nM DA, although levels of 10 μM and higher can be reached in cytosol following L-DOPA. 122

Amperometric recordings from cultured neurons further show that the amount of DA stored within a synaptic vesicle can be regulated by a variety of relevant means, including altered cytosolic DA levels (as occurs when L-DOPA is administered), altered VMAT as mentioned above, altered vesicular pH gradients (which occurs with amphetamine), or changes in synaptic vesicle size. 83,84 The demonstration of these modulatory mechanisms indicates that they might contribute to increased presynaptic DA accumulation in the disease state and almost certainly modify presynaptic DA accumulation under conditions such as when amphetamine is ingested.

Additional possibilities for enhanced presynaptic DA are suggested by the enhanced labeled L-DOPA accumulation observed in the disease. The rate of accumulation of [18F]DOPA in the striatum depends on multiple factors, including transport through the blood-brain barrier, transport into cells by a possible L-amino acid transporter, 85 and the activity of AADC that converts L-DOPA into DA. Once converted into DA, the synaptic vesicular storage capacity is important, as is the number of uptake-competent synaptic vesicles within a terminal. Importantly, intracellular patch electrochemistry measurements in DA neuronal culture suggests that the rate-limiting step for L-DOPA-derived DA accumulation likely occurs at AADC, with important additional effects on resulting cytosolic DA due to monoamine oxidase metabolism. 122 To our knowledge, AADC activity has not been directly assessed in schizophrenia.

Studies of amphetamine sensitization may also provide important clues. Amphetamine-sensitized rats show greater amphetamine-induced displacement of striatal [3H]raclopride 86 and so may participate in the same mechanism that leads to high DA accumulation. Experiments on chromaffin cells indicate that prolonged methamphetamine increases the amount of catecholamine stored in vesicles via a rebound hyperacidification of vesicle pH gradients. 122 Amphetamine can also stimulate DA synthesis, which contributes to some of its effects on DA release. 18

**Cortical and Limbic Regulation Studies**

Could altered limbic or cortical activity associated with schizophrenia 87 underlie increased striatal presynaptic DA? In rodents, the frontal cortex can drive DA neuronal activity, 35 but this drive may be much smaller in primates,
who have little direct cortical input. Even in primates, however, the cortical activity modulates DA neuronal activity via cortical-basal ganglia loops, as nearly all cortical areas topographically project to the striatum and drive MSN activity, which in turn modulate the substantia nigra reticulata. Perhaps, this would promote greater DA neuronal activity and enhance DA turnover in the synaptic terminals.

Integrating the effects of all this regulation is complex, however. Within the striatum, ipsilateral motor area corticostriatal terminals are selected presynaptically, so that weaker inputs are inhibited by D2 DA receptors, possibly via a retrograde endocannabinoid signal from MSNs. Overall, it appears that ipsilateral cortical projection neurons project to D2-containing indirect pathway MSNs and show decreased activity with elevated DA release, as do tonically active cholinergic neurons in the striatum. In contrast, D1-containing direct pathway MSNs stimulated by both ipsilateral and contralateral projections show increased activity with DA release. Classic “box-and-arrow” models of the basal ganglia suggest that striatal DA release acting at both pathways might inhibit ventral midbrain GABA interneurons that disinhibit DA cell bodies and increase DA neuronal activity. Such a model is attractive, as it could explain how acute amphetamine challenge could maintain stimulation-dependent DA release and enhanced l-DOPA accumulation.

However, the disinhibition of DA neurons might be cancelled because high cortical activity can exert a long-lasting inhibition of striatal DA release by activating an inhibitory metabotropic glutamate type I presynaptic heteroreceptor. Cortical potentiation of acetylcholine released from tonically active cholinergic striatal neurons may enhance DA release via presynaptic nicotinic receptors on DA terminals, although its consequences on presynaptic DA stores are unknown. It is possible that corticostriatal homeostasis is altered by chronically elevated DA, as DA regulates the response of striatal and corticostriatal neurons via a variety of long-term changes of receptor and channel activity, including a very long-lasting depression of corticostriatal activity after repeated methamphetamine.

An alternate possibility to greater DA neuronal activity via reduced cortical “drive” is greater activity due to enhanced hippocampal output. Activation of the ventral hippocampus increases the firing of ventral tegmental neurons and enhances DA release in rodents. The effect on the nucleus accumbens may alter DA neuronal activity via a variation of the “box-and-arrow” basal ganglia model. It is not clear if either the altered cortical or limbic drive can explain the extent and characteristics of changes in presynaptic DA accumulation found in the imaging studies.

To provide even more complications, the decreased cortical or increased limbic input suspected to alter striatal DA may itself be a result of changes in the basal ganglia. Ventral tegmental area DA neurons that project to the cortex exhibit very different properties than those that project to various striatal areas, including low levels of D2 autoreceptors and DATs, and effects on these different populations of DA neurons could affect the corticostriatal and mesostriatal projections in complex ways. Transgenic mice overexpressing striatal DA D2 receptors show increased prefrontal cortical DA levels and decreased rates of DA turnover as a result of a developmental compensation.

Animal Model Studies

The complex relationship of cortical and basal ganglia systems with DA can be revealed further by studying animal models of the disease. Indeed, the concept that striatal DA dysregulation might follow abnormal limbic and cortical input in schizophrenia has been very influential in the development of animal models. Neonatal ventral hippocampal lesions (NVHLs) lead to postpubertal emergence of phenotypes relevant to schizophrenia, including increased responsivity to the behavioral effects of amphetamine. Importantly, the increased behavioral response to amphetamine does not emerge until after puberty, a developmental pattern that may have high construct validity for the onset of psychosis in schizophrenia. The regulation of striatal DA release in this model is complex, with an increase in amphetamine-evoked DA efflux in the nucleus accumbens core but an attenuation of DA efflux in the nucleus accumbens shell.

In the methyloxazymethanol acetate model (MAM), a DNA methylator is administered at E17, which damages the hippocampus, prefrontal cortex, and other brain regions. Similar to the NVHL model, abnormal response to amphetamine does not emerge until after puberty. Promisingly, the MAM E17 rats possess increased amphetamine-induced DA efflux in the medial and ventral striatum (H.M., unpublished results, 2004), and there is an increased rate of firing by ventral tegmental area DA neurons.

Lesioning DA terminals in adult prefrontal cortex with 6-hydroxydopamine enhances striatal DA transmission, possibly via increased stress-induced DA turnover in the nucleus accumbens regulated by noradrenergic inputs to the prefrontal cortex. Thus, several animal models suggest that aberrant cortical or limbic input to the striatum might underlie increased striatal presynaptic DA.

The near future is likely to bring us additional animal models, including those that exhibit alterations of proteins encoded by genes that contribute to the development of schizophrenia, and newly developed electrochemical and optical methods promise to be important for understanding the complex relationship between the neuronal pathways that may underlie psychosis in schizophrenia.
Conclusions

Classic investigations by Carlsson and others suggested that abnormal DA input is involved in the positive symptoms of schizophrenia, and recent imaging studies have now focused these observations to show evidence for increased presynaptic DA stores in the striatum, with the apparent exception of catatonic patients. As summarized in Figure 1, there are a multiple, likely interacting, mechanisms regulating accumulation of DA in the presynaptic terminal and its excess in schizophrenia. Many of these mechanisms are downstream of the decreases in prefrontal cortical activity and increases in temporolimbic cortical activity (and their respective effects on DA neuron spike activity) that are predominantly featured in current rodent models of the pathophysiology of schizophrenia. Thus, far more work needs to be done to characterize local, presynaptic factors regulating presynaptic DA stores. The new resources for studying presynaptic DA accumulation and release\(^1\)\(^9\),\(^12\) and the continuing development of new animal models promise that future research could elucidate these mechanisms.

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