

Pharmacogenetic Tools for the Development of Target-Oriented Cognitive-Enhancing Drugs

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Abstract: The identification of the anatomical and physiological substrates involved in the regulation of the dorsolateral prefrontal cortex function in humans provided the basis for the understanding of mechanisms involved in cognitive and executive function under normal as well as pathological conditions. In this context, substantial evidence indicates that alterations in monoaminergic function in the dorsolateral prefrontal cortex significantly contributes to the cognitive impairments present in schizophrenia, attention deficit disorders, and other neuropsychiatric conditions. The development of a number of compounds that selectively increase extracellular dopamine (DA) concentrations in the dorsolateral prefrontal cortex but not in subcortical areas by either blocking its metabolism or reuptake, or increasing its release, or that directly activate postsynaptic DA-1 receptor mechanisms provided powerful pharmacother-

apeutic tools to mitigate the cognitive deficits brought about by the dopaminergic alterations of the prefrontal cortex. More recently, the findings that polymorphisms of the catecholamine-O-methyl-transferase gene may also modify the effect of these drugs on the prefrontal cortex points toward a more specific genotype-based neuropsychopharmacology for the treatment of cognitive deficits in schizophrenia as well as in a number of other neuropsychiatric conditions. The ability of these compounds to increase DA load selectively in the frontal cortex and not on subcortical systems allows a targeted intervention without the stimulant-like effects observed with older drugs used to treat those conditions. **Key Words:** Dorsolateral prefrontal cortex, dopamine, schizophrenia, attention deficit disorder, dopamine-1 receptors, Tolcapone, Modafinil, Atomoxetine, DAS-431.

INTRODUCTION

A growing body of evidence supports the notion that the dorsolateral prefrontal cortex (DLPC) plays a major role in a number of cognitive processes including executive function, learning, nonverbal memory, vigilance, and attention among others.¹⁻⁴ Because prefrontal cortex dysfunction is a frequent concomitant of psychiatric disorders, cognitive deficits in such patients are common and prevent them from maintaining or developing new skills necessary for reintegration into community life. The DLPC is innervated by a number of neurotransmitter systems and modulators that have been popular targets for drug development, mainly the monoaminergic system.^{5,6} Disruption of the mesocortical dopaminergic

(Me-DA) system, a premier catecholaminergic pathway involved in the regulation of frontal lobe function,⁷ leads to impairments in executive function and other cognitive difficulties. A primary defect in the Me-DA system may, in turn, lead to a number of compensatory modifications in interconnected pathways^{8,9} resulting in functional changes in brain areas subserved by those interrelated neuronal systems. Over the last few years, pharmacological strategies pointing toward treatment of cognitive deficits in psychiatric disorders have been pursued. Historically, stimulants such as amphetamines and methylphenidate were shown to affect cognitive function both in experimental animals¹⁰ and in humans¹¹ by increasing extracellular dopamine (DA) and norepinephrine concentrations through presynaptic neurotransmitter release or reuptake blockade.^{12,13} More recent evidence indicates that atypical antipsychotics are able to improve cognitive function¹⁴⁻¹⁷ in patients with schizophrenia, possibly related to the serotonin (5-HT) 2a receptor blocking properties of these agents. The blockade of 5-HT 2a receptors induces an activation of the dopami-

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nergic neurons projecting to the DLPFC with ensuing increase in extracellular dopamine concentrations.¹⁸

Another strategy for improving cortically mediated cognition, this one based on the proposed role of glutamate on schizophrenia, has been the use of glutamatergic agonists to facilitate NMDA transmission and target cognitive symptoms in patients with schizophrenia. Early trials with NMDA agonists such as D-cycloserine and milacemide, produced mixed results on cognition including positive effects¹⁹ or no effects.^{20–22} The new generation of glutamatergic drugs acting on the AMPA-type receptor such as the AMPAkines seems more promising. In fact, the AMPAkine receptor agonist CX516 improves cognitive abilities both in humans and experimental animals.²³ In the last few years, there has been a mounting interest in the contribution of gene polymorphisms in the efficiency of response with which cognition-enhancing drugs improve cognitive function in humans and experimental animals. In this context, catecholamine-*O*-methyltransferase (COMT) and brain-derived neurotrophic factor (BDNF) represent major genes of interest for the field.

In the present review, we will focus on new pharmacotherapeutic approaches in psychiatry involving the dopaminergic system in the prefrontal cortex and genotype-based targeted pharmacology.

THE DOPAMINERGIC INNERVATION OF THE PREFRONTAL CORTEX

The use of anti-TH antibodies provided a powerful tool to selectively identify dopaminergic terminals in the dorsolateral prefrontal cortex both in monkeys and human samples.⁵ At odds with the dopaminergic innervation in rodents, in the mammalian neocortex, dopaminergic neurons are widely distributed in every cortical region. Interestingly, the distribution of these terminals indicates preferential innervation of motor over sensory regions, sensory associations over primary sensory regions, and auditory associations over visual associations.⁶ Gradients of distribution show that, in the dorso-medial convexity of the frontal lobe, fiber density increases in a rostral to caudal manner from the prefrontal to the premotor region. Additionally, the medial cortical surface displays a ventral to dorsal gradient of increasing fiber density. The laminar distribution of TH-containing terminals showed that lightly innervated areas of the human prefrontal cortex tend to have fibers distributed in a bilaminar manner, whereas the more densely innervated regions such as the dorsomedial region have a more uniform distribution.⁶

Dopamine neurons in the ventral tegmental area provide a critical modulatory role on behavior (facilitation of reward seeking) and cognition.^{24,25} The DA neuron projections to the DLPFC regulate cognitive processes including executive functions, planning and atten-

tion.^{26,27} Although there is some controversy about their functional properties, the Me-DA system is believed to have a faster firing rate, have more action potentials in bursts, higher dopamine turnover,²⁸ and exhibit enhanced sensitivity to stress^{29,30} when compared with the mesolimbic and nigrostriatal dopamine systems. Also, at odds with other forebrain areas, in the Me-DA system terminals, dopamine exhibits greater extrasynaptic diffusion and slower clearance.^{31,32} Furthermore, transcerebral dialysis studies showed that the ratio of DA in the dialysate is considerably higher in the DLPFC than in the striatum and the accumbens, suggesting that a proportionally greater amount of extracellular DA relative to intraneuronal sites.^{31,33} Factors affecting dopamine synthesis, autoreceptor density, and other features have been ascribed as possible factors to explain these findings.^{34,35} Studies on mice lacking dopamine transporter (DAT) gene expression, however, indicated that those factors had less impact on extracellular dopamine accumulation than the DAT protein itself.³⁶ Thus, the lack or paucity of DATs in the cortical synaptic cleft may provide a rational explanation to understand the higher extracellular dopamine concentrations and greater diffusion space at the level of the Me-DA system terminals when compared with the mesolimbic or nigrostriatal systems. The limited efficacy of dopamine reuptake blockers in the DLPFC when compared with other dopaminergic areas further substantiates this view.^{37–39}

A number of studies aiming at identifying the mechanism(s) by which the DLPFC terminate the actions of dopamine revealed that reuptake by the norepinephrine transporter (NET) diffusion, as well as enzymatic processing, are responsible for eliminating DA from the cortical synaptic cleft.^{33,39,40} Both *in vivo* and *in vitro* evidence indicates that extracellular DA in the DLPFC is cleared primarily by NET mechanisms. In a cerebral dialysis model, studies using the norepinephrine (NE) inhibitor desipramine revealed that this compound elevates both DA and NE levels in the prefrontal cortex, an area where the NET is more concentrated than the DAT.^{37,41} Furthermore, *in vitro* studies using a knockout mice model for the NET further confirmed that the uptake of DA in the frontal cortex depends primarily on the NET.⁴²

A second mechanism by which DA is cleared from the DLPFC, in this case a volume diffusion mechanism, has been more recently identified. “Volume” transmission refers to three-dimensional signal diffusion within the brain extracellular fluid. Volume transmission includes short- (~20 nm) and long-distance diffusion of signals through the extracellular and CSF. The slow uptake of DA in the DLPFC, apparently due to the low density of NET when compared with other brain areas, enables diffusion over dimensions approaching 100 μm .^{43,44} This diffusion increases the likelihood of volume trans-

mission, with subsequent catabolism of DA by different means, including an extrasynaptic DAT mechanism, in an area far from the original synapse where the release took place.

A third mechanism by which the action of DA is terminated in DLPFC involves the enzyme COMT (EC 2.1.1.6). COMT is a ubiquitous enzyme that catalyzes the transfer of a methyl group from substrates carrying the catechol moiety⁴⁵ and plays a critical role in the metabolism of catecholamine neurotransmitters such as dopamine, adrenaline, and noradrenaline. The structures of the two isoforms of COMT and the gene were characterized almost 15 years ago.^{46–48} Both rat and human soluble (S)-COMTs contain 221 amino acids, and the molecular masses are 24.8 and 24.4 kDa, respectively. The human S-COMT peptide sequence is about 81% identical to the rat enzyme.^{49,50} The respective molecular masses of membrane-bound COMT are 29.6 and 30.0 kDa. One single gene was found for COMT, which encodes both the soluble and membrane-bound COMT.^{46,48} Using *in situ* hybridization and cell hybrid techniques, the COMT gene in humans was localized in chromosome 22, band q11.2.^{51,52} Early pharmacological studies⁴⁰ revealed that the catabolic flux of synaptic dopamine through the COMT pathway is much greater in the prefrontal cortex as compared to other brain DA pathways. More recent studies in the COMT knockout mice, when compared with the wild-type mice, revealed that DA concentrations are increased in the DLPFC but not in the striatum.^{53,54} Our group has also shown that COMT mRNA is expressed primarily in neurons in the prefrontal cortex in both rodent and human tissue. Furthermore, COMT mRNA concentrations in the DLPFC are significantly higher than in the striatum of both species.⁵⁵ The high levels of COMT and the lack or scarcity of DAT at synapses in the DLPFC most likely contribute to the relative functional specificity for COMT to prefrontal DA metabolism. In contrast, in the striatum, the very efficient dopamine neuronal uptake mechanism terminates the synaptic action of dopamine and COMT plays little functional role.^{31,36,56–59}

The level of COMT enzyme activity is genetically polymorphic in human red blood cells and liver, with a trimodal distribution of low, intermediate, and high levels of activity.^{60–63} This variation is largely explained by a single-nucleotide polymorphism at base 158 in the COMT gene. This genetic polymorphism results in substantial, three- to four-fold differences in COMT activity in red blood cells and liver,^{60,62} which may explain individual variation in metabolism of catecholamines and catecholaminergic drugs.^{64–66} In postmortem DLPFC, however, determination of COMT activity using standard laboratory methods revealed that, in human subjects, the activity of the COMT-Val allele is approximately 40% higher and shows higher thermostability at

37°C than the COMT-Met allele. Similar results were confirmed in human lymphocytes under the same experimental conditions, indicating that the findings in postmortem tissue are not artifacts of potential differences in postmortem protein stability based on genotype.⁶⁷

GENES AND DOPAMINE MODULATION OF PREFRONTAL COGNITIVE FUNCTION

Neuropharmacological intervention with monoaminergic drugs in healthy subjects can have an individual-specific response. A psychopharmacological study with bromocriptine (a D2-receptor agonist)⁶⁸ reported improvement in performance of a visuospatial working memory task only in subjects with lower working memory capacity. On the other hand, subjects with high capacity performed more poorly on the drug. They suggested that these population differences (subjects with high vs low working memory capacity) might be related to different (high vs low) endogenous metabolism of dopamine. This notion is consistent with monkey studies that examined dose-response relationships and can be summarized as biphasic effects of D1 antagonists, with low-dose facilitation and medium- to high-dose impairment^{10,69–72} and triphasic effects of D1 (and D2) agonists, with low-dose impairment, medium-dose facilitation, and high-dose deterioration.^{10,71,72} Consistent with the findings of Kimberg et al.,⁶⁸ it was observed in six subjects performing the N-Back working memory task that dextroamphetamine improved performance only in those subjects that had relatively low working memory capacity at baseline. In the subjects that had high working memory capacity at baseline, performance deteriorated on amphetamine.⁷³

It is possible that these population differences in working memory capacity (high vs low working memory capacity) and the differential effects of dopaminergics may be related to allelic variation of dopamine system genes, e.g., COMT enzyme activity with resulting variation (high vs low) in extracellular concentrations of catecholamines. In this line, we have recently found that this polymorphism in the COMT gene accounts for 4% of the variance in performance of working memory tasks in humans.^{74,75} The val allele produces the high-activity enzyme, which presumably breaks down synaptic dopamine more rapidly compared to the met allele, resulting in lower dopaminergic activity at critical synapses in the prefrontal cortex. Subjects with the val/val genotype have relatively impaired performance relative to met/met subjects, both in normal controls and schizophrenic patients. This genetic finding is consistent with prior data in animals showing a region-specific effect of COMT activity in prefrontal dopamine metabolism⁵³ and that COMT knockout animals have improved working memory.⁷⁶

The COMT gene polymorphism findings suggest that increasing the availability of DA in the DLPFC either by decreasing DA metabolism through COMT inhibition, by reducing DA reuptake through blockade of noradrenaline (NA) reuptake sites, or by increasing DA release, particularly in subjects with the val/val genotype, will most likely improve working memory and prefrontal efficiency both in normal individuals and in patients with other cognitive difficulties including schizophrenia, attention deficit problems, and dementing processes.

COMT INHIBITORS AND DLPFC FUNCTION

In the last few years, a number of compounds with inhibitory activity on COMT were discovered.⁷⁷ Among them, tolcapone^{78,79} proved to be the most potent *in vitro* and can penetrate the blood brain barrier. Tolcapone inhibits brain COMT activity *in vivo* with an ID₅₀ value of 26–28 mg/kg.^{2,80} More recently, PET studies also have shown that tolcapone acts as a central COMT inhibitor in Parkinson's disease.^{81,82} Tolcapone seems to be a quite specific inhibitor of COMT because it neither affects the reuptake of tritiated amines nor interacts with neurotransmitter receptors.^{78–80} In behavioral studies, intraperitoneal administration of tolcapone after pretraining facilitated spatial working memory in intact rats. Similarly, tolcapone improved the performance of senescent poor performers in the spatial memory task but was unable to counteract memory performance deficits in rats treated with scopolamine.^{83,84} These data suggest that tolcapone may improve some aspects of cognition, in particular working memory, by reducing dopamine catabolism in the prefrontal cortex.

Clinical studies showed that tolcapone decreases COMT activity and increases L-dopa availability when both compounds are given in combination.^{85,86} Interestingly, the addition of tolcapone to L-dopa improved function in attentional tasks, auditory verbal short-term memory, visuo-spatial recall, and constructional praxis.^{84,87} Tolcapone by itself was also effective for the treatment of major depression. Tolcapone by itself was also effective for the treatment of major depression.

In normal volunteers, we have recently found tolcapone effects and genotype effects on measures of cognitive function. Tolcapone significantly enhanced performance in normal subjects on tasks that demand speed of response in the context of working memory, namely *Trails B* (in which the sequencing of letters and numbers proceeds in alternating manner) and *N Back* at high loads (in which updating of the working memory buffer is near continuous).⁸⁸ For *ID shifting* from the CANTAB test battery, and *episodic memory* tasks, the response to tolcapone was modulated by COMT genotype. In the *ID shifting* test, a task that requires rule generalization in the face of a salient distractor, a significant drug × genotype

interaction was present, such that individuals with val/val genotypes markedly improved while receiving tolcapone, whereas individuals with met/met genotypes worsened. A genotype effect was also observed in verbal episodic memory tests in which individuals with val/val genotypes markedly improved, although not statistically significant, while receiving tolcapone, whereas individuals with met/met genotypes worsened. These results are consistent with previous studies on cognitive tasks in which dextroamphetamine, a drug that releases dopamine from nerve terminals, improved performance only in those subjects that had relatively low working memory capacity at baseline (val/val). In the subjects that had high working memory capacity at baseline (met/met), performance deteriorated on amphetamine.⁷³ An effect of tolcapone on the physiologic response during working memory was also observed on functional magnetic resonance imaging (fMRI) studies. In fact, tolcapone improved DLPFC efficiency in normal volunteers without observing overall significant genotype by drug effects on fMRI-based efficiency measures.⁸⁹

MODAFINIL AND DLPFC FUNCTION

The psychoactive drug modafinil is an agent marketed for the treatment of excessive day sleepiness associated with narcolepsy, sleep apnea, and idiopathic hypersomnia.^{89–92} The mechanism of action of modafinil has not yet been clearly identified but several studies have shown an interaction with α_1 adrenergic receptors and with DAergic, GABAergic and glutamatergic transmission mechanisms.^{93–96} Recent studies showed that modafinil increases dopamine release at wake-promoting doses through an action on the dopamine transporter (DAT). In fact, homozygous DAT knockout mice were shown to be unresponsive to the normally wake-promoting effect of modafinil and methamphetamine but were hypersensitive to the wake-promoting effects of adenosine receptor antagonist caffeine.^{96,97}

Microdialysis studies in the rat⁹⁸ showed a significant increase in extracellular DA, 5-HT, and NA in the prefrontal cortex following modafinil administration. The increase of extracellular NA in the prefrontal cortex, however, was shorter lasting and lower when compared with the increase in extracellular DA and 5-HT in the same area. The higher levels of DA in the frontal cortex may indicate that, in part, dopaminergic neurotransmission in the prefrontal cortex mediates the pharmacological effects of modafinil.⁹⁶ In turn, the increase in synaptic DA in the prefrontal cortex after modafinil may be responsible for the therapeutic effects of this drug on cognitive function. In fact, administration of modafinil in normal mice induces a dose-dependent improvement short-term working memory without changes in explor-

atory latencies or in activity in a four hole-board apparatus.⁹⁸

Neuropsychological studies in normal volunteers revealed that Modafinil at a dose of 100–200 mg/d significantly improved the performance in tests of digit span, visual pattern recognition memory spatial planning and stop-signal reaction, and a slowing in latency on delayed matching to sample and the spatial planning task. In these studies, however, no effect of modafinil was observed on spatial memory span, attentional shifting or spatial working memory.^{99,100}

Recent studies on modafinil and narcolepsy found an association between COMT genotype and narcolepsy. This association, however, was observed only on measures of narcolepsy severity and were dependent on gender. In fact, female narcoleptics with the met/met genotype were less severely affected and also responded to lower doses of modafinil.¹⁰¹ In line with the investigations on COMT polymorphisms and cognition,⁷⁴ it could be proposed that the effect of Modafinil on working memory and other neurocognitive tests may be associated with a specific COMT polymorphism. The ability of modafinil to raise DA concentrations in the prefrontal cortex and to improve working memory in experimental models raises the question of whether administration this drug in humans will improve working memory and physiological efficiency of the prefrontal cortex in a manner similar to that induced by COMT inhibitors and whether this effect may be genotype dependent. If this were the case, it could be hypothesized that the most prominent effect of modafinil will be observed in subjects bearing the val/val genotype in which the high COMT activity in the DLPFC keeps synaptic dopamine levels at a concentration lower than those bearing the met/met genotype. Initial investigations are being performed in normal volunteers and patients with schizophrenia to test this hypothesis.

SELECTIVE NOREPINEPHRINE REUPTAKE BLOCKERS AND DLPFC FUNCTION

In the last few years, a series of studies were designed to elucidate how norepinephrine reuptake inhibitors work and exert their therapeutic action on the prefrontal cortex. Among these compounds, atomoxetine was found to be effective for the treatment of attentional problems related to attention deficit disorder (ADD) with or without hyperactivity.¹⁰² The precise mechanism by which atomoxetine improves ADD is not totally fully understood, although it is thought to be related to selective inhibition of the presynaptic NET, as determined by neuronal uptake and transmitter depletion studies.¹⁰³ Blockade of the NET increases extracellular levels of NE and DA in the prefrontal cortex, a region involved in attention and memory¹⁰⁴ without modifying DA levels in

other areas such as the accumbens or the striatum where NE terminals are scarce.¹⁰⁵ The ability of atomoxetine to improve attentional deficits problems without activating the mesolimbic or nigrostriatal DA pathways explains the lack of a psychotomimetic effect or abuse potential of this drug.¹⁰⁶

The capacity of norepinephrine reuptake blockers (NRIs) to enhance dopamine neurotransmission in the frontal cortex provides a rational base to use atomoxetine for the treatment of attention difficulties.¹⁰² Historically, stimulant medications have been used for the treatment of cognitive difficulties associated with attention deficit disorders. There is general consensus that, similar to NRIs, stimulants modulate attention by potentiating monoaminergic activity in the DLPFC.¹⁰⁷ However, they also increase dopamine output in other brain areas inducing motor and affective changes as well as increasing the potential for drug abuse. Recent studies showed that stimulants, namely amphetamine, enhanced the efficiency of the DLPFC function assayed with fMRI during an N-Back, working memory task.¹⁰⁸ Interestingly, this effect seemed to be associated with a COMT (*val158met*) polymorphism. In subjects with the high enzyme activity *val/val* genotype, which presumably have relatively less prefrontal synaptic dopamine, amphetamine significantly increased DLPFC efficiency at all levels of N-Back load. In contrast, in subjects with the low activity *met/met* genotype who perform better on baseline prefrontal working memory tasks, the drug had no effect on cortical efficiency at low-to-moderate working memory load and caused deterioration at high working memory load.¹⁰⁸

The possibility that NRIs such as atomoxetine may be able to 1) improve cognitive functions other than attention deficit disorder and, 2) discriminate this effect through a COMT polymorphism related mechanism merits investigation. Enhanced dopaminergic output in the DLPFC may improve attentional difficulties possibly through indirect activation of DA-1 receptors known to modulate working memory and other executive functions.¹⁰⁹ Atomoxetine, as well as other potential NRIs, hold some potential as cognitive enhancers because of their ability to target very specifically prefrontal cortex dopaminergic neurotransmission.

DOPAMINE-1 AGONISTS AND DLPFC FUNCTION

Dopamine activity at D1 receptors mediates many of the cognitive processes subserved by the prefrontal cortex (PFC) discussed above, particularly working memory. Anatomical data indicates that the D1 receptor is the most abundant dopaminergic receptor in the mammalian PFC.¹¹⁰ Indeed, medications that directly activate the D1 receptor may remediate cognitive deficits in rats enhance

ing the accuracy of attentional performance and the speed of making correct responses.¹¹¹ By contrast, the use of dopaminergic antagonists, especially those more selective for the D1 receptor such as SCH23330, worsens performance in attentional tasks and in the delayed response paradigm.^{110–112}

Based on the above data, it is also reasonable to approach the therapeutics of cognitive dysfunction by directly stimulating the D1 receptor in the PFC. Although D1 receptor agonists are in experimental phase, one clinical trial has taken place in patients with schizophrenia using the selective partial D1 agonist SKF-38393. Interestingly, in line with previous findings using amphetamine,¹¹³ about one third of the patients had an improvement in perseverative errors in the WCST, whereas the other two thirds had a worsening of the perseverative errors,¹¹⁴ suggesting the possibility that the effect of direct DA-1 agonists on cognitive function may be also mediated through a genotype-mediated event. Another promising drug is dihydroxidine, a selective and full D1 agonist that is being developed for the treatment of Parkinson's disease. Animal studies have demonstrated cognitive enhancing properties of this drug,¹¹⁵ which appear mediated by D1 activation and by interaction with the cholinergic system.

In the last few years, a new class of D1 agonists typified by ABT-431 (DAS-431), with long-term solid-state stability, was synthesized.¹¹⁶ DAS-431 is fully converted to A-86929 rapidly by nonspecific plasma esterases with a $t_{1/2}$ of less than 60 s. A-86929 exhibits 20-fold higher (nanomolar) affinity for D1 and Dopamine-5 (D5) receptors when compared with the D-2 receptor and exhibits full agonist efficacy on D1-stimulated cAMP formation.¹¹⁷ A-86929 is also over 400 times more selective for dopamine D1 than D2 receptors.¹¹⁸ Both DAS-431 and A-86929 produced contralateral rotation in rats bearing unilateral 6-hydroxydopamine lesions and improve the behavioral disability scores and increased locomotor activity in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) marmoset model of Parkinson's disease. When administered chronically, A-86929 significantly improved disability scores during the duration of the study.^{117,118} DAS-431 has also been reported to reverse haloperidol-induced cognitive deficits such as spatial working memory and object working memory in monkeys, suggesting that it may be an effective treatment for the cognitive dysfunction associated with aging and disease.¹¹⁹

Preclinical studies showed that DAS-431 could attenuate the ability of cocaine to induce cocaine-seeking behavior and does not induce cocaine-seeking behavior in a rodent model of cocaine craving and relapse.¹²⁰ In human cocaine abusers, intravenous DAS-431 reduces cocaine craving and other cocaine-induced subjective effects. The results of animal abuse liability studies in-

dicate that DAS-431 is unlikely to have abuse potential in man.¹²¹

As the first full dopamine D1 receptor agonist to show efficacy in improving Parkinson's disease and cognitive function and to reduce the craving and subjective effects of cocaine in cocaine abusers, DAS-431 represents an important tool to understand the pharmacotherapeutic potential of DA-1 receptor agonists in different pathological conditions including cognitive function.

IMPLICATIONS FOR THE TREATMENT OF COGNITIVE DYSFUNCTION IN SCHIZOPHRENIA

The study of cognitive dysfunction associated with impairment of the DLPFC has been one of the hallmarks in schizophrenia research.^{121–126} Over the last two to three decades, a growing number of studies on DLPFC cognitive function^{127–129} and functional neuroimaging^{130–137} consistently revealed significant abnormalities in patients with schizophrenia. Exaggerated mesolimbic dopaminergic transmission has been proposed as the primary mechanism that contributes to the presence of positive symptoms such as delusions and hallucinations. This dopaminergic deregulation, however, does not seem to explain the prominent cognitive difficulties observed in patients with schizophrenia. Instead, more conclusive evidence suggests that the cognitive difficulties and negative symptoms (e.g., blunted affect, lack of motivation, poverty of speech, avolition, etc.) present in schizophrenia are related to a decreased connectivity and reduced functional activity of the mesocortical afferents innervating the DLPFC.^{138,139}

Dysfunction in prefrontal activation during working memory tasks in patients with schizophrenia has been reported by number of groups indicating that the working memory deficits are most likely related to the decreased connectivity and reduced activity of the mesocortical system.^{132,139–142} From a molecular pharmacological perspective, a reduction in presynaptic activity in the mesocortical dopaminergic system would decrease the input into postsynaptic sites with ensuing increase in the number or the affinity in postsynaptic receptors. In this context, when compared with control subjects, either a significant decrease or increase in the number of DA-1 receptors in the DLPFC of patients with schizophrenia was reported in studies using positron emission tomography^{143,144} and both changes have been associated with impaired cognition. The increase in DA-1 receptors strongly predicted poor performance in N-Back task, a reliable test of working memory.¹⁴⁴

Pharmacological intervention aiming at increasing synaptic dopamine in the synaptic cleft or activating directly the DA-1 receptor in the prefrontal cortex may represent a useful tool to target DLPFC mechanisms and

improve working memory and other cognitive deficits in patients with schizophrenia. Dopaminomimetic drugs (e.g., stimulants), however, may also produce a number of unwanted effects (i.e., worsening of psychosis, motor side effects) due to their effect on mesolimbic and nigrostriatal systems other than on the Me-DA pathway. The use of medications that specifically target the Me-DA system without acting on the other dopaminergic pathways may provide new avenues in the treatment of cognitive dysfunction in schizophrenia. This fact, coupled to the recent findings that patients with schizophrenia showed similar COMT allelic variations as in normal volunteers, and that genetic variation in this gene also predicts performance on tests of cognitive function, points toward a more targeted, genotype-based pharmacology for the treatment of cognitive disorders in schizophrenia.⁷⁴

Blockade of the catabolic mechanisms involved in DA regulation in the frontal cortex by inhibition of COMT may result in a very circumscribed and selective increase of dopamine in the frontal cortex without affecting subcortical regions¹⁴⁵ and represent another pharmacological mechanism for improving cortical cognitive function in schizophrenia.

The fact that modafinil, a drug used for the treatment of daytime sleepiness is also able to increase significantly extracellular dopamine in the prefrontal cortex⁹⁷ and to enhance short-term working memory in experimental animals may also lead to expanded strategies with this medication for the treatment of cognitive dysfunction in schizophrenia.¹⁴⁶ Modafinil proved to have a low side-effect profile and limited clinically relevant drug interactions, making this drug even more interesting from the perspective of its use in a combined therapeutic strategy with antipsychotics and other medications regularly prescribed in schizophrenia.

Early neuropsychological studies showed that an acute dose of dextroamphetamine, which increases dopamine levels in the DLPFC, has positive effects on affect and cognition in schizophrenic patients. However, because of its potential negative effects on psychosis, abnormal movements and behavior secondary to its effect on subcortical areas, the authors did not advocate the use of amphetamine as a routine clinical treatment of cognition problems in schizophrenia.¹⁴⁷ In the last few years, the specific NE reuptake inhibitors (e.g., atomoxetine) have been shown also to be effective in increasing extracellular dopamine concentrations in the DLPFC without effects on subcortical dopaminergic systems¹⁰⁴ and, hence, with no psychotomimetic effect or abuse potential. This very specific pharmacological action led to the development of this drug as a tool for the treatment of inattentiveness in attention deficit spectrum disorders.^{102,103} However, its spectrum of pharmacological action makes atomoxetine also a potential candidate to treat individu-

als with working memory difficulties including schizophrenia, mood disorders, and cognitive deficits of different origin. It is to be expected that, similar to the effect of stimulants in the DLPFC,¹⁴⁷ atomoxetine administration should result in an enhanced performance on WCST and improved activation in the DLPFC.

The evidence that impairment in cognitive function is connected with a deficit in the dopaminergic input to the DA-1 receptors in the DLPFC¹¹¹ opened new avenues for the development of specific dopaminergic agonists with activity on the DA-1 receptors.¹¹⁶ Even though no available compounds exist for routine clinical trials, recent phase I and phase II trials with DAS-431, a DA-1 agonist that is delivered by intravenous route, provides future options for more comprehensive clinical studies with direct DA-1 compounds with agonistic action. Changes in the number of DA-1 receptors in the PFC in patients with schizophrenia, irrespective of their medication status,^{143,144} and their association with impaired performance in tests that measure working memory,¹⁴⁴ supports the critical role of these receptors in cognitive control in schizophrenia. Drugs with DA-1 receptor agonistic activity would be of major interest for the treatment of the cognitive deficits of schizophrenia, and could be administered in conjunction with selective DA-2 antagonists for the combined treatment of positive and negative symptoms of schizophrenia.

CONCLUSIONS

The understanding of the pathophysiological mechanisms involved in the cognitive deficits observed in schizophrenia provided new grounds for the development of therapeutic strategies aiming at improving the DLPFC function. Preclinical studies identified a number of Food and Drug Administration (FDA)-approved drugs able to specifically regulate dopaminergic transmission in the terminals of the mesolimbic dopaminergic system

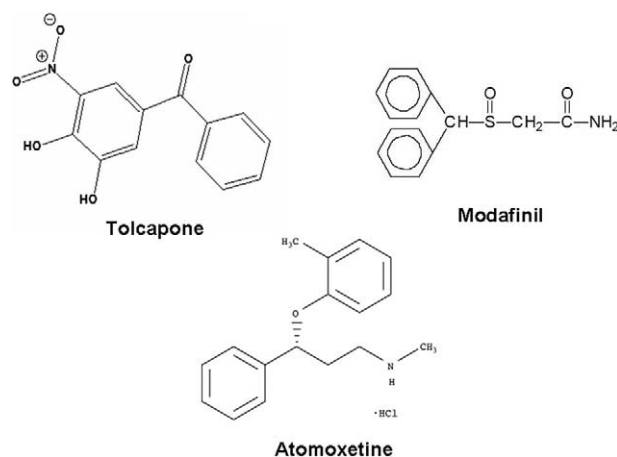


FIG. 1. FDA-approved drugs with selective prodopaminergic action at the level of the dorsolateral prefrontal cortex.

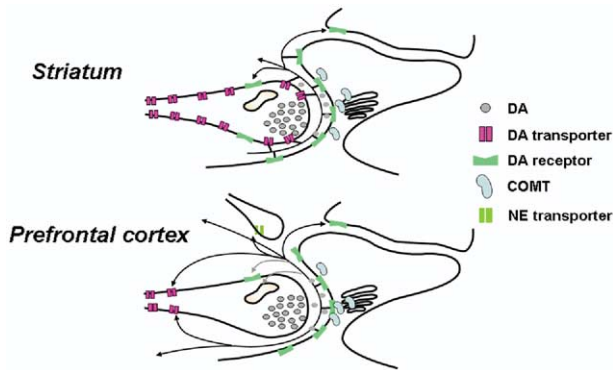


FIG. 2. Mesocortical dopamine terminals in the prefrontal cortex lack specific dopamine reuptake sites.

without affecting the neurotransmission on subcortical systems (see FIGS. 1 and 2). The ability of these compounds to improve cognitive function by increasing DA load to the DLPFC without stimulant-like effects provides an example of targeted pharmacology for the treatment of cognitive deficits in schizophrenia as well as other illnesses in which the DLPFC function is compromised. On the other hand, the preliminary evidence indicating that the effect of these drugs may be modified by interactions with the COMT genotype, the main catabolic mechanism in the prefrontal cortex, also illustrates the this new pharmacological approach may be also genotype-based.^{92,100,112,126,146}

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