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COMMENTARY

Mesoprefrontal Dopaminergic Neurons: Can Tyrosine Availability Influence Their Functions?

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ABSTRACT. The dopamine (DA) neurons projecting to the prefrontal cortex (PFC) are thought to be involved in working memory, stress response, and the pathogenesis of schizophrenia. In this commentary, we review the current evidence supporting a precursor tyrosine dependence of these mesoprefrondal DN neurons. Several studies in rats employing different experimental paradigms [i.e. experimental diabetes and early-treated phenylketonuria (PKU) model] have shown that reduced tyrosine levels in brain can affect markedly the physiology and functions of these DA neurons. However, supplemental tyrosine is effective in enhancing functional transmitter outflow from mesoprefrontal DA neurons only under conditions where their physiological activity is enhanced and DA synthesis and release are uncoupled from intrinsic regulatory controls. Recent studies in humans have also suggested that variations in brain tyrosine levels can affect significantly higher cortical functions subserved by the PFC. In early-treated PKU patients with mildly reduced tyrosine levels, marked impairments in cognitive functions dependent on the dorsolateral PFC could be detected. In drug-treated schizophrenic patients, supplemental tyrosine was shown to have a disruptive effects on the smooth-pursuit eye movement performance task. Furthermore, tyrosine administration was effective in restoring impaired working memory in humans following a cold stress paradigm, as assessed by a computer-based delayed matching-to-sample memory task. These human studies, together with the current evidence obtained from animal experiments, suggest that the functions of the mesoprefrontal DA neurons can, under certain circumstances, be readily influenced by the availability of the precursor tyrosine. BIOCHEM PHARMACOL 53;4:441–453, 1997.

KEY WORDS. dopamine neurons; prefrontal cortex; neurotransmitter precursor; tyrosine; phenylalanine; phenylketonuria; cognition; stress; dietary supplement

Whether varying the levels of tyrosine in the blood can affect higher cortical function in brain, and thus behavior, remains a controversial issue. Tyrosine is a neutral aromatic amino acid that is either supplied in our diet or derived from Phe,§ an essential amino acid found in foods. The hydroxylation of tyrosine to DOPA, catalyzed by the enzyme tyrosine hydroxylase, is the rate-limiting step in the biosynthesis of the catecholamines such as DA and NE. As summarized in an earlier commentary by Milner and Wurtman [1], entitled "Catecholamine Synthesis: Physiological Coupling to Precursor Supply," which appeared in *Biochemical Pharmacology* a decade ago, there had been convincing evidence obtained from extensive animal studies to support the idea that tyrosine availability can affect brain catecholamine synthesis and release. Importantly, it was known at that time that the state of physiological activity of the catecholamine neurons is the crucial determinant for the extent to which tyrosine availability can exert its effect on these neurons. It appeared that a given catecholamine neuron would only respond to more or less tyrosine when the physiological activity or firing rate of the neuron was enhanced [1]. Few attempts were made to extrapolate these experimental findings in animals to humans in order to determine whether tyrosine could affect behavior in healthy human subjects or in those with neurologic or psychiatric disorders. Since that time, neurochemical studies in rats identifying the DA neurons projecting to the medial PFC as one of the most susceptible targets of tyrosine availability in the brain have been reported [2, 3]. The rapid firing rate of these DA neurons in their basal state appears to account for the susceptibility of these neurons to be influenced by precursor availability [4]. These observations have provided the rationale for subsequent studies to assess whether tyrosine availability could influence some of the higher cortical functions subserved by the PFC. More recently, several studies reported by independent groups of investigators in the areas of child psychology, psychiatry, and stress-modified neuropsychological performance have

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§ *Abbreviations:* Phe, phenylalanine; DA, Dopamine; PFC, prefrontal cortex, NE, norepinephrine; VTA, ventral tegmental area; 5-HT, serotonin; PKU, phenylketonuria; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindole acetic acid; DOPA, 3,4-dihydroxyphenylalanine; GABA, γ-aminobutyric acid; SPEM, smooth-pursuit eye movements; and DOPAC, 3,4-dihydroxyphenylacetic acid.

provided converging evidence indicating that varying tyrosine levels in the brain can affect cognitive functions that are mediated by the PFC and its DA innervation in humans [5–9]. This commentary highlights some of the recent obserevations on the structure and characteristics of the mesoprefrontal DA system and their regulatory controls, and reviews the recent findings on the tyrosine dependence of this particular subset of the midbrain DA systems.

MESOPREFRONTAL DOPAMINERGIC NEURONS

Unique Characteristics

The DA neurons innervating the medial PFC originate from the A10 cell group in the ventral mesencephalon. Extensive biochemical and physiological studies have revealed that these DA neurons possess distinct characteristics as compared with other midbrain DA neurons such as the mesolimbic and nigrostriatal DA neurons. These unique characteristics include a faster firing rate and more bursting activity, increased transmitter turnover, diminished responsiveness to DA agonists and antagonists, lack of tolerance development following chronic antipsychotic drug administration, and resistance to development of depolarization-induced inactivation following chronic treatment with antipsychotic drugs [10]. These mesoprefrontal DA neurons also exhibit enhanced susceptibility to activation by stress or conditioned fear [11], and are selectively activated by systemic administration of anxiogenic benzodiazepine inverse agonists (β-carbolines) [12–15].

Regulatory Controls

A number of studies have provided evidence suggesting that the unique characteristics of the mesoprefrontal DA neurons can be attributed to some of the distinct intrinsic and extrinsic regulatory controls that are operating in these DA neurons (see Table 1).

INTRINSICS. DA autoreceptors are found on the soma, dendrites, and nerve termainals of midbrain DA neurons. Three functional types of DA autoreceptors have been identified: somatodendritic impulse-modulating autoreceptors; nerve-terminal synthesis-modulating autoreceptors; and nerve-terminal release-modulating autoreceptors. Stimulation of these DA autoreceptors exerts feedback regulatory influences in a concerted manner on the firing rate, DA synthesis, and DA release of these DA cells. The nigrostriatal and mesolimbic DA neurons possess all three types of autoreceptors, whereas the mesoprefrontal DA cells appear to lack or have diminished numbers of both the impulse-regulating and synthesis-regulating autoreceptors. The absence of these DA autoreceptors on the mesoprefrontal DA neurons has been postulated to be responsible, in part, for some of the unique characteristics of these neurons (reviewed in Ref. 16).

EXTRINSIC. Exposure to acute stress results in a preferential activation of DA metabolism and DA release in the mesoprefrontal DA system. Numerous studies attempting to characterize the various afferent inputs to DA and non-DA neurons in the VTA have been reported to date (reviewed in Refs. 17 and 18). Some of these afferent inputs to the VTA have been shown to play a role in mediating the stress-induced activation of the mesoprefrontal DA system (see Fig. 1). The various neurotransmitters identified in those VTA afferents thought to influence mesoprefrontal DA cell functions include: 5-HT, NE, acetylcholine, GABA (via GABA/benzodiazepine receptors), opioid peptides such as dynorphin B and Met-enkephalin, excitatory amino acids such as glutamate, and neuropeptides such as neurotensin, substance P, and calcitonin gene-related peptide [19, 20].

TABLE 1. Regulatory controls of mesoprefrontal DA neurons

(1)	Impulse flow-dependent	
	(a) Intrinsic regulatory controls	
		–Release modulation autoreceptors
		–Firing pattern
		–End-product inhibition of synthesis
		–Transmitter re-uptake
		–Neuropeptide coexistence (NT, CCK)
	(b) Extrinsic regulatory controls	
		(i) Afferent inputs that innervate the somatodendritic regions of mesencephalic DA neurons
		–acetylcholine
		–neuropeptides (SP, opioids, NT, CGRP)
		–monoamines (5-HT, NE)
		–inhibitory amino acids (GABA, GABA/benzodiazepine)
		–excitatory amino acids (NMDA)
		(ii) Precursor availability (tyrosine)
(2)	Impulse flow-independent	
	(a) Intrinsic prefrontal cortical GABAergic interneurons	
	(b) Afferent inputs to DA terminals (NT)	

Abbreviations not defined previously: NT, neurotensin; CCK, cholecystokinin; SP, substance P; CGRP, calcitonin gene-related peptide; and NMDA, *N*-methyl-D-aspartic acid.

Heterogeneity and Interspecies Differences

Berger *et al.* recently identified two classes of DA afferents that project to the cerebral cortex of the rat. Class I DA afferents are found predominantly in the deep cortical layers, whereas Class II afferents distribute mainly in the superficial cortical layers. These two classes of DA afferents can be distinguished further by their differences in time of development, regional distributions, morphology, coexistence with neurotensin, site of origin in the mesencephalon, collateralization, DA content, and rate of DA turnover (reviewed in Ref. 21). To further complicate our views on the organization of the mesocortical DA systems, recent studies have shown that DA afferents to the cerebral cortex in primates are distinct from their counterparts in rodents. In primates including humans, the cortical DA projections

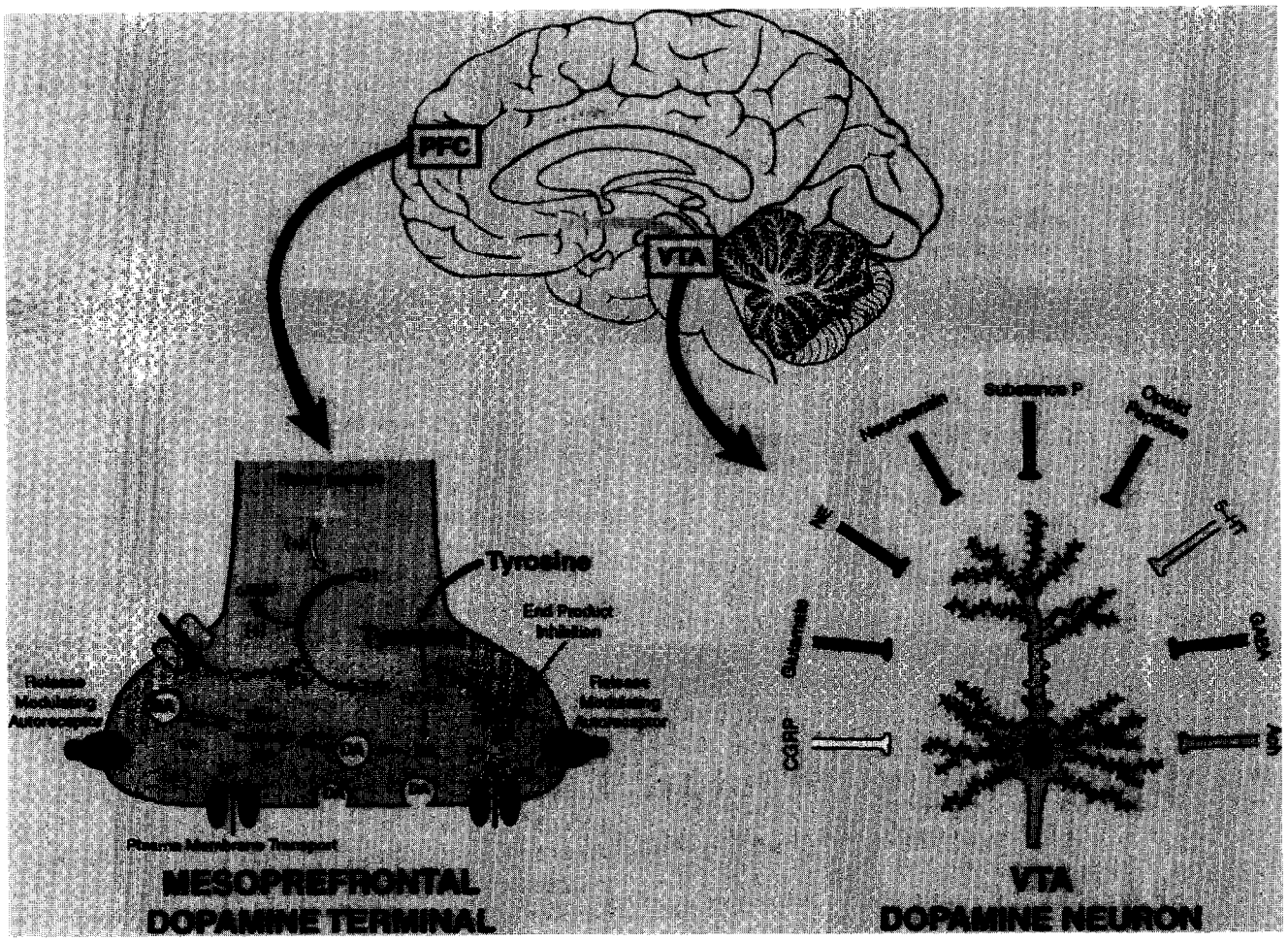

FIG. 1. Schematic diagram illustrating the anatomy of the mesoprefrontal DA system depicting the afferent inputs to the VTA believed to play a role in the modulation of mesoprefrontal DA function and the intrinsic regulation of DA in the prefrontocortical nerve terminal. Abbreviations: Ach, acetylcholine; CGRP, calcitonin gene-related peptide; DA, dopamine; DOPA, 3,4-dihydroxyphenylalanine; GABA, γ-aminobutyric acid; 5-HT, 5-hydroxytryptamine; NE, norepinephrine; PFC, prefrontal cortex; TH, tyrosine hydroxylase; and VTA, ventral tegmental area.

appear to reach their full development by the first half of the prenatal period, and the mesoprefrontal DA neurons do not appear to co-localize with either neurotensin or cholecystokinin [21]. These findings will probably have significant impact on the future directions of research in this area. Importantly, the apparent heterogeneity observed with these mesocortical DA systems poses further questions on how the two distinct classes of mesoprefrontal DA neurons are regulated. Distinct regulatory controls could also explain, in part, some of the interspecies differences noted. Furthermore, biochemical and physiological studies of the regulatory controls of the mesoprefrontal DA systems using non-human primates may prove to be essential in understanding the critical roles of these DA neurons in the pathogenesis of certain psychiatric disorders such as schizophrenia and post-traumatic stress disorder.

Functional Implications

The intense attention focused on the prefrontal DA innervation has been stimulated by the possible involvement of these neurons in higher cognitive function and their possible roles in the etiology of schizophrenia.

WORKING MEMORY. The PFC plays an important role in the processing of working memory. Working memory is defined as the ability to remember relevant events for a short period of time, and can be distinguished from semantic or procedural memories that are acquired by repeated association between stimuli and responses. Working memory has been studied in non-human primates and in rodents, using delayed alternation task paradigms. Using such an experimental model, it has been shown that deficits in working memory can result from destruction or removal of the PFC, and such deficits can be mimicked further by depletion of DA or blockade of DA receptors in the PFC. These studies thus suggest that the mesoprefrontal DA system is critical for the processing of working memory, a higher cortical function that is dependent on the PFC (reviewed in Ref. 22). More recent studies have demonstrated that there is also a critical balance in dopaminergic tone required for optimal PFC-dependent working memory. Ex-

cessive DA tone induced by stress can lead to severe dysfunction of the PFC and cognitive impairment [15].

SCHIZOPHRENAI. The PFC has also emerged as a key brain region thought to be critically involved in the pathogenesis of schizophrenia. Functional *in vivo* brain imaging studies have consistently indicated a strong prevalence of hypoprefrontality among schizophrenic patients. Based on the speculation that schizophrenia is associated with an abnormality of brain development, Weinberger [23] put forward a neurodevelopmental model for the etiology for schizophrenia. In light of the many important roles the mesoprefrontal DA system palys in cognitive and physiological functions that are dependent on PFC, it was hypothesized that a developmentally specific abnormality of the mesoprefrontal DA afferents occurs in schizophrenic subjects, and this dysfunction or diminished activity of the mesocortical DA system renders a corresponding enhancement in mesolimbic DA activity. The resulting decreased activity of the frontal lobe and the disinhibition of the limbic functions have been speculated to account for some of the negative and positive symptoms observed in schizophrenic patients [24]. Consistent with this hypothesis, recent studies have reported abnormalities in neuronal organization of the PFC of schizophrenic brains. Benes and coworkers [25] reported a reduced density of small interneurons and an increase in GABA receptor binding activity in PFC in schizophrenic patients. A recent study by Akbarian *et al.* [26] has indicated a decrease in expression of the mRNA encoded for glutamic acid decarboxylase in prefrontal cortical neurons in schizophrenic subjects, without any apparent cell loss in the PFC. In contrast, recent reports by Daviss and Lewis [27] and by Selemon *et al.* [28] have demonstrated an increased density of nonpyramidal and pyramidal neurons in the schizophrenic PFC, suggesting that neuronal atrophy may play an important role in the pathophysiology of this disease [28]. On the other hand, some of the cognitive, emotional, and motivational deficts associated with schizophrenia are very similar to those symptoms observed in patients with physical damage in the PFC (reviewed in Ref. 28). These findings are consistent with the view that further investigation of how the activity of the PFC is regulated by DA and other neurotransmitters is essential for providing additional insight toward understanding the etiology of schizophrenia.

Presursor Control of DA Synthesis and Release in Mesoprefrontal DA Neurons

The ability of precursor availability to influence the synthesis of neurotransmitters in the brain has been demonstrated in neurons utilizing 5-HT, NE, DA, and acetylcholine (reviewed in Refs. 29 and 30). Administration of low doses of tryptophan readily elevates brain levels of 5-HT and its metabolite, 5-HIAA. However, the synthesis of catecholamines in, and their release from, catecholamine neurons, appear to be unaffected by administration of the precursor tyrosine under normal circumstances. However, if experimental manipulations were employed to increase the firing rates and transmitter turnover in catecholamine neurons, tyrosine administration was shown to be effective in enhancing catecholamine metabolism in these neurons [1, 29, 30]. Similarly, in the retina, tyrosine hydroxylase activity in DA-containing amacrine cells is activated in response to light stimulation. Supplemental tyrosine increases *in vivo* tryosine hydroxylation and DA metabolite levels in amacrine cells when the animals are exposed to light, but not when they are kept in the dark [31].

In 1984, biochemical and electrophysiological studies were published revealing that the mesoprefrontal DA neurons are distinct from the other midbrain DA neurons in their higher basal firing rate and their rapid transmitter turnover [4]. Based on these findings, we asked the question that seemed rather obvious at the time: If the tyrosine dependence of catecholamine synthesis is observed only in catecholamine neurons that have enhanced physiological activity, would transmitter synthesis in and release from mesoprefrontal DA neurons, which exhibit very high basal firing frequency, be readily influenced by variations in the levels of tyrosine in the brain? Surprisingly, the answer is both yes and no. The answer is yes, as it was shown subsequently that the mesopreformal DA neurons are the most susceptible subset of midbrain DA neurons to the effects of a chronic reduction in brain levels of tyrosine on DA synthesis and DA metabolism. However, this answer had to be qualified since tyrosine administration failed to increase functional outflow of the mesoprefrontal DA system under normal circumstances, even though it could be demonstrated that aphysiologically relevant dose of tyrosine can elevate DA synthesis and DA levels in these mesocortical neurons.

Effects of Increased Brain Tyrosine Levels on Mesoprefrontal DA Synthesis and Release

NORMAL QUIESCENT PHYSIOLOGICAL CONDITIONS. Doses of 25 and 50 mg/kg of tyrosine were initially used to examine the effects of tyrosine administration on *in vivo* tyrosine hydroxylase activity in selected DA terminal field regions [2], as previous studies had indicated that low doses of tyrosine were more effective in enhancing catecholamine synthesis in rat brain than doses of 50 mg/kg and above [32]. The effects of tyrosine administration on DA synthesis and release in the mesoprefrontal DA neurons were found to exhibit several distinct features. On the one hand, the tyrosine effect on the *in vivo* tyrosine hydroxylation in these DA neurons followed a somewhat paradoxical dose–response relationship. A higher dose of tyrosine (50 mg/kg) was found to elevate tyrosine hydroxylation rapidly, and the resultant increase in intracellular DA levles in these neurons appeared to normalize subsequent tyrosine hydroxylation by end-product inhibition. On the other hand, a low dose of tyrosine (25 mg/kg) was shown to cause a more persistent enhancement of *in vivo* tyrosine hydroxylation, as

endogenous DA levels were not sufficiently elevated by a small tyrosine dose to exert a negative feedback control on tyrosine hydroxylase activity. Indeed, in almost all of our studies using these two doses of tyrosine, there appeared to be an inverse reciprocal relationship between the degree of enhancement of DOPA accumulation (index of *in vivo* tyrosine hydroxylase activity) and the amount of endogenous DA detected concurrently in the PFC (unpublished data). These findings suggest that DA synthesis in mesoprefrontal DA neurons is readily influenced by precursor availability, but it appears at the same time to be tightly regulated by end-product via alterations in endogenous DA levels. A recent study confirmed our findings that high levels of brain tyrosine do not affect significantly *in vivo* tyrosine hydroxylation in the PFC [33]. In rats that had ingested isocaloric diets containing 2, 5, 10, or 20% protein for 2 weeks, brain levels of tyrosine were increased to 6-fold above controls. The rate of *in vivo* tyrosine hydroxylation in retina and hypothalamus, but not the PFC, was found to be elevated significantly, although a trend towards an increase could be detected in PFC [33]. In our previous study [2] tyrosine administered acutely at 25 mg/kg (the dose that was effective in enhancing tyrosine hydroxylase activity in the PFC) caused only a 60% increase in tyrosine levels in the PFC.

In catecholamine neurons that were experimentally manipulated to fire more rapidly, tyrosine administration was found to elevate catecholamine metabolite levels in these neurons (reviewed in Ref. 1). However, in our studies on the PFC under basal control conditions, no changes in the levels of DA metabolites such as DOPAC and HVA were observed in the PFC following acute tyrosine administration, even at doses of tyrosine that enhance tyrosine hydroxylase activity (25 mg/kg) or higher doses that are ineffective (200 mg/kg) [2]. Thus, the functional outflow of the mesoprefrontal neurons is not affected significantly by tyrosine supplementation under normal circumstances.

ACTIVATED PHYSIOLOGICAL CONDITIONS. In view of the susceptibility of the mesoprefrontal DA system to various forms of stress, it was of interest to assess whether the functional output of these DA neurons could be altered by tyrosine supplementation under conditions in which the activity of these neurons was further enhanced. The mesoprefrontal DA neurons were selectively activated by administration of the anxiogenic β-caboline FG 7142, and the ability of small doses of tyrosine to alter prefrontal DA metabolite levels was examined. Our previous studies with FG 7142 have indicated that the level of enhancement in prefrontal DA metabolite levels elicited by this drug (+50%) is significantly less than that observed when the mesoprefrontal neurons are activated by mild footshock stress (>100%) [11–13]. Interestingly, tyrosine administration was shown to elevate dramatically DA metabolite levels in FG 7142-activated mesoprefrontal DA neurons to the levels observed in mild footshock stress paradigm, thus suggesting that a further enhancement of the physiological activity of these DA neurons can render them much more dependent on tyrosine availability for maintenance of transmitter output. However, in another series of experiments, when mesoprefrontal DA neurons were activated by a mild footshock stress paradigm [11], tyrosine administration did not appear to further elevate the enhanced DA metabolite levels observed in the PFC (unpublished data). This latter finding, together with other evidence obtained from our footshock stress experiments, has led us to speculate that the DA outflow from the mesoprefrontal DA neurons may have reached the maximum level under the footshock stress paradigm, and an increase in tyrosine availability can elicit no further enhancement of the stress-induced increase in DA metabolism. A recent study by Morrow *et al.* [34] has provided some additional evidence supporting this speculation. Using an aversive conditioning or conditioned fear paradigm, rats were conditioned to fear a tone that was paired with footshock. When rats were subsequently exposed to the tone, they exhibited typical fear responses with elevated DA metabolism in both the PFC and the nucleus accumbens. Interestingly, tyrosine administered at 25 mg/kg did not further enhance prefrontal DA metabolism in the conditioned group, but significantly elevated the DOPAC/DA ratio in the PFC of non-conditioned animals to the levels observed with the conditioned groups [34]. The observed tyrosine effects with the non-conditioned group can most likley be accounted for by the fact that these animals have experienced a mild level of stress when they were handled and subsequently exposed to a novel environment as required for a negative control for conditioning. This less severe stressor may have resulted in a submaximal activation of the mesoprefrontal DA system.

In contrast to the PFC, tyrosine treatment did not alter DA metabolism in the nucleus accumbens in control rats in the above study but elicited a significant increase in DA utilization in animals subjected to conditioned stress as compared with those observed with other experimental groups [34]. These findings are consistent with a partial activation of the mesoaccumbens neurons by the conditioning paradigm, making these neurons more susceptible to precursor regulation without attaining a ceiling effect as observed in the PFC DA neurons. Consistent with what has been shown previously, the enhancement effect after tyrosine supplementation observed in the PFC of control animals in this study indicates that a mild form of stress (handling) selectively activates DA metabolism in the PFC but not in the nucleus accumbens. However, a more severe form of stress (conditioned fear) appears to elicit a maximal rate of DA utilization in the mesoprefrontal neurons, resulting in a ceiling effect on DA metabolism and turnover in the PFC, but a submaximal effect on the mesolimbic DA system. Thus, tyrosine administration is unable to further enhance this increase in the PFC, but it is able to enhance the submaximal effect in the nucleus accumbens. Different intrinsic regulatory controls operating in these two DA systems may account, in part, for the difference in the ways these two DA systems respond to stress and to tyrosine

supplementation. The absence of synthesis and impulse modulating DA autoreceptors may make the mesoprefrontal DA neurons, in contrast to the mesoaccumbens DA neurons, more responsive to regulation by afferent inputs to the VTA.

Effects of Reduced Brain Tyrosine Levels on Mesoprefrontal DA Synthesis and Release

ACUTE VALINE ADMINISTRATION. It has been shown previously that administration of valine, a large neutral amino acid that competes with tyrosine for uptake into the brain, causes a dramatic reduction in brain tyrosine levels. In one of our earlier studies, we examined the effects of an acute dose of valine on DA synthesis and metabolism in the PFC. Interestingly, although valine administration significantly reduced the rate of *in vivo* tyrosine hydroxylation in the PFC, the prefrontal DA metabolite levels were not altered significantly by valine [2]. These data are again consistent with the concept that the functional outflow of the mesoprefrontal DA neurons under normal circumstances is not affected significantly by acute fluctuations in brain tyrosine levels. Recently, an acute tyrosine depletion paradigm employing oral administration of a mixture of amino acids lacking both tyrosine and Phe has been developed [35]. This paradigm was shown to lower brain tyrosine levels rapidly in rats following intubation of this mixture and to reduce the rate of *in vivo* tyrosine hydroxylation in both the retina and hypothalamus. Although the PFC was not examined in these studies, on the basis of the valine study it is quite likely that DA synthesis in the mesoprefrontal DA neurons would respond to acute tyrosine depletion in a similar fashion. No evidence has been provided that the acute short-term depletion of tyrosine induced by this technique or administration of competing amino acids and the small reduction of transmitter synthesis are accompanied by a reduction in the outflow of transmitter in the PFC. A longer term depletion of tyrosine appears to be required (see below).

EXPERIMENTAL DIABETES. The level of tyrosine in the brain is determined by the relative ratio of plasma concentration of tyrosine to that of other large neutral amino acids that compete for a common transport carrier across the blood–brain barrier. In diabetes, the plasma levels of the competing branched-chain amino acids are greatly elevated, resulting in a marked and prolonged reduction of tyrosine levels in the brain. To assess how the functional output of the mesoprefrontal DA system would be affected by a prolonged and persistent reduction in tyrosine availability, Bradberry *et al.* [3] used streptozotocin to induce experimental diabetes in rats, which resulted in a significant and chronic decrease in the levels of brain amino acids, including tyrosine. Although the rate of *in vivo* tyrosine hydroxylation and the DA metabolite levels are decreased significantly in certain subsets of midbrain DA neurons in animals treated with streptozotocin, the mesoprefrontal DA neurons appear to be the most susceptible to the diabetes-induced effects on DA synthesis and metabolism. Indeed, there was a significnat positive correlation between PFC tyrosine levels and the levels of DOPA accumulation detected in the PFC. Insulin treatment of the diabetic rats was shown to normalize tyrosine levels in the brain, and to restore the levels of tyrosine hydroxylase activity and DA metabolism to normal values [3]. These studies thus provide good evidence indicating that the DA synthesis and metabolism of the mesoprefrontal DA neurons are highly sensitive to, and profoundly affected by a chronic reduction in tyrosine levels in the brain.

EXPERIMENTAL MODEL OF EARLY-TREATED PKU. PKU, an autosomal recessive disorder with a frequency of 1/10,000 to 1/20,000 in live births, is caused by mutations in the gene encoding phenylalanine hydroxylase, the enzyme that converts Phe to tyrosine. The mutations result in the absence or markedly reduced activity of the enzyme phenylalanine hydroxylase in the liver, leading to an elevation of Phe levels in the bloodstream and decreases in the levels of tyrosine in the blood (reviewed in Ref. 36). This genetic disorder, if untreated, can often lead to brain damage and severe mental retardation. The current treatment consists of a diet regimen low in Phe given early after birth, and has been shown to be successful in reducing Phe levels in the bloodstream in these patients and in averting the prognosis from mental retardation and other neurological dysfunctions to relatively normal development [37]. However, some recent studies have revealed that in children who have been treated early and continuously since birth for PKU but whose plasma Phe levels were approximately 3–5 times above normal, certain impairments in cognitive functions can still be detected. Specifically, children treated early for PKU exhibit deficits in problem solving, especially those requiring abstract reasoning and executive functions, reaction time or speed of mental processing, and sustained attention (reviewed in Ref. 38). Interestingly, several earlier studies have implicated that the cognitive deficts exhibited by these patients are linked to prefrontal dysfunction ([39, 40] and reviewed in Ref. 5). Thus, it was hypothesized by Welsh *et al.* [5] that the cognitive deficits observed with these PKU patients are caused by prefrontal dysfunction, which, in turn, is caused by DA depletion in the brain due to less precursor tyrosine availability.

Based on the observations that DA synthesis and metabolism in the mesoprefrontal DA neurons are most sensitive to a chronic mild reduction in tyrosine levels in the brain as compared with other midbrain DA systems [2, 3], Diamond *et al.* [41] reformulated the prefrontal dysfunction hypothesis. They suggested that the various cognitive deficits that are dependent on the PFC and which are observed in early-treated PKU patients may represent the functional consequences of a reduction in DA outflow from the mesoprefrontal DA system due to elevated blood Phe levels and, therefore, less entry of precursor tyrosine into these DA neurons. As Phe and tyrosine compete for the same transporter protein to cross the blood–brian barrier and the trnasporters exhibit higher affinity for Phe than for tyrosine, a slight imbalance in the plasma Phe:tyrosine ratio

found in some early-treated PKU patients would lead to less tyrosine crossing into the brain. To test their hypothesis, Diamond *et al.* [41] developed an animal model of early-treated PKU. In this model, rats were treated (i) prenatally and postnatally, or (ii) postnatally, with Phe and α-methylphenylalanine, which serves as a phenylalanine hydroxylase inhibitor. These two different treatment schedules resulted in a significant elevation of the plasma Phe levels to six times above the control levels, accompanied by significant behavioral and neurochemical effects observed with these animals. Specifically, the two treatment groups were shown to exhibit impairment on the delayed alternation task test, which is indicative of prefrontal cortical function, and the levels of the DA metabolite, HVA, were reduced markedly in the PFC (−50%l) and in the cingulate cortex (−70%) [41]. In addition, levels of the 5-HT metabolite 5-HIAA were reduced significantly in all brain regions examined. However, of all the neurochemical changes that were observed, the diminished levels of HVA in the PFC observed in the treatment groups represent the only neurochemical effect that was found to be significantly correlated with all measures of performance on the delayed alternation task [41]. Thus, these studies indicate that a mild elevation of Phe levels in the plasma can result in a diminished DA metabolism and function in the mesoprefrontal DA system, accompanied by a significant impairment in the delayed alternation task that is dependent on prefrontal cortical functioning. These data are consistent with the hypothesis that the behavioral deficits observed in early-treated PKU patients are caused by specific changes in function of the DA system projecting to the PFC as a consequence of alterations in the plasma Phe:Tyr balance and diminished tyrosine transport into the brain [41]. However, more direct evidence may be necessary to further substantiate the validity of this animal model in supporting the prefrontal dysfunction hypothesis of early-treated PKU. For example, it will be of interest to determine if brain tyrosine is actually diminished and if normalizing brain tyrosine will be effective in reversing the noted deficits in PFC function and, importantly, in restoring the reduced DA metabolism in the PFC to normal in the treated animals showing improved function.

Recently, using *N*-ethyl-*N*-nitrosourea as a germline mutagen, McDonald and coworkers [42] developed a mouse model for human PKU. Several mutant mouse lines have been produced, and two lines, PAH^{ENU-2} and PAH^{ENU-3}, in particular, exhibit phenotypes characteristic of classical PKU seen in humans. Thus, it will be of interest to examine if the prefrontal dopaminergic function is compromised in these mutant mice, as seen in rats with experimental diabetes or the rat model of early-treated PKU and if tyrosine administration can normalize PFC function in these mouse mutants.

Perspectives

Based on a large body of convincing evidence, Wurtman *et al.* [29] formulated the principle that tyrosine supplementation can increase catecholamine synthesis in and release from catecholamine neurons only under conditions in which the physiological activity of the catecholamine neurons is enhanced. Consistent with this hypothesis, a physiologically relevant dose of supplemental tyrosine can enhance tyrosine hydroxylation in mesoprefrontal DA neurons, which have been found previously to possess a high basal level of firing frequency [2, 4]. Similarly, DA synthesis and metabolism in the mesoprefrontal DA neurons are most sensitive among all the midbrain DA neurons to a chronic reduction in brain tyrosine levels [3]. The enhanced responsiveness to the availability of tyrosine in these DA neurons is consistent with the findings that a greater proportion of the tyrosine hydroxylase enzyme associated with the mesoprefrontal DA neurons is in an activated form than that found in other midbrain DA neurons, apparently as a consequence of their rapid firing rate [43].

Although tyrosine hydroxylation in the mesoprefrontal DA neurons is readily influenced by tyrosine supplementation, transmitter turnover, as reflected by DA metabolite levels in these neurons, is not affected significantly by tyrosine administration under normal circumstances [2]. Thus, these findings are not consistent with previous findings showing increased catecholamine metabolite levels in activated catecholamine neurons following tyrosine administration [1, 29]. Similarly, valine administration significantly reduces brain tyrosine levels and tyrosine hydroxylation in the PFC, but does not appear to affect metabolite outflow from the mesoprefrontal DA neurons [2]. It appears that under basal conditions, the mesoprefrontal DA neurons possess autoregulatory mechanisms to maintain transmitter homeostasis. The increase in tyrosine hydroxylation induced by tyrosine administration in the PFC appears to be rapidly offset by inhibitory feedback effects exerted by elevated levels of endogenous DA [2]. Moreover, studies by Galloway *et al.* [44] have indicated that changes in DA synthesis and release are tightly coupled in mesoprefrontal DA neurons, as a consequence of their rapid firing rate and small transmitter pool. Therefore, as DA synthesis returns to basal level, due to increased feedback inhibition as a result of elevated DA levels, DA release is normalized, and no further increase in metabolite levels can be detected. This view is consistent with the findings reported by During *et al.* [45] for mesoaccumbens and nigro-striatal DA neurons. Using the brain microdialysis technique, it was shown that a systemic administration of tyrosine (200 mg/kg) significantly enhances DA release from the nucleus accumbens and striatum under normal basal conditions. However, the effect is short-lived, suggesting that a feedback mechanism may function to normalize DA release in these DA projection regions as well. Moreover, the rapid cessation of the tyrosine effect in these DA terminals may explain some of the previous findings showing the lack of tyrosine effects on tissue DA levels measured in these brain areas [45]. Thus, a similar scenario can be applied to the mesoprefrontal DA neurons, and it would be interesting to determine,

using the similar microdialysis method, whether tyrosine can enhance DA release from the PFC under basal or activated conditions.

Several mechanisms have been proposed to account for the relationship between precursor dependence and firing frequency or degree of bursting in DA neurons. It was suggested that the link between these two phenomena may involve tyrosine hydroxylase existing in a kinetically activated form or the absence of DA autoreceptor regulation [29]. However, the apparent lack of effect of tyrosine supplementation on DA metabolism in the mesoprefrontal DA system is not consistent with some of these proposed mechanisms. Although mesorprefrontal DA neurons are found to possess high basal firing frequency, to lack both synthesis-modulating and impulse-modulating autoreceptors, and to contain tyrosine hydroxylase in actived forms, supplemental tyrosine is ineffective in enhancing DA outflow form these neurons under normal unperturbed circumstances [2]. However, when these neurons are further activated by an apparent mild form of stress or the administration of β-carbolines, DA metabolism is greatly enhanced in these DA neurons [2, 34]. Thus, the critical factor that determines the dependence of DA metabolism on supplemental tyrosine in the mesoprefrontal neurons appears directly related to the non-homeostatic state of physiological or pharmacological activation of these neurons. For example, the activation of the afferent inputs regulating these DA cells following mild stress or β-carboline administration may override some of the cellular homeostasis mechanisms functioning in the quiescent neurons, including the intrinsic regulatory controls governing DA synthesis and release.

An acute tyrosine depletion is also ineffective in altering DA metabolism in these mesoprefrontal DA neurons, whereas the transmitter outflow and the functional response of these DA neurons are affected significantly by a chronic reduction in brain tyrosine levels [2, 3, 41]. The high basal firing rate and the rapid basal transmitter turnover render the mesoprefrontal DA neurons less capable in compensating for the deleterious effect of a chornic precursor depletion on transmitter synthesis and release. Thus, it is reasonable to speculate that the reduction in DA metabolism and the impairment in prefrontal functioning observed in experimental diabetes and in experimental PKU reflect the dysfunctioning of these DA neurons as a consequence of dysregulated DA synthesis and release due to a chronic reduction in tyrosine supply.

Effects of Precursor Tyrosine Availability on Behaviors in Animals and in Humans

ANIMAL STUDIES. Some earlier and several recent studies have suggested that tyrosine supplementation can reverse stress-induced cognitive and behavioral deficits in animals. It has been shown in rodents that tyrosine administration reverses the stress-induced inhibition of subsequent exploratory behavior in an open field, the hypothermia-induced behavioral depression, the intruder aggression, and the immobility after swim test (reviewed in Ref. 1). Recent studies have shown that tyrosine pretreatment prior to cold stress is effective in improving cold stress-induced decrements in delayed matching-to-sample performance [46], and in reversing the cold stress-induced impairment in a differential-reinforcement-of-low-rate reinforcement schedule [47]. Using a conditioned fear paradigm, Morrow *et al.* [34] have shown that tyrosine administration enhances the fear-induced immobility, which may represent a coping strategy to minimize the adverse effects in response to stress.

HUMAN STUDIES. Although many animal studies have shown that tyrosine supplementation can influence behavior in rodents, the relevance of these findings to human health and diseases was not investigated extensively. During the last few years, however, several research groups have reported interesting evidence in humans, indicating that tyrosine availability can influence specific cognitive functions that are subserved by the mesoprefrontal DA neurons (see Table 2).

Early-treated PKU. As discussed above, it has been shown that in children who were treated early and continuously for PKU and whose blood levels of Phe were 3–5 times above normal, significant cognitive deficits could be detected (reviewed in Ref. 38). It was suggested by early studies that a deficit in information processing may be the underlying cause of these developmental problems. To date, many studies have indicated that treated patients with PKU consistently exhibit deficits in problem solving, particularly abstract reasoning and executive function, reaction time or speed of mental processing, and sustained attention [38]. In particular, attention has been focused on the deficits in executive function of these patients. Executive function is thought to be indicative of prefrontal functioning in developing humans and nonhuman primates, and is defined as the mental ability to organize an appropriate problem-solving set for carrying out goal-directed activity [49]. Several earlier studies have suggested a dysfunction in PFC in early-treated PKU patients ([39, 40] and reviewed in Ref. 5). It was hypothesized that the mild elevations in Phe levels exhibited in these patients result in a mild depletion of DA in the PFC, which, in turn, causes prefrontal dysfunction [5]. To test this hypothesis, Welsh *et al.* [5] examined the cognitive functions of 11 preschool early-treated children using a battery of executive function measures indicative of dorsolateral prefrontal functions. The study showed that PKU patients exhibited significant impairment on an executive function composite score. Moreover, there was a negative correlation between the composite score and the Phe levels detected among the PKU children. These findings therefore support the prefrontal dysfunction hypothesis of early-treated PKU and suggest further that the executive function deficits observed in these patients are directly dependent on the plasma Phe levels and thus can

TABLE 2. Effects of tyrosine availability on mesoprefrontal dopaminergic metabolism and functions

Species	State of mes-PFC physiological activity	Experimental paradigm: brain tyrosine change	Effects on meso-PFC DA metabolism	Effects on behavior or cognitive functions subserved by PFC	Ref(s).
Rat	Basal	+Tyrosine (i.p.): ↑	DOPA ↑ DA ↑ DOPAC (±)	ND	2
Rat	Basal	+Protein-enriched diet: ↑↑↑	DOPA (±)	ND	33
Rat	Basal	+Valine (i.p.): ↓	DOPA ↓ DOPAC (±)	ND	2
Rat	Partially activated (β-carboline administration)	+Tyrosine (i.p.): ↑	HVA ↑	ND	2
Rat	Fully activated (acute electric footshock)	+Tyrosine (i.p.): ↑	DOPAC (±)	ND	Unpublished data
Rat	Partially activated? (acute cold stress)	+Tyrosine (i.p.): ↑↑	ND	Delayed matching-to-sample memory task ⇑	46
Rat	Partially activated? (exposure to novel environment)	+Tyrosine (i.p.): ↑	DOPAC/DA ↑	ND	34
Rat	Deactivated	Experimental diabetes: ↓	DOPA ↓ DOPAC ↓	ND	3
Rat	Deactivated	Animal model of early-treated PKU: ↓	HVA ↓	Delayed alternation task ⇓	41
Human	Dysregulated or deactivated? (schizophrenia)	+Tyrosine (orally): ↑	ND	Smooth-pursuit eye movement task ⇓	8
Human	Partially activated (cold stress)	+Tyrosine (orally): ↑	ND	Delayed matching-to-sample memory task ⇑	9
Human	Deactivated	Early-treated PKU children: ↓	ND	Battery of tests selective for PFC function ⇓	5, 6
Human	Deactivated	Early-treated PKU children + tyrosine (orally): ↓	ND	Executive function tasks ⇓	48
Human	Deactivated	Adolescents and young adults with early-treated PKU: ↓	ND	Some executive function tasks ⇓	7

Key: +Tyrosine = administration of supplemental tyrosine; ↑, ↓ = increase or decrease in tyrosine levels; ⇑, ⇓ = improvement or deficit in behavioral measure; (±) = no change; and ND = not determined.

be improved by stricter dietary compliance or pharmacological intervention [5]. A recent longitudinal study by Diamond [6], employing a large number of human subjects and neuropsychological tests that are highly specific to prefrontal functioning, has extended this analysis and shown that infants and young children with early-treated PKU were impaired on some specific cognitive tests dependent on the PFC. Specifically, Diamond examined 37 children with PKU who were placed on a dietary regimen, and 25 hyperphenylalaninemic children who had mild increases in plasma Phe levels, but did not exhibit classical PKU and were not placed on a dietary regimen [6].

Among these patients, PKU children with Phe levels 3–5 times above normal (6–10 mg/dL) were found to be impaired on all cognitive tests that are dependent on both the working memory and inhibitory control functions subserved by dorsolateral PFC [6]. However, these patients appeared to exhibit no deficit in performance on other prefrontal tasks that only require working memory but not inhibitory control,* and on control tests indicative of functions of other cortical areas [6]. Furthermore, a negative correlation between concurrent plasma Phe levels and the impairment in cognitive performance on measures of dorsolateral PFC could be observed with these PKU children, suggesting that the deficits may be reversible by dietary or pharmacological treatments [6]. In line with the prefrontal dysfunction hypothesis, a recent study has shown that in adolescents and young adults with early-treated PKU who had discontinued the restricted diet for 3 years since age 10, significant impairment on some executive function tasks could be detected [7]. Taken together, these consecutive studies strongly suggest that the cognitive deficits exhibited by the early-treated PKU patients with moderately reduced

* Diamond A, personal communication. Cited with permission.

blood levels (and presumptive brain levels) of tyrosine are specific to prefrontal cortical functions and implicate the critical importance of tyrosine availability in maintaining the functions of the mesoprefrontal DA system in humans.

Although there are good indications that reduced brain tyrosine levels can affect brain functions in humans, it remains controversial whether supplemental tyrosine can reverse some of the cognitive deficits observed with early-treated PKU patients. Based on the reasoning that reduced levels of brain precursor tyrosine could decrease catecholamine synthesis in the brain [50], some earlier studies by Lou *et al.* have shown that young adult PKU patients on an unrestricted diet exhibited reduced concentrations of HVA and 5-HIAA in cerebrospinal fluid and impairment in a reaction time task [51, 52]. Supplementation of the unrestricted diet with large doses of tyrosine resulted in a normalization of DA metabolite levels in the cerebrospinal fluid and an improvement of test performance in some of these patients. Although a subsequent study by Mazzocco *et al.* examining the effect of tyrosine supplementation on cognitive performance on executive function measures among school-aged children with early-treated PKU indicated that tyrosine supplementation did not improve cognitive performance significantly in these patients [48], serum tyrosine levels were unchanged in this study. More recently, in a study by Pietz *et al.* [53], the effect of high dose tyrosine supplementation on cognitive functions was examined in young adults with early-treated PKU who had relaxed or stopped dietary restriction. Consistent with what has been reported previously, these patients were impaired in some behavioral tasks indicative of certain aspects of brain function. Although tyrosine supplementation was found to increase significantly serum tyrosine levels in this study, it did not appear to affect test performance among these patients [53]. Thus, at present, there are insufficient existing data to fully evaluate the efficacy of tyrosine supplementation in reversing some of the cognitive deficits observed with early-treated PKU patients.

As discussed earlier, the DA-containing amacrine cells in the retina exhibit high firing frequncy, and tyrosine supplementation has been shown to enhance DA synthesis and turnover (metabolite levels) in these neurons [31]. Depletion of DA in the retina is associated with impaired contrast sensitivity, and this visual deficit is found in patients with Parkinson's disease [54]. Based on these findings, Diamond and Herzberg have assessed contrast sensitivity of 12 early-treated PKU children. All subjects examined showed impaired contrast sensitivity, suggesting that a reduced supply of precursor tyrosine to the brain can also compromise the function of the human retina [55]. These findings are consistent with and strengthen the concept that tyrosine availability can influence brain dopaminergic functions in humans. Furthermore, as the contrast sensitivity testing is a relatively simple procedure, it was suggested that this testing may be useful as a preliminary diagnostic tool to screen for cognitive deficits in early-treated PKU patients, and to monitor the efficacy of various treatments used to normalize plasma Phe levels in these patients [55].

Schizophrenia. It has been proposed with the dopamine hypothesis of schizophrenia that the deficit symptoms and cognitive dysfunctions of schizophrenia may be attributed to a diminished activity of the mesoprefrontal DA neurons, which, in turn, leads to an enhanced activity of mesolimbic DA neurons [34]. In the studies reported by Deutsch *et al.* [8], tyrosine was administered in conjunction with molindone, an antipsychotic drug, to 11 schizophrenic patients. As the tyrosine transport across the blood–brain barrier may be impaired in schizophrenic patients resulting in reduced precursor tyrosine levels in the brain, this group speculated that tyrosine intervention might be able to restore brain tyrosine levels to normal, enhance DA outflow in the mesoprefrontal DA system, and improve therapy. However, schizophrenic patients receiving the 6-week cross-over treatment of tyrosine did not improve their scores in the psychosis, movement rating, or neuropsychological tests [8]. Interestingly, during the course of tyrosine supplementation, all of the 8 patients selected for the SPEM performance task consistently scored worse in every SPEM measure used in the study, indicating that tyrosine intervention did exert some central effects in these human subjects. Previous studies have suggested that the frontal lobe is involved in mediating SPEM preformance [56–58]. SPEM deficits are prevalent among schizophrenic patients [59], and the disruptive effects of tyrosine supplementation on SPEM in these patients are consistent with the idea that tyrosine can influence the function of the mesoprefrontal DA system in humans [8].

Cold stress. Acute exposure to environmental stressors such as cold stress is often associated with an impairment of short-term or working memory. Since it is known that cold stress depletes brain catecholamines, it has been suggested that the reduced levels of DA and NE in the brain following exposure to stress may account for the stress-induced cognitive deficits. An earlier study indicated that supplemental tyrosine is effective in reducing symptom intensities and adverse moods and in reversing some cognitive deficits in human subjects who have been exposed to a simulated high-attitude cool environment [60]. A recent study reported by Shurtleff *et al.* [9] examined whether a supplemental dose of tyrosine could restore working memory in humans following a cold stress paradigm, as assessed by a computer-based delayed matching-to-sample (DMTS) memory task. It was found that tyrosine pretreatment significantly improved the matching accuracy observed after cold exposure to the same levels as observed following placebo or tyrosine administration given to control subjects at room temperature [9]. These studies thus show that tyrosine supplementation is effective in reversing or preventing the stress-induced working memory deficit in humans. As the mesoprefrontal DA projection is thought to be involved in working memory and in emotional responses to stress, the beneficial effects of tyrosine on working memory impair-

ment elicited by stress may reflect the rapid restoration or maintenance of DA levels in these prefrontal DA neurons by the supplemental tyrosine. After the immedicate cessation of the stress response, the sustained activation of the enzyme tyrosine hydroxylase, together with reduced levels of endogenous prefrontal DA, should result in an increase in DA synthesis and a restoration of transmitter supply in these mesoprefrontal neurons when local precursor tyrosine levels are elevated. Further studies using an experimental stress paradigm in rats, where direct measures of prefrontal DA release and turnover can be made, will be useful in testing this hypothesis.

FUTURE PERSPECTIVES

The manifestation of deficits in cognitive abilities mediated by the PFC in early-treated PKU children, which correlate with the mildly elevated levels of Phe, has provided a convincing argument for the importance of tyrosine availability in the maintenance of functions in mesoprefrontal DA neurons in humans [5–7]. These studies have further confirmed the validity of some of the previous animal studies and their predictions, suggesting that the mechanisms underlying the tyrosine dependence effects in the PFC may be very similar in both humans and animals (see Table 2). Furthermore, as it has been pointed out by many investigators, the identification of specific cognitive deficits dependent on the PFC in these patients may help to design therapeutic treatments specifically targeted to prefrontal functions for these patients. Although large doses of tyrosine were found to alleviate some cognitive impairments in PKU patients that were on unrestricted diets [51, 52], the cognitive functions of some early-treated PKU children and young adults with PKU were not improved by tyrosine supplementation [48, 53]. However, the results of the latter studies do not necessarily rule out the possibility that tyrosine supplementation can have a beneficial effect on these patients. Future studies should be directed at investigating whether tyrosine administration at different dosages and treatment schedules could influence cognitive functions in patients with early-treated PKU. On the other hand, in view of the importance of numerous afferent controls that regulate the mesoprefrontal DA neurons (cf. Fig 1) and thus influence the function of the PFC, alternative therapeutic targets directed at these VTA afferents may have utility in the treatment of the tyrosine non-responsive deficits noted in those PKU patients resistant to tyrosine supplementation.

In light of the recent findings in humans indicating that tyrosine administration can prevent working memory deficit after cold stress [9], future studies should be directed at assessing how tyrosine supplementation can be pursued as a therapeutic strategy to enhance or to sustain optimal functional transmitter outflow in the mesoprefrontal DA neurons. Supplemental tyrosine should be particularly beneficial in situations where the mesoprefrontal DA neurons are activated, DA synthesis is enhanced, and the intraneuronal DA is rapidly or chronically depleted. However, in view of the finding that excessive DA release from mesoprefrontal neurons can also be detrimental to the functions of the PFC [15], the rationale of using supplemental tyrosine as a therapeutic strategy for dysregulated states such as schizophrenia remains to be determined. In fact, the findings of Deutsch *et al.* [8] indicating that tyrosine supplementation is not a useful adjuvant treatment strategy for schizophrenic patients, when administered in combination with neuroleptic drugs, argue against this strategy. On the other hand, the adverse effects of tyrosine supplementation on a performance task dependent on the prefrontal cortical function noted in schizophrenic patients are intriguing [8]. The results are consistent with a possible dysregulation of mesoprefrontal DA neurons in these patients and an inability to control the transient increases in DA synthesis and release elicited by an increased tyrosine supply. These observed changes may reflect impairments of the regulatory controls modulating the mesoprefrontal DA projection in schizophrenic patients.

In summary, converging data are accumulating from both animal and human studies to suggest that the functions of the mesoprefrontal DA system can be readily influenced by the availability of the precursor tyrosine. Studies directed toward further assessment of how tyrosine supplementation can be utilized as a therapeutic strategy to optimize functional outflow in the mesoprefrontal DA projection in conditions that influence PFC function seem warranted. Moreover, the elucidation of the cellular mechanisms by which tyrosine availability regulates DA functions in the PFC may lead to a better understanding of some of the regulatory controls influencing these DA neurons and, importantly, some of the abnormal cellular processes underlying the pathogenesis of stress disorders and schizophrenia.

A recent study by Smith et al. (Smith ML, Klim P, Mallozzi E and Hanley WB, A test of the frontal-specificity hypothesis in the cognitive performance of adults with phenylketonuria. *Dev Neuropsychol* **12:** 327–341, 1996) has reported that adult patients with PKU, who were off diet or in poor diet control, exhibited impaired performance in cognitive tasks indicative of functions of the PFC and in those tasks indicative of functions of the parietal cortex or the temporal lobe. However, a significant negative correlation between plasma Phe levels and test performance was found only in cognitive tasks that were dependent on the PFC.

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