1985

Potentiation of the discriminative stimulus cues elicited by methylphenidate and imipramine by amino acid :: implications for the treatment of attention deficit disorder.

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POTENTIATION OF THE DISCRIMINATIVE STIMULUS CUES ELICITED BY METHYLPHENIDATE AND IMIPRAMINE BY AMINO ACID: IMPLICATIONS FOR THE TREATMENT OF ATTENTION DEFICIT DISORDER

A Thesis Presented
By
SANDRA KAY WOODS

Submitted to the Graduate School of the University of Massachusetts in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
September 1985
Psychology
POTENTIATION OF THE DISCRIMINATIVE STIMULUS CUES ELICITED BY METHYLPHENIDATE AND IMIPRAMINE BY AMINO ACID: IMPLICATIONS FOR THE TREATMENT OF ATTENTION DEFICIT DISORDER

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ACKNOWLEDGEMENT

There are many people without whose support this thesis would not have been possible.

I am indebted to Robert Feldman, my advisor and thesis committee chairman, for his significant input and encouragement and his willingness to let me pursue my own research interests. I particularly wish to thank him for his enduring belief in my ability throughout the years which enabled me to begin my career as a graduate student and scientist.

I am very grateful to Jerrold Meyer who has willingly given me much of his time throughout this process and whose genuine concern and thoughtful feedback as a scientist has been invaluable.

I am thankful to John Donahoe for serving on my committee on such short notice and for his scholarly comments and suggestions.

The excellent technical assistance provided by Thomas Burke is greatfully acknowledged. I am especially thankful for his enthusiasm, sense of humor and commitment to this project in the face of all of the many other demands on his time.

To my office mates, Sue Anne Assimon and Joan Hamilton, goes my enduring thanks for their friendship

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and support during my graduate career and their willingness to listen and comment on my work.

Finally, I am indebted to my parents, Harry and Frances, without whom I would not have been able to reach my goals.
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Attention Deficit Disorder

Attention deficit disorder (ADD), formerly known as hyperkinetic syndrome or minimal brain dysfunction, is characterized predominantly by developmentally inappropriate inattention and impulsivity, as well as hyperactivity which is of a poorly organized and non goal-directed nature. Associated features vary as a function of age and may include mood lability, a low frustration tolerance, poor self-esteem, stubbornness, negativism, bossiness, bullying, obstinacy, temper outbursts and a lack of response to discipline. "Soft" neurological signs such as clumsiness as well as perceptual-motor dysfunctions, e.g., poor eye-hand coordination, may also be present (American Psychiatric Association [APA], 1980). Prevalence rates for ADD vary from 3% (APA, 1980) to as high as 5-10% of prepubertal children (Wender, 1975). Although typically thought of as a disorder of childhood and, not uncommonly, adolescence, recent reports in the literature suggest its persistence into adulthood where its existence may be

Huey et al. (1978) and Bellak and Charles (1979) have treated adult patients previously diagnosed as schizophrenic with symptoms characteristic of ADD who were poor responders to antipsychotic medications yet did well on medication known to be effective in the treatment of ADD, e.g., the catecholamine agonist methylphenidate and the tricyclic antidepressant imipramine. The APA (1980), reflecting this relatively new awareness of the persistence of symptoms into adult life, has created a diagnostic category for the disorder in adults called ADD, Residual Type.

In children, the most effective treatment regimen typically includes stimulant medication (methylphenidate, pemoline, or d-amphetamine) in combination with an operant conditioning approach to the management of behavior (Wender, 1971). In adults, central nervous system stimulants have also proved effective but often the preferred medication seem to be tricyclic
antidepressants, most typically imipramine (Huessy et al., 1979). In some cases, imipramine is the drug of choice because it is less subject to abuse than d-amphetamine or methylphenidate.

Amphetamine and amphetamine-like compounds (methylphenidate) act as central nervous system stimulants in that they potentiate the release of the neurotransmitters dopamine (DA) and norepinephrine (NE) from presynaptic nerve terminals and inhibit their intracellular reuptake (Kuczenski, 1983). Whereas amphetamine seems to release, preferentially, newly synthesized pools of DA, methylphenidate facilitates the release of stored or vesicular pools (Chieuh and Moore, 1975). In so doing these compounds facilitate neurotransmission in select groups of neurons by making more of the transmitter available at the synapse.

The tricyclic antidepressants are believed to exert their pharmacologic effect via reuptake inhibition of NE and serotonin (5-HT), another central nervous system neurotransmitter (Iversen and Mackay, 1979). Imipramine, in particular, has been found to be a more potent NE than 5-HT reuptake inhibitor (Green and Nutt, 1983), although in rat brain high affinity 3H-imipramine binding sites have been found to be selectively located presynaptically.
on 5-HT neurons (Gross, Gothert, Ender, and Schumann, 1981).

On the basis of the favorable response of children and adolescents to the psychostimulants and antidepressant drugs, and in view of what is known about the mechanism of drug action both behaviorally and neurochemically, Wender (1971, 1975, 1978) hypothesized that ADD involves a functional underactivity of one or more of the central nervous system monoamines (NE, DA, and/or 5-HT) due to decreased synthesis, release or receptor sensitivity. There have been some attempts to document this hypothesized neurochemical abnormality in humans. Shawitz, Cohen and Bowers (1977) found significantly lower levels of homovanillic acid (HVA) in the cerebral spinal fluid (CSF) of a clinically homogeneous group (N=6) of ADD children as compared with controls (N=26). Both groups had been pretreated with probenecid (100-150 mg/kg, orally), a drug which blocks the removal of acidic monoamine metabolites from CSF. CSF concentrations of 5-hydroxyindolecetic acid (5-HIAA), the principle metabolite of 5-HT, were not significantly different between groups. The results suggest that there may be an abnormality in DA turnover in the brains of ADD children.
This presumed biochemical dysfunction affects behavior primarily by the impairment it produces in the putative reward and punishment centers of the brain. This impairment is expressed as a diminished capacity for positive and negative affect, the subjective nature of which is a reduction in the capacity to experience both psychic pain, e.g., guilt, remorse, and psychic pleasure (anhedonia). Outwardly, it is manifested as a decreased responsitivity to reward and punishment, i.e., to operant conditioning. This hypothesis is consistent with a large body of animal data on the reward and punishment centers of the brain including the potentiation of previously rewarded behavior, and the further suppression of previously punished behavior by the administration of substances which increase the levels of one or more of these central nervous system monoamines (Stein, 1964; Stein and Wise, 1970; Wise, Berger and Stein, 1973; Stein, Wise and Belluzzi, 1977).

Since the initial discovery by Olds and Milner (1954) that electrical stimulation of certain discrete areas of the brain could serve as a reward, and by Delgado, Roberts and Miller (1954) that stimulation of different discrete areas had punishing effects, researchers have attempted to investigate the
neurochemical basis of these phenomena, and evidence has accumulated that monoaminergic systems are involved. Work by Dewit and Wise (1977), Routtenberg (1978) and Wise (1980) have suggested that DA plays the critical role in brain reward. Anatomically, brain reward has been found at specific locations extending from the frontal cortex to the midbrain and into the hindbrain, and passing through the medial forebrain bundle in the hypothalamus. Although both NE and DA systems overlap in much of the area giving rise to intracranial self-stimulation behavior in rats, only DA fibers are confined to areas that mediate brain reward, whereas NE fibers extend into other regions (Routtenberg, 1978). Wise (1980) found self-stimulation to be uniquely associated with the layer of DA-containing cells in the substantia nigra and ventral tegmentum. The occurrence of robust, low threshold stimulation from DA cells and the lack of occurrence of self-stimulation from bordering areas suggest a unique involvement of DA cells or their afferents in brain stimulation reward in at least these anatomical sites.

The neurochemical basis for punishment, on the other hand, appears to involve serotonergic systems (Stein and Wise, 1974; Stein, Wise and Belluzzi, 1977). Wise et al.
(1973) observed suppression of self-stimulation and increased suppression of punished behavior in rats with intraventricular injections of 5-HT and the alpha-noradrenergic antagonist phentolamine, whereas injections of p-chlorophenylalanine (PCPA), a 5-HT synthesis inhibitor, produced increased rates of punished behavior. This latter effect was reversed after intraperitoneal injections of the serotonin precursor, 5-HTP.

Although known to be effective in relieving the symptoms associated with ADD, drugs which potentiate monoaminergic systems often have undesirable side effects. The most common side effects seen in children who are taking methylphenidate are anorexia and insomnia (Bassuk and Schoonover, 1977) Tachycardia, abdominal pain, and a lowering of the seizure threshold in children with a history of seizures or EEG abnormalities have also been observed. Common imipramine side effects include dry mouth, decreased intestinal motility, urinary retention, blurred vision and sedation (Bassuk and Schoonover, 1977). These side effects, understandably, are areas of concern to parents and professionals involved in the treatment of ADD children.
Precursor Control of Neurotransmitter Synthesis and Release

Recently, researchers in the area of nutrition and behavior have demonstrated an increase in the synthesis and release of the monoamine neurotransmitters with the administration of the appropriate amino acid precursor, e.g., tyrosine in the case of DA and NE; tryptophan in the case of 5-HT (Fernstrom and Wurtman, 1971, 1974; Wurtman, Larin, Mostafapour and Fernstrom, 1974; McBride, Hyde, Smith, Lane and Aprison, 1976; Wurtman, 1976; Gibson and Wurtman, 1977, 1978; Sved, Fernstrom and Wurtman, 1979; Melamed, Hefti and Wurtman, 1980; Wurtman, 1982; Conlay and Zeisel, 1982).

Since tyrosine and tryptophan are readily available from protein products normally present in the diet, and as nutritional supplements in many health food stores, a question now emerges, "Can these amino acid precursors be used in place of or as a supplement to pharmacotherapy in the treatment of ADD?" If so, then the potentially harmful side effects of the central nervous system stimulants and the tricyclic antidepressants may be reduced or avoided altogether. In addition, parents, teachers, and physicians now opposed to medicating young
children may be more open to a nutritional approach to therapy thus making effective treatment available for a larger number of ADD children.

Since the late 60's and early 70's, the relationship between nutrition and brain function has been the focus of intense investigation by Richard Wurtman and his colleagues. One of their earliest findings (Shoemaker and Wurtman, 1973) was that the brains of 24 day old weanling rats given a diet deficient in protein (8% protein) contained abnormally low levels of DA and NE as compared with controls (24% protein). This observation suggested that precursor availability may play a significant role in the control of CA synthesis.

In a subsequent study (Wurtman et al., 1974), the effect of tyrosine injections (i.p.) on brain CA synthesis was estimated by measuring 3,4-dihydroxyphenylalanine (DOPA) accumulation after administration of the aromatic amino acid decarboxylase inhibitor R04-4602. Administration of 50 mg/kg of tyrosine produced an 81% increase in brain tyrosine and a 13% increase in DOPA (P<.05). In contrast, the brains of rats given either tryptophan (50 mg/kg) or leucine (100 mg/kg), amino acids known to compete with tyrosine for uptake into brain, contained significantly less tyrosine
and DOPA than controls. These findings supported the hypothesis that nutritional factors may play an important role in CA synthesis by controlling the availability of tyrosine.

Confirming these findings, Gibson and Wurtman (1977) looked at the relationship between DA and NE synthesis and brain tyrosine concentration. CA synthesis was again estimated by DOPA accumulation and measurements were taken 30 or 60 minutes after the administration of R04-4602. In general, treatments that raised or lowered brain tyrosine concentrations were found to cause significant parallel changes in DOPA accumulation rates.

The possibility exists that exogenous tyrosine may differentially effect DA and NE synthesis. Oishi and Szabo (1984) found that tyrosine injection (51.2 mg/100 g) significantly increased DA levels in rat striatum and cortex without markedly altering NE levels. Using DOPA accumulation as the only measure of CA synthesis may, as they have suggested, tend to disregard these differences.

Brain tyrosine concentrations have been shown to influence not only the synthesis but also the release of CAs. In a study by Gibson and Wurtman (1978), injections of tyrosine (100 mg/kg, i.p.) produced a significant increase in the accumulation of the major NE metabolite,
3-methoxy-4-hydroxy-phenylethleneglycol-sulfate (MOPEG-SO₄), after treatment with probenecid, a drug which blocks the efflux of MOPEG-SO₄ from brain.

In this same study, Gibson and Wurtman looked at the effect of cold stress (4°C for 1 h) on MOPEG-SO₄ accumulation after pretreatment with tyrosine (125 mg/kg) or after the consumption of a single protein rich meal (40% casein). (Placing rats in a cold environment is known to increase NE turnover [Meek and Neff, 1973]). Significant increases in brain MOPEG-SO₄ levels were seen under both conditions.

Morre, Hefti and Wurtman (1980) investigated regional variations in tyrosine concentrations in the brains of rats given l-tyrosine (100 mg/kg, i.p.) and the time course of these changes. Tyrosine administration significantly increased tyrosine concentrations in all areas of the brain. However, relative increases in the various regions differed; high relative increases were observed in areas of low initial concentrations and vice versa, resulting in a more uniform distribution of tyrosine in brain tissue. Increases were maximal 1 hour after tyrosine administration and gradually declined over the next 3 hours. The highest relative increases were observed in cortex and hippocampus. Co-administration of
l-valine (500 mg/kg), a large neutral amino acid known to compete with tyrosine for transport into brain, completely blocked this effect.

Increased tryptophan availability has also been shown to increase neurotransmitter synthesis; specifically 5-HT synthesis. Fernstrom and Wurtman (1971) found significant elevations in both brain tryptophan and 5-HT one hour after rats received injections of l-tryptophan (12.5, 25, 50, or 125 mg/kg, i.p.). McBride, Hyde, Smith, Lane and Aprison (1976) analyzed nerve ending fractions from the telencephalon of pigeons given intramuscular injections of l-tryptophan (300 mg/kg) and found significantly increased levels of tryptophan, 5-HT, and 5-HIAA, suggesting that both synthesis and release can be influenced by tryptophan pretreatment.

An unexpected finding (Fernstrom and Wurtman, 1974) was that rats fed a diet rich in carbohydrate and low in protein had increased brain levels of tryptophan and 5-HT, whereas a diet rich in protein and low in carbohydrate produced decreased levels. This was contrary to expectation, as tryptophan is an essential amino acid and, as such, cannot be synthesized in vivo. Whatever tryptophan one has can only be obtained through
the ingestion of dietary protein. This is not true for all amino acids; tyrosine, for example, can be synthesized in the liver from phenylalanine, another essential amino acid.

This apparent paradox was finally resolved by the consideration of the following: First, tryptophan is a large, neutral amino acid which cannot cross the blood-brain barrier and so is transported across it by a carrier molecule. Second, as a neutral amino acid, it competes with five other large neutral amino acids (leucine, isoleucine, valine, phenylalanine and tyrosine) for the same transport molecule for uptake into brain. Third, as much as 80% of all plasma tryptophan molecules are bound to albumin, a protein normally present in blood. Fourth, it is the total amount of tryptophan in plasma, i.e., albumin-bound plus free tryptophan, that determines the amount that is potentially accessible to the brain. Fifth, a diet rich in carbohydrate stimulates the secretion of insulin which in turn lowers the plasma concentrations of competing amino acids more than it does that of tryptophan. Because approximately 80% of the available tryptophan is bound to albumin, it is essentially free from the effect of insulin; more tryptophan is therefore available, proportionately, for
transport into brain. Fifth, a protein-rich meal, on the other hand, will reduce brain levels of 5-HT by elevating the relative levels of the other neutral amino acids in the bloodstream which compete for transport into brain. The net effect is that less tryptophan is carried across the blood-brain barrier and less reaches the neurons where 5-HT synthesis takes place.

An additional part of this paradox involves the role of non-esterified fatty acids (NEFA) which appear to modulate the binding of tryptophan to albumin in plasma (Madras, Cohen, Messing, Munro, and Wurtman, 1974). The affinity of NEFA for albumin is apparently greater than that of tryptophan for albumin. When insulin is secreted, plasma levels of NEFA fall because insulin facilitates the uptake of NEFA into fat-producing cells. As these NEFA are stripped off the circulating albumin, the affinity of albumin for tryptophan increases; hence, plasma levels of albumin-bound tryptophan rise. When albumin-bound tryptophan molecules pass through brain capillaries, they readily dissociate from albumin because the affinity of the blood-brain transport system for tryptophan is considerably greater than the affinity of albumin for tryptophan (Wurtman and Pardridge, 1979; Wurtman, Hefti, and Melamed, 1981).
Although the intake of both tyrosine and tryptophan may influence the synthesis and turnover of their respective neurotransmitters, the mechanism by which they do so appears to differ. Understanding this difference involves, among other factors, knowledge of the steps involved in the biosynthesis of the CAs and 5-HT. In both cases, the initial step involves hydroxylation of the precursor by enzymatic reaction. These reactions are catalyzed by two specific enzymes, tyrosine hydroxylase and tryptophan hydroxylase, respectively. The activities of these enzymes have been demonstrated to be rate-limiting in nature in the sense that reductions in the activities of these enzymes parallel decreases in neurotransmitter synthesis (Lovenberg, Jequier and Sjoerdsima, 1968).

It has been demonstrated that both enzymes use a reduced pteridine as a cofactor (Nagatsu, Levitt and Udenfriend, 1964; Lovenberg, Jequier and Sjoerdsma, 1968). Apparently CAs can compete with this cofactor for attachment to the binding site on tyrosine hydroxylase thereby inhibiting the hydroxylation of tyrosine and therefore CA synthesis (Udenfriend, Zaltman-Nireenberg, and Nagatsu, 1965; Weiner, 1970). In contrast, serotonin does not appear to exert a significant direct inhibitory
effect on tryptophan hydroxylase so it does not suppress its own biosynthesis (Jacoby, Colmenares, and Wurtman, 1975). As tryptophan hydroxylase is a low affinity enzyme and therefore not saturated with its substrate in vivo, increased levels of tryptophan can lead to increased levels of brain 5-HT. This suggests that one important regulator of 5-HT synthesis may simply be the intake of dietary tryptophan (Fernstrom and Wurtman, 1971).

Although tyrosine hydroxylase is also of the low affinity type, i.e., not saturated with its substrate in vivo, increased levels of its precursor do not necessarily produce increased levels of neurotransmitter due to the influence of endproduct inhibition. Rather it appears that certain conditions must be present for increased tyrosine intake to induce increases in CA synthesis and release. Wurtman (1982) and Melamed et al. (1980) have raised the possibility that, in CA neurons, tyrosine's effect on neurotransmitter synthesis and release may vary with neuronal activity, becoming important when neurons are firing frequently but not when they are relatively quiescent. In an earlier study, Scally, Ulus and Wurtman (1977) found that levels of HVA were significantly elevated in the striata of rats given
tyrosine (100 mg/kg) and the DA receptor blocker, haloperidol (2 mg/kg) but not in animals given tyrosine plus probenecid (200 mg/kg). Haloperidol increases DA turnover due to receptor blockade. Probenecid, on the other hand, blocks the egress of organic acids from CSF and has no effect on DA turnover. The failure of HVA levels to vary with brain tyrosine in probenecid animals was attributed to end-product inhibition; the increase in HVA accumulation in haloperidol animals, to an inhibition of this feedback mechanism. To examine the possibility that the failure of tyrosine administration to accelerate HVA accumulation in probenecid animals resulted from a feedback mediated change in the kinetic properties of tyrosine hydroxylase, they measured the enzyme's affinity for tyrosine and for its pteridine cofactor, DMPH₄ (2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine hydrochloride), on brain samples from each experimental group. Haloperidol administration with or without tyrosine significantly reduced the $K_m$ of tyrosine hydroxylase for DMPH₄ as compared to probenecid administration.

Badawy and Williams (1982) have suggested that the ability of exogenous tyrosine to enhance CA synthesis may be dose related. In rats, tyrosine doses of up to 40
mg/kg were found to produce significant increases in DOPA accumulation in brain but only the 20 mg/kg dose increased brain DA, DOPAC, and NE to a significant degree. Doses of 50 mg/kg and over abolished this enhancement. The fact that small doses enhanced CA synthesis is consistent with the idea that tyrosine hydroxylase is normally unsaturated with its substrate in vivo and can become more fully saturated by increasing the availability of tyrosine. Badawy and Williams suggest that the failure of larger doses to enhance CA synthesis may be due to substrate inhibition of tyrosine hydroxylase activity.

Melamed et al. (1980) examined the relationship between the apparent firing rates of nigrostriatal neurons, DA release and tyrosine availability. Partial, unilateral nigrostriatal lesions of varying severity were produced in rats by injecting graded doses of 6-hydroxydopamine into the substantia nigra. In surviving ipsilateral nigrostriatal neurons, the formation of the DA metabolites, DOPAC and HVA, increased significantly in rats with severe lesions (DA concentrations less than 25% of control values in contralateral unlesioned striata) but remained unchanged in rats with less severe lesions. In animals with significantly enhanced DA metabolite
formation, i.e., those with severe lesions, administration of tyrosine (250 mg/kg) but not valine (500 mg/kg) lead to further significant increases in HVA and DOPAC. These findings suggest that after severe damage to nigrostriatal neurons, remaining populations of striatal neurons increase their firing rate and accelerate DA synthesis and release. Under these conditions, increased tyrosine availability appears to enhance DA synthesis and release.

Having established that exogenous supplies of at least two nutrients, tyrosine and tryptophan, could influence the synthesis of their neurotransmitter products, it became possible for Wurtman (1982) to formulate some general principles for predicting whether a given neurotransmitter might be under this type of precursor control. First, the exogenously supplied precursor must significantly elevate the plasma level of the precursor and the level cannot be held constant by feedback mechanisms like those regulating plasma pH for example. Second, the precursor must be able to penetrate the blood-brain barrier and the concentration of the precursor in brain must depend on and fluctuate with the concentration in plasma. Third, carrier molecules that transport the precursor across the blood-brain barrier
must be of the low-affinity type, i.e., the transport mechanism must be unsaturated with its substrate so that it can become more fully saturated when plasma levels rise. Fourth, the enzyme that catalyzes the conversion of the precursor to neurotransmitter must also be of the low-affinity type. Finally, the catalyzing enzyme must be relatively free of end-product inhibition when the intracellular level of its product, the neurotransmitter, rises.

All these biochemical conditions have been demonstrated to be fulfilled in the case of 5-HT. All but the last condition has been shown to be fulfilled in the case of the CAs where, as previously stated, tyrosine's effect on synthesis and release appears to vary with neuronal activity, becoming important when neurons are firing frequently but not when they are relatively quiescent.

**Amino Acid Precursors in the Treatment of Psychiatric Disorders**

It is interesting that the responsive neurotransmitters include the CAs and 5-HT, transmitters known to be affected by drugs used to treat psychiatric
disorders such as depression and ADD. There already have been several reports in the literature of the use of amino acid precursor to treat depression (Coppen, Whybrow, Noguera, Maggs, and Prange, 1972; Jensen et al., 1975; Walinder, Skott, Carlsson and Roos, 1976; Gelenberg, Wojcik, Growdon, Sved and Wurtman, 1980; Walinder, Carlsson, Persson and Wallin, 1980; Young, Chouinard, and Annable, 1981; van Praag, 1983; van Praag and Mendlewicz, 1983). Gelenberg et al. (1980) employed a double-blind, placebo-controlled cross over trial of tyrosine in a 30 year old woman who had suffered from chronic and recurrent depression for several years and was diagnosed as having primary depression, unipolar type. L-tyrosine (100 mg/kg) was administered orally each day in three doses for two weeks, then placebo at the same schedule for eighteen days, and finally tyrosine for an additional five weeks. Symptoms of depression reportedly decreased dramatically during tyrosine administration and recurred when placebo was substituted as measured by the Hamilton Depression Rating Scale and the Zung Self-Rating Depression Scale. Comparisons between mean ratings during pretreatment, tyrosine administration and placebo were statistically significant.
In a study by Gibson and Gelenberg (1983), a four week trial of tyrosine (100 mg/kg/day) was effective in alleviating the symptoms of depression without any of the adverse side effects which often accompany the use of tricyclic antidepressants in four out of six unipolar depressed patients as measured by the Hamilton Rating Scale.

In a double-blind study by Coppen et al. (1972) the efficacy of l-tryptophan (9 g/day, n=22) vs. imipramine (150 mg/day, n=20) in the treatment of primary depression was compared. Patients reportedly responded as well to l-tryptophan as they did to imipramine as measured by their scores on the Hamilton Rating Scale. In agreement with this finding is a double-blind study by Jensen et al. (1975) in which both tryptophan patients (n=22) and imipramine patients (n=20) showed statistically significant reductions in symptoms at the end of three weeks of treatment; however, improvements were more rapid in the imipramine group.

Walinder et al. (1976) investigated whether the antidepressant action of clomipramine could be enhanced by tryptophan. In a double-blind study it was shown that patients given clomipramine plus dl-tryptophan (0.1 g/kg) improved more rapidly than the group given clomipramine
alone. Clomipramine alone produced an approximately 50% decrease in CSF 5-HIAA levels, presumably due to a feedback inhibition of 5-HT turnover induced by reuptake inhibition. DL-tryptophan produced an up to threefold increase in tryptophan levels in plasma and CSF sufficient to prevent the clomipramine-induced decrease in CSF 5-HIAA. An unexpected finding was that in the clomipramine plus tryptophan group, the level of HVA in CSF was significantly increased after 12 days of treatment. The possibility was raised that the change in 5-HT neurotransmission induced by this combination may have produced a secondary change in DA neurotransmission via neuronal connections.

Chouinard et al. (1983) present a review of several studies which used tryptophan in combination with antidepressant drugs in the treatment of depression. Of the six studies which supplemented a reuptake inhibitor with tryptophan, only one found a significant potentiation of the antidepressant effect. This study was the previously cited study by Walinder et al. (1976).

Whether tyrosine or tryptophan therapy will ultimately prove to be safe and effective in a significant number of patients suffering from depression remains to be seen. If it is, it might be an attractive
alternative to the tricyclic antidepressants and the monoamine oxidase inhibitors which often produce unwanted side effects.

Similarly, tyrosine and/or tryptophan therapy may be a more attractive and palatable alternative to methylphenidate or imipramine therapy in children and adolescents with ADD. To date, as far as can be determined, there have been no reports in the literature on the use of either nutrient in the treatment of ADD.

Some indirect evidence that short-term alterations in diet composition may influence hyperactive behavior in humans was reported by Chiel and Wurtman (1981) using an animal model of hyperactive motor behavior. We have already seen from previously reported studies how the proportions of protein and carbohydrate in the diet can affect the amounts of tryptophan and tyrosine transported into the brain and consequently, the synthesis of 5-HT and the CAs. Wurtman's study attempted to investigate changes in the spontaneous nocturnal activity patterns of rats fed diets in which the ratio of carbohydrate to protein was systematically varied. One group of 20 rats received a diet containing 18% protein (casein), 57% carbohydrate (dextrose, sucrose, and dextrin), and 15% vegetable fat; a second group of 20 rats received no
protein, 75% carbohydrate, and 15% fat. The results indicated that as this ratio increased, rats were more continuously active as measured by the total number of infrared photocell beam interruptions in successive 20-minute periods during dark hours when rats are normally active. Results were statistically significant (P<.01). Animals eating the 18% protein diet showed a highly irregular pattern of activity during darkness; periods of intense activity were followed by periods of complete quiescence. In contrast, rats fed the no protein diet were almost continuously active during darkness, with few or no periods of quiescence. No correlation was found between the number of calories an animal ate, the fat content of the diet or weight and its activity pattern.

Another indirect indicator that tyrosine therapy may be beneficial in the treatment of ADD comes from a study by Heffner and Seiden (1980). This study measured the synthesis of DA and NE from tyrosine in brain during the performance of operant behavior. It is well known that ADD children are difficult to discipline, i.e., to condition. The threat of punishment or the expectation of reward seems to have little or no effect on their behavior. Often a dramatic improvement is seen in their responsiveness to reward and punishment upon the
administration of pharmacotherapy (Wender, 1971). As previously indicated, the medications of use in the treatment of ADD children increase the levels of DA and NE in brain. These same neurotransmitters are thought to underly synaptic activity in the putative reward centers of the brain, and thus they serve as part of the neurochemical basis for the process of operant conditioning. Direct evidence for the involvement of NE and DA in the operant behavior of rats was found by Heffner and Seiden.

In their study, synthesis rates of DA and NE were estimated after the intraventricular injection of radioactive [3H] tyrosine. A conversion index was used which expressed the levels of [3H] DA or [3H] NE accumulated in various brain regions as a function of the specific activity of [3H] tyrosine. In rats lever pressing on a fixed ratio 5 schedule of reinforcement, the DA conversion index in the caudate putamen was 66% higher than in controls but was not different from control values in the mesolimbic area and hypothalamus. The NE conversion index in operant-performing rats was 48% higher in the hypothalamus than in controls but was unchanged in the mesolimbic area, telencephalon and brain stem. Changes in the NE and DA conversion indices seen
during operant performance were associated with increases in the brain levels of [3H] DA or [3H] NE. The relative increment in the DA conversion index seen in the caudate putamen was directly proportional to the number of lever presses emitted following [3H] tyrosine administration. Thus it appears that operant behavior is associated with increases in the rate of the synthesis of DA and NE within select populations of neurons.

On the basis of data presented thus far, it appears that tyrosine and/or tryptophan therapy is worthy of serious consideration in the treatment of ADD.

Specific Aims

The purpose of the current study, was to assess whether drugs used in the treatment of ADD, specifically methylphenidate and imipramine, could be potentiated by exogenous supplies of nutrients which serve as precursors to the neurotransmitters on which these drugs ultimately act. Moreover, this study examined the feasibility of an animal model to investigate these phenomena under more objective conditions than those afforded by using clinical patients.
CHAPTER II

METHOD

Subjects

Thirty male Holtzman Albino rats (Holtzman Company, Madison, WI) approximately 160 days old at the start of the study and weighing between 300-400 g served as subjects for this experiment. All animals were housed singly at a constant temperature (21° C) with a daily cycle of 12 h light and 12 h dark. Each day immediately following training and testing procedures, animals were fed approximately 20 g of Ralston Purina Rodent Lab Chow 5001. Tap water was available ad libitum.

Apparatus

Three standard operant chambers enclosed in sound-attenuated and fan-ventilated cubicles were used in this study. Each chamber was equipped with two levers, one on either side of and equidistant from a centrally located dipper which delivered 0.1 cc of liquid reinforcement consisting of a mixture of 25% evaporated milk, 25% Similac and 50% water. Lighting was provided by a 15 W
bulb located on the back wall of each chamber. The room in which the experiment took place was unlit. Standard electromechanical programming equipment and cumulative counters were used to control and record behavior.

**Procedure**

**Phase I - Preliminary Training**

Animals were randomly assigned to one of three operant chambers. Lever pressing for liquid food reinforcement was shaped according to the method of successive approximations. Once bar pressing was well established on one of the levers, the food delivery mechanism was inactivated for that lever and activated for responses on the opposite lever. Practice trials alternated between levers in this manner for the duration of each 10 minute training period. In this way animals were trained to alternate between response levers with reinforcement initially being delivered on a continuous reinforcement schedule. Gradually, the reinforcement contingency was changed to a fixed ratio 10 (FR 10) schedule of reinforcement with every 10th press on the appropriate lever resulting in the presentation of 0.1 cc
liquid reinforcement for a period of 3 s. This initial training procedure was carried out on a daily basis Monday through Friday for approximately 10 minutes/day at roughly the same time of day between the hours of 11:00 a.m. and 2:00 p.m..

Phase II - Drug Discrimination Training

After animals were consistently alternating between levers and pressing on a FR 10 schedule of reinforcement, drug discrimination training began. Animals were trained to discriminate either methylphenidate (Ritalin) or imipramine (Tofranil) from vehicle (physiological saline). (Methylphenidate hydrochloride and imipramine hydrochloride were graciously supplied by the CIBA Pharmaceutical Company, Summit, New Jersey.) Fifteen animals were randomly assigned to the methylphenidate group and fifteen to the imipramine group. On days when an animal received drug, only responses on the drug lever were reinforced; on saline days, only responses on the saline lever were reinforced. For half the animals in each group, the right lever was assigned as the drug lever and the left as the saline lever. The lever assignment was reversed for the remaining animals.
For the methylphenidate group, an initial starting dose of 2.5 mg/kg was selected; for the imipramine group, the starting dose was 10 mg/kg. The starting dose for methylphenidate was based on a study by Huang and Ho (1974) who found that the interoceptive cues used by rats previously trained to discriminate 0.8 mg/kg d-amphetamine from saline were generalizable (90% amphetamine lever choices) to methylphenidate when 2.5 mg/kg was injected. Drug was administered intraperitoneally (i.p.) 30 minutes prior to training. The rationale for this injection-training interval was based on previous research (Silverman and Ho, 1977) indicating that for methylphenidate at least, discrimination begins to diminish one hour post injection.

Drug discrimination sessions, 10 minutes in duration, were carried out at approximately the same time of day, once a day, for a minimum of five consecutive days per week. Drug and saline days were varied so that no animal was likely to select the correct lever because of sequence repetition. Discrimination training continued until an animal reached a final criterion of no more than three incorrect lever presses prior to the delivery of the first reinforcement on each daily 10 minute trial for nine out of ten consecutive sessions.
Data were recorded in terms of percent drug lever selection (\% DLS). On drug days, the specific criterion was 10/13 or 77\% DLS; on saline days it was 3/13 or 23\% DLS. Thus, low scores indicated a preference for the saline lever; high scores, a preference for the drug lever. The probability of reaching these criteria by chance was less than .05. If an animal was unable to discriminate drug from saline at the initial starting dose, this dose was increased in increments of .5 mg/kg for methylphenidate and 5 mg/kg for imipramine until the final criterion was met.

Phase III - Subthreshold Dose Determination

Once an animal had reliably learned to discriminate drug from saline as demonstrated by reaching criterion, testing was conducted to determine its subthreshold of discrimination. For example, if a particular animal had learned to discriminate methylphenidate from saline at 3 mg/kg, this dose was reduced in increments of .5 mg/kg to that dose which produced a \% DLS score which approached chance levels (~50\%). Similarly, imipramine doses were reduced by 5 mg/kg until responding approached chance levels. On test days the animal was placed in the
operant chamber and allowed to bar press until 10 responses had been made on one of the levers. If an animal met the drug lever criterion on a particular test, it was allowed to continue bar pressing for the duration of the 10 min trial. If not, it was immediately removed from the chamber and returned to its home cage.

**Phase IV - Amino Acid Drug Potentiation Testing**

After an animal's subthreshold dose had been determined, it was given its subthreshold dose along with either tyrosine or tryptophan on the test day and allowed to bar press until 10 responses were made on one of the levers. Again, if an animal correctly discriminated that it had drug as demonstrated by meeting criterion, it was allowed to continue bar pressing for the remainder of the 10 minute trial; if not, it was returned to its home cage. Methylphenidate animals were injected i.p. with 150 mg/kg dl-tyrosine suspended in saline and sonified to further refine the suspension and facilitate injection. Imipramine animals were given either 250 mg/kg l-tryptophan or 150 mg/kg dl-tyrosine. Tryptophan was suspended in propylene glycol and sonified.

Subthreshold dose and amino acid drug potentiation
test sessions were usually carried out on Fridays allowing for drug discrimination practice trials to continue prior to testing. This insured that the discrimination was still in place before the test day.

Animals whose drug lever selection was enhanced by supplementing their subthreshold dose with amino acid were subsequently tested with gradually reduced drug doses, i.e., below subthreshold values, while the amino acid dose was kept constant until % DLS scores again fell to chance levels (~50%). On subsequent test days, these animals were given the drug dose that produced chance responding plus a higher dose of amino acid to see if drug lever selection could once again be potentiated. Tyrosine doses were increased stepwise by 25 mg/kg to a maximum of 250 mg/kg; tryptophan, by 50 mg/kg to a maximum of 300 mg/kg. Interspersed among the drug plus amino acid trials were trials of the various amino acid doses alone. On amino acid alone test days, animals were always removed from the chamber after 10 responses had been made on one of the levers.
CHAPTER III

RESULTS

In order for animals to be considered as having reliably discriminated drug from saline, they had to meet a criterion of no more than three errors prior to the delivery of the first reinforcer on each daily ten minute trial for nine out of ten consecutive days. Mean ± SEM % DLS data for those ten criterion days for methylphenidate and imipramine animals are presented in Fig. 1.

Only data from animals that completed the first three phases of the study are represented. Of the original 15 methylphenidate animals, 1 died five months into the study of unknown causes. The remaining 14 met criterion and went on to complete the first three phases. Of the original 15 imipramine animals, only 6 completed the first three phases and all 6 met criterion. The remaining 9 animals died at various times apparently from drug related causes during the approximately sixteen months it took to complete the study.

With respect to side effects, imipramine has been known to decrease intestinal motility and increase urine retention, side effects thought to be due to its anticholinergic properties (Bassuk and Schoonover, 1977).
Fig. 1. Mean ± SEM % drug lever selection at the time of the first reinforcement for the 10 drug discrimination criterion days. Saline vs. drug. High scores indicate a preference for the drug lever; low scores, a preference for the saline lever.
For a few days prior to death, imipramine animals showed the following symptoms: darkened and sometimes pus-laden urine, dried blood around the nostrils, and hardened and bloated abdomens. An autopsy on one of the animals revealed a bloated and apparently obstructed intestinal tract filled with semidigested food. Occasionally, death could be postponed by eliminating drug trials for a period of several days to a week. Most often, however, once these symptoms appeared death soon followed. Those animals that died demonstrated varying thresholds for the development of these symptoms. Several animals developed symptoms within two months. A few tolerated drug for as long as eight months. All imipramine animals had developed some symptoms by the time their participation in the study ended. The limited ability of rats to tolerate chronic administration of imipramine has been noted elsewhere (Schecter, 1983).

The mean discriminable dose for the ten day criterion period was 3.32 mg/kg for methylphenidate animals and 19.17 mg/kg for imipramine animals. Methylphenidate animals took an average of 66 ± 10 days to meet criterion; imipramine animals an average of 75 ± 18 days.

Once an animal reached criterion, its subthreshold
dose was determined. Mean ± SEM % DLS data for the day subthreshold doses were established for methylphenidate and imipramine animals are presented in Fig. 2. Fig. 2 clearly illustrates that drug lever selection for both groups was well below chance (50%) and indicates a significant preference for the saline lever. The mean subthreshold dose for methylphenidate and imipramine animals was 1.5 mg/kg and 11.67 mg/kg, respectively.

Mean ± SEM % DLS data for the ten consecutive days prior to the establishment of subthreshold doses are presented in Fig. 3, and demonstrate that animals were reliably discriminating drug from saline prior to the test day on which their subthreshold dose was established.

Following this phase of the study, amino acid potentiation testing began. Initially, each animal was given its previously established subthreshold dose plus amino acid. Methylphenidate animals received i.p. injections of drug followed by 150 mg/kg tyrosine; imipramine animals, drug plus 250 mg/kg tryptophan or 150 mg/kg tyrosine, i.p.. Mean ± SEM % DLS data for this initial phase of amino acid drug potentiation testing for both groups are presented in Fig. 4. Fig. 4 shows that supplementation by amino acid of a subthreshold dose of
Fig. 2. Mean ± SEM % drug lever selection during the test that established the subthreshold dose for methylphenidate and imipramine. Low scores indicate a preference for the saline lever.
Methylphenidate

Imipramine

N = 14

N = 6

MEAN % DRUG LEVER SELECTION
Fig. 3. Mean ± SEM % drug lever selection during the 10 consecutive days just prior to the test day on which the subthreshold dose was established.
The graph shows the mean % drug lever selection for different substances. The x-axis represents the substances: Methylphenidate and Imipramine. The y-axis represents the percentage of drug lever selection. The graph compares Saline and Drug conditions. The Drug Criterion and Saline Criterion are indicated on the graph. The results indicate a significant difference in drug selection between Saline and Drug conditions for both substances.
Fig. 4. Mean ± SEM % drug lever selection when subthreshold drug doses were supplemented with amino acids, tyrosine or tryptophan vs. subthreshold dose alone. (Methylphenidate was supplemented with tyrosine; imipramine with tyrosine or tryptophan.)
Subthreshold Dose

Drug + Amino Acid

Methylphenidate

Imipramine

Mean % Drug Lever Selection

0 20 40 60 80 100

Methylphenidate Imipramine
drug enhanced drug lever selection in both methylphenidate and imipramine groups as compared to a subthreshold dose alone. In terms of the possible differential effects of tyrosine vs. tryptophan on imipramine potentiation, tyrosine potentiated a subthreshold dose of drug in 2 of 3 imipramine animals; tryptophan did so in 3 of 3 imipramine animals.

In order to determine whether amino acid by itself would produce drug lever selection, all animals were given the same kind and dose of amino acid they had received on the amino acid drug potentiation test day. Group means in terms of % DLS are presented in Fig. 5. As can be seen, low DLS scores indicate that amino acid alone produced relatively high levels of responding on the saline lever for both methylphenidate and imipramine groups. In terms of the possible differential effects of tyrosine alone vs. tryptophan alone in eliciting a drug response in imipramine animals (n=6), tyrosine alone failed to elicit a drug response in 3 of 3 imipramine animals (mean % DLS = 0.00); tryptophan alone elicited a drug response in 1 of 3 imipramine animals (mean % DLS = 33.3).

Subthreshold dose, subthreshold dose plus amino acid, and amino acid alone data (% DLS) were submitted to
Fig. 5. Mean ± SEM % drug lever selection after amino acid alone administration. Methylphenidate vs. imipramine.
HI

Methylphenidate Group

Imipramine Group

Tyrosine or Tryptophan

MEAN % DRUG LEVER SELECTION

Tyrosine

Tyrosine or Tryptophan
a two-way mixed design analysis of variance. Raw data were first transformed in accordance with the Arcsin transformation procedure (Snedecor and Cochran, 1967). The main effect for groups (methylphenidate vs. imipramine) was not found to be significant (P>0.05). The main effect for trials (subthreshold dose vs. subthreshold dose plus amino acid vs. amino acid alone) was found to be significant (F_{2,36}=14.78, P<0.001). The interaction of trials by groups was not significant (P>0.05).

Post hoc tests involving orthogonal comparisons between trial means indicated that trial two (subthreshold dose plus amino acid) was significantly different (F_{1,36}=29.56, P<0.001) from the average of trials one (subthreshold dose) and three (amino acid alone), which did not differ significantly from each other (P>0.05). Mean ± SEM % DLS (untransformed data) for trials one through three is presented in Figs. 6 (combined group data) and 7 (methylphenidate vs. imipramine).

Upon completion of the initial amino acid alone trials, an attempt was made to investigate whether even lower subthreshold doses of drug would enhance drug lever selection when supplemented by amino acid. Accordingly,
Fig. 6. Comparison of mean ± SEM % drug lever selection across trials (combined group data). Subthreshold dose vs. subthreshold dose plus amino acid vs. amino acid alone.
Mean % Drug Lever Selection

Subthreshold Dose
Subth. Dose + Amino Acid
Amino Acid Alone
Fig. 7. Mean ± SEM % drug lever selection across trials (methylphenidate vs. imipramine).
subthreshold doses were further decreased in stepwise fashion by .5 mg/kg for methylphenidate and 5 mg/kg for imipramine while the amino acid dose was kept constant. When % DLS again fell to 50% or below, the same subthreshold dose that produced this level of responding was given while the amino acid dose was increased by 25 mg/kg for a maximum of 250 mg/kg tyrosine. Interspersed among these subthreshold dose plus amino acid trials, were trials of the various doses of amino acid alone. Not all animals received this procedure due to time constraints, the age of the animals by the time of testing, and the high mortality of imipramine animals.

Data on several animals that completed most of this process in a systematic fashion are presented in table 1. An inspection of the data in table 1 reveals the following: (1) There is a clear indication that even lower subthreshold doses of drug in the same animal can be potentiated by amino acid supplementation. This effect was demonstrated in 7 of 11 animals. (2) When a subthreshold dose of drug plus amino acid no longer produced a clear cut preference for the drug lever in an animal, drug lever selection could often be enhanced by increasing the amino acid dose while keeping the drug dose constant. This effect was demonstrated in 5 of 8
Table I

% Drug Lever Selection (DLS) From Subsequent Testing in Which Subthreshold Doses of Drug Were Decreased and Varying Doses of Amino Acid Were Administered Alone and in Combination With Drug (doses are given in mg/kg).

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Note. S = subject, Tyr = tyrosine, MPD = methylphenidate, IMIP = imipramine.
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Note. S = subject, Tyr = tyrosine, MPD = methylphenidate, IMIP = imipramine.
animals. (3) While amino acid alone sometimes produced drug lever selection, this effect did not appear to be dose related nor was it consistent within or between animals.

Finally, in order to determine whether animals' subthreshold doses were subject to change over time due to the development of tolerance or increased sensitivity, subthreshold doses were re-evaluated for some animals. In most instances, this testing took place several weeks to a few months after the initial subthreshold dose had been established. Of the 13 animals tested in this way, the subthreshold dose had decreased in 8, remained the same for 2, and increased in 3.
CHAPTER IV
DISCUSSION

The purpose of this study was to examine whether a drug that enhances the action of a neurotransmitter can be potentiated by an exogenous supply of a nutrient which serves as a precursor for that neurotransmitter. In this case, we examined whether the action of methylphenidate and imipramine could be enhanced by supplying exogenous sources of tyrosine or tryptophan. The behavior of rats bar pressing for food reward in a drug-controlled two-lever choice situation was used to evaluate drug and amino acid effects.

The results indicate that the interoceptive cues used by animals to discriminate methylphenidate or imipramine from vehicle were potentiated by i.p. injections of tyrosine or tryptophan when subthreshold doses of these drugs were administered. This potentiation was able to control responding on the drug lever. Moreover, it appears that drug effects cannot be produced in this paradigm solely by administering the appropriate amino acid precursor; some drug, albeit in subthreshold amounts, has to be present. In trials in which subthreshold doses were reduced until the
combination of drug plus amino acid no longer produced a drug induced response, simply increasing the dose of amino acid in several animals reinstated the response. In other trials in which larger doses of amino acid alone were tested, a few drug responses occurred but this effect was sporadic and did not appear to be dose related. It may be that the amino acid alone doses were, in general, too large. Badawy and Williams (1982) have suggested that the ability of exogenous tyrosine to enhance CA synthesis and release may be dose related with smaller doses being more effective than larger ones. In their study, tyrosine doses of up to 40 mg/kg were found to produce significant increases in DOPA accumulation in rat brain but only the 20 mg/kg dose increased brain DA, DOPAC, and NE to a significant degree. Doses of 50 mg/kg and over abolished this enhancement. Badawy and Williams suggest that this may have been due to substrate inhibition of tyrosine hydroxylase activity.

With respect to tyrosine, our finding that subthreshold amounts of drug, e.g., methylphenidate, must be present for amino acid potentiation to occur was not contrary to expectation as the enzyme which catalyzes the key step in the conversion of tyrosine to neurotransmitter is subject to end-product inhibition.
arising both from intracellular and extracellular events (Wurtman et al., 1981). Increasing the level of neurotransmitter by precursor loading ultimately serves to inhibit synthesis and release. In this study we may have effectively overcome this type of feedback inhibition on tyrosine hydroxylase by supplying low doses of drug which may have served to increase synaptic activity. Under these conditions, increased levels of precursor in brain may have led to more neurotransmitter release and thus a potentiation of drug effects. This finding is consistent with the results of other studies (Melamed et al., 1980; Wurtman et al., 1981) suggesting that tyrosine's ability to influence the synthesis and release of CAs may depend upon neuronal activity - increasing when populations of neurons are firing frequently and decreasing when they are relatively quiescent.

This does not preclude the possibility that tyrosine administration alone may be useful in the treatment of ADD or for that matter other disorders thought to be due to decreased functioning within select populations of CA neurons. For example, tyrosine injection has been shown to significantly lower blood pressure in spontaneously hypertensive rats (Sved et al., 1979), and to increase
blood pressure in hypotensive rats (Conlay, Maher and Wurtman, 1981). There is also some indication that tyrosine may be useful in the treatment of unipolar depression (Gibson and Gelenberg, 1983).

Perhaps those patients who will ultimately respond to tyrosine therapy alone represent a subgroup of patients who have abnormally low levels of plasma and/or brain tyrosine to begin with due perhaps to dietary influences or an as yet unidentified deficit in the transport system which carries tyrosine across the blood brain barrier.

In terms of tryptophan, our results are more equivocal with respect to whether tryptophan can elicit a drug effect in the absence of low doses of imipramine. Tryptophan was difficult to get into an injectable solution. Because of this as well as the high mortality of imipramine animals due to the side effects of this drug, imipramine animals did not have many tryptophan alone trials. More data are needed in order to determine more fully the efficacy of tryptophan alone in producing a drug response.

Unlike tyrosine hydroxylase, tryptophan hydroxylase is not reported to be significantly affected by end-product inhibition (Jacoby et al., 1975) so that
increased amounts of precursor should lead to increased synthesis and release. There is some evidence to support this (Fernstrom and Wurtman, 1971; McBride et al., 1976). On the other hand, imipramine has been reported to be a more potent NE than 5-HT reuptake inhibitor (Green and Nutt, 1983). If the interoceptive cues produced by imipramine were primarily related to its effects on NE rather than 5-HT, this would be consistent with our preliminary finding that tryptophan alone does not consistently elicit a drug response. The fact that tryptophan was able to potentiate subthreshold doses of imipramine in 3 out of 3 animals tested in this way suggests that drug effects involve a more complex interaction between 5-HT and NE. This is supported further by the finding that tyrosine and tryptophan were both effective in producing a drug response in the presence of subthreshold doses of imipramine.

It should be pointed out, however, that the tyrosine dose (.83 mmol/kg) was considerably less than the tryptophan dose (1.22 mmol/kg). There has been at least one report in the literature on the differential effects of tyrosine vs. tryptophan on the same physiological phenomenon (Sved et al., 1979). In their study, Sved et al. found that tryptophan injection (225 mg/kg) lowered
blood pressure in spontaneously hypertensive rats but only by about half as much as an equivalent dose of tyrosine.

Another possible explanation, as well as a more parsimonious one for the failure of tryptophan to elicit a drug response, is the following. Imipramine is known to have effects on multiple neurotransmitter systems, e.g., cholinergic and histaminergic as well as monoaminergic (Iversen and Mackay, 1979). Amino acids on the other hand affect only the neurotransmitter systems for which they serve as precursors although it is possible that there may be indirect effects on other neurotransmitter systems as well. If the interoceptive cues used by our animals to discriminate imipramine were related to its effects on multiple neurotransmitter systems, then the failure of tryptophan alone to produce a drug response can be easily understood.

Finally, the failure of tryptophan to produce a drug response does not preclude the possibility that tryptophan therapy may be effective in the treatment of disorders associated with decreased functioning in populations of 5-HT neurons, especially if the putative deficits are specifically confined to this system. Tryptophan has been used alone and in combination with
antidepressant drugs in clinical trials for the treatment of depression although, as previously mentioned, the results have been inconsistent (Chouinard et al., 1983; Walinder, 1983).

One possibility that was not investigated was that the potentiation of drug effects by amino acid was due to a non-specific amino acid effect. In order to test this hypothesis, another amino acid such as valine which uses the same transport system into brain as tyrosine and tryptophan but which does not serve as a precursor for the CAs or 5-HT could have been given along with subthreshold doses of drug to a control group of animals. However, Morre et al. (1980) have shown that increases in brain tyrosine produced by tyrosine injection (100 mg/kg) can be blocked by the co-administration of valine (500 mg/kg). In addition, it has been demonstrated (Wurtman et al., 1974) that tyrosine (50 mg/kg) but not leucine (100 mg/kg) or tryptophan (50 mg/kg) can produce significant increases in DOPA accumulation in rat brain following decarboxylase inhibition. These studies suggest that in the case of tyrosine at least we are not dealing with a non-specific amino acid effect.

It is also possible that in the intervening time (~1-2 weeks) between the establishment of the
subthreshold dose for each animal and the amino acid plus subthreshold dose trials, animals' thresholds changed and they "got better" at discriminating drug. For some of our animals, several weeks to a few months after the first phases of testing were completed, we repeated the procedure for determining the subthreshold dose to assess the possibility that either tolerance or an increased sensitivity to the drug had developed. Of the 13 animals tested, the subthreshold dose had remained the same in 2, increased in 3 and decreased in 8. Those animals whose subthreshold dose had increased or remained the same happened to have had fewer (Mean ± SEM = 14.8 ± 3.1) drug trials during the intervening period than those whose subthreshold dose had decreased (Mean ± SEM = 28.6 ± 2.7). Thus it appears that subthreshold doses may have changed over the course of repeated trials perhaps increasing in the short term and decreasing over the long term. When we tested 4 of the animals whose subthresholds had subsequently decreased with their new subthreshold dose plus 150 mg/kg tyrosine, drug lever selection was not enhanced; however, we reinstated the enhancement effect in 3 of these 4 animals by increasing the dose of amino acid while keeping the new subthreshold dose constant. These findings support the supposition
that we are dealing with a true potentiation effect and not an artifact of increased sensitivity to drug.

An interesting phenomenon which was observed in the majority of our animals and which may point to possible side effects accompanying the administration of doses of amino acids substantially above those normally present in diet was the following: Almost immediately after being injected with tyrosine or tryptophan, animals appeared to become sedated, often lying down on their sides in their home cages. If approached by an experimenter they would often struggle to get up and appeared unsteady. When handled they were somewhat flaccid to the touch and seemed to have experienced a loss of muscle tone. If placed on a flat surface at this time and allowed freedom of movement, they often wobbled about and had difficulty staying on their feet. These effects were temporary however, and animals appeared to be back to normal by the time they were to be tested, 30-45 minutes later. Animals seemed to vary in the degree to which they manifested these symptoms. Over the course of repeated amino acid injections, a few animals appeared to develop a tolerance to this effect. Of interest is the observation that these behavioral symptoms occurred whether amino acid was injected alone or in combination
with drug.

There is evidence that excessively large doses of tryptophan are toxic (Gullino, Winitz, Birnbaum, Cornfeld, Otey and Greenstein, 1956). Symptoms of toxicity which appear as early as 10 minutes post injection include dyspnea, hypothermia, and extreme prostration often connected with uncoordinated movement. The LD$_{50}$ for adult male rats is 8 mmol/kg body weight for the l-isomer. This dose represents more than 1.5 g/kg of l-tryptophan or 80-120 g for an adult human (Sourkes, 1983). When an LD$_{99.9}$ (12 mmol/kg) of l-tryptophan was given to rats, a severe but transient hyperglycemia resulted and animals ultimately died in a hypoglycemic state (Winitz, Gullino, Greenstein, and Birnbaum, 1956).

A dose of 750 mg/kg of l-tryptophan administered i.p. to both fed and food deprived (36 h) rats produced an initial hyperglycemia after 1.5 h followed by a return to normal blood glucose concentrations after 3 h, followed by a period of severe hypoglycemia; blood glucose levels then slowly returned to initial values (Smith and Pogson, 1976). The hypoglycemic effect of tryptophan was potentiated in animals pretreated with pargyline, a monoamine oxidase inhibitor and blocked in animals pretreated with the 5-HT antagonist,
methysergide, suggesting the involvement of 5-HT. This effect was found to be dependent on the presence of functional pancreatic beta cells suggesting that insulin is also involved. Perhaps what we observed in our animals given 250 mg/kg l-tryptophan was a mild and transient hyperglycemic state. This possibility is currently under investigation in our lab.

Finally, the question arises as to whether the interoceptive cues used by animals in this drug discrimination paradigm were central or peripheral. Indirect evidence that the methylphenidate cue at least may be central comes from two experiments by Richards, Harris and Ho (1973) on the discriminative stimulus properties of d-amphetamine, a drug which is closely related, pharmacologically, to methylphenidate. In the first experiment, two groups of rats implanted with intraventricular cannulae were trained to discriminate d-amphetamine (0.5 and 1.5 mg/kg, i.p.) from saline in a two-lever choice situation. Both groups responded on the saline lever to i.p. injections of 2 mg/kg p-hydroxyamphetamine (p-OHA), a drug which has peripheral effects similar to amphetamine but possess little central activity due to its limited ability to cross the blood-brain barrier (Silverman and Ho, 1977). Central
administration of 50 ug or more of d-amphetamine in the 0.5 mg/kg group and 75-100 ug or more in the 1.5 mg/kg group produced responding on the drug lever. In the second experiment, two groups of rats were trained to discriminate intraventricular injections of amphetamine (150 ug) from saline and then tested to see if drug lever responding would generalize to i.p. injections of 0.5 and 1.5 mg/kg amphetamine, 1 mg/kg saline, and 2.0 mg/kg (p-OHA). Again, animals responded on the drug lever to d-amphetamine but not to p-OHA. The results of both experiments demonstrate that central rather than peripheral cues are involved in the discriminative stimulus properties of d-amphetamine.

In summary, the following conclusions seem to be warranted on the basis of our findings: First, psychoactive drugs such as methylphenidate and imipramine used in the treatment of ADD may be potentiated by exogenous supplies of amino acids which serve as precursors to the neurotransmitters on which these drugs ultimately act. As such, it may be possible to attenuate or eliminate some of the side effects produced by these drugs while still maintaining their therapeutic efficacy. This possibility may be of considerable interest to parents and professionals who are resistant to medicating
young children and adolescents often because of these side effects. To date, as far as can be determined, no studies have attempted to use tyrosine or tryptophan in combination with methylphenidate or imipramine in the treatment of ADD, although our results suggest that such studies may be warranted. Second, the experimental paradigm used in this study seems to offer a viable animal model within which to study this drug potentiation phenomenon as a whole, offering as it does a better degree of control than is typically possible with clinical populations. Third, follow-up studies are needed to determine if the potentiation of the behavioral effects of these drugs by amino acid can be supported by neurochemical studies demonstrating an increase in neurotransmitter turnover in the presence of a dose of drug plus amino acid when compared to that same dose of drug alone. This type of investigation is now in progress in our lab.

Finally, Wender (1975) has hypothesized that ADD may involve a functional underactivity in one or more of the monoamine neurotransmitter systems due to decreased synthesis, release or receptor sensitivity. It is also possible that this presumed functional underactivity may be a result of a lower than normal number of neurons in
one of these systems leading to secondary deficits in neurotransmitter and biosynthetic enzyme activity within brain. There is evidence, for example, that genetically related natural variations in the number of DA neurons within brain are responsible for observed neurochemical (tyrosine hydroxylase activity), morphological (receptor density; size of target organ), pharmacologic (sensitivity to the effects of d-amphetamine) and behavioral (spontaneous motor activity and exploration) differences found in two different strains of mice (Reis, Fink, Baker, 1983). Perhaps the ADDchild or adult represents an extreme of a temperament continuum, the underlying physiological basis for which may involve genetically related natural variations in numbers of CNS neurons of a particular neurochemical type. In any case, supplying more precursor while simultaneously priming the system with drug could theoretically compensate for this hypothetical deficit.
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