Strategies for Study of the Neurochemistry of Attention Deficit Disorder in Children

by Robert D. Hunt, Donald J. Cohen, Sally E. Shaywitz, and Bennett A. Shaywitz

Abstract
The interpretation of neurochemical studies of attention deficit disorder (ADD) with hyperactivity is complicated by the variability in diagnosis and by the limitations of single neurochemical measures—whose levels may be compensated by chronic feedback mechanisms. This article reviews previous neurochemical studies of ADD and proposes the use of single-dose neurochemical probes. Administration of a provocative agent with subsequent sequential measures of plasma levels of neurotransmitters, their metabolites, and hormones may help define the responsivity of neurochemical systems. Blood levels of drugs and neurotransmitters following single doses of methylphenidate and clonidine are studied as a strategy to explore the responsivity of dopaminergic and noradrenergic mechanisms in ADD.

The ability to sustain and shift attentional focus is a major component of cognitive and emotional maturation. Many disciplines have pursued an understanding of the psychological and neurophysiological mechanisms of attentional and cognitive processes. Yet considerable ambiguity remains about the nature of attention and the neuronal mechanisms that underlie a child’s ability to focus and shift his thoughts and orientation.

There is no single measure—behavioral, cognitive, physiological, or chemical—that uniquely spans the breadth of, or is adequately diagnostic of, attentional processes. Lacking a unique or sufficient marker or correlate of these processes, investigators sample a variety of phenomena along the attentional path. Perception, discrimination, and visual-motor integration are all components of a system that rapidly links internal and external events with behavioral output. Physiological measures of central nervous system (CNS) evoked response or of peripheral response to an orienting stimulus (e.g., heart rate, peripheral vascular resistance, and skin conductance) provide inferences about the processes of attention and arousal. Yet, no measure stands independently as a barometer of the multiple and simultaneous events that comprise attention. Investigators seek neurochemical and neurophysiological threads that will link the clinical behavior of a child restless-gazing about the classroom to his performance on laboratory tests of sensory discrimination, reaction time, vigilance, and memory.

Selective neurochemical measures and neuropharmacological responses suggest some neurotransmitter components of attentional processes. Interpretation of individual chemical measures, however, is clouded by the imprecise relationship between the state or trait status of selected measures (e.g., enzymes, neurotransmitters, and hormones), and the cognitive or behavioral processes with which they may correlate. Furthermore, given the nervous system’s ability to alter the sensitivity of chemoreceptors to their neurotransmitters, individual chemical levels cannot easily be interpreted without assessment of their receptor sensitivity. A growing litera-

Reprint requests should be sent to Dr. D.J. Cohen at the Child Study Center, 333 Cedar St., New Haven, CT 06510.
tecture assigns significance to catecholaminergic mediation of information processing and the modulation of arousal, attention, and activity. The pursuit of these leads with more refined cognitive tasks, and more precise pharmacological probes, may illuminate mechanisms of the development and disturbance of attention.

**Attention Deficit Disorder—Clinical Constellation and Diagnostic Considerations**

The diagnostic criteria for attention deficit disorder (ADD) with hyperactivity as defined in DSM-III (American Psychiatric Association 1980) include the constellation of hyperactivity (running, climbing, fidgeting, and difficulties in staying seated or still); inattention (characterized by failing to finish tasks, not listening, easy distractibility, difficulty concentrating on school work or staying with a play activity); and impulsivity (including a tendency to act before thinking, to shift activities rapidly and to be disorganized in work or to require supervision). These symptoms can be considered indicative of ADD only if they appear before age 7 and persist for longer than 6 months. A primary diagnosis of ADD is not applied to the cognitive disturbances which occur within pervasive developmental disorder, schizophrenia, or manic-depressive disorder. But there are complex diagnostic and nosological connections between the symptoms of ADD and other neuropsychiatric conditions, such as Tourette's syndrome or borderline personality disorders (Cohen et al., in press).

The phenotypic expression of the attention deficit disorder (American Psychiatric Association 1980) changes with development. Careful history may disclose that the ADD child was an unusual infant. He cried a lot, was irritable, and slept less than most infants. He did not sustain play or exploration with one toy or object. He destroyed and lost even the most "childproof" toy. He wore out his clothes, his toys, and his mother's patience earlier than most toddlers. Many of these children are identifiable before they enter school because of their difficulty in modulating attention, aggression, and excitement. Their monitoring of internal stimuli and feelings may be as impaired as their difficulties in sustaining external attention (Cantwell 1975a; Millichap 1975; Cohen 1977; Lazor and Chandler 1978; Weiss and Hectman 1979).

By elementary school, these children frequently exhibit learning difficulties. Some appear to have primary perceptual problems, such as enhanced tendency to reverse letters and numbers. Others have reading difficulties (dyslexia), perhaps secondary to the impulsivity of their visual scanning. Learning difficulties also may reflect impairment in other aspects of cognition. For example, the correct sequencing of information or the discrimination of a relevant pattern or concept often requires sustained focusing on subtle aspects of a stimulus, subject, or thought. The impulsive child may miss sequences and patterns through repeated distraction. Similarly, the abstract or idiomatic meaning of a word which is partially defined by its context may be lost to the child who processes information in rapidly shifting fragments.

A specific learning disability exists when cognitive functioning in one modality or skill is impaired relative to other more general intellectual abilities. Many children with ADD may have specific reading or arithmetic disabilities (Clemmens and Kenny 1972; Gittelman-Klein and Klein 1976; Harris 1976; Rosenbloom 1972).

Attentional dysfunction frequently includes an insensitivity to social cues. ADD children often ignore facial expressions, are unaware of danger, and fail to anticipate the effect their behavior will have on others. Social difficulties reflect the impetuousness with which they approach relationships. Attentionally impaired children may have difficulty integrating discrepant cues from different sensory modalities and hence miss subtle discrepancies between what is said and what is implied (Whalen et al. 1979). ADD children try to intrude into and take over groups, and they may insist on being the leader. They often have little capacity for fairness, reciprocity, and taking turns. Recess provides too many opportunities for fights as the children bully or are bullied. ADD children may initially appear to be friendly, but they lose friends as quickly as they are made.

Frequently these children have a delay in development of fine and gross motor coordination, causing them to appear slow and awkward. Handwriting is usually sloppy; gross motor coordination is often floppy, loose, or disheveled. In spite of their bravado and provocation, these children frequently are poor athletes. Boys, probably because of their high activity level,
are much more likely to manifest the behavioral and motoric components of this disorder (Shaffer 1978; Shaywitz 1982). Some attentionally impaired children are fidgety and impulsive; others are slow but competent in information intake and visual motor performance, and appear grossly hypoactive. Thus, ADD often occurs in association with other difficulties in modulation, including motoric hyperactivity, learning or cognitive disability, and poor social adjustment (Loney, Langbourne, and Paternite 1978; Loney et al. 1978).

By middle childhood, attentionally impaired children are often enmeshed in conflict. Parents are unable to "make them mind," teachers have difficulty making them learn and behave in class; peers are annoyed by immature, attention-seeking behavior; neighbors may complain of their negligence, destruction of property, or "bad influence" on other children. By middle childhood, symptoms of depression and low self-esteem may complicate adaptation. Failure to channel efforts into a meaningful sequence of accomplishments often leads children to achievement levels below their abilities and a lack of gratification and pride (Das, Leong, and Williams 1978; Huessy, Metoyer, and Townsend 1974).

Attentional deficit and impulsivity may persist into adulthood, although the motoric hyperactivity usually diminishes to manageable levels of restlessness. While some outgrow the effects of ADD, others have severe impulsivity and other difficulties as adults. They may develop personality disorders or have substantial antisocial and legal difficulties. ADD may be a precursor to some later forms of major affective disorder or psychosis (Mendelson, Johnson, and Stewart 1971; Minde, Weiss, and Mendelson 1972; Huessy, Metoyer, and Townsend 1974; Cantwell 1975a; Quinn and Rapoport 1975; Wood et al. 1976; Lerer and Lerer 1977; Hopkins et al. 1979).

Environmental Influence. Attention provides continuity of the linkage of a child’s inner life to his external environment. The child’s development of the capacity for sustained attention is enhanced by interaction with calm and predictable parents. An environment in which affection and rules are reliably provided allows safety for a child to direct his attention to his “work” of practicing and exploration. A chaotic family, prone to explosiveness and disaster, creates an endless whirl of distraction requiring the child’s continued vigilance or worry. The child may not be able to focus energy into persistent pursuit of a goal (Sandburg, Rutter, and Taylor 1978; Rollins and Thomas 1979). Children from chaotic home environments may fail to develop the continuity of focus and the reinforced pursuit of meaningful goals which sustain investment of intellectual and emotion.

Transient variations in level of the child’s arousal, alertness, and motivation (his experience of the rewards and incentives for focusing on a task) all may alter attentional functioning. Clinical disturbances of attentional mechanisms may result from internal psychological conflicts which lead to preoccupation and an inward shift of attention. Attention may be diffused by a highly distracting environment or by anxiety about performance of a difficult academic task. Attentional deficit and hyperactivity may be specific to a particular task or setting for some children (Ellis et al. 1974; Barkley and Jackson 1977). Klein and Gittelman-Klein (1975) found that of 155 subjects who were hyperactive in the classroom, only 25 percent were hyperactive at home.

Attentional disturbance is a frequent component of more pervasive disorders such as autism, atypical development, and Tourette’s syndrome (Cohen 1980; Jagger et al. 1982). In some cases, this attentional deficit may reflect a disturbance in attributing meaning to stimuli, or as shown by recent data, disturbances in physiological mechanisms that modulate arousal and intake of external stimuli in developmentally delayed children (Kootz and Cohen 1981).

Given the diversity of children whose attentional disturbance reflects a disruptive environment or a more global impairment of intelligence or personality, it is difficult to find a homogeneous population in which attentional disruption appears as a primary trait. Yet, the quest for clinical homogeneity, both as a behavioral pattern and a personality type, must precede and complement exploration of a common biological substrate.

Familial and Genetic Studies of ADD

Familial studies suggest a genetic contribution to the development of this disorder. The presence and severity of ADD in a specific child may reflect a response to either genetic vulnerability or environ-
mental chaos and emotional deprivation. Clinical observations suggest that parents of hyperactive children have an increased prevalence of mixed psychopathology (schizophrenia, affective disorders, and sociopathy) (Wender 1971) and a greater incidence of neurosis, antisocial behavior, "nervous breakdown," suicide, and alcoholism (Satterfield et al. 1973). Stewart et al. (1966) found that more than half of 37 hyperactive children had a first or second degree relative with serious legal, psychiatric, or employment difficulties. Heavy drinking was associated with behavioral difficulties in 22 percent of the fathers and 4 percent of the mothers. A history of learning difficulties was evident in nearly a fourth of fathers and 10 percent of mothers of 83 hyperactive children (Mendelson, Johnson, and Stewart 1971).

In a more systematic psychiatric examination of the parents of 50 hyperactive children and 50 matched controls, Cantwell (1975) found that nearly half of the parents of hyperactive children met clinical criteria for diagnosis of a psychiatric disorder; this was in marked contrast to the virtual absence of psychiatric diagnoses in parents of controls. The main psychiatric diagnoses found in the parents of hyperactive children were alcoholism and sociopathy among fathers, and hysteria among both parents. Sixteen percent of the fathers of hyperactive children gave a history of having been hyperactive themselves, as children. This subgroup of previously hyperactive fathers demonstrated an equally high incidence of alcoholism and sociopathy as adults. Similar findings were reported by Morrison and Stewart (1971) in their evaluation of parents of 59 hyperactive and 41 normal children. One third of the parents of hyperactive children exhibited a psychiatric or behavioral disorder usually consisting of alcoholism, sociopathy, or hysteria; over 10 percent of the hyperactive children had at least one parent with a history of childhood hyperactivity.

Adoption, sibling, and twin studies have also added further evidence of a genetic transmission of vulnerability to this disorder. Using a systematic psychiatric examination, Morrison and Stewart (1973) and Cantwell (1975b) compared the incidence of psychiatric disorder found in the nonbiological parents of adopted hyperactive children to that of the biological parents of nonadopted hyperactive children. (The biological parents of adopted hyperactive children were not available for examination.) These studies found no greater prevalence of a psychiatric disturbance or of childhood hyperactivity among the adoptive parents than existed in the parents of control children. A comparison of the incidence of "minimal brain dysfunction" (MBD) in full- and half-siblings of 17 MBD children found that while 52.6 percent of their full-siblings appeared to demonstrate MBD, only 9.1 percent of their half-siblings were symptomatic (Safer 1973). Comparisons of activity ratings by parents of 93 sets of monozygotic or dizygotic twins suggested a substantial genetic component to activity levels (Willerman 1973). However, this study did not distinguish activity level from the diagnosis of attention deficit as a complete syndrome.

While these studies do not determine the extent of genetic and environmental influences in the transmission and expression of ADD, they do suggest a possible genetic component to the vulnerability for this disorder in some children. The mechanism of inheritance is not clear since father-son transmission and variable penetrance appear likely. Familial studies have not yet clarified what is transmitted: an impaired ability to modulate impulses, an increased level of energy or aggression, or a more specific disruption of attentional or motoric integration. The evidence for substantial genetic contribution to chronic multiple tics of Gilles de la Tourette syndrome, in which motor and attentional disturbances coexist (Kidd, Prusoff, and Cohen 1980), and the suggestion that obsessive-compulsive disorders may have an inherited component further buttress the impression that some biological substrate affects these modulating processes.

Whether or not the diverse components of activity and attention in this disorder are inherited as separate but potentially coexisting factors is unclear. Sexual differences in activity and aggressiveness may contribute to differences in hyperactivity among children with similar attentional diffusion. The suggestion of a genetic factor in this disorder or in its specific components provides impetus for further study of chromosomes, enzymes, and neurotransmitters which may convey this vulnerability.

**Biological Factors in ADD.** Other lines of evidence suggest a possible biological substrate for ADD with hyperactivity (ADDH). The
findings of increased minor physical anomalies among attentionally impaired, hyperactive children may evidence a genetic disturbance or an in utero disruption of physical as well as cognitive-integrative integrity. Waldrop and Halverson (1971) found an increased incidence of minor physical anomalies (stigmata) among those nursery school children having higher levels of activity and aggressivity. Rapoport, Quinn, and Lamprecht (1974) documented the presence of multiple minor physical anomalies in 76 severely hyperactive boys. High stigmata scores were associated with teachers' ratings of "hyperactivity" and conduct problems. High stigmata scores were also associated with fathers' history of childhood behavior disorders and with mothers' history of obstetrical difficulties. In addition, plasma dopamine-β-hydroxylase (DBH) showed a significant positive relationship with stigmata scores. These findings suggest the possibility of a genetic disorder that may be mimicked (phenocopied) by a traumatic event in early pregnancy.

The frequency of delayed development of gross and fine motor coordination in children with ADDH provides another possible marker of a biological substrate. However, not all children with attentional deficit have impaired motor coordination, and many children with coordination difficulties are intact cognitively, or exhibit no other psychiatric diagnoses (Rie et al. 1978; Shaffer 1978). "Soft" neurological signs may be developmentally or age-related (Adams, Koesis, and Estes 1974) and are reliably elicited, persistent, but nonspecific; their clinical significance is not clear (Shaywitz 1982). Dysgraphia has been identified in 10 percent, dysdiadochokinesia in 8 percent, and mirror movements in 14 percent, and choreiform movements have been elicited in 11 percent of children without learning or behavioral difficulties (Stine, Saratsioter, and Mosser 1975; Shaffer 1978).

Several investigators have reported that hyperactive children have a higher incidence of nonspecific EEG abnormalities which are not influenced by stimulant medications (Klinkerfuss et al. 1965; Capute, Niedermeyer, and Richardson 1968; Klinkerfuss et al. 1965; Satterfield et al. 1973a; Satterfield, Cantwell, and Satterfield 1974). Computer-assisted spectrum analysis of the EEG has suggested some drug effects (Itil and Simeon 1974).

Differences in peripheral autonomic nervous system responses may also suggest a physiological basis for this disorder in some ADD children. Investigators have reported lower levels of spontaneous skin conductance, increased variability and delay in reaction time, and decreased responsivity to an orienting stimulus in ADD. Furthermore, the finding of normal brain morphology using computerized axial tomography in approximately 100 ADD children (Caparulo et al. 1981) suggests that if abnormalities in CNS morphology do exist in ADD, they are not apparent using current techniques. New neuroradiological diagnostic procedures (positron emission tomography, nuclear magnetic resonance) may be useful in demonstrating abnormalities in ADD.

**Effect of Stimulants on Catecholamines.** Stimulant medications have been widely used in the treatment of ADDH. While amphetamine and methylphenidate have similar effects, amphetamine is approximately twice as potent clinically as methylphenidate. Although stimulants do not improve all aspects of the clinical problem, understanding their pharmacological mechanisms may illuminate some neurochemical components of ADDH. In animal studies, d-amphetamine and methylphenidate also produce similar behavioral and CNS activation. Stimulants affect animal learning and activity levels. In high doses they increase stereotyped gnawing behavior in rats, probably a dopamine-related phenomenon.

Stimulant medications have multiple effects on CNS neurotransmitters (Smith and Davis 1977). Amphetamine releases newly synthesized (reserpine-resistant) dopamine and blocks presynaptic reuptake of dopamine and norepinephrine (Clemens and Fuller 1979). Although d-amphetamine has more potent effects on norepinephrine release and reuptake than does methylphenidate, they are nearly equipotent in stimulating dopamine release (Ferris, Tong, and Maxwell 1972). Methylphenidate preferentially stimulates release of previously synthesized (reserpine-sensitive) dopamine from vesicular stores (Scheel-Kruger 1971; Davis 1973; Costall and Naylor 1974).

Serotonin modifies and may mediate some of the effects of stimulants. The locomotor hyperactivity induced in rats by intraperitoneal amphetamine was reduced by serotonin injection into the nucleus accumbens, a site rich in dopamine receptors. Lesions of the me-
Biochemical Studies of ADD. Given the clinical homogeneity of ADD, the vagueness of the concept of attention, and the variability in activity level among attentionally impaired children, it is not surprising that attempts to define a neurochemistry remain rudimentary. Stimulant effects are not a chemical mirror of the mechanisms of attentional deficit since the medications provide only partial improvement of attentional and behavioral disturbance.

Enzyme Studies of ADD. Dopamine-β-hydroxylase (DBH), the catalytic enzyme which converts dopamine to norepinephrine, is released from the dendritic vesicles into the circulation where it may be assayed. Its wide variability (over 100-fold) in normal populations and its increasing levels during early childhood complicate the determination of normal values (Freedman et al. 1972; Young et al. 1980). Rapoport, Quinn, and Lamprecht (1974) found that while hyperactive children with the highest scores for minor physical anomalies had increased levels of plasma DBH, no direct correlation existed between DBH levels and hyperactivity. The finding of normal DBH levels in a group of children with ADD was verified by subsequent investigation by our group (B.A. Shaywitz et al., in press). Methylphenidate and imipramine treatment appear to increase DBH levels (Rapoport, Quinn, and Lamprecht 1974), but our observations suggest that this is uncertain.

Platelet monoamine oxidase (MAO) is a well-studied enzyme under genetic control. Low levels of platelet MAO may be a source of nonspecific vulnerability to various major psychiatric illnesses (Murphy and Donnelly 1974). MAO, Type A, found in plasma, oxidizes norepinephrine and serotonin, but not dopamine. MAO, Type B, is found in platelets and oxidizes dopamine (Youdim et al. 1972). Platelet MAO activity varies with age and sex; it is greater in girls than boys. MAO levels tend to decrease from infancy throughout childhood, and then increase from late adolescence throughout the adult years (Roth, Young, and Cohen 1976; Robinson and Nies 1980; Young et al. 1980). A similar pattern exists with brain MAO.

In contrast to reduced levels of platelet MAO in chronic schizophrenia (Wyatt and Murphy 1976), normal levels have been found in our studies of autistic children (Cohen, Young, and Roth 1977). In our recent measures of MAO, it is suggested that most children with ADD exhibit normal platelet concentration, although a subgroup with reduced activity may exist.

Cerebrospinal Fluid (CSF) Studies. Determination of amines and metabolite levels in the CSF offers the possibility of obtaining specimens more closely reflective of brain than of peripheral function. The low concentrations of these substances require sensitive assay methods, usually using gas chromatography-mass spectrometry or high performance liquid chromatography (HPLC). Levels of the acid metabolites (HVA and 5-HIAA) can be increased by competitive blocking of their egress through prior administration of probenecid. The levels of these metabolites will increase in approximate proportion to the CSF concentration of probenecid until blockage becomes nearly complete. Subsequent metabolite accumulation may provide an index of dopamine and serotonin turnover rate (Young et al. 1982).

Shetty and Chase (1976) measured CSF homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), the major metabolites of dopamine and serotonin, respectively, in 24 hyperactive children (19 boys and 4 girls, ages 2–13) without probenecid loading and compared these to six control subjects. Assays were performed by a fluorometric method. Shetty and Chase (1976) found no difference between patients and controls in baseline levels of either acid metabolite. Following 2–14 days of treatment with 0.5 mg/kg d-amphetamine in 10 patients, they found a 34 percent reduction of CSF HVA; a slight but inconsistent increase occurred in 5-HIAA. The degree of clinical improvement on medication correlated highly with the reduction of HVA. These changes in HVA with treatment may reflect feedback inhibition of dopaminergic activity and inhibitory serotonergic compensation.

Shaywitz, Cohen, and Bowers (1977) examined CSF levels of HVA and 5-HIAA in six children diagnosed as MBD (boys, ages 5–11; mean = 7.5 years) compared to 16 controls who had other neurological difficulties. After probenecid loading (100–150 mg orally), a lumbar puncture was performed. A significant positive correlation between probenecid and HVA levels was found. While the average level of CSF HVA in
MBD children was only slightly below that found in controls, the concentration of HVA (ng/ml) per unit of probenecid (μg/ml) was found to be significantly lower in children with MBD (mean = 9.8 ± 1.5) than in controls (16.5 ± 1.5). CSF 5-HIAA levels did not significantly differ in MBD children and controls.

**Urinary Metabolite Measures in ADD.** Since urinary samples contain the composite excretions of amines originating predominantly from peripheral rather than brain metabolism, the implications of urinary findings for CNS function are obscure. Although 3-methoxy-4-hydroxyphenylglycol (MHPG), is the primary metabolite of brain norepinephrine, it also reflects peripheral noradrenergic metabolism. Recent estimates suggest that about 60 percent of urinary MHPG is of central origin (Maas et al. 1979). Even though much of urinary MHPG is of peripheral origin, its relative amount may reflect the influence of central noradrenergic activity on peripheral function.

In the initial study of urinary amine metabolites, Wender et al. (1971) measured the 24-hour excretion in nine children (7 to 13 years old) diagnosed as having minimal brain dysfunction (MBD) on the basis of behavior difficulties at home and learning difficulties in school. Their amine excretion was compared to levels from normal age- and sex-matched controls. No differences were found between the groups in the 24-hour excretion of the serotonin metabolite, 5-HIAA, in the DA metabolite, HVA, or in the noradrenergic metabolites, MHPG, vanillylmandelic acid, metanephrine, and normetanephrine. Following a 1-week treatment with d-amphetamine, 10–20 mg/day, the MBD group demonstrated an increased excretion of epinephrine and metanephrine but no change in urinary MHPG and HVA levels.

Noradrenergic mechanisms have been investigated further in ADD by Shekim, Dekirmenjian, and Chapel (1979), who compared 15 hyperactive boys to 13 controls, ages 7–12, all with Wechsler Intelligence Scale for Children (WISC–R) IQ scores above 80. Diagnosis of ADD with hyperactivity was established according to DSM–III and the Children’s Diagnostic Scale of NIMH. Soft neurological signs were quantified using the Physical and Neurological Exam for Soft Signs (PANESS). Two 24-hour urine collections for MHPG were obtained before and 2 weeks after treatment with d-amphetamine .5 mg/kg/day. MHPG was analyzed utilizing electron-capture gas-liquid chromatography according to the methods of Maas et al. (1973).

The MBD group excreted lower pretreatment levels of MHPG than did controls (mean ± SD = 1.35 ± .49 micrograms per milligram of creatinine vs. 1.69 ± .33). Following amphetamine treatment, the mean MHPG levels decreased to .95 ± .44. Nine of the 15 MBD children demonstrated improved scores on the Connors Teachers Rating Scales (Factors I and IV) following d-amphetamine treatment, while the remaining six showed no significant change. Treatment with d-amphetamine decreased urinary MHPG production in the responders (1.40 to .78) while no significant change occurred in the MHPG of nonresponders. In three of the nonresponders, the MHPG actual-
an increase in peripheral 5-HIAA, suggesting an association between diminished serotonin and hyperactivity. Rapoport, Quinn, and Lamprecht (1974) found normal blood serotonin levels in hyperactive children. While both methylphenidate and imipramine improved behavior, imipramine alone lowered blood serotonin levels. Other studies in children who were both hyperactive and retarded showed that they had lower blood serotonin levels than controls. Serotonin appeared to increase as behavior improved following nondrug treatment regimens (Greenberg and Coleman 1976).

Recent methodological advances in the preparation of platelets and the assay of serotonin may clarify further the role of indoleamines in this disorder. Previous techniques of platelet centrifugation to obtain platelet-rich plasma suffered from variability in the type and percentage of platelets recovered. Improved ability to lyse platelets, and thereby free all serotonin, and the development of a simplified assay of plasma serotonin using HPLC provides renewed impetus for further examination of serotonin levels in ADD (Anderson and Young 1981; Young et al., in press).

Recent analytical techniques enable concurrent assays of several neurotransmitters within and across neurotransmitter systems. Simultaneous indices of synthesis, release, catabolism, and receptor response along the pathway of a functional neurotransmitter system can be obtained. Such a map or equation is best generated in response to a controlled pharmacological stress or stimulus which has considerable neurochemical specificity of action. By stimulating or inhibiting the system at a specific point and monitoring the response or impact at other moments, one can generate a map of neuronal sensitivity. Concurrent or sequential measures of crucial loci along a functional system allow individual determinations to be placed within the context of the tone or responsiveness of their neurotransmitter system. This strategy attempts to identify alterations in the modulation and sensitivity of a neurotransmitter system rather than a high or low concentration.

An index of presynaptic functioning can be obtained by determination of synthesizing enzymes (e.g., tyrosine hydroxylase, dopamine-β-hydroxylase), by dietary loading (tryptophan enhancement of serotonin), or by precursor administration (L-dopa stimulation of dopamine). Depletion of the precursor (e.g., reserpine release) or inhibition of catecholamine synthesis through destruction of selected neuronal systems (e.g., inhibition via 6-OHDA administration, electrolytic lesions, on enzyme blockade with FLA-63, or disulfiram) can alter presynaptic synthesis. Turnover of presynaptic neurotransmitters can be estimated by the accumulation of the acid metabolites following probenecid blockade from the CSF. Timed urinary collections of MHPG provide an estimate of CNS noradrenergic release (Maas et al. 1979). Postsynaptic receptor sensitivity may be estimated by measures of response to secondary messengers, such as amine-specific cyclic 3',5'-adenosine monophosphate (AMP) to receptor stimulation, or by determination of physiological response, such as platelet aggregation (Daly 1976). Pharmacological probes and neuroendocrine strategies may provide another window into CNS amine functioning.

**Pharmacologic Probes and Pharmacokinetics**

Approximately 200,000 to 500,000 children in the United States receive stimulant medication for attentional and behavioral control. The recent development of a sensitive assay for methylphenidate enables the quantification of blood levels and the determination of the pharmacokinetics of methylphenidate in children. Blood level determination may have therapeutic utility in identifying children who absorb methylphenidate poorly or excrete it rapidly and thereby fail to achieve adequate levels. Blood levels of methylphenidate, when coupled with sequential plasma measures of catecholamines and hormones, may provide a pharmacological probe of the responsiveness of these neurochemical systems. Repeated measures of methylphenidate absorption and neurotransmitter or peptide response during the course of drug treatment may identify shifts in drug absorption and metabolism which affect its bioavailability. An alteration of subsequent neurochemical response during the course of methylphenidate treatment may indicate a drug-induced change in amine synthesis, release, or receptor sensitivity.

In recent studies in our laboratory, 12 boys (age 6.5 to 15, mean ± SD = 11.3 ± 2.8 years) who met DSM-III criteria for ADD received oral methylphenidate, .3 mg/kg ± .05 mg/kg. Blood samples were
drawn every half hour for 4 hours, then less frequently until 8 hours after medication. The peak level of methylphenidate was reached at a mean time of 2½ hours (± 20 minutes) following oral administration. Considerable individual variance was evident since peak times for individuals ranged from 1 to 4 hours. The mean (±SD) of the peak blood levels achieved was 8.3 ± 3.0 ng/ml. A large variability of peak levels (5.4–14.8 ng/ml) existed across individuals receiving similar methylphenidate doses. There was a tendency for early peaks (those occurring at or before 1½ hours after administration) to be associated with high peak levels, suggesting more rapid absorption. The excretion half-life (t½) of methylphenidate was 2.5 ± .5 hours. Repeated blood level curves obtained following the same dose of methylphenidate in children treated for 6 weeks showed a high replicability when compared to blood levels obtained after their first dose.

Substantial blood levels of methylphenidate persisted at 4, 5, and 6 hours following administration; measurable levels were common at 8 hours. Thus, the blood level remained significant even after the optimal clinical effect had passed. This lack of a simple correlation between blood level and clinical effect probably reflects the mechanism of action of methylphenidate. It rapidly facilitates the release of stored catecholamines and has relatively less direct stimulating effect on postsynaptic receptors. Thus, high methylphenidate levels persisting after the initial release of catecholamines might be expected to have diminished effect. These results are compatible with a pharmacological study of methylphenidate (Hungund et al. 1978) on four children with ADD.

In a related study of methylphenidate blood levels during chronic treatment, single methylphenidate measures were obtained at 1 and 2 hours following the child’s regular outpatient dose. Children whose behavior ratings improved during treatment had significantly higher blood levels of methylphenidate than those who did not show behavioral improvement (S.E. Shaywitz et al., in press).

A somewhat different pharmacokinetic profile was noted in studies of d-amphetamine performed at the National Institute of Mental Health using a reliable, sensitive radioimmunoassay (Cheng et al. 1973; Ebert, van Kammen, and Murphy 1976). The pharmacokinetics of d-amphetamine was evaluated in 16 ADD boys following a single dose of about .5 mg/kg. Peak d-amphetamine concentrations (mean ± SD = 65.9 ± 4.6 ng/ml) occurred 4 hours after oral administration. The elimination half-life (t½) was about 6.8 ± 0.5 hours. Behavioral improvement, rated on the Conners abbreviated 10-item scale, was significant from 1 to 4 hours after medication. The decrease in restless motor activity usually occurred before the peak level of amphetamine was reached. The greatest variability in blood level occurred during the absorption phase. Since the maximal behavioral improvement occurred during the absorption phase, no simple correlation between absolute blood level and behavioral response emerged. Retest of six children with the same d-amphetamine dose showed no significant difference in absorption or elimination 1 week later (Brown et al. 1979a).

**Neuroendocrine Response to Stimulant Medications**

Neuroendocrine provocative tests provide a means of assessing the responsivity of the hypothalamic-pituitary axis. Hormonal responsivity to a pharmacological stimulus with well-defined neurotransmitter effects may provide an index of receptor sensitivity and may reflect the integrity of processes affecting peptide synthesis, storage, or release. This strategy has been used in a preliminary manner to characterize ADD.

**Neurotransmitter Regulation of Prolactin and Growth Hormone.** Increasing evidence illuminates the prominent role of neurotransmitters in the regulation of pituitary hormone release (Anton-Tay and Wurtman 1971; Sachar 1976; Martin 1976; Rose and Ganong 1976). Pharmacological agents with specific well-defined effects on neurotransmitter systems have been used to dissect these mechanisms (MacLeod and Lehmeyer 1972; Donoso, Bishop, and McCann 1973).

Prolactin and growth hormone are regulated by catecholamines and indoleamines. Prolactin secretion is inhibited by dopamine stimulation (McCann et al. 1972; Hyppa and Wurtman 1973; Sachar and Clemens 1974). Both synthesis and secretion of prolactin are inhibited by norepinephrine (MacLeod and Lehmeyer 1972), although large doses may have the opposite effect. Serotonin appears to stimulate prolactin secretion when given intraventricularly or systemically. Thyroid-releasing
factor (TRF) also stimulates release of prolactin (PRL). This effect is decreased by thyroxine and L-dopa and enhanced by estrogens. PRL may be under dual regulation, with prolactin inhibitory factor (PIF) being stimulated by dopamine and prolactin-releasing factor (PRF) being inhibited by serotonin.

Human growth hormone (HGH) is stimulated by dopamine and norepinephrine (Martin 1973; Eddy et al. 1974; Lovinger et al. 1975; Martin et al. 1975). α-Adrenergic stimulation, as with clonidine, increases HGH (Leckman et al. 1980). Phentolamine, an α-adrenergic antagonist, suppresses the L-dopa-stimulated rise in HGH (Kansel et al. 1972), while propranolol, a β-adrenergic blocker, enhances the rise in HGH which follows a variety of stressors (Frohman and Stachura 1975).

Dopaminergic and serotonergic drugs promote HGH release; drugs which block or inhibit these neurotransmitters usually decrease growth hormone (Sachar 1975).

Drugs which diminish synaptic availability of dopamine by depleting dopamine stores—for example, reserpine (Ratner, Talwalker, and Meites 1976)—or which block dopamine postsynaptic receptors—for example, haloperidol (Dickerman et al. 1974) or chlorpromazine (Kleinberg, Noel, and Frantz 1971)—elevate prolactin or diminish growth hormone (Clemens, Smalstig, and Sawyer 1974). Drugs which stimulate dopamine receptors—for example, apomorphine (Brown and Reichlins 1972; Martin et al. 1974; Lal et al. 1975)—or increase synthetically available dopamine—for example, L-dopa (Donoso, Banzan, and Barcagioni 1974)—decrease prolactin and stimulate growth hormone (Kansel et al. 1972; Lal et al. 1975).

**Hormonal Effects of Stimulant Medication.** The effect of stimulant medication on pituitary hormone response has been studied in animals and in psychiatric illnesses of man. Marantz et al. (1976) noted growth hormone stimulation following i.v. d- and l-amphetamine administration in monkeys. Brown and Williams (1976) observed a marked elevation of HGH in schizophrenic patients who were not receiving neuroleptics following i.v. administration of .5 mg/kg methylphenidate. Growth hormone response to stimulation with L-dopa has been found to be relatively attenuated in depressed patients (Sachar 1976). Langer et al. (1976) found that patients with endogenous depressions demonstrated a smaller HGH release following amphetamine administration than did patients with reactive depression or schizophrenia. Brown (1977) found HGH release in 17 normal males following methylphenidate administration (10 or 20 mg orally) correlated highly with their subjectively experienced euphoria. Janowsky, Parker, and Leichner (1976) observed a significant elevation of HGH in schizophrenic patients, many of whom were receiving neuroleptics, following i.v. administration of .5 mg/kg methylphenidate. Janowsky and Davis (1976) found a similar significant increase in plasma HGH following i.v. methylphenidate in a mixed group of psychiatric patients. Brown et al. (1978) gave oral methylphenidate (10 or 20 mg) and d-amphetamine (10 or 20 mg) to 50 normal male volunteers and found that their HGH was stimulated by both drugs. At the higher amphetamine dose, HGH levels correlated with amphetamine blood levels and with subjectively reported euphoria. Methylphenidate decreased the elevated prolactin levels in schizophrenic patients receiving neuroleptics. Cortisol was elevated following amphetamine alone and also correlated with subjective arousal. PRL is inhibited by dopamine and increased by dopamine-blocking agents. Amphetamine (5 mg/kg), but not methylphenidate (10 mg/kg), blocks the increase of serum prolactin in reserpine pretreated male rats (Clemens and Fuller 1979). Amphetamine lowered, while methylphenidate increased, brain 3,4-dihydroxyphenylacetic acid (DOPAC) in control rats.

**Neuroendocrine Studies in ADD.** Few neuroendocrine studies have been performed in children with ADD. Aarskog, Fevang, and Klove (1977) gave single doses of either L-dopa (sinemet), approximately 10 mg/kg, methylphenidate, 20 mg, or d-amphetamine, 15 mg, to 20 hyperactive children and measured HGH response at 30-minute intervals for 3 hours. They noted a similar pattern of HGH release and peak (at 60 to 90 minutes) in all treatment conditions. After 6- to 8-month treatment with methylphenidate, seven children were retested with L-dopa and d-amphetamine provocation 24 hours after their last methylphenidate dose. Their baseline HGH levels after chronic treatment with methylphenidate were elevated and response to d-amphetamine was diminished at 30 to 60 minutes.
Using methylphenidate as a pharmacological probe in ADD boys to examine the responsivity of the hormone and amine systems, we obtained sequential half-hourly measures of HGH, PRL, norepinephrine; epinephrine, and dopamine from plasma and correlated these with the concurrent levels of methylphenidate. HGH concentration increased and PRL decreased concurrent with the peak level of methylphenidate; plasma catecholamines tended to decline following administration of this stimulant (S.E. Shaywitz et al., in press).

Clonidine in ADD. Clonidine, an α-adrenergic agonist, given as a single oral dose with subsequent plasma measures of HGH, catecholamines, and their metabolites, may help define the responsivity of noradrenergic and dopaminergic systems in ADD. In low doses, clonidine acts preferentially on the presynaptic norepinephrine receptor to diminish endogenous release of norepinephrine (Stark and Montel 1973). The effect of clonidine on plasma norepinephrine and MHPG in ADD children compared to age-matched controls may be indicative of their noradrenergic tone or sensitivity (Leckman et al. 1980). Responsivity may be altered by treatment or withdrawal from stimulant medication. In our preliminary observations on boys before and during treatment with methylphenidate, the degree of stimulation of HGH release following administration of a controlled dose of clonidine may be an index of the sensitivity of this neuroendocrine axis. This could further clarify the mechanism of growth suppression in children chronically treated with high-dose stimulant medication (Cohen et al. 1979; Gil-Ad, Topper, and Laron 1979). The responsivity of the neuroendocrine system to acute clonidine, however, may not parallel the response of central brain noradrenergic receptors.

Clonidine is also a potential therapeutic agent for some children with ADDH. The need for therapeutic alternatives to stimulant medication is evident from the side effects of stimulants (irritability, growth impairment), contraindications (e.g., tics), and from the limited benefit often seen from treatment with medication alone (lack of improvement in academic achievement). A double-blind, crossover study of clonidine and placebo in 37 children with ADD suggested clinical improvement in ratings of hyperactivity, attention, learning, and impulsive behavior (Lechin et al., in press). We have completed an open therapeutic pilot study of four children (9-14 years) with ADDH given clonidine 3-4 μg/kg/day for 2-5 months. Parent and teacher ratings suggested improvement in the Hyperactivity Index from the Conners Scale. Parents noted that their children were calmer, more compliant, and had increased frustration tolerance. Distractibility often continued, but subjects were more able to return to and complete a task (R.D. Hunt, D.J. Cohen, and S.E. Shaywitz, personal communication, 1982).

Summary and Speculations

Attention deficit disorders (ADD) describe several clusters of children who share symptoms of short attention, impulsivity, and motoric hyperactivity. The role of attention and cognition as a core influence in personality development makes the study of ADD germane to understanding many psychiatric syndromes. Human and animal studies suggest the importance of catecholamines, indoleamines, acetylcholines, and hormones in the interlocking processes which constitute attention, activity, arousal, and memory. Since attentional disorders may follow disruption of perceptual or integrative functions, there may not be a unique neurochemistry underlying the complex syndrome of ADD. The pattern of neurochemical disturbance may reflect the severity as well as the source of disturbance within a predominant clinical subtype. Among children whose symptoms fall under the broad umbrella of ADD, some have primary disturbance in activity level; others may be primarily explosive, impulsive, or episodically aggressive; still others are primarily inattentive and distractible but demonstrate minimal disturbance in activity, aggression, or mood; and another subgroup exhibits predominant fluctuation in mood. The phenotypic expression of the varied genotypes or substrates may be difficult to dissect in the classroom or to evaluate in the playroom but may reflect varying neurochemical equilibriums within the brain.

Static or state measures of these and other neurotransmitters may not reflect their functional significance. The use of discrete pharmacological probes with well-established neurochemical mechanisms of actions, correlated with acute behavioral, attentional, and chemical response, may define further the relationship between neurochemical substrates and attentional processes.
References


Starke, K., and Montel, H. Involvement of alpha-receptors in
clonidine-induced inhibition of transmitter release from central monoamine neurons.


Acknowledgments

We acknowledge the editorial assistance of Margrethe Cone and Laura Zeff. Dr. J. Gerald Young collaborated in many of the studies and we acknowledge his contributions, as well as those of Dr. George M. Anderson.

The Authors

Robert D. Hunt, M.D., is Assistant Professor of Child Psychiatry; Donald J. Cohen, M.D., is Professor of Pediatrics, Psychiatry, and Psychology; Sally E. Shaywitz, M.D., is Associate Professor of Pediatrics; and Bennett A. Shaywitz, M.D., is Associate Professor of Pediatrics and Neurology, Yale University School of Medicine, New Haven, CT.