Strategies for Detecting Subclinical Monoamine Depletions in Humans

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INTRODUCTION

Certain lesions of the central nervous system (CNS) have obvious manifestations that are readily detected by an experienced clinician. For example, a right-handed individual who develops sudden right-sided weakness and language problems is likely to have pathology involving the left cerebral hemisphere. A careful neurological examination might yield more specific information regarding the anatomical distribution of the lesion, which often can be confirmed radiologically using imaging techniques such as computerized tomography or magnetic resonance imaging (MRI). Unfortunately, other lesions of the CNS may remain undetected for long periods, both because they do not have obvious clinical manifestations under ordinary conditions and because behavioral functions served by the involved brain areas may be subtle and difficult to test and measure.

Parkinson's disease, a neurodegenerative disease involving brain dopamine neurons, provides an example of a naturally occurring CNS lesion that can exist in a preclinical or asymptomatic form and that can go undetected for years (Koller 1992). Patients with late-stage Parkinson's disease are readily diagnosed by a well-defined symptom complex that includes slow movement, rigidity, resting tremor, and postural instability. Often, there is also a loss of facial expression and some degree of cognitive impairment. However, none of these impairments becomes apparent until 60 to 70 percent of dopamine neurons have degenerated and brain dopamine concentrations are depleted by 80 to 90 percent (Hornykiewicz 1975; Forno and Alford 1987; Koller 1992). The absence of symptoms in the face of such large dopamine depletions underscores the problem of preclinical diagnosis. This problem is magnified severalfold when brain structures with more subtle functions (e.g., serotonin or mesocortical dopamine systems) are involved.

Several recreationally used amphetamine analogs, including methamphetamine (MA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyethylamphetamine (MDEA), have the potential to

damage CNS dopamine and serotonin neurotoxins in a variety of animal species (Seiden and Ricaurte 1987). Whereas the toxic effects of MA involve dopamine and serotonergic neurons, the toxic effects of MDMA appear to be specific for serotonergic neurons (Stone et al. 1986; Battaglia et al. 1987; Ricaurte et al. 1988*a*). Moreover, it appears that nonhuman primates are at an increased risk for developing MDMA neurotoxicity (Ricaurte et al. 1988*b*; Ricaurte and McCann 1992), and there is increasing concern that humans who use amphetamines recreationally may also incur damage to brain dopamine and serotonin neurons. Because the recreational use of MDMA and related drugs may be on the rise (Noticeboard 1992), it is important to determine their neurotoxic potential in humans and develop reliable methods for detecting preclinical monoamine neurotoxicity, if it exists. This chapter reviews the strategies available for detecting partial depletions of CNS dopamine or serotonin in humans. Strengths and weaknesses of each strategy are discussed, and potential tools for enhancing current methodologies are proposed.

CEREBROSPINAL FLUID ANALYSIS

Cerebrospinal fluid (CSF) bathes the brain and spinal cord. Because of the CSF's close proximity to brain structures and its isolation from other major organs, changes in the neurochemistry of CSF are thought to reflect changes specific to the CNS (Wood 1980). Furthermore, because the total volume of CSF (100 to 150 mL) undergoes complete replacement approximately six times per day (approximately 600 to 700 mL per day), CSF measures can be used to detect relatively short-term dynamic changes in the CNS milieu. Although plasma and urine measurements also have been utilized as indices of CNS monoamine neurotransmitter function, these are generally considered to be less specific because multiple organ systems (including the adrenal medulla) contribute considerably to their components (Kagedal and Goldstein 1988).

CSF measures not only are used routinely for the determination of neuropathological processes in clinical populations but also have been used as a strategy for detecting suspected neurotransmitter alterations in neuropsychiatric diseases. More specifically, CSF collected by lumbar puncture has been used to measure concentrations of relevant neurotransmitter metabolites (Moir et al. 1970). With regard to amphetamine neurotoxicity, measurements of lumbar CSF homovanillic acid can be used as an indirect measure of brain dopamine activity (Kagedal and Goldstein 1988), whereas 5-hydroxyindoleacetic acid (5-HIAA) can be used to detect changes in brain serotonin (Ricaurte et al. 1988*a*; Banki and Molnar 1981). Decreases in either

of these metabolites in individuals who had been exposed to amphetamines provides support for the occurrence of amphetamine neurotoxicity in humans.

The advantages of using CSF measures to detect subclinical neurotoxicity include easy accessibility of CSF, reproducibility of the assay (Menachem et al. 1989), and that it is a dynamic measure. Drawbacks of this strategy include that CSF composition provides an indirect reflection of brain neurochemical activity, that it is nonspecific (numerous factors are known to influence the composition of CSF) (Moir et al. 1970; Post and Goodwin 1977; Wood 1980), that it is not particularly sensitive (i.e., concentration changes in the cervical spine underestimate changes in the forebrain) (Sourkes 1973; Sjostrom et al. 1975; Ricaurte et al. 1988*a*), and that the lumbar puncture procedure can be unpleasant and can have complications (e.g., "lumbar puncture headache").

NEUROENDOCRINE STUDIES

A second strategy for detecting subclinical damage to dopamine and serotonin systems takes advantage of the important roles of these systems in the hypothalamic-pituitary-adrenal (HPA) axis. Both dopamine and serotonin are involved in the normal regulation of prolactin and growth hormone secretion (for reviews, see Kato et al. 1985; Arimura and Culler 1985), and damage to either monoaminergic system can sometimes be detected using neuroendocrine measures. For example, dopamine is known to tonically inhibit prolactin release at the level of the pituitary (Weiner and Bethea 1981). When dopamine levels are severely depleted, or postsynaptic dopamine stimulation is decreased, abnormally high baseline prolactin levels result. In instances where depletion of dopamine is not extensive, it may be necessary to pharmacologically challenge the dopamine system before endocrine disturbances are noted. For example, in a normal individual, an oral dose of L-dihydroxyphenylalanine (L-dopa), the precursor to dopamine, leads to a rise in growth hormone levels. In an individual with damaged dopamine neurons, there might be a blunted growth hormone response to L-dopa because fewer dopamine neurons would lead to reduced conversion of L-dopa to dopamine. By contrast, the same individual given a postsynaptic dopamine receptor agonist (e.g., bromocriptine) might be expected to have an abnormally robust rise in growth hormone, because of postsynaptic dopamine receptor supersensitivity.

In the case of serotonin, similar neuroendocrine strategies have been utilized to measure neurotransmitter dysfunction. Because serotonin plays a role in regulating normal secretion of prolactin, growth hormone, and cortisol, damage to serotonin pathways in the HPA axis can be detected through disturbances in the homeostatic mechanisms of these hormones (Heninger et al. 1984). For

example, both direct and indirect serotonin agonists (e.g., m-chlorophenylpiperazine and L-tryptophan) cause acute elevations in serum prolactin concentrations (Meites and Sontag 1981; Charig et al. 1986), possibly via a prolactin-releasing agent at the level of the hypothalamus (Garthwaite and Hagen 1979). In an individual with severe serotonin damage in the hypothalamus, the rise in prolactin following administration of L-tryptophan might be expected to be diminished (assuming that postsynaptic supersensitivity is not sufficient to mask presynaptic dysfunction). If the damage is partial, or if a postsynaptic 5-hydroxytryptamine agonist is administered, a supraphysiologic response might be anticipated. Because of the possibility of compensatory mechanisms over time (e.g., postsynaptic receptor supersensitivity or decreased inhibitory feedback), timing of the neuroendocrine challenge relative to the time of the suspected neurotoxic insult may be crucial for proper interpretation of the neuroendocrine challenge.

The previous examples of neuroendocrine methods for detecting preclinical monoaminergic damage are not exhaustive and are intended to illustrate the general neuroendocrine strategy. The advantages to neuroendocrine studies include their sensitivity, low cost, relatively noninvasive nature, and the ease with which procedures can be performed. However, like CSF measures, neuroendocrine measures are indirect and nonspecific. Furthermore, because of the possibility of compensatory mechanisms, results of the neuroendocrine challenge may not be consistent over time. Therefore, although potentially lending support for the diagnosis of neurotoxicity, neuroendocrine disturbances are not definitive.

NEUROIMAGING TECHNIQUES

Recent advances in imaging technology and the development of neurotransmitter-specific ligands hold promise for the detection of subclinical neurotoxic injury. As further advances are made, imaging techniques that can be used to this end will include single photon emission computerized tomography, positron emission tomography (PET), and possibly MRI (at least in cases where the extent of neuronal volume loss is sufficient). However, to date only PET has been used successfully in humans for the detection of neurotoxic damage, and only in the dopamine system (Calne et al. 1985). The reasons for this have been largely caused by the limited number of suitable ligands available rather than because of limitations of imaging techniques. However, in the case of serotonin, which is present in the brain at much lower concentrations than dopamine, sensitivity of imaging techniques also may prove to be problematic in detecting subtle neurotoxic lesions.

Because it is known that neurotoxic amphetamines damage the presynaptic element, the ideal ligand for detecting amphetamine neurotoxicity would be a presynaptic marker. Postsynaptic receptor markers, although possibly demonstrating receptor down- or up-regulation, would not provide compelling evidence of neurotoxicity. Of the various possible presynaptic markers, ligands for the uptake site may prove to be superior for the detection of neurotoxic injury because they label a "structural" macromolecular element of the nerve terminal (as opposed to neurotransmitter precursors such as 6-fluorodopa that can be altered by metabolic events). In the case of the dopamine system, several such ligands exist and include GBR-12909, nomifensine, and WIN-35428. Neurotoxic damage in humans caused by the potent neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahyropyridine has been detected using 6-fluorodopa, and this ligand also should be useful in detecting dopaminergic damage caused by amphetamines such as methamphetamine, which damages dopamine nerve terminals.

Development of presynaptic markers for serotonin neurons has been more difficult, although there are several promising candidates in the development phase. In particular, a variety of ligands that label the serotonin transporter complex (e.g., paroxetine derivatives) may prove to be ideal for studies in individuals exposed to potential serotonin neurotoxins such as MDMA or MDEA.

Advantages to PET and other neuroimaging techniques are that they provide evidence of neuronal alteration, they can be anatomically and pharmacologically specific, and they are relatively noninvasive (intravenous catheters are required). Disadvantages to neuroimaging studies are their potential lack of sensitivity, their expense, the large number of personnel required for completion of the study, and the inability to perform studies without special equipment and personnel.

PHARMACOLOGICAL CHALLENGE

A specific pharmacological challenge to a compromised neurotransmitter system has not been widely used in the detection of subclinical monoaminergic neurotoxicity but has potential utility. This method would take into account that the neurotransmitter system in question, although not eradicated, has limited reserves. In essence, under normal conditions, a compromised neurotransmitter system might be able to function, but if it is stressed, abnormalities might become apparent. An analogy can be drawn with the use of the cardiac stress test in instances where coronary artery insufficiency is suspected but unconfirmed. In an individual with "silent" coronary artery disease, cardiac abnormalities are inapparent under ordinary physical

conditions. Abnormalities such as irregular heart rhythms or angina pectoris may become manifest when the individual is subjected to physical stress such as the treadmill test (Astrand 1976; Kilpatrick 1986).

A pharmacologic neuronal stress test might be useful in the evaluation of patients with suspected subclinical neurotoxicity. For example, in an individual with a history of significant methamphetamine use, diminished dopaminergic neurotransmission might be expected but, under ordinary conditions, might be clinically inapparent because it was not sufficiently severe. If that individual were given a drug that specifically interfered with dopaminergic neurotransmission, it might be anticipated that the subject would demonstrate exquisite sensitivity to its effects. Drugs that could be used to challenge the dopaminergic system include α-methyl-para-tyrosine, a synthesis inhibitor, or haloperidol, a postsynaptic dopamine receptor blocker. Endpoints that could be used to measure drug effects include parkinsonian symptoms and elevations in serum prolactin levels, as mentioned above.

Specific challenges of the serotonin system might be accomplished using the serotonin synthesis inhibitor parachlorophenylalanine. Unlike challenges to the dopamine system, challenges to the serotonin system have no obvious and easily measured functional endpoint. However, because serotonin has been implicated in the regulation of sleep, mood, anxiety, impulsivity, and pain sensitivity, disturbances in these behavioral spheres might be expected.

SUMMARY

Given the reported increase in the recreational use of controlled substance analogs such as MDMA and related drugs, it is important to determine whether these drugs produce neurotoxic effects in humans. Several strategies available for detecting preclinical neurotoxicity to dopamine and serotonin neurons have been discussed, and their strengths and limitations have been listed. In addition, some promising strategies that are still in the development stage (e.g., PET) have been mentioned. None of the available methods for detecting neurotoxicity is conclusive; therefore, converging lines of evidence will be essential to provide convincing indication of subclinical neurotoxicity in humans. Such studies will help define the public health risk of recreationally used drugs. Furthermore, documentation and determination of drug-induced neurotoxic changes may shed light on the pathophysiology of idiopathic neurodegenerative diseases involving monoaminergic neurons in humans and could be useful in the development of new treatment strategies. Finally, detailed neuropsychiatric evaluation of individuals with confirmed subclinical serotonergic neurotoxicity may enhance knowledge regarding the functional role of brain serotonin neurons in health and disease.

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DISCUSSION

Q: George, do you think that we will ever be able to get a clear answer by comparing people who use MDMA with control groups? Are we going to need to use people as their own controls?

A: If we compare results of an MDMA cohort with those of a control group, the comparison is compromised for a number of reasons, including the fact that invariably people have used other drugs as well. Also, there may be differences in educational background as well as a host of other differences that make intergroup comparisons difficult. One strategy that might be used to get around such problems would be an epidemiologic one, where numbers are such that you can begin to factor out some of the variables; but still the evidence is going to be indirect. Between-group comparisons are flawed, but I am afraid for now it is really the only viable option given the fact that to try to do before-and-after comparisons using subjects as their own controls is difficult unless (1) you have the authorization to do such a study and (2) you are willing to do such a study because (as you know) there are risks associated with testing the effects of MDMA on humans.

Q: How far do you think you have to go without doing that?

A: If you can develop a consistent line of argument with numerous lines of evidence where various results all point to the same conclusion, I think you can go reasonably far. I doubt that we will ever reach the point where we can definitely state that there is such a percent depletion of serotonin in a given individual's brain. However, using the strategies I have discussed in combination, looking for converging lines of evidence, I think you can probably develop a reasonable case for the presence or absence of neurotoxicity in a human cohort. In that regard, the human situation is not terribly different from the preclinical situation, where converging lines of evidence also are necessary to reach the conclusion that you are in fact dealing with neurotoxicity. None of the measures is going to make a compelling case by itself. But if different measures are considered together, a picture begins to emerge.

Q (Miller): Has anyone tried to look at the same issues with the fenfluramine populations in Europe?

A: Not that I know of. There is one disturbing result in the literature. Fenfluramine is often used instead of L-tryptophan as a challenge agent to study serotonin systems in humans. The disturbing observation is that when Coccaro and colleagues[1] used fenfluramine as the challenge drug, they found a shift in the baseline of their "control group," so that when a control group was rechallenged with fenfluramine 1 week later, the prolactin response appeared blunted, suggesting a change in serotonin system as a function of fenfluramine exposure. Aside from that one study, fenfluramine-exposed individuals have

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[1] Coccaro, E.F.; Siever, L.J.; Klahr, H., et al. Diminished prolactin responses to repeated fenfluramine challenge in man. *Psychiatry Res* 22(3):257-259, 1987.

not been studied either with CSF or neuroendocrine challenge strategies, to my knowledge.

COMMENT (Seiden): There was one study done in Europe that was published in Lancet, I think last year—a fairly large study of people who were getting fenfluramine for a whole year. They were testing various neurological and psychological outcomes in these patients who had taken fenfluramine for weight reduction. There was nothing marked in that study. But the only way a factor would come up in that study was if the patient reported to the physician that he was experiencing x, y, or z effect. Patients weren't given questionnaires. So it is a little biased toward nonreporting.

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