EDITORIAL

Functional Studies in Substance Abuse: Imaging and Beyond

Substance abuse continues to be one of the compelling public health problems of our times. The medical and social consequences provide a powerful incentive for attempting to understand the neurochemical basis and sequelae of substance abuse, particularly cocaine abuse. The last decade has seen an explosion of basic neuroscience research in this regard. Radiopharmaceutical tools have proven indispensable in advancing our basic knowledge of the mechanisms of cocaine's effects and consequences, and emission computed tomographic imaging has afforded great strides forward in this effort. Such imaging has provided information about specific cocaine receptor sites in the brain, effects of chronic cocaine abuse, as well as effects of potential treatment strategies. Additionally, developments in this field have important clinical applications beyond substance abuse.

Cocaine and Dopamine

Since cocaine is a highly addictive drug, determining the neurochemical basis for its addictive potential is fundamental to understanding and potentially treating cocaine dependence. Several years ago, it was proposed that the neurotransmitter dopamine plays a critical role. Cocaine blocks the dopamine transporter, a presynaptic binding site on dopaminergic neurons responsible for dopamine reuptake, which may mediate the reinforcing properties of cocaine (*1–3*). Depletion of brain dopamine in chronic cocaine abuse may be equally important in producing drug craving and dependence (*4*). PET studies in humans have shown reduced striatal postsynaptic dopamine receptor binding (*5*) and reduced striatal presynaptic dopamine precursor uptake (*6*) in chronic cocaine abusers. In addition, there is PET evidence of preferential striatal uptake of cocaine, associated with the dopamine reuptake site in normal humans and baboons (*7*).

Cocaine Abuse and Imaging

Clinical investigation regarding cocaine abuse has focused on well known medical and neurological sequelae. Anatomic imaging has been useful mainly in instances of acute neurological events such as cerebral infarction or hemorrhage (*8*). Functional imaging, on the other hand, has provided new insights into the chronic and acute effects of cocaine on brain systems. Volkow and colleagues were the first to demonstrate alterations in cerebral perfusion (*9*), glucose metabolism (*10*) and dopamine receptor function (*5*) in chronic cocaine abusing men. Since then, small focal abnormalities in cerebral perfusion have been demonstrated using SPECT in cocaine-dependent men (*11,12*), but not in women (*13*), who improve with buprenorphine drug addiction treatment (*14,15*). In addition, both PET (*16*) and SPECT (*17*) studies have shown global hypometabolism and regional hypoperfusion immediately following acute administration of cocaine. In general, perfusion and metabolic abnormalities are seen in dopaminergic projection areas (*9, 10, 12, 13*).

Cocaine Receptors and Imaging

Other investigations have focused on the neuroanatomical localization and pharmacological specificity of cocaine binding in the brain and how this relates to addiction. In this issue of the *Journal*, Volkow et al. (*18*) address an interesting issue with regard to the nature of central targets that mediate cocaine's behavioral effects: namely, whether cocaine recognition sites, which have been shown to exist as both high and low affinity binding sites* in vitro (*2,19–22*), can be differentiated pharmacologically in vivo. Defining the nature of these sites may be an important step in understanding cocaine's behavioral effects because: (1) the low affinity site approaches full occupancy only at behaviorally relevant cocaine concentrations and (2) its density determined in vitro is typically several times larger than that of the high affinity recognition site, and at full occupancy its effects should predominate.

Volkow et al. used PET to examine cocaine recognition sites in the baboon brain. A fixed concentration of ^{11}C-cocaine and two different concentrations of unlabeled cocaine (a subpharmacological and a pharmacologically relevant dose) were used to determine the distribution and pharmacological specificity of ^{11}C-cocaine binding. The basic assumption with this methodology is that subpharmacological doses are expected to highlight high affinity sites, while pharmacological dose studies are expected to highlight predominantly low affinity sites. At subpharmacological doses, Volkow et al. provided evidence for selective accumulation of ^{11}C-cocaine in striatum but not clearly in other brain regions. This selective accumulation was inhibited by cocaine and selective dopamine uptake inhibitors. In studies conducted at the pharmacological cocaine dose, however, selective ^{11}C-cocaine accumulation in brain was not demonstrated and a more homogeneous pattern of uptake was seen. Additionally, unlike subpharmacological dose studies, pretreatment with dopamine reuptake inhibitors, including cocaine, had no measurable effect on striatal accumulation of labeled cocaine. This suggests that radiotracer accumulation at the pharma-

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*Data from in vitro studies suggest that striatal cocaine recognition sites exist as either a single protein entity with two affinity states or as two protein entities with differing affinities. For clarity, we refer to these sites/states as sites.

cological dose was unrelated to cocaine recognition sites.

These results are consistent with two main possibilities: either low affinity sites do not exist in vivo, or low affinity sites exist but were simply not detected under these experimental conditions. Volkow et al. favor the latter explanation, and attribute their inability to characterize the low affinity site in part to the poor sensitivity of PET. While this may have played a role, the resultant low specific activity used in pharmacological dose studies may also have been a significant limitation. Recent findings by Madras and Kaufman, who conducted ex vivo autoradiographic studies with high specific activity [^{3}H]cocaine, provide evidence for selective accumulation of behaviorally relevant cocaine doses in striatum, hippocampus, amygdala, locus coeruleus, and other regions in nonhuman primates (*23*). Use of a higher specific activity of ^{11}C-cocaine in PET studies may provide more favorable results. Dosimetric concerns, however, may preclude higher specific activity studies in vivo.

Other assumptions merit consideration as well. Are assumptions regarding specific binding using the cerebellum as a standard valid, especially at high cocaine doses? Can in vitro binding data be extrapolated to in vivo conditions? In this regard, in vitro binding studies conducted at physiologic temperature suggest that differences may exist between in vitro and in vivo affinities of cocaine congeners at striatal cocaine recognition sites (*24*). Perhaps just as important, does excess synaptic dopamine or other monoamines, induced by high synaptic concentrations of cocaine in pharmacological dose studies, compete with ^{11}C-cocaine, thereby reducing radiotracer binding at cocaine recognition sites, as the authors suggest? Such interactions have been observed in PET measurements of dopamine receptor occupancy following amphetamine or methylphenidate administration (*25,26*), and may significantly limit studies employing low affinity compounds such as cocaine. Resolution of the distribution and pharmacological specificity of striatal cocaine recognition sites in vivo may require the use of radiolabeled compounds of higher affinity for cocaine recognition sites than cocaine itself.

Cocaine Congeners

Much effort has been devoted towards the development of high affinity cocaine congeners for radiotracer studies of cocaine recognition sites. One fluorinated congener with exceptionally low levels of nonspecific binding and high potency at striatal cocaine recognition sites is WIN 35,428 or CFT (*21*). It was shown to be selective for dopamine-rich brain regions (*27–29*) and for the dopamine transporter (*30*), and its in vivo distribution closely parallels that of cocaine (*23*). PET imaging studies have demonstrated its usefulness in vivo (*31,32*). Additionally, an iodinated congener useful as a SPECT or PET probe, RTI-55 (or β-CIT), has been shown to be more potent than WIN 35,428 at the dopamine transporter, but essentially equipotent at the dopamine and serotonin transporters (*33,34*). It is important to note that these high affinity cocaine congeners bind, like cocaine, to high and low affinity sites, and as such, may be particularly useful in characterizing both types of sites. One recently developed compound, difluoropine, maintains an over 320-fold selectivity for the dopamine versus the serotonin transporter (*35*). Such developments may facilitate pharmacodynamic characterization in vivo.

Beyond Cocaine

These issues are of concern to the field of substance abuse research. Radiolabeled forms of cocaine and related congeners have been applied as diagnostic probes to monitor neurological processes associated with monoamine nerve terminal changes. Carbon-11-labeled cocaine was recently utilized to detect an age-related decline in striatal accumulation, a pattern which may reflect reduced dopamine nerve terminal density (*36*). More selective and higher affinity cocaine congeners may be superior to cocaine for examining age-dependent or disease-dependent processes associated with dopamine nerve terminal loss because their accumulation patterns primarily reflect dopamine nerve terminal distribution and are less susceptible to perturbation by endogenous substances such as dopamine. Tritium-WIN 35,428 has been shown in vitro to detect the full extent of dopamine terminal loss postmortem in Parkinson's disease striatum (*37*). Subsequent in vivo studies using [^{11}C]WIN 35,428 (*38*) as well as [^{123}I]RTI-55 (*39*) have demonstrated dopamine nerve terminal loss in the nonhuman primate model of Parkinson's disease. Recent studies with both PET and SPECT have confirmed similar findings in humans with Parkinson's disease (*40,41*). Cocaine congeners may also have utility in PET or SPECT evaluation of treatment efficacy in Parkinson's disease, including the efficacy of fetal tissue transplantation procedures (*42*). Additionally, they may prove useful in the detection or diagnosis of other neurological (*43*) or psychobiological disorders (*44,45*).

CONCLUSION

Cocaine use and abuse is associated with immediate and long-term functional brain changes and abnormalities that can be directly observed with a variety of imaging modalities not available just a decade ago. PET and SPECT imaging facilitate in vivo investigation of the mechanisms underlying functional and structural changes associated with cocaine use, including pharmacological studies of receptor function. The study by Volkow and colleagues clearly illustrates some of the difficulties and limitations of the current methods and technology, particularly for examining low affinity recognition sites, and we should not be disappointed with these results. Use of protocols with higher radiotracer specific activities or higher affinity cocaine congeners may enhance our ability to perform difficult pharmacological characterization studies in vivo. The knowledge acquired in these pursuits can be expected to be valuable in the development of clinical tools for diagnosis and for monitoring

therapeutic intervention in addictive and other neurologic disorders.

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