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Perspective

How Might Cocaine Interfere with Brain Development?

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Women addicted to cocaine often continue drug use through pregnancy, despite risks to the fetuses they are carrying. Multiple studies have attempted to identify the effects of cocaine and other commonly abused drugs on fetal brain development and behavior in clinical populations. The attribution of risk to specific drugs remains challenging, however, because women addicted to cocaine often use other illegal drugs as well as alcohol and tobacco. Moreover, they tend to have poor nutrition, low levels of prenatal care, and other problems that confound analysis.

Not surprisingly, as the outcomes of different cohorts of pregnant women who use drugs are examined, diverse findings have been reported. Overall, however, children exposed to cocaine prenatally appear to have neurological and cognitive deficits. For example, at six months of age children exposed to cocaine in utero have been observed to have abnormalities of tone and posture [1]. A longitudinal, prospective, comparison birth cohort study that measured drug levels in urine and meconium found that children exposed to cocaine showed significant cognitive deficits and a higher rate of developmental delay during the first two years of life [2]. Another cohort of children exposed to cocaine and other drugs had deficits in sustained attention and problems with impulsivity at age four [3].

Animal and Cellular Models of Prenatal Cocaine Exposure

Animal and cell culture models have been widely used to investigate effects of cocaine and the mechanisms by which it might act during brain development. In this issue of *PLoS Medicine*, Chun-Ting Lee and colleagues use both cell culture and fetal rat models to investigate mechanisms by which cocaine might decrease the number of neurons in the brain [4].

Animal models have the advantage of permitting the study of individual drugs such as cocaine with far fewer confounding variables than those that bedevil human studies. Animal models also allow for well-controlled behavioral studies in the laboratory [5] and invasive anatomic, physiological, and biochemical studies [6,7]. Studies of cocaine effects in tissue culture facilitate well-controlled investigations of molecular mechanisms of drug action, especially mechanisms that are cell-autonomous. The brain is characterized by complex interactions of neural circuits and multiple neurotransmitters. In the mature brain, cocaine inhibits the uptake of dopamine, norepinephrine, and serotonin by their respective transporters, thus increasing synaptic levels of these neurotransmitters. Despite the limitations of cell culture—it is difficult to recreate the cellular and chemical environment of the brain—Lee and colleagues used cell culture to examine neurotransmitter-independent actions of cocaine on a neuronal precursor cell line, AF5, and on human primary neural progenitors [4].

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Linked Research Article

This Perspective discusses the following new study published in *PLoS Medicine*:

Lee C-T, Chen J, Hayashi T, Tsai S-Y, Sanchez JF, et al. (2008) A mechanism responsible for the inhibition of neural progenitor cell proliferation by cocaine. PLoS Med 5(6): e117. doi:10.1371/journal.pmed.0050117

Investigating the mechanism of cocaine's effect on fetal brain development, Chun-Ting Lee and colleagues find that down-regulation of cyclin A by a cocaine metabolite inhibits neural proliferation.

Mechanism by Which Cocaine Inhibits Cell Proliferation

Primate studies have shown that intrauterine cocaine exposure (during a period corresponding to the second trimester in humans) results in a decrease in the number of neurons in the cerebral cortex and disorganization of the normal laminar structure of the cortex [6,7]. Based on such findings, Lee and colleagues investigated mechanisms by which cocaine might inhibit the proliferation of neural progenitor cells. Of course, decreased cell number might also result from cell death, but based on evidence that cocaine can inhibit proliferation without killing neurons [8], Lee and colleagues focused on nonlethal actions of cocaine on neurons [4]. It should be acknowledged, however, that in noncortical regions, such as the locus coeruleus of the brainstem, cocaine has been reported to produce apoptosis [9].

In an impressive series of experiments, the researchers showed that cocaine inhibits cell proliferation without affecting cell survival in AF5 cells, and does so in the absence of dopamine, norepinephrine, or serotonin. This antiproliferative effect occurs by blocking the cell cycle, specifically the G1/S phase transition beyond which cells are committed to division. Using microarray technology,

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the researchers found that of the 93 cell cycle genes they examined, only *cyclin A2* was significantly down-regulated by cocaine. They then showed that cocaine also suppressed cyclin A2 in primary human fetal cells (largely neuronal by their markers), derived from the cerebral cortices of second-trimester human fetuses, and also in fetal rat brains during early (embryonic day 13–15) or middle (embryonic day 15–17) periods of neurogenesis.

Cocaine appears to cause down-regulation of cyclin A2 by generating reactive oxygen species, which in turn causes an endoplasmic reticulum stress response. The generation of reactive oxygen species depends on *N*-oxidation of cocaine by cytochrome P450. This led the investigators to test cytochrome P450 inhibitors, including the widely available drug cimetidine (originally developed as a histamine H2 receptor antagonist to treat peptic ulcers), in both AF5 cells and in cocaine-exposed fetal rats. In cell culture, the P450 inhibitors SKF-525A and cimetidine blocked cocaine-induced formation of reactive oxygen species and significantly diminished the inhibitory effects of cocaine on cell proliferation. When pregnant rats were pretreated with cimetidine prior to each cocaine administration, the effects of cocaine on cyclin A2 down-regulation and on the inhibition of cell proliferation were significantly blocked.

Clinical Implications

Both animal and cell culture studies trade control of variables and the opportunity for invasive analysis for questions about "real world" relevance in humans. Two critical questions in examining the developmental effects of an environmental toxin such as cocaine, whether in humans or in animal models, are the dose and route of administration and the timing of the exposure. Even brief exposure at a particularly vulnerable time in brain development may have lasting deleterious effects of greater magnitude than greater exposures at other times [10]. Moreover, the postnatal age at which the effects of cocaine are measured, whether in humans or animals, may show evolving outcomes. In humans, the attribution of outcomes to drug effect is complicated by the observation that the circumstances under which children have been raised subsequent to cocaine exposure affect their behavior [11].

An important strength of this study was the use of cocaine concentrations in both animal and cell culture experiments that are likely to correlate with those found in human fetal brains. Its most important strength was that it examined a possible mechanism by which cocaine might suppress the proliferation of neurons and used this mechanistic analysis to identify a drug that might block this deleterious effect. It will be a challenging matter of further research to determine whether the inhibition of proliferation is relevant to the human situation and whether this research provides ideas about preventive interventions for those pregnant women who cannot abstain from cocaine. This early research is exciting, but the reader must keep in mind the complexity of factors that might contribute to cognitive and emotional abnormalities in children exposed to cocaine and other dangerous drugs in utero. ■

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