The rampant use of cocaine as a recreational drug in the 1980s prompted the anticocaine commercial “The thrill can kill.” The mood-enhancing effect of cocaine in the user is swift and fleeting. In contrast, the unfortunate children of crack cocaine mothers often inherit developmental and psychological disorders, such as low birth weight and learning disabilities (Bauer et al., 2005). They also show a higher incidence of heart failure and ischemic injury in adulthood. Although its exact cause remains unknown, a recent study reported in this issue of Molecular Pharmacology by Zhang et al. (2007) suggests a provocative molecular mechanism. These authors administered cocaine to pregnant laboratory rats and studied their offspring. Compared with pups of control rats not exposed to cocaine, those of cocaine-exposed mothers showed much lower cardiac levels of protein kinase Cε (PKCε), an enzyme known for its cardioprotective power. The mRNA level of PKCε was reduced even more dramatically. We were surprised to find that the nucleotide sequence of the PKCε promoter was unchanged, pointing to a possible “epigenetic” mechanism, defined by heritable changes in gene regulation without a change in DNA sequence. The search eventually led to a methylation event in which a methyl group placed on a sequence near the PKCε promoter prevented binding of the essential transcription factor activator protein-1 (AP-1), resulting in abrogation of PKCε transcription. It is clear that the specific methylation in the fetal genome, triggered by the mother’s use of cocaine, led to the loss of PKCε expression, resulting in cardiac hypertrophy and susceptibility to ischemia and reperfusion injury later in life (Fig. 1). These results have important implications in the etiology and management of crack-cocaine children.

Cocaine, an alkaloid, prepared from the leaves of the plant Erythroxylon coca and first isolated by the German chemist Friedrich Gaedcke in 1855, has a long history of both medicinal and recreational use in the human society (http://en.wikipedia.org/wiki/Cocaine). World annual cocaine use currently stands at around 600 metric tons, with Americans consuming roughly half and Europeans, about a quarter. In its various forms (mainly “freebase” and “crack”) cocaine is second only to cannabis as the most popular illegal recreational drug in the United States, amounting to an annual street business of approximately $40 billion. Like most psychostimulants and antidepressants, cocaine causes transient euphoria through the relatively well documented biochemical stimulation of the dopaminergic system. In contrast, little is known about the mechanism of the more lasting and heritable effects of cocaine, which makes the studies of Zhang et al. (2007) significant.

Cocaine kills thousands of its users every year. Within minutes of administration, cocaine is found in a variety of organs, including brain, lung, kidney, and heart (Benveniste et al., 2005). At the clinical and physiological levels, the most common cardiac disorders are ischemia, acute coronary syndrome, myocarditis, cardiomyopathy, and arrhythmias. Past studies have revealed some effects of cocaine at the genetic level in the heart tissue, but their exact clinical correlations...
await further study. These include reduction of sarcoplasmic reticular Ca\(^{2+}\)-ATPase (SERCA2a) levels (Wang et al., 2002a), induction of various forms of cytochrome P450 proteins (Wang et al., 2002b), and transcriptional up-regulation of harmful complements, possibly by reactive oxygen species (Tanhehco et al., 2000). In recent studies, the Zhang group also showed that prenatal cocaine exposure increased apoptosis or programmed cell death in neonatal rat heart and increased the susceptibility to ischemia-reperfusion injury in month-old rats (Bae and Zhang, 2005; Li et al., 2005). What sets the current study (Zhang et al., 2007) apart is its ability to offer an in-depth molecular mechanism based on known premises.

A major strength of the study derives from its focus on the offspring rather than the cocaine-using mother and the demonstration of a long-lasting epigenetic effect. The key molecular event is the specific methylation in a non–CpG-island region that normally functions as the enhancer for AP-1 binding. The abrogation of binding was demonstrated in a direct assay in vitro as well as by immunoprecipitation analysis of chromosomes in vivo. A number of control experiments were in place. For example, cocaine showed no effect on the binding of AP-1 on unmethylated PKC\(_e\) enhancer sequence, showing that AP-1 itself was unaffected. Reporter gene assays provided in vivo confirmation of the loss of AP-1-dependent transcription when the methylated enhancer was used. Enhancers of a number of other transcription factors were not methylated, ruling out their role. Finally, methylation of the promoter region of another PKC (i.e., PKC\(_\beta\)) was unaffected. Although not specifically shown in this article, there is a large volume of literature on the cardioprotective role of PKC\(_e\) that probably operates via phosphorylation of multiple downstream molecules (Liu et al., 1999; Inagaki et al., 2006; Morrow et al., 2006; Yue et al., 2006).

As with any leading study, the article brings up as many questions as answers. An important question is whether the observed effect is due to cocaine in the fetal blood or to a secondary effector produced by the mother’s system in response to cocaine, which then traveled to the fetus. Cocaine itself is known to freely traverse from the mother’s bloodstream to the fetal circulation (Benveniste et al., 2005), and the same is possible for other small molecule effectors. Because the placental transport cannot be obstructed without harming the fetus, a better therapeutic target may be the putative methyltransferase activity itself, which needs to be characterized. A relatively unique activity with specialized regulatory roles will be a desirable drug target for short-term intervention during each episode of cocaine use. Another question is the stability of the methylation through adulthood and old age. Zhang et al. (2007) correctly speculate that as the cardiomyocytes do not divide after birth the acquired methylation is likely to persist. However, this should be tested at various ages because methylation can be a reversible process.

The article also leaves the door open as to what activates the methylation in the first place. By far the two most common mechanisms of epigenetics are post-translational modifications of histone (e.g., acetylation, phosphorylation) and
methylation of DNA. Both play important roles in a variety of inheritable phenomena in health and disease, including tissue-specific and parental imprinting, X-chromosome inactivation, development and cancer (Couture and Trivel, 2006; Wood and Oakey, 2006). It would be interesting to determine whether histone modification precedes and actually regulates the accessibility of the DNA to the methylation process discovered by Zhang et al. (2007). Pioneering studies have revealed that sets of adjacent genes are often regulated jointly, most likely through ultrastructural remodeling of the chromosome, mediated by local modification of histone (Spellman and Rubin, 2002). Each set may cover 20 to 200 chromosome, mediated by local modification of histone revealed that sets of adjacent genes are often regulated discovered by Zhang et al. (2007). Pioneering studies have undertaken. Comprehensive genome-wide analyses may unveil the epigenetic signature of each intrauterine and/or environmental factor, which will affect both basic and clinical sciences.

References

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