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4 National Institute on Drug Abuse Conference report on 5 placental proteins, drug transport, and fetal development

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The use of illicit and licit drugs during pregnancy is a major public health concern because of potential adverse effects on the fetus and the risk to maternal health. Because the placenta is the primary link between the mother and the conceptus and is essential for the growth and survival of the fetus, abnormalities in placental formation and function resulting from drug use could have a major influence on pregnancy outcome. At present, little information is available on the impact of abused drugs on placental biology alone or in combination with other "host" factors (eg, stress, infections). This prompted the National Institute on Drug Abuse (NIDA) to convene a meeting of experts in placental biology to review cutting-edge research with the mission to translate existing information to new clinical and research initiatives in the drug abuse field. This report summarizes the presentations and research recommendations resulting from the workshop discussions.

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30 The placenta is an extra embryonic tissue that is
 31 essential for the growth and survival of the fetus as it

serves as the primary link between the mother and the developing fetus. Because the placenta performs numerous physiologic functions that are essential for the maintenance of pregnancy and fetal growth and development, abnormalities in placental formation and function are often associated with human pregnancy complications. Preeclampsia, placenta previa, and placental abruption complications seen in the general population, which are associated with adverse or poor fetal outcomes, frequently occur in women who abuse drugs during pregnancy, which represents a major

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public health concern.¹⁻³ The clinical literature to date reports that infants born of mothers who used drugs, licit and illicit, have impaired somatic growth and development as well as neurobehavioral deficits.⁴⁻⁸ Other studies show that cocaine, marijuana, heroin, and other abused drugs can cross the placental barrier.⁹⁻¹¹ However, at present, there is little information about the effects of these drugs on placental biology. Also, little information is available as to whether the detrimental effects seen in drug-exposed offspring are the direct result of perturbations in the development of placenta and its functions or caused by "host" factors such as poor prenatal care, stress, infection, and poor maternal nutrition, which are common comorbid factors in drug abusing women.^{4,12}

Recent advances in the field of placental biology and the limited investigation of drugs of abuse related to the development of placenta prompted the National Institute on Drug Abuse (NIDA) to assemble a group of biomedical researchers to discuss cutting-edge research with the mission of translating these findings to clinical application and to identify technologies that could facilitate research on the effects of drugs of abuse on placental function. The research areas covered in the conference included the development of the placenta, its role in immune-endocrine interactions during pregnancy, its function in the transfer of molecules (transporter systems), and the influence of infectious agents and xenobiotics on placental biology. The workshop was held in Bethesda, Md, on August 27 and 28, 2003, and this conference report summarizes the major findings and the issues discussed by the workshop participants. This report also highlights the gaps and research opportunities in placental biology and substance(s) abuse as identified by the meeting participants and attendees. A program book with conference participants' presentation will be posted on the NIDA Web site (<http://www.nida.nih.gov>) in the near future.

Drug effects on the developing organism are dependent on the activity and retention of the parent drug, its metabolites in the maternal-fetal unit as well as on the duration and time of exposure during pregnancy.^{13,14} Environmental and other host factors such as stress and infections can also influence drug effects on the fetal development. Dr Strauss started the meeting with an overview of the placenta as an organ with specialized transport and metabolic functions that on one hand delivers key nutrients to the fetus and on the other, catabolizes or excludes/extrudes potentially noxious substances. He discussed probable relationships between adult onset diseases and fetal origins as a consequence of early insult(s). He presented data that showed cocaine elicits decreases in uteroplacental blood flow, and that the fetus is directly exposed to cocaine as a result of accumulation of the drug in amniotic fluid via transport across the chorion-amnion.^{15,16} He noted that

although most work on drug effects and drug transport has been conducted on the placenta (mostly the term placenta) and choriocarcinoma cells, the placenta and trophoblast cells are not the only organ/cells that influence embryo/fetal exposure to drugs or other exogenous chemical agents. Exposure to xenobiotics could also occur in other compartments (yolk sac, fetal membranes, fallopian tubes, and uterine lumen) and the transport mechanisms involved in delivering drugs into these compartments may be passive or active and can be specific to pregnancy stage. He suggested that transport mechanisms in these compartments need further investigation. To illustrate his point, Dr Strauss presented recent data that showed the expression of the family of ATP-binding cassette (ABC) transporters, some of the key proteins involved in disposition of drugs, is greater in the uterus than in placenta.^{17,18} These transporters are also expressed in the fallopian tubes. The central role of ABC transporters in controlling drug access to the fetus has been documented in mice deficient in placental P-glycoprotein (P-gp), *mdr1a* gene, in which there is increased delivery of P-gp substrates into the fetus.¹⁹ Thus, genetic variation in the transporters could be 1 factor that affects fetal drug exposure. The analysis of polymorphisms in these genes and their impact on placental drug transport deserves investigation.

Dr Unadkat reported that although considerable evidence is available from knockout mice that P-gp is important in excluding drugs from the privileged compartments, the fetus, and the brain, the importance of this transporter at other biologic barriers in primates has not been quantified. To address this issue, he described the noninvasive and quantitative imaging technique that his group has developed to measure P-gp activity in the placenta in nonhuman primates using positron emission tomography (PET) and [¹¹C]-verapamil as the P-gp substrate. He also presented evidence that the expression of P-gp in the human placenta is regulated by gestational age (Unadkat, unpublished results). He discussed the significance of these findings in relation to clinical studies of disposition of antihuman immunodeficiency virus (HIV) protease inhibitors (substrates of P-gp and CYP3A) that suggest that P-gp and CYP3A activity may be upregulated during pregnancy.²⁰ Dr Audus described the use of in vitro cell models, BeWo cells, to assess the expression of enzymes and carrier systems. He observed responses of cytochrome P450s and transporters (BCRP and P-gp) in this cell line that were similar to trophoblast cells in vivo. This suggested that in vitro cell models are useful for the investigation of drug metabolism in human placenta.^{21,22}

Other studies presented showed functional changes in the placental amino acid and monoamines transport systems induced by cocaine, amphetamine, and nicotine use. For example, Dr Ganapathy presented evidence that the use of cocaine and amphetamine interferes with

the clearance of vasoactive serotonin and norepinephrine from the intervillous space. A compromise in the monoamines clearance induced by these drugs could increase vasoconstriction and reduce blood flow as well as diminish the transfer of oxygen and nutrients to the fetus leading to intrauterine growth retardation.²³ Dr Novak showed cocaine-induced inhibition of amino acid (AA) transport system A in placental vesicles,²⁴ whereas the effect of other addictive substances (alcohol, tobacco, and marijuana) on AA transport systems were not as clear-cut. For instance, high doses of nicotine inhibited AA uptake into placental villi in vitro, whereas placental villi isolated from smokers demonstrated enhanced AA uptake suggesting not only the complexity of the in vivo studies but also the concern that contributions of other noxious agents present in tobacco could be altering AA uptake.^{25,26} Dr Novak also discussed the importance of nutrition during pregnancy as a low protein diet was found to have an effect on the AA transport system.²⁷

To characterize drug effects on the placenta, a clear understanding of the cellular and molecular processes involved in the normal placental development is essential. As it is difficult to conduct this type of research in humans, various animal models have been employed to study placental biology. Dr Dey reported that in a mouse model, anandamide, an endogenous cannabinoid ligand, and its receptors play an important regulatory role in the establishment of normal pregnancy and these effects are dose and gestational stage-specific as lower levels of endocannabinoids and CB receptors were found to be beneficial for implantation whereas higher concentrations were detrimental to this process.²⁸ Furthermore, anandamide within a very narrow range regulates blastocyst function and implantation by differentially modulating mitogen-activated protein kinase (MAPK) signaling and Ca⁺⁺ channel activity via CB1 receptors. Anandamide, at a low concentration, induces extracellular regulated kinase (ERK) phosphorylation and nuclear translocation in trophoblast cells without influencing Ca⁺⁺ channels, and renders the blastocyst competent for implantation in the receptive uterus. In contrast, anandamide at a higher concentration inhibits Ca⁺⁺ channel activity and blastocyst competency for implantation without influencing MAPK signaling. Besides uncovering a potentially important regulatory mechanism for synchronizing blastocyst and uterine competency to implantation, this observation has high clinical relevance as elevated levels of anandamide could induce spontaneous early pregnancy losses in women who smoke marijuana.²⁹

Molecular and genetic studies presented by Dr Cross revealed that in a mouse model, distinct transcription factor genes, bHLH-*Hand1* and *Mash 2* regulate the differentiation of trophoblast giant cells. Furthermore, in *Hand1* mutant mice the expression of the giant cell-

specific hormone, placental lactogen-I (PLI) gene was dramatically reduced suggesting that the absence of *Hand1* or its mutation could have profound effects on the giant cell differentiation.³⁰⁻³² In this context, Dr Sadovsky also reported observing alterations in the expression of transcription factors in human placental biopsy samples obtained from pregnancies complicated by intrauterine growth retardation and in human trophoblasts exposed to hypoxia.³³ Dr Salafia's presentation addressed how human placental data obtained at several levels, gross anatomic inspection, and histologic and molecular analyses allow researchers to "reconstruct" the tissue structure and identify, to a certain degree, the pathophysiology that can stress or potentially damage a fetus and create long-term health risks. These findings led to discussions of how this approach combined with the study of mutant animal models could be used to discover drug-induced perturbations at the cellular, molecular, and genetic levels as well as during gestation to identify at risk pregnancies or interventions that could prevent long-term health effects of drug exposure.

Because interactions between the endocrine and immune systems play a vital role in the maintenance of pregnancy, several presentations at the meeting addressed how placental hormones/cytokines of pregnancy may regulate processes of mammalian gestation because the synthesis and secretion of these molecules are tightly regulated in a temporal and cell-specific manner during this period. In addition, insults such as stress, nutritional deprivation, infection, and/or drug exposure are likely to influence the expression of these molecules and their interactions with other compartments, thus altering specialized functions of trophoblast cells and the maternal-fetal immune systems. A compromise in any of these gestational processes would likely result in impaired fetal growth and development. Dr Linzer reported that the synthesis of 2 prolactin-like proteins (PLP-E and PLP-F) in the mouse placenta differs in terms of gestational stage and trophoblast cell type, yet these 2 PLPs have the same biologic activities. They both act on the myeloid blood lineages to enhance cell growth and differentiation.^{34,35}

Dr Soares reported that a species-specific subpopulation of trophoblast cells show differential invasion pattern into the endometrium during the last week of gestation in rodents. Furthermore, this subpopulation of cells can synthesize a unique set of prolactin-like hormones/cytokines, PLP-A, PLP-L, PLP-M, and PLP-N, and the timing of the expression of these cytokines is precise and coincides with the disappearance of uterine mesometrial NK cells. This suggested the presence of factors that modulate their migratory behavior.^{35,36} Dr Erlebacher reported that in pregnant mice, administration of an anti-CD40 monoclonal antibody that serves as an agonist for CD-40, an activating surface molecule on immune cells, early in gestation

caused complete fetal resorption, leading to pregnancy failure. This pregnancy failure was found to be due to luteal insufficiency and ovarian prolactin resistance (unpublished results). His results suggest that the cause of pregnancy failure after exposure to anti-CD40 monoclonal antibodies is the result of progesterone insufficiency rather than the direct rejection of the fetus by immune cells at the maternal-fetal interface.³⁷

Pregnant women who use drugs of abuse often consume a variety of abused substances and are prone to the exposure to various environmental toxins and infections. The last 3 presentations addressed how these factors may influence placental immunomodulatory molecules and thereby, the fetal outcome. Dr Coats reported no differences in expression of the proinflammatory cytokines interferon- γ (IFN- γ) and interleukin-1 β (IL-1 β), the anti-inflammatory cytokine IL-10, or the chemokine receptor CXCR4 when term placentas from cats infected with feline immunodeficiency virus (FIV) were compared with those of control animals. However, increased expression of IFN- γ and IL-1 β was detected in placentas from resorbed fetuses of FIV-infected cats when compared with placentas from nonresorbed fetuses (Coats, unpublished results). She discussed the significance of these findings as similar results have been reported in women with pregnancy failure³⁸ and the usefulness of this feline model in studying in utero drug exposure and transmission of HIV and other viruses in the context of placental immunology. Dr Anderson continued on this theme by presenting evidence that the placental barrier and natural defense mechanisms are not only fetal age and pregnancy stage-specific, but can also vary with the type of infection (eg, bacterial vs viral infection). She reported that in 90% of HIV-exposed gestations, the fetus is not infected with HIV even though the placenta may be infected. HIV is incorporated into DNA but RNA production does not occur in placental trophoblasts. The remarkable natural resistance of the placenta to HIV needs further investigation. Dr Miller showed that environmental toxins induce alterations in placental and yolk sac function and that the magnitude and type of perturbation in fetal outcome was dependent on the time of exposure. For instance, an exposure to ethylnitrosourea before implantation causes embryonic loss, whereas postimplantation exposure induces fetal central nervous system (CNS) malformation, raising again the issue as to how little information is available on the barrier functions of various compartments in the maternal-fetal unit under both normal and abnormal conditions.

Because the objective of this workshop was to identify gaps in our knowledge and topics for future research in the drug abuse field related to placental physiology, and how current technology could advance NIDA's mission, the discussion centered on the emerging research in the placental drug abuse arena. Although existing data

clearly suggest that drugs of abuse do produce alterations in the placental function that can adversely affect the fetus, a number of key issues require attention. Workshop participants identified the following deficiencies in our knowledge base and made recommendations for a research agenda to address these deficiencies:

1. Information as to how "host" factors, including genetic variation, recreational drug use, infection, and malnutrition influence the placental barrier and its function is lacking;
2. Another topic identified for further investigation is how the embryo/fetus is protected in different compartments;
3. Studies to characterize the cellular and molecular processes involved in the transport of drugs and other molecules by various transporter systems (ABC, AA, monoamines) are needed;
4. Investigations designed to study interactions of the endocrine and immune systems, both from a maternal and fetal perspective, as well as related to placental development and the impact of drugs of abuse are needed;
5. Elucidation of the effects of drugs of abuse on growth and angiogenic factors using animal and model cell systems are also encouraged;
6. There is a need to develop new technology to examine drug distribution and pharmacokinetics in vivo in small animals;
7. There is a pressing need for biomarkers in biologic fluids (serum, urine, saliva) and placental tissue that can be used to assess drug exposure in pregnant women by using newer tools of genomics and proteomics.

It was believed that ultimately, studies addressing these needs would generate information that would advance our understanding as to how development of the placenta is affected by drugs of abuse alone or in combination with other host factors, and how these effects relate to the growth and development of the fetus and postnatal health.

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The National Institute on Drug Abuse held a multidisciplinary workshop on Placental Proteins, Drug Transport and Fetal Development at the NIH campus in August 2003. This article contains a brief summary of the presentations and the priorities for research, which were recommended for consideration by NIH by clinicians and scientists attending the meeting. The American Journal of Obstetrics and Gynecology considers that the event and the conclusions are important.

Condensation: An NIDA report summarizing the effect of [Q2] drug abuse on fetal development.