

Prenatal Exposure to Drugs of Abuse in Humans: Effects on Placental Neurotransmitter Receptors

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PERRY, B. D., D. J. PESAVENTO, P. H. KUSSIE, D. C. U'PRICHARD AND S. H. SCHNOLL. *Prenatal exposure to drugs of abuse in humans: Effects on placental neurotransmitter receptors.* NEUROBEHAV TOXICOL TERATOL 6(4) 295-301, 1984.—Recently, the concept of behavioral teratology has evolved [32]. In animal models, prenatal exposure to low doses of psychotropics (e.g., methadone, diazepam) results in abnormal development of behavior. Furthermore, in animals, pre- and perinatal exposure to psychoactive drugs results in altered brain neurochemistry [27]. In humans, similar behavioral and neurological disruptions have been reported [5]. The mechanisms responsible for these effects are unknown; however, all of these psychotropics have high affinity, specific interactions with various neurotransmitter receptors. Furthermore, normal development of nervous tissue appears to be mediated through neurotransmitter receptors. Disruption of normal, receptor-mediated "signals" by psychoactive agents would be expected to alter development of brain. In order to examine the regulation of neurotransmitter receptors by centrally acting agents, we have compared placental neurotransmitter receptors from "control" and substance-abusing women, hypothesizing that (1) placental neurotransmitter receptor regulation may mirror fetal brain neurotransmitter receptor regulation and (2) placental neurotransmitter receptors themselves may play a role in development. Standard radioligand binding methods were employed. Control values were relatively consistent while substance-abusing values were inconsistent, with wide variation in each substance-abusing group, possibly indicative of "regulation" of neurotransmitter receptors, yet certainly complicated by other variables surrounding gestation and birth (e.g., drug dosage and schedule, length of labor). In this regard, levels of neurotransmitter receptors were consistently higher (20-100%) in caesarian section tissue, possibly reflecting "down-regulation" of neurotransmitter receptors in placentas by the known excess sympathetic and opiate activity during labor. These preliminary results demonstrate (1) endogenous agonist exposure during labor may "regulate" neurotransmitter receptors in placenta (fetal brain as well?) and (2) psychoactive agents may regulate placental neurotransmitter receptors and, if present during gestation, may also regulate developing fetal neuroreceptor-neurotransmitter systems.

Psychotropics Placental neurotransmitter receptors Humans Prenatal exposure

IT has been known for some time that use of drugs or exposure to chemicals during pregnancy can result in disruption of fetal growth, often resulting in some developmental abnormality in the newborn [1]. More recent studies have determined that changes can occur in the fetus which do not manifest themselves as grossly obvious physical abnormalities but as subtle behavioral changes in the newborn [32]. Among the hypothesized mechanisms which may underlie these behavioral changes in the newborn are persistent drug effects in the newborn from decreased ability to metabolize and excrete the drugs [23], postnatal withdrawal [9] and alterations in brain neurochemistry [27].

Numerous investigators have demonstrated that exposure to chemicals during pregnancy can result in alterations in the

development of catecholamines and other neurochemical systems [13, 24, 27]. Although the mechanisms mediating these alterations are not known, many of the substances studied in pregnant animals act via high affinity interactions at neurotransmitter receptors, possibly resulting in the changes found. These changes in the development of the central nervous system could result in behavioral changes in the newborn.

Studies on infants born to mothers who have been using opiates during pregnancy show behavioral abnormalities when tested on the Brazelton Neonatal Assessment Scale and a prolonged withdrawal syndrome [3,12]. Dinges *et al.* [5] have shown that babies exposed to narcotics during pregnancy show disturbed sleep, with more REM sleep and more

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restless sleep than the control infants. Wilson *et al.* [34], in a follow-up of 3- to 6-year-old children of heroin-addicted mothers, found that these children were smaller than their peers, less well adjusted and differed from controls on perceptual tests.

Behavioral abnormalities have been demonstrated in infants born to mothers using drugs other than opiates during pregnancy. Streissguth [28], in studies of infants born to alcohol-abusing mothers, found effects on behavioral and neurological development. A recent study completed in our program [4] shows that babies born to polydrug-addicted mothers differ from babies born to opiate-addicted mothers and controls. The opiate-addicted babies were smaller than the polydrug-addicted babies and the controls, and had smaller head circumferences. On the Brazelton Scale, the opiate-dependent group had more depression of interactive behaviors and state controls than the polydrug group, although the polydrug group had more depression in these scales than controls.

To determine whether the effects of prenatal exposure to drugs result in central nervous system neurotransmitter receptor changes which, in turn, result in behavioral teratology, would require the study of brain tissue. This approach is not feasible in humans. However, studies of human placental tissue have demonstrated the presence of opiate receptors [21, 22, 31] and β -adrenergic receptors [15, 26, 33]. Preliminary studies in our laboratory have demonstrated that in addition to the above receptors, α_1 - and α_2 -adrenergic, D_2 -dopaminergic, 5HT₂ serotonergic and μ -opiate receptors may also be located on placental tissues.

The presence of neurotransmitter receptors on placental tissue may represent (1) a control mechanism of a placental function [7, 14, 15, 33] or (2) a reflection of receptors in fetal tissue. In either situation, alterations of these receptors by maternal drug use could result in profound effects on fetal and newborn development.

The present study examined and compared placental neurotransmitter receptors from control and substance-abusing mothers.

METHOD

Population

Experimental placental tissue was collected from women who participated in the Perinatal Addiction Project of the Chemical Dependence Program at Northwestern Memorial Hospital. The Perinatal Addiction Project monitors and treats women addicted to all types of abusable drugs from early in pregnancy through delivery and follows the mothers and newborns post-partum. Control placentas are available from routine deliveries at Prentice Women's Hospital and Maternity Center of Northwestern Memorial Hospital.

Collection of Placental Tissue

Placentas were received from normal vaginal, normal caesarian, experimental vaginal and experimental caesarian births and placed in ice-cold saline within 15 minutes of afterbirth.

Tissue Preparation

Placentas were dissected free of major vessels, washed of blood in ice-cold Tris HCl buffer (pH 7.7, 25°C), quickly frozen in a dry ice/acetone bath and stored at -70°C until use. Frozen or fresh tissues were prepared for receptor bind-

ing assays by modification of the methods of Shocken *et al.* [26]. The frozen placental tissue was brought to room temperature by placing it in ice-cold 25 mM Tris HCl buffer (pH 7.4 at 4°C) containing 23 M sucrose. This tissue was thoroughly minced and homogenized with a Brinkmann Polytron (setting 7, 60 sec). The homogenate was filtered with two-ply cheesecloth; the filtrate was centrifuged at 300×g for 15 min and the collagenous pellet was discarded. The supernatant was centrifuged at 50,000×g for 15 minutes. The resulting pellet was resuspended in fresh Tris HCl/sucrose buffer, rehomogenized (Polytron, setting 7, 15 sec) and recentrifuged (50,000×g, 15 min). The resulting pellet was washed by centrifugation, as above, in Tris HCl buffer (pH 7.7, 25°C). The final pellet was suspended in 50 mM NaKPO₄ buffer, pH 7.5, in a final tissue concentration of 1.0 mg prot/ml or roughly 30 mg original wet weight/ml. Pellets were used immediately or frozen in a dry ice/acetone bath and stored at -70°C until assayed.

Frozen placental samples (intact tissue or pellets of homogenates from samples prepared as described above) were thawed in 50 mM Tris-HCl buffer and homogenized using a Brinkmann polytron. Homogenates were centrifuged once at 50,000×g for 10 min at 4°C with intermediate resuspension in fresh Tris-HCl buffer. Final pellet suspensions were in 50 mM Tris HCl (pH 7.7, 25°C) for [³H]-p-aminoclonidine (³H-PAC) or in 50 mM Na₂HPO₄/KH₂PO₄ buffer (pH 7.4) for [³H]-naloxone ([³H]-NAL), [³H]-rauwolscine ([³H]-RAUW and [¹²⁵I]-BE 2254 ([¹²⁵I]-HEAT) assays.

Binding Assays

Radiological binding assays are all modifications of previously published methodologies originally developed for brain tissue. Original methodologies are described for [³H]-prazosin by Greengrass and Bremner [10], for [³H]-rauwolscine by Perry and U'Prichard [17], for [¹²⁵I]-iodocyanopindolol by Engel *et al.* [6] for [³H]-lysergic acid diethylamine by Peroutka and Snyder [16], for [³H]-spiroperidol by Peroutka and Snyder [16], for [³H]-quinidclidinyl benzilate by Yamamura and Snyder [35] and for [³H]-naloxone by Pert and Snyder [20].

Adrenergic Binding Assays

Alpha-2 receptors, like other adenylate cyclase-coupled receptors [2, 29, 30] occur in two or more affinity states. We currently utilize the agonist ligand [³H]-p-aminoclonidine ([³H]-PAC), which at low concentrations selectively labels states of the α_2 receptor with high affinity for agonists and low affinity for antagonists (α_2 [H]): and the specific antagonist ligand [³H]-rauwolscine ([³H]-RAUW), an isomer of yohimbine, which labels states of the receptor with low affinity for agonists and preferential high affinity for some antagonists including RAUW itself (α_2 [L]).

A recently developed iodinated antagonist radioligand [¹²⁵I]-BE-2254 (BE: 2-[4-hydroxyphenyl]-ethylamino-methyl] tetralone or HEAT) is potent (K_D 100 pmol) and very selective at the brain α_1 receptor, with high specific radioactivity (S.A. 2,000 Ci/nmole). Similarly, β -adrenergic receptors will be labeled in these studies with [¹²⁵I]-iodocyanopindolol (ICYP), according to the methods of Engel *et al.* [6]. Binding assays were routinely performed in triplicate using a 1.0 ml assay volume containing 10 mg original tissue (c. 0.2 mg protein) when using [³H]-ligands, and 1 mg tissue (c. 40 μ g protein) when using [¹²⁵I]-ligands. We have demonstrated

that a satisfactory signal (20–70% specific binding at K_D concentrations) is obtained in placenta with these tissue concentrations. Some initial experiments have determined that equilibrium binding was being examined for all ligands in the placenta at our standard assay times and temperatures. All subsequent experiments were performed under equilibrium conditions. Nonspecific binding of α -receptor ligands was determined by measuring parallel triplicate samples containing (–)norepinephrine (0.01 mM for [3 H]-PAC; 0.1 mM for [3 H]-RAUW and [125 I]-HEAT). Nonspecific binding in β -receptor assays was determined using (–)isoproterenol. Assays were terminated by rapid filtration under vacuum, and rinsed filters were counted in minivials in a Packard 460C scintillation counter (47% [3 H] efficiency) for tritium samples, or a Beckman gamma counter for [125 I] samples. Specific binding data (cpm) was converted to fmoles bound/mg protein. Protein concentrations were determined by the method of Lowry.

Analysis of Receptor Binding Data

Monophasic saturation isotherms (data from [125 I]-HEAT, [3 H]-PRAZ, [125 I]-ICYP and [3 H]-RAUW over narrow concentration ranges) were converted to Eadie-Hofstee plots (reciprocal of Scatchard plots) and were subjected to weighted linear regression analysis using a TI-59 calculator program [36]. From each individual isotherm, corrected values for the K_D and B_{max} of the radioligand were obtained (\pm S.D.), together with a term, $E(rad)$, which is an index of the extent of data scatter. Saturation isotherms for which $E(rad)$ 0.1 were omitted from further data reduction. Polyphasic saturation isotherms (PAC and RAUW) over extended concentrations and agonist competition data were subjected to computer-assisted iterative analysis using the BIPHAS program. The BIPHAS program, written by Dr. Steven Grill (Department of Physiology, Northwestern University Medical School), uses a nonlinear, least-square curve-fitting methodology based upon mass action interactions of ligand and receptor [8], assuming a two-site model as represented below:

$$\text{Bound} = \frac{B_{max_1} \times \text{Free}}{\text{Free} + K_{D_1}} + \frac{B_{max_2} \times \text{Free}}{\text{Free} \times K_{D_2}}$$

BIPHAS, written in FORTRAN for a PDP11/60, analyzes curves for the binding of the radioligand (or unlabelled competitor) to one or more binding sites by the “robust” method of iteratively determining best estimates of K_D and B_{max} values within a preselected range for each component of binding until the lowest sum of differences between actual and predicted binding values is obtained. Testing for significant differences between models of one or two binding sites was performed by comparing (f-test) the lowest sums of differences with each model.

Competition data was transformed such that bound=% inhibition and free=concentration of unlabelled drug.

Drugs, Reagents and Radioligands

Drugs were obtained from the following sources: (–)epinephrine, (–)norepinephrine, Sigma Chemical Co., (St. Louis, MO); (–)isoproterenol, (–) α -methyl norepinephrine, (–)phenylephrine, Sterling Winthrop, (New York); (–)propranolol, Ayerst Research Laboratories (Montreal, Canada); clonidine, Boehringer-Ingelheim (Ingelheim, West

Germany). Other reagents are obtained from the pharmaceutical company of origin or from commercial sources. All radioligands except [3 H]-prazosin were obtained from New England Nuclear (Boston, MA). [3 H]-Prazosin was obtained as a gift from Amersham Corp. (Arlington Heights, IL). All radioligands were stored at -20°C and diluted in 10^{-3} N HCl.

RESULTS

Screening Assays

Initial experiments were carried out to determine the specific binding of a variety of radioligands [3 H] rauwolscine, [3 H] prazosin, [3 H] quinonuclinybenzilate, [3 H] spiroperidol, [3 H] naloxone, [3 H] lysergic acid diethylamine, [125 I] iodocyanopindolol [18] to “control” placental membranes. Following these preliminary determinations, we selected four radioligand/receptor systems ([3 H] rauwolscine: α_2 , [3 H] prazosin: α_1 , [125 I] iodocyanopindolol: β , and [3 H] naloxone: μ -opiate) for more complete characterization, as well as for experimental comparisons.

Specific binding (at a radioligand concentration near the K_D value) of the tritiated radioligands to control membranes was between 30–40% and demonstrated some variability. Under similar conditions, specific binding of iodocyanopindolol was higher (80%) and resulted in more consistent results. Using single concentrations of the radioligands to screen experimental (caesarian, substance-abuse) placentas resulted, in many cases, in values different from controls [18]. These results implied a potential regulation of placental neurotransmitter receptors, and we initiated a series of experiments to investigate this possible regulation.

Saturation Experiments, Control

The first series of experiments we performed was designed to confirm that the antagonist radioligands we employed were labeling, in fact, the specific receptor subtype in control placentas for which each radioligand has selectivity. With each of the radioligands, association, dissociation and saturation studies were performed; in addition, competition of a variety of cold compounds at sites radiolabeled by [3 H] rauwolscine (RAUW), [3 H] prazosin (PRAZ), ICYP and [3 H] naloxone (NAL) was performed to determine pharmacological specificity of the binding site. These results are in the process of preparation for publication (Perry *et al.*, in preparation).

In summary, each of the adrenergic radioligands (PRAZ, RAUW, ICYP) binds to central placental binding sites in a stereospecific, saturable and reversible manner, resulting in radioligand affinities (K_D values, Table 1) close to those reported for other tissues. The opiate radioligand, NAL, with morphine or levorphanol defining specific binding, also labeled binding sites in a saturable, reversible manner with K_D values close to those found in other tissues. Table 1 summarizes the results of saturation experiments in control (vaginal) and caesarian (scheduled, no labor) deliveries. PRAZ, labeling α_1 -binding sites, has a range of K_D values (0.43 to 0.58 nM) close to the many reports from brain tissue [10,19]. The number of PRAZ sites is relatively low in vaginal delivery placental membranes (30.0 fmol/mg prot) but almost twice as high (68.5 fmol/mg prot) in no-labor caesarian tissue.

Alpha-2 adrenergic binding sites in both vaginal and caesarian delivery placentas are labeled by RAUW with K_D

TABLE 1
ADRENERGIC AND OPIATE RECEPTOR BINDING SITES IN HUMAN PLACENTAL MEMBRANES: CONTROLS

Nal Tissue (n)	³ H] PRAZ		³ H] RAUW		¹²⁵ I] CYP			³ H]	
	K _D	B _{max}	K _D	B _{max}	K _D	β	B _{max}	K _D	B _{max}
		α ₁		α ₂				μ opiate	
Control									
Vaginal (5)	0.43±0.03	30.0±15.0	2.4±0.78	24.5±7.9	0.07±0.02	132.0±12.0		1.7±0.6	26.9±1.7
Caesarian (4)	0.58±0.06	65.5± 8.0	1.9±1.0	37.6±4.6	0.09±0.05	119.9±86.0		0.66	38.1

Values represent means±S.E.M. from 3–5 experiments, each performed in triplicate. Five to seven concentrations of radioligand were employed as described in the Method section, and for each ligand, concentration ranges were selected to represent the “high affinity” component of binding.

K_D values for all ligands are in nM and all B_{max} values are in fmol/mg prot.

TABLE 2
ADRENERGIC AND OPIATE RECEPTOR BINDING SITES IN HUMAN PLACENTAL MEMBRANES: SUBSTANCE ABUSERS

Tissue Delivery (n)	³ H] PRAZ		³] RAUW		¹²⁵ I] CYP			³ H] NAL	
	K _D	B _{max}	K _D	B _{max}	K _D	β	B _{max}	K _D	B _{max}
		α ₁		α ₂				μ opiate	
Opiate									
Vaginal (5)	0.48±0.21	12.0± 5.6	1.6±1.3	42.0±27.3	0.13±0.10	51.3±10.2		1.8±0.8	57.0±34.2
Ethanol									
Vaginal (3)	1.0 ±0.4	12.0±12.7	2.2±0.5	18.0±15.5				1.8±0.3	33.0±19.7
Amphetamine									
Vaginal (1)			2.0	180	0.14	106.0		0.7	90.9
Phencyclidine									
Vaginal (1)			1.1	61.5	no binding detected			1.6	51.3

Values represent mean±S.E.M. (or a single experiment) from 3–7 experiments, each performed in triplicate. Five to seven concentrations were employed as described in the Method section, and for each ligand, concentration ranges were selected to represent the “high affinity” component of binding.

K_D and B_{max} values for all ligands are in nM, and all B_{max} values are in fmol/mg prot.

values of 1.9–2.4 nM, again very close to those reported for brain tissue [17, 19, 29, 30]. It should be pointed out that the range of RAUW concentration employed (0.1–3.0 nM) for these assays will select for the high affinity component of binding. In cases where extended concentrations (0.1–15.0 nM) are employed in placenta (experiment not shown) a biphasic Scatchard is observed with computer-derived K_D and B_{max} estimates for the components of binding, similar to those derived in brain [19,30].

As in the case of PRAZ, RAUW labeled more sites (37.6 fmol/mg prot) in the caesarian delivery placental membranes than in the vaginally delivered placentas (24.5 fmol/mg prot). The binding of ICYP to β-receptors resulted in the most consistent results. ICYP K_D values (70–90 pmol) are close to those found in brain [6], and the B_{max} values are much higher (119–132 fmol/mg prot) than those for the α₁, α₂-adrenergic or μ-opiate ligands. NAL, labeling μ-opiate binding sites,

labeled (again, with K_D values similar to other, well-characterized systems) roughly 30 fmol/mg prot of sites. In the one case where comparison could be made, the B_{max} value in the caesarian delivery membranes (38.1 fmol/mg prot) was higher than those for the vaginally delivered placental membranes (26.9 fmol/mg prot) (Table 1).

Saturation Experiments, Substance Abuse

Following the initial characterization of the binding sites in “control” tissues, we began our analysis of the saturation of these radioligands (PRAZ, RAUW, NAL, ICYP) in placental tissues collected from substance-abusing women. All deliveries were vaginal. In many cases, the B_{max} values obtained from the binding sites in the substance abuse group were different from controls (Table 2, Fig. 1). In all cases, however, the K_D values from the substance abuse group

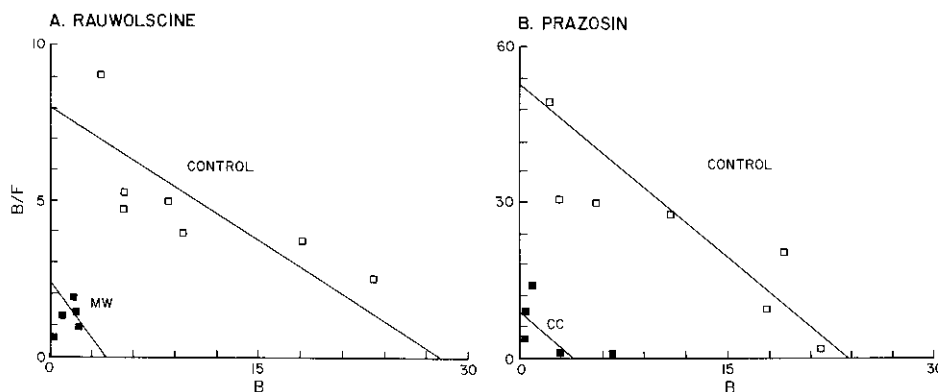


FIG. 1. Scatchard transformations of alpha-adrenergic radioligand saturation isotherms in placentas from substance-abusing and "control" women. A. Rauwolscine binding. B. Prazosin binding. Placental membranes from control and substance-abusing women were incubated with increasing concentrations of radioligand (see the Method section). Saturation isotherms were transformed and analyzed by the method of Zivin and Waud [36]. MW and CC both represent placental tissue from ethanol-abusing patients. Values represent one experiment performed in triplicate.

were within the control range. In our present study, the largest group of substance abusers ($N=7$), opiate (Table 2), demonstrated the greatest apparent changes in radioligand binding sites, with fewer PRAZ and ICYP sites, and more RAUW and μ -opiate sites. The wide variation in values from patient to patient (as evidenced by the large S.E.M. values) complicated analysis, and no obvious trend appeared (i.e., no consistent, specific, single receptor demonstrated changes in each individual within an experimental group). Individual B_{max} values for each radioligand from a given opiate-abusing patient generally were within normal limits with only one or two radioligands demonstrating numbers of binding sites apparently out of the normal range. However, when the B_{max} values were measured from all individuals within the experimental group, the opiate group emerged as different (Student's t -test) from normal for each of the radioligands employed. The ethanol group, somewhat smaller ($N=3$) than the opiate group, demonstrated relatively normal values for all radioligands employed, except PRAZ, where the number of sites was somewhat lower than "control" (12.0 vs. 30.0 fmol/mg prot).

One placenta was obtained from a woman who abused amphetamine during her pregnancy. Saturation studies with RAUW and NAL revealed much higher levels of binding than seen in controls (RAUW, 106 vs. 24, and NAL 90.9 vs. 26.9 fmol/mg prot). ICYP binding in this placenta was within the normal range. One placenta was obtained from a woman abusing phencyclidine (PCP). Radioligand binding on this placenta was also different from controls, with the most interesting finding being the apparent lack of ICYP binding detected (Table 2).

DISCUSSION

The present study presents three preliminary findings which are of interest: (1) the demonstration and partial characterization of α_1 , α_2 and μ -opiate radioligands in "normal" placental membranes; (2) preliminary evidence for the regulation of adrenergic and opiate receptors by endogenous agonist compounds during labor and delivery and (3) the demonstration of the regulation (or disruption) of placental neurotransmitter receptors by prenatal exposure to centrally

acting drugs of abuse. While the variation in K_D and B_{max} values from both control and experimental placentas remains a problem, the ability to radiolabel α_1 -, α_2 - and β -adrenergic, and μ -opiate (among other) receptors implies a functional role for these receptors. A number of previous studies have identified and characterized placental β -adrenergic receptors [7, 14, 15, 26, 33] and κ -opiate receptors [21, 22, 31]. Our findings and characterization of the β -adrenergic receptor replicate these studies.

We are unaware of any studies which have directly demonstrated α_1 - and α_2 -adrenergic, and μ -opiate receptor binding sites in human placenta. The presence of the adrenergic receptors may be related to the high vascularity of placental tissues as α_1 -, α_2 - and β -adrenergic receptors are known to be localized to a wide variety of human peripheral vascular tissues, including arteries, veins and arterioles as well as the blood elements (e.g., red blood cell β -receptors and platelet α_2 -adrenergic receptors). Indeed, the very high concentration of β -adrenergic binding sites reported by our laboratory and others [14, 15, 26, 33] may represent, at least partially, red blood cell membrane β -receptors. The possibility is under investigation presently. The presence of opiate receptors (μ - and κ -opiate) in placental membranes has not obviated any functional role. The presence of these placental receptors in the light of the large increases in maternal β -endorphin during labor and delivery indicate that the placenta may be a locus of action for opioid compounds, both endogenous and exogenous.

The more interesting findings of the present study are those relating to the regulation (up-regulation and down-regulation) of placental receptors. Agonist-induced down-regulation of adrenergic receptors (and opiate receptors), in concert with desensitization of the receptor-mediated response, is a well-known, thoroughly characterized phenomenon in the brain and periphery. During labor and delivery, there is a massive outpouring of the adrenergic agent epinephrine and endogenous opiates (e.g., β -endorphin). The process of labor, particularly for primigravid women, can take many hours (18 to 48), a period certainly long enough to induce down-regulation of the receptors exposed to higher levels of these agonists [29,30]. Our current findings seem to indicate that this is taking place (Table 1). In caesarian de-

liveries where no labor has taken place, the levels of α_1 - and α_2 -adrenergic and μ -opiate receptors are much higher than traditional vaginal deliveries. In caesarian deliveries which have taken place due to the excessive duration of labor, the number of α_1 - and α_2 -adrenergic and μ -opiate receptors is as low, and in many cases, much lower, than control values (data not shown). We are in the process of further investigating this regulation of placental receptors during labor and delivery. Two main issues arise from these preliminary findings (assuming our interpretation of agonist-induced down-regulation of receptor number is correct) (1) a major complicating factor in the process of establishing "control" radioligand binding values will be the length and severity of labor and delivery; (2) what are the functional concomitants of the potential desensitization of the receptor-mediated responses in the mother, placenta and newly born infant? In regards to establishing a "control" pool of radioligand binding values, we have recorded a wide range of information (age, number of births, length of pregnancy, length of labor, drugs during labor, etc.) from each control and experimental delivery to help make reasonable comparisons and enable multiple analysis of variance following the collection of a large enough pool of data. We are not at present speculating on the functional significance of the apparent receptor down-regulation surrounding birth.

The major purpose of the present investigation was to compare the initial results of radioligand binding studies in placentas from substance-abusing women with those from "control" women. The hypothesis of this work being that the locus of action of the drugs of abuse for inducing the observed behavioral teratogenesis may be at specific receptor sites in the developing brain and that, in the absence of brain tissue, placental tissue receptor regulation may mirror brain receptor regulation. All of the drugs of interest (opiates, stimulants, sedative-hypnotics and hallucinogens) have specific, high-affinity interactions at various neurotransmitter receptors (e.g., μ - and κ -opiate, α_1 -, α_2 - and β -adrenergic; D_1 - and D_2 -dopaminergic, GABA, muscarinic cholinergic, and S_1 - and S_2 -serotonergic).

Our preliminary studies implied the presence of many neurotransmitter receptors in placental membranes [18]. The present studies established the presence of α_1 -, α_2 - and β -adrenergic and μ -opiate receptors in placental membranes from women abusing a variety of drugs during pregnancy. More important was the demonstration of many, often major, differences from "control" in the number of binding sites, but apparently not the affinity of radioligand for those binding sites in these substance-abuse placentas. Of prime importance, however, was the *lack* of consistent differences, implying that many superimposed variables can influence the number of binding sites observed. In this regard, for each substance-abusing mother, we have collected the dosage, schedules and period of the pregnancy during which the drug was administered and will, when populations are large enough, begin multiple analysis of variance. These preliminary results, however, confirm the hypothesis that prenatal exposure to drugs of abuse can alter the way in which placental neurotransmitter receptors are expressed. These alterations may or may not have functional significance; indeed, if similar alterations are taking place in the developing fetal brain, functional changes would be difficult to minimize.

In summary, these results provide preliminary evidence for the regulation of neurotransmitter receptors by the low level administration of drugs of abuse. Further investigation of this clearly complex human model may provide a very useful method by which both brain development and the molecular processes of receptor down-regulation may be studied.

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