Norepinephrine Transporters in Rat Placenta Labeled with [$^{3}$H]Nisoxetine$^{1}$

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ABSTRACT

Previous research has identified a norepinephrine (NE) transporter in brush-border membranes from human placental syncytiotrophoblastic cells. In the present study, we used the selective ligand [$^{3}$H]nisoxetine to demonstrate the presence of an NE transporter in rat placental membranes, determine the binding characteristics of the transporter and ascertain its localization by means of in vitro film and dry-emulsion autoradiography. Additional membrane binding studies were performed with [$^{3}$H]GBR 12935 to determine whether a dopamine transporter also was present in rat placenta. Saturation analyses carried out on washed membrane fractions from whole rat placentas at gestational day 20 showed saturable [$^{3}$H]nisoxetine binding (mean $K_{d}$ = 1.00 nM, $B_{max}$ = 1.24 pmol/mg of protein) but no saturable binding of [$^{3}$H]GBR 12935. When various monoamine uptake inhibitors were tested for their potency to inhibit placental [$^{3}$H]nisoxetine binding, the results supported the conclusion that the radioligand was labeling an NE transporter. Autoradiographic studies showed the presence of [$^{3}$H]nisoxetine binding in all three cellular zones of the rat placenta: the decidua, junctional zone and labyrinth. Binding was greatest in the junctional zone, particularly in the giant syncytiotrophoblastic cells. These findings indicate the presence of a high density of NE transporters in the late-gestation rat placenta. Catecholamine uptake probably has a multifunctional role in placental physiology, and blockade of the NE transporter by certain drugs such as cocaine may therefore contribute to the adverse effects of such compounds on pregnancy outcome and offspring development.

The placenta serves as a vital interface between the mother and fetus. Its functions include the transport of nutrients (e.g., amino acids and metabolic fuels) and oxygen from the maternal to the fetal circulation, elimination of waste products from the fetus, metabolism of some chemicals (including potential toxicants), protection of the fetus from maternal immune rejection and secretion of hormones such as chorionic gonadotropin and placental lactogen. Of particular interest to pharmacologists is the growing evidence that placental NE transport appears to be mediated by the NE transporter rather than by a specific DA uptake system (Ramamoorthy et al., 1993). Brush-border membranes are also capable of transporting DA, but such transport appears to be mediated by the NE transporter rather than by a specific DA uptake system (Ramamoorthy et al., 1992). Other evidence for placental NE uptake comes from the work of Bzoskie et al. (1995, 1997), who demonstrated that this organ contributes significantly to the clearance of catecholamines from the fetal circulation.

It is important to investigate further the properties of the placental NE transporter, not only because of its possible role in normal placental and fetal physiology (see Discussion) but also because it is a potential target of certain psychoactive drugs. For example, tricyclic antidepressants are potent inhibitors of NE uptake, and the NE transporter is also blocked by the abused psychostimulants cocaine and amphetamine. We selected the rat placenta as a model for studying the NE transporter and other neuronally-related markers. With respect to placental structure, both human and rat placentas belong to the general category of hemochorial placentas; that is, trophoblastic cells are in direct contact with maternal

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ABBREVIATIONS: DA, dopamine; DBH, dopamine b-hydroxylase; E, embryonic day; GD, gestational day; hCG, human chorionic gonadotropin; 5-HT, 5-hydroxytryptamine; NE, norepinephrine.
blood without an intervening endothelium (Leiser and Kaufmann, 1994). However, there are several structural differences between human and rat placentas. The human placenta is hemochorial, which means that there is only a single layer of trophoblastic cells between the maternal and fetal circulations. These cells form numerous villi that project into the maternal blood spaces, thereby facilitating the transfer of substances to and from the mother. It is also interesting to note that the trophoblasts of the human placenta do not form individual cells but rather constitute a syncytiotrophoblast (hence, the term “syncytiotrophoblasts”). In contrast, the rat placenta is of a hemochorial, labyrinth type (Leiser and Kaufmann, 1994). This denotes the presence of three layers of trophoblasts between the maternal and fetal circulations and also indicates that instead of forming villi, the chorion is laced with numerous channels containing either maternal blood or fetal capillaries. Interestingly, the outer layer of trophoblast (the layer in contact with maternal blood) is cellular, whereas the middle and inner layers appear to be syncytial (Enders, 1964). Thus, the rat placenta is not structurally identical to that of humans, but it nevertheless possesses the same types of cells and same basic hemochorial organization. This suggests that rats might serve as a useful model species for the investigation of placental pharmacology.

We investigated the pharmacological characteristics and localization of NE transporter binding sites in the GD 20 rat placenta. Saturation analyses were performed with the NE transporter-selective ligand [3H]nisoxetine to determine the density and affinity of putative NE transporters in whole rat placenta. Drug competition experiments were conducted with several monoamine reuptake inhibitors to characterize placental [3H]nisoxetine binding. In vitro film and dry-emulsion autoradiography were also performed to provide an anatomical and cellular localization of [3H]nisoxetine-labeled NE transporters. Finally, saturation analyses were also performed with the DA transporter-selective ligand [3H]GBR 12935 to determine whether specific binding of this compound could be detected in the rat placenta.

Materials and Methods

Animals. Sprague-Dawley albino rats were bred in our laboratory from a Charles River (Wilmington, MA) CD stock and housed under a 14:10-hr light/dark cycle (lights on at 6:00 a.m.) at an ambient temperature of 23° to 24°C. Food (Purina Rodent Chow, St. Louis, MO) and tap water were available ad libitum. Timed breedings were carried out by combining females (70–100 days of age) individually with stud males in large metal hanging cages. The first day a sperm plug was found was defined as GD 1. After mating, females were transferred into individual metal cages and periodically inspected for weight gain until they were killed on GD 20.

Tissue source and preparation. Placentas were obtained from pregnant dams on GD 20. This time point, which was near full term, yielded fully developed placentas and permitted comparison of the results with our previous studies of [3H]cocaine and [125I]RTI-55 binding sites in GD 20 fetal brain (Meyer et al., 1993; Shearman et al., 1996). Each dam was killed by decapitation, its uterus was exposed and the placentas were rapidly removed. After harvesting, each placenta was immediately frozen on dry ice and stored at −70°C for later use.

For each membrane-binding assay, tissue from four placentas (average weight, 0.4 g each) was pooled from two or three different animals. Frozen placentas were thawed, weighed and minced on a cold glass plate. The tissue was dispersed with a Polytron (Brinkmann Instruments, Westbury, NY) at setting 6 for 40 sec in 10 volumes of ice-cold assay buffer (see below for buffer composition) containing 0.25 M sucrose. The homogenate was brought up to 20 volumes and then centrifuged at 1000 × g for 10 min at 2°C. The supernatant was decanted into fresh tubes and centrifuged at 20,000 × g for 20 min. The resulting crude P2 pellet was resuspended in 20 volumes of assay buffer (without sucrose) and dispersed with the Polytron at setting 6 for 15 sec. After recentrifugation at 20,000 × g for 20 min, the pellet was resuspended in 20 volumes of assay buffer to yield its final dilution. Protein concentrations of each tissue preparation, which were determined with the Bradford dye-binding method using bovine γ-globulin as the standard (Bradford, 1976), averaged 0.5 mg/ml.

[3H]Nisoxetine saturation analyses. The selective NE uptake inhibitor [3H]nisoxetine [N-methyl-3-(o-methoxyphenoxy)-3-phenylpropylamine] (Wong et al., 1982; Wong and Bymaster, 1976), which has been used to study NE transporters in the rat brain (Tejani-Butt, 1992), was selected to label these sites in the rat placenta. Although Tris-containing buffers have previously been used with this radioligand, we performed a preliminary experiment to compare the amount of total and nonspecific binding observed with phosphate vs. Tris buffer with either a low or high sodium concentration. The best results were obtained with a buffer consisting of 10 mM NaHPO4, 120 mM NaCl and 5 mM KCl, pH 7.4, and this buffer was therefore used in all subsequent studies.

[3H]Nisoxetine binding assays were carried out as described by Tejani-Butt (1992), with minor modifications. An initial time course study was conducted to determine when binding reached equilibrium in placental tissue samples. Tissue (0.4 ml) was incubated with 1 nM [3H]nisoxetine in a final volume of 0.5 ml for either 30 min or 1, 2, 3, or 4 hr at 4°C. Parallel tubes also contained 1.0 μM mazindol to define nonspecific binding. Incubation mixtures were rapidly filtered through Whatman GF/B filters that had been presoaked with 0.05% polyethyleneimine in buffer. Filters were washed twice with 5 ml of ice-cold assay buffer, transferred into minivials and filled with 5.0 ml of scintillation cocktail (Opti-Fluor; Packard, Downers-Grove, IL). The following day, radioactivity was measured with a Packard 1900CA scintillation counter with a counting efficiency of ≈45%.

[3H]Nisoxetine saturation analyses were performed by incubating tissues as described above with 14 concentrations of [3H]nisoxetine (ranging from 0.1 to 12 nM) in triplicate. Three saturation experiments were conducted with placental tissue preparations from different subjects.

Drug competition experiments. To clarify whether [3H]nisoxetine was indeed labeling an NE transporter in the placenta, drug competition experiments were performed to pharmacologically characterize this binding site. Drugs tested included the NE uptake inhibitors nisoxetine, desipramine and nomifensine; the DA uptake inhibitors GBR 12909 and bupropion; the 5-HT uptake inhibitor O-620 (O-620), which was developed as a potential selective DA uptake inhibitor (Meltzer et al., 1993). The novel benztropine and cocaine analog difluoropine (O-620) was selected to label these sites in the rat placenta. Although Tris-containing buffers have previously been used with this radioligand, we performed a preliminary experiment to compare the amount of total and nonspecific binding observed with phosphate vs. Tris buffer with either a low or high sodium concentration. The best results were obtained with a buffer consisting of 10 mM NaHPO4, 120 mM NaCl and 5 mM KCl, pH 7.4, and this buffer was therefore used in all subsequent studies.

[3H]Nisoxetine uptake studies were conducted by incubating tissue (0.4 ml) with a fixed concentration of [3H]nisoxetine (2.0 nM) in the presence of 14 concentrations of each displacing drug. Three separate experiments were performed with placental tissue preparations from different subjects.

In vitro autoradiography. Localization of placental [3H]nisoxetine binding sites was determined using both film and dry-emulsion autoradiography. GD 20 placentas were transversely sectioned at 20...
μm in a cryostat, thaw-mounted onto gelatin/chrome alum-subbed slides, dried at ~0°C under reduced pressure for 2 hr and then stored with dessicant for no more than a few days at ~70°C before use. [3H]Nisoxetine in vitro autoradiography was performed according to the procedure of Tejani-Butt (1992), except for a change in buffer. Sections of placenta were incubated with 3.0 nM [3H]nisoxetine in the buffer described above for 4 hr at 4°C. For some sections, the incubation medium also contained 1.0 μM mazindol to define nonspecific binding. After the incubation, sections were washed three times in ice-cold buffer for 5 min each, dipped briefly in cold deionized water and then quickly dried under a stream of cool air.

Film autoradiography was first carried out to obtain information on the gross distribution of [3H]nisoxetine binding sites in the placenta. For this purpose, the slides were placed in cassettes and exposed to tritium Hyperfilm (Amersham, Arlington Heights, IL) in total darkness. Cassettes were stored at room temperature for 3 weeks and then developed with Kodak D-19 for 4 min at 22°C. Film images were captured and digitized using an imaging system described previously (Shearman et al., 1996). Tissue sections were stained with hematoxylin and eosin for histological examination.

Dry-emulsion autoradiography was subsequently performed in an attempt to ascertain the cellular localization of the binding sites. However, instead of using the classic emulsion-coated coverslip technique in which the coverslip is glued to the slide (Young and Kuhar, 1979), we chose a recently reported variation of the procedure in which a piece of adhesive-backed, Teflon-reinforced aluminum foil serves as a flexible hinge that allows the coverslip to swing away from the slide to facilitate eventual emulsion development and tissue staining (Rhodes et al., 1993). Briefly, adhesive-backed aluminum foil (0.3 mil; Cole Parmer, Chicago, IL) was attached to the bottom, end and first 3 to 5 mm of unfrosted slides bearing the [3H]nisoxetine-labeled placental sections. Coverslips (25 × 75 mm) were acid-washed, dried and dipped into nuclear-track emulsion (Kodak NTB-2, diluted 1:1 with high-performance liquid chromatography-grade water) under sodium-vapor safe light conditions. Emulsion-coated coverslips were dried overnight, attached to the slides with the foil hinges and clamped on with a binder clip. The assemblies were packaged in slide boxes, wrapped light-tight and exposed in the dark for 6 weeks at 4°C. The coverslips were then separated from the slides and developed in Kodak D-19 (diluted 1:1 with water) for 4 min at 22°C, rinsed in distilled water for 10 sec and then fixed without hardener for 5 min. Custom-made Plexiglas chambers were used to process the coverslips without immersing the tissue sections in the developing and fixing reagents (see Rhodes et al., 1993). The tissue was then fixed in 10% formalin for 2 hr, washed twice for 5 min in water and left to dry overnight. The following day, the sections were stained, and the coverslips were attached to the slides with Permount.

Before examination of the slides, the dried emulsion was scraped off the top of each coverslip. For preliminary localization of cellular binding sites, silver grains were visualized under darkfield illumination using the method of Richfield (1991), as modified by Collins and Meyer (1996). As described in these papers, 0.75 μM transfuraptenolt was added to the incubation medium to block binding of the ligand to nontransporter piperazine acceptor sites, and nonspecific binding was defined with 25 μM mazindol.

Materials. Cocaine HCl and desipramine were obtained from Sigma Chemical (St. Louis, MO). Mazindol, bupropion HCl, GBR 12909, 1-[2-bis-(4-fluorophenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine dihydrogen chloride, nomifensine and zimelidine dihydrochloride were purchased from Research Biochemicals (Natwick, MA). The following drugs were generously donated: nisoxetine HCl (Eli Lilly Research Laboratories, Indianapolis, IN), cilostazol HBr (H. Lundbeck/Amersham, Copenhagen, Denmark), difluoropine (O-620) (Dr. Bertha K. Madras, New England Regional Primate Research Center, Southborough, MA; and Dr. Peter Meltzer, Organon, Inc., Woburn, MA) and RTI-55 [β-(4-iodophenyl)-tropane-2-carboxylic acid methyl ester] (Dr. F. Ivy Carroll, Research Triangle Institute, Research Triangle Park, NC). [3H]Nisoxetine (78.4 and 80.6 μCi/mmol) and [3H]GBR 12935 [1-β-(2-fluorophenyl)ethyl]-4-(3-phenylpropyl)piperazine (44.5 μCi/mmol) were purchased from Du Pont-New England Nuclear (Boston, MA) and stored at ~20°C. [3H]GBR 12935 was diluted in ethanol on receipt. All other chemicals used were of analytical grade.

Data analysis and statistics. Data from the saturation experiments were analyzed and subjected to nonlinear curve-fitting using EBDA/LIGAND software (Biosoft, Ferguson, MO) to obtain representative binding isotherm and the corresponding Scatchard transformation were plotted by means of Prism graphics software (GraphPad Software, San Diego, CA). Lundon ReceptorFit Competition software (Lundon, Chagrin Falls, OH) was used to analyze data from the [3H]nisoxetine drug competition studies. Statistical analyses were performed with InStat (GraphPad Software).

Results

Saturation analyses. Results from the initial time course study indicated that [3H]nisoxetine binding reached equilibrium within 3 hr and remained stable for up to 6 hr (data not shown). Therefore, incubations for all subsequent membrane-binding studies with this radioligand were carried out for 3 or 4 hr.

EBDA/LIGAND analysis of the saturation experiments showed that [3H]nisoxetine bound to a single population of binding sites in rat placenta with a Kd value of 1.00 ± 0.09 nM (mean ± S.E.M.) and a Bmax value of 1.24 ± 0.07 pmol/mg of protein (n = three separate experiments). The binding isotherm and Scatchard plot from a representative experiment are presented in figure 1.

In contrast, no saturable (specific) binding was detected in two experiments with rat placental membrane fractions using the DA transporter-selective ligand [3H]GBR 12935 (data not shown).

Drug competition experiments. Drug competition experiments were carried out to investigate the pharmacological profile of [3H]nisoxetine binding to rat placenta. As shown in figure 2 and table 1, drugs with a high affinity for NE uptake sites such as desipramine, nisoxetine and nomifensine were the most potent displacers of [3H]nisoxetine binding to rat placental membranes. Among the cocaine-related compounds, cocaine itself was the least potent inhibi-
tor of rat placental [3H]nisoxetine binding; the novel benztrpine and cocaine congener difluoropine was approximately twice as potent as cocaine and RTI-55 was by far the most potent. Finally, selective 5-HT or DA uptake inhibitors (i.e., citalopram, zimelidine, GBR 12909 and bupropion) were the least potent inhibitors of [3H]nisoxetine binding.

In vitro autoradiography. Davies and Glasser (1968) identified three distinct cellular zones in the rat placenta: (1) the decidua basalis, which is the maternal contribution to the placenta and is reduced to a very thin layer during the late stages of pregnancy; (2) the basal (also called junctional) zone, which is adjacent to the decidua; and (3) the labyrinth. The decidua and the junctional zone contain only maternal blood vessels, whereas the labyrinth functions as the zone of interaction between the fetal and maternal circulations. In the present study, [3H]nisoxetine autoradiography demonstrated substantial NE transporter binding in all three zones, although the binding was clearly greatest in the junctional zone (fig. 3, top). Mazindol (1.0 μM) blocked most of the [3H]nisoxetine binding, leaving a low and relatively homogeneous level of nonspecific binding (fig. 3, bottom).

The dry-emulsion autoradiography confirmed the film results and further demonstrated that NE uptake sites are present on several different cell types, including various individual cells, small trophoblastic cells in the labyrinth and giant trophoblastic cells in the junctional zone. For the purpose of quantitative analysis and presentation, nonspecific grain densities (which averaged 13% of the total grain density values) were subtracted from the total density values. Although these findings must be considered preliminary due to the small sample size, the data show that the heaviest [3H]nisoxetine labeling was seen over the giant trophoblastic cells of the junctional zone, whereas somewhat lower grain densities were observed over cells in the decidua and labyrinth (table 2). Statistical analyses were carried out by first subjecting the grain density values to a log transformation (because of the unequal sample variances) and then performing paired Student's t tests comparing each placental area. Paired tests were considered appropriate given that density measurements for all areas were taken from the same placentas. The results of these analyses indicated a statistically significant difference between the junctional zone and the labyrinth (t = 60.08, P = 0.01), a nonsignificant trend toward a difference between the junctional zone and decidua.
Quantification of regional placental \(^{3}H\)nisoxetine binding (3.0 nM) by grain counting

Silver grains in the dry-emulsion autoradiograms were counted as described in Materials and Methods. For presentation purposes, specific binding data were derived by subtracting grain densities associated with nonspecific binding (defined with 1.0 \(\mu M\) mazindol; four sections per placenta) from the total binding data (four or six sections per placenta). The results were pooled across sections to yield a single grain density value for each region of analysis in each placenta. The values shown are the mean and (in parentheses) individual grain densities obtained from two normal GD 20 placentas.

<table>
<thead>
<tr>
<th>Placental zone</th>
<th>Grain density</th>
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<tbody>
<tr>
<td>Decidua</td>
<td>220 (183, 256)</td>
</tr>
<tr>
<td>Junctional zone (giant cells)</td>
<td>391 (391, 391)</td>
</tr>
<tr>
<td>Labyrinth</td>
<td>271 (269, 273)</td>
</tr>
</tbody>
</table>

* Significantly different from the junctional zone (\(P < .05\)) by paired \(t\) test.

and no difference between the decidua and labyrinth. Examination of the data suggests that the higher variability in grain density for the decidua probably accounts for the lack of a statistically significant difference between this area and the junctional zone.

**Discussion**

To our knowledge, the present study is the first to investigate the NE transporter in rat placenta and the first to image any placental transporter by autoradiography. Membrane-binding studies revealed a high level of \(^{3}H\)nisoxetine binding with the characteristics expected of an NE transporter. In the autoradiographic experiments, we observed NE transporter binding in all cellular zones of the placenta, although its distribution was not uniform across these zones.

\(^{3}H\)Nisoxetine bound with high affinity to a single population of sites with a \(K_d\) value similar to the 0.7 nM value reported for rat cortical membranes by Tejani-Butt (1992). In contrast, the affinity of human placental brush-border membranes for \(^{3}H\)nisoxetine was >10-fold lower (mean \(K_d = 13.8\) nM) (Jayanthi et al., 1993). This disparity may reflect a species difference in NE transporter binding characteristics, although a comparison of neural tissues would be desirable in order to confirm this hypothesis. The rat placenta was found to express a high density of NE uptake sites. The mean \(B_{\text{max}}\) value of 1.24 pmol/mg of protein is >4-fold greater than the \(B_{\text{max}}\) value reported for rat frontoparietal cortical tissues and is not much lower than the amount of binding found in the locus ceruleus of rat brain sections incubated with 3.0 nM \(^{3}H\)nisoxetine (Tejani-Butt, 1992). This finding implies that rat placental cells may avidly take up NE and EPI, perhaps from both the maternal and fetal plasma (see below). Furthermore, because both the rat and human NE transporters readily transport DA (Di Chiara et al., 1982; Ramamoorthy et al., 1992), it is possible that some uptake of plasma DA also occurs.

In contrast to the results obtained with \(^{3}H\)nisoxetine, membrane-binding assays using the DA transporter ligand \(^{3}H\)GBR 12935 revealed no specific binding. Based on these results, it is likely that few if any DA transporters are present in the rat placenta. Nevertheless, we cannot rule out the possibility of a very low level of DA transporter expression (which might not be detected against the background of nonspecific binding) or higher levels of expression in a very limited population of cells.

The rank order of potency of various monoamine uptake blockers to inhibit \(^{3}H\)nisoxetine binding to the rat placental NE transporter paralleled that found in human placenta (Jayanthi et al., 1993), except that the compounds were up to 11 times more potent in rat placenta. These results support the previously mentioned idea of a species difference in NE transporter pharmacology. As previously shown for the human placenta (Jayanthi et al., 1993), rat placental \(^{3}H\)nisoxetine binding sites are sensitive to inhibition by cocaine and the potent cocaine congener RTI-55, as well as by the classical antidepressant desipramine and the atypical, NE-selective antidepressants nisoxetine and nomifensine. On the other hand, selective inhibitors of either 5-HT (citalopram and zimelidine) or DA (GBR 12909 and bupropion) uptake showed low affinities for placental \(^{3}H\)nisoxetine binding sites. Combined with the results from the saturation analyses, these findings are fully consistent with the view that \(^{3}H\)nisoxetine is labeling an NE transporter in the rat placenta.

Some of the compounds examined in the drug competition study were previously tested for their ability to inhibit radio-labeled NE uptake by HeLa cells transfected with a human NE transporter cDNA (Pacholczyk et al., 1991). Although estimated \(K_I\) values were reasonably similar for desipramine, nomifensine and citalopram, cocaine and particularly GBR 12909 exhibited much less potency in the present study than in the experiments of Pacholczyk et al. (1991). Because GBR 12909 is a very weak inhibitor of NE uptake by brush-border membrane vesicles prepared from human synaptotrophic blast cells (Ramamoorthy et al., 1993), it seems likely that these disparities are at least partly related to methodological differences between membrane binding studies using a synthetic radioligand and studies of neurotransmitter uptake by living cells. Another possibility is that NE transporter pharmacology is modulated \textit{in vivo} by phosphorylation or other regulatory processes.

It is noteworthy that both film and dry-emulsion autoradiography revealed evidence of NE uptake sites in all areas of the placenta. A particularly high density of sites was
observed in giant trophoblastic cells of the junctional zone. Because previous studies of the human placental NE uptake system used membrane fractions enriched in syncytiotrophoblast brush-border membranes, the results are the first evidence that the giant cells, which serve as important hormone-producing cells in the placenta (e.g., see Soares et al., 1991), also possess NE transporters. This finding not only indicates that the NE transporter is present in more than one cell type in the rat placenta but it also implies that NE uptake may play multiple roles in placental functioning.

Because the animals were not perfused before tissue harvesting, blood cells were undoubtedly present in the placental sections and also contributed to the membrane preparations. Nevertheless, it is unlikely that blood elements contributed significantly to the [3H]nisoxetine binding. Although catecholamines are taken up by both erythrocytes and lymphocytes, such uptake is thought to occur by way of a choline transporter and a 5-HT transporter, respectively (Azou et al., 1996; Faraj et al., 1994).

The functional significance of placental NE uptake is currently a matter of conjecture. Given that NE transporters are present at a high density in the syncytiotrophoblast brush-border membranes of the human placenta, Ganapathy and coworkers (Ganapathy and Leibach, 1995; Ramamoorthy et al., 1993) hypothesized that one function of NE uptake from the maternal blood is the maintenance of a low concentration in the intervillous space. These investigators argued that because catecholamines exert constrictive effects on vascular smooth muscle, reducing catecholamine levels in the intervillous space could be important in maintaining adequate circulation within the uteroplacental vascular bed. Placental catecholamine uptake probably has little effect on the tone of the chorial arteries because these vessels are upstream from the intervillous space (Ramsey, 1962). On the other hand, the downstream vessels (i.e., the chorionic veins that collect blood draining from the intervillous space) are also ensheathed by smooth muscle and exhibit a contractile response to NE (Maigaard et al., 1986; Reviriego et al., 1990). Hence, brush-border membrane NE uptake may be important in reducing the resistance of these vessels, thereby ensuring adequate maternal blood flow through the placenta.

It is likely that the placental NE transporter serves other roles in addition to removal of catecholamines from the intervillous space. For example, there is growing evidence that the placenta actively removes catecholamines from the fetal circulation (Bzoskie et al., 1995; 1997; Jones, 1980). This is consistent with the possibility that syncytiotrophoblast NE uptake sites are present not only in the maternal-facing brush-border membrane but also in the fetal-facing basal membrane. One reason why placental catecholamine clearance might be important is to protect the fetal cardiovascular system from the potentially harmful effects of high levels of these compounds (Bzoskie et al., 1995; 1997). Moreover, catecholamines exert vasoconstrictive effects on umbilical arteries (Dyer, 1970; Nair and Dyer, 1974), thereby reducing blood flow from the placenta to the fetus. The fetal plasma is probably the main source of catecholamines that reach umbilical smooth muscle, as the umbilical cord is not innervated (Fox and Khong, 1990; Walker and McLean, 1971).

Two other possible functions of placental NE uptake can be hypothesized. The first involves transport of NE to the fetus during early development, before maturation of the fetal sympathoadrenal system. Most of the catecholamines taken up by the placenta are metabolized by the enzymes monoamine oxidase and catechol-O-methyltransferase (Chen et al., 1974; DeMaria, 1964). Nevertheless, several early studies reported that small but detectable amounts (typically 5–10%) of unmetabolized NE were transferred from the maternal to the fetal side of the human and guinea pig placentas (Morgan et al., 1972; Saarikoski, 1974; Sandler et al., 1963; Sodha et al., 1984). Although it has previously not been clear whether this placental transport plays a role in fetal development, recent work by Thomas et al. (1995) has shed new light on this question. These investigators studied mutant mice lacking expression of DBH, the enzyme that catalyzes the synthesis of NE from DA. In pregnant female mice homozygous for the disrupted DBH gene, all homozygous offspring died in utero by E13.5. Lethality was shown to be related to the absence of NE because survival was enhanced after treatment with dihydroxyphenylserine, a compound that is converted to NE through a DBH-independent pathway. Moreover, analysis of whole-body catecholamines in wild-type fetuses showed a marked rise in NE levels from E10.5 to E13.5, whereas EPI remained consistently low during this period. Most importantly for the present discussion, the survival of homozygous mutant fetuses carried by heterozygous mothers (who expressed some DBH activity) was enhanced over that of mutant fetuses carried by homozygous mothers. This enhancement was noted as early as E11.5 and continued throughout pregnancy such that 12% of homozygous offspring from heterozygous mothers survived to term. Thomas and coworkers interpreted these results to indicate rescue of some fetuses by placental transferred NE. Such an interpretation is supported by the additional finding that whole-body NE was undetectable in homozygous E11.5 fetuses carried by homozygous mothers, whereas small but measurable NE levels were observed in homozygous fetuses from heterozygous mothers. We hypothesize that even in normal subjects, there may be a significant developmental role for placentally transported catecholamines during early development, before the fetal sympathoadrenal system begins to synthesize and secrete significant amounts of these substances. As fetal development proceeds and circulating catecholamine concentrations begin to rise, the role of the placenta may shift from that of transfer to clearance, thereby protecting the fetus from possible deleterious effects of these compounds.

Finally, another possible function of the placental NE transporter may be to regulate the availability of catecholamines to stimulate placental beta adrenergic receptors. Various studies have demonstrated the presence of beta receptors as well as a beta receptor-sensitive adenyl cyclase in the human placenta (see Strauss et al., 1992). In the syncytiotrophoblastic cells, this signaling system has been found in the basal but not the brush-border membrane (Matsumura et al., 1987; Whitsett et al., 1979, 1980). It seems possible that beta adrenergic receptors in this location could be stimulated either by fetal catecholamines or (hypothetically) by catecholamines taken up from the maternal blood spaces and released in a paracrine manner on the fetal-facing side of the syncytiotrophoblasts. In either case, NE transporters on the fetal side of the placenta are well-positioned to regulate catecholamine concentrations in the vicinity of these receptors, which may be important in light of the fact that the
beta receptor/adenyl cyclase system is known to stimulate the secretion of placental hormones such as hCG (Grullon et al., 1995; Nulsen et al., 1988; Oike et al., 1989).

A number of psychoactive drugs bind to and inhibit the NE transporter, including the abused drugs cocaine, amphetamine and methamphetamine, as well as certain antidepressant medications. The presence of a high density of NE uptake sites in the placenta therefore raises concerns that maternal exposure to such drugs could adversely affect pregnancy outcome and/or offspring development (Bzowski et al., 1997; Jayanthi et al., 1993; Ramamoorthy et al., 1993, 1995a). Maternal cocaine exposure did not produce any gross alterations in the placental perfusion system (Simone et al., 1997; Jayanthi et al., 1993; Ramamoorthy et al., 1993, 1995a). Further studies are needed to determine whether this effect is related to the influence of cocaine on placental NE uptake.

In summary, the present study has shown that the human placenta, the rat placenta contains a high density of [3H]norisotrine-labeled NE transporter sites. These binding sites are found throughout the placenta and appear to be present on both syncytiotrophoblastic and giant trophoblastic cells. The significance of placental NE uptake is still unknown; however, it seems likely that this process plays a multifunctional role in both placental physiology and fetal development.

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References


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