Relationship between $^{[125}I]RTI-55$-labeled cocaine binding sites and the serotonin transporter in rat placenta

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Relationship between $^{[125}I]RTI-55$-labeled cocaine binding sites and the serotonin transporter in rat placenta. Am. J. Physiol. 275 (Cell Physiol. 44): C1621–C1629, 1998.—We investigated the characteristics of cocaine-like binding sites in rat placenta using $^{[125}I]RTI-55$, $^{[3}H]paroxetine$ binding and immunocytochemical staining for serotonin $5$-hydroxytryptamine (5-HT)) and for the 5-HT transporter were also used to obtain evidence for rat placental 5-HT uptake. $^{[125}I]RTI-55$ saturation analyses with membranes from normal gestational day 20 placentas yielded curvilinear Scatchard plots that were resolved into high- and low-affinity components (mean dissociation constants of 0.29 and 7.9 nM, respectively). Drug competition studies with various monoamine uptake inhibitors gave rise to complex multiphasic displacement curves, although the results obtained with the selective 5-HT uptake inhibitor citalopram suggest that the 5-HT transporter is an important component of placental high-affinity $^{[125}I]RTI-55$ binding. The presence of a rat placental 5-HT uptake system was additionally supported by the $^{[3}H]paroxetine$ binding experiments and by the presence throughout the placenta of immunoreactivity for 5-HT and the 5-HT transporter. Immunostaining with both antibodies was most intense in the junctional zone, whereas the density of $^{[125}I]RTI-55$ binding sites was greater in the placental labyrinth. This discrepancy may be due to the fact that $^{[125}I]RTI-55$ appears to be labeling additional cellular components besides the 5-HT transporter. The presence of cocaine- and antidepressant-sensitive 5-HT transporters in the placenta has important implications for the possible effects of these compounds on pregnancy and fetal development.

Studies by Ganapathy and colleagues (15) have indeed demonstrated the presence of both 5-HT and NE transporters in brush-border membranes isolated from human placental syncytiotrophoblastic cells. The structure of the cloned human placental 5-HT transporter is identical to those found in human platelets and brain (30). Moreover, the placental transporter is similar to the platelet and neuronal transporters in terms of ion dependence and pharmacology. It is sodium and chloride dependent, and it is inhibited by imipramine, 5-HT-selective antidepressants such as fluoxetine, and cocaine (15).

Study of the physiology and pharmacology of placental monoamine transporters would benefit greatly from the development of appropriate animal models. In this regard, Padbury and co-workers (28) have recently cloned ovine placental transporters for 5-HT and NE, and have also begun investigating the physiological role of placental catecholamine clearance from the fetal circulation (8). We have chosen to focus instead on the rat placenta, which is structurally more similar to the human placenta than is the sheep placenta (22). Furthermore, much is known about the effects of prenatal cocaine exposure on rat neurobehavioral development, and some of these effects could be secondary to a drug-induced inhibition of monoamine uptake by the placenta (25).

Monoamine transporters can be labeled by structurally diverse compounds, including cocaine and its congeners as well as various non-cocaine-like drugs. Although the sites labeled by these different compounds may overlap, they are not identical either at the level of the transporter protein (3) or in terms of neuroanatomic distribution (39). Hence, a full understanding of the ability of cocaine to influence placental function requires the pharmacological characterization and localization of cocaine-associated binding sites in this tissue. For this purpose, we chose the potent cocaine congener 3-[4-iodophenyl]tropane-2-carboxylic acid methyl ester (RTI-55), which we previously used to characterize cocaine-like binding sites in fetal and adult rat brain (32). Both membrane binding and autoradiographic techniques have shown that $^{[125}I]RTI-55$ labels 5-HT and DA transporters in rat (5, 32), monkey (20), and human (36) brain. Like the human placenta (15), the rat placenta appears to have few, if any, specific DA uptake sites (33). Consequently, we anticipated that $^{[125}I]RTI-55$ would selectively label the 5-HT transporter (if present) in this tissue. One disadvantage of this ligand (compared with cocaine itself) is its insensitivity to the NE transporter at low concentrations. For
Experiments were also conducted with the 5-HT transporter to determine the density and affinity of \[125\text{I}\]RTI-55 binding sites in the rat placenta (33).

MATERIALS AND METHODS

In the present study, saturation analyses were performed to determine the density and affinity of \[125\text{I}\]RTI-55-labeled cocainelike binding sites in membranes prepared from whole rat placenta. Additionally, we characterized these binding sites by measuring the potencies of various monoamine uptake blockers to inhibit \[125\text{I}\]RTI-55 binding. In contrast to previous studies, which primarily focused on transporter biochemistry with the use of cultured cell lines, brush-border membrane vesicle preparations, and tissue homogenates, we used in vitro autoradiography to determine the localization of \[125\text{I}\]RTI-55 binding sites. Experiments were also conducted with the 5-HT transporter-selective ligand \[3\text{H}\]paroxetine (24) to confirm the presence of placental 5-HT transporters. Finally, immunocytochemistry for 5-HT and the 5-HT transporter was performed to visualize cellular sites of presumptive 5-HT uptake in the rat placenta.

MATERIALS AND METHODS

Materials. Cocaine HCl, desipramine, and benztoprine methane-sulfonate were obtained from Sigma Chemical (St. Louis, MO); Mazindol, clonopine HCl, imipramine HCl, and zimelidine dihydrochloride were purchased from Research Biochemicals (Natick, MA). The following drugs were generously donated: nisoxetine HCl was acquired from Eli Lilly Research Laboratories (Indianapolis, IN); citalopram HBr was supplied by H. Lundbeck/Amersham (Copenhagen, Denmark); unlabeled RTI-55 was generously provided by Dr. F. Ivy Carroll (Research Triangle Institute, Research Triangle Park, NC). N-[1-(2-benzo(b)thiophenyl)cyclohexyl]piperydine (BTCP) HCl was provided by Research Biochemicals, as part of the chemical synthesis program of the National Institute of Mental Health [Contract 278-90-0007 (BS)]. \[125\text{I}\]RTI-55 (sp act 2,200 Ci/mmol) and \[3\text{H}\]paroxetine (sp act 20 Ci/mmol) were purchased from DuPont NEN (Boston, MA) and stored at −20°C. \[125\text{I}\]RTI-55 and \[3\text{H}\]paroxetine were diluted in ethanol on receipt. All other chemicals used were analytical grade.

Tissuetsource and preparation. Sprague-Dawley albino rats were bred in our laboratory from Charles River (Wilmington, MA) CD stock and kept under a 14:10-h light-dark cycle (lights on at 0600) at 23°C. Food (Purina Rat Chow) and tap water were available ad libitum. Timed breedings were carried out by placing 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 gestational day (GD) 1. After mating, females were transferred into individual metal cages and inspected for weight gain until they were killed on GD 20 for harvesting of placentas. For the membrane binding and autoradiographic studies, each dam was decapitated, its uterus was exposed, and the placentas were immediately removed. The placentas were frozen over powdered dry ice and stored at −70°C for later use.

For each membrane binding assay, four placentas (average wt ∼ 0.4 g each) were pooled from two or three different females. Frozen placentas were thawed, weighed, and minced on a cold glass plate. Tissue was dispersed with a Polytron (Brinkmann Instruments, Westbury, NY) at setting 6 for 40 s in 10 volumes of ice-cold 10 mM sodium phosphate buffer (pH 7.4) containing 0.25 M sucrose. Tissue was brought up to 20 volumes after homogenization and then centrifuged in 50-mL Sorvall tubes in an SS34 rotor at 1,000 g for 10 min. The supernatant was decanted into fresh tubes and centrifuged at 20,000 g for 20 min. The crude P2 pellet was resuspended in 20 volumes of phosphate buffer (without sucrose) and dispersed with a Polytron at setting 6 for 15 s. The tissue was recentrifuged at 20,000 g for 20 min, and the pellet was resuspended in 20 volumes of phosphate buffer to yield its final dilution. The Bradford dye-binding method (6) was used to determine the protein concentration of each tissue preparation (∼0.5 mg/ml).

\[125\text{I}\]RTI-55 membrane binding assays. Saturation analyses with \[125\text{I}\]RTI-55 were conducted according to the procedure described in Shearman et al. (32). Briefly, membranes were incubated with a fixed concentration of 10 µM \[125\text{I}\]RTI-55 along with 15 concentrations of unlabeled RTI-55 (0.3 µM to 100 nM) in 10 mM sodium phosphate buffer (pH 7.4; total volume 0.5 ml). Incubations were carried out at room temperature (22°C) for 50 min with gentle shaking. Nonspecific binding was defined with 50 µM cocaine in parallel assay tubes and subtracted from total binding to yield specific binding. Reactions were terminated by rapid filtration of samples through polyethyleneimine-pretreated Whatman GF/B glass fiber filters. The filters were washed three times with 5 ml ice-cold assay buffer, and radioactivity was assessed with a Packard 1900 CA liquid scintillation spectrometer at a counting efficiency of 71%. Four separate saturation analyses were carried out with different tissue pools.

\[125\text{I}\]RTI-55 drug competition experiments. Drug displacement studies were performed to determine the potency of nine monoamine uptake inhibitors to displace \[125\text{I}\]RTI-55 (10 µM) binding to rat placental membranes (32). Each unlabeled drug was tested at 12 concentrations ranging from 1 nM to 100 µM, and each experiment was replicated 3 or 4 times. Binding determinations were performed as above, and results were expressed as the percentage of total binding remaining in the presence of the displacer.

\[3\text{H}\]paroxetine membrane binding assays. Saturation experiments with \[3\text{H}\]paroxetine were performed based on a modification of the methods of Cool et al. (12) and Marcusson et al. (24). An initial buffer test was carried out in which \[3\text{H}\]paroxetine binding (0.2 nM \[3\text{H}\]paroxetine with and without 100 µM imipramine to define nonspecific binding) using the standard Tris-HCl buffer (in mM: 50 Tris, 120 NaCl, and 5 KCl, pH 7.4) was compared with the binding obtained with a sodium phosphate buffer (in mM: 10 Na2HPO4, 120 NaCl, and 5 KCl, pH 7.4). The sodium phosphate buffer yielded the best ratio of total to nonspecific binding, and therefore this buffer was used in all subsequent assays. For the saturation analyses, 0.4 ml of placental tissue was incubated with 10 concentrations of \[3\text{H}\]paroxetine (0.02–2 nM) in a total volume of 0.5 ml for 1 h at room temperature. Nonspecific binding was defined with 100 µM imipramine in parallel assay tubes and was subtracted from total binding to determine specific binding. Reactions were terminated by rapid filtration and washing as previously described. The counting efficiency for \[3\text{H}\]paroxetine was ∼45%. Four saturation analyses were performed with separate tissue pools. In addition, competition studies were carried out to determine the potencies of unlabeled cocaine (10 nM to 30 µM) and RTI-55 (1 nM to 1 µM) to displace \[3\text{H}\]paroxetine (0.3 nM) binding to rat parental membranes (n = 3 experiments for each drug).

\[125\text{I}\]RTI-55 in vitro autoradiography. The animals and tissue collection were the same as those described above. Twenty-micrometer placental sections were cut with a cryostat at −18°C in both the transverse (across the placental disc) and horizontal (parallel to the placental disc) planes.
Sections were thaw mounted onto gelatin-chrome alum-subbed slides, dried at -10°C under reduced pressure for 2 h, and then stored with desiccant for no more than a few days at -70°C before use. In vitro \[^{125}\text{I}\text{RTI-55}\] autodiography was carried out as described in Shearman et al. (32). Slide-mounted sections were incubated for 120 min at room temperature in 10 mM sodium phosphate buffer (pH 7.4) containing 10 pM \[^{125}\text{I}\text{RTI-55}\]. Nonspecific binding was determined with 100 \text{µM} cocaine. Sections were washed twice for 20 min with ice-cold buffer, dipped briefly in cold deionized water, and then rapidly dried with a stream of air. Slides were apposed to tritium Hyperfilm (Amer sham, Arlington Heights, IL) and developed after 5 or 7 days with Kodak D-19 developer. Film images were captured and digitized using a COHU camera, Northern Light lightbox, Macintosh PowerPC computer with a Scion video capture board, and Image 1.57 software (Dr. Wayne Rasband, National Institutes of Health).

Sections from quick-frozen placentas did not provide ideal histological material, even when fixed before staining. Consequently, we anesthetized several pregnant dams at GD 20 and perfused them transcardially with PBS followed by buffered 4% paraformaldehyde to fix the placentas. These placentas were subsequently cryoprotected in 30% sucrose, frozen, sectioned, and stained with hematoxylin and eosin to provide better material for the identification of different cell types.

Immunocytochemistry. 5-HT immunocytochemistry was performed using the experimental conditions recommended by the commercial supplier of the anti-5-HT antiserum (Incstar, Stillwater, MN). Two pregnant dams on GD 20 were deeply anesthetized with pentobarbital sodium and then perfused transcardially with 0.1 M sodium phosphate buffer (pH 6.5), 4% paraformaldehyde in 0.1 M phosphate buffer (pH 6.5), and finally 4% paraformaldehyde in 0.1 M borate buffer (pH 10.0). The maternal brains and placentas were removed, postfixed for 1.5 h at 4°C in 4% paraformaldehyde in 0.1 M borate buffer (pH 10.0), and then stored in cryoprotectant (30% sucrose in phosphate buffer) for at least 3–4 days until sectioning. For the 5-HT transporter immunocytochemistry, three pregnant dams on GD 20 were deeply anesthetized and then perfused transcardially with 0.1 M sodium phosphate buffer (pH 7.2) followed by 5% acrolein in 0.2 M phosphate buffer. Maternal brains and placentas were removed, postfixed for 2 h at 4°C, and then stored in cryoprotectant.

In both experiments, two placentas from each dam were sectioned, along with one of the brains for use as a positive control. Forty-micrometer frozen sections were cut in the transverse plane and collected in buffered 30% sucrose. The sections were rinsed three times in 50 mM Tris-buffered saline (TBS), pretreated with 1% sodium borohydride, and then rinsed four times in 50 mM TBS. Sections were then incubated for 20 min in blocking serum (1% hydrogen peroxide, 20% goat serum, and 1% BSA in TBS). In the first experiment, sections were incubated for 17 h at 4°C in four different dilutions (1:500, 1:1,000, 1:2,000, and 1:3,000) of a rabbit anti-5-HT antibody (Incstar). In the second experiment, sections were incubated for 72 h at 4°C in three different dilutions (1:500, 1:1,500, and 1:3,000) of an affinity-purified 5-HT transporter antibody (5-HTT-N) generated and characterized by Zhou and colleagues (41). Primary antibodies were prepared in TBS containing 0.1% gelatin, 0.02% sodium azide, and 0.02% Triton X-100 (pH 7.6 at 4°C). Additional placental tissue sections were processed without the primary antibody as a negative control. For a positive control, several maternal brains were cut, and sections were prepared that contained raphe nuclei for staining of serotonergic cell bodies or hypothalamic areas for staining of serotonergic fibers. Tissue sections were subsequently processed using the avidin-biotinylated enzyme complex (ABC) method (Vector Elite kit; Vector Laboratories, Burlingame, CA) and then reacted with 0.05% dianobenzidine and 0.05% hydrogen peroxide to visualize the immunoreactivity. Finally, the sections were mounted onto slides and coverslipped with Permount. Photomicrographs of selected areas were taken using a Nikon F3 camera mounted on a Nikon Labophot microscope. The resulting prints were scanned at high resolution, imported into Adobe Persuasion presentation software, and then recorded on T-Max 100 film using a Polaroid 3000 digital palette.

Data analysis and statistics. Data from the saturation experiments were analyzed by computerized nonlinear curve fitting using EBDA/LIGAND software (Biosoft, Ferguson, MO) to obtain \(K_d\) and \(B_{\text{max}}\) values for one- and two-site binding models. Lundon ReceptorFit Competition software (Lundon, Chagrin Falls, OH) was used to analyze data from the drug competition studies. Student’s \(t\)-tests were performed with InStat (GraphPad Software, San Diego, CA).

RESULTS

\[^{125}\text{I}\text{RTI-55}\] saturation experiments. Saturation analyses of \[^{125}\text{I}\text{RTI-55}\] binding to rat placental membranes consistently yielded curvilinear Scatchard plots (Fig. 1). Table 1 shows the results from both one- and two-site binding models and compares these results to previously published data from normal adult rat brain (32). In both tissues, the two-site model was preferred significantly over the one-site model (\(P < 0.05\)), even though the mean Hill coefficient for placental membranes was close to unity. Resolution of the binding isotherms into two components yielded a high-affinity site with a mean \(K_d\) of 0.29 nM and a low-affinity site with a \(K_d\) of 7.9 nM. The high-affinity \(K_d\) and \(B_{\text{max}}\) values for placental tissue are similar to those reported for adult rat brain using the same assay conditions (32). In contrast, the placental low-affinity site \(K_d\) is significantly lower than that found in adult brain, whereas...
the density of these sites (B_max) is approximately eight times greater.

Pharmacological characterization of [125I]RTI-55 binding sites. Drug displacement studies were carried out to investigate the pharmacological profile of [125I]RTI-55 binding to rat placental tissue. Displacement curves from representative experiments are shown in Fig. 2, and the numerical data are presented in Table 2. Based on IC50 values, cocaine and its congener benztpine were the most potent displacers of [125I]RTI-55 binding, followed by the catecholamine uptake inhibitors mazindol and desipramine. Other 5-HT and NE uptake inhibitors such as clomipramine, BTCP, citalopram, and desipramine all displayed a relatively weak ability to compete for [125I]RTI-55 binding sites in placental membranes. Except for cocaine, desipramine, and clomipramine, all displacers had relatively low Hill coefficients (<0.8), suggesting binding to multiple sites. This is confirmed by visual examination of the displacement curves, which in many cases are nearly shallow and multiphasic (Fig. 2).

The drug competition data thus support the results of the saturation analyses in suggesting that [125I]RTI-55 binds to more than one type of site in the rat placenta. Consequently, these data were reanalyzed using a two-site model. Not all displacement curves could be resolved into two sites, nor was the two-site model statistically preferred in all cases. Nevertheless, one striking finding from these analyses was that in three of four separate experiments, displacement curves for the potent and highly selective 5-HT uptake inhibitor citalopram yielded two sites with mean high- and low-affinity Michaelis-Menten inhibition constant (K_i) values of 2.1 and 3,341 nM, respectively. The high-affinity K_i value is similar to the reported affinity of citalopram for the rat 5-HT transporter (31), which is consistent with the hypothesis that one component of rat placental [125I]RTI-55 binding is to this transporter.

Table 2. Potencies of monoamine uptake inhibitors to inhibit [125I]RTI-55 (10 pM) binding to GD 20 placental membranes

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>IC50, nM</th>
<th>Hill Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>4</td>
<td>379 ± 45</td>
<td>0.95 ± 0.02</td>
</tr>
<tr>
<td>Benztpine</td>
<td>4</td>
<td>431 ± 111</td>
<td>0.76 ± 0.06</td>
</tr>
<tr>
<td>Mazindol</td>
<td>4</td>
<td>842 ± 157</td>
<td>0.44 ± 0.01</td>
</tr>
<tr>
<td>Desipramine</td>
<td>4</td>
<td>1,088 ± 302</td>
<td>0.92 ± 0.04</td>
</tr>
<tr>
<td>Clomipramine</td>
<td>4</td>
<td>1,517 ± 244</td>
<td>0.90 ± 0.06</td>
</tr>
<tr>
<td>BTCP</td>
<td>3</td>
<td>1,671 ± 119</td>
<td>0.77 ± 0.05</td>
</tr>
<tr>
<td>Citalopram</td>
<td>4</td>
<td>2,182 ± 601</td>
<td>0.60 ± 0.15</td>
</tr>
<tr>
<td>Zimelidine</td>
<td>3</td>
<td>2,601 ± 157</td>
<td>0.76 ± 0.05</td>
</tr>
<tr>
<td>Nisoxetine</td>
<td>3</td>
<td>4,296 ± 880</td>
<td>0.47 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE for no. of experiments indicated; 2 drugs were tested in each experiment, using a tissue pool derived from 4 placentas from 2 or 3 different dams. BTCP, N-[1-(2-benzo(b)thio-phenyl)cyclohexyl]piperidine.
fmol/mg protein (means ± SE). In drug displacement experiments with a fixed concentration (0.3 nM) of [3H]paroxetine, cocaine yielded a mean IC50 of 270 nM and a Ki of 67 nM. RTI-55 was much more potent than cocaine at inhibiting [3H]paroxetine binding, with mean IC50 and Ki values of 2.1 and 0.48 nM, respectively. Note that the estimated affinity (Ki, 0.48 nM) of RTI-55 for [3H]paroxetine-labeled placental 5-HT transporters is reasonably similar to its affinity (Ki, 0.29 nM) for the high-affinity binding component determined from the [125I]RTI-55 saturation analyses. On the other hand, there is a greater than twofold difference in mean Bmax values between [125I]RTI-55 and [3H]paroxetine. As discussed below (DISCUSSION), these findings suggest that high-affinity binding of [125I]RTI-55 to the rat placenta includes the 5-HT transporter but also one or more additional sites not labeled by more selective ligands.

Autoradiographic localization of [125I]RTI-55 binding sites. Examination of the autoradiograms revealed a heterogeneous distribution of [125I]RTI-55 binding to the placenta (Fig. 4). The decidua basalis and the junctional (also called basal) zone exhibited a relatively low degree of labeling, with the exception of a thin, heavily labeled layer. Microscopic examination of the tissue sections revealed that this heavy labeling corresponded to an area of maternal blood spaces containing large blood clots. In general, the labyrinth was more heavily labeled than the other regions of the placenta. There was also an unexpected gradient within the labyrinth such that labeling was more intense toward the maternal side (nearest the junctional zone) and less intense toward the fetal side (nearest the chorionic-allantoic plate). This gradient can be seen most clearly in transverse sections (Fig. 4C). Cocaine (100 µM) occluded essentially all [125I]RTI-55 binding, leaving nearly invisible film images (Fig. 4B).

Immunocytochemistry. Under the conditions of the present study, optimum immunostaining was observed with antibody dilutions of 1:1,000 or 1:2,000 for the 5-HT antibody and 1:1,500 for the transporter antibody. Tissue sections exposed to these dilutions were therefore used for analysis of the results and for presentation purposes. The patterns of placental staining obtained with the 5-HT and 5-HT transporter antibodies were quite similar. In both cases, immunoreactivity was found in all zones of the placenta, although the most intense staining tended to be seen in the junctional zone (Fig. 5, A and B, and Fig. 6, A and B). Within this area, many small trophoblastic cells and an occasional giant cell (Fig. 6C) showed strong staining. In the first set of placentas studied, we also frequently observed 5-HT-like immunoreactivity in blood cells and presumed clots within maternal blood spaces, presumably due to either nonspecific peroxidase activity (in the case of erythrocytes) or the presence of a 5-HT transport system (in the case of blood platelets). Blood cells and clots were not seen in the placentas used for the 5-HT transporter immunostaining, probably because of more efficient clearance of blood during the perfusions. Staining with both antibodies was found throughout the labyrinth, although it clearly had a heterogeneous appearance (Figs. 5C and 6D). Such heterogeneity could be associated with the different types of cells within the labyrinth; however, this hypothesis is difficult to validate with the present material and may require double staining with antibodies that can distinguish among these various cell types. In the absence of the 5-HT primary antibody, there was a low level of staining in the junctional zone but virtually none in the labyrinth (Fig. 5D). Essentially no staining occurred at
all in the second set of placental sections when the 5-HT transporter antibody was omitted (not shown). Finally, staining of brain sections as a positive control revealed immunoreactivity for both 5-HT and the 5-HT transporter in cells of the raphe nuclei and in fibers of brain areas known to contain 5-HT (e.g., hypothalamus; not shown).

DISCUSSION

These studies demonstrate that the rat placenta expresses a 5-HT transporter that can be labeled either with \[^{125}\text{I}]\text{RTI-55}\), a potent cocaine congener, or with \[^{3}\text{H}]\text{paroxetine}\), a selective 5-HT uptake-inhibiting antidepressant drug. Similar to previous findings with cocaine or its analogs (5, 23, 32), \[^{125}\text{I}]\text{RTI-55}\) binding to rat placental membranes yielded curvilinear Scatchard plots, suggesting binding to multiple sites. The placental high-affinity \[^{125}\text{I}]\text{RTI-55}\) binding site had a \(K_d\) very similar to that found for the brain, although the \(B_{\text{max}}\) was somewhat lower (32). In contrast, the low-affinity binding site in placenta had a lower \(K_d\) than that of brain, but its density was much greater.

Drug competition studies were performed to help resolve the identity of \[^{125}\text{I}]\text{RTI-55}\) binding sites; however, the results are somewhat difficult to interpret. All of the \(IC_{50}\) values are rather high, indicating that none of the monoamine uptake inhibitors tested was very potent in displacing all of the \[^{125}\text{I}]\text{RTI-55}\) binding. Yet, the multiphasic displacement curves obtained with many of these compounds, along with the curvilinear \[^{125}\text{I}]\text{RTI-55}\) Scatchard plots, indicate that the low potency values may be related, at least in part, to the fact that the drugs are displacing the ligand from several different binding sites with differing affinities. The DA uptake inhibitors benztropine and mazindol were among the most potent displacers of placental \[^{125}\text{I}]\text{RTI-55}\) binding, but it is unlikely that such binding was to a DA transporter, based on previous studies of both rat (33) and human (15) placenta.

The most interesting result from the drug competition studies comes from citalopram, a highly selective 5-HT uptake inhibitor. The displacement curves produced by this compound could be resolved into high- and low-affinity components, and the high-affinity component yielded a \(K_v\) value consistent with the affinity of citalopram for the 5-HT transporter (31). Moreover, the reported affinities of RTI-55 for the 5-HT transporter in two different cultured cell lines are essentially identical to the \(K_v\) value we obtained for the high-affinity \[^{125}\text{I}]\text{RTI-55}\) binding site in rat placenta (3, 17). Taken together, these results are consistent with the hypothesis that a 5-HT transporter constitutes at least part of the high-affinity binding of \[^{125}\text{I}]\text{RTI-55}\) to the rat placenta.

Experiments performed with the more selective ligand \[^{3}\text{H}]\text{paroxetine}\) provide additional evidence for the presence of a placental 5-HT transporter, thereby strengthening the case for a possible interaction of \[^{125}\text{I}]\text{RTI-55}\) with this site. \[^{3}\text{H}]\text{paroxetine}\) bound to rat placental membranes with a \(K_v\) of 88 pM, which is in close agreement with the reported \(K_v\) for \[^{3}\text{H}]\text{paroxetine}\) in purified human placental brush-border membranes (72 pM) (12). Furthermore, unlabeled cocaine and RTI-55 both potently displaced \[^{3}\text{H}]\text{paroxetine}\) binding to rat placental 5-HT transporters, which is consistent with previous results from human placenta (29). On the other hand, the density of \[^{3}\text{H}]\text{paroxetine}\)-labeled binding sites (96 fmol/mg protein) was almost threefold less than the density of high-affinity \[^{125}\text{I}]\text{RTI-55}\) binding sites. Although there are several possible reasons for this difference, the results of the drug competition studies suggest that the best explanation is probably that high-affinity \[^{125}\text{I}]\text{RTI-55}\) binding in the placenta includes one or more sites in addition to the 5-HT transporter.

There was a very high density of low-affinity \[^{125}\text{I}]\text{RTI-55}\) binding sites in the rat placenta. It is interesting
that the $K_i$ for unlabeled RTI-55 displacement of $[3\text{H}]$nisoxetine binding to the rat placental NE transporter is 7.6 nM (33), which is virtually identical to the $K_d$ for the placental low-affinity $[125\text{I}]$RTI-55 binding site (7.9 nM). Given its high density in the rat placenta, the NE transporter is probably an important contributor to the low-affinity $[125\text{I}]$RTI-55 binding found in the present study. Yet the low-affinity $B_{\text{max}}$ value for $[125\text{I}]$RTI-55 is severelfold greater than the $B_{\text{max}}$ for placental $[3\text{H}]$nisoxetine binding (33). This raises the possibility of other contributions to the low-affinity binding of $[125\text{I}]$RTI-55, which remain to be identified.

In vitro autoradiography showed a heterogeneous distribution of $[125\text{I}]$RTI-55 binding sites in the rat placenta. One area of relatively high binding was a thin layer of maternal blood spaces filled with blood clots. This is consistent with the idea that at least some of the $[125\text{I}]$RTI-55 binding to the tissue sections was to the 5-HT transporter, since this transporter is known to be present on the membranes of blood platelets (19). The placental labyrinth, which contains both fetal and maternal blood vessels, generally exhibited a higher level of $[125\text{I}]$RTI-55 binding than the junctional zone, which contains only maternal vessels. This could indicate either that the trophoblastic cells of the labyrinth are specialized for greater 5-HT uptake or that some of the transporters are being expressed by fetal endothelial cells.

Immunocytochemistry for 5-HT and the 5-HT transporter provided additional insight into the possible cellular sites of 5-HT uptake. The presence of 5-HT in the placenta presumably depends on transporter activity, as the placenta does not appear to synthesize this substance (40). Indeed, we found a similar distribution of immunoreactivity for 5-HT and for the 5-HT transporter, although the staining intensity was unexpectedly higher in the junctional zone than in the labyrinth. This finding is consistent with the demonstration of 5-HT uptake by giant trophoblastic cells in cultured mouse placenta (40) and also with the recent preliminary report of 5-HT transporter mRNA in rat placental giant cells using in situ hybridization (18). Given that the giant cells seem to avidly accumulate 5-HT, it is necessary to explain why the junctional zone, which contains the majority of the giant cells, exhibited a lower level of $[125\text{I}]$RTI-55 binding than the labyrinth, which has few of these cells. One obvious reason is that the distribution of placental $[125\text{I}]$RTI-55 binding appears to represent multiple sites, only one of which is the 5-HT transporter (see above). It is also possible that the trophoblastic cells of the labyrinth could metabolize 5-HT more rapidly than those of the junctional zone, thereby leading to a lower steady-state concentration and hence weaker staining.

A placental 5-HT transporter (or at least evidence for 5-HT uptake) has now been demonstrated in four different species: humans (15), sheep (28), mice (40), and rats (Ref. 18 and the present study). The widespread occurrence of placental 5-HT uptake raises important questions concerning the possible role of this process in placental functioning and fetal development. Bengel et al. (4) recently reported that mice with a targeted disruption of the 5-HT transporter gene were fertile and produced apparently normal litters when mated together. Although these findings indicate that 5-HT uptake is not essential for placental functioning, it may still serve some physiological purpose that is not evident from the initial gene “knockout” studies. For example, 5-HT has been shown to exert a marked contractile effect on human chorionic veins (16) and on the umbilical artery and vein (37). Thus one function of trophoblastic 5-HT uptake may be to help maintain an optimal flow through these downstream vessels that carry blood away from the placenta as well as to and from the fetus.

Another possible role of this uptake mechanism may be to transfer 5-HT from the mother to the fetus. Although the placenta contains substantial monamine oxidase activity (11) and therefore probably metabolizes most of the 5-HT accumulated from the blood, at least a small amount of this neurotransmitter might reach the fetus. Some evidence for maternal-fetal transport of radiolabeled 5-HT was reported in Essman and Cooper (13). Moreover, the enterochromaffin cells in the gut, which are the major source of circulating 5-HT, do not express 5-HT immunoreactivity until embryonic day 16 in mice (7). Yet Lauder and co-workers (21) observed a transient period of 5-HT uptake by craniofacial tissues of the mouse embryo at embryonic day 12, and more recent studies demonstrated a role for 5-HT in cranial neural crest cell migration between embryonic days 9 and 12 (27). 5-HT has also been implicated in regulating the astroglial trophic factor S-100 during early development (38). The developing embryo may thus be dependent on the placenta to provide maternal 5-HT until its own sources of 5-HT have become functional.

Finally, it is important to recognize that placental 5-HT uptake is likely to be inhibited by several important classes of drugs, including the abused drug cocaine as well as selective 5-HT uptake inhibitor antidepressants such as fluoxetine, paroxetine, sertraline, fluvoxamine, and citalopram. It is possible that some of the adverse effects of maternal cocaine use on pregnancy outcome and offspring development result from interference with placental 5-HT transport (35). Furthermore, although most reports suggest that maternal fluoxetine use during pregnancy is safe for the offspring (2), a recent study found that third trimester exposure to fluoxetine was associated with an increased incidence of premature birth, intrauterine growth retardation, and postnatal complications requiring admission to the special care nursery (10). It is important to determine whether these effects are related to blockade of placental 5-HT uptake sites during the late stages of pregnancy.

In summary, the present results demonstrate the presence of a 5-HT transporter in the rat placenta. These transporters can be labeled in vitro with either $[3\text{H}]$paroxetine or the cocaine congener $[125\text{I}]$RTI-55, although the latter compound also labels other sites that have not yet been identified. Placental $[125\text{I}]$RTI-55
binding sites are heterogeneously distributed, with the greatest density in the labyrinth. Unexpectedly, however, immunoreactivity for both 5-HT and the 5-HT transporter is more intense in the junctional zone than in the labyrinth. The placental 5-HT transporter is a potential site of action of cocaine and of selective 5-HT uptake inhibitors, which raises questions concerning possible adverse effects of these compounds on pregnancy and offspring development. Furthermore, the placenta may prove useful as a model tissue for examining the effects of such drugs on 5-HT transporter regulation.

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