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Cocaine up-regulates norepinephrine transporter binding in the rat placenta

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Abstract

We investigated the influence of 3 days of continuous cocaine exposure on norepinephrine transporter binding in the rat placenta. On gestational day 17, pregnant rats were implanted subcutaneously with two cocaine-containing Silastic capsules. There were two control groups, one that received capsules with vehicle only and was pair-fed to the cocaine-treated females, and a second group that was untreated and fed ad libitum. Placentas and fetal brains were harvested and frozen on gestational day 20, and subsequently subjected to saturation analyses for norepinephrine transporter binding using the selective ligand [^{3}H]nisoxetine. There was a marked increase in the density (B_{max}) of norepinephrine transporter binding sites in the placentas of the cocaine-treated animals compared to both control groups, but no change in the fetal brain. The mechanism underlying this up-regulation of the placental norepinephrine transporter is not yet known, but it could involve a β-adrenoceptor- and cAMP-mediated induction of transporter gene expression.

Keywords: Norepinephrine transporter; Placenta, rat; Cocaine, chronic

1. Introduction

Specific transporter proteins located on nerve terminal plasma membranes are responsible for the clearance of norepinephrine, dopamine, and serotonin (5-hydroxytryptamine, 5-HT) after their release into the synaptic cleft (Amara and Kuhar, 1993). Norepinephrine and 5-HT transporters have also been identified in the placentas of several species. Studies by Ganapathy et al. (1993) found these transporters on brush-border membranes purified from human placenta, which suggests monoamine uptake from maternal blood. In contrast, work by Bzoskie et al. (1995) in sheep clearly demonstrated that the placenta plays a major role in catecholamine clearance from the fetal circulation.

Recent studies of the rat placenta in our laboratory have characterized and localized the binding of [^{3}H]nisoxetine to the norepinephrine transporter (Shearman and Meyer, 1998) and of [^{125}I]3-[4-iodophenyl]tropane-2-carboxylic acid methyl ester ([^{125}I]RTI-55) and [^{3}H]paroxetine to the 5-HT transporter (Shearman et al., 1998) using both membrane-binding and autoradiographic techniques. Membrane-binding studies showed a much greater abundance of norepinephrine compared to 5-HT transporters in normal rat placenta at gestational day 20. Autoradiographic localization experiments demonstrated high-affinity radioligand binding in both the basal (junctional) zone and labyrinth of the placenta, although the distribution was different for the two transporters.

One important feature of monoamine transporters is that they are blocked by cocaine. Indeed, it is possible that blockade of placental transporters mediates some of the adverse effects of maternal cocaine use on pregnancy outcome and offspring development (Ramamoorthy et al., 1993; Prasad et al., 1994). Yet, little is known about the influence of cocaine on placental transporter regulation. Bzoskie et al. (1997) recently reported that women with pregnancy complications, including cocaine use in some

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cases, showed large elevations in umbilical cord plasma norepinephrine concentrations, and that placental mRNA for the norepinephrine transporter was inversely related to umbilical cord norepinephrine levels. On the other hand, there are no previous studies examining the specific effect of cocaine on placental transporter expression either in humans or laboratory animals.

In the present study, we investigated the influence of cocaine administration from gestational day 17 to gestational day 20 on rat placental norepinephrine transporter binding. We used a Silastic capsule treatment regimen originally developed by Lipton et al. (1991) and previously shown in our laboratory to produce a down-regulation of striatal dopamine transporter binding in offspring at postnatal day 10 (Collins and Meyer, 1996). Because of the possibility of differential regulation in fetus compared to placenta, we also measured the effects of cocaine on whole-brain transporter binding in fetuses obtained from the same dams.

2. Materials and methods

2.1. Chemicals

[^{3}H]Nisoxetine (78.4 or 80 Ci/mmol) was purchased from Dupont/New England Nuclear (Boston, MA) and stored at −20°C. Cocaine HCl and mazindol were obtained from Sigma (St. Louis, MO) and Research Biochemicals (Natick, MA), respectively. Cocaine base was generously provided by the NIDA drug supply program. All other chemicals used were reagent grade.

2.2. Animals

Sprague–Dawley albino rats were bred in our laboratory from Charles River (Wilmington, MA) stock. The animals were maintained under a 14:10 light–dark cycle (lights on at 0600 h) at an ambient temperature of approximately 23°C. Food (Purina Rat Chow) and tap water were available ad libitum except as indicated below. 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 that a sperm plug was found was designated gestational day 1. After mating, females were transferred to individual metal cages, switched to a powdered diet to facilitate pair-feeding (see below), and periodically inspected for weight gain until capsule implantation on gestational day 17. All animal experimentation was carried out in accordance with the United States Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals, and the procedures were approved by the University of Massachusetts Institutional Animal Care and Use Committee.

2.3. Surgery and drug treatment

Pregnant dams were randomly assigned one of three groups ($n = 8$ per group): cocaine-treated, vehicle-treated, and untreated controls. Vehicle-treated animals were pair-fed to members of the cocaine group, whereas untreated control animals were fed ad libitum. Maternal body weight and food intake data were recorded during the treatment period from gestational day 17 to 20.

Preparation and subcutaneous implantation of Silastic capsules was carried out as previously described (Lipton et al., 1991; Meyer and Dupont, 1993). Dams were anesthetized with methoxyflurane (Metofane, Pitman-Moore, Mundelein, IL) and were implanted subcutaneously with two capsules, each containing either 0.62 ml of polyethylene glycol vehicle alone (vehicle group) or 60 mg of cocaine base dissolved in the same volume of polyethylene glycol (cocaine group). We previously found that this treatment regimen produces average circulating cocaine levels of approximately 250 ng/ml (Meyer, unpublished observations), which is well within the range reported for human cocaine users (Isenschmid et al., 1992; Evans et al., 1996). Untreated control dams did not receive anesthesia or capsule implantation, although they were subjected to daily weighing and measurement of food intake from gestational day 17 to 20.

2.4. Radioligand binding assays

Animals were sacrificed by decapitation on gestational day 20. Placentas and fetal brains were rapidly removed and frozen on powdered dry ice. Binding assays were performed on tissues from six of the eight animals in each group. For each placental assay, four placentas from the same female were weighed, thawed, minced on an ice-cold glass plate, and homogenized in 10 volumes of ice-cold buffer using a Polytron at setting 6 for 40 s. The buffer contained 0.25 M sucrose, 10 mM Na_2HPO_4, 120 mM NaCl, and 5 mM KCl, pH 7.4 (Shearman and Meyer, 1998). Subsequent tissue processing was carried out as described in Shearman and Meyer (1998), yielding a final tissue suspension in 20 volumes of homogenization buffer without sucrose. Preparation of fetal brain membranes was carried out using the same procedures, except that 10 brains from the same litter were pooled for each assay and the tissue did not require mincing prior to homogenization. Protein content of the washed membrane preparations was determined using the Bradford dye-binding assay (Bradford, 1976), with bovine γ-globulin as the standard.

Saturation analyses of norepinephrine transporter binding were carried out as described in Shearman and Meyer (1998), with minor modifications. Membranes were incubated with 10 different concentrations of the selective ligand [^{3}H]nisoxetine (0.15 nM to 9 nM) in triplicate for 3 h at 4°C. Parallel tubes contained 1.0 μM unlabeled mazindol to define nonspecific binding.

Table 1
Maternal body weight data as a function of treatment condition
Data represent the mean ± S.E.M. for eight animals per group.

Group	Body weight at gestational day 17 (g)	Percentage body weight gain from gestational day 17–20
Untreated controls	354 ± 11	13.9 ± 0.8
Vehicle controls	393 ± 9[a]	3.0 ± 1.8[b]
Cocaine	399 ± 10[a]	6.7 ± 1.2[c]

[a] $P < 0.05$ compared to untreated controls.
[b] $P < 0.001$ compared to untreated controls.
[c] $P < 0.01$ compared to untreated controls.

2.5. Data analysis

Data from the saturation experiments were analyzed using EBDA/LIGAND software (Biosoft, Ferguson, MO) to obtain K_D and B_{max} values. One-way analyses of variance and Student's *t*-tests were calculated with Instat (GraphPad software, San Diego, CA). According to a Bartlett test, the placental B_{max} data showed heterogeneity of variance across the three treatment groups and were therefore subjected to a reciprocal transformation prior to the analysis of variance. A two-way analysis of variance was performed on the maternal food intake data (with Group as a between-subject variable and Day of Treatment as a repeated measure) using CRUNCH Interactive Statistical Package, Version 4.0, 1991 (Oakland, CA). Tukey–Kramer post-hoc tests were conducted when statistically significant effects ($P < 0.05$) were obtained from the initial analysis.

3. Results

3.1. Food intake and growth

Maternal body weight data are shown in Table 1. Despite random assignment of dams to the three treatment groups, the untreated control group had a significantly lower mean body weight than the other two groups on gestational day 17 (Treatment effect: $F(2,21) = 5.51$, $P = 0.012$, followed by post-hoc testing). This group also showed a greater percentage increase in weight from gestational day 17 to 20 (Treatment effect: $F(2,21) = 16.60$, $P < 0.001$, followed by post-hoc testing). Maternal food intake data were measured in all three groups during the treatment period from gestational day 17 to 19. We only analyzed the data from the untreated and cocaine-treated animals, however, because the vehicle group's food consumption was not independent of the cocaine group's intake. Food intake values collapsed across the three days were 27.8 ± 0.6 and 17.0 ± 1.3 g/day (mean ± S.E.M.) for the untreated and cocaine groups, respectively. This difference was highly significant by analysis of variance ($F(1,14) = 23.24$, $P < 0.001$). There was also a significant Day main effect and a Treatment × Day interaction, such that the cocaine dams ate less food on gestational day 18 (the day following surgery) than on the other 2 days (data not shown). Thus, the differential weight gain shown in Table 1 can be explained by the anorexic effect of the cocaine treatment and the associated pair feeding of the vehicle control group.

Placental and fetal brain weights are shown in Table 2. Although there was a tendency for both the placental and brain weights to be somewhat lower in the vehicle group than in the other two groups, the differences were not significant by analysis of variance. Hence, the treatment effects on maternal food intake and weight gain did not lead to a reliable reduction in either placental or fetal brain growth.

3.2. Radioligand binding

Results of the placental [^{3}H]nisoxetine binding assays are shown in Table 3. The Scatchard plots generated by LIGAND analysis were linear for all three treatment groups, which is reflected in mean Hill coefficients very close to unity. Placentas from the cocaine dams exhibited a modest increase in K_D compared to the two control groups (Treatment effect: $F(2,15) = 17.37$, $P < 0.001$, followed

Table 2
Effect of maternal cocaine treatment on placental and fetal brain weights on gestational day 20
Data represent the mean ± S.E.M. for the number of litters shown (four to eight placentas and 10 fetal brains represented from each litter).

Group	Placental weight (g) (n = 8 litters/group)	Fetal brain weight (g) (n = 6 litters/group)
Untreated controls	0.512 ± 0.015	0.138 ± 0.002
Vehicle controls	0.482 ± 0.018	0.133 ± 0.001
Cocaine	0.514 ± 0.020	0.139 ± 0.002

Table 3
Effect of maternal cocaine treatment on [^{3}H]nisoxetine binding to gestational day 20 placental membranes
Data represent the mean ± S.E.M. of six litters per group (four placentas pooled from each litter). To correct for heterogeneity of variance across groups, the B_{max} data were subjected to a reciprocal transformation prior to analysis.

Group	K_D (nM)	B_{max} (fmol/mg protein)	Hill coefficient
Untreated controls	1.03 ± 0.02	1172 ± 53	0.99 ± 0.01
Vehicle controls	1.07 ± 0.05	891 ± 15[a]	1.01 ± 0.01
Cocaine	1.32 ± 0.04[b]	1888 ± 188[b]	0.98 ± 0.004

[a] $P < 0.01$ compared to the untreated controls.
[b] $P < 0.001$ compared to both control groups.

by post-hoc testing), which indicates a reduction in binding affinity of the radioligand for the norepinephrine transporter. Even more striking was the effect of cocaine on the mean placental [^{3}H]nisoxetine B_{max} value, which was increased by 61% and 112% over the untreated and vehicle control groups, respectively (Treatment effect: $F(2,15) = 42.64$, $P < 0.001$). Post-hoc analysis indicated again that the cocaine group differed significantly from both control groups, but in this case the pair-fed vehicle group was significantly lower than the untreated control group. Representative Scatchard plots for all three treatment groups are presented in Fig. 1.

It was important to ensure that the differences between the cocaine and control groups did not result from residual cocaine remaining in the tissue preparations from the drug-treated animals. Consequently, four additional binding assays were conducted using the same procedures as described above but with an extra wash step. During membrane preparation, the tissues were also left at room temperature for 30 min to allow for dissociation of any residual cocaine. No differences in binding were found between the samples treated in this manner and those processed in the normal way (data not shown).

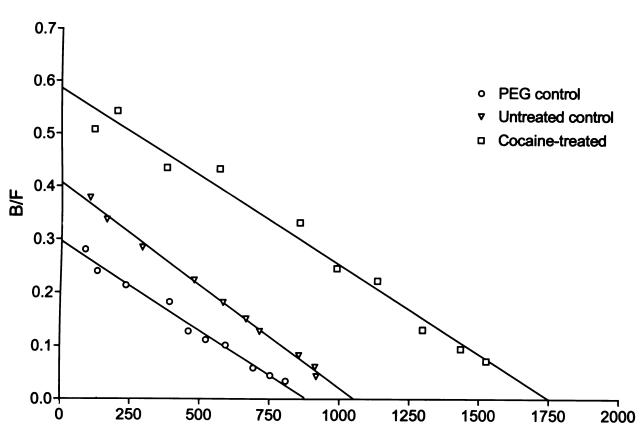

Fig. 1. Representative Scatchard plots of [^{3}H]nisoxetine binding to gestational day 20 placental membranes as a function of maternal treatment. Each plot depicts the results from a single animal. PEG, polyethylene glycol vehicle-treated controls.

Table 4
Effect of maternal cocaine treatment on [^{3}H]nisoxetine binding to gestational day 20 whole fetal brain membranes
Data represent the mean ± S.E.M. of six litters per group (10 brains pooled from each litter).

Group	K_D (nM)	B_{max} (fmol/mg protein)	Hill coefficient
Untreated controls	1.07 ± 0.07	69.6 ± 5.7	0.97 ± 0.06
Vehicle controls	1.05 ± 0.02	70.3 ± 4.8	0.96 ± 0.05
Cocaine	1.14 ± 0.08	74.0 ± 6.6	1.04 ± 0.07

Fetal brains obtained from the same treated and control dams were also assayed for [^{3}H]nisoxetine binding. As shown in Table 4, fetal brain K_D values and Hill coefficients were quite similar when compared to those obtained from placentas, whereas the density of binding sites was over 10-fold lower. Most importantly, there was no suggestion at all of a treatment-related change in norepinephrine transporter binding in the fetal brain.

4. Discussion

To our knowledge, this is the first study to investigate the influence of maternal cocaine administration on placental monoamine transporters in rats. We found a striking up-regulation of [^{3}H]nisoxetine-labeled norepinephrine uptake sites after just 3 days of continuous maternal cocaine treatment. There was also a smaller but statistically significant reduction in affinity of the transporter for the radioligand. These effects were specific to the placenta, as no effect was observed for fetal brain norepinephrine transporter binding. Moreover, the results cannot be attributed to either the stress of surgery and capsule implantation or the anorexic properties of cocaine, because placentas from the vehicle-implanted, pair-fed dams differed from the cocaine group even more than did placentas from untreated control dams.

We hypothesize that the observed up-regulation of placental norepinephrine transporter binding is a compensatory response to the chronic blockade of placental catecholamine uptake produced by the cocaine treatment regimen. Although no direct evidence is available yet in support of this hypothesis, there exists a potential signaling pathway involving β-adrenoceptors that are expressed on the trophoblastic cells (Moore and Whitsett, 1982). We propose that blockade of the placental norepinephrine transporter by cocaine increases local extracellular catecholamine concentrations in the vicinity of these receptors. As placental β-adrenoceptors show the normal positive coupling to adenylyl cyclase (Grullon et al., 1995), cocaine enhancement of β-receptor activation should increase cAMP formation within the cells. Nguyen et al. (1999) recently reported that incubation of SK-N-SH neuroblastoma cells with dibutyryl-cAMP or forskolin substantially

increased norepinephrine transporter mRNA levels following 24 h of treatment. Although the rodent norepinephrine transporter promoter does not possess a cAMP response element, it does have an activator protein-1 (AP-1) site (Fritz et al., 1998), and immediate-early gene products that bind to this site (for example, Fos and Jun) can be induced by cAMP (Herdegen, 1996). According to this model, therefore, continuous cocaine treatment may increase transcription of the placental norepinephrine transporter through a signaling cascade involving β-adrenoceptors, cAMP, and AP-1 transcription factors.

Three days of continuous cocaine treatment failed to produce a change in fetal brain norepinephrine transporter binding, although because we sampled the entire brain, we cannot exclude the possibility of a treatment effect in a small subset of noradrenergic neurons. Burchett and Bannon (1997) recently reported that a ''binge'' pattern of cocaine administration to adult rats increased the levels of norepinephrine transporter mRNA within the locus coeruleus. Szot et al. (1993) obtained similar results following long-term treatment of rats with desipramine, a relatively selective norepinephrine uptake inhibitor. In contrast, other investigators have found that chronic treatment with desipramine decreases the density of [^{3}H]nisoxetine binding sites either in vivo (Bauer and Tejani-Butt, 1992) or in cell culture (Zhu and Ordway, 1997; Zhu et al., 1998). Even though these findings are not consistent with respect to the direction of change, they do suggest either that fetal brain noradrenergic neurons are not yet competent to regulate their norepinephrine transporter expression at gestational day 20, or that the regulatory mechanisms differ from those observed in adult placenta, adult brain, and several cell culture models.

We do not yet know whether cocaine administration also alters the expression of placental 5-HT transporters. When additional placentas from the dams in the present study were analyzed for binding of the radiolabeled cocaine congener [^{125}I]RTI-55, there was no significant effect of the treatment (Shearman and Meyer, unpublished observations). Because this ligand appears to label one or more placental constituents in addition to the 5-HT transporter (Shearman et al., 1998), however, no definite conclusions can be drawn at this time.

The ability of maternal cocaine treatment to influence the placental norepinephrine transporter adds to the growing evidence that the placenta is a target of cocaine action. Placentas obtained from cocaine-using women showed reduced densities of β-adrenoceptors (Wang and Schnoll, 1987), opiate receptors (Wang and Schnoll, 1987), and sigma binding sites (Flynn et al., 1993). Cocaine has also been reported to inhibit placental secretion of human chorionic gonadotropin (Simone et al., 1996), reduce placental amino acid uptake (Dicke et al., 1993, 1994), and alter placental prostaglandin production (Monga et al., 1994). Along with blockade of the norepinephrine and 5-HT transporters, these effects of cocaine on the placenta may contribute to the adverse effects of maternal drug use on pregnancy outcome and offspring neurobehavioral development.

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