Clozapine Protection against Gestational Cocaine-Induced Neurochemical Abnormalities

Elena Yablonsky-Alter, Eleonora Gashi, Theodore I. Lidsky, Hoau-Yan Wang, and Shailesh P. Banerjee

Department of Physiology and Pharmacology, the City University of New York Medical School, New York, New York (E.Y.-A., E.G., H.-Y.W., S.P.B.); Department of Biology and Neuroscience, Graduate School of the City University of New York, New York, New York (E.G., T.I.L., H.-Y.W., S.P.B.); and Department of Psychobiology, New York State Institute for Basic Research, Staten Island, New York (T.I.L.)

Received July 12, 2004; accepted September 20, 2004

ABSTRACT

Clozapine was found to be effective in attenuating cocaine-induced neurochemical effects. We investigate whether clozapine influences in utero cocaine exposure-induced changes in striatal dopamine levels and cortical N-methyl-D-aspartate (NMDA) receptor density in mouse and rat brains. Pregnant mice or rats were injected with cocaine (5 or 10 mg/kg intraperitoneally) or saline every 24 h throughout gestation and continued for 6 weeks following the delivery. Striatal dopamine levels measured by high-pressure liquid chromatography were found to decrease 24 to 33% in gestational cocaine exposed between the ages of 3 to 15 days, but not in 42-day-old pups. The cortical NMDA receptor densities assessed either in the presence of 100 μM glutamate or 30 μM glycine were significantly increased in 15-day-old gestational cocaine-exposed rats. Simultaneous daily administration of 3 mg/kg clozapine with 5 mg/kg cocaine to pregnant mice protected against the decrease in striatal dopamine levels or an increase in the concentration of NMDA receptor measured in the presence of 100 μM glutamate in 15-day-old pups. Clozapine did not affect striatal dopamine levels by itself or when coadministered with cocaine in 42-day-old pups. The results show gestational cocaine may induce neurochemical abnormalities in brain exhibited as an increased glutamate NMDA receptor density together with a decreased striatal dopamine level. These effects of gestational cocaine exposure may be prevented by simultaneous administration of clozapine. Thus clozapine, which is a partial agonist at the NMDA receptor, may be of value in protecting against gestational cocaine-induced adverse effects in the brain.

Many studies have documented that cocaine exposure during embryonic development alters subsequent neurobiological and behavioral functions in the offspring (Dow-Edwards et al., 1990; Henderson and McMillen, 1993). The mechanisms for cocaine-induced neurobehavioral effects, however, remain to be elucidated. Previous investigators have reported developmental changes in several neurotransmitters including dopamine (Miller et al., 1995; Salvatore et al., 2004), serotonin (Snyder-Keller and Keller, 1993), and acetylcholine (Tyrala et al., 1992) following gestational exposure to cocaine. The mechanism for developmental changes in monoaminergic systems after prenatal cocaine exposure may be attributed to its ability to block neurotransmitter uptake in the fetal brain or the vasoconstrictor activity of the drug. Cocaine is known to inhibit high-affinity neurotransmitter uptake at the presynaptic nerve terminals in both adult animals and fetus brains (Pitts and Marwah, 1987; Meyer et al., 1994); therefore, cocaine enhances synaptic levels of dopamine, norepinephrine, and serotonin (Ng et al., 1991; Keller et al., 1994) following systemic and gestational administration. Cocaine has also been reported to reduce the fetal blood flow to decrease the supply of oxygen and other nutrients available to the fetus in the pregnant dams (Woods et al., 1987). Hypoxia or diminution of oxygen levels in the fetal brain has been shown to alter development of brain monoaminergic systems (Silverstein and Johnston, 1984).

Numerous studies in fetal and mature animals indicate a role of changes in the dopaminergic system in the neurological effects of cocaine. Cocaine-induced increases in synaptic concentrations of dopamine may slow glutamate uptake at its presynaptic sites (Kerkerian et al., 1987) resulting in a heightened glutamatergic transmission. Also, it may augment this amino acid transmission at the postsynaptic site by the activation of D3 receptors (Cepeda and Levine, 1998). In the retinal ganglion and bipolar cells, activation of postsynaptic NMDA receptors...
aptic D1 receptors enhances glutamate-mediated currents by a G protein-coupled mechanism involving activation of adenylyl cyclase (Maguire and Werblin, 1994). Therefore, higher synaptic levels of dopamine may increase glutamatergic transmission by acting at the pre- and postsynaptic sites of this amino acid. Again, cocaine-induced vasoconstriction due to augmentation sympathetic outflow in mature animals or pregnant dams may cause ischemia with consequent hypoxia leading to extra release of glutamate (Krajnc et al., 1994). These cocaine-mediated effects may independently, or in combination, cause cellular damage to the adult and fetal brain.

Thus, it is our hypothesis that cocaine-induced adverse neurobehavioral activity is mediated by the excitotoxicity of glutamate, which may occur by both peripheral and central actions of cocaine. In this investigation, we examine changes in the striatal dopamine levels at different stages of the postnatal period following gestational cocaine exposure. In addition, we also measured striatal NMDA receptor concentration. Using changes in dopamine level and NMDA receptor density induced by prenatal cocaine exposure as the guide, we evaluate the ability of clozapine, an atypical antipsychotic agent with partial NMDA agonist activity, in blocking cocaine-induced effects.

Materials and Methods

Materials. [3H]MK-801 (24 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). Unlabeled MK-801, (5)g)–cocaine HCl, Tris base, L-glutamate, L-glycine, and monochloroacetic acid were purchased from Sigma/RBI (Natick, MA). GF/8 glass fiber filters were purchased from Whatman (Maidstone, UK). Micro nylon filters were purchased from Millipore Corporation (Billerica, MA).

Animals. Pathogen-free, male and female CD-1 mice and Sprague-Dawley rats were obtained from Charles River Laboratories, Inc. (Wilmington, MA) and were allowed to be acclimated to the colony room with daily handling and weighing for 7 days. Animals were housed individually at 21–23°C with 40 to 60% relative humidity and a 12-h light/dark photoperiod with lights on at 7:00 AM. They were maintained on Purina Rodent Laboratory Chow and tap water ad libitum. All animal procedures were in compliance with the Animal Welfare Act, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, the National Institutes of Health Guide for the Use and Care of Laboratory Animals, and the City University of New York (CUNY) institutional guidelines and were approved by the CUNY Animal Care and Use Committee.

A pair of 10-week-old male and female rats or mice were placed in a cage overnight. The presence of sperm-positive vagina plug is considered E0. The pregnant female animals were housed individually without disturbance other than the daily injection with indicated drugs. Every experimental series consists of six pregnant rats or mice with two receiving one of three treatment paradigms.

In utero cocaine treatment was performed by injecting pregnant mice and rats with 5 and 10 mg/kg (5)g)–cocaine hydrochloride dissolved in sterile physiological saline (2 mg/ml) intraperitoneally (i.p.) at 9:00 AM, respectively, at an interval of 24 h throughout 21 days of gestation. Control female mice were injected with an equal volume of physiological saline. The dose of cocaine used in this investigation was based on a series of preliminary experiments in which this dose of cocaine was found to be effective in a wide variety of behavioral and biochemical parameters comparable with those reported by other investigators without overt growth effects (Dow-Edwards et al., 1990). Extra precaution was made to prevent injecting directly into the womb and injury to the fetuses. Administration of saline or cocaine to control or drug-treated groups, respectively, was continued for another 6 weeks following delivery, and the mothers were allowed to feed milk to their newborn pups up to 42 days. The treatment schedule did not alter body weight gains of the dams or pups. To study protective effects of clozapine against cocaine-induced central nervous system toxicity, clozapine (3 mg/kg i.p.) was simultaneously administered with cocaine hydrochloride (5 mg/kg i.p.) during the entire pregnancy (approximately 21 days) and up to 6 weeks after delivery. The control groups received equal volumes of saline. Animals were decapitated between 3 and 42 days of age. In general, all animals were decapitated within 12 to 15 h of reaching the indicated ages.

Receptor Binding of [3H]MK-801 to NMDA Glutamate Receptor. Pups were decapitated at the age of 15 days, and the brains were removed and placed on a glass plate. Striata and cerebrocortical were dissected out, and the tissue was frozen in liquid nitrogen and stored at −100°C until use. We assessed the functional activity of the NMDA receptor in the frontal cortex of control and experimental groups by measuring the displacement of [3H]MK-801 by nonradioactive MK-801 in the presence of either glycine or glutamate by adopting a procedure previously described (Banerjee et al., 1995). Fifteen-day-old rat pups were sacrificed, and the brains were removed. The frontal cortices were dissected on ice and immediately frozen at −80°C, and thawing at 4°C on the day of the experiment, the tissue was washed to reduce residual levels of glutamate and glycine. The tissue was homogenized with a Brinkman Polytron PT-20 homogenizer in 20 volumes of ice-cold 0.05 M Tris buffer (pH 7.4 at 25°C) and centrifuged at 49,000g for 20 min. The supernatant was discarded, and the pellet was rehomogenized in the same buffer and centrifuged as before. This last procedure was repeated twice, and the resulting membrane pellet was frozen 1 more day at −80°C. For assays, pellet was resuspended, homogenized in Tris (20 volumes, 5 mM), centrifuged at 49,000g for 20 min, and repeated two additional times. Receptor binding assays were carried out by incubating 800 µl of crude fractions containing (0.8 µg of tissue) with radioligand [3H]MK-801 (about 100,000 cpm) with either 100 µM glutamate or 30 µM glycine for 45 min at 25°C. To determine the nonspecific binding, a parallel set of samples were incubated with large excess of MK-801 ligand (100 µM MK-801). Specific binding is defined as the difference between total binding and nonspecific binding. Incubation was terminated by rapid filtration through GF/B glass fiber filters. The filters were rinsed twice with 4-ml ice-cold Tris buffer to remove unbound radioactive ligand. They were then dried and placed in vials containing 3 ml of scintillation fluid and counted by scintillation spectroscopy at 60% efficiency. The receptor binding was conducted to develop a displacement curve of [3H]MK-801 binding to the NMDA receptor by conducting the binding assays with eight different concentrations of unlabeled MK-801 in the presence of 100 µM glutamate or 30 µM glycine. The displacement curves were introduced to the Beckman program. The AccuFit Competitive Two-Site program was used to compute the Scatchard analysis to estimate the maximal number of binding sites. The program is based on the principle of nonlinear least-squares regression analysis to solve the equations describing the binding of labeled ligand to receptor proteins. In a well washed tissue with no residual endogenous amino acids, [3H]MK-801 binding is minimal to negligible (Reynolds et al., 1987; Banerjee et al., 1995). In accordance with a previous report (Reynolds et al., 1987), addition of either glycine or glutamate increased an ionophore opening that caused several-fold enhancement of [3H]MK-801-specific binding. This functional assay of NMDA activity was then used to measure specific binding of [3H]MK-801 to cortical tissue obtained from control and experimental 15-day-old pups in the presence of either 100 µM glutamate or 30 µM glycine.

The protective effect of clozapine on in utero cocaine-mediated activities was also assessed by determining changes in striatal NMDA receptor density. Six pregnant rats were divided into three groups: control (saline), cocaine (5 mg/kg), and cocaine (5 mg/kg) plus...
clozapine (3 mg/kg). Pups born to the dams in different groups were sacrificed 15 days after birth. The number of glutamate receptors was estimated by measuring specific binding of $[^3H]$MK-801 to striatal membranes in the presence of 100 μM glutamate.

**Determination of Dopamine Levels by High-Pressure Liquid Chromatography (HPLC).** Pups were decapitated at the age of 3, 7, 11, 15, or 42 days, and the brains were removed and placed on a glass plate. Striatum was dissected out from the sagittal section. The tissue was frozen in 0.07 M monochloroacetic acid and stored at −100°C. Each striatum obtained from mouse pup was sonicated in 0.07 M monochloroacetic acid using ultrasonic cell disrupter and centrifuged for 20 min. The supernatant was filtrated through a 0.22-μm micro nylon filter. Samples were injected in HPLC.

In a separate set of experiments, the effect of clozapine cotreatment on the dopamine level was determined. Sixteen pregnant mice were divided into the following four groups: control (saline), cocaine (5 mg/kg i.p.), clozapine (3 mg/kg i.p.), and cocaine (3 mg/kg) plus clozapine (3 mg/kg). Pups born to the dams in each of the treatment groups were sacrificed at either 15 or 42 days after birth, and striatal dopamine levels were measured by HPLC.

A bioanalytical HPLC system (BAS Bioanalytical Systems, West Lafayette, IN) consisting of a refrigerated CMA/200 autosampler, a LC4B electrochemical detector, and a PerkinElmer series 410 pump was used for analysis. The column used was a 3-μm reverse-phase column (phase separation Spherisorb column S3 ODS2, 10 × 4.6 cm). Mobile phase consisted of 0.07 M monochloroacetic acid, 0.5 M sodium ethylenediaminetetraacetic acid, 1 mM sodium dodecyl sulfate, and 1.5% acetonitrile adjusted to pH 4.1 and was delivered at a rate of 1.0 ml/min. Twenty microsplits of each sample were injected onto a column for analysis. A potential on-flow cell of electrochemical detector was set at +0.65 V versus Ag/AgCl. Chromatograms were integrated, compared with standards, and analyzed using PerkinElmer Turbochrom software. The approximate sensitivity limits of the assay with these detector settings and this chromatographic separation were 10 pg for dopamine and norepinephrine and 20 pg for 5-hydroxyindole acetic acid and 3,4-dihydroxyphenylacetic acid.

**Statistical Analysis.** All data were presented as mean ± S.E.M. Two-tailed Student’s t test was used to compare particular responses between two groups. In addition, analysis of variance followed by Newman-Keuls test for multiple comparisons was also used to evaluate the differences in each of the age groups. The threshold for significance was $p < 0.05$.

**Results**

**Effect of in Utero Cocaine Exposure on Striatal Dopamine Levels in Mouse Brains.** To define a marker for neurochemical effects of cocaine, we measured striatal dopamine levels by HPLC in offspring derived from a group of 20 pregnant mice (10 control and 10 cocaine-exposed). There was no further decrease in the levels of striatal dopamine in the control group compared with the cocaine-exposed groups (Table 1). This suggests that the decline of striatal dopamine following gestational cocaine exposure seen at an early postnatal period was reversed in 42-day-old pups. The magnitudes of decreases in dopamine were similar in the pups from the age of 3 to 15 days. However, despite the absence of a decline in dopamine levels in the control groups, there was a significant decrease in dopamine in 42-day-old pups obtained from control and 10 mg/kg/day gestational cocaine-exposed female mice. There was a significant decrease in the striatal levels of dopamine at all ages except in the 42-day-old pups. The data are expressed as mean ± S.E.M. derived from 8 to 10 individual determinations at each age. Statistical differences were evaluated using two-factor ANOVA followed by Newman-Keuls test for multiple comparisons.

**Effect of Gestational Cocaine Exposure on Cortical NMDA Receptor Concentrations in Rat Brains.** We further determined the basis for the diminution of striatal dopamine in the gestational cocaine-exposed pups since cocaine may inhibit dopamine reuptake in the fetal brains to increase the levels of this neurotransmitter in the synaptic cleft. This extracellular increase in dopamine concentration may decrease the rate of dopamine synthesis by a feedback mechanism resulting in diminution of striatal dopamine after gestational cocaine exposure. Prenatal cocaine administration has been shown to reduce cortical GABA cell count (Yablonsky-Alter et al., 1993); a decrease in striatal dopamine may represent reduction in dopaminergic terminals induced by the excitotoxicity of glutamate caused by gestational cocaine exposure. To test this possibility, we measured the density of NMDA receptors in the cortical membranes obtained from control and experimental groups of female rats by a $[^3H]$MK-801 binding assay. Results shown in Fig. 2 indicate that the densities of NMDA receptors significantly increase in the frontal cortex of 15-day-old pups that were exposed to gestational cocaine compared with control animals both in the presence of glutamate as well as glycine. These observations suggest that gestational cocaine exposure may enhance gluta-matergic transmission by increasing ionophore opening to facilitate the occurrence of excitotoxicity.

**Prevention of in Utero Cocaine Exposure-Induced Diminution of Striatal Dopamine by Clozapine in Mouse Brains.** In these experiments, the levels of dopamine in striata following in utero cocaine exposure were assessed. A decrease in the striatal levels of dopamine produced by gestational exposure to cocaine was completely abolished by clozapine in 15-day-old pups (Table 1). At the dose given, clozapine by itself did not significantly alter the levels of striatal dopamine (Table 1). Again, there was no discernible difference between the four groups in the levels of striatal dopamine in 42-day-old pups (Table 1).
Prevention of an Increase in Striatal NMDA Receptor Levels Following Gestational Cocaine Exposure by Clozapine in Rat Brains. To further confirm that clozapine protects against gestational cocaine-induced neurochemical effects, we also investigated whether clozapine prevents augmentation of striatal NMDA receptors after gestational cocaine exposure. The results again indicate that there was a significant increase in the number of NMDA receptors in the striatal membranes of pups born to dams exposed to cocaine compared with the control group (Fig. 3). This cocaine-mediated effect was prevented by pretreatment with clozapine (Fig. 3). The increase in NMDA receptor density in prenatal cocaine-exposed rats may result from a decrease in NMDA neurotransmission.

Discussion

The present data demonstrate a diminution of striatal dopamine levels in pups born to cocaine-exposed mice compared with the control group between the ages of 3 to 15 days. In contrast, the 42-day-old pups showed no significant difference in the levels of striatal dopamine between control and cocaine-treated groups (Fig. 1). This suggests that despite initial loss of striatal dopamine at early ages, there is a recovery in the concentration of this neurotransmitter in the striatum during the course of brain maturation after gestational exposure to cocaine. These data are in accord with previously reported developmental recovery in terms of extracellular striatal dopamine levels in rats (Keller et al., 1994), auditory brain stem transmission in human newborns (Salamy et al., 1990) and postnatal rats (Church et al., 1990), and neurobehavioral indices in human infants (Chasnoff et al., 1992) following prenatal cocaine exposure.

Our results showing that gestational cocaine lowered striatal dopamine level in early postnatal ages are also consistent with another study with human neonates where prenatal cocaine exposure showed significantly lower levels of cerebrospinal fluid, homovanillic acid, the principal metabolite of dopamine (Needelman et al., 1993). The diminution of striatal dopamine may be due to excessive release of this monoamine and/or a reduction of striatal dopamine transporter protein following gestational cocaine exposure. Consistent with this notion, an increased potassium-stimulated rat striatal dopamine release concurrent with a decrease in dopamine transporter proteins were observed in brains from the offspring of gestational cocaine-exposed rats (Salvatore et al., 2004). Moreover, the release of striatal dopamine after an acute cocaine injection was found to be greater and more prolonged in the prenatal cocaine-exposed rat pups compared with the vehicle control group (Keller et al., 1994). Interestingly, Keller and his associates (1994) observed that basal extracellular dopamine levels, as well as its metabolites, were increased in the prenatal cocaine-exposed pups compared with control pups. Therefore, a diminution of striatal dopamine transporter protein after prenatal cocaine exposure (Salvatore et al., 2004) may decrease the endogenous levels of dopamine (Fig. 1) and increase its concentration in the extracellular fluid (Keller et al., 1994). A reduction of endogenous dopamine concentration in the striatum may result in an elevated dopamine synthesis rate as a feedback mechanism in the gestational cocaine-exposed pups that, in turn, account for the recovery of dopamine concentrations observed at the later postnatal age. This assumption is consistent with the demonstrations that prenatal cocaine administration increased fetal brain or cortical tyrosine hydroxylase activity or immunoreactivity (Akbari and Azmitia, 1992; Meyer and Dupont, 1993). In contrast to the recovery of striatal dopamine levels at 42 days of postnatal age in prenatal cocaine-exposed pups reported here, the forebrains of the mature offspring following prenatal cocaine exposure showed a reduction of dopamine concentration in male mice (Miller et al., 1995). Such discrepancy may result from differences in doses of cocaine used (5 mg/kg daily versus 30 mg/kg daily). The present data reported a 30% decrease in extracellular dopamine levels at 42 days of postnatal age in prenatal cocaine-exposed pups compared with control pups. This discrepancy may be due to the difference in the gestational cocaine dose used in these studies.

Table 1

Clozapine cotreatment prevents cocaine-induced changes in striatal dopamine levels

<table>
<thead>
<tr>
<th>Striatal Dopamine Level</th>
<th>Control</th>
<th>Cocaine</th>
<th>Clozapine</th>
<th>Cocaine + Clozapine</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-Day-old pups</td>
<td>219.5 ± 2.2</td>
<td>154.5 ± 3.1</td>
<td>238.2 ± 1.8</td>
<td>230.1 ± 3.2</td>
</tr>
<tr>
<td>42-Day-old pups</td>
<td>200.4 ± 5.4</td>
<td>169.4 ± 12.3</td>
<td>186.5 ± 10.0</td>
<td>186.1 ± 11.1</td>
</tr>
</tbody>
</table>

*p < 0.01 when compared with controls in each age group.

*p < 0.01 when compared with cocaine-treated animals in each age group.
The procedure to measure NMDA receptor binding was similar to that described under Materials and Methods or the legend to Fig. 2. All binding assays were conducted in the presence of 100 μM glutamate. The results are expressed as moles per milligram of wet weight x 10^-10 (mean ± S.E.M.) derived from four independent determinations. The data show a significant increase in the density of NMDA binding sites in the cocaine-exposed group compared with control and cocaine- plus clozapine-exposed groups. The control and cocaine- plus clozapine-exposed groups showed no significant difference. Also, there were no significant differences in the apparent dissociation constants between the three groups. Statistical differences were evaluated using one-factor ANOVA followed by Newman-Keuls test for multiple comparisons. *, p < 0.01 when compared with the control group. †, p < 0.01 when compared with the cocaine-treated group.

mg/kg twice daily), route of administration (i.p. injection versus intragastric intubation), and/or treatment paradigms (E0 to E21 versus E8 to E19) between these two studies.

In addition to dopaminergic neurotransmission, glutamatergic systems, especially the NMDA receptor, are likely involved in cocaine-mediated neurochemical and/or neurobehavioral effects. Previous investigators have shown that MK-801 prevents the development of sensitization to the convulsive response in rats and mice (Itzhak and Stein, 1992) and the augmentation of lethality rate in mice (Itzhak and Stein, 1992) following repeated administration of cocaine in adult animals. Moreover, up-regulation of cortical NMDA receptors following repeated administration of cocaine to mice appears to parallel the sensitization phenomenon (Itzhak and Stein, 1992). These observations suggest that some of the cocaine-induced toxicity may be mediated through glutamatergic neurons. To test the possibility that gestational cocaine exposure affects the levels of cortical NMDA receptors, we measured [3H]MK-801 binding to rat cortical membranes either in the presence of glutamate or glycine obtained from 15-day-old pups of either control or experimental groups. This experiment was based on a previous finding that in the presence of either glycine or glutamate, an increase in ionophore opening results in several-fold enhancement of [3H]MK-801-specific binding (Reynolds et al., 1987). There was a significant increase in the density of the NMDA receptors either in the presence of glycine or glutamate in the cortical membranes obtained from pups exposed to gestational cocaine compared with the control group (Fig. 2). Although the precise underlying mechanism for the increased NMDA receptors is currently not clear, it is possible that this may be a compensatory response to either diminution of extracellular glutamate levels or down-regulation of postsynaptic NMDA receptor activity in the experimental group. Although there is no information on the effects of gestational cocaine exposure on the cortical extracellular glutamate levels or postsynaptic NMDA receptor sensitivity, a decrease in the levels of extracellular glutamate in the nucleus accumbens following repeated cocaine administration has been reported (Bell et al., 2000). Alternatively, disruption of the NMDA receptor subunit composition and/or their interaction with signal regulators that are critical to the pharmacological and electrophysiological activities in the cerebral cortex may have occurred during cocaine exposure to developing brains.

The above results raise the possibility that attenuation of glutamatergic transmission may protect against adverse neurochemical changes mediated by gestational cocaine. Olney and his associates have shown that noncompetitive NMDA receptor antagonists such as MK-801 are neurotoxins and of limited value as therapeutic agents (Olney et al., 1991). This MK-801-mediated neurotoxic effect may be derived from blocking glutamatergic transmission mediated via the NMDA receptor system located on GABA neurons leading to a tonic inhibitory influence over excitatory tone in the cortex (Olney and Farber, 1994). Reduction or blockade of this tonic inhibition by NMDA antagonist may result in imbalanced excitation and cell death. Additionally, MK-801 may also block the presynaptic NMDA receptors to increase release of glutamate that would eventually cause excitotoxicity. Despite such difficulties with noncompetitive NMDA antagonists, drugs useful to tone down glutamatergic transmission are available. For example, GM1 ganglioside has been shown to prevent glutamate-induced excitotoxicity without affecting normal synaptic transmission (Favaron et al., 1990).

Reduction in glutamate-induced excitotoxicity may also be achieved using partial agonists of the NMDA receptors such as clozapine (Banerjee et al., 1995). This hypothesis is supported by the results shown in Table 1 and Fig. 3 indicating that clozapine protects against a gestational cocaine-induced decrease in the striatal dopamine concentration and an increase in striatal NMDA receptor density in 15-day-old mouse or rat pups, respectively. These data suggest that the mechanism for the protection against cocaine-induced toxicity may be related to its ability to reduce glutamatergic transmission. Alternatively, clozapine may reduce cocaine-mediated vasoconstriction and hypoxia by acting as an antihypertensive by blocking D1 receptor subtype (Witkin et al., 1993). Together with the reports demonstrating clozapine effectively blocks another NMDA noncompetitive antagonist, phencyclidine-induced hyperlocomotion in rats (Phillips et al., 2001), these data support the notion that clozapine could prevent prenatal cocaine-induced neurochemical changes by modulating glutamatergic activity. The pivotal role of NMDA receptors in mediating cocaine- or phencyclidine-induced neurochemical and behavioral changes is further strengthened by demonstration that despite the fact that clozapine blocks D1, 5-HT2, and muscarinic cholinergic receptors, more specific blockers of these receptors do not prevent increased locomotion caused by cocaine or phencyclidine unless very high dosages are used (Phillips et al., 2001; Moy and Breese, 2002).

The clinical relevance of clozapine in preventing neurochemical and neurobehavioral changes elicited by gestational cocaine exposure is supported by reports demonstrating clo-
zapine is effective in the treatment of cocaine abuse with or without schizophrenia (Farren et al., 2000; Zimm et al., 2000). Similarly, another atypical antipsychotic, olanzapine, has also been shown to prevent locomotor sensitization induced by chronic phencyclidine administration in rats and cocaine abusers with schizophrenia (Moy and Breese, 2002; Tsuang et al., 2002). Since modafinil, which increases brain glutamate and inhibits GABA release, attenuated some aspects of cocaine withdrawal including blunting of cocaine-induced euphoria (Dackis and O’Brien, 2003), the possibility that clozapine protects against gestational cocaine-induced neurotoxicity may be expressed by the enhancement of glutamatergic transmission cannot be ignored. In addition, the attenuated glutamatergic neurotransmission may also be improved by directly stimulating the postsynaptic NMDA receptors because clozapine is also a partial agonist at the NMDA receptors. Alternatively, the ability of clozapine in blocking cocaine-induced neurotransmission changes mediated by other systems, especially the dopaminergic receptors, may also be involved. Although future research is required to elucidate the precise mechanism of action for clozapine in its protection against gestational cocaine-induced neurotoxicity, our data presented here directly demonstrate that coadministration of clozapine with cocaine during the gestational period prevents cocaine-induced neurochemical changes and support the notion that clozapine and other agents that modulate the NMDA neurotransmission may be used in the management of neurochemical effects induced by prenatal cocaine exposure.

References


