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PRENATAL COCAINE EXPOSURE ENHANCES RESPONSIVITY OF LOCUS COERULEUS NOREPINEPHRINE NEURONS: ROLE OF AUTORECEPTORS

J.D. ELSWORTH^{1,2}, B.A. MORROW¹, V.-T. NGUYEN², J. MITRA², M.R. PICCIOTTO^{1,2}, and R.H. ROTH^{1,2}

¹Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA

²Department of Pharmacology, Yale University School of Medicine, New Haven, CT, USA

Abstract

Children exposed to cocaine during gestation have a higher incidence of neurobehavioral deficits. The neurochemical bases of these deficits have not been determined, but the pharmacology of cocaine and the nature of the abnormalities suggest that disruptions in catecholaminergic systems may be involved. In the current study, we used a rat model of prenatal cocaine exposure to examine the impact that this exposure has on the locus coeruleus (LC) noradrenergic system in offspring. Pregnant rats received twice-daily intravenous injections of cocaine (3 mg/kg) or saline between gestational days 10 and 20, and progeny were tested as juveniles. Exposure to a mild stressor elevated an index of norepinephrine turnover in the prefrontal cortex and also increased Fos expression in tyrosine hydroxylase-positive LC neurons in rats exposed to prenatal cocaine but not in rats exposed to prenatal saline. No change in the number of tyrosine hydroxylase-positive neurons in the LC was observed between the two prenatal treatment groups. Specific binding of [¹²⁵I]-para-iodoclonidine, a radioligand with specificity for high affinity α_2 A-adrenergic receptors, was decreased in the LC of rats exposed to prenatal cocaine compared to prenatal saline controls. As α_2 -adrenergic receptors on LC norepinephrine neurons function as autoreceptors, their down-regulation by prenatal cocaine exposure provides a plausible mechanism for the observed heightened reactivity of norepinephrine neurons in these animals. These data indicate that prenatal cocaine exposure results in lasting changes to the regulation and responsivity of rat LC norepinephrine neurons. A similar dysregulation of LC norepinephrine neurons may occur in children exposed to cocaine during gestation, and this may explain, at least partly, the increased incidence of cognitive deficits that have been observed in these subjects.

Keywords

alpha-2 adrenergic receptor; ADHD, Fos; prefrontal cortex; stress; tyrosine hydroxylase

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Correspondence: John D Elsworth PhD, Yale University School of Medicine, 300 George Street, Suite 8300, New Haven, CT 06511, USA, Phone: 203 785 4506, Fax: 203 785 5275, john.elsworth@yale.edu.
Neuropharmacology Section Editor for *Neuroscience*: Dr Yoland Smith, Yerkes Primate Research Center, Emory University, Atlanta, GA 30329

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Recent well-controlled and detailed clinical studies on large cohorts of children exposed to prenatal cocaine have elaborated on the enduring cognitive deficits that they exhibit. The profile of behavioral effects associated with fetal cocaine exposure includes alterations in modulation of arousal, attentional control and reactivity to stressful conditions. The syndrome includes deficits that have been related to prefrontal cortex (PFC) function, such as sustained focus, inhibitory control, working memory and short-term memory (Mayes and Fahy, 2001; Bennett et al., 2002; Singer et al., 2002; Schroder et al., 2004; Noland et al., 2005; Savage et al., 2005). Animals exposed to prenatal cocaine also exhibit cocaine-induced deficits in sustained and selective attention, short term memory and increased reactivity to stress (Romano and Harvey, 1996; Garavan et al., 2000; Morgan et al., 2002; Morrow et al., 2002; Gabriel et al., 2003; Gendle et al., 2003; Thompson et al., 2005). As catecholamine neurotransmission has a critical influence on PFC function (Coull, 1994; Murphy et al., 1996; Dalley et al., 2004; Seamans and Yang, 2004; Arnsten and Li, 2005; Floresco and Magyar, 2006) it is reasonable to suspect that the impact of prenatal cocaine on these developing transmitter systems may contribute to the behavioral abnormalities described above.

The impact of prenatal cocaine exposure in animals on the dopamine (DA) systems of the offspring has been studied by several groups (Keller and Snyder-Keller, 2000; Harvey, 2004), including our own findings that DA neurons innervating the PFC show an enhanced reactivity to stress in rats exposed to prenatal cocaine (Elsworth et al., 2001; Morrow et al., 2001). It is also logical to implicate the norepinephrine (NE) system in the behavioral deficits associated with prenatal cocaine exposure, as cocaine is known to have high affinity for the NE transporter, which is expressed early in development (Ren et al., 2003), and because the NE system is particularly sensitive to stressful conditions and novel environmental stimuli, and is known to exert a modulatory action on higher cognitive processes, such as attention and working memory (Aston-Jones et al., 1999; Berridge and Waterhouse, 2003; Arnsten and Li, 2005). Several older studies using the rat subcutaneous model of prenatal cocaine reported altered indices of NE function. Thus, rats exposed to prenatal cocaine exposure were found to have an increase in forebrain NE turnover (Seidler and Slotkin, 1992), an age-dependent increase in NE transporter binding in the bed nucleus of the stria terminalis (McReynolds and Meyer, 1998), and an age-dependent increase in β -adrenoceptor binding in the parietal cortex (Henderson et al., 1991). Two more recent studies, using the rat intravenous model of prenatal cocaine exposure, have also suggested an impact on the NE system. Specifically, prenatal cocaine exposed rats were demonstrated to have altered sensitivity to the effects of a NE α_2 antagonist on certain facets of an attention task (Bayer et al., 2002), and an increase in α_2 adrenergic binding in the parietal cortex (Booze et al., 2006). Additionally, evidence from culture studies indicated that cocaine exposure reduced neurite outgrowth of locus coeruleus neurons (Snow et al., 2001). While these few rather disparate studies together suggest an effect of prenatal cocaine on the NE system of the offspring, we decided it was appropriate to measure several indices that relate to NE PFC function in our intravenous rat model of prenatal cocaine exposure in which we have documented deficits in short-term memory (Morrow et al., 2002). We hypothesized that NE neurons innervating the PFC of rats exposed to prenatal cocaine would be hyperactive compared with appropriate controls.

EXPERIMENTAL PROCEDURES

Prenatal cocaine administration

Timed-pregnant females (Sprague-Dawley) were obtained from Charles River Laboratory (Wilmington, MA), and housed singly in a temperature-controlled colony room (21–22°C) with lighting between 7 am and 7 pm. On embryonic day (E)7 or E8, an indwelling jugular catheter for cocaine/saline administrations was implanted in each rat as described before (Morrow et al., 2002). Cocaine or saline was administered for 11 days starting on E10 and

ending on E20. Rats received cocaine (3 mg/kg) or an equal volume of saline (1 ml/kg) twice a day at approximately 10 am and 4 pm. Within 24 h of birth, the pups from the saline- and cocaine-treated dams were moved to foster dams that had also just given birth, in order to control for any changes in maternal behavior due to the prenatal treatment. The number of pups was reduced to 12, if the litter was larger than that. After fostering, the surgically prepared dams were given an overdose of pentobarbital (100 mg/kg) and the location of the catheter tip was verified during necropsy.

On postnatal day (P) 21, pups were weaned, and housed with their same-sex littermates. A maximum of 5 rats were permitted in a cage; however, no rat was individually housed. Rats were used at the late adolescent/young adult stage, to match the juvenile ages when cognitive effects have been identified in humans exposed to cocaine in utero. Each experimental group contained no more than one male or one female from each litter (Holson and Pearce, 1992; Spear and File, 1996). All animal procedures were approved by the Yale University Institutional Animal Care and Use Committee, and designed to minimize animal use and suffering.

Mild stress and prefrontal cortex NE turnover

Rats (male and female) were habituated to the test environment by exposing each one to the footshock apparatus for 30 min. On the following day (P40–42), rats were subjected either to footshock (“threshold” at 0.4 mA, or “mild” at 0.8 mA) paired with audible tones, or tones alone. The “threshold” level of shock is barely discernable to the human hand. Ten tones of 2.8 kHz each lasting 5 sec were delivered randomly, 1 to 4 min apart over 30 min, and in cases where footshocks were given, each tone co-terminated with a footshock lasting 0.5 sec. This procedure is essentially the acquisition session of our established conditioned fear paradigm (Morrow et al., 1999). Animals were sacrificed immediately after removal from the apparatus by decapitation. The brain was rapidly removed, and the medial PFC dissected bilaterally on a thermostatically controlled refrigerated surface (1–2 °C), frozen on dry ice, and stored at –70 °C. Tissue was assayed for NE by alumina extraction followed by HPLC with electrochemical detection using dihydroxybenzylamine as internal standard (Elsworth et al., 1989). MHPG and the internal standard [²H₃]MHPG were quantified by gas chromatography-mass spectrometry, monitoring m/e 362 and 365 ions of the 4-acetyl-di-trifluoroacetyl derivatives (Elsworth et al., 1983). The ratio of MHPG/NE was calculated, and used as an index of NE turnover (Cooper et al., 2003).

Mild stress and Fos expression in LC neurons

In this paradigm, male rats (P45) were exposed to a novel environment. Each animal in the test group was separated from their littermates, handled and placed alone in a plexiglass cage (23×20×45 cm) with fresh bedding (Sharp et al., 2002). A white noise generator minimized external noises. After 30 min rats were returned to their home cage. Animals were not subjected to a more severe stress paradigm as this might have induced a near-maximal increase in Fos expression in control animals and obscured the ability to detect small differences in responsiveness between the prenatal groups. Two hours after exposure to the novel environment, rats were anesthetized and then perfused with saline, followed by phosphate-buffered paraformaldehyde solution (4%, pH 7.4). In the control group, animals were removed from the home cage, then sacrificed and perfused as described above. Brains were removed, post-fixed in 15 ml of phosphate-buffered paraformaldehyde solution for 2 days, and then placed in 0.1 M phosphate buffer containing 30% sucrose for 2 days. A slab containing the LC was cut coronally on a freezing microtome into 40 µm sections and stored as five serially collected sets of tissue.

To assess the extent of NE neuron activation in response to a novel environment, tissue sections were double-stained for tyrosine hydroxylase (TH) immunoreactivity, to identify NE neurons, and for immunoreactivity to the immediate early gene product, Fos, to assess neuronal activation, as previously described (Morrow et al., 2001). Briefly, one of the five sets of tissue was used, consisting of 40 μm sections taken every 200 μm starting rostral to the LC and continuing past the caudal edge of the LC. Immunohistochemical staining was carried out in 2 batches using identical methods, timing, reagents, buffers, and antibodies over a 2-week period to minimize any potential differences (Taylor and Levenson, 2006). Each batch contained tissues from half of the rats in each treatment group, and they were coded to blind the investigators. Tissues were first immunostained for Fos immunoreactivity using a nickel-intensified diaminobenzidine reaction to yield a purple-black nuclear stain, and then immunostained for TH immunoreactivity using an unmodified diaminobenzidine reaction to yield a red-brown cytosolic stain. This protocol allowed complete penetration of the antibodies, as indicated by good immunostaining of neurons throughout the 40 μm sections (Morrow et al., 2005). Tissue sections were also stained with cresyl violet, to permit identification of the nucleolus for cell counting purposes. Counts of LC TH-immunoreactive (ir) cells and of LC TH-ir cells expressing Fos immunoreactivity were made unilaterally, starting with sections anterior to the appearance of the LC and continuing caudally until no LC TH-ir cells were seen. As the LC is a compact nucleus, we followed the simple profile method; all identifiable LC TH-ir neurons on one side of the brain (unilateral) were counted rather than using a sampling technique within the region (Howard and Reed, 1998). Cell counts were alternated between the right LC of one rat and the left LC of the next one to be estimated. A double-stained cell was counted when Fos-ir staining was observed within a TH-ir cytoplasm in the unilateral LC being counted. A TH-ir cell was counted if a clear, Nissl-stained nucleolus was visible in the nucleus of a TH-ir cell. As nuclear Fos immunoreactivity obscures the nucleolus, double-stained cells were also counted as TH-ir cells. The total bilateral number of LC TH-ir cells in each animal was estimated by taking the total TH-ir cells counted from the tissue set and correcting for the total number of sets of tissue from the animal (by multiplying by 5) and for the unilateral count (by multiplying by 2). The number of double-stained cells was expressed as a percent of counted LC TH-ir cells. In this way, the same tissue section was used to determine both the number of TH-ir cells in the LC and the number of TH-ir cells that also expressed Fos-ir.

Radioligand binding and autoradiography

To investigate changes in α_2 -adrenergic receptors in the LC, radioligand binding to these sites was examined by autoradiography in brains from male rats (P42–45) exposed in utero to either cocaine or saline. Following decapitation and brain removal, the hindbrain containing the LC was blocked coronally on a thermostatically controlled refrigerated surface. Brain slabs were immediately frozen on dry ice and stored at -70°C . Subsequently, each tissue block was cut on a cryostat and serial sections (12 μm thickness) containing the LC (Paxinos and Watson, 1997) were thaw-mounted on gelatin-subbed slides and organized into sets, with sections in each set being 156 μm apart. Analysis of a set of tissues ensured an unbiased representation of sections from the entire locus coeruleus, which may be important considering the reported posterioranterior heterogeneity in α_2 -adrenergic binding in region (Chamba et al., 1991). The sections were stored in an airtight container at -70°C . The clonidine derivative, [^{125}I]-para-iodoclonidine (NEX-253; Dupont NEN, Boston, MA, 2200 Ci/mmol), was used to label the high-affinity α_2 -adrenergic receptor sites from a set of tissues, which comprised a mean of 4 slides from each rat containing LC for specific binding. Evidence indicates that this radioligand preferentially labels the high affinity state of the $\alpha_2\text{A}$ -adrenergic receptor subtype in the LC (Baron and Siegel, 1990; Alburges et al., 1993; Wallace et al., 1994). The assay (Alburges et al., 1993) was initiated with a 20 min preincubation in 170 mM Tris-HCl buffer (pH 7.6) containing 20 mM MgCl_2 , followed by 90 min incubation at room temperature with the

radioligand at twice its K_D (1.1 nM) in a humidified chamber. The radioligand was removed with two successive 5-min rinses in fresh cold buffer. Non-specific binding was established by inclusion of 10 μ M phentolamine (Sigma Chemical Company, St. Louis, MO) with the radioligand on 1–2 additional sections from the set. Sections were dried under a cool stream of air, and then loaded into autoradiography cassettes, each with a [125 I] microscale autoradiographic standard (GE Healthcare Bio-Sciences Corp., Piscataway, NJ), and exposed for 3 days at -70°C to Hyperfilm MP (GE Healthcare Bio-Sciences Corp.). After hand-development in GBX developer and fixer solutions (Eastman-Kodak, Rochester, NY), films were placed on a Northern Light illuminator (InterFocus Imaging Ltd., Linton, England) and images digitized using a monochrome CCD Camera (Cohu model 4913, San Diego, CA) with a Nikon Micro-Nikkor lens (55 mm f2.8). Quantitative analysis of autoradiographs was performed using NIH Image (version 1.6.2, <http://rsb.info.nih.gov/nih-image>).

Statistics

The data were analyzed by ANOVA using 1- or 2-factor models (prenatal treatment, stress) where appropriate (SuperANOVA; Abacus Concepts, Berkeley, CA).

RESULTS

Effect of prenatal cocaine on pregnancy

As reported previously, the twice daily i.v. administration of 3 mg/kg cocaine did not produce any alterations in maternal weight gain, length of gestation, number of pups in a litter, male/female ratio in a litter, or weight of the offspring (data not shown, see Morrow et al., 2002).

Effect of prenatal cocaine exposure on NE turnover

An initial planned comparison revealed no significant effect of sex on NE turnover, so data from males and females were merged in the analysis of prenatal treatment and stress factors on NE turnover. Rats exposed to prenatal cocaine exhibited elevated NE turnover compared with prenatal saline controls (Fig. 1).

Effect of prenatal cocaine on number of LC TH-ir neurons

There was no significant difference in the mean number of TH-ir neurons ($\pm$ S.E.M.) in the LC between prenatal saline (2068 ± 242 , $n = 4$) and prenatal cocaine (2313 ± 123 , $n = 4$) groups.

Effect of prenatal cocaine on stress-induced Fos-ir expression in TH-ir cells

When taken directly from their home cage, there was no difference in the number of Fos-ir/TH-ir neurons in the LC of rats exposed prenatally to either cocaine or saline (Fig. 2 and Fig. 3). However, compared with their respective home cage controls, a novel environment resulted in a significant increase in LC TH-ir neurons positive for Fos-immunoreactivity in rats exposed to prenatal cocaine, but not in rats exposed to prenatal saline (Fig. 3). This exposure to a novel environment resulted in an approximately 5-fold increase in the percentage of LC TH-ir neurons expressing Fos-immunoreactivity in prenatal cocaine rats compared to the saline controls (Fig. 3).

Both measures of NE activation (NE turnover in the prefrontal cortex and expression of Fos-immunoreactivity in LC TH-ir cells) were elevated in prenatal cocaine rats and not in saline controls following stress; however, that the relative magnitude of change in these indices cannot be meaningfully compared in the present studies as they were made in at different times after different stressors.

Effect of prenatal cocaine on [¹²⁵I]-p-iodoclonidine binding

A preliminary study in a small number of subjects indicated a 40% reduction in specific binding of [¹²⁵I]p-iodoclonidine to LC of rats exposed to prenatal cocaine compared with prenatal saline controls (Mitra, 2003). The present data confirmed this significant decrease in specific [¹²⁵I]p-iodoclonidine binding to LC of rats exposed to prenatal cocaine compared with prenatal saline controls, albeit to a lesser extent (Fig. 4 and Fig. 5). No between-group difference was seen in the binding of the [¹²⁵I]-p-iodoclonidine to the parabrachial nucleus in the same sections (Fig. 5).

DISCUSSION

The data reported here, using 3 different techniques, indicated that prenatal cocaine exposure results in lasting changes to the LC NE system. Compared with controls, juvenile rats that had been exposed to cocaine in utero exhibited increased NE turnover in the PFC, enhanced Fos activation in LC NE neurons in response to a mild stress, and decreased α 2-adrenergic receptor binding in the LC. Together these findings suggest a more active and responsive NE system in rats exposed to prenatal cocaine, which may play a role in the cognitive deficits observed in such rats.

Our initial indication of a change in the NE system of rats exposed to prenatal cocaine was an elevated MHPG/NE ratio in the PFC. We had hypothesized an exaggerated effect on mild footshock on NE turnover in prenatal cocaine rats, but analysis of the data revealed a main effect of prenatal treatment suggesting an elevated NE turnover under both baseline and stressed conditions. However, it should be noted that rats not subjected to footshock were still handled and placed in the testing equipment. Thus, it is possible that either prenatal cocaine control rats were stressed enough to activate the NE system, or that the NE neurons in these rats permanently have a higher turnover rate than normal rats. An earlier study using the subcutaneous model of prenatal cocaine administration (Seidler and Slotkin, 1992) also reported an increased forebrain NE turnover in offspring that were not intentionally stressed, yet these rats also were handled and injected prior to sacrifice. While neither the present investigation nor the work of Seidler and Slotkin (1992) provide a resolution on the basal state of activity of the NE system in prenatal cocaine rats, data from our Fos studies (below) do address this issue, and favor the explanation that the NE system in rats exposed to prenatal cocaine is not activated in a truly unperturbed state and is exceptionally sensitive to stress.

Regarding the magnitude of elevation in NE turnover, we have previously found that even electrical stimulation of the locus coeruleus at 20 Hz for 30 mins only produces an 80% increase in cortical MHPG concentration (Crawley et al., 1980). We also know though that stress- or pharmacologically-induced changes in cortical NE turnover become greater with periods of activation greater than 30 mins (Tanaka et al., 1982; Ida et al., 1991), and 3–4 fold increase in NE turnover been reported in rat frontal cortex after restraint for 4 hours (Ando et al., 2000). Thus, it is likely that the increase in prefrontal cortex NE turnover in prenatal cocaine rats would have been much greater in response to a more severe stressor or to a more prolonged exposure to the mild stressor.

The immunohistochemical studies provided several insights into the effects of prenatal cocaine exposure on the LC NE system. No differences were seen in the number of LC TH-ir neurons between the prenatal cocaine- and saline-treated groups, and our estimate of the population agrees with previous counts of NE neurons in the LC (e.g., Monji et al., 1994). A previous *ex vivo* study suggested that prenatal cocaine exposure induced a loss of LC NE neurons, as a significantly lower number (20%) of adhering unstained LC neurons were observed 2 days after dissection and plating of tissue harvested from offspring at P0–1 (Snow et al., 2001). The

apparent difference between this culture study and the present one may be due to differences in model systems, specificity or sensitivity.

As the pattern and magnitude of c-fos induction following acute challenges have been used to reveal stress-related circuitry (Cullinan et al., 1995; Kovacs, 1998), we used the expression of the transcription factor, Fos, to evaluate the responsivity of LC NE neurons in prenatal cocaine rats and controls. When taken directly from their home cage, there was no difference in the expression of Fos in LC NE neurons between rats exposed to prenatal cocaine or prenatal saline, indicating that under baseline conditions the level or pattern of stress was insufficient to activate c-fos. In addition, handling rats and placing them in a novel environment did not significantly alter Fos expression in LC NE neurons of rats exposed to prenatal saline. However, this relatively mild stress did elicit a marked increase in the number of Fos-ir/TH-ir neurons in the LC of rats exposed to prenatal cocaine. Thus, these data indicate that LC NE neurons in animals exposed to prenatal cocaine are hyper-responsive to stressful stimuli.

The autoradiography studies revealed a possible mechanism by which the responsivity of the LC NE neurons may have been increased. As somatodendritic α_2 -adrenergic receptors present on NE neurons in the LC serve as autoreceptors, a reduction in function of α_2 -adrenergic receptors would be expected to compromise auto-inhibitory control of NE neurons in the LC of prenatal cocaine exposed animals, which could become more apparent during stress-induced activation of LC NE neurons (Svensson et al., 1975; Cooper et al., 2003). One mechanism by which α_2 -adrenergic binding in the LC may be decreased in rats exposed to prenatal cocaine is a response to elevated synaptic levels of NE, resulting from blockade of NE uptake in the LC by cocaine, the region with the highest density of NE transporters during development (Sanders et al., 2005). Thus, the observation of elevated NE turnover in a terminal region of LC NE neurons, and decreased threshold for stress-induced Fos expression in LC NE neurons may be explained by a partial loss of autoreceptor control of LC NE neuron activity.

In considering whether the loss of α_2 -adrenergic binding sites in the LC of prenatal cocaine rats can explain the observed increase in stress-induced Fos expression in the LC and elevated NE turnover in the PFC, it is useful to review other animal models in which there is a down-regulation of α_2 -adrenergic receptors. Mice with a genetic deletion of α_2A -adrenoceptors have a heightened fear response compared with wild type controls, and a greater Fos expression in LC neurons (Davies et al., 2003). More relevant to the degree of loss of α_2 -adrenergic receptor function reported here are a series of studies in Maudsley rats. The Maudsley reactive strain have a greater reactivity to stress than rats from the Maudsley non-reactive strain (Broadhurst, 1975), and investigations of the basis for this behavioral difference have revealed that the reactive strain have 30–40% less [125 I]para-iodoclonidine binding sites in the LC (Sara et al., 1993), a decreased behavioral response to clonidine (Sara et al., 1993), and an increased noradrenergic response to stress (Buda et al., 1994). Thus, these studies support the contention that the degree of loss of α_2 -adrenergic binding observed in rats exposed to prenatal cocaine may be linked with the altered Fos and NE turnover measures reported here, and play a role in the altered behavior documented in other studies (Spear et al., 1998, and citations provided in the Introduction).

In contrast to the finding in the LC, there was no difference between the groups in [125 I]- p-iodoclonidine binding in the parabrachial nucleus. High density of α_2 -adrenergic binding sites have been noted previously in the parabrachial nucleus (Unnerstall et al., 1984), a region that is innervated by A1 and A5 cell groups (Byrum and Guyenet, 1987; Woulfe et al., 1990) and is involved in cardiovascular regulation (Gutterman and Goodson, 1996). The unchanged [125 I]-p-iodoclonidine binding in the parabrachial nucleus in the current study indicates that there was some CNS regional specificity to the effect of prenatal cocaine exposure on α_2 -adrenergic receptors. It is interesting, though, that a decrease in α_2A -adrenergic receptor

mRNA has been observed in the intestine of rat fetuses exposed to cocaine (Ward et al., 2002), suggesting that rats exposed to prenatal cocaine may have a dysregulation of NE function in the periphery. However, no firm comparisons can be made with the present data, as Ward et al. did not measure receptor protein expression and limited the study to the fetal stage of development. Recently, an increase in binding to α 2-adrenergic sites in the parietal cortex, identified by [3 H]RX821002 binding, was reported in rats exposed to prenatal cocaine (Booze et al., 2006). [3 H]RX821002 binding has been reported to recognize the α 2A and α 2C subtypes of the adrenergic receptor, whereas [125 I]- p-iodoclonidine is more selective for the α 2A form (Wallace et al., 1994). High levels of the α 2A-adrenergic receptor and its mRNA are present in the LC (McCune et al., 1993; Nicholas et al., 1993; Scheinin et al., 1994; Talley et al., 1996), where it functions as an autoreceptor on NE neurons (Norenberg et al., 1997; Callado and Stamford, 1999). Low levels of the α 2C subtype are expressed in the LC, yet higher density occurs in other regions including cerebral cortex (McCune et al., 1993; Nicholas et al., 1993; Scheinin et al., 1994; Rosin et al., 1996). Thus, the reported change in [3 H]RX821002 binding in the parietal cortex could reflect alteration in either the A or C subtypes. Clearly, further studies will be necessary to delineate fully the specificity of the effect of prenatal cocaine exposure on α 2-adrenergic binding in different brain regions.

Early studies had discovered that stress and arousal activate LC NE neurons and a role for them was proposed in anxiety (Redmond and Huang, 1979). More recent studies have suggested that the general function of this widespread efferent system is the facilitation of processing of relevant or salient information (Aston-Jones et al., 1999; Berridge and Waterhouse, 2003; Arnsten and Li, 2005). It is known that LC NE neurons can fire in both a tonic mode and in a mode where there are brief episodes of phasic firing, so that the relationship between firing rate of the neurons and a particular behavior is complex. It has been proposed that for vigilance, or tasks requiring focused attention, optimum performance occurs when there is phasic activity together with moderate tonic activity. However, high tonic activity without phasic activity favors a state of high arousal and behavioral flexibility with poor focused attention (Aston-Jones et al., 1999). Stress activation of tonic firing, or inappropriate activation of tonic firing would result, according to the hypothesis, in compromised cognitive performance on tasks requiring focused attention and working memory. Thus, the current finding that prenatal exposure to cocaine reduces binding to LC α 2-adrenergic receptors suggests that mild stress in these animals may result in a relatively premature transition to high rates of tonic activity and a loss of selective attention and working memory function under conditions that would not affect untreated rats.

There are some interesting parallels between the effects of prenatal cocaine exposure in animal or children, and ADHD (Sumner et al., 1993; Scherling, 1994; Dow-Edwards et al., 1999). For example, one of the cardinal signs of ADHD is inattentiveness, and impaired attention is a characteristic cognitive deficit that occurs following exposure to prenatal cocaine in children (Noland et al., 2005; Savage et al., 2005) and experimental animals (Garavan et al., 2000; Gendle et al., 2003). Another similarity between ADHD and prenatal cocaine exposure may be enhanced PFC noradrenergic activity, which occurs following prenatal cocaine exposure (Fig. 1 and Seidler and Slotkin, 1992) and has been postulated to occur in ADHD (Arnsten, 1998; Solanto, 1998; Russell et al., 2005). In fact, clonidine, which decreases NE activity, has been reported to be of benefit in ADHD and is now in a Phase III clinical trial for treatment of ADHD (<http://clinicaltrials.gov/show/NCT00031395>). Our evidence suggests that a decrease in α 2-adrenergic autoreceptor control of NE activity underlies noradrenergic hyperfunction in our rat model of prenatal cocaine, so it is interesting that a similar deficit appears to occur in the spontaneously hypertensive rat model of ADHD (Russell, 2002) and that a decreased density and decreased affinity of binding to α 2-adrenergic sites have been reported in ADHD (Shekim et al., 1994; Deupree et al., 2006). Moreover, there is evidence supporting association and linkage of α 2A-adrenergic receptor gene polymorphisms with ADHD (Park et al., 2005).

Thus prenatal cocaine exposure appears to model certain aspects of ADHD, although it is not known to what degree it fulfills the many other requirements of an animal model of ADHD (Sagvolden et al., 2005).

In conclusion, measurement of both NE turnover in PFC and Fos expression in LC NE neurons indicate that the LC NE system is more responsive to stimuli in rats exposed to prenatal cocaine compared with controls. Autoradiography studies revealed a down-regulation of $\alpha 2$ -adrenergic receptor sites in the LC of prenatal cocaine rats, and as these sites serve as autoreceptors on NE neurons, their compromised function provides a plausible explanation for the increased reactivity of NE neurons in prenatal cocaine rats. A similar dysregulation of LC NE neurons may occur in children exposed to cocaine during gestation, and this may explain, at least partly, the increased incidence of cognitive deficits that have been observed in these subjects. These findings suggest that the NE system may be a suitable target for pharmacological intervention aimed at improving the neurobehavioral deficits associated with prenatal cocaine exposure.

Abbreviations

DA	dopamine
E	embryonic day
ir	immunoreactive
LC	locus coeruleus
NE	norepinephrine
P	postnatal day
PFC	prefrontal cortex
S.E.M.	standard error of the mean
TH	tyrosine hydroxylase

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Figure 1.

Rats exposed to prenatal cocaine exhibit elevated NE turnover compared with prenatal saline controls (P40–42, $N=7-8$ in each group). Mean values are shown $\pm$ standard error of the mean (S.E.M.). ANOVA revealed significant main effects of prenatal cocaine treatment ($F_{1,45}=11.40$, $p<0.005$) and shock ($F_{2,45}=4.44$, $p<0.02$) and no interaction between these factors. * denotes the significantly elevated NE turnover found in the group exposed to prenatal cocaine compared with prenatal saline group.

Figure 2. Immunohistochemical staining for TH and Fos in the LC. In this field, there are examples of cells staining for TH-ir alone, Fos-ir alone or TH-ir with Fos-ir. This section was taken from anterior LC where the TH-ir cell density is lower and the staining is observed more easily in a grayscale image.

Figure 3.

Prenatal cocaine exposure increases reactivity of LC TH-ir neurons to novel stimuli (P45, $n=4$ in each group). The mean number ($\pm$ S.E.M.) of TH-ir/Fos-ir neurons is shown as a percentage of the total number of LC TH-ir neurons. ANOVA revealed significant main effects of prenatal treatment ($F_{1,12}= 16.41$, $p< 0.002$) and housing prior to sacrifice ($F_{1,12}= 27.02$, $p< 0.0002$) on expression of TH-ir/Fos-ir, and a significant interaction between these factors ($F_{1,12}= 33.24$, $p< 0.001$). Contrast analysis indicated that TH-ir/Fos-ir expression in the prenatal cocaine-novel cage group was significantly greater (*) than in both prenatal cocaine-home cage ($F=48.18$, $p<0.0001$) and prenatal saline-novel cage ($F=60.10$, $p<0.0001$) groups.

Figure 4. Autoradiograph showing binding to α_2 -adrenergic sites in the LC and parabrachial nucleus (PB), using [125 I]-para-iodoclonidine. Lowest binding is white, and highest binding is black. This image is slightly over-exposed for illustrative purposes, so that other regions of the brain besides LC and PB can be discerned.

Figure 5.

Prenatal cocaine exposure decreases specific binding to α_2 -adrenergic sites in the LC compared with saline-exposed controls, while no difference was observed in the adjacent dorsal parabrachial nucleus (PD42–45, $n=9-10$ in each group). Mean specific binding ($\pm$ S.E.M.) is shown. ANOVA revealed a significant effect of prenatal treatment in the LC ($F_{1,17} = 7.0$, $p < 0.02$), but not in the parabrachial nucleus. * denotes significant difference from binding in the LC of the prenatal saline group.