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Pharmacology, Biochemistry and Behavior 69 (2001) 451–459

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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The role of NMDA receptors in neonatal cocaine-induced neurotoxicity

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Received 18 August 2000; received in revised form 23 February 2001; accepted 7 March 2001

Abstract

The present study assessed the ability of *N*-methyl-D-aspartate (NMDA) receptor antagonist, dizocilpine (MK-801), to modulate neonatal cocaine-induced neurobehavioral changes in the rat. Sprague–Dawley rats were randomly assigned on postnatal day 0 (PND 0) to one of four treatment groups. Treatments began on PND 4 and continued until PND 10. Treatments consisted of an oral bolus of either cocaine HCl (40 mg/kg), (+)MK-801 (0.4 mg/kg), (+)MK-801 (0.4 mg/kg) followed 30 min later with cocaine HCl (40 mg/kg) or 0.9% saline. On PND 21, 30, 40 and 60, males and females were examined for stress response using the cold-water swim test. Cocaine-treated male and female rats exhibited significantly diminished tolerance to cold-water stress compared to control and MK-801/cocaine-treated groups. In addition, neonatal exposure to cocaine was associated with increased severity of motor symptoms (tail twitches, wet dog shaking and convulsions) following the administration of NMDA (35 mg/kg). Treatment groups were also tested for pain sensitivity using the tail flick (TF) and hot plate (HP) methods. The results indicated that neonatal cocaine exposure altered pain sensitivity in both tests. NMDA receptor binding studies showed a significant increase in receptor densities in the hippocampus and hypothalamus of the cocaine-treated group compared to control. MK-801 administered to rat pups before cocaine treatment blocked the increase in receptor density. The results indicated that neonatal cocaine exposure was associated with altered responses to NMDA, stress tolerance and pain sensitivity. Moreover, the pretreatment with NMDA receptor antagonist, MK-801, abolished or attenuated these cocaine-induced neurobehavioral changes.

Keywords: Cocaine; NMDA; MK-801; Neonatal; Stress; Pain

1. Introduction

Cocaine is a powerful reinforcement agent that when abused during pregnancy affects maternal physiology (Plessinger and Woods, 1993; Woolley et al., 1990), but crosses the placenta (DeVane, 1991), enters the fetal circulation and affects the central and peripheral nervous systems of the fetus (Szeto, 1993; Wiggins, 1992). In utero cocaine exposure has been associated with a variety of developmental, behavioral and neurochemical alterations (Choi et al., 1998; Delaney-Black et al., 1998; Gingras et al., 1992; Lidow, 1998; Minabe et al., 1992; Nassogne et al., 1991) that have led to deficits in learning and memory (Choi et al., 1998; Heyser et al., 1995), stress coping mechanisms (Spear, 1996; Spear et al., 1998) and social interaction skills (Coles and Platzman, 1993).

Recently, the glutaminergic system, in particular *N*-methyl-D-aspartate (NMDA) receptors, has received a great deal of attention due to its modulatory role in various brain regions. From a quantitative standpoint, glutamate and aspartate are the major excitatory neurotransmitters in the human central nervous system (CNS), while classical neurotransmitter systems (acetylcholine, dopamine, norepinephrine, histamine and 5-hydroxytryptamine) account for transmission at a small percentage of central synaptic sites (Cooper et al., 1996). NMDA receptors have become a major focus due to evidence suggesting their involvement in a wide range of neurophysiological and pathological processes, including memory acquisition (Conway, 1998; Escobar et al., 1998; Murray and Ridley, 1997; Riekkenin et al., 1998), developmental plasticity (Curras and Dao, 1998), epilepsy (DeLorenzo et al., 1998; Rice et al., 1998), inflammation following ischemia (Nair et al., 1998) and

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0091-3057/01/$ – see front matter
PII: S0091-3057(01)00521-4

excitotoxicity following overstimulation or prolonged activation (Sonsalla et al., 1998; Zhang et al., 1998).

Although meager information exists linking prenatal/neonatal cocaine exposure to alterations in the NMDA receptor system, there is growing evidence regarding prenatal ethanol exposure and NMDA receptors. Recent studies have shown that prenatal ethanol exposure decreases NMDA receptor densities in the hippocampus and cortex (Karler et al., 1989) and decreases NMDA-stimulated increases in intracellular Ca^{2+} in the cortex, hippocampus and cerebellum (Karler et al., 1989). Similar results have been shown due to neonatal ethanol exposure (Gloor and Fariello, 1988). Another study shows that NMDA receptors play an important role in the developmental toxicity of prenatal lead exposure by increasing NMDA receptor binding in the hippocampus and entrorhinal cortex at postnatal day (PND) 14 (Jett and Guilarte, 1995).

By administering cocaine neonatally, we gain many advantages over prenatal treatment methods. First, by treating rat pups from PND 4 to 10, the drugs are introduced during a period of rapid CNS development known as the ''neuronal spurt period,'' analogous to third trimester in humans (Delaney-Black et al., 1998; DeSarro et al., 1985). Next, by administering the drugs neonatally, it will eliminate maternal contributions during gestation (i.e., hypoxia and anorexia). Finally, the neonatal model will allow a dizocilpine (MK-801)/cocaine-treated group. Previous studies in our laboratory have shown poor results in maternal parturition in animals treated with MK-801 during pregnancy. Thus, the present study will investigate the role NMDA receptors play in long-term behavioral and neurochemical alterations associated with neonatal cocaine exposure.

2. Materials and methods

2.1. Animals

Twenty-eight timed-pregnant Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN), gestational day 7 (GD 7), were housed individually in polyethylene maternity cages. Environmental conditions were controlled and kept under a 12-h light/dark cycle with lights on at 06:00 h and an ambient temperature of $21 \pm 1^{\circ}C$. Animals had free access to rat chow (Teklad Harlan) and tap water. Pregnant dams were left undisturbed until parturition. All 28 dams gave birth to a litter greater than 12 pups, and 27 dams gave birth to a litter with at least five males and five females. Twenty-four of the 27 litters were used for this study giving six animals in each treatment group. The Institutional Animal Care and Use Committee at Florida A&M University approved all experimental protocols used in this study.

2.2. Chemicals

[^{3}H]MK-801 (specific activity, 22.5 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Unlabeled MK-801 was donated by Merck (Whitehouse Station, NJ) and cocaine HCl and other chemicals, unless otherwise stated, were purchased from Sigma (St. Louis, MO).

2.3. Experimental procedure

Pregnant dams gave birth on GD 22. At PND 0, litters were weighed, sexed and culled to 10 with an even ratio of males to females. Litters were randomly assigned to one of four treatment groups–saline control (0.9%, 200 μl po), cocaine HCl (40 mg/kg, 200 μl po), (+)MK-801 (0.4 mg/kg, 200 μl po) and (+)MK-801 (0.4 mg/kg, 100 μl po) followed 30 min later with cocaine HCl (40 mg/kg, 100 μl po). Treatments began on PND 4 and continued through PND 10. Total time of separation from mother was less than 2 min per dosage in order to prevent separation anxiety. On PND 21, rat pups were weaned from mothers, separated by sex and housed with members of their litter.

An individual male rat from each litter was used for cold-water swim test, NMDA response, radioimmunoassay and NMDA receptor binding. Tail flick (TF) and hot plate (HP) pain sensitivity measurements were conducted on the same animal (HP, in the morning; TF, in the afternoon). All experiments conducted on females used an individual subject from each litter for each experiment tested.

2.4. Cold-water swim test

Sex-balanced pairs of rat pups ($n = 6$/gender/treatment) were used for the cold-water swim test on PND 21, 30, 40 and 60. Rat pups were weighed and placed into an aquarium ($28 \times 30 \times 50$ cm) filled to a 28-cm depth with cold water ($4 \pm 1^{\circ}C$). Depth of the water was adequate to prevent pups from touching the bottom of the floor with their tails. Water was replaced between each test and temperature was monitored constantly. Endurance in the cold-water swim test was evaluated based on duration of time the rat pup maintained an angular position such that both the animal's nose and head were above surface of the water. Submersion of the animal's nose below surface level of the water represented termination of timed latency response. Total latency time was indicative of endurance and measured by total time accrued between placement into water and submersion of the animal. After each test, rat pups were removed from the cold water, dried and returned to their cages.

2.5. Immobilization study

On PND 30, male offspring from each litter ($n = 6$/treatment) were placed in a restraint and held immobilized for a 20-min period. A 250-μl sample of blood was withdrawn from tail vein at 0, 20, 60 and 120 min via a small tail nick. A total of 1 ml of blood was drawn from the animal over a 120-min period.

Blood was collected in EDTA-coated tubes and immediately placed on ice. Tubes were centrifuged at $1000 \times g$

for 15 min at 4°C. Plasma was then separated and stored at −80°C. All experiments were initiated at 08:00 h and the last sample was collected by 10:30 h. Plasma corticosterone levels are lowest during morning hours and maximum levels of corticosterone response should be provoked during the morning (Choi et al., 1998). Plasma was tested for levels of adrenocorticotropin hormone (ACTH) and corticosterone using radioimmunoassay procedures.

2.6. Radioimmunoassay determination of ACTH concentration

Assay was conducted using an ACTH radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA). Anti-ACTH was generated in rabbits using a purified porcine ACTH conjugate. Concentration of hormone antigen determined by a radioactive-labeled antigen hormone [^{125}I] antibody complex. With the addition of increasing amounts of hormone, a corresponding decreasing fraction of hormone antigen–antibody complex remained bound. After separation of the bound hormone from the free hormone, radioactivity in the free fraction was counted using a Titertek gamma counter (Huntsville, AL). Assay required 20 μl of plasma per sample and run in duplicate. Samples and standards were diluted 1:5.

2.7. Radioimmunoassay determination of plasma corticosterone concentrations

Assay was conducted using a rat corticosterone radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA). Bovine serum albumin was the antigen used to generate anticorticosterone antibody in rabbits. Concentration of hormone antigen determined by a radioactive-labeled antigen hormone [^{125}I] antibody complex. With the addition of increasing amounts of hormone, a corresponding decreasing fraction of hormone antigen–antibody complex remained bound. After separation of the bound hormone from the free hormone, radioactivity in the free fraction was counted using a Titertek gamma counter. Assay used 5 μl of plasma per sample and run in duplicate. Samples were diluted 1:20 and standards were diluted according to protocol at 1:200.

2.8. NMDA response

On PND 30, male and female rats ($n=6$/treatment) were injected intraperitoneally with a single bolus of NMDA (35 mg/kg, 200 μl). Animals were observed for 100 min following injection. Behavior was observed every 5 min and scored using a seizure rating from 0 to 5, modified from Petit et al. (1992):

(0) assigned if no observable seizure behaviors were noticed
(1) tail twitching
(2) wet dog shakes, rear body shakes
(3) running, body biting, intermittent seizures
(4) tonic seizure activity
(5) death

Scores were taken blind to treatment group.

2.9. Tail flick (TF) test

On PND 30, male rats ($n=6$/treatment) were used to assess pain sensitivity. Baseline latency was established at 3–4 s and cut-off time was set at 15 s to avoid undue injury to the tail. Five measurements were taken with a 1-min interval between measurements. Latency of TF response to a radiant heat source was measured using a Tail Flick Analgesia Meter (TF6) (EMDIE Instruments, Maidens, VA).

2.10. Hot plate (HP) test

On PND 30, male rats ($n=6$/treatment) were used to assess pain sensitivity. Nociception was measured using a HP (Columbus Instruments International, Columbus, OH). Temperature was set at a constant 55 ± 0.5°C and cut-off time was set at 30 s. Animals were tested five times with a 1-min interval between measurements. Animals were placed onto HP apparatus and the time required to lick their hind paw was measured.

2.11. NMDA receptor binding assay

On PND 30, male rats ($n=6$/treatment) were anesthetized with halothane and decapitated. Hippocampus, cortex and hypothalamus were dissected on ice and stored at −80°C. On the day of the assay, brain regions were homogenized in 1.5 ml of ice-cold 0.32 M sucrose using a Virtishear homogenizer (setting 70) (Virtis, Gardiner, NY). Homogenates were centrifuged at $700\times g$ for 12 min at 4°C and the supernatant was centrifuged at $40{,}000\times g$ for 12 min at 4°C. Pellet was resuspended in 1.5-ml binding buffer using a vortex and washed three more times. Membrane pellet was resuspended in 4-ml binding buffer. A small aliquot of membrane sample was used to determine protein concentration using Lowry's method (Lowry et al., 1951).

MK-801 binding assays were performed in triplicate in a total volume of 300 μl, containing 10 mM HEPES, various concentrations of [^{3}H]MK-801 (specific activity, 22.5 Ci/mmol), 100 μM glutamate, 100 μM glycine, 100 μM spermidine and membrane protein. Nonspecific binding was determined in the presence of 10-μM unlabeled MK-801. Incubations were run at room temperature for 3 h and terminated by filtration using a Brandel M-48 Cell Harvester (Gaithersburg, MD) and Whatman GF/B glass fiber filters presoaked in 0.5% polyethylenimine. Filters were washed with three 3-ml aliquots of ice-cold 10 mM Tris–HCl buffer (pH 7.4). Radioactivity was determined in a Beckman LS6500 multipurpose scintillation counting system (Beck-

man Instruments, Fullerton, CA) at efficiency of 60%. K_d and B_{max} were calculated using Scatchard plot analysis.

2.12. Statistical analysis

Statistical analyses of data for the animal weights during treatment, stress endurance latency times and NMDA administrations were analyzed using three-way analysis of variance (ANOVA) with repeated measures (Gender - × Treatment × Time). Corticosterone and ACTH were analyzed using two-way ANOVA with repeated measures (Treatment × Time). All other data obtained were analyzed using one-way ANOVA. Significant differences between mean estimates were determined using Tukey's post-hoc test. Variability was expressed as mean ± S.E.M.

3. Results

3.1. Neonate viability

Administration of only MK-801 produced significant decreases in weight gain and had to be exempted from the study due to high mortality rate (24 of 60 pups lived to PND 10) (Fig. 1) (treatment: $_{.99}F_{3,4408} = 3.78$, $F_{calc} = 5.61$). Cocaine-treated, MK-801/cocaine-treated and control pups showed no difference in weight gain during the treatment period. No significant interactions were observed.

3.2. Cold-water swim test

For male rat pups, cocaine treatment showed a significant decrease in swim endurance when compared to both control and MK-801/cocaine-treated groups at PND 21 and 30 (Fig. 2a) (treatment: $_{.95}F_{2,120} = 3.07$, $F_{calc} = 3.18$). There was no significant difference in swim endurance between any treatment group at PND 40 and 60.

Male pups treated neonatally with cocaine exhibited significant decline ($P < .01$) in weight gain following repeated stress as compared to both control and MK-801/cocaine-treated groups at PND 40 and 60 (Fig. 2b) (treatment: $_{.95}F_{2,120} = 3.07$, $F_{calc} = 3.56$).

For female rat pups, cocaine treatment showed a significant ($P < .01$) decrease in swim endurance at PND 21 and 30 when compared to control and MK-801/cocaine-treated groups (Fig. 3) (treatment: $_{.95}F_{2,120} = 3.07$, $F_{calc} = 4.33$). There was no significant difference in swim endurance between any treatment group at PND 40 and 60. No significant difference was seen between MK-801/cocaine-treated and control rat pups on any day tested.

Female pups did not show any significant difference in weight gain among the three treatment groups as the cold-water swim test progressed. Statistical analysis, using three-way ANOVA, determined significant interactions between (Treatment × Sex: $_{.99}F_{2,120} = 4.787$, $F_{calc} = 63.23$ and Treatment × Day × Gender: $_{.99}F_{6,120} = 2.94$, $F_{calc} = 9.31$); however, no interaction was determined between (Treatment × Day: $_{.95}F_{6,120} = 2.18$, $F_{calc} = 1.19$ and Day × Gen-Gender: $_{.95}F_{3,120} = 2.68$, $F_{calc} = 0.286$).

3.3. Immobilization study

All treatment groups had equivalent basal plasma levels of ACTH and corticosterone. Following a 20-min restraint, all treatment groups showed a significant increase in ACTH levels (treatment: $_{.99}F_{3,400} = 3.78$, $F_{calc} = 5.20$) and the cocaine-treated group had a significant increase in corticosterone (treatment: $_{.99}F_{3,400} = 3.78$, $F_{calc} = 4.53$). The investigation also showed that cocaine-treated rats sustained a significantly higher level of plasma corticosterone than the basal hormonal level throughout the remainder of the study. Both control and MK-801/cocaine groups had an elevation in stress hormones at the 20-min point and returned to basal level by 120 min. There was no significant difference seen

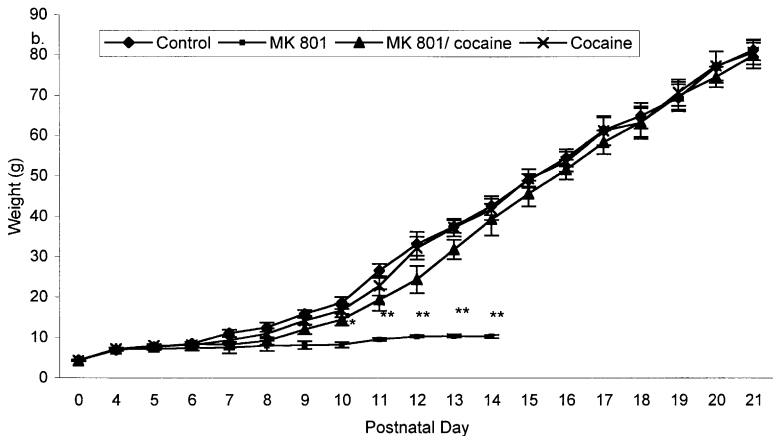

Fig. 1. Neonatal weight gains for (a) male and (b) female treatment groups from PND 0 to 21. MK-801 treatment alone caused a severe lack of weight gain and a higher mortality than the other treatment groups (no. of living at PND 10/total no. treated). Each point represents mean ± S.E.M. *$P < .05$ and **$P < .01$ indicate significantly different from control.

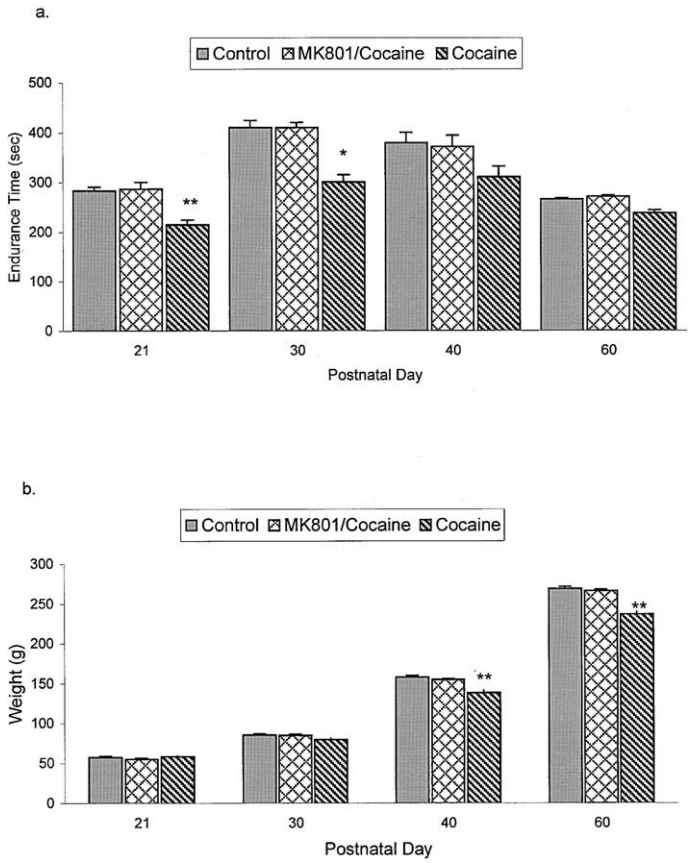

Fig. 2. (a) Effect of neonatal cocaine exposure on postnatal male endurance in a cold-water swim test on PND 21, 30, 40 and 60. (b) Effect of neonatal cocaine exposure on weight gain in rats subjected to a cold-water swim test on PND 21, 30, 40 and 60. Each data bar represents mean ± S.E.M. ($n = 6$). **$P < .01$ indicates significantly different from control.

in hormone levels between control and MK-801/cocaine group at any period of the study (Fig. 4a and b). No significant interactions were determined.

3.4. NMDA response

In male rats, a 35-mg/kg dose of NMDA given on PND 30 showed a significant increase in NMDA sensitivity in cocaine-treated group when compared to control group at 90 and 100 min (Fig. 5a) (treatment: $_{.95}F_{2,600} = 3.00$, $F_{\text{calc}} = 3.16$). MK-801/cocaine-treated group showed no significant difference in NMDA sensitivity from control group at any interval of the study. Each group exhibited signs of intermittent and tonic muscle twitching activity with peak response activity occurring at 10 min following injection. After the 10-min period of the study, all groups began to steadily decrease in their NMDA sensitivity until 30 min into study at which point all three groups plateaued until 70-min mark.

For female rat pups, a 35-mg/kg dose of NMDA produced a quick onset of muscular activities in all treatment groups. Each group exhibited signs of intermittent and tonic seizure activity with peak seizure activity occurring at 15 min following injection. After 15 min, the control and MK-

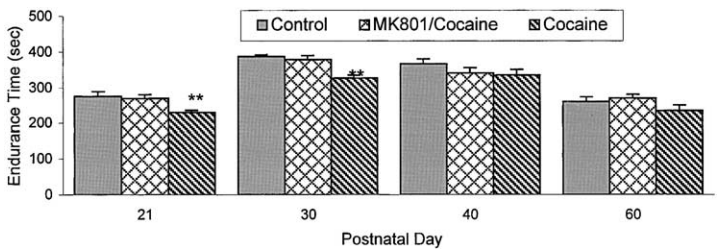

Fig. 3. Effect of neonatal cocaine exposure on postnatal female endurance in a cold-water swim test on PND 21, 30, 40 and 60. Each data bar represents mean ± S.E.M. ($n = 6$). ** $P < .01$ indicates significantly different from control.

801/cocaine groups began to steadily decrease in NMDA response scores with a plateau from 30 to 70 min. From the 70-min point, both control and MK-801/cocaine groups sharply declined nearing zero by 100 min. The cocaine-treated group began a plateau period immediately following the peak at 15 min and maintained near the peak level until 90 min. From 90 to 100 min, the cocaine-treated group began to decline. There was a significant increase in NMDA response in the cocaine group as compared to both the control and MK-801/cocaine groups for the time periods 10, 40, 50, 60, 70, 80, 90 and 100 min of the study (Fig. 5b) (treatment: $_{.99}F_{2,600} = 4.60$, $F_{calc} = 7.75$).

There were no significant interactions in NMDA sensitivity and no animals died during the study due to NMDA administration.

3.5. Pain sensitivity study

In the TF test, there was a significant ($P < .05$) decrease in latency time in the cocaine-treated group (2.13 ± 0.014) compared to both the MK-801/cocaine-treated (3.05 ± 0.15) and control group (3.47 ± 0.16) (treatment: $_{.95}F_{2,30} = 3.31$, $F_{calc} = 4.29$). No significant difference was seen between the control group and the MK-801/cocaine-treated group in TF latency times.

In the HP test, there was a significant ($P < .05$) increase in latency time shown in the cocaine-treated group (19.9 ± 1.7) when compared to both the MK-801/cocaine-

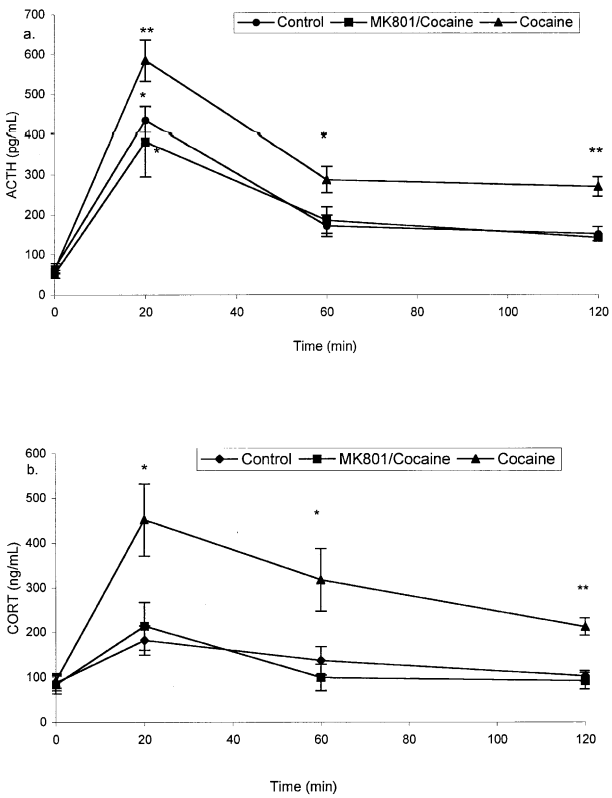

Fig. 4. Effect of neonatal cocaine exposure on plasma (a) ACTH and (b) corticosterone levels in male rats during a 20-min immobilization study at PND 30. Blood samples were obtained at 0 (basal level), 20, 60 and 120 min. Each data point represents mean ± S.E.M. ($n = 6$). * $P < .05$ and ** $P < .01$ indicate significantly different from control basal hormone concentration.

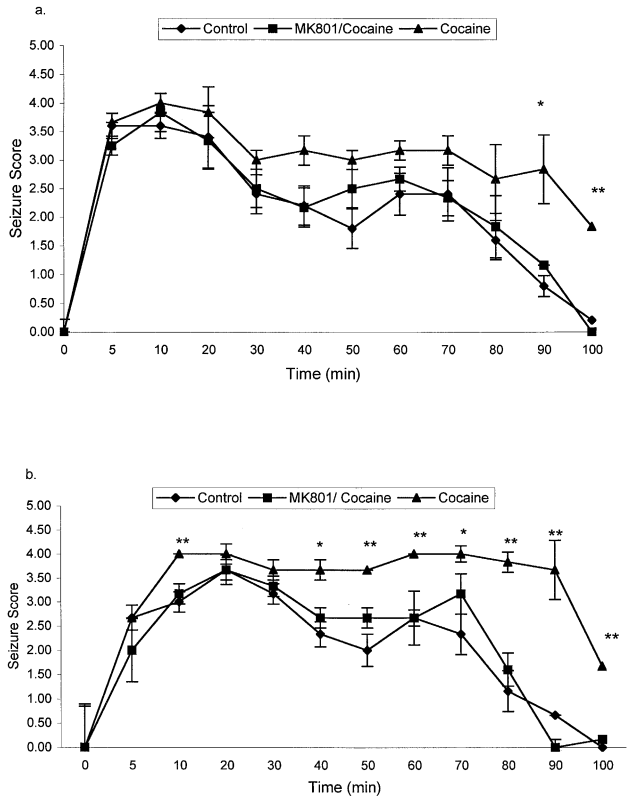

Fig. 5. Effect of neonatal cocaine exposure on NMDA sensitivity in (a) male and (b) female rat pups at PND 30. Rat pups were administered a single 35-mg/kg dose of NMDA and observed for 100 min. Each data point represents mean ± S.E.M. ($n = 6$). * $P < .05$ and ** $P < .01$ indicate significantly different from control at the time period.

Table 1
NMDA receptor binding assay at PND 30 using the noncompetitive antagonist, [^{3}H]MK-801, on the hypothalamus, hippocampus and frontal cortex of rats following neonatal treatment from PND 4 to 10

	K_d (nM)	B_{max} (fmol/mg protein)
Hypothalamus		
Control	2.18 ± 0.34	249.88 ± 18.29^a
MK-801/cocaine	2.08 ± 0.21	223.46 ± 11.71^a
Cocaine	2.58 ± 0.47	592.54 ± 13.20^b
Hippocampus		
Control	1.98 ± 0.16	2158.24 ± 78.55^a
MK-801/cocaine	2.08 ± 0.29	2145.13 ± 51.08^a
Cocaine	2.15 ± 0.09	3331.47 ± 62.96^b
Frontal cortex		
Control	4.44 ± 0.58	2585.14 ± 157.49
MK-801/cocaine	4.71 ± 0.29	2523.91 ± 87.52
Cocaine	4.80 ± 0.37	2616.35 ± 164.82

Means followed by a different superscript (within brain region) differ significantly ($P < .01$) from each other.

treated group (12.5 ± 1.2) and the control group (12.6 ± 1.3) (treatment: $_{.95}F_{2,30} = 3.31$, $F_{calc} = 5.12$). There was no significant difference between the control and MK-801/cocaine-treated group in HP endurance times.

3.6. Receptor binding studies

NMDA receptor binding assays showed that on PND 30, cocaine-treated group displayed a significant ($P < .01$) increase in receptor density in the hypothalamus (treatment: $_{.99}F_{2,30} = 5.39$, $F_{calc} = 6.50$) and hippocampus (treatment: $_{.99}F_{2,30} = 5.39$, $F_{calc} = 8.43$) compared to both control and MK-801/cocaine-treated groups (Table 1). No difference in binding affinity (K_d) was seen in any of the brain regions assayed.

4. Discussion

The present study shows that neonatal cocaine administration, during a critical CNS developmental period (PND 4 to 10), significantly reduced the animal's ability to handle stress incurred weeks after initial cocaine exposure. Blockade of the NMDA receptors prior to cocaine administration effectively abolished many of the observed behavioral and neurochemical alterations.

Results from cold-water swim test combined with quantification of ACTH and corticosterone levels showed that the administration of MK-801 before cocaine abolished the diminished stress adaptive response seen in both male and female cocaine-treated offspring. Exposing cocaine-treated males to restraint stress revealed a heightened, sustained elevation in plasma corticosterone and ACTH levels. MK-801 pretreatment appeared to abolish the heightened hormonal response seen following restraint stress.

Acute immobilization can heighten HPA axis response leading to an elevated plasma ACTH and corticosterone levels (Pacak et al., 1995). In the present study, baseline plasma ACTH and corticosterone levels were not significantly different among treatment groups. Immobilization for 20 min, led to elevated HPA axis response in all treatment groups with the cocaine-treated group having a greater increase in plasma ACTH and corticosterone levels. Thus, it may be concluded from these data that neonatal cocaine exposure induces long-term alterations to both hormonal and behavioral responses to stress in the rat. Furthermore, these effects appear to be effectively abolished with NMDA receptor blockade.

An important facet of NMDA receptor functions are the induced changes following chronic administration of high levels of corticosterone (Woolley et al., 1990) or prolonged stress (Watanabe et al., 1992), which include reduced numbers of branch points of dendrites on CA3 pyramidal neurons of the hippocampus (Arbel et al., 1994; Sapolsky et al., 1985; Uno et al., 1989). Corticosterone-induced changes in dendritic plasticity require the activation of the NMDA receptors (Margarinos and McEwen, 1995). The NMDA receptor binding assay showed cocaine-treated animals had a significant increase in NMDA receptor levels in the hypothalamus and hippocampus at PND 30 compared to control animals. When NMDA receptors were blocked before cocaine administration, receptor density (B_{max}) and binding affinity (K_d) were similar to control animals, thus, MK-801 treatment appears to act as a neuroprotectant to cocaine-induced toxicity. The increase in receptors seen in the cocaine-treated group may, in part, account for the stress-induced behavioral deficits. Increases in stress-induced HPA axis activity will increase glutaminergic activity (Abraham et al., 1998; Gabr et al., 1995) and NMDA receptor activation (Armanini et al., 1990; Nair et al., 1998), thus, an increase in NMDA receptor density may lead to a hypersensitive HPA axis and a prolonged stress recovery period.

Many in vivo (Gloor and Fariello, 1988; McNamara et al., 1985) and in vitro (Herron et al., 1985; Taylor and Dudek, 1982) studies support the role of NMDA receptors in the development and expression of convulsant actions in adult rats. Glutamate and aspartate have been found to cause convulsions (Hayashi, 1952) and elevated concentrations of glutamate have been found in human epileptic foci (Perry and Hansen, 1981). One of the most widely studied models of convulsant activity is the kindling model, which can be used to study epileptogenesis and seizure expression (McNamara et al., 1985). Using the kindling model, it was discovered that the noncompetitive NMDA antagonist, MK-801, could suppress seizure expression in a dose-dependent manner (DeSarro et al., 1985). NMDA receptor activity can be directly enhanced by an increase in receptor density or neurotransmitter release and indirectly by changes in inhibitory processes (McNamara et al., 1988). An increase in NMDA receptor activity can also lead to an alteration in agonist-induced decreases in extracellular Ca^{2+} concentration (Plessinger and Woods, 1993). In the present study, cocaine treatment during neonatal development induced an increase in NMDA receptor densities (which was blocked by MK-801 pretreatment), especially in the hippocampal region, which may account for the hypersensitive response to NMDA administration and appears to increase seizure-like activities.

The present study also examined long-term alterations in pain sensation from neonatal cocaine exposure. Results indicate that rat pups exposed to cocaine from PND 4 to 10 showed a hyperalgesic response to the TF test and a hypoalgesic response to the HP test. Previous studies in adult animals have determined that NMDA antagonists (MK-801 and memantine) attenuate long-term spinal hyperalgesic changes due to neurochemical alterations resulting from long-term drug abuse, stress or mechanical injury (Borowsky and Kuhn, 1991; Gruol et al., 1998; Perry and Hansen, 1981). In addition, NMDA antagonists have been shown to attenuate central hypoalgesic effects due to prenatal opiate exposure (McNamara et al., 1985) and condi-

tioned behavioral responses (Delaney-Black et al., 1998). Although pain sensation involves many different mechanisms, it appears that NMDA receptors are involved in cocaine-induced alterations.

In summary, this study indicates that neonatal cocaine exposure induces long-term behavioral and neurochemical effects. Some of which can be linked to the activation of NMDA receptors. Animals exposed to cocaine neonatally during the "neuronal spurt" period showed a diminished response to stress and NMDA challenge and an altered response to painful stimuli. ACTH and corticosterone quantification suggests that cocaine-treated animals have a sensitized HPA axis to stress and a sustained period of recovery is necessary. NMDA receptor binding analysis indicates that neonatal cocaine exposure produces a significant increase in receptor densities in the hippocampus and hypothalamus, which may help explain the hypersensitive behavior these animals displayed. Administration of MK-801 prior to cocaine treatment abolishes or attenuates the behavioral and neurochemical responses seen in the cocaine-treated animals, thus, further implicating the role of NMDA receptors in cocaine-induced neurotoxicity.

Acknowledgments

The authors would like to acknowledge grants support by the National Institutes of Health (GM 08111 and RR 03020).

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