Pharmacology Biochemistry & Behavior, Vol. 40, pp. 291–295. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Psychopharmacological Effects of MK-801 in Infant and Preweanling Rat Pups

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Received 25 March 1991

RAJACHANDRAN, L., G. A. GOODWIN AND L. P. SPEAR. *Psychopharmacological effects of MK-801 in infant and preweanling rat pups*. PHARMACOL BIOCHEM BEHAV **40**(2) 291–295, 1991.—Neonatal (3–4-day-old) and preweanling (17–18-day-old) Sprague-Dawley rat pups were tested following SC administration of saline, 0.01, 0.05, 0.1, 0.5, or 1.0 mg/kg MK-801. In neonatal rat pups, reductions in a number of behaviors (forward locomotion, mouthing) were seen at the higher (0.5 and 1 mg/kg) doses. In contrast, evidence of behavioral stimulation in forward locomotion at 30 min postinjection was seen at a lower dose (0.1 mg/kg). In preweanling rat pups, marked sedative effects of MK-801 were seen at higher doses (decreases in forward locomotion, headlift and sniff), with signs of behavioral stimulation (increases in forward locomotion and mouthing) evident at low doses. Thus, as in adults, low doses of MK-801 may be behaviorally stimulatory and higher doses inhibitory to both neonatal and preweanling pups, although the stimulatory effects appear to be somewhat less pronounced in these young animals than has been previously reported in adulthood.

MK-801 Neonatal Preweanling Behavioral stimulation NMDA antagonist

EXCITATORY amino acids (EAAs) not only play an important role in activity-dependent synaptic plasticity (long-term potentiation) and learning and memory [for review, see (3)], but also are presumed to have additional functions during development of the central nervous system. EAAs appear to trophically influence differentiating neurons and to modulate the formation of neuronal circuitry and early synaptic plasticity [see (11) for review]. There is evidence that glutamate receptors of the N-methyl-D-aspartate (NMDA) type as well as markers of EAA synaptic terminals are transiently overexpressed in a number of brain regions (11). This transient overproduction prior to synaptic elimination and stabilization may be critical for synaptic plasticity. For instance, blocking NMDA receptors with the competitive antagonist AP5 during this period of over-expression prevents the ocular dominance shift in the visual cortex seen in response to monocular visual experience (9) and disrupts early olfactory learning and growth in certain areas of the olfactory bulb which usually occurs in conjunction with this early learning (10). Such transient overexpression may also be related to the increased susceptibility of young animals to NMDA neurotoxicity (11), and the greater effectiveness of NMDA antagonists in protecting against hypoxia/ischemia-mediated neurotoxicity in young animals than in adults (5,12).

NMDA antagonists have been used extensively to investigate the neural mechanisms underlying excitotoxicity in the neonate and to examine the role of NMDA receptors in trophic actions, neuronal plasticity and learning during ontogeny [see (11)]. Yet, little is known of the behavioral consequences of acute administration of NMDA antagonists in developing animals, although the psychopharmacological profile of NMDA antagonists has been well characterized in adulthood. For instance, the selective, noncompetitive NMDA antagonist (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801) induces a number of behavioral alterations in adult rodents, including ataxia, headweaving, sniffing, turning, backpedaling and a decrease in rearing [e.g., (4, 13, 17)]. Hyperlocomotion is characteristically seen following low doses of MK-801, whereas suppression in activity (akinesia) has been consistently observed after administration of higher doses of the drug (13, 17, 18).

Due to the extensive use of NMDA antagonists to examine the role of EAAs in neuronal plasticity during development, as well as the potential use of these antagonists as therapeutic agents in excitotoxic clinical disorders to which infants are particularly susceptible (11), it is important to characterize the profile of the acute psychopharmacological effects of these antagonists in developing animals. Consequently, the present study investigated the behavioral effects of the NMDA antagonist MK-801 in rat pups at two critical stages: during the neonatal period (prior to the time of maximal expression of EAA pathways and NMDA binding) and during the late preweanling period (when maximal expression of these markers is seen in a number of brain regions) (12).

METHOD

Subjects

Offspring (n = 192) of Sprague-Dawley rats bred in our laboratory were used in these experiments. All breeding pairs and

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their offspring were maintained on ad lib food and water in a vivarium under a 12/12-hour light-dark cycle with light onset at 0700 h. The day of birth was designated as postnatal day 0 (P0). Litters were culled to ten pups at P1. No litters containing less than 8 pups were used in this experiment.

Testing Procedures

All testing was conducted between 1100 and 1600 hours. Neonatal (P3–4) and preweanling (P17–18) pups were deprived of the dam and food for 4 h prior to testing via placement with littermates in a temperature-controlled incubator maintained at 34 ± 1°C. To allow accurate assessment of weight gain, neonatal pups were voided 30 min prior to testing by gentle stroking of the anogenital region with a warm, moist cotton swab; this region was then coated with a nonirritating sealant (nail polish) to prevent further voiding during the test. The neonates were weighed following sealing and again immediately following testing to monitor weight gain as a measure of consumption. Preweanling pups were weighed prior to and after testing but not voided or sealed (as pups of this age can spontaneously micturate, and coating with a sealant does not deter this process at this age). Body temperatures were assessed prior to and at the completion of testing using a YSI 511 probe, with axillary temperatures being used for P3–4 pups and rectal temperatures for P17–18 preweanlings. Each pup received a subcutaneous injection (SC) of one of five doses of the noncompetitive antagonist MK-801 (0.01, 0.05, 0.1, 0.5, or 1.0 mg/kg/5 ml) or the vehicle (0.9% saline, 5 ml/kg). Pups were then placed in a holding incubator maintained at approximately 34 ± 1°C (P3–4) or 32 ± 1°C (P17–18) until the onset of testing at 30 or 60 min postinjection.

Pups were tested individually in both the absence (low mouthing baseline) and presence (high mouthing baseline) of milk in order to assess both potential facilitatory and inhibitory effects of MK-801 on mouthing and milk ingestion [see (8) for further discussion]. At the onset of testing, each pup was placed in a clear Plexiglas chamber housed within a temperature/humidity-controlled incubator maintained at 34 ± 1°C for 3–4-day-old pups and 32 ± 1°C for 17–18-day-old preweanlings. The chamber used for the 3–4-day-old pups was cylindrical (15 cm diameter × 11 cm height) with a wire mesh floor. Preweanling pups were placed in a (18 × 12 × 12 cm) test apparatus with sides and bottom composed of Plexiglas. During this 5-min premilk test period, behavior of each pup was recorded using a time sampling procedure whereby every 20 s the pup was observed for 5 s and any behaviors observed were recorded [see (8) for further details]. Behavioral categories included mouthing (opening and closing of the oral cavity), probing (pushing with the snout against the floor of the apparatus), forward locomotion, rearing, wall climb (treading with the forelimbs against the side of the apparatus with the body held in a near vertical plane), lie still, sniff, and grooming. An "other" category was used for describing and quantifying additional behaviors. Immediately following the 5 min of baseline testing, each pup was placed in a similar Plexiglas container with a terrycloth-covered floor saturated with approximately 100 ml of warm "Half and Half" dairy cream. This test procedure has been shown to elicit significant milk ingestion and increases in mouthing in deprived rat pups (6). The temperature of this test apparatus was elevated to approximately 38 ± 1°C for P3–4 pups and 36 ± 1°C for P17–18 pups in an attempt to counteract the cooling effect of milk evaporation from the skin. Each pup was observed for 5 min in this "milk" testing situation, using the same time sampling method and behavioral categories outlined above. Immediately following testing, each pup was gently dried and its body temperature and posttest weight determined.

Pups were tested at only one age and one postinjection test interval. A total of 8 pups, each from a different litter, were tested in each of the 12 [6 dose × 2 test interval (30 vs. 60 min)] testing conditions at each age. Sex of the subjects was equated as much as possible within test condition. Testing was conducted by experimenters who had no knowledge of the contents of any of the given test solutions.

Data Analysis

Preliminary analyses revealed no reliable sex differences in the data, so the data were collapsed over this variable prior to further analysis. Behavioral data were analyzed by a 2 condition (premilk vs. milk) × 2 test interval (30 min vs. 60 min postinjection test interval) × 6 dose analysis of variance (ANOVA) at each age. Body temperature data were analyzed by a 2 (pre- vs. posttest) × 6 dose × 2 (30 vs. 60 min) ANOVA with data on percent body weight gain being analyzed by a 6 dose × 2 (30 vs. 60 min) ANOVA. Dunnett's and Tukey's tests were used for post hoc analyses to assess the locus of significant dose effects and other comparisons of interest, respectively. Reported differences were significant at $p<0.05$.

RESULTS

P3–4 Pups

The ANOVA of lie still revealed a significant main effect of dose, $F(5,95)=3.349$, $p<0.01$. As can be seen in Fig. 1a, lie still was increased at the two highest doses (0.5 and 1.0 mg/kg) of MK-801 relative to saline control.

A significant main effect of dose was observed in the analysis of grooming (forelimb paddle/face wash), $F(5,95)=5.961$, $p<0.001$. As can be seen in Fig.1b, pups given the highest dose of MK-801 exhibited more of these behaviors than saline controls.

The ANOVA of the mouthing data revealed significant main effects of dose, $F(5,95)=9.136$, $p<0.001$, and condition, $F(5,95)=106.69$, $p<0.001$, along with a significant dose × condition interaction, $F(95,5)=8.639$, $p<0.001$. Mouthing was decreased compared to saline by doses of 0.1, 0.5, and 1.0 mg/kg only in milk testing (see Fig. 1c). Mouthing was rarely seen in the premilk condition, thus it is possible that a floor effect may be masking reductions in mouthing in this condition. Concomitant with this decrease in milk-induced mouthing, a significant decrease in body weight was also seen as a function of increasing dose, $F(5,84)=2.836$, $p<0.02$. Pups given doses of 0.05 (mean ± SEM) (0.39 ± 0.16), 0.5 (0.30 ± 0.16), and 1.0 (0.48 ± 0.46) mg/kg exhibited decreased weight gain relative to saline control pups (1.54 ± 0.32). There was no effect of postinjection test interval on percent body weight gain.

The effects of MK-801 on forward locomotion were characterized by an inverted U-shaped function. A significant dose effect was observed in the ANOVA of forward locomotion, $F(5,95)=4.554$, $p<0.001$, with forward locomotion being decreased at the 1.0 mg/kg dose of MK-801. Separate ANOVAs conducted at each test interval revealed a significant main effect of dose at 30 min, $F(5,47)=3.800$, $p<0.01$, with forward locomotion being significantly increased at the 0.1mg/kg dose over saline control (see Fig. 1d). There was no main effect of dose on forward locomotion at the 60-min test interval.

No significant main effects or interactions were revealed in the ANOVAs of the probing, rearing, wall climbing, or sniffing data at this age. The only significant finding in the body temperature ANOVA was a main effect of pre- vs. posttest, $F(1,52)=32.103$, $p<0.01$, with temperatures pretest (35.6 ± 0.13) being

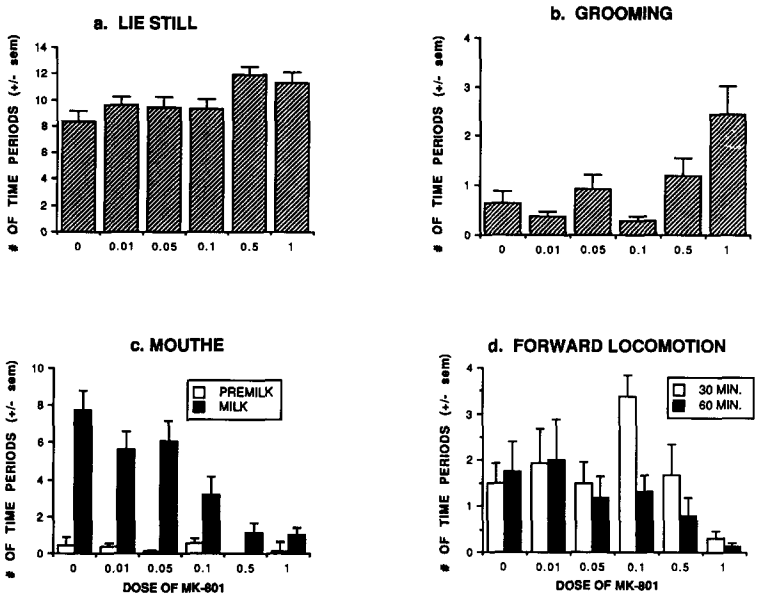

FIG. 1. Mean number of time periods (±S.E.M.) that 3–4-day-old rat pups spent (a) lying still, (b) grooming, (c) mouthing, and (d) exhibiting forward locomotion following doses of 0 (saline), 0.01, 0.05, 0.1, 0.5 and 1.0 mg/kg/5 ml MK-801. Data in (a) and (b) are collapsed across test condition (premilk, milk) and test interval (30 vs. 60 min), with data in (c) collapsed across test interval and data in (d) collapsed across test condition.

significantly higher than those posttest (34.6±0.14).

P17–18 Pups

The ANOVA of the lie still data revealed significant main effects for dose, $F(5,95)=16.49$, $p<0.001$, and condition, $F(1,95)=8.48$, $p<0.01$, and a significant interaction between these two variables, $F(5,95)=2.55$, $p<0.05$. As can be seen in Fig. 2a, in both the premilk and milk testing conditions, pups given a dose of 1 mg/kg MK-801 exhibited more lie still behavior than saline controls. The only indication of the locus of the significant interaction was seen when comparing the two levels of milk condition separately at each dose. Tukey's comparisons revealed that at the 0.05 mg/kg dose, lie still was less frequent in the milk than in premilk testing.

The ANOVA of the sniffing data revealed a significant main effect of dose, $F(5,95)=9.191$, $p<0.01$, with the two highest doses significantly decreasing sniffing compared with saline controls (see Fig. 2b).

A significant main effect of dose, $F(5,95)=7.544$, $p<0.001$, was seen in the ANOVA of the headlift data, with doses of 0.1, 0.5, and 1.0 mg/kg of MK-801 significantly decreasing head lifting (see Fig. 2c).

The ANOVA of the mouthing data revealed a significant main effect of dose, $F(5,95)=4.030$, $p<0.05$, and testing condition, $F(1,95)=38.03$, $p<0.001$, along with a significant interaction between these two variables, $F(5,95)=2.94$, $p<0.05$. Although Dunnett's tests revealed no significant differences of any dose relative to saline control, Tukey's tests showed that in the milk condition, mouthing was more frequent at doses of 0.05 and 0.1 mg/kg than at 1 mg/kg (see Fig. 2d). No indication of such an inverted U-shaped function was seen in premilk testing.

The ANOVA for forward locomotion revealed a significant effect for dose, $F(5,95)=4.40$, $p<0.001$, and for condition, $F(1,95)=4.94$, $p<0.05$. Forward locomotion was slightly but significantly increased in the premilk condition relative to the milk condition. Whereas post hoc analysis by Dunnett's tests to determine the locus of the main effect of dose did not indicate any significant effects, Tukey's tests revealed an inverted U-shaped function of MK-801 on this behavior, with forward locomotion being significantly greater at the 0.1 and 0.5 mg/kg than the 1.0 mg/kg dose of MK-801 (see Fig. 2e).

No significant main effects or interactions were observed in the ANOVAs of the probing, rearing, wall climbing, or grooming data at this age. There was also no significant effect of dose on body temperature or body weight at this age.

DISCUSSION

The noncompetitive NMDA antagonist MK-801, when administered SC across a dose range of 0.01–1.0 mg/kg, induced behavioral alterations in both neonatal and late preweanling rat pups. At both ages, as in adults, higher doses of the drug induced akinesia, as reflected by increases in the amount of time spent lying still at both ages, along with decreases in forward locomotion and mouthing at 3–4 days of age and attenuations in sniffing and head lifting at 17–18 days postnatally. However, in contrast to the general behavioral suppression and increase in lie still seen at these higher doses, dose-dependent increases in grooming were seen in 3–4-day-old pups.

Some indication of a stimulation of locomotion was also seen at both ages, although this effect appears less robust than that reported in adulthood (4, 13, 17, 18). At 3–4 days of age, an increase in forward locomotion was seen following administration of 0.1 mg/kg MK-801 at 30 min, but not 60 min. At 17–18 days of age, although no dose of MK-801 significantly increased

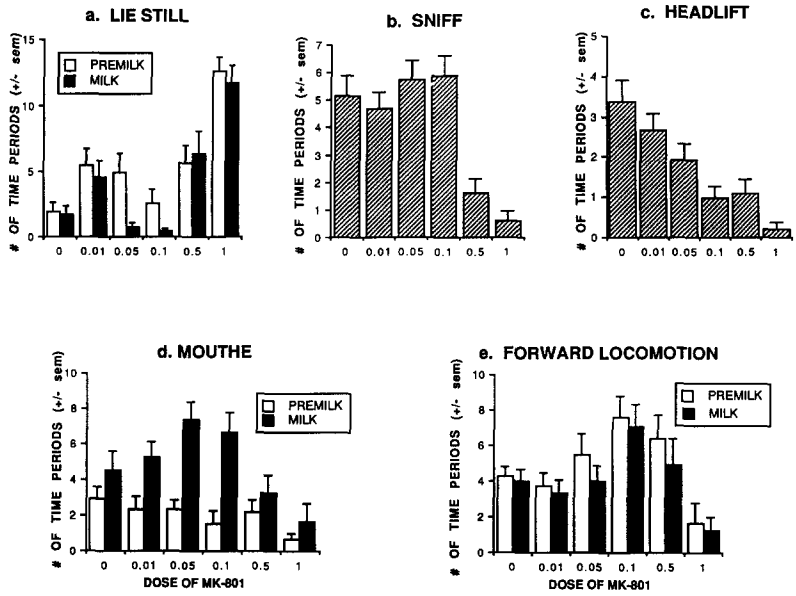

FIG. 2. Mean number of time periods (±S.E.M.) that 17–18-day-old rat pups spent (a) lying still, (b) sniffing, (c) headlifting, (d) mouthing and (e) exhibiting forward locomotion following doses of 0 (saline), 0.01, 0.05, 0.1, 0.5 and 1.0 mg/kg/5 ml MK-801. Data in (b) and (c) are collapsed over test condition (premilk, milk) and test interval (30 vs. 60 min), with data in (a), (d) and (e) collapsed across test interval.

forward locomotion relative to saline controls, pups given doses of 0.1 and 0.5 mg/kg MK-801 exhibited significantly more forward locomotion than pups given the highest (1.0 mg/kg) dose. This inverted U-shaped curve of locomotor activity is similar to that seen in neonates, although the low dose stimulation of locomotion appears to be less evident in older pups than neonates. This attenuated low dose effect in the late preweanling animals could be related in part to the higher baseline levels of locomotor activity seen at this age. Rate-dependent effects have been frequently reported in ontogenetic studies. For instance, in studies examining age-related differences in amphetamine responsiveness, drug effects were reported to be relatively attenuated at ages when baseline activity levels were the highest (14,16).

Although age-related differences in pharmacokinetics [and differences in the route of administration used in this study (SC) versus those typically used in adulthood (IV or IP)] preclude direct comparison of dose levels across age, the inverted U-shaped dose response profile of MK-801-induced alterations in locomotor activity seen in neonates and late preweanling pups is also characteristically reported in MK-801-treated adult animals (13, 17, 18). However, whereas pups at both test ages exhibited clear akinesia at a high dose of MK-801, the hyperlocomotion response to low doses in young animals, particularly during the late preweanling period, was observed to be less robust than reported in adulthood. It is not clear why this should be the case. The neural substrates underlying these nonmonotonic dose-dependent locomotor effects of MK-801 have yet to be characterized. Although MK-801 has been shown to activate catecholaminergic neurons (7), presumably via NMDA receptor blockade per se (2), the hyperlocomotion induced by MK-801 is not dependent upon catecholaminergic activity. This conclusion is based on the observation that MK-801-induced hyperlocomotion is seen in mice depleted of monoamines via pretreatment with alpha-methyl-tyrosine and reserpine (1,2). Thus it is unlikely that ontogenetic patterns of catecholaminergic activity are related to the less robust induction of locomotor activity by low doses of MK-801 in neonates and preweanlings.

Behaviors other than forward locomotion and akinesia (lie still) were altered by MK-801 administration in both neonatal and late preweanling rat pups. These behaviors generally varied from those observed following MK-801 administration in adults. In neonates, dose-dependent decreases in mouthing and weight gain and an increase in grooming in response to the highest dose of MK-801 were observed. In the late preweanlings, an inverted U-shaped dose-response effect on mouthing was observed, along with decreases in sniffing and head lifting at higher doses of the drug. Some of these behavioral alterations may be related to the nature of the testing situation used for these young animals. For instance, alterations in mouthing were observed in milk testing (when baseline levels of mouthing were high), but not in testing in the absence of milk (a situation more analogous to those in which adults have been typically tested). The pups exhibited no significant increases, however, in a number of behaviors that are reported following MK-801 administration in adults, including head weaving, sniffing, turning and backpedaling (4, 11, 17). This may not be surprising given that both the spontaneous and drug-induced response repertoire of young rat pups often differs in terms of specific behavioral components from that seen later in life [e.g., see (14) for review]. It is not simply the case, however, that young rat pups are incapable of emitting these behaviors. Sniffing behavior is emitted frequently by rat pups during the late preweanling period, and turning (pivoting on hindlimbs) is a predominant component of the movement repertoire of even neonatal rat pups.

Taken together, the results of this study demonstrate that the NMDA antagonist MK-801 alters the behavior of both neonatal and late preweanling rat pups with a general dose-response profile of low dose stimulation and high dose suppression of locomotor movements reminiscent of that reported following MK-801 administration in adulthood (4, 11, 17). These findings may have implications for any potential experimental or therapeutic use of NMDA antagonists in young organisms. For instance, in

studies examining the influence of NMDA antagonists on learning early in ontogeny, drug-induced alterations in activity during conditioning may influence the ability or propensity of the pups to process stimuli, and hence may influence conditioning indirectly. Systematic attention to the acute behavioral effects of NMDA antagonists should be considered in studies assessing the neural and cognitive consequences of these drugs when administered early in ontogeny.

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