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Suppression of Cortical Epileptic Afterdischarges by Ketamine is not Stable During Ontogenesis in Rats

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KUBOVÁ, H. and P. MAREŠ. *Suppression of cortical epileptic afterdischarges by ketamine is not stable during ontogenesis in rats.* PHARMACOL BIOCHEM BEHAV 52(3) 489–492, 1995. – Anticonvulsant action of ketamine, a noncompetitive NMDA antagonist, was studied in three groups of immature rats (12-, 18-, and 25-day-old) using cortically elicited epileptic afterdischarges (ADs) as a model. Rats with implanted electrodes were used, so that EEG and motor phenomena could be recorded. Stimulation (bipolar pulses of 1-ms duration and 8-Hz frequency) lasting 15 s was repeated four times with intervals of 10 min. Ketamine was administered IP 5 min after the first AD in doses of 5, 10, 20, or 40 mg/kg. Control groups did not receive any drug. Ketamine shortened ADs and suppressed motor correlates of stimulation as well as of ADs in a dose-dependent manner in 12- and 25-day-old rats. No significant changes were observed in 18-day-old animals, demonstrating thus a rather complicated development of anticonvulsant action of ketamine. Not only NMDA antagonism, but also other possible effects of ketamine must be taken into account.

Epileptic seizures Cortex Ontogeny Rat Ketamine

KETAMINE, a noncompetitive antagonist of *N*-methyl-D-aspartate (NMDA) type of excitatory amino acid receptors (1,7) exhibits an anticonvulsant action in some models of epileptic seizures. Although the data from adult animals are numerous (3–5,9,12,18,21), ontogenetic development of ketamine anticonvulsant action in vivo was studied only in our laboratory (24,25). These studies demonstrated a specific action of ketamine against generalized tonic-clonic seizures induced by pentylenetetrazol, picrotoxin, or bicuculline with higher efficacy of ketamine at early developmental stages than in adult rats. Minimal, clonic seizures remained uninfluenced. The higher sensitivity of immature rats is in agreement with the developmental profile of NMDA receptors (11). We have examined the selectivity of anticonvulsant action of ketamine in other types of seizures in adult rats (23). Specific anticonvulsant action of ketamine was demonstrated: in addition to suppression of generalized tonic-clonic seizures induced by different chemoconvulsants, it was effective against a model of complex partial seizures – hippocampal afterdischarges. On the contrary, ketamine did not suppress activity of cortical epileptic foci, minimal metrazol seizures and spike-and-wave rhythm elicited by low doses of pentylenetetrazole. Epileptic afterdischarges induced by cortical electrical stimulation, representing probably a model of one type of human myoclonic seizures, were among the seizures sensitive to ketamine (14). We started to study the ontogeny of the action of ketamine against those models of epileptic seizures that could be suppressed by this drug in adult animals to verify if higher anticonvulsant action at early developmental stages is a general property of ketamine.

METHODS

Experiments were performed in Wistar albino rats held at 12 L : 12 D h cycle. Dams were fed by Altromine 1310 diet ad lib. Three age groups were used: animals 12-, 18-, and 25-day-old, i.e., all the animals were in the preweaning period. Surgical preparation was performed under ether anesthesia.

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Flat silver electrodes were implanted epidurally; two stimulation electrodes over the right sensorimotor, frontal cortical area (coordinates AP −1 and +1, L 2 mm in relation to bregma), recording electrodes over left sensorimotor region (AP 0, L 2), right and left visual, occipital areas. The coordinates for occipital electrodes were calculated from the adult values of AP 6 and L 4 mm. The recalculation was based on the actual bregma-lambda distance, the background value of 8 mm for adult rats was taken. An indifferent electrode was placed on the nasal bone. All electrodes were cemented to the skull by a fast-curing dental acrylic. After the interruption of ether anesthesia the rat pups were placed into Plexiglas boxes (in isolation) and allowed to recover for at least 1 h, then they were fed by sucrose solution; in such a way, suckling reflex was examined. In addition, righting and placing reflexes were tested and only if normal the animals entered the experiment. Body temperature of the rat pups was maintained during the recuperation period as well as during the experiment by means of a pad heated to 35°C (i.e., the normal temperature in the nest). The total time of isolation of pups from mothers (surgical preparation, recuperation, and experiment) did not exceed 2 h.

Stimulation was performed by means of a constant current stimulator of our own construction. Series of biphasic rectangular pulses of 1-ms duration and 8-Hz frequency were applied for 15 s. The intensity was reliably higher than the threshold for elicitation of afterdischarges (13), mostly 3 mA. The stimulation series were repeated four times with an interval of 10 min between the end of ADs and the beginning of the next stimulation. Control animals did not receive any treatment, they did not differ from physiological saline injected animals used in a study with another drug performed at the same time (Világy et al., submitted). Ketamine (Narcamon® Spofa, 5% water solution) was administered IP 5 min after the end of the first AD in doses of 5, 10, 20, or 40 mg/kg. Eight to ten rat pups were used for each dose in each age group. At least two different doses were administered to animals from one nest, i.e., the siblings never formed one dose group. The experiments were performed between 0900 h and 1600 h (i.e., during the light part of the diurnal cycle) in a sound-isolated room. The animals were used only once, each age and dose group consisted of eight to ten animals.

Motor phenomena elicited by stimulation and accompanying ADs were recorded and quantified using the slightly modified five-point scale of Racine (19). Classification was as follows: 0—animals sat motionless; 1—behavioral activities not in phase with stimulation or spike-and-wave AD; 2—head nodding; 3—clonus of forepaws; 4—clonus of forepaws and rearing; 5—clonus of forepaws, rearing, and falling. Clonic movements of head or forepaws were synchronous with stimuli or sharp elements of the spike-and-wave rhythm. Electrocorticogram was recorded in both reference and bipolar connections before and during stimulation, during an AD, and 1 min after the AD. The duration and pattern of ADs was evaluated.

Statistical analysis of the data was performed using analysis of variance models [BMDP Program (6)] with grouping factors dose (five levels—controls, and four doses tested) and age groups (three levels). Simple effects in the model were computed from restricted models under the condition of fixed level of some factors (26). Logarithmic transformation of AD values was used to stabilize variance in cells (2). The level of statistical significance was set at 5% and was adjusted according to Holm's multiple test procedure (10).

RESULTS

Electrocorticographic Afterdischarges

Cortical stimulation elicited ADs in all animals included in this study. Electrographically ADs were formed by spike-and-wave rhythm in 18- and 25-day-old rats, by rhythmic sharp waves in 12-day-old rat pups. Under control conditions, repeated stimulations led to a progressive increase in duration of ADs. This phenomenon was especially expressed in 12-day-old rats (Fig. 1). Ketamine blocked the progression of ADs in all

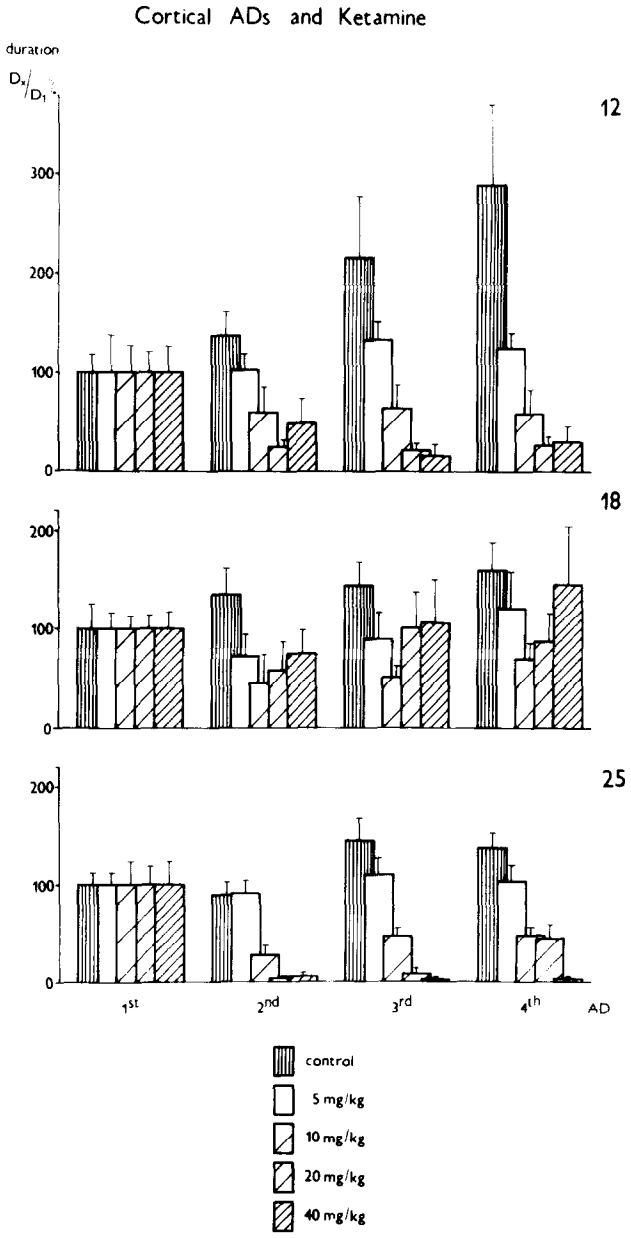

FIG. 1. Effects of ketamine on duration of afterdischarges (ADs; mean ± SEM) in 12-, 18-, and 25-day-old rats (from top to bottom). Abscissa = first, second, third, and fourth ADs; ordinates = relative duration of the ADs, the first AD was always taken as 100%. Explanation of individual columns can be found in the key.

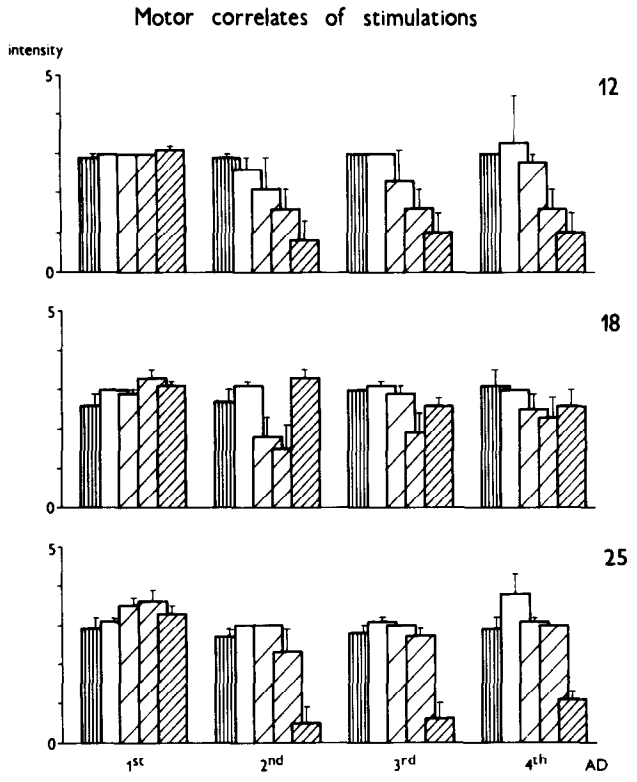

FIG. 2. Effects of ketamine on movements accompanying stimulation (mean intensity ± SEM). Details as in Fig. 1, only ordinates = five-point scale of intensity of motor phenomena according to Racine (19).

age groups even at the 5 mg/kg dose. Dose-dependent shortening or suppression of postdrug ADs in comparison with controls was observed in 12- and 25-day-old animals with doses of 10 mg/kg and higher. The 20 and 40 mg/kg doses resulted in significant shortening of postdrug ADs in comparison with the first, predrug AD in both these age groups. ADs in 18-day-old rats were never blocked by ketamine, significant shortening was observed only after the second stimulation, i.e., 5-min after ketamine administration, with all doses used.

Motor Phenomena

Under control conditions stimulation was accompanied by clonic movements of facial and forelimb muscles (Racine's stage 3) synchronous with individual stimuli. Rearing of rats was seen only exceptionally with the intensities of stimulation used. The intensity of these movements did not change significantly with repeated stimulations (Fig. 2). Ketamine exhibited a dose-dependent suppression of these movements in 12-day-old rats, the differences being significant for 20 and 40 mg/kg doses. Similar suppression was observed in 25-day-old rats only after the highest dose used. These significant differences were found in comparison with both control values and the first, predrug stimulation. Eighteen-day-old rats exhibited only a tendency to suppression of clonic movements accompanying the second stimulation after the 10 and 20 mg/kg doses (Fig. 2).

Movements accompanying ADs were identical to those bound to stimulation, only their frequency was lower because of synchrony with sharp elements of the ECoG; spikes in 18- and 25-day-old animals and sharp waves in 12-day-old rats. The intensity of movements in individual animals was never higher than that of stimulus-bound movements so that the averages tended to be lower (compare Figs. 2 and 3). The effects of ketamine in 12- and 25-day-old rats were similar to those described above: the 20 and 40 mg/kg doses led to significantly lower intensities of motor seizures than those in corresponding ADs of nontreated controls. The doses of 10 mg/kg and higher resulted in significantly less intense motor phenomena accompanying postdrug ADs than those accompanying the first, predrug AD. The 40 mg/kg dose resulted often in a complete block of motor seizures in both these groups, the 20 mg/kg dose exhibited the same effect in 25-day-old animals only. This means that in the mentioned dose and age groups some ADs lacked a motor counterpart. There were no significant changes in 18-day-old rats (Fig. 3), the seizures in some animals were less affected than movements accompanying stimulation (compare Figs. 2 and 3).

DISCUSSION

Ketamine exhibited an anticonvulsant action against cortically induced ADs even in immature rats. The developmental profile of ketamine differed substantially from our previous data. The efficacy of ketamine against pentylenetetrazol-induced tonic-clonic seizures clearly increased with decreasing age, whereas our present data demonstrated high activity of ketamine in 12- and 25-day-old rats but not in 18-day-old animals. Similar results were found also in a study of hippo-

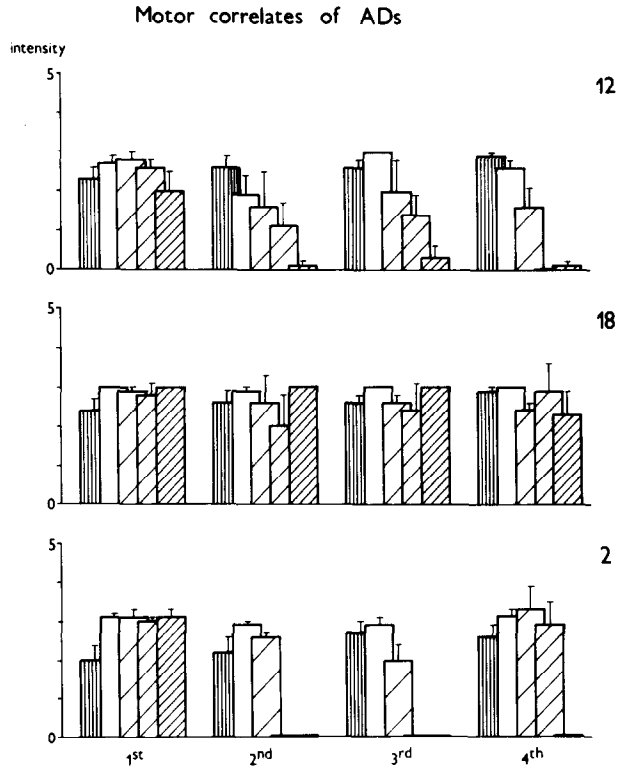

FIG. 3. Effects of ketamine on movements accompanying afterdischarges (mean ± SEM). Details as in Figs. 1 and 2.

campal afterdischarges (15). This complicated development of ketamine action cannot be explained at present but similar developmental "discontinuities" were described, e.g., for catecholamines in the periadolescent period [rats 32–38 days old; for review (20)]. Ketamine is a noncompetitive antagonist of NMDA receptors [for review (7)], but the development of these receptors exhibiting an overshoot of adult level between postnatal days 14 and 28 (11) does not fit with our data. We also have to take into account other effects of ketamine (binding to sigma opioid receptors, interaction with other neurotransmitter systems). To decide if NMDA antagonistic properties or other effects of ketamine are substantial for its action in our model, the experiments with more specific NMDA antagonists are in progress.

Ketamine was found to antagonize both EEG ADs and their motor correlates. The motor seizures were more sensitive so that EEG ADs without clonic movements were recorded. It might be due to the action of ketamine on the spread of epileptic activity, but our data about the inefficacy of ketamine against projected discharges of cortical epileptic foci (23) speak against this possibility. An alternative explanation might be a direct action of ketamine on the motor system. The finding that ketamine is able to suppress the movements accompanying stimulation speaks in favor of this possibility. Another supporting evidence may be the high concentration of NMDA receptors in basal ganglia (17); an adult level of these receptors could be demonstrated at postnatal day 1 (11). On the other hand, the connectivity (8) and properties of striatal neurons (16,22) mature toward the end of the second and beginning of the third postnatal week in rats.

This action on motor phenomena accompanying stimulation as well as ADs is at variance with our data from adult rats where these motor phenomena remained practically uninfluenced by ketamine—only the 40 mg/kg dose transitorily suppressed clonic seizures (correlates of ADs). That means that during the development, the role of NMDA transmission is not stable and may change unevenly in different systems or parts of the brain. This conclusion is supported by our data on the action of ketamine against hippocampal ADs in developing rats, where behavioral correlates of ADs were profoundly influenced in immature animals but not in adult rats (15).

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