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Picrotoxin-Induced Tonic-Clonic Seizures and Lethality are Decreased by MK-801 in Developing Rats

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VELÍŠKOVÁ, J. AND L. S. VELÍŠEK. *Picrotoxin-induced tonic-clonic seizures and lethality are decreased by MK-801 in developing rats.* PHARMACOL BIOCHEM BEHAV **43**(1) 291–295, 1992. – The action of MK-801 (NMDA antagonist; 0.1 and 0.5 mg/kg, IP) was tested against picrotoxin-induced seizures (3–6 mg/kg, IP) in rats aged 7, 12, 18, 25, and 90 days. We found MK-801 only inconsistently affected clonic seizures in 12- and 25-day-old rats, whereas tonic-clonic seizures were suppressed or delayed in almost all age groups. In addition, the lethality of picrotoxin was diminished by the higher dose of MK-801 in all age groups. The results suggest: a) different generators for both seizure patterns (clonic and tonic-clonic), b) an involvement of NMDA receptors in the genesis of tonic-clonic seizure pattern, and c) an interaction of MK-801 with GABAergic transmission throughout the entire development studied.

Seizure Neural development Picrotoxin MK-801

MK-801 is a relatively specific blocker of the NMDA-operated ionic channel (26). This action results in various effects, for instance, a blockade of long-term potentiation in the hippocampus, learning and memory impairments, protective effects against hypoxia-ischemia-induced damage, and anticonvulsant effects (3,4,7,13).

The anticonvulsant action of MK-801 has been tested in different models of seizures. A good anticonvulsant activity of MK-801 was demonstrated against maximal electroshock and pentylenetetrazol-, bicuculline-, and strychnine-induced seizures (3). There is also an extensive literature dealing with the action of MK-801 in kindled seizures; however, the results of theses reports are not quite consistent (6,10,11,15).

There are few data demonstrating the anticonvulsant effects of MK-801 during ontogenesis, although the NMDA receptor system changes during development (21,22). These changes in sensitivity of the NMDA receptor system also were shown in the experiments where convulsant effects of excitatory amino acids were studied (17). In that article, of all excitatory amino acid antagonists tested MK-801 was found to be the most potent antagonist of seizures induced by NMDA in baby rats. In our recent study, we described the action of MK-801 against pentylenetetrazol-induced seizures during ontogenesis in the rat (24). We demonstrated that MK-801, although ineffective against clonic seizures, was able to suppress tonic-clonic pentylenetetrazol-induced seizures during the entire developmental interval studied (age 7–90 days; 12,16). Moreover, the effectiveness of MK-801 decreased with increasing age of animals.

In another study (25), we tested the effects of another NMDA-operated ionic channel antagonist, ketamine (1), against the seizures induced by bicuculline and picrotoxin (drugs blocking GABAergic transmission) during ontogenesis of the rat. We found that ketamine slightly suppressed tonic-clonic seizures induced by both drugs and predominantly decreased the lethality, suggesting a possible interaction with GABA-ergic transmission. A recent study in adult rats demonstrated that MK-801 is able to block picrotoxin-induced lethality but not picrotoxin-induced tonic-clonic seizures (8). While ketamine is a relatively nonspecific drug with described effects on different neurotransmitter systems (9,18), we decided to test the more specific antagonist MK-801 against seizures induced by picrotoxin during ontogenesis to address the following questions:

1. Is there any ontogenetic difference in the effects of MK-801 against picrotoxin-induced seizures?
2. Is there any difference in the action of MK-801 against clonic and tonic-clonic seizures?

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TABLE 1

EFFECTS OF MK-801 ON THE INCIDENCE OF CLONIC PICROTOXIN-INDUCED SEIZURES

Age (days)	Dose of Picrotoxin	Controls	MK-801 (mg/kg) 0.1	0.5
7	4	2/8	2/8	5/8
12	4	0/8	7/8*	5/8*
18	3	7/8	8/8	7/8
25	5	8/8	8/8	7/8
90	6	7/8	6/8	7/8

Numbers in the fields of the table indicate phenomenon in question/number of animals in the group.

*Significant difference ($p < 0.05$; Fisher's exact test) in comparison with an appropriate control group. Dose of picrotoxin used in mg/kg.

3. Is MK-801 in low doses (which are supposed specifically to antagonize the NMDA receptor system) able to suppress picrotoxin-induced lethality throughout ontogenesis?

METHOD

Male Wistar rats were used for the experiments ($N = 120$). They formed five age groups: 7, 12, 18, 25, and 90 days old.

Control animals were injected intraperitoneally with picrotoxin (Sigma Chemical Co., St. Louis, MO) freshly dissolved in physiological saline. The doses of picrotoxin (see Table 1) were derived from our previous studies (19,25). Experimental groups were pretreated with MK-801 (a gift from Research Institute for Pharmacy and Biochemistry, Praha) in the doses 0.1 or 0.5 mg/kg intraperitoneally 30 min prior to picrotoxin administration. The doses of MK-801 represent the range found effective against pentylenetetrazol-induced seizures in our previous study (24).

Rats were observed in separated cages for 60 min after administration of picrotoxin; rat pups (7, 12, and 18 days old) were heated by electric pad.

We registered the incidence and latencies of the following phenomena: a) clonic seizures; b) tonic-clonic seizures; and c) Picrotoxin-induced lethality.

Moreover, each animal was assigned a seizure score, which represented the maximal seizure severity observed during the session. The following scale was used (14): 1, isolated myoclonic jerks; 2, imperfect (often unilateral) clonic seizure; 3, full clonic seizure; 4, incomplete tonic-clonic seizure (i.e., the loss of posture and clonus of four limbs); and 5, fully developed tonic-clonic seizure.

Statistical evaluation was performed by analysis of variance (ANOVA) with posthoc Scheffe test (latencies), by Kruskall-Wallis nonparametric test (scores), and by Fisher's exact test (incidence). The level of significance was set to 5%.

RESULTS

MK-801 pretreatment caused moderate dose-dependent ataxia predominantly of hindlimbs connected with enhanced

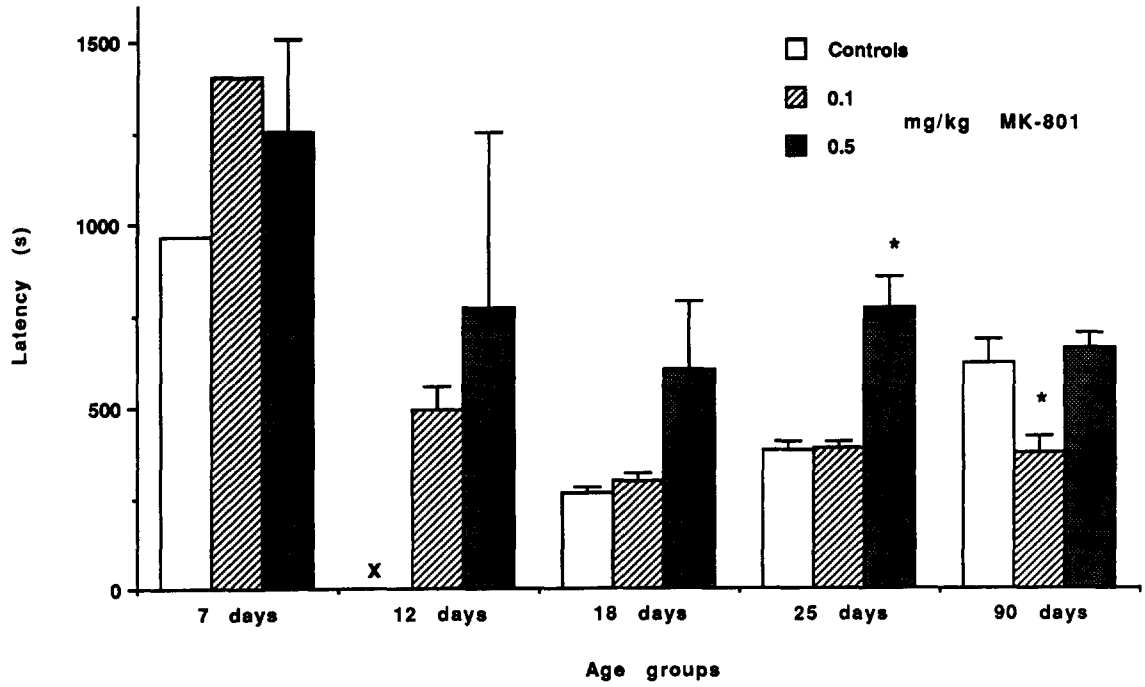

FIG. 1. Effects of MK-801 on the latency of picrotoxin-induced clonic seizures (mean ± SEM). Horizontal, age groups; vertical, latency in seconds; x, a phenomenon was not observed. *Significant difference ($p < 0.05$; ANOVA with posthoc Scheffe test) in comparison with the appropriate control group. Where the SEM bars are absent, sufficient amount of data for the statistics was not available.

TABLE 2

EFFECTS OF MK-801 ON THE INCIDENCE OF TONIC-CLONIC PICROTOXIN-INDUCED SEIZURES

		MK-801 (mg/kg)	
Age (days)	Controls	0.1	0.5
7	7/8	3/8	5/8
12	8/8	7/8	2/8*
18	8/8	5/8	3/8*
25	8/8	8/8	5/8
90	7/8	5/8	2/8*

For details, see Table 1.

*Significant difference ($p < 0.05$; Fisher's exact test) in comparison with an appropriate control group.

explorating activity of animals (sniffing, orientation, rearing in adults). The ataxia was greater in rat pups than in adults.

Clonic seizures consisted of a facial clonus, clonus of forepaws, and tail erection while the animal preserved the righting ability. It should be noted that in pups clonic seizures were often imperfect (e.g., unilateral).

Clonic seizures were induced by picrotoxin in all age groups with the exception of 12-day-old animals. The incidence of clonic seizures (demonstrated in Table 1) was relatively low in 7-day-old animals; however, it increased nearly to 100% in 18-, 25-, and 90-day-old rats. Incidence of clonic seizures was not influenced by MK-801 pretreatment with the exception of the 12-day-old group, where MK-801 increased the incidence of clonic seizures.

The action of MK-801 on the latency of clonic seizures was inconsistent (Fig. 1). In 25-day-old rats, MK-801 in the dose of 0.5 mg/kg delayed clonic seizures, $F(2, 20) = 20.7$, $p < 0.05$, whereas in 90-day-old rats the dose of 0.1 mg/kg MK-801 significantly decreased the latency of clonic seizures, $F(2, 17) = 7.77$, $p < 0.05$.

Tonic-clonic seizures began usually with running. After the loss of the righting ability, the tonic phase occurred for seconds, followed by clonus of all four limbs lasting for minutes, very often until the death of the animal.

The incidence of tonic-clonic seizures was between 87.5–100% in control rats. The higher dose (0.5 mg/kg) of MK-801 decreased the incidence of tonic-clonic seizures significantly in 12-, 18-, and 90-day-old rats (Table 2).

Tonic-clonic seizures were significantly delayed by lower dose of MK-801 in 12-day-old, $F(2, 14) = 20.2$, $p < 0.05$, and 18-day-old, $F(2, 13) = 75.09$, $p < 0.05$, pups. In the latter group, the 0.5-mg/kg dose of MK-801 had the same effect (Fig. 2).

The seizure severity score reached nearly 5 points in all age control groups (Fig. 3). MK-801 pretreatment caused a dose-dependent decrease of the score in all age groups with the exception of 7-day-old animals. Significant decreases were noted after 0.5 mg/kg MK-801 in 12-, 18-, and 90-day-old animals. It should be noted that MK-801, although it reduced the severity of seizures, did not change the motor pattern of both clonic and tonic-clonic picrotoxin-induced seizures in comparison with control animals.

In the controls, mortality varied between 50–100%. The lower dose of MK-801 (0.1 mg/kg) significantly decreased mortality in 7-, 18-, and 25-day-old animals, while the higher

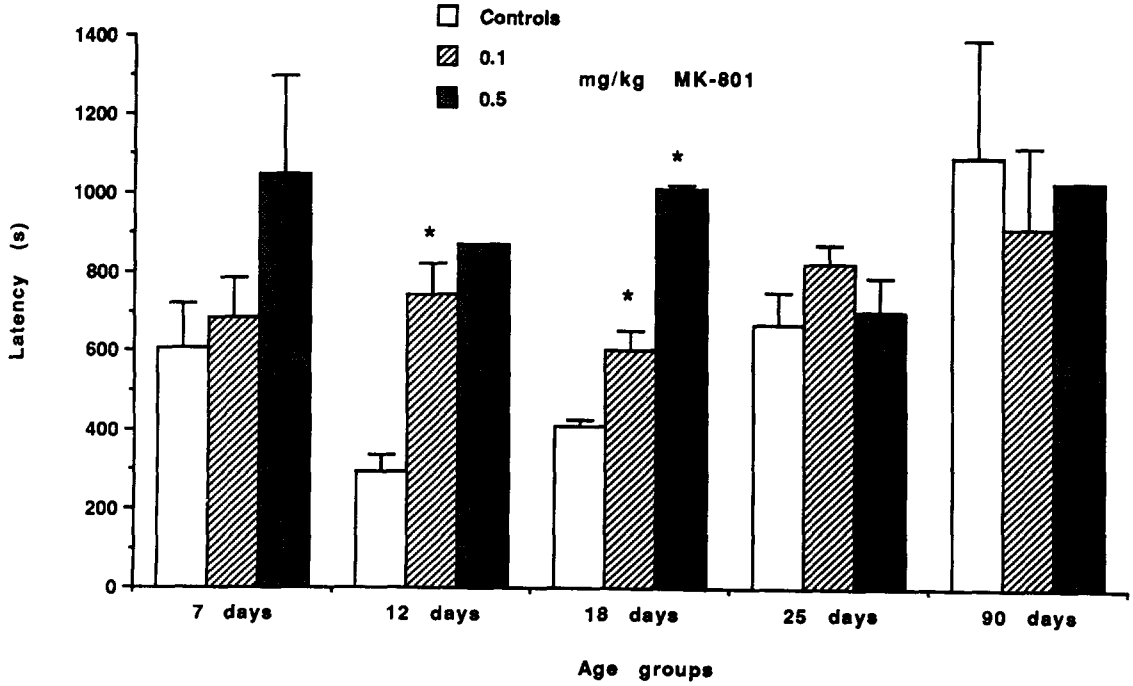

FIG. 2. Effects of MK-801 on the latency of picrotoxin-induced tonic-clonic seizures (mean ± SEM). For details, see Fig. 1.

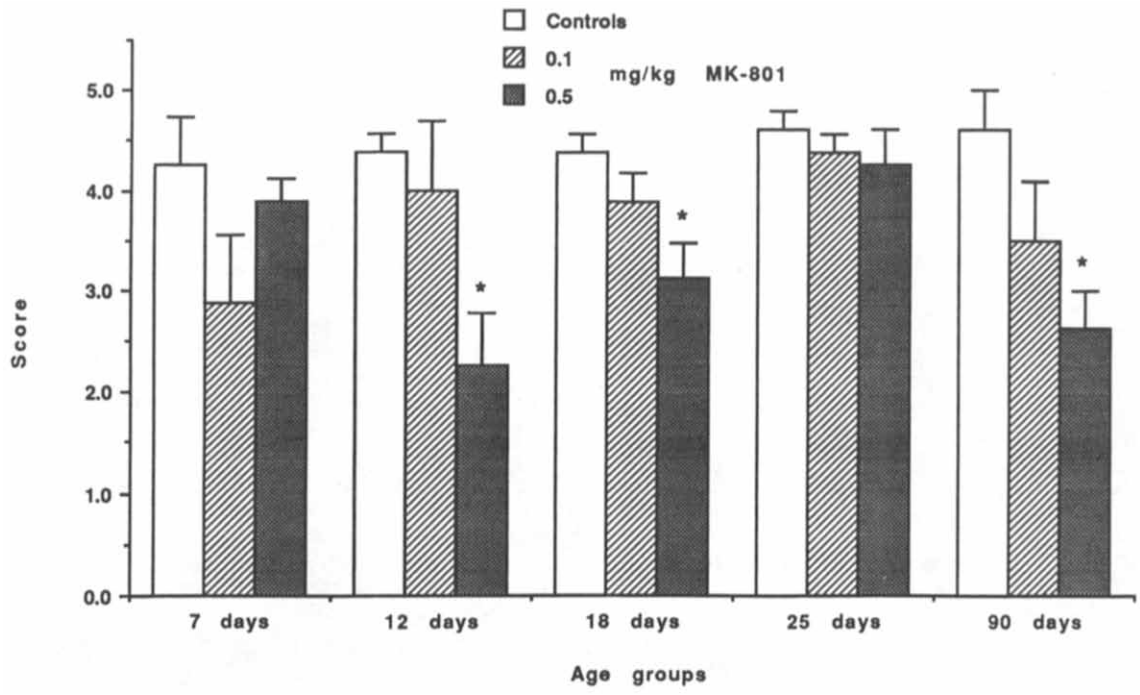

FIG. 3. Effects of MK-801 on the severity of seizures induced by picrotoxin (mean ± SEM). Horizontal, age groups; vertical, score in points; *, Significant difference ($p < 0.05$; Kruskall-Wallis test) in comparison with appropriate control group.

dose of MK-801 significantly suppressed the mortality in all age groups (Table 3).

DISCUSSION

Our study demonstrated that relatively low (8) doses of MK-801 do not suppress clonic picrotoxin-induced seizures throughout ontogenesis. In contrast, tonic-clonic seizures were suppressed nearly in all age groups studied, as well as picrotoxin-induced lethality.

This dissociative effect of MK-801 supports the theories describing different generators for clonic and tonic-clonic seizures in general (2,5) based either upon lesion studies or on the local application of pharmacological agents. Moreover, it appears that while the generator of clonic seizures does not operate through NMDA receptors the NMDA receptor action is essential for the generation of tonic-clonic seizures (23,24). The unsolved question is whether NMDA receptors are involved directly in this hypothetical generator, in the input to the generator, and/or in the generator's output.

The action of MK-801 on clonic and tonic-clonic seizures was almost consistent throughout ontogenesis with the exception of 25-day-old pups, which represent the prepubertal group of animals.

An increase in the incidence of clonic convulsions in 12-day-old pups is similar to the same phenomenon observed earlier. In our recent articles dealing with the action of ketamine against picrotoxin- and bicuculline-induced seizures (25) and MK-801 against pentylenetetrazol-induced seizures (24), we described similar findings in 12-day-old rats. The phenomenon is probably caused by the delay of tonic-clonic seizures, which unmasks clonic seizures.

Our results concerning tonic-clonic seizures, however, are different from the data presented in the previous study dealing with MK-801 vs. picrotoxin in adult animals (8). This difference was probably due to the different doses of picrotoxin used. In the article cited, the dose of 10 mg/kg picrotoxin was used, a supramaximal dose for the induction of tonic-clonic seizures, whereas our dose is close to the CD95 for tonic-clonic seizures (19). Moreover, we cannot exclude the varying sensitivity of the different rat strains to the convulsant.

The suppression of picrotoxin-induced lethality in almost all age groups by low-dose MK-801 is very interesting. We published similar results obtained with ketamine against

TABLE 3

EFFECTS OF MK-801 ON THE INCIDENCE OF PICROTOXIN-INDUCED LETHALITY

		MK-801 (mg/kg)	
Age (days)	Controls	0.1	0.5
7	4/8	0/8*	0/8*
12	4/8	2/8	0/8*
18	8/8	0/8*	0/8*
25	8/8	3/8*	1/8*
90	7/8	5/8	2/8*

For details, see Table 1.

*Significant difference ($p < 0.05$; Fischer's exact test) in comparison with an appropriate control group.

picrotoxin and bicuculline-induced seizures (25). An action of NMDA agonists on GABAergic transmission has been demonstrated (20). It appears, therefore, that noncompetitive NMDA antagonists may interfere with GABAergic transmission as well and this effect is worth studying in the future.

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