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Effects of Ketamine on Metrazol-Induced Seizures During Ontogenesis in Rats

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VELÍŠEK, L., R. MIKOLÁŠOVÁ, S. BLANKOVÁ-VAŇKOVÁ AND P. MAREŠ. *Effects of ketamine on metrazol-induced seizures during ontogenesis in rats.* PHARMACOL BIOCHEM BEHAV 32(2) 405–410, 1989.—An anticonvulsant action of ketamine, a noncompetitive NMDA receptor antagonist (1–40 mg/kg IP), on the metrazol-induced seizures was assessed in male Wistar rats aged 7, 12, 18, 25 and 90 days. Ketamine alone caused ataxia even in the lowest dose used. As concerens its interaction with metrazol it exerted a clearcut anticonvulsant effect against generalized tonic-clonic seizures at all developmental stages. On the contrary, the effects on clonic (i.e., minimal) seizures were only moderate or absent. Higher efficacy of ketamine was observed in young animals. Our results suggest a role of excitatory amino acids in the generation of generalized tonic-clonic metrazol seizures, but their share on the induction of clonic (minimal) seizures seems to be very small.

Ketamine Metrazol Epileptic seizures Rat Ontogenesis NMDA receptors

THE metrazol-induced seizures are most commonly used for modeling generalized seizure activity either tonic-clonic seizures or (in small doses) absence models (31). The effects of metrazol in developing rats were described previously (23,24). Briefly, high doses of metrazol elicit generalized tonic-clonic seizures in all age groups studied (i.e., starting with the postnatal day 5) whilst clonic seizures ("minimal metrazol seizures") induced by low doses of metrazol are reliably induced since the 3rd postnatal week of the rat. Until now no complete explanation exists for mechanisms of action of metrazol on the cellular and/or molecular level; direct excitatory effects on the membrane level and/or antagonism of the receptors for GABA and benzodiazepines are suggested (40).

Ketamine is a dissociative anesthetic commonly used in the young and elderly for limited surgical procedures (39). It is the derivative of phencyclidine, a drug with anesthetic and psychotomimetic properties which later became a street drug (28). Both drugs were reported to suppress metrazol-induced seizures in adult mice and rats (17, 30, 33). The phencyclidine treatment causes an inhibition in the rate of development of amygdaloid-kindled seizures, probably by means of increased kindled seizure thresholds (7,15). Other authors demonstrated that phencyclidine (21,39), as well as ketamine (6), have proconvulsant properties in laboratory animals. Similar proconvulsant action of ketamine is described in humans (4). The relation between anticonvulsant and proconvulsant activity of ketamine is probably dependent on the dosage (29).

An antagonistic action of ketamine on the N-methyl-D-aspartate (NMDA) receptors for excitatory amino acids was described recently (2, 8, 34). NMDA receptors are considered to be responsive for a long-term potentiation in the limbic system (14), where their high density is found (11,38) and their role in epilepsy is studied both in vivo and in vitro (1, 8, 10, 32, 41).

We have not found any data concerning the influence of ketamine on seizures in immature animals. For that reason we decided to study this question and the possible role of NMDA receptors in the generation of metrazol-induced seizures during the development of rats.

METHOD

Our experiments were carried out on 259 male Wistar albino rats of SPF breeding. The animals were divided into five age groups (7, 12, 18, 25 and 90 days old). Day of birth was taken as zero.

Drugs

Experimental groups were pretreated with ketamine (Narkamon® Spofa; KET) in the doses varying from 1 to 40 mg/kg intraperitoneally (for detailed distribution of the doses see Table 1). The subcutaneous administration of metrazol (pentamethylenetetrazol; PTZ) at a dose of 100 mg/kg followed after 10 minutes. One additional group of adult (90-day-old) animals received 120 mg/kg of PTZ SC for reliable induction of generalized tonic-clonic seizures.

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TABLE 1
DISTRIBUTION OF ANIMALS USED

Age/Dose	Numbers of Rats C	1	5	10	20	40
7	16	8	8	9	—	—
12	13	8	8	8	7	—
18	8	8	7	10	8	—
25	11	7	8	8	9	8
PTZ 100						
90	12	8	8	8	8	8
PTZ 120						
90	14	—	—	—	8	8

Rows—age of animals in days (two groups of adult animals represent rats receiving 100 or 120 mg/kg of PTZ).
Columns—doses of KET in mg/kg used.
C—denotes control animals which received PTZ only.
Values in the fields of the table indicate the numbers of animals used in the age group for each dose of KET.

The animals were observed in separate cages for 30 minutes after PTZ administration and their behavior was continuously recorded. The young animals were placed on an electrically heated pad and the temperature was held at 34°C.

We evaluated the incidences and latencies of two different seizure patterns:

Minimal metrazol seizures (mS) consisted of clonic convulsions of the head muscles and forelimbs. The animal did not lose the righting ability. This type of seizure lasted for tens of seconds.

Generalized tonic-clonic seizures (major seizures, MS) began, as a rule, with running fits followed by a short tonic phase. At the beginning of the tonic phase the animals lost their righting ability. The tonic phase consisted of the flexion and/or extension of forelimbs and/or hindlimbs and it was followed by a clonic phase which represented the longest part of seizure. The whole seizure lasted several minutes, exceptionally tens of minutes. It should be noted that all the forementioned signs of the generalized tonic-clonic seizure were not always present (23,25).

The score for each animal (representing maximal behavioral pattern accomplished) was calculated according to the following scale: 0—no changes, 0.5—abnormal behavior (e.g., sniffing, orientation), 1—isolated myoclonic jerks, 2—atypical minimal seizure (often seen in rat pups), 3—typical minimal seizure, 4—generalized seizure without the tonic phase, 5—generalized seizure including the tonic phase.

Results were compared with the control groups injected with corresponding doses of PTZ, part of controls (N=48) were already published in our previous paper (25).

Statistical analysis was computed in terms of analysis of variance (18) and results were compared by the Tukey studentized range method (27). Score values for both experimental and control animals were unified into the 2×2 contingency tables. The tables were evaluated by means of Fisher's F (20). Statistical significance was set at 5% level.

RESULTS

Ketamine alone produced ataxia which was well pronounced even after the lowest dose used in the youngest group of the rat pups.

Abnormal Behavior

In control animals receiving only PTZ we never observed any abnormal behavior with the exception of the muscle jerks and the seizure phenomena described above. Ataxia, rearing, orientation, sniffing, running and jerks of muscles over the entire body was often registered after the combination of KET and PTZ. The motor pattern of this behavior as well as that of the seizures always corresponded with the developmental stage of the animals, i.e., slow running movements, or rather swimming of all four limbs were observed in 7-day-old rats. The behavioral stereotype formed by repeated "wet dog shakes" was observed in the majority of adult animals in experiment groups; this phenomenon was never seen in controls receiving only PTZ.

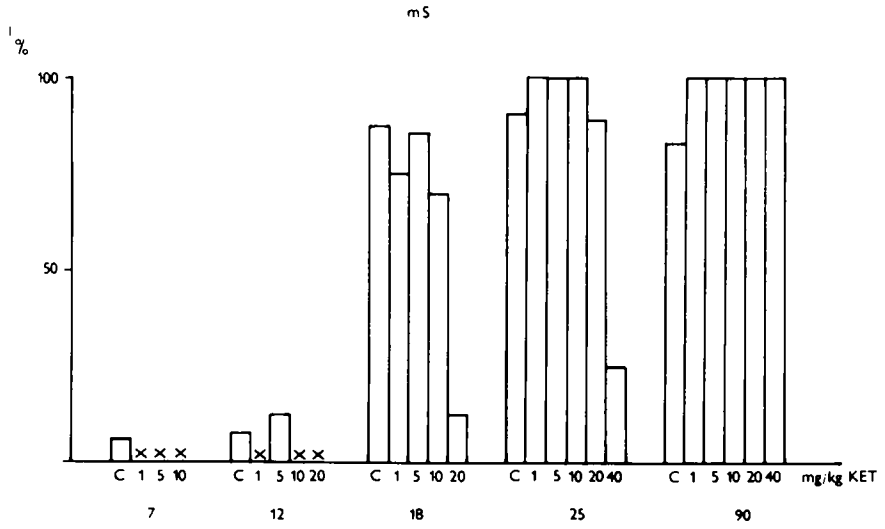

FIG. 1. Incidence of minimal metrazol seizures. Abscissa: number for each group of columns represents the age of animals, number under each column informs about the dose of KET used in mg/kg. C—denotes control animals receiving PTZ only. Ordinate: incidence in percents. x—denotes that illustrated sign was not observed.

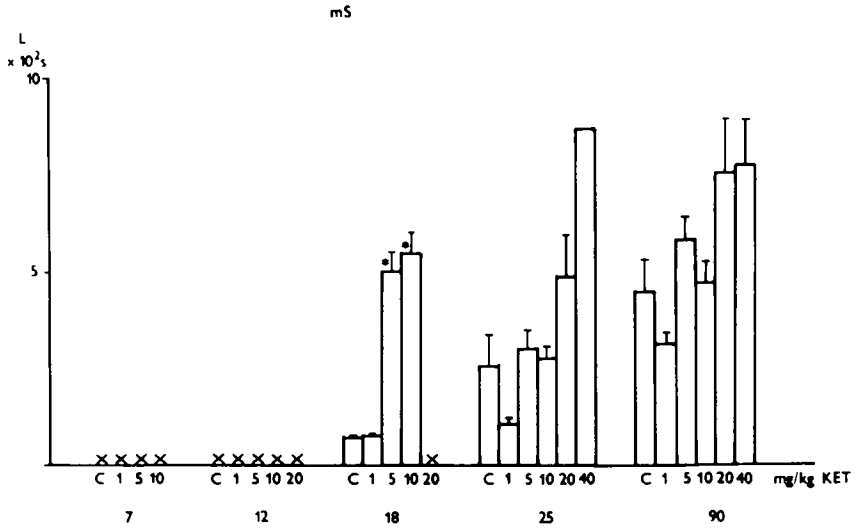

FIG. 2. Latency of the beginning of minimal metrazol seizures (mean±SEM). Ordinate: latency in hundreds of seconds. Where the bars representing SEM are absent, only about two values in the group were measured and taken into consideration. An asterisk denotes the significant difference in comparison with the control group (5% level). Other details as in Fig. 1.

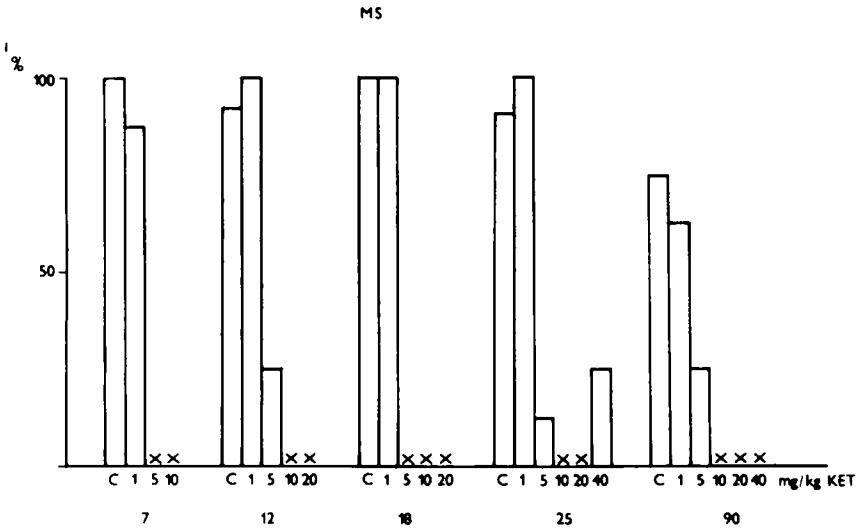

FIG. 3. Incidence of major seizures. For details see Fig. 1.

Minimal Seizures

The incidence of minimal seizures is presented in Fig. 1. Nearly all the rats aged 18 days and up in control groups exhibited minimal metrazol seizures. Minimal seizures in 18-day-old rats were often evaluated as imperfect because they often appeared to be unilateral or incomplete (i.e., consisting only of chewing and a Straub tail). Minimal seizures were rarely seen in control groups of 7- and 12-day-old rats. Ketamine in the doses used had minimal effects on the incidence of minimal seizures, only the highest doses of KET in 18- (20 mg/kg) and 25-day-old rats (40 mg/kg) markedly stressed the incidence of the minimal seizures. The incidence of minimal seizures tended to increase after all doses of KET in adult rats.

The latencies of minimal seizures were lengthened in three older age groups (Fig. 2). The doses of 20 and 40 mg/kg of KET were effective only in 25-day-old and adult animals, whereas in 18-day-old animals the latency increased following the administration of 5 and/or 10 mg/kg of KET. Analysis of variance revealed the differences among latencies in the subgroups of 18-day-old and 90-day-old (adult) rats treated with 120 mg/kg of PTZ (Fig. 5).

Seven- and 12-day-old rat pups exhibited minimal seizures only exceptionally even after KET pretreatment. That is why we were not able to compare the latencies.

Major Seizures

The incidence of major seizures (Fig. 3) reached 80–100% in controls of all age groups with exception of adult rats where the 120 mg/kg dose of PTZ was necessary (Fig. 5). The 10 and 20 mg/kg doses of KET abolished major seizures at all developmental stages. An effective suppression was also ac-

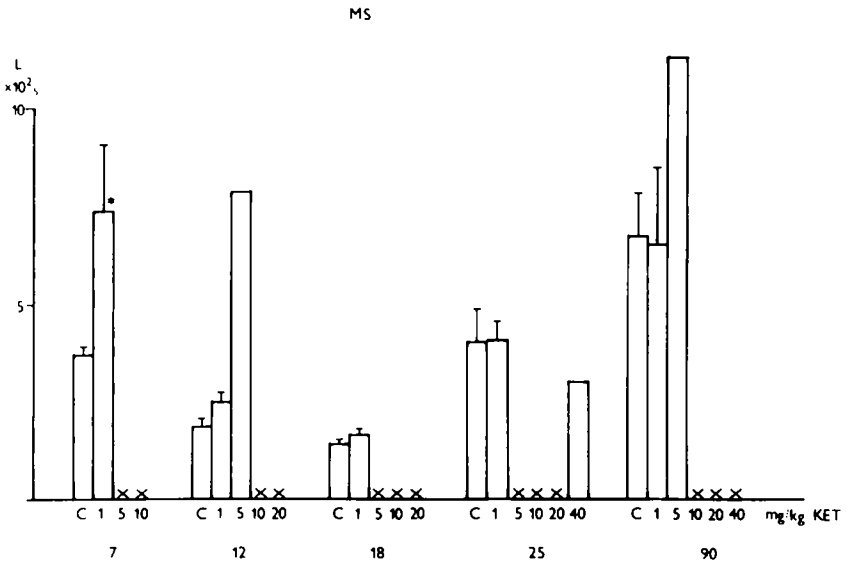

FIG. 4. Latency of the beginning of the major seizures (mean±SEM). For details see Fig. 2.

complished in 7- and 12-day-old rats with a dosage of 5 mg/kg of KET. On the contrary, the dose of 40 mg/kg of KET did not abolish major seizures in 25-day-old rats as well as in the group of adult rats with the 120 mg/kg dose of PTZ (Figs. 3 and 5).

The latencies of major seizures if observed were prolonged (Fig. 4), but due to a low incidence statistical significance was reached only in the case of 7-day-old animals between the dose of 1 mg/kg of KET and controls (without KET pretreatment). The only exception was the dose of 1 mg/kg of KET in adult rats treated with 100 mg/kg of PTZ which tended to decrease the latency of the major seizures.

Severity of Seizures (Fig. 6)

The score of each animal represents maximal behavioral or seizure patterns observed. In 7-, 12- and 18-day-old rats KET pretreatment led to the dose-dependent decrease of the mean score. On the contrary, abolition of major seizures without affecting minimal seizures resulted in nearly uniform decreases to the average score of 3.0 in 25- and 90-day-old animals.

Further statistical analysis presented decreasing of the efficacy of KET with the increasing age of animals.

DISCUSSION

On the basis of our experiments we can summarize results into the following points:

1) We observed moderate ataxia even after the lowest doses of KET used during all development. It is in concordance with the recent experiments (7), which revealed some ataxia after low doses of phencyclidine and KET. It is interesting that the dose sufficient to produce ataxia in 7-day-old pups is of two orders lower than the dose required for reliable induction of sleep in adult rats [Marešová *et al.* (26)]. It may lead to the conclusion that the atactic action of KET is highly specific and is mediated via specialized mechanism different from that responsible for anesthetic effects.

2) We did not observe any direct convulsant action of KET within the range of doses used. The conclusion is supported by the other authors (21) who demonstrated that KET

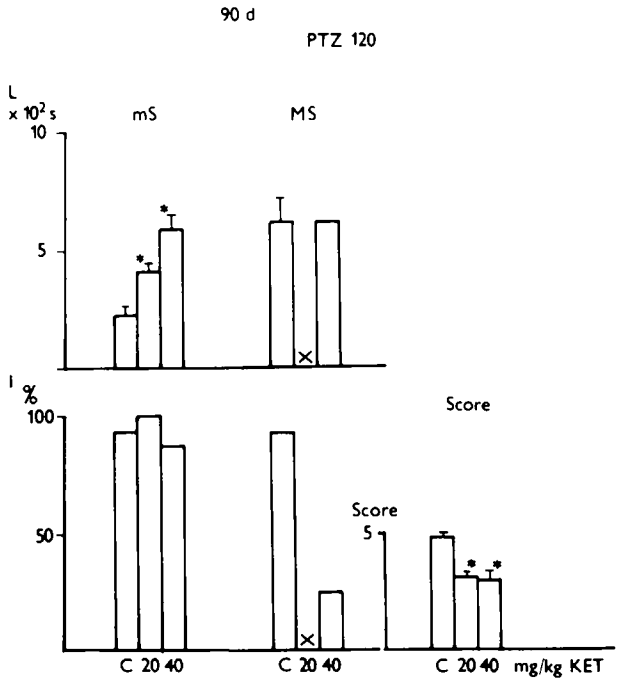

FIG. 5. Results of adult rats treated with 120 mg/kg of PTZ. Abscissa: doses of KET in mg/kg. C —represents control animals treated with PTZ 120 mg/kg only. Ordinate: (from top to bottom) latency of signs in hundreds of seconds; incidence of signs in perdents; score, respectively. mS—minimal metrazol seizures. MS—major seizures (tonic-clonic). Score—score (see the Method section). x—illustrated sign was not observed. An asterisk denotes the significant difference in comparison with the control group (5% level).

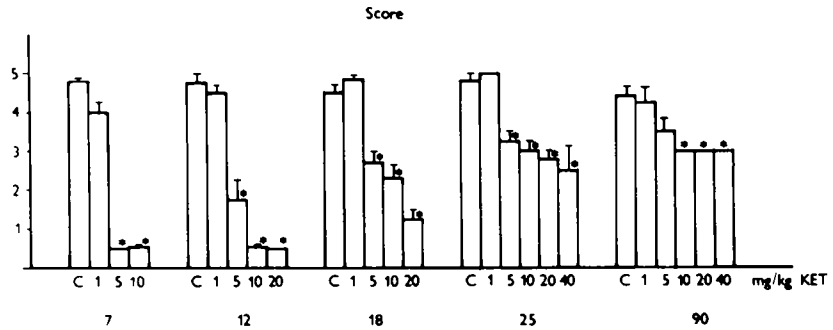

FIG. 6. Score (maximal behavioral pattern accomplished, mean±SEM). Abscissa: see Fig. 1. Ordinate: score according to the scale in the Method section. Asterisks denote significant differences in comparison with the control groups (5% level).

was not convulsive up to the lethal doses. We were not interested in proconvulsant effects of KET, which are described elsewhere (6). An attenuation of the anticonvulsant action of KET, i.e., reappearance of major seizures after the 40 mg/kg dose of KET in 25-day-old rats and adult rats treated with 120 mg/kg of PTZ, was not originally a part of our experimental program. But it may be explained on the basis of the Winters' scheme of the mechanism of action of anesthetics (39). Winters suggests a convulsant effect in some anesthetics (including phencyclidine and KET). This effect was substituted by an attenuation of anticonvulsant action of KET in our experiments. Such dissociation of effects (anticonvulsant versus proconvulsant) suggests more than one mechanism of action of KET. Our proposal results from the earlier studies (16,36), which demonstrated both serotonergic and dopaminergic activity of KET. Further exploration of these effects is necessary.

3) Ketamine caused dramatic reduction of major seizures but only a slight or nonconsistent decrease in the incidence of minimal seizures. We have confirmed the study of Hayes and Balster (17); these authors did not observe any effect of phencyclidine and its analogues on clonic seizures. But the same drugs prolonged the onset or prevented the occurrence of tonic-clonic metrazol-induced seizures in mice. This can be understood on the basis of antagonistic action of KET on NMDA receptors. Davies and Watkins (12) reported 2-amino-5-phosphonovalerate (which is a specific antagonist of NMDA receptors) to be a more effective antagonist of polysynaptically- than monosynaptically-evoked excitation. If we accept Browning's theory that seizure activity during tonic-clonic seizures begins in the brain stem and spreads from there to the whole brain (9), it seems to be clear that generation and spread of seizure activity in polysynaptic system is much more vulnerable than more simple generator of "forebrain" or clonic seizures, which is probably restricted in a part of the brain. An alternative explanation is that only the generator for tonic-clonic (maximal) seizures uses excitatory amino acids as neurotransmitters.

4) It is possible that high effectiveness of KET as an anticonvulsant as well as in induction of loss of righting reflexes (26,37) in rat pups and its decrease with age parallels the supersensitivity of NMDA receptors in young rats (3) as well as the higher percentage of visual cortical neurons excited by NMDA in kittens in comparison with adult cats (35). On the other hand, an unspecific effect of KET could not be excluded. Another study (13) found that higher doses of 2-amino-5-phosphonovalerate also reduced responses to kainate and quisqualate. The similar action of KET might take place. The higher density of the kainate receptors begins in the third week of the postnatal life of the rat (5), and that is why their role in seizure generation increases. Eventually, we should not exclude simple pharmacokinetic reasons.

5) We confirmed the role of excitatory amino acids in the generation of PTZ-induced seizures. Our results suggest an involvement of excitatory amino acids mainly in the generation of the major tonic-clonic seizures induced by metrazol. But we cannot conclude if it is true only for PTZ-induced tonic-clonic seizures or generally for the tonic-clonic seizure pattern. The role of excitatory amino acids in minimal metrazol seizures seems to be negligible.

It is generally accepted that some KET effects are mediated by NMDA receptors for excitatory amino acids. The proofs on microlevels were presented earlier (2, 8, 34) and the behavioral changes after KET administration correspond well with the behavioral changes induced by 2-amino-5-phosphonovalerate (19). But we should not exclude possible further mechanisms of action of KET (22). The question of the real mechanism of action of metrazol should also be studied.

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