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BRIEF COMMUNICATION

Ethosuximide Suppresses Seizures and Lethality Induced by Picrotoxin in Developing Rats

J. VELÍŠKOVÁ,* L. VELÍŠEK,*‡[1] P. MAREŠ,†‡ R. ROKYTA* AND D. MIČIANIKOVÁ‡

**Departments of Physiology and Clinical Physiology and †Pathophysiology, 3rd Medical Faculty, Charles University, Prague, Czechoslovakia*
‡Institute of Physiology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia

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VELÍŠKOVÁ, J., L. VELÍŠEK, P. MAREŠ, R. ROKYTA, AND D. MIČIANIKOVÁ. *Ethosuximide suppresses seizures and lethality induced by picrotoxin in developing rats.* PHARMACOL BIOCHEM BEHAV 44(4) 975-979, 1993.— The action of ethosuximide (125 or 250 mg/kg, IP) against picrotoxin-induced seizures (3-6 mg/kg, IP) was assessed in rats 12, 18, 25, and 90 days old. In 18-day-old and older controls, picrotoxin regularly elicited clonic seizures; tonic-clonic seizures were induced in all age categories with high consequent mortality. Only the higher dose of ethosuximide (250 mg/kg) increased the latency of clonic seizures in 18- and 25-day-old pups. Tonic-clonic seizures were delayed by ethosuximide in 12-, 18-, and 90-day-old rats. Picrotoxin-induced lethality was suppressed only in 18- and 90-day-old rats by the 250-mg/kg dose of ethosuximide. In contrast, ethosuximide pretreatment increased the incidence of clonic seizures in 12-day-old rats. The results suggest that only high doses of ethosuximide can suppress clonic seizures, and this action is not consistent. Tonic-clonic seizures probably have model-specific sensitivity to ethosuximide because in previous studies ethosuximide completely suppressed pentylenetetrazol-induced tonic-clonic seizures but had no effect on kainic acid-induced tonic-clonic seizures. The suppression of mortality rates is probably due to nonspecific effects of high doses of ethosuximide.

Ethosuximide Picrotoxin Seizures Development Rat

ETHOSUXIMIDE is a drug frequently used against human epileptic seizures of the absence type (6,14,24). The effect of ethosuximide is specific against this type of seizure in humans. These seizures appear in young children and often disappear in puberty (5). In the experiment of Farrance and Halpern (7), other types of seizures were either unaffected or worsened by ethosuximide.

In our recent study (25), we described that ethosuximide has effects against both kainic acid- and pentylenetetrazol-induced clonic seizures, suggesting that: a) There may be a common part of the clonic seizure substrate (i.e., structures, pathways, or circuits) that is involved in the induction of seizures by both drugs; and b) this substrate can be affected by ethosuximide. In contrast, ethosuximide abolished only pentylenetetrazol-induced generalized tonic-clonic seizures and did not affect this type of seizure induced by kainic acid. This suggests that the suppression of tonic-clonic seizures by ethosuximide may be specific for pentylenetetrazol. To decide whether clonic seizures in general may be affected by ethosuximide and whether the superior action of ethosuximide against pentylenetetrazol-induced tonic-clonic seizures is a unique feature of ethosuximide, we used another model, picrotoxin-induced seizures, for the experiments.

Picrotoxin, a blocker of chloride channels associated with $GABA_A$ receptors, elicits both types of seizures mentioned above (i.e., clonic and tonic-clonic) when administered systemically in rats (10,19,22). Picrotoxin-induced seizures result from suppressed GABAergic inhibition, but their clinical motor patterns are closely similar to those seizures induced by both pentylenetetrazol and kainic acid. Picrotoxin-induced seizures may change during development (17,23,28).

Our working hypothesis was that clinically similar or identical seizures may share (at least partially) common substrates at the level of structure, pathway, or circuit. This may be

[1] Current address and address to which requests for reprints should be sent: Libor Velíšek, M.D., Ph.D., Department of Neurology, Albert Einstein College of Medicine, Kennedy Center, Room 316, 1410 Pelham Parkway South, Bronx, NY 10461.

expressed as a similar or identical response of the seizures to the antiepileptic drug therapy if the antiepileptic drug targeted the common substrate. Therefore, we performed the experiments with picrotoxin-induced seizures to determine the effects of ethosuximide in another model of similar seizure patterns (clonic and tonic-clonic).

In our previous study (15), we found that ethosuximide is only moderately active against pentylenetetrazol-induced seizures in 18-day-old rats whereas it is fully effective in adult rats. Therefore, we decided to study the action of ethosuximide during development in a picrotoxin-induced seizure model.

METHOD

The experiments were performed on 106 male Wistar albino rats 12, 18, 25, and 90 days old. Day of birth was taken as zero; 90-day-old animals were considered to be adult. The age groups represent our standard age groups that from the developmental point of view roughly correspond with the prematurely born (7 days), young child (12 days), preschool (18 days), prepubertal (25 days), and adult (90 days) human nervous system (12). We concentrated on early stages of development a) because of exclusive appearance of human absences (which respond to ethosuximide) in childhood (5) and b) because the developing nervous system is more prone to seizures than the adult nervous system (18). Experimental groups were pretreated with ethosuximide (ESI; a gift from VEB Arzneimittelwerke Dresden) in doses of either 125 or 250 mg/kg IP 15 min prior to administration of picrotoxin (PX). The doses of PX were 4, 3, 5, and 6 mg/kg IP for rats 12, 18, 25, and 90 days old, respectively. Both drugs were dissolved in normal saline. The dosage of both drugs, the delay between the administration of ESI and PX, and the duration of the observation period (below) were based upon our previous data to get high incidence of seizures in all age groups (15,23,26).

After administration of picrotoxin, animals were placed into separate cages and observed for 60 min. Young rats were placed on an electric pad heated to 34°C. We registered the latency and incidence of clonic seizures, tonic-clonic seizures, and mortality of rats. The latencies to the seizure onsets were compared by analysis of variance with posthoc Tukey's test within each age group separately (because of different doses of picrotoxin). The variable was the pretreatment. Only valid data were included in the analysis (see Table 1 for n in each subgroup). Incidence of seizures and mortality were compared by Fisher's exact test in each age group separately. Incidences are presented as numbers of rats with observed phenomenon of the n for each pretreatment subgroup. For those interested, percentual values of incidence are presented. It should be noted that comparison has been always performed between control and pretreated groups within each age group. The level of significance was set to 5%.

RESULTS

Pretreatment with 125 mg/kg ESI did not elicit any disturbances in behavior. The dose of 250 mg/kg of ESI elicited ataxia in 12- and 18-day-old rats; only slight sedation was present in 25- and 90-day-old rats after this dose.

In control animals, picrotoxin induced both clonic and tonic-clonic seizures. Clonic seizures consisted regularly of chewing, head nodding, facial and forelimb clonus, and tail erection. Animals preserved the righting reflex during the seizure. In 12-day-old pups, picrotoxin in the dose used was not able to elicit clonic seizures. In 18-day-old rats, clonic seizures were usually evaluated as imperfect because only unilateral clonus or head nodding and tail erection were present. Tonic-clonic seizures were consistently elicited in all age groups studied. They usually began with wild running. Animals lost their righting reflexes right before the beginning of the tonic phase. The tonic phase lasted for several seconds and consisted of tonic flexion and/or extension of fore- and/or hindlimbs. Following the tonic phase, a long clonus (duration in minutes) of all four limbs appeared. These seizures were sometimes terminated by the death of the animal. All 25- and 90-day-old rats that displayed tonic-clonic seizures died within the observation period. In younger animals, this correlation decreased; 80% of 18-day and 50% of 12-day-old rats died after having a tonic-clonic seizure.

Pretreatment with ESI did not change the motor pattern of clonic seizures and this seizure type was only inconsistently affected by ESI. The incidence of clonic seizures is demonstrated in part A of Table 1. In contrast to controls, clonic seizures were registered in 12-day-old pups pretreated with ESI. Following the higher dose of ESI (250 mg/kg), clonic seizures were detected in all 12-day-old pups. This was the only age-dependent effect of ESI pretreatment. In older animals (18 and 25 days old), ESI was not able to influence the incidence of clonic seizures; however, an increase in their latency was registered following the dose of 250 mg/kg of ESI (Fig. 1A). In adult animals, no effects of ESI against clonic seizures were observed.

After ESI pretreatment, the motor pattern of tonic-clonic seizures was unchanged and ESI suppressed this seizure type in three age groups. The incidence of tonic-clonic seizures, however, was not significantly influenced by ESI (Table 1, part B). A tendency to suppression of tonic-clonic seizures was seen in 12-day-old and adult rats following pretreatment with 250 mg/kg ESI. The latency of tonic-clonic seizures increased in 12-day-old animals (125 mg/kg ESI), 18-day-old rats (both doses), and adult rats (250 mg/kg ESI). The latency of tonic-clonic seizures did not differ between controls and ESI-pretreated 25-day-old rats (Fig. 1B).

The protective action of ESI against the lethal effects of picrotoxin was more marked than the action against tonic-clonic seizures (Table 1, parts B and C).

ESI pretreatment (250 mg/kg) significantly decreased the mortality in 18- and 90-day-old rats (Table 1, part C). Survival time (i.e., the latency to the death of rats) was enhanced only in adult animals following the 250-mg/kg dose of ESI (Fig. 1C). A high correlation of tonic-clonic seizures and death of rats was preserved only in adults following ESI pretreatment (88.9%); in other age groups, it was decreased: in 25-day-old rats to 77.8%; in 18-day-old pups to 26.7%, and in 12-day-old rats to 30%. However, the correlation decrease was significant only in 18-day-old pups.

DISCUSSION

Our study demonstrates moderate effects of high (borderline ataxic) doses of ethosuximide against clonic (facial and forelimb clonus) picrotoxin-induced seizures in 18- and 25-day-old animals. Tonic-clonic seizures were either suppressed or delayed in 12-, 18-, and 90-day-old rats. Ethosuximide decreased mortality of rats or increased survival time following picrotoxin in 18- and 90-day-old animals. There were no age-specific effects of ethosuximide except the increase of the incidence of clonic seizures in 12-day-old rats.

The action of ethosuximide against clonic picrotoxin-induced seizures correlates well with our previous results with

TABLE 1

A: INCIDENCE OF CLONIC PICROTOXIN-INDUCED SEIZURES AND EFFECTS OF ETHOSUXIMIDE

	Age (days)			
Treatment	12	18	25	90
Controls	0/10 0%	9/10 90%	10/10 100%	9/10 90%
ESI 125	3/8 37.5% $p = 0.069$	7/8 87.5% $p = 0.710$	9/12 75 $p = 0.440$	8/8 100% $p = 0.556$
ESI 250	6/6* 100% $p = 0.00013$	8/8 100% $p = 0.56$	7/8 87.5% $p = 0.440$	8/8 100% $p = 0.556$

B: INCIDENCE OF TONIC-CLONIC PICROTOXIN-INDUCED SEIZURES AND EFFECTS OF ETHOSUXIMIDE

	Age (days)			
Treatment	12	18	25	90
Controls	8/10 80%	10/10 100%	10/10 100%	9/10 90%
ESI 125	7/8 87.5% $p = 0.588$	7/8 87.5% $p = 0.444$	11/12 92.7 $p = 0.545$	5/8 62.5% $p = 0.206$
ESI 250	3/6 50% $p = 0.242$	8/8 100% $p = 1.000$	7/8 87.5% $p = 0.440$	4/8 50% $p = 0.089$

C: MORTALITY OF DEVELOPING RATS FOLLOWING PICROTOXIN AND AFTER ETHOSUXIMIDE PRETREATMENT

	Age (days)			
Treatment	12	18	25	90
Controls	4/10 40%	8/10 80%	10/10 100%	9/10 90%
ESI 125	0/8 0% $p = 0.069$	3/8 37.5% $p = 0.082$	9/12 75 $p = 0.140$	5/8 62.5% $p = 0.206$
ESI 250	3/6 50% $p = 0.550$	1/8* 12.5% $p = 0.0076$	5/8 62.5% $p = 0.069$	3/8* 37.5% $p = 0.032$

Controls were treated only with picrotoxin. ESI 125, 150, groups pretreated with 125 or 250 mg/kg ethosiximide, respectively. In format x/n, x represents animals with expressed phenomenon of total subgroup n. Also percentual incidence is shown. p values were computed using Fisher's exact text.

*Significant difference in comparison with age-matched control group.

pentylenetetrazol and kainic acid (15,25). In those as well as in the present study, we found only moderate effects of ethosuximide. The results suggest that clonic seizures (with preserved righting reflexes) may not be an appropriate model of human absences because sufficient levels of ethosuximide control about 80% of absence seizures in humans (21). In this study, even behaviorally toxic doses of ethosuximide were not able to control clonic seizures completely. The suggestion that clonic seizures in rodents (induced by any convulsant) represent a model of human myoclonic seizures appears to be more realistic (14,16). It appears that the substrates (i.e., structures, pathways, and circuits) participating in the genesis of clonic seizures in general are only moderately affected by ethosuximide. That probably means that clonic seizures induced by different drugs may have in common several pathways, structures, and/or receptor system(s) that form the seizure substrate. From the anatomic point of view, previous studies suggested that the clonic seizure substrate is probably localized in

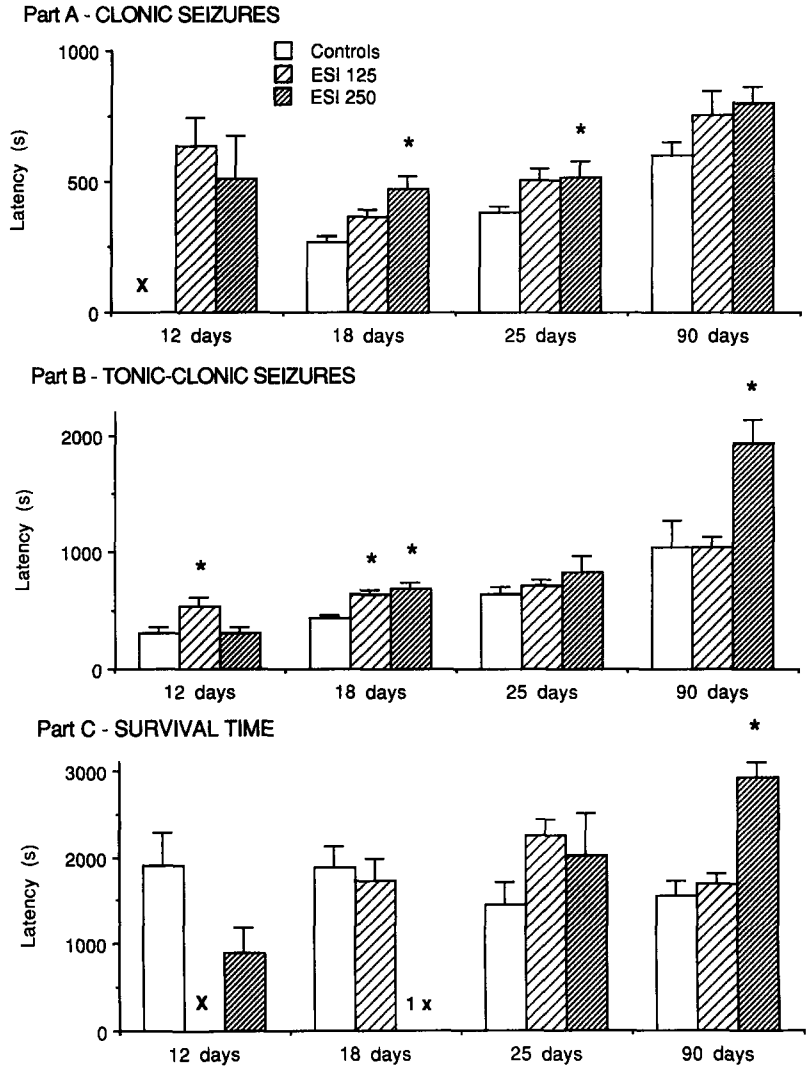

FIG. 1. Effects of ethosuximide against picrotoxin-induced phenomena in developing rats (Mean ± SEM). (A) Latency of clonic seizures. (B) Latency of tonic-clonic seizures. (C) Duration of survival time following picrotoxin administration. Abscissa, age of rats; ordinates, latencies of phenomena in seconds. Controls, groups treated only with picrotoxin. ESI 125, 250, groups pretreated with 125 or 250 mg/kg ethosuximide, respectively. X, a phenomenon was not observed. 1x, a phenomenon was observed only in one animal in the subgroup. *Significant difference in comparison with the appropriate age-matched control group (ANOVA with posthoc Tukey's test; $p < 0.05$).

the forebrain (3,11). In the case of ethosuximide action, the common part of the substrate might be represented by thalamic relay neurons (4).

An increase in the incidence of clonic seizures in 12-day-old pups was an interesting finding. This effect was probably caused by the suppression or delay of tonic-clonic seizures that masked the clonic seizures. Similar unmasking of clonic seizures when tonic-clonic seizures are blocked or delayed has been described following various pretreatments [e.g., NMDA antagonists (26,27); carbamazepine and hydroxycarbamazepine (13); and flumazenil (20)].

Ethosuximide was not as effective against tonic-clonic picrotoxin-induced seizures as against tonic-clonic pentylenetetrazol-induced seizures (25). However, the effects of ethosuximide against picrotoxin-induced seizures were better than against kainic acid-induced generalized tonic-clonic seizures (25). The inferior action of ethosuximide against picrotoxin-induced tonic-clonic seizures in comparison with the pentylenetetrazol model may be caused by the higher dosage of picrotoxin in the present study, although the results of our pilot study suggested the doses used to be equally potent in the seizure induction in different age groups (23). Another explanation originates from the suggested substrate responsible for tonic-clonic convulsions, which is believed to be located in the brain stem (2). Different drugs used as convulsants in this as well as in previous studies (15,25) may activate this hypothetical seizure substrate by different means (i.e., pathways or receptor systems), each with a different sensitivity to ethosuximide. One might speculate that, for example, tonic-clonic seizures induced by kainic acid that generalize from a limbic focus (1) are almost completely resistant to ethosuximide action, whereas tonic-clonic seizures induced by other drugs may use ethosuximide-sensitive substrate for generalization (25).

The suppression of picrotoxin-induced tonic-clonic seizures

by ethosuximide in 18- and 90-day-old rats was followed also by the suppression in mortality. Although the differences between control and ethosuximide-pretreated groups were not large, they were significant, suggesting a relation between tonic-clonic seizure and seizure-induced death. The effects of ethosuximide against picrotoxin-induced lethality was mediated probably by nonspecific mechanisms induced by high doses of ethosuximide.

In conclusion, ethosuximide inconsistently suppressed clonic seizures. In contrast, their incidence in 12-day-old rats was significantly increased after ethosuximide pretreatment. This increase was probably caused by the suppression or delay of tonic-clonic seizures that masked the clonic seizures. The action of ethosuximide against tonic-clonic seizures seems to be seizure model specific. The exact mechanisms of action of ethosuximide on structural and/or cellular level remains to be elucidated; nevertheless, several attempts have been made suggesting that thalamic relay neurons may form a part of a hypothetical substrate involved in absence seizures (4,8,9).

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