Similar effects of lamotrigine and phenytoin against cortical epileptic foci in immature rats

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Short title: Pharmacology of cortical epileptic foci in immature rats

Abstract

Action of antiepileptic drugs in immature brain may differ from that in adult brain. The aim of our study was to study an anticonvulsant action of lamotrigine and phenytoin, i.e. two drugs active against partial seizures in adult experimental animals as well as human patients, in a model of simple partial seizures in immature rats. Epileptic foci were induced by local application of bicuculline methiodide on sensorimotor cortical area of 12-day-old rat pups. The animals were pretreated with lamotrigine (LTG, 10 or 20 mg/kg i.p.) or phenytoin (PHT, 15, 30 or 60 mg/kg i.p.). Control rats for LTG received saline, controls for PHT solvent composed of propyleneglycol, ethanol and water. Influence of either drug on interictal activity was negligible. High doses of both LTG and PHT suppressed the transition into ictal phases and shortened the duration of persisting seizures. The tricomponent solvent exhibited moderate activity against ictal activity if compared with saline controls. The two drugs exhibited similar action in our model – suppression of secondary generalization from epileptic focus. This action is comparable to that described for human patients and adult experimental animals. In favour of lamotrigine speaks an absence of serious side effects.

Key words: cerebral cortex – epileptic focus – bicuculline methiodide – immature rat – antiepileptic drugs

The goal of therapy of epilepsies is to keep the patients free of seizures without compromising their brain function. The preservation of normal function is especially important in the developing brain because pathological changes may derange brain development. Studies of epileptic phenomena and the action of antiepileptic drugs during brain development are therefore necessary because of very high incidence of epileptic seizures in infancy and childhood (Hauser et al. 1993).

The two common experimental models of seizures used to test efficacy of possible new anticonvulsant drugs - maximal electroshock seizures (a model of generalized tonic–clonic seizures) and minimal metrazol seizures (a model of myoclonic seizures) do not cover the whole spectrum of human epileptic seizures. It has been proposed that the maximal electroshock seizure test might also predict efficacy against partial seizures but serious doubts arose. Thus a valid model of complex partial seizures with secondary generalization is represented by amygdala kindled seizures (Lőscher and Schmidt 1988) but there is no generally accepted model of simple partial seizures in spite of the fact that partial seizures represent a common type of seizures in the immature brain.

Interictal discharges as the main EEG phenomena in partial seizures represent a simple, most common sign of epileptic process (de Curtis and Avanzini 2001) during which thousands of neurons in the focus exhibit paroxysmal depolarization shift with superimposed burst of action potentials. This phenomenon was experimentally analyzed mostly in the model of PNC-induced foci. Similar model could be formed by local application of a GABA-A antagonist bicuculline in its water soluble form – bicuculline methiodide (for review Prince 1985, Velíšek 2006). This model was elaborated in our laboratory in adult as well as immature rats (Velíšková et al. 1991, Soukupová et al. 1993) and it was verified in adult animals by Eder et al. (1997). This model allows to study not only discharges of a primary epileptic focus, but also spread of focal discharges through the cortex and a transition of an interictal activity into ictal phases. Unfortunately, developmental data on pharmacological sensitivity of this model are not yet available in spite of the marked developmental differences in pharmacokinetics as well as pharmacodynamics of antiepileptic drugs (Morselli et al.

1983). We decided to start with testing of two antiepileptic drugs with main mechanism of action connected with voltage-gated sodium channels: phenytoin - a classical antiepileptic drug that serves epileptic patients for many decades and lamotrigine - a drug that belongs to the third generation of antiepileptics (DeLorenzo and Sun 2002, Leach et al. 2002).

A preclinical profile of LTG anticonvulsant action is in some respect similar to that of PHT. LTG blocks electrically or chemically induced maximal (tonic) seizures but has no effect on the threshold of chemically induced seizures in rodents (Miller et al.1986). As cerebral cortex is concerned, LTG is active in cortical kindling model in rats (O'Donnell and Miller 1991) and it attenuates local field potentials elicited by somatosensory stimulation (Kida et al. 2006). It inhibits repetitive high frequency firing in depolarized neurons by selectively prolonging slow inactivation of the sodium channel, thereby suppressing the release of excitatory amino acids, mainly glutamate (Leach et al. 1986, 2002). In addition, LTG influences also calcium and potassium channels (Stefani et al. 1996, Grunze et al. 1998, Zona et al. 2002). Similar action of the two anticonvulsants against pentetrazol-induced generalized tonic-clonic seizures was found also in immature rats (suppression of the tonic phase – Stanková et al. 1992). There is not only similarity but also a difference between the actions of the two drugs – LTG is efficient against human absence seizures (Stephen and Brodie 2002) as well as against their animal models (Hosford and Wang 1997, Gibbs et al. 2002), PHT is without effect or aggravates these seizures (Gurbanova et al. 2006). The most important difference - convulsant effects of an overdose of PHT in early postnatal rats (7- and 12-day-old ones - Mareš et al. 1987) - led us to test the effects of both LTG and PHT in 12-day-old rat pups.

Methods

The experiment was carried out in 12-day-old male rat pups of albino Wistar strain, housed under standard conditions (mothers were given food and water ad libitum, 12:12 light:dark cycle, temperature 22±1˚C). The day of birth was counted as zero and all litters were reduced to eight pups. Body weight of 12-day-old animals was 28±0.7 g (mean±SEM). The experiments were approved by the Animal Care and Use Committee

of the Institute of Physiology of the Academy of Sciences of the Czech Republic to be in agreement with the Animal Protection Law of the Czech Republic (fully compatible with European Community Council directives 86/609/EEC).

Surgical preparation and recording

Surgical implantation of the electrodes and cannula was done under light ether anaesthesia. Four openings (made by means of a razor blade) for flat silver epidural electrodes were placed symmetrically on the two hemispheres, two over the frontal sensorimotor cortex (AP=0; L=2 mm) and two over the occipital visual area (AP=3; L-2 mm). The indifferent electrode was placed into the nasal bone. The opening for the plastic cannula (7 mm long and 1 mm of outer diameter) was made beside the left frontal electrode. The whole assembly was fixed to the scull with a fast-curing dental acrylic (Duracrol® Dental, Prague).

The pups were allowed to rest on a heating pad for one hour after the surgery. They were fed with 5% glucose solution and simple neurological tests (righting and placing reflexes) were checked. After that, the rats were intraperitoneally injected either with lamotrigine (LTG - a gift from Glaxo Smith Kline - in the dose of 10 or 20 mg/kg; it was freshly dissolved in warm physiological saline and because it is a weak base it was acidified with two drops of 0.1 mmol HCl;) and/or with phenytoin (PHT - Sigma, St.Louis, MO – freshly dissolved in a tricomponent solvent; in doses of 15, 30 and/or 60 mg/kg). Control group for LTG received saline, controls for PHT were injected with solvent (propylene glycol, ethanol and water in a ratio of 3:2:5). The same volume (1ml/kg) of drug solution or solvent was always administered. This administration was done 30 minutes before the bicuculline methiodide (BMI, Sigma, St.Louis, MO) application. BMI (1 mmol solution) was also freshly prepared immediately before each experiment. A microsyringe was used to apply 2.5 µl of the BMI solution through the cannula on the sensorimotor cortical area. A total of 33 animals was used in LTG experiments (N=11 in each group), 35 rat pups in PHT experiments (N=8-9 in each group).

Control electrocorticographic recording was performed for 3 minutes before the BMI application. Then BMI was applied and electrocorticographic activity (in both reference and bipolar connections) was registered for 30 minutes. Motor phenomena (isolated jerks of contralateral forelimb accompanying interictal discharges, clonic

seizures of both forelimbs characteristic for ictal episodes) were marked directly into the recording during the whole registration period. No animal died during the registration period.

Evaluation of ECoG recordings

The interictal (negative spikes), and ictal phenomena were assessed in the ECoG recordings (Fig.1). Latency of the first focal interictal discharge, latency of the first generalised interictal discharge and number of interictal discharges in the interval between the 10th and 15th minute after BMI application (i.e. during stable focal activity – Soukupova et al., 1993) were evaluated. As ictal episodes were concerned latency and duration of the first ictal episode, incidence, number and mean duration of ictal episodes were evaluated. Statistical analysis (SigmaStat® SPSS) was performed using One Way Analysis of Variance (ANOVA) and ANOVA on Ranks with subsequent pairwise comparison by Dunn's method or Dunnett´s test. The incidence of ictal episodes was evaluated by means of Fisher Exact test. The level of statistical significance was set at 5 %.

Results

The first focal epileptic discharge appeared in the ECoG some tens of seconds to minutes after local application of bicuculline on the sensorimotor cortex. Its main part was a negative spike lasting 50 ms with amplitude markedly surpassing the background activity. Focal activity quickly projected to other cortical regions (generalized interictal discharges). The first motor correlate of focal interictal discharges was a jerk of a contralateral forepaw. As the interictal discharges progressed the jerks of both forelimbs were recorded. Transititon of the interictal discharges into seizure activity was regularly registered (Fig.1). The ECoG ictal activity was accompanied by clonic seizures of head and forelimb muscles. Seizures were repeated several times during the recording period (30 min).

Lamotrigine

Interictal discharges were not significantly changed by either dose of LTG (data not shown). The latencies of both the first focal and first generalized discharge as well as the number of interictal discharges between the 10th and 15th minute did not differ from

the controls. In contrast, the incidence of seizures decreased significantly after the 20-mg/kg dose of LTG. Number of seizures in rats exhbiting ictal activity was not influenced but the duration of seizures was significantly shortened by either dose of LTG (Fig.2). Motor phenomena accompanying interictal as well as ictal activity remained unchanged by LTG.

Phenytoin

Tricomponent solvent for PHT did not influence interictal activity and did not change the percentage of rats exhibiting seizures but some anticonvulsant effect was observed – it decreased the number and duration of ictal episodes (Fig.3). The difference from saline-pretreated controls for LTG was significant.

Comparison of the three PHT groups with solvent controls demonstrated a significant increase of latencies of the first focal and generalized interictal discharges after the 15-mg/kg dose but not after the higher doses. The number of interictal discharges was also not influenced by PHT (Fig.3).

A dose-dependent effect of PHT on the incidence of ictal activity was outlined but only the highest dose of PHT (60 mg/kg) was able to suppress transition of interictal into ictal activity significantly – six out of eight rat pups were protected. Latency of ictal episodes was not influenced by any dose of PHT whereas number of seizures was decreased by the highest dose and duration of seizures by the 30-mg/kg dose (Fig.4). Difference between controls and rats with the highest dose of PHT stayed just below the level of significance ($p=0.062$). Again, isolated jerks as well as clonic seizures (accompanying interictal and ictal discharges, respectively) were not influenced by the anticonvulsant drug.

Discussion

Our study demonstrated a failure of the used doses of LTG and PHT to prevent generation of an epileptic focus by local application of bicuculline methiodide on the sensorimotor cortical area. On the other hand transition of interictal into ictal activity was suppressed by high doses of both LTG and PHT. In addition, LTG was able to shorten persistent seizures.

Local application of bicuculline methiodide induced an epileptic focus in all animals. Previously we demonstrated that this competitive antagonist at GABA-A receptors is able to elicit epileptic focus in somatosensory cortex even in 5- and 7-day-old rat pups (Velíšková et al. 1991), that means that the GABA-A receptors in the rat sensorimotor neocortex function as inhibitory at the end of the first postnatal week. It is in agreement with data on development of brain GABAergic system (Coyle and Enna 1976) as well as with a change from depolarizing to hyperpolarizing action of GABA in hippocampus (Cherubini et al. 1990, Khalilov et al. 1999). It allows to use this model even at very early stages of postnatal development (Velíšková et al. 1991, Soukupová et al. 1993).

The two drugs studied in present experiments have the same basic mechanism of action – use-dependent blockade of voltage-gated sodium channels. Either drug has also other mechanisms of action much better known in the case of phenytoin (de Lorenzo and Sun 2002) than LTG (Leach et al. 2002). The similarity of the main mechanism of action can explain the basic similarity of effects on neocortical foci in our experiments. Unfortunately, there are nearly no data on pharmacology of LTG and PHT in immature animals. Our previous study demonstrated basic similarity of the action of the two drugs also against generalized tonic-clonic seizures induced by pentetrazol – they specifically suppressed the tonic phase of these generalized seizures (Stanková et al. 1992). There is a difference in unwanted effects of the two drugs in developing animals: prenatal PHT administration induces sex-related changes in motor behavior (Mullenix et al. 1983), single injection of PHT in the dose of 60 mg/kg compromises motor performance in 12-day-old rats (Mares et al. – data on file). Repeated postnatal injections of LTG do not significantly change motor behavior of developing rats (Mikulecka et al. 2004). Marked difference was also described in induction of cell death – PHT in daily doses of 35 mg/kg induced cell death in postnatal dentate gyrus and cerebellum (Ohmori et al. 1999, Ogura et al. 2002) whereas a huge dose of LTG (100 mg/kg) had to be used to elicit neuronal degeneration (Katz et al. 2007).

Both drugs affected generalization of epileptic activity from the focus but not focal interictal activity and its spread over the cortex. The effect of LTG was exhibited at lower

dose than that of PHT and it does not exhibit neurotoxic action in the doses active in present experiments.

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Text to Figures

1. Electrocorticographic recording from a 12-day-old rat 17 min after application of bicuculline methiodide on the right frontal (sensorimotor) cortical area. Interictal discharges in the right frontal area are not constantly projected to the left frontal region, ictal activity is clearly seen in both frontal regions. From top to bottom: RF – right frontal; LF – left frontal; RO – right occipital (visual); LO – left occipital cortical area always in reference connection. A downward deflection means negativity of cortical electrode.
2. Effects of lamotrigine on ictal activity. From top to bottom: incidence, number (mean + S.E.M.) and duration (mean + S.E.M.) of ictal episodes. Abscissa: doses of lamotrigine, C means controls; ordinates from top to bottom: percentage of rats exhibiting seizures, number of seizures and duration in seconds. Asterisks denote significant difference in comparison with controls.
3. Effects of phenytoin on latencies of the first focal (upper graph) and the first generalized (lower graph) interictal discharge (mean + S.E.M.). Abscissa: doses of phenytoin, S denotes controls injected with tricomponent solvent; ordinates: latencies in seconds. Asterisks denote significant difference in comparison with controls.
4. Effects of phenytoin on ictal activity. Details as in Figs. 2 and 3.

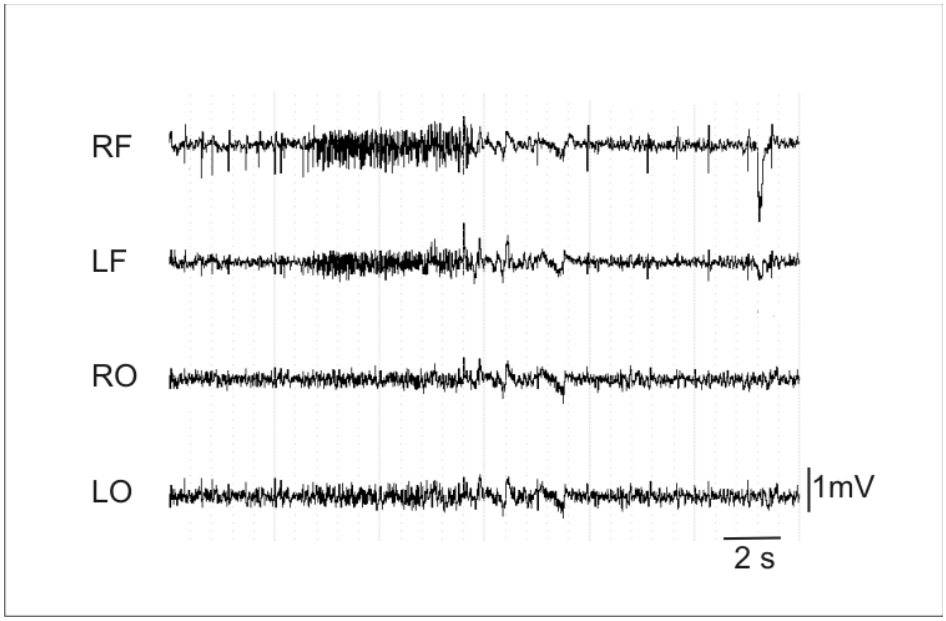

Fig. 1

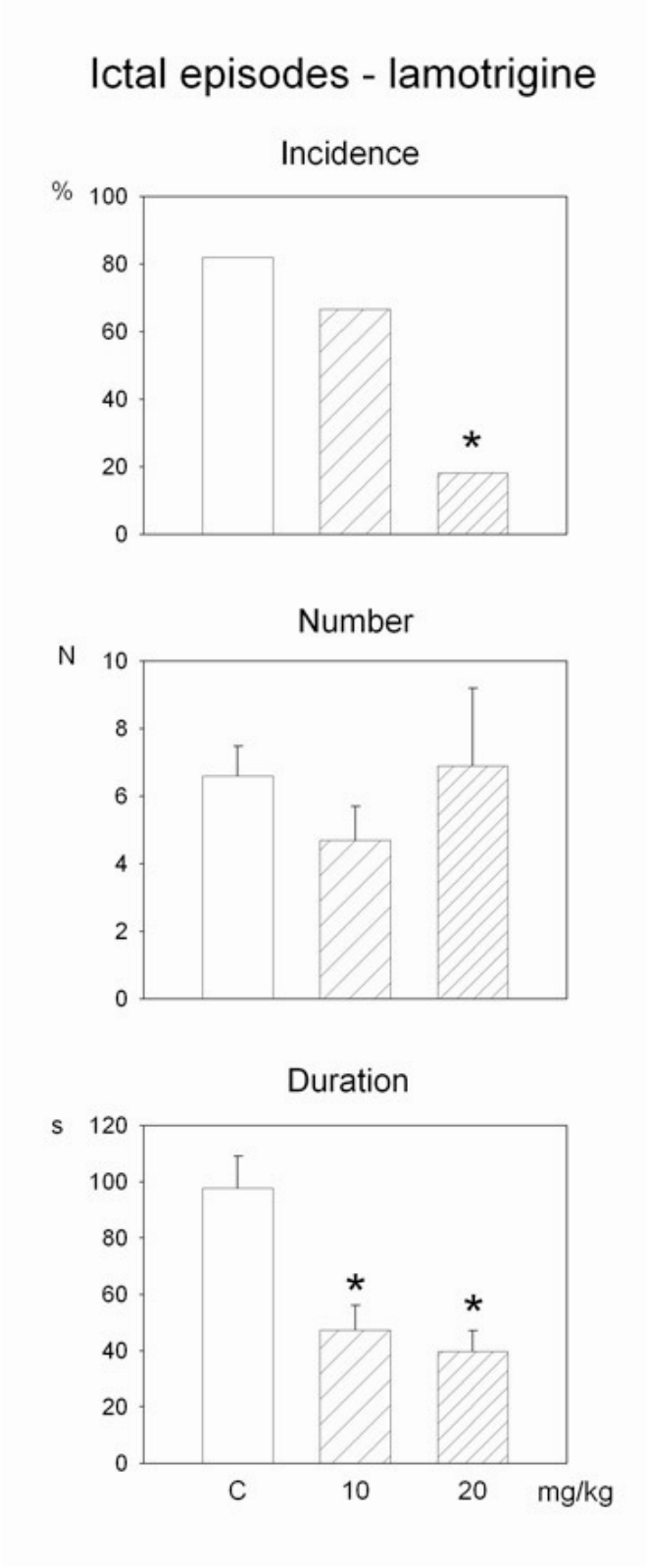
Ictal episodes - lamotrigine
Incidence
%
100
80
60
40
20
0
*
Number
N
10
8
6
4
2
0
Duration
s
120
100
80
60
40
20
0
*
*
C
10
20
mg/kg

Fig. 2

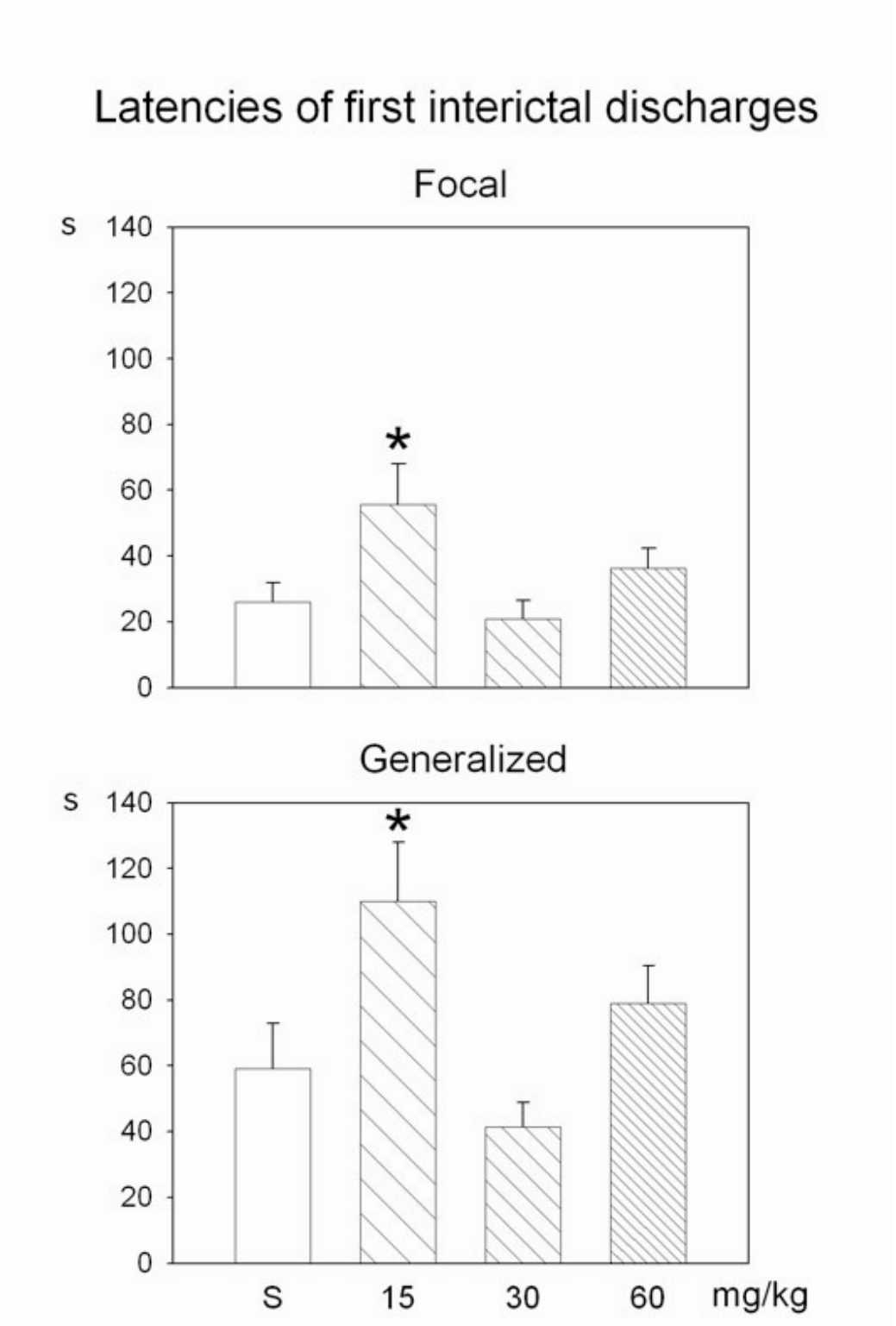
Latencies of first interictal discharges
Focal
s
140
120
100
80
60
40
20
0
*
Generalized
s
140
120
100
80
60
40
20
0
*
S
15
30
60
mg/kg

Fig. 3

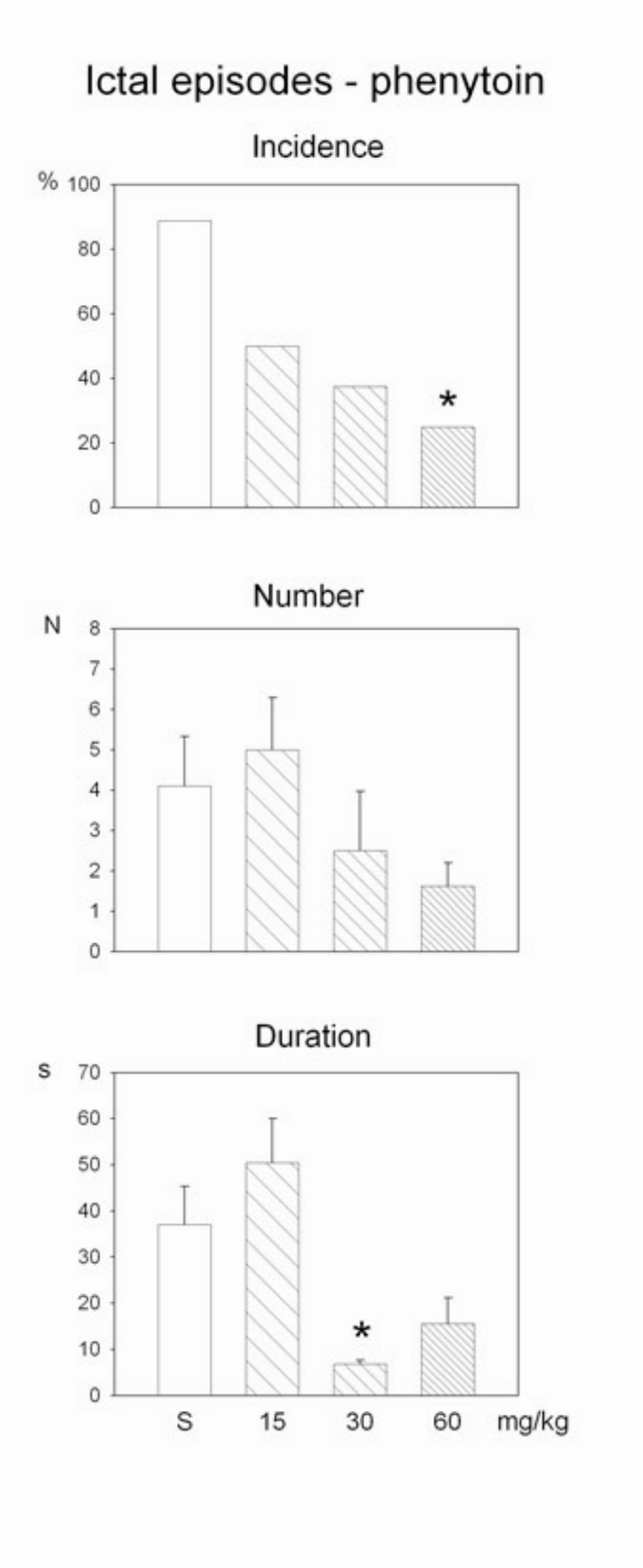
Ictal episodes - phenytoin
Incidence
%
100
80
60
40
20
0
*
Number
N
8
7
6
5
4
3
2
1
0
Duration
s
70
60
50
40
30
20
10
0
*
S
15
30
60
mg/kg

Fig. 4