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Antinociceptive effect of phenytoin in rats

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Abstract:
Phenytoin is an anticonvulsant agent of the first-generation that blocks voltage-gated Na^+-channels. Systemic administration of phenytoin induces anticonvulsant effect in humans and in experimental animals. Moreover, it was demonstrated that this drug also inhibited neuropathic and post-stroke pain.
The present study was undertaken in order to determine the effect of a direct phenytoin administration into the lateral brain ventricle (*icv*) on pain perception in rats exposed to noxious thermal stimuli and to compare its probable effect with recently reported antinociceptive effect of lidocaine, another sodium channel blocker. Moreover, the effect of intraperitoneally (*ip*) injected phenytoin on pain perception was checked. A transient antinociceptive effect of phenytoin applied *icv* at doses of 0.13 and 0.65 μmol and no effect of phenytoin injected *ip* was observed. Antinociceptive effect of phenytoin was confirmed but it was less pronounced in comparison with similar activity of lidocaine. The obtained results also indicate that a single *icv* dose of phenytoin is less effective in inducing analgesia in the model of thermal pain in comparison with its effect in neuropathic pain reported in several papers.
In conclusion, phenytoin is the drug of lesser importance in the study of the mechanism of the thermal pain perception in the brain.

Key words:
pain, phenytoin, intracerebroventricular administration, rat

Abbreviations: *icv* – intraventricular (into the lateral brain ventricle), *ip* –intraperitoneal, *iv* –intravenous

Introduction

Phenytoin is a classical anticonvulsant drug used in the therapy of epilepsy [3, 6, 19, 36, 37]. It increases the threshold for focal seizures in amygdala-kindled rats [17, 20] or for electroconvulsions in mice [14]. Phenytoin blocks voltage-sensitive Na^+ channels and in this way inhibits neural firing in the brain [5, 19] and seizure activity [19]. The study performed on isolated small cells from adult rat dorsal ganglia showed that two antiepileptics phenytoin and carbamazepine inhibited tetrodotoxin-sensitive Na^+ currents [21]. It was also shown that phenytoin acted on other types of ion channels, which resulted in anticonvulsant activity. Moreover, it appears to evoke other pharmacological effects [37] among them antinociceptive and neuroprotective effect [37]. Antinociceptive effect of phenytoin in neuropathic pain [1, 12, 15, 16, 31], and in post-stroke pain was demonstrated [8].

However, there are no reports on antinociceptive effect of phenytoin applied directly into the lateral brain ventricle in rats exposed to the thermal nociceptive stimulus. *Ip* injection of phenytoin at the dose up to 40 mg/kg was without any antinociceptive effect determined by the formalin test in rats [2]. Subcutaneous (*sc*) injection of this drug in the range between

1–100 mg was ineffective in exerting antinociceptive effect in the test of acute and chronic pain in rats [10]. It was proved by Borowicz et al. that a single phenytoin injection at the dose of 9.5 mg/kg, *ip* resulted in detectable constant phenytoin levels in plasma and brain tissue of rats [12]. Moreover, Scholz et al. reported that the frequency of painful sensations in epileptic seizures varies from 0.3 to 2.8%. They reported a casual evidence of an effective treatment with anticonvulsants of short-lasting painful attacks in the right arm [26]. They considered this type of central pain as a manifestation of partial epileptic seizures [26]. Teriakidis et al. demonstrated inhibition by phenytoin and other anticonvulsants of antidromic hippocampal action potentials induced by electrical stimulation [33].

On the other hand, it was demonstrated that a local anesthetic, lidocaine, commonly used in clinical practice as a local anesthetic drug or as antiarrythmic drug blocked voltage-gated sodium channels [9, 13, 30] and to a lesser degree K^+ and Ca^{2+} channels [35]. Lidocaine also acts on sodium channels in neurons of the central nervous system [4]. Intravenous administration of this anesthetic inhibited different kinds of pain in patients [28, 29]. Experimental studies revealed that *ip* lidocaine injection was without any effect on mechanical or cold allodynia in the model of neuropathic pain in rats [7], while its intracerebroventricular administration exerted significant antinociceptive effect in rats determined in two tests recording pain induced by noxious thermal stimuli [22].

The present study was undertaken in order to determine the effect of a direct phenytoin administration into the lateral brain ventricle (*icv*) on pain perception in rats exposed to noxious thermal stimuli, to compare its probable effect with recently reported effect of lidocaine [22], and to test usefulness of phenytoin, as a sodium-channel blocker in the brain of rats.

Materials and Methods

The protocol of this study was approved by the Ethics Committee of the Medical University of Silesia.

Animals

The study was performed on adult (280–320 g) male Wistar rats, obtained from Animal Farm of the Medical University of Silesia. Animals were kept under 12 h light: 12 h dark cycle with free access to standard food and water.

Experimental protocol

A week before experiments, polyethylene cannulas (TOMEL, Tomaszów Mazowiecki, Poland) were implanted into the lateral brain ventricle (*icv*) of anesthetized rats using the same method as in our previous papers [22–24]. Briefly, animals were anesthetized with chloral hydrate (POCH, Gliwice, Poland) injection (300 mg/kg, *ip*). The skin of the skull was cut and skull bones were uncovered. Polyethylene cannulas were implanted *icv* using the following coordinates: the depth of 4 mm from the surface of the skull, 2 mm to the right from the sagittal suture, and 2 mm behind the coronary suture. Cannulas were attached to the skull bones with dental cement (Duracryl, Spofa Dental, Czech Republic).

On the day of experiment, every dose of phenytoin (Warszawskie Zakłady Farmaceutyczne Polfa, Poland) dissolved in a volume of 5 µl of 0.9% NaCl was administered *icv* using a Hamilton microsyringe. Antinociceptive effect was determined by two methods: by the method of the hot-plate test [18] using HP-41 apparatus (COTM, Białystok, Poland) and the tail immersion test [11]. In both tests, the latency time of rats' reaction to nociceptive stimuli was recorded before and at the following time intervals: 5, 15, 30, 45, 60, 90, 120 min and 24 h after phenytoin administration. The determined latency time of each animal in the hot-plate test was converted to the coefficient: percent of analgesia according to the formula:

$$\text{\% of analgesia (\% of maximal antinociceptive effect)} = \frac{T_x - T_o}{20 - T_o} \times 100$$

Latencies determined in the tail immersion test were converted to percent of analgesia according to the formula:

$$\text{\% of analgesia (\% of maximal antinociceptive effect)} = \frac{T_x - T_o}{10 - T_o} \times 100$$

Where:

T_x – individual latency time determined at time intervals after phenytoin administration;

T_o – individual latency time determined before phenytoin administration;

20 – maximal latency time in the hot-plate test (in s);

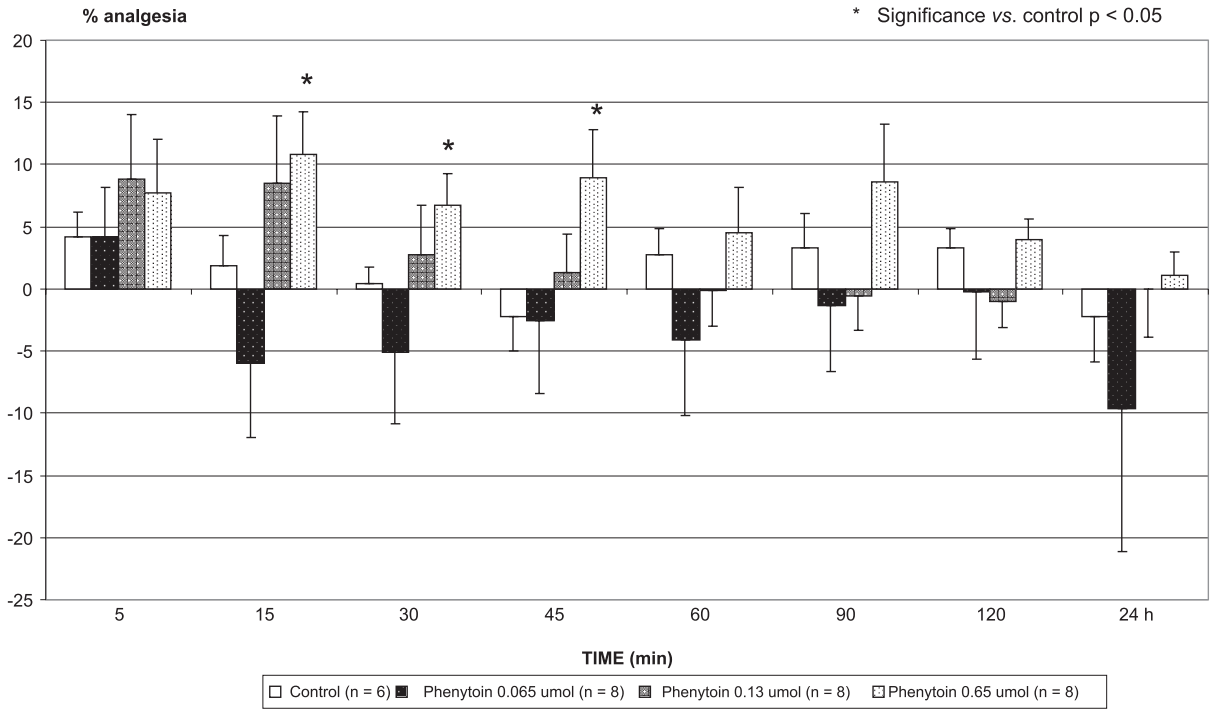

Fig. 1. Antinociceptive effect of phenytoin in the hot-plate test

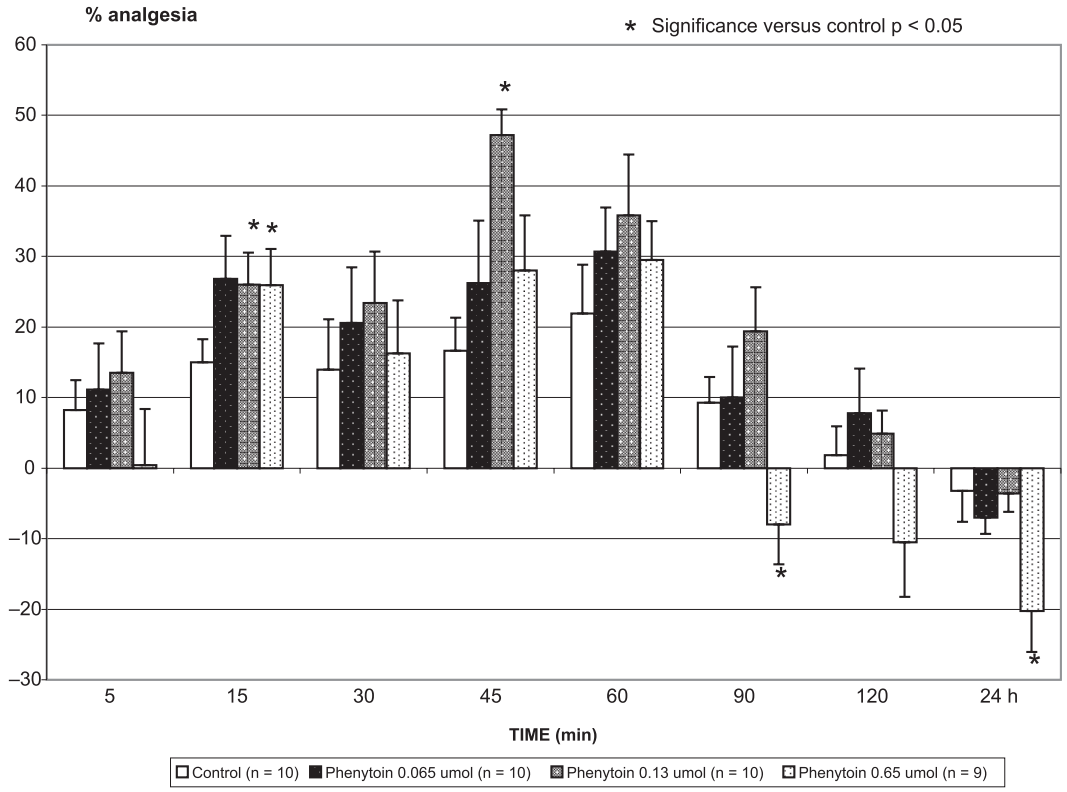

Fig. 2. Antinociceptive effect of phenytoin in the tail immersion test

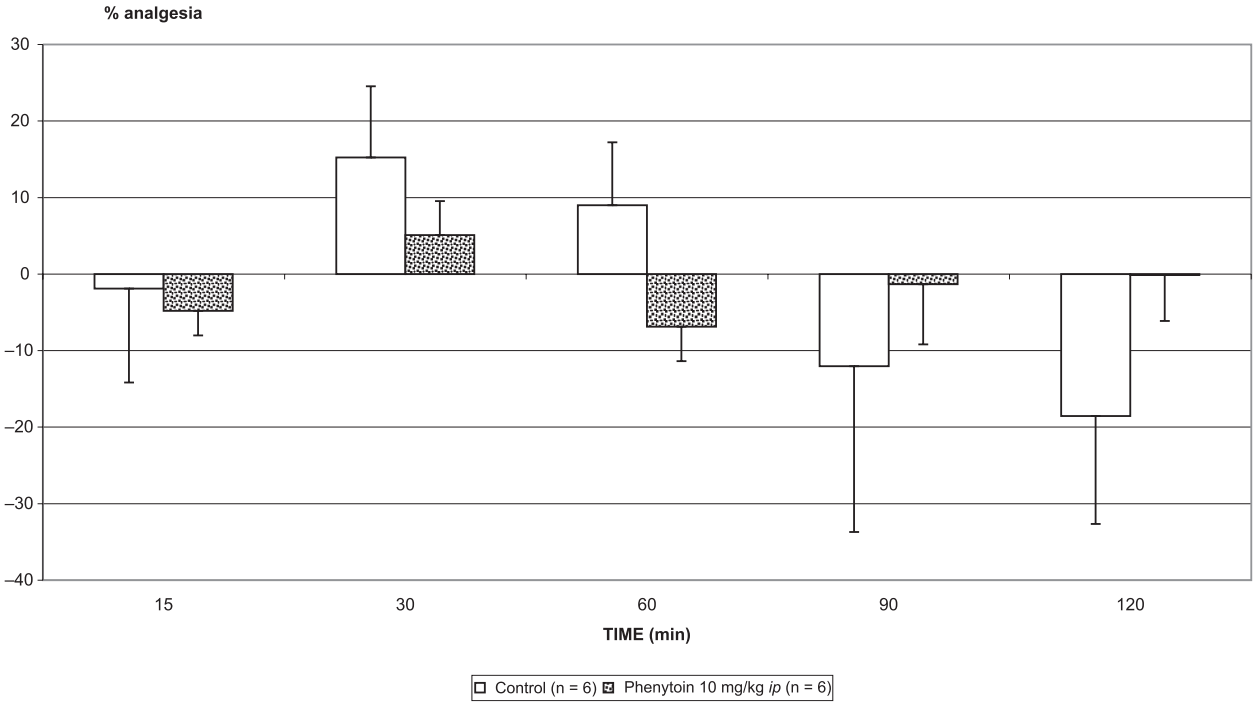

Fig. 3. Effect of phenytoin administered intraperitoneally in the tail immersion test

10 – maximal latency time in the tail immersion test (in s).

At the end of the experiment, the rats were sacrificed by chloral hydrate overdosing (900 mg/kg, *ip)* and placement of the tips of the cannulas was controlled by *icv* injection of Indian ink solution and visual inspection of the lateral brain ventricle.

An additional group of rats was used to study antinociceptive effect of *ip* phenytoin injection at a dose of 10 mg/kg in the tail immersion test. The latency time of rats' reaction was recorded at the following time intervals: before and 15, 30, 60, 90 and 120 min after the administration.

Data were subjected to ANOVA and the post-ANOVA Dunnett test [32]. All these experiments were performed in accordance with guidelines for investigations of experimental pain in conscious animals [38].

Results

Phenytoin injected *icv* at the dose of 0.65 μmol (178.1 μg) induced significant antinociceptive effect determined by means of the hot-plate test at 15, 30 and 45 min after its administration (Fig. 1). The lower applied doses of 0.065 μmol (17.8 μg) and 0.13 μmol (35.6 μg) were without any significant effect (Fig. 1). Similar antinociceptive effect of *icv* injected phenytoin was determined by the tail immersion test. The dose of 0.13 μmol induced significant effect only 15 and 45 min after administration (Fig. 2). Higher dose of phenytoin of 0.65 μmol revealed antinociceptive effect only at 15 min after injection (Fig. 2). Surprisingly, the highest dose of phenytoin of 0.65 μmol *icv* induced in rats significant hyperalgesia at the last time intervals of the experiment (Fig. 2). The lower phenytoin dose of 0.065 μmol was without any effect (Fig. 2). *Ip* administration of phenytoin at the dose of 10 mg/kg did not induce any significant antinociceptive effect as determined by a tail immersion test (Fig. 3).

Discussion

The results presented here indicate that *icv* injection of phenytoin induced a significant but transient antinociceptive effect in rats. This effect was not accidental as it was detected by two methods used for the

determination of antinociceptive effect: the hot-plate and tail immersion test. *Icv* administration of this drug allows to confirm that it exerts antinociceptive effect mainly at the level of the brain. *Icv* phenytoin administration allows also for the exact definition of the applied dose. The significant antinociceptive effect of this drug was observed at the *icv* dose of 0.13 and 0.65 μmol determined by the tail immersion test (Fig. 1), and only at the dose of 0.65 μmol in the hot-plate test (Fig. 2). It means that the effect of phenytoin was lesser in comparison with recently reported effect of lidocaine, which induced significant effect in the whole range of *icv* applied doses from 0.065 to 1.3 μmol [22]. Moreover, ip phenytoin administration at the dose of 10 mg/kg was without any significant antinociceptive effect in spite of its good penetration into the brain in rats [3].

Sakue et al. demonstrated that several sodium channel blockers, including phenytoin applied *sc*, exerted antinociceptive effect against acute thermal stimulation in mice by elevation of the threshold for thermal nociception determined by the plantar test. They observed short-lasting, significant effect of the highest used dose of phenytoin of 100 μmol/kg, *sc* (25.2 mg/kg) and transient effect of its dose of 30 μmol/kg, *sc* (7.5 mg/kg) only at one time interval [25]. They attributed this effect to local anesthetic action [25].

It was proved by Todorovic et al. that phenytoin applied peripherally into the rat paw induced a dose-dependent analgesia [34].

On the other hand, it was found that phenytoin did not exert any analgesic effect in the formalin test in rats [27]. On the contrary, the effect of peripherally applied phenytoin in neuropathic pain in humans and in experimental models is well documented [1, 12, 15, 16, 31].

It is possible to compare the antinociceptive effect of both these drugs, sodium channel blockers: phenytoin and lidocaine despite that the effect of lidocaine was determined in another experiment [22]. Antinociceptive effect of both these drugs was estimated in the same experimental model, i.e. on rats of the same strain, by the same methods of determination of antinociceptive effect and the same way of administration (*icv*) of equimolar doses of these drugs were used.

Systemic administration of phenytoin induces central biological effects mainly anticonvulsant effect in humans and experimental animals [3, 6, 19, 36].

At present it is not possible to explain the mechanism of paradoxical hyperalgesia induced by the highest dose of phenytoin of 0.65 μmol *icv* in the last time intervals of the experiment (Fig. 2). It seems that it was due to an increased sensitivity of rats' tail nociceptors during the experiment when central inhibitory/antinociceptive effect of phenytoin was diminished. Further study is necessary to determine the mechanism of this interesting phenomenon.

Thus, presently observed antinociceptive effect of phenytoin applied *icv* supports conclusion that this drug inhibits perception of the thermal nociceptive stimuli in the central nervous system, but to much lesser degree than lidocaine. The stronger antinociceptive effect of lidocaine perhaps was due to involvement of other additional mechanisms, like blocking also K^+ and Ca^{2+} channels.

Conclusions

1. The results of the present study confirmed previous reports of analgesic effect of phenytoin.
2. A significant but transient antinociceptive effect of the single intracerebral dose of phenytoin, a sodium channel blocker, indicates that this drug is less important in the study of the mechanism of the thermal pain perception in the brain.

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