PENICILLIN EPILEPSY IN RATS

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The purpose of this study is to test, in a model of experimental penicillin epilepsy, a hypothesis of primary and dominant role of cortex in genesis of epileptic discharges, by using recordings of electrocorticograms (EcoG). The experiments were made with around two and a half old male rats. The rats were divided into four groups of five animals each. In the case of the first group, antibiotics were applied intraperitoneally (i.p.). Epileptic generalized discharges of this group were not registered until administering a dosage of antibiotics higher than 1.8 x 10^6 IU/kg. In the second group, application of a dosage of 1.2 x 10^6 IU/kg resulted in individual discharges. In the third group, antibiotics were applied intracortically (i.c.) in a dosage of 50 IU and caused individual discharges without big seizures. The fourth group needed a dosage of 100 IU/kg of penicillin applied i.c. to develop a big seizure. Discharges in the first three groups were registered first as unilateral with a subsequent development of bilateral synchrony. In the case of the fourth group, immediately and abruptly after the application of penicillin big seizures developed violently and repeated several times. We consider that the very implantation of electrodes provokes occurrence of discharging. *Acta Medica Medianae 2004; 43 (4): 19–23.*

***Key words**: experimental epilepsy, penicillin, rats*

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Introduction

Epileptogenic effect of penicillin has been known as far back as 1945 when Walker and Johnson (1) announced occurrence of epileptiform activity in the case of application of penicillin to the cortex of the cat, dog and monkey. From among the models of experimental epilepsy, the one with penicillin is the most frequently used.

According to Edmonds (2), advantages of that model are the following:

1. Penicillin causes focal seizures in vertebrate ranging widely from fish to the man.

2. Seizure inducing is rapid and easily registered. The activity begins in the initial 15 minutes after the application and continues for a number of hours that follow.

3. Pathomorphological changes after the local application of penicillin are rarely found.

4. The speed of activity spreading from the focus and intensity of clinical manifestation of the seizure are directly dependent on the dosage of penicillin administered.

5. Penicillin induced seizures are not resistant to anticonvulsants.

6. The induced epileptiform activity completely disappears 24 hours after the application of penicillin.

Ways of the penicillin application are diverse. Depending on the model one wants to get the administration of penicillin can be intraperitoneal (i.p.), intramuscular (i.m.), intravenous (i.v.) or intracortical (i.c.).

In addition to penicillin, aluminium compounds are most frequently used as epileptogenic agents for provoking experimental epilepsies. Insoluble compounds, such as aluminium hydroxide or soluble salts of other metals such as lead, mercury, cobalt, zinc, bismuth and copper can also be applied. We wanted to compare in this study two ways of applying penicillin in a model of penicillin-induced epilepsy in rats. In the first way, the i.p. application is used and that model is known in literature as the multifocal one (centrencephalic). In the second way, the i.c. application is used and that model is predominantly known as cortical. We set the following direct targets:

1. To register electrocorticogram (EcoG) of bio-electric activity of the rats' brain before and after i.p. or i.c. penicillin application.

2. To determine penicillin dosages necessary for development of a big seizure (grand mal), separately for i. p. and i. c. applications respectively.

Methods

Experiments were performed on rats Wistar, 2–3 months old, male, body weight of 200–250 grams. Rats were awake during the experiment. By means

of a stereotactic device experimental animals had permanent monopolar electrodes in different brain structures implanted. We placed electrodes made of stainless steel in the form of a screw in apertures 1.5 mm in diameter that had been made by a dentist's drill in the skull bones. Four electrodes were used, one in the middle occipitally, one left and right each parietally and one on the nose bone (indifferent electrode). In the case of a group of animals we made an additional opening in the parietal area for implantation of a cannula through which the i. c. application of penicillin was made. Registration of bioelectric activity was carried out 4–5 days after the implantation of electrodes. An EEG apparatus of RIZ-Zagreb made recorded activity. During the registration, the animal was awake and freely mobile. We applied crystal potassium salt of penicillin G, produced by ICN Galenika. In i.c. application, penicillin was injected with the help of a Hamilton syringe, which was inserted by means of a thin cannula to the cortex so that the tip of the needle was at the level of the cortex surface. We did not calculate the dosage of penicillin per kilogram of body weight in i.c. applications. We made exclusively visual analysis of the EcoG recording. The total of 20 rats was divided into two groups of ten animals each. Penicillin was applied i.p in one group and i.c. in the other group respectively.

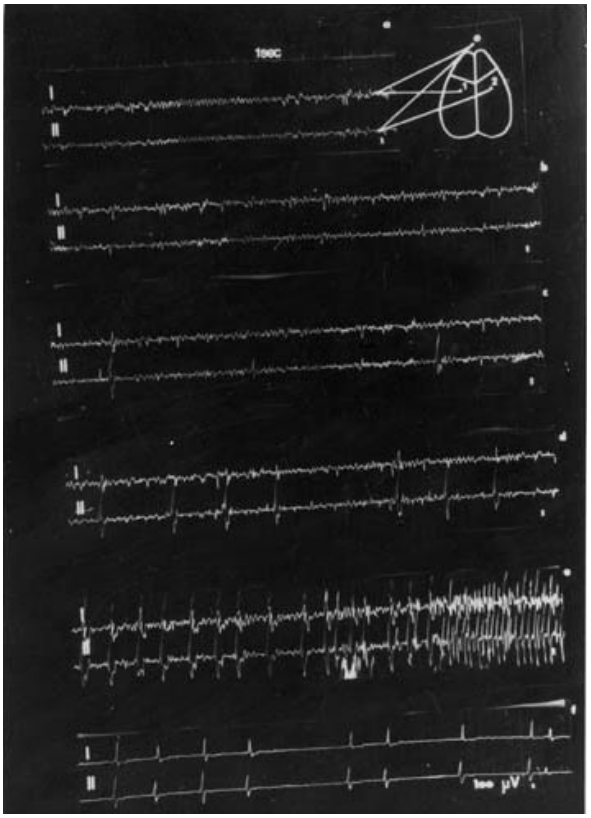

Figure 1. Two drainages were recorded – the left and right parietal ones. A. Control. B. Ten minutes after administering 2 x 10^6IU/kg of penicillin i.p. C. Twelve minutes after administering penicillin. One larger discharge on the parietal right. D. Discharges become more frequent. E. Seventeen minutes after application of penicillin a big seizure begins. F. The animal recovers an hour and a half after administration of penicillin

Results

Already in preliminary experiments we noticed that big doses of penicillin are necessary for development of epileptic event in the case of i.p. application. In 5 rats that received more than 1. 800.000 international units per kilogram (IU/kg) (Figure 1) big seizures developed. Otherwise, the range of dosages was from 1.8 to 2.5 million per kilogram. After a latent period that lasted 6–28 minutes and in the course of which the bioelectric activity was totally normal, epileptic discharges took place registered on the EcoG recordings in the form of spikes. Discharges always began unilaterally. The frequency and amplitude of spikes gradually increased. Following 5–10 minutes of unilateral discharges spikes began to appear also on the opposite hemisphere but completely asynchronously at the onset. After the next few minutes, gradual bilateral synchrony occurred, accompanied with further rise in the amplitude and frequency of discharge. Discharges were accompanied with a mild mioclonus affecting neck muscles and manifesting itself in the form of even jerks of the head and bristling of hair. Breathing became heavier and even cyanosis appeared from time to time. In that phase, animals are lethargic, cataleptic, with marked reduction in vigilance. Animals react to audiogenic stress (strong clap of hands, etc.) by a quick jerk of the whole body. Following 20–40 minutes, a big seizure develops, being manifested as a series of discharges of big amplitude (more than 500 microV) and high frequency (2–8/s). Discharges that make a big seizure begin synchronously in both hemispheres and spread over the cerebellum. Simultaneously with discharges motor phenomena occur in the form of intensive convulsions that affect especially extremities so that it happens that the animal is sometimes thrown out of the cage it is situated in. A big seizure lasts for 15–50 seconds and ceases suddenly. After the end of the big seizure EcoG records electric silence lasting for 8–16 seconds and representing the sign of the maximum desynchrony of bioelectric activity. The cause of the seizure arrest is considered to be, in addition to exhaustion and other factors, electrogenic pump inhibition (5). Gradually, bilateral synchronous discharges resume. After 15 to 20 minutes a new seizure occurs, which can be either stronger or weaker than the preceding one. Such seizures repeat several times in the course of 2 to 3 hours and then stop. Only individual discharges remain that are less frequent and accompanied with gradual reduction in amplitude. The animal recovers.

Five rats received less than 1.800.000 IU/kg of penicillin (the range of dosages in this group was from 1.200.000 to 1.700.000 IU/kg). This group, like the previous one, had a gradual development of discharges that appeared unilaterally and then were followed by bilateral synchrony. However, due to the insufficient dosage of penicillin, this group did not have a big seizure developed.

In 5 rats that received 40–50 IU of penicillin discharges took place immediately on the same side

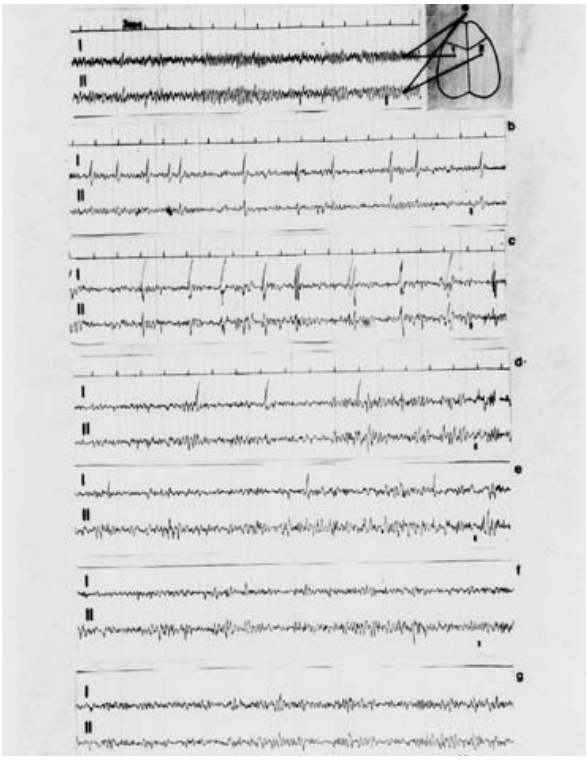

Figure 2. A. Control. B. Five minutes after 40 IU of penicillin had been given i.c. Discharges on the parietal left occur. C. Twenty minutes after penicillin was administered. Discharges are somewhat more frequent and have bigger amplitudes. The right parietal side is calm. D, E and F. Thirty minutes up to an hour since penicillin was administered, discharges gradually disappear and the animal recovers completely. A big seizure did not develop

and then soon, after 2–4 minutes, bilateral synchrony followed, accompanied with microclonus (Figures 2 and 3). In the case of this group other signs of epileptic activity were not recorded. The described results are presented summarily in Table 1.

To induce epilepsy in the case of the i.c. application, very small doses of penicillin are sufficient. In 5 rats that received 90–100 IU of penicillin development of extremely violent big seizure immediately followed (Figure 4). Seizures were repeating one after another and after 36 hours seizures and epileptic discharges ceased and animals recovered.

Discussion

In our experiments, both in the case of the i.p. and i.c. applications, discharges always occurred first unilaterally but as a secondary effect bilateral synchrony followed. This mechanism covers cortico-subcortico-cortical projection systems. The cortical spike serves

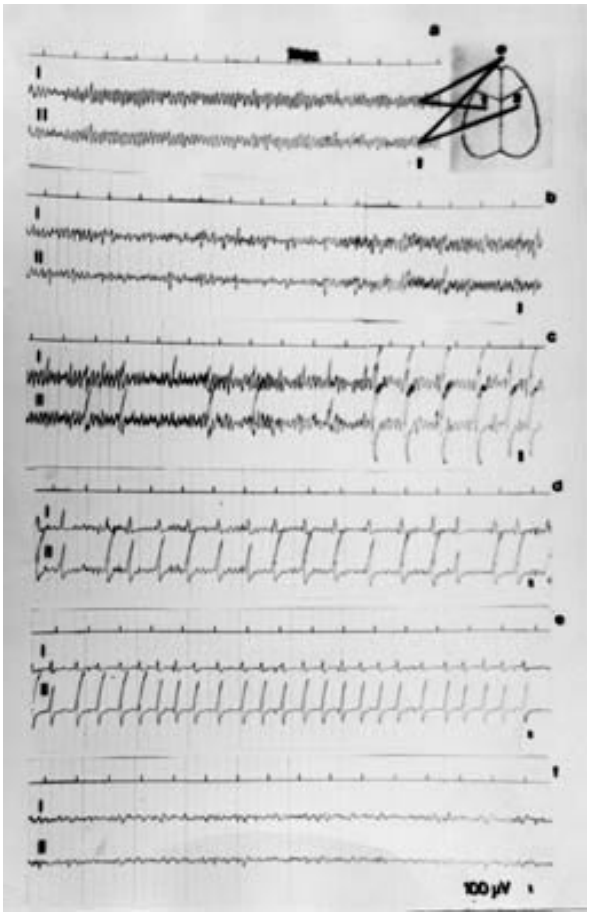

Figure 3. A. Control. B. A minute after 60 IU/kg of penicillin was administered i.c. C. Two minutes after the penicillin was administered. Synchronous bilateral discharges of a large amplitude begin. D. Five minutes after penicillin was administered. E. Ten minutes after penicillin was administered. Bilateral synchronous discharges continue. Much bigger right parietal amplitude is marked. F. Sixteen minutes after the administration and discharges the animal recovers. A big seizure did not take place due to a small dosage of antibiotics

as a trigger that activates through corticofugal pathways the centrally situated neurone network which then discharges paroxysmally and encompasses both hemispheres by way of bilateral projection pathways (6). Mares (7) applied locally small quantities of Na penicillin to dura mater in the occipital, temporal and frontal regions of the rat, which led to discharge after 1.5 – 2.5 minutes. Projected discharges occurred simultaneously in the symmetrical region of the contra lateral hemisphere. While some authors (8,9,10) are of the opinion that corpus callosum plays a major role in the contra lateral projection of the focus, Ottino (11) considers that deeper inter-hemisphere connections are also involved, such as thalamus and mesencephalon. Findings similar to ours were published by Chen (12).

Table 1

Mode of application	Dosage	Effects
i. p. application	Doses 1.2–1.7x10^6IU/kg	Development of individual discharges
	Doses 1.8–2.5x10^6IU/kg	Development of big seizure
i. c. application	Doses 40–50 IU	Development of individual discharges
	Doses 90–100 IU	Development of a big seizure

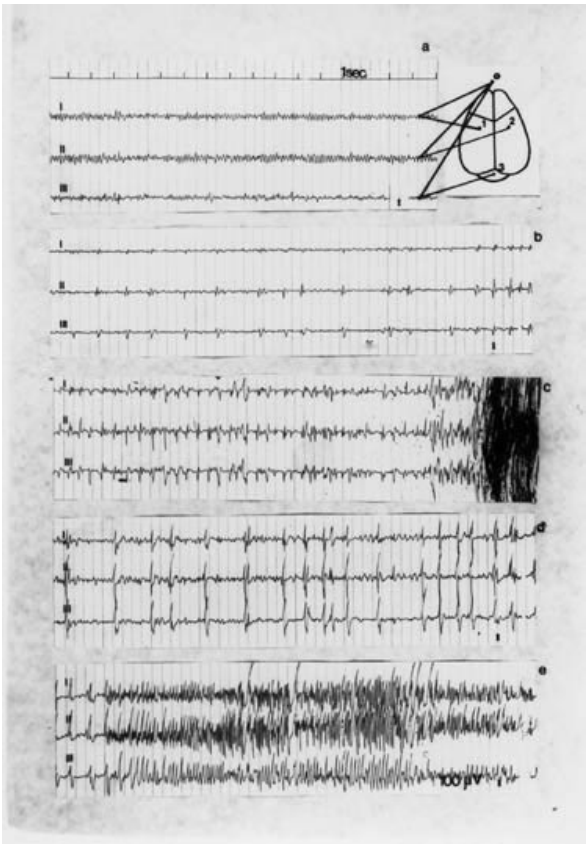

Figure 4. Three drainages were recorded – the left and right parietal ones and an occipital one. A. Control. B. and C. The onset of a big seizure 15 seconds after the i.c. administration of 100 IU penicillin. Both parietal and the occipital cortices were affected. D and E. The onset of a new, somewhat weaker, big seizure 5 minutes after the application of penicillin

With i.p. penicillin doses of 2.5 – 5 million IU/kg this researcher developed in rats first individual spikes and than a big seizure followed. In relation to our results the difference is in the time of the occurrence of epileptic activities. Thus, individual spikes appeared only after 45.7 minutes (mean value) while the big seizure occurred after 71.5 minutes. In these experiments, dosages that necessarily provoke a big seizure were used. Our experiments confirmed results (13,14) according to which very big doses of penicillin are necessary in the model with the i.p. application. We consider that in the case of the i.p. application a gradual resorption of penicillin into the circulation takes place in the course of the latent period. Necessary big doses ensure that sufficient quantity of penicillin reaches the cortex. The general excitability of the cortex increases under the influence of penicillin. This increase can be asymmetrical explaining thereby unilateral appearance of first discharges. The very process of the electrodes implantation is also likely to create the locus minoris of resistance that reduces considerably the threshold of cortex stimulation. In the i.c. application we had effects with dosages that were smaller than those mentioned by other authors (2,15). In order to check that a possible damage to the surface of cortex by a mechanical effect of the needle point in the i.c. application is irrelevant, we applied the physiological solution in the same way. On that occasion no abnormal bioelectrical activity occurred. We are of the opinion that extremely small doses of penicillin are efficient in the i.c. application because the very technique of application directly into the cortex makes it possible to provide sufficient quantities of antibiotic that form a focus of strong excitation (16).

Conclusion

Multifocal epileptic model with secondary generalization of tonic-clonic convulsions developing after the i.p. or i.c. penicillin application has its primary origin in the cerebral cortex. Discharges always begin unilaterally, with subsequent development of the secondary bilateral synchrony. Dosages necessary for provoking a big seizure or individual discharges depend to the largest extent on the mode of antibiotics application. In the i.c. application, doses of around 50 for individual discharges and around 100 IU for development of a big seizure are sufficient. Doses of 1.2 million IU/kg are necessary in the i.p. application in order to have individual discharges developed. Only application of a dose higher than 1.8 million IU/kg leads to the occurrence of a big seizure.

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PENICILINSKA EPILEPSIJA KOD PACOVA

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Cilj ovog ispitivanja jeste testirati, na modelu eksperimentalne epilepsije, hipotezu o dominantnoj ulozi kore u genezi epileptičnih poremećaja, koristeći mogućnost registrovanja pomoću elektrokortigrama. Eksperimenti su rađeni na pacovima, muškog pola. Životinje su bile podeljene u četiri grupe, gde se u svakoj grupi nalazilo po 5 životinja. U prvoj grupi, antibiotici su davani intraperitonealno. Epileptični poremećaji životinja u ovoj grupi su detektovani kada je dat antibiotik u koncentraciji većoj od 1.8 x 10^6 IU/kg. U drugoj grupi, aplikacija doze od 1.2 x 10^6 IU/kg dovela je do pojedinačnih poremećaja. U trećoj grupi, antibiotici su aplikovani intrakortikalno u dozi od 50 IU/kg i doveli do pojedinačnih poremećaja. U četvrtoj grupi doza penicilina od 100 UI/kg, aplikovana intrakortikalno, dovela je do pojave velikih napada. U ovoj grupi gotovo odmah nakon aplikacije pojavili su se napadi sa učestalim ponavljanjem. *Acta Medica Medianae 2004; 43(4): 19–23.*

***Ključne reči**: eksperimentalna epilepsija, penicilin, pacovi*