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REVIEW

How can neuroprotective drugs (FK506 and CSA) affect epileptogenesis and susceptibility to status epilepticus in the normal and pathological brain?

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Symptoms characteristic of epilepsy have been recognised relatively well at the very beginning of medicine. Despite that fact, mechanisms underlying the disease still remain unknown. The brain injury has been commonly considered as one of the most powerful triggers for epileptogenesis. It appeared, therefore, obvious that the application of neuroprotective drugs minimising effects of injury would also protect the brain from epileptogenesis. However, a question has been raised that the neuroprotective treatment might not always be beneficial to the injured brain, since it would also promote the survival of neurons of functional features permanently modified under pathological condition. Therefore, the neuronal survivors themselves might constitute the source of initial seizures or show an increased susceptibility to seizuregenic stimuli. Indeed, it has already been demonstrated that long-term effects of neuroprotective drugs might not always have a positive influence on further susceptibility of the injured brain to seizures. On the contrary, application of neuroprotectants during the course of epileptic seizures may instead result in smaller structural and functional impairments. Finally, effective stimulation of endogenous neuroprotective potential existing in the injured brain also became the subject of experimental verification. Apparently, physical exercise is a promising example of this therapeutic approach.

Key Words

Animal models of epilepsy; Neuroprotection; Brain injury

For many years, epilepsy has been a serious clinical problem. According to a WHO estimate, about 50 million people suffer from this disease worldwide. In recent years, there has been a rapid progress in its diagnosis and therapy due to the engagement of experts from various fields of pharmacology, surgery, neurology and neurobiology. New antiepileptic drugs, such as felbamate, gabapentin, lamotrignine, vigabatrin and many others[1] were introduced thanks to the co-operation of interdisciplinary teams. Unfortunately, exact causes and mechanisms of epileptic discharges are still unknown, which greatly hinders attempts to efficiently counteract the development of this disease.

Animal models of epilepsy

Studies on epileptogenesis or new antiepileptic drugs require the use of animal models of epileptic seizures. In the search for an ideal model of epilepsy, more than 50 different methods of seizure induction have already been described. An ideal model should present a picture possibly the most closely resembling clinical symptoms in humans[2]. Seizures should be accompanied by the changes in electroencephalographic activity of the brain. If a model fulfils these criteria, it can be used for studies of the mechanisms underlying epileptogenesis, and for the development of new treatment strategies.

The pilocarpine model of temporal lobe epilepsy has been in use for about 25 years. This model was created by Waldemar Turski, a Polish scientist, who was the first to observe that the administration of the cholinergic agonist, pilocarpine, to rats induced limbic seizures manifested by an array of behavioural symptoms and characteristic changes in EEG. After acute phase of seizures, isolated seizures in animals which survived status epilepticus could even be observed for several years[3]. Histological analysis of brains of pilocarpine-treated animals demonstrated a number of pathological changes in the olfactory bulb, amygdala, thalamus, hippocampus and neocortex[3,4]. An important weakness of the pilocarpine model is the high mortality of animals which can, however, be prevented by lowering pilocarpine doses and its combination with lithium chloride[5].

Treatment with the glutamatergic agonist, kainic acid, is another method used to trigger epileptic seizures. Such treatment elicits several characteristic behavioural and electroencephalographic changes. Histological analysis shows lesions in brain regions similar to those caused by pilocarpine, but the changes are less conspicuous[6].

Seizures can be provoked also by strychnine, pentetrazole, excitatory amino acids and many other substances[5].

Recently, a research team led by Turski[7] proposed a new model of epilepsy. Methomyl was used as a seizurogenic substance. It is a carbamate commonly used as an insecticide which can reversibly inhibit choline esterase activity. Methomyl in combination with lithium evokes limbic seizures followed by neurodegenerative changes and characteristic EEG alterations, but does not cause high mortality[7]. For this reason, this model can be an alternative to the models of epilepsy used so far.

Beside treatment with various substances, seizures can be provoked by a single electrical stimulation via ear clip electrodes[8] or by repeated subthreshold stimulation (kindling). The latter method requires implantation of electrodes which *per se* injures the brain and may be epileptogenic.

The models of epileptic seizures presented above are most frequently used in experimental studies. Besides, there are a number of other models of epilepsy, e.g. models utilising visual or auditory stimulation, or genetic manipulation[2].

Post-traumatic epilepsy

Brain injury following ischemia or trauma is one of the possible causes of epileptic seizures. Developmental malformations in the brain leading to permanent changes in its structure may also underlie epileptogenesis[9].

Clinical studies demonstrated that 50% of patients who suffered a penetrating brain injury developed epilepsy[10-13]. The risk of post-traumatic epilepsy increased with lesion severity, nevertheless, epilepsy could develop even in patients experiencing very small lesions[14]. Clinical studies, however, do not always guarantee effective exploration of effect of the injury on epileptogenesis since etiology of this disease in humans is usually not completely clear. Therefore, animal models of epilepsy or mechanical brain injury are used to elucidate this problem in more detail[15-17]. In these models, an effort is made to unambiguously define all parameters of seizures. Usually these studies are carried out on adult animals, therefore, it is difficult to speculate on that basis what effects such injury would elicit in the immature brain.

On the other hand, clinical studies report that children and teenagers are the groups in greatest danger of head injuries. The resulting brain injuries are the most frequent cause of neurological disorders (including post-traumatic epilepsy) in children[17,18].

For these reasons, recent studies of epileptogenesis focus on the search for means how to prevent development of epilepsy of different etiologies, particularly post-traumatic epilepsy in children and adults.

3. Experimental models of epilepsy

3.1. Pilocarpine model of epilepsy

In order to elucidate how mechanical injury influences brain susceptibility to epileptic seizures, a well-known model of penetrating injury of the brain hemisphere was used. In this model, the integrity of cranium and meninges was broken showing, therefore, a good analogy to sudden and traffic accidents[19-22]. Two animal groups were subjected to this kind of injury on postnatal days 6 or 30 (P6 and P30, respectively, Fig. 1 shows a general scheme of the experiment):

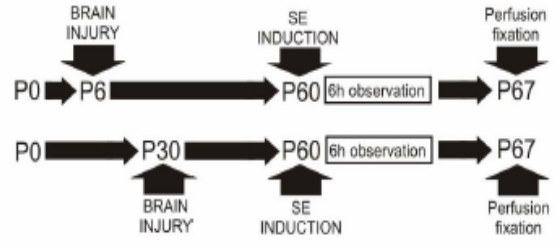

Fig. 1.

When the animals became 60 days old (P60s), they received pilocarpine injections to induce status epilepticus (SE)[23]. Thereafter, all behavioural symptoms were assessed. It was proven that susceptibility to the seizures depended on the developmental stage at which the mechanical injury was performed.

In rats injured on P6, the course of seizures evoked on P60 did not differ from that observed in controls. On the contrary, brain injury on P30 severely increased susceptibility to seizures. Moreover, the proportion of survivors on the seventh day of the experiment in animals injured on P6, was higher than in the control group. On the contrary, mortality of animals injured on P30 was extremely high, and reached 97%[23]. The evidence that injury on P6 did not increase the susceptibility to epileptic seizures evoked in mature animals does not agree with clinical data showing that paediatric insults are most epileptogenic[12,24].

Probably, diverse predisposition to seizures in animals injured on P6 or P30 is partially a result of different, age-dependent structure of the injury-induced glial scar. This is in agreement with pioneering research of Penfield[25], who first indicated the relation between astrogliosis and epilepsy. Maxwell and co-workers[26] proved that in animals injured in the first postnatal week, glial scar remains dispersed and permissive to neuronal regeneration process[27]. Following the similar brain injury made in one-month-old animals, the glial scar has compact structure constituting a physical barrier for growing axons[28] making the restoration of damaged neural connections impossible. Apart from this, the scar is also a potent biochemical barrier producing axonal growth inhibitors[29]. All the injury-induced structural and biochemical changes could be responsible for the significant differences in susceptibility to seizures between the two age groups. It is worth remembering that astrocytes have their own calcium-dependent mechanisms of excitation, with surface receptors and ion channels, like in neuronal cells. For these reason, actrocytes can be considered as a primary source of epileptic activity[30]. It may depend on the changes in proportion and functional interfaces between astrocytes and neurons, especially after injury-induced glial proliferation and modification of extracellular environment.

Moreover, many cells within the region of injury undergo apoptotic or necrotic changes[31]. However, if they can survive, they may also be able to create additional pathological connections underlying increased susceptibility to spontaneous epileptic discharges or to seizuregenic stimuli and, therefore, facilitating occurrence of status epilepticus.

3.2. Kainic acid model of epilepsy

In clinical settings, we are not sure enough which neurotransmitter systems are involved in epileptogenesis and/or seizure processes. Animal models of seizures improve the situation by application of appropriate seizuregenic substances that can activate selected neuronal populations. Nevertheless, definition of specific neural subsystems responsible for changes in susceptibility to seizures can be much less effective. It is always necessary to keep in mind that the changes are observed in a given animal model following application of a specific seizure-generating substance that activates selected functional subsystems. Consequently, all the above-presented phenomena can rather be considered as characteristic of the pilocarpine model of epilepsy[23]. In order to resolve, if the activation of another neurotransmitter systems can produce similar effects, another model of epileptic seizures using kainic acid was applied in the same experimental paradigm[32]. Since kainic acid is a glutamatergic agonist, its dosage was calculated on the basis of data from experiments by other authors, to produce similar histopathogical and behavioural changes[6,33]. Contrary to previously described results obtained using pilocarpine model of epilepsy[23], adult rats with brains injured on P30 showed no change in their susceptibility to kainic acid-induced seizures. Furthermore, brain injury performed on P6 led to significant amelioration in the course of seizures in the same experimental conditions. The results indicate that the brain injury may have different influence on long-term susceptibility to seizures when agents used to evoke the seizures activate functionally different neuronal subsystems. Changes in susceptibility to seizures following brain injuries performed at different developmental stages may also present different profiles depending on the experimental model, i.e. when pilocarpine or kainic acid were chosen as seizuregenic agents[23,32]. Each of the two models of epilepsy might display different aspects of the same age-dependent process triggered by the brain injury. These facts show clearly that no general and unequivocal conclusion concerning mechanisms of epipletogenesis can be obtained on the basis of experiments using a single experimental model of epilepsy since the process appears to depend on multiple functional subsystems.

Potential for use of CsA and FK506 in antiepileptic therapy

Since, as mentioned earlier, each brain injury may have irreversible consequences, factors that

could prevent development of post-traumatic changes have been tested for many years. Compounds that can minimise the effects of brain injury by increasing the ability of nervous cells to survive under pathological conditions are defined as neuroprotective factors.

In experimental studies, such substances can either be administered systemically or the organism itself can be stimulated to produce neuroprotective factors. If such factors are considered to be used in clinical practice, they should be thoroughly examined in preclinical studies. Unfortunately, only a few of them fulfil requirements for being used to treat humans.

Immunosuppressive action of CsA and FK506

CsA was first described as neuroprotective compound based on experimental studies by Shiga et al[34]. Until then, it had been exclusively used as an immunosuppressant. CsA is a lipophilic undecapeptide isolated from the fungus *Tolrypocladium infantum* Gams in 1976[35]. It has a wide-ranging biological action as a fungicide, and an antiparasitic or anti-inflammatory agent. Finally, it is used as an immunosuppressant in transplantology[36,37], in autoimmune diseases of the skin[38] and of the nervous system[39] and in rheumatism[40].

FK506 (Tacrolimus, commercial name Prograf), isolated from the fungus *Streptomyces tsukubensis* in 1984, shows similar actions to CsA[41]. It has been successfully used for the prevention of liver and heart graft rejection. When this drug is used, it is not necessary to administer so high doses of corticosteroids as in posttransplantational therapy with CsA[42].

The main therapeutic effect of the two compounds consists in the inhibition of lymphocyte T activation[43,44]. When CsA or FK506 have entered the cell, they bind to cyclophilins (CyPs) or FK binding proteins (FKBPs), known also as immunophilins[45]. CyPs or FKBPs complexes are able to inhibit the activity of calcineurin, which is an enzymatic protein with phosphatase activity. Because of its ability to cleave phosphate groups, it is engaged in many metabolic reactions critical for cell function.

Calcineurin is involved in the regulation of ion channel activity[46], and controls neurotransmitter release[47] and nitric oxide synthase activity[48], moreover, it is engaged in signal transmission in the cell[49].

The activated calcineurin dephosphorylates nuclear factor of activated T cells (NFAT), among other molecules, which leads to conformational changes exposing site of NFAT transport to the nucleus. NFAT has relatively low affinity for DNA and interacts with other transcription factors to activate such promoters as IL-2,IL-3, GM-CSM, TNF and Fas ligand, which initiate T cell activation[50]. Therefore, if calcineurin is inhibited, IL-2 cannot be activated, which disables full activation of T cells[51,52]. These phenomena are the basis of well-established immunosuppressant action of CsA and FK506.

By inhibiting calcineurin activity, CsA and FK506 affect also the activity of another transcription factor CREB (cAMP response element-binding), which amplifies their immunosuppressant activity[53,54].

4.2. Neuroprotective action of immunosuppressive compounds

Many years of the studies of neuroprotective action of the above-mentioned immunosuppressive compounds paved the way for their use in different models of brain (traumatic brain injury, global and partial ischemia) or peripheral nervous system injury.

4.2.1. Neuroprotectine action of cyclosporine

The experiments presented below illustrate the action of neuroprotective compounds in the selected models of brain injury that most closely simulate clinical situations.

CsA (10 mg/kg) administration for seven days before ischemia induction significantly reduced infarct area. Most probably, it was attributable to the activation of TrKa receptor of brain-derived neurotrophic factor (BDNF)[55]. CsA injection significantly increased the level of phosphorylated CREB (pCREB), which was earlier observed in hippocampal cells resistant to hypoxia-induced damage[56,57]. CsA at 20 mg/kg decreased number of destroyed striatal dopaminergic neurons in an animal model of Parkinson's disease[58]. CsA (10 mg/kg) administered after hypoxia induced by middle carotid artery occlusion (MCAO) significantly reduced degeneration area[59].

In the peripheral nervous system, CsA (2.5 mg/kg) administered 72 h before injury and an hour afterwards decreased demyelisation and number of vanishing nervous cells and supported recovery of motor functions[60]. It also lowered proliferative activity of astrocytes in the injured brain (our unpublished studies).

4.2.2. Neuroprotective action of FK506

FK506 (1 mg/kg) administered after sciatic nerve section facilitated regeneration of pe-

ripheral axons by increasing the level of growth factors[61]. Likewise, a single injection of FK506 (3 mg/kg) 30 min before traumatic brain injury significantly diminished number of damaged neurons[62]. It reduced the area of hypoxia-induced damage in MCAO model even by 58% but only when it was administered in the dose range from 1 mg/kg to 10 mg/kg and not later than 2h after hypoxia[63,64]. The latest experiments have indicated that the mechanism of neuroprotective action of immunosuppressive drugs can depend on non-neuronal components of the nervous tissue. Specifically, it was noted that post-injury FK506 treatment modulated activity of astrocytes and microglia which was accompanied by lowering of astrocytic IL-1b mRNA and TNFa mRNA levels[65]. Moreover, astrocytic proliferation was reduced in the brains of animals that were treated with FK506 after mechanical brain injury (our unpublished results). It is probable that a combined treatment with FK506 and tissue plasminogen factor can be helpful in clinical treatment of brain ischemia[66].

A few examples of studies of neuroprotective action of CsA and FK506, presented above, provide evidence that these two compounds show neuroprotective activity in adult animals only when they are applied at appropriate time after injury. The time elapsing between the injury and treatment should be short enough to allow for onset of neuroprotective agent's action before irreversible changes actually occur (therapeutic time window[67]). Obviously, the neuroprotective drugs should also be administered at appropriate doses that assure the most beneficial therapeutic effects with tolerable negative side effects for the organism.

Effect of neuroprotective compounds on epilepsy progression

5.1. Posttraumatic neuroprotection

Experimental studies repeatedly demonstrated neuroprotective action of CsA and FK506. Administration of these compounds after brain injury, which minimizes injury consequences, i.e. decreases necrosis area, reduces number of vanishing neurons, inhibits development of glial scars, could be the basis for more advanced clinical therapy.

Taking into consideration epileptogenicity of brain injury, known from clinical practice[13], and varying post-injury vulnerability to epileptic seizures in animals[23], we decided to check whether the administration of neuroprotective compounds after brain injury would change predisposition to manifestation of seizure activity. To attain this goal, animals, whose brains were injured at different periods of postnatal life (P6 or P30), were administered cyclosporine A or FK506. Subsequently, when the animals were 60 days old, they were administered pilocarpine to induce seizures. We observed that pilocarpine-evoked seizure activity in 60-day-old animals with brains injured on P6 administered FK506 did not significantly differ from those which did not receive any neuroprotectant after injury. However, animals injured on P6 but treated with CsA after injury, showed aggravated seizures elicited on postnatal day 60 and mortality in this group was extremely high. On the other hand, post-injury FK506 treatment following brain injury performed on P30 did not significantly affect seizures evoked in adulthood but survival rate was increased. CsA treatment after injury in this age group prolonged seizure activity but markedly increased survival time after experimentally induced epilepsy[68].

These results did not provide full confirmation of the initial hypothesis assuming lighter seizures after treatment with neuroprotectants, but they proved that CsA and FK506 induced persistent changes in the damaged nervous tissue thereby changing its functional properties. It was manifested by the increased susceptibility to seizures, particularly after CsA treatment, whereas FK506 injection did not cause such changes but significantly prolonged survival after acute phase of seizures[68].

The question arises, therefore, whether final effects of neuroprotective intervention, evaluated only quantitatively (larger number of neurons protected from degeneration) is always functionally beneficial, since this is functional but not anatomical (structural) improvement which would justify implementation of neuroprotective strategy. Results of experimental studies usually are considered to be quite satisfactory if the number of surviving neurons in some damaged areas of the brain is increased or necrotic area is reduced. However, the studies presented above showed that the post-injury administration of compounds with well-defined neuroprotective action could have increased brain susceptibility to seizures. Therefore, it is possible that neuroprotection, whose efficacy is conspicuous at quantitative level and demonstrable by histological studies, not always leads to functional improvement of damaged brain, but, surprisingly, can even cause functional deterioration, since characteristics of 'rescued' neurons had been permanently modified by pathological posttraumatic conditions.

It should be remembered that cyclosporin A

and tacrolimus have non-polar molecules and thus they are not soluble in water. In post-transplational therapy, these drugs are administered in appropriate pharmaceutical formulations to achieve therapeutic effect[69]. Substance in which an active substance is dissolved or suspended is called vehicle. In this case, a mixture of NaCl, Cremofor and ethyl alcohol was used as vehicle. Cremofor (modified castor oil) is the main component of the vehicle. Cremofor is obtained from *Ricinus communis* seeds and is characterised by very high viscosity and contains glycerides of ricinoleic acid, stearates and dihydroxystearic acid[70]. Such vehicle composition increases stability of the drug stored in the form of solution. Moreover, Cremofor is a surface-active agent increasing solubility of the active compounds[71]. The vehicle contains also 0.25% ethanol in 0.9% NaCl. It improves drug solubility and uptake[72].

It seems obvious that in order to be able to unequivocally assess CsA and FK506 potentials as the agents preventing seizure development after brain injury, it is crucial to test biological effects of vehicle in the same experimental design.

5.2. Effect of CsA and FK506 on acute phase of epilepsy

Clinical studies and animal models of epilepsy indicate that even a short episode of epileptic convulsions can produce local or remote neurodegenerative changes[5]. Neuronal death in vulnerable brain structures in rats can be observed already after epileptic discharges (in EEG recording) of 4-16 min duration[73,74]. Seizure activity probably leads to the activation of postsynaptic NMDA receptors due to excessive glutamate release from presynaptic structures, and excessive calcium influx inside neurons, which can elicit numerous transformations, even cell death.

Hence, minimisation of the changes triggered by spreading seizures is the important goal of clinical neurology. Since apoptosis in one of processes occurring in epileptogenic focus and remote areas, treatment with such neuroprotective compounds as CsA and FK506 could minimise post-epileptic tissue deterioration.

In order to test this hypothesis, CsA or FK506 were administered to animals with pilocarpine-induced seizures when seizures were of intermediate intensity according to Racine's scale[23,68]. Subsequently, the animals were observed for seizure progression. These experiments demonstrated that FK-506-treated animals showed lighter seizures than control animals and survived significantly longer. CsA administration also alleviated seizure intensity and prolonged survival time after seizure initiation[75]. Therefore, it can be assumed that each of the used compounds had a beneficial effect on the course of experimentally induced seizures, making them milder. It seems that the compounds under discussion can be efficient only when they are administered in the acute phase of seizures. Thus, they can presumably be considered to be primary neuroprotectants, since they counteract initial phenomena leading to neuronal death consequent to the spread of excitatory wave triggered probably by activation of voltage-gated Na^+ and Ca^{2+} channels or glutamate receptors[76].

The above-described experiment does not unequivocally answer the question whether the studied compounds acted as primary neuroprotectants or by inhibiting the cascade leading to cell death, thereby contributing to secondary neuroprotection. The studies were carried out only for the first six hours after seizure initiation, thus, they should be linked with phenomena occurring in the early phase of epileptic activity, like excessive stimulation or primary neurodegenerative changes[77]. Since the used compounds have neuroprotective activity, it can be assumed that they can save neurons in epileptogenic focus or its vicinity from death or degeneration[78]. However, characteristics of those cells can be permanently changed, which may constitute a source of epileptic activity or susceptibility to seizures in the future. This hypothesis can be verified by further experiments with longer survival time after seizure initiation. If we envisage using compounds of similar type (calcineurin inhibitors) in clinical practice, we will also have to check whether they do not elicit undesirable effects in combination with currently used antiepileptic drugs.

6. Alternative forms of neuroprotective intervention: endogenous neuroprotection

It should be remembered that numerous complex processes occurring in damaged nervous tissue (degeneration, glial activity, regeneration) activate different tissue factors. Some of them can afford neuronal protection, particularly under pathological conditions. The presence of these factors or their absence can be decisive for death or survival of neurons at risk. Since these factors are produced by the nervous tissue itself, they are endogenous agents, therefore, their positive effect can be termed endogenous neuroprotection. It was discovered that moderate physical exercise can be one of situations inducing release of such endogenous neuroprotective factors.

As well as improving physical fitness in humans, moderate physical exercise improves psychical well-being, elevates mood, alleviates depression and decreases aggression[79]. Motor training elicits also a number of changes in the nervous system, alters the levels of neurotransmitters: glutamate, aspartate and serine[80], and modifies expression of endogenous growth factors[81,82]. Furthermore, exercise promotes brain vascularisation[83-86].

Many experiments investigating electrical activity of the brain have proven that exercise can terminate non-physiological discharges but they return when training is over[87].

Setkowicz and Mazur[88] investigated whether regular long-term exercise of moderate intensity (45-day training on treadmill and swimming) changed susceptibility of adult rats to pilocarpine-induced epilepsy. The obtained results unequivocally confirmed that moderate exercise elicited a significant positive effect, since the course of experimentally induced seizures was milder in trained animals than in controls (Fig. 2).

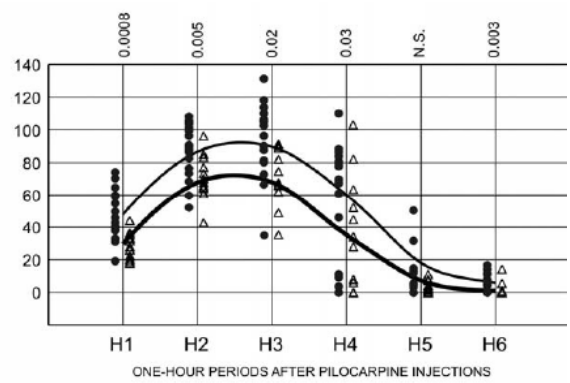

Fig. 2. Changes in the intensity of behavioural symptoms evoked by pilocarpine injections in trained and control, non-trained rats (thick and thin curves, respectively). Rating scores of the symptoms were summarised separately for each of six consecutive one-hour periods (H1-6) following pilocarpine injection. Triangles and circles represent the trained and control groups, respectively. Each decimal index shows statistical significance of difference between the two groups (Mann-Whitney test).

The neuroprotective effect of running may possibly be attributed to the formation of greater number of synapses[89], which results from the increased BDNF mRNA and BDNF expression in the hippocampus[90]. BDNF was observed to inhibit excitotoxic changes in neuronal cultures[91], and to reduce ischemia consequences *in vivo*[92]. Therefore, it is believed that elevation of its level during exercise is accompanied by neuroprotective effect[93]. It is also possible that exercise-induced rise in BDNF level triggers regulatory processes leading to the increase in neuropeptide Y level, which suppresses epileptic discharges[94]. In addition, exercise enhances neurite growth factor (NGF) level. NGF can protect cells against hypoglycemia and excitotoxicity by reducing intracellular calcium excess[95]. BDNF and NGF show also ability to boost activity of free radical scavengers[96], making the organism less endangered by their harmful effects. It is, thus, possible that, owning to the above-described properties, the NGF released during exercise abrogates pilocarpine-induced epileptic changes.

In other models of seizures, e.g. in the kindled seizures, trained animals were observed to require greater number of stimulations in order to elicit stage 5 seizures according to Racine's scale than non-trained animals[97], which corroborates positive effect of exercise.

Opposite effects were described by Ramsden et al.[98], who noted aggravation of neuronal damage after kainic acid-induced seizures in females subjected to physical exercise.

Advantages of endogenous over exogenous neuroprotection

Based on the above-described facts, it can be inferred that the application of substances commonly known to be neuroprotective after brain injury not necessarily has to have positive functional effects. Therefore, despite histological demonstration of distinctly increased neuronal survival in a damage area, this positive change may not be beneficial in terms of future susceptibility to epileptic attacks. The studies discussed in this review did not investigate such changes, so further experiments are necessary to clarify this issue. It is also required to examine the effects of vehicle components on development of epilepsy. Moreover, it is important to distinguish to what extent the neuroprotectant and the injury itself contribute to the observed changes. Therefore, CsA and FK506 effects should be examined also in the uninjured brain. Obviously, it is essential to take account of developmental changes occurring in the brain in the period when neuroprotective drugs are administered. Surprisingly, despite lack of these data, the analysed drugs have been used in posttransplational therapy in children for many years.

The administration of neuroprotective compounds in acute phase of seizures in animals brought positive effects and lowered seizure activity[75]. However, long-term consequences of their use are uncertain, although their long-lasting use in post-transplational therapy is known to be associated with the risk of different side effects[99,100].

Luckily, the stimulation of endogenous neuroprotection, like through exercise, seems propi-

tious. Endogenous neuroprotection has no side effects accompanying the activation of various intracellular factors promoting neuronal survival. Furthermore, experiments refuted long-standing conviction that epileptic patients should avoid exercise. It may be expected that regular exercise of moderate intensity can counteract the increased susceptibility to seizures after injury. This hypothesis unquestionably requires experimental verification. Contemplating clinical use of neuroprotection free of side effects or invasive therapies, namely, endogenous neuroprotection, one should check whether the stimulation of intracellular repair systems is efficient in any experimental model of epilepsy. While attempting to apprise endogenous treatment strategies, one should take notice of the fact that all experiments presented here were carried out on males. Actually, epilepsy incidence in women and men is comparable, but experimental data indicate that seizure attacks in women are milder [101]. Numerous researchers reported neuroprotective action of estrogen and progesterone[102,103]. These hormones influence not only seizure intensity, but estrogen protects neurons from death by activation of *bcl* family genes or growth factors, and by inhibition of caspase-3, IL-1 and IL-6 activities[104]. Furthermore, data from experiments on an animal model of traumatic brain injury indicate that progesterone significantly reduces neuronal damage in CA1 and CA2 and brain oedema[105].

Based on the above-presented facts, it can be assumed that the substances produced by the organism (neuropeptides, growth factors and even hormones) are more promising neuroprotectants for therapeutic prevention of generation and spread of epileptic stimulation in the brain than exogenous neuroprotective agents like FK506 or CsA. Researchers and clinicians need only to find a proper stimulation, assuring the adequate change in their level at critical time for seizure occurrence.

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