TÉZISEK

AGYI INGERÜLETI FOLYAMATOK SZEREPE FELNŐTT ÉS FEJLŐDŐ IDEGRENDSZER AGYKÉRGI EPILEPTIKUS TEVÉKENYSÉGBEN

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INTRODUCTION

Epilepsy is a functional disorder of the central nervous system, which can be characterized by excessive abnormal, synchronized rythmic firing of large populations of neurons termed seizures. The fragile balance between physiological excitation and inhibition fall during seizure activity.

Fast excitatory neurotransmission involving glutamate plays important role in the initiation, maintenance and spread of seizre activity. There are two major groups of glutamate receptors: ionotropic receptors (iGluR) which contain internal ion channels within the receptor complex, and metabotropic receptors (mGluR) which activate the second messenger system through G proteins.

(1) Contribution of both ionotropic and metabotropic glutamate receptors to the seizure discharges and epleptogenesis is well studied. A question with grear significance is what role do the specific subtypes of glutamate receptor play in the induction, expression and propagation of epileptic activity in the brain. Therefore, we were interesed to know, wheather non-NMDA, NMDA and mGluRs are involved in the initiation, maintenance or propagation of focal periodic ictal activity in *in vivo* rat neocortex, as well as to analyse the retrograde, feed-back effects of the secondary focus on the epileptiform activity in the primary one.

(2) Influence of metabolic process on the cortical excitability and 4-amiopyridine (4-AP)-induced epileptic activity was also studied.

(3) It is known, that the seizure susceptibility is higher in childhood than in adults. The enhanced epileptogenicity might be explained by such a neurobiological features of developing nervous system like increased synaptic density, enhanced synaptic plasticity, hipersensitivity of NMDA receptors, appearance of bursting neurons or GABA excitatory effect can also be responsible for the higher excitability of developing nervous sytem. Therefore, we investigated the seizure susceptibility of developing rats on 4-AP-induced epilepsy modell. The depolarizing or excitatory effects of GABA in early postnatal life is thought to be associated with reversal transmembrane chloride gradient. Precipitation of chloride with silver is a potential way to immobilize and visualize chloride ions in biological tissue. We examined the applicability of light microscopic histochemistry, based on trapping tissue chloride with silver ions during freeze-substitution or aldehyde fixation, to visualize the chloride distribution in hippocampal slices.

METHODS

Electrophysiological experiments

In vivo experiments were carried out on pentobarbital-anaesthethized (i.p. 50 mg/kg) rats, ictal epileptiform activity was induced by local application of 4-aminopyridine to the cortical surface. ECoG was recorded fromfour points: from the primary focus (Pf), from the mirror focus (Mf) and from two other points posterior direction to the foci. ECoG was stored on computer memory for the off-line analyse. Somatosensory evoked responses were also recorded from the cortical surface and from deeper cortical layers, as well.

We have choosen to use GYKI 52466 ((4-aminophenyl)-4-methyl-7,8-methylene-dioxy-5H-2,3-benzodiazepine.HCl), a highly selective, non-competitive antagonist of the AMPA glutamate receptor responses, as pharmacological tool to study the importance of these receptors in the induction, maintenance and expression of cortical ictal epileptic discharges.

Data analysis

Latency of the first ictal event, numbers and averaged duration of ictal episods and the summated ictal activity was determined. We also measured the amplitudes of epileptiform discharges, and studied the ratio of different epileptiform pattern and the probability of seizure propagation.

Pharmacological experiments

In order to study the role of AMPA, NMDA and mGluR receptors in the cortical ictal activity selective or non-selective receptor agonists/antaginusts were used.

- GYKI 52466 is a highly selective, non-competitive AMPA receptor antagonist
- L-AP5 is a selective NMDA antagonist
- 1S,3R-ACPD is a broad spectrum mGluR agonist
- DCG-IV II is a specific mGluR agonist
- 3,5-DHPG is a selective I mGluR agonist
- AOAA (aminooxyacetic acid) is a non-specific inhibitor of several transaminase

Light histochemistry

Hippocampal slices were prepared from new born and adult rats. The chloride distribution of the tissue was wxamined by trapping tissue chloride with silver ions during freeze-substitution or aldehyde fixation. For the analyse of the AgCl precipitation electron spectoscopic imaging (ESI) and electron energy loss spectroscopy (EELS) were used.

RESULTS AND DISCUSSION

(1) The results demonstrates that GYKI 52466 exerts anticonvulsive effects on both the induction and the expression of epileptiform activity, by delaying the onset of the first ictal event, decreasing the duration of ictal periods, as well as the amplitudes of epileptiform discharges both in the primary and mirror foci. However, seizure propagation to other cortical areas seemed to be facilitated. It is supposed that AMPA receptors are probably more dominant in the induction of epileptiform activity than maintenance of it, mainly through the activation of cortico-thalamo-cortical networks.

(2) It is suggested that the activation of AMPA receptors of GABAergic interneurons in the epileptigenic focus contribute to the surrounding inhibition. Our results show, that the mirror focus has a remarkable control effect on the site of the origin of the primary epileptic activity.

(3) The results show, that L-AP5 also supresses the epileptic activity. It is supposed that NMDA receptors are not necessary in the induction of seizure discharges, however they are dominantly involved in the expression of the epilepic phenomena by activity-dependent manner. NMDA receptors also play an important role in the propagation of seizure discharges in the cortico-cortical, and vertical extended cortico-thalamo-cortical networks. For the synchronized activity between the two foci NMDA receptors are prominantly needed.

(4) We demonstrated that activation of group I mGluRs play important role in the neuronal hypersynchronisation. Group I mGluRs facilitate the induction of epileptic activity by 4-AP, increase the duration of ictal periods, enhance the amplitudes of epileptiform discharges and potentiate the propagation of abnnormal activity in the whole cortical surface.

(5) DCG-IV has a potent anticonvulsive effect on 4-AP-induced epielptic activity as delay the onset of the first ictal event, reduce the duration of seizure periods, decrease the amplitudes and propagation of epileptiform discharges. Is supposed that activation of group II mGluRs significantly decrease the synchronized firing of neurons, prevent the generation of paroxysmal activity, remarkably suppress the expression and propagation of seizure activity.

Based on these observations we can conclude that AMPA and group II mGluRs can be considered seriously as potential targest for antiepileptic drugs.

(6) AOAA at low concentration probably increase the efficacy of the NMDA receptor excitatory system and decreases GABA-sythesis, resulting neuronal hyperexcitation. However, AOAA at high concentration can lead to an effective cortical inhibition through intra- and extracellular accumulation of GABA. It is supposed that different metabolic process, like transmitter sythesis or degradation can control and modify the excitability of neurons and the seizure susceptibility.

(7) Progressive changes in the basic ECoG and the 4-AP-induced activity were observed in the early postnatal life. The suscepptibility to seizure is highest in young animals (age 16-17 days) and decreases progressively with age between18 and 24 and reaches a level comparable to adult in 4-5 weeks.

(8) The enhanced seizure susceptibility can be rlated with GABA excitatory effect. The GABA exciting effect may be associated with increased intracellular chloride ditribution. The histochemistry method, based on trappiung chloride with silver ions, was able to visualize a difference chlorid distribution between newborn and adult. This method can be a usefull tool for studing the increased epieleptoginicity of developing brain

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