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Molecular mechanism of DNA fragmentation without cell loss in the epileptic mutant EL mice brain

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In EL mice, ictogenesis has been established at around 10 weeks of age, whereas epileptogenesis will be established through experience of repetitive seizures during development. It is suggested that an "abnormal neural plasticity" may play a crucial role in the establishment of ictogenesis and epileptogenesis, and therefore, can be a potential target for the development of a new pharmacotherapy to the epilepsy.

Recently, antiapototic, proapoptotic factors and neurotrophic factors are suggested to play a key role not only in the cell survival but also in the epileptogenesis. In the brain of EL, DNA fragmentation without cell loss was observed in the parietal cortex and hippocampus which play the key role in the seizure initiation and generalization respectively.

To investigate the role of antiapototic, proapoptotic factors and neurotrophic factors in the ictogenesis and epileptogenesis in EL mice brain, developmental changes of these factors are determined.

METHODS

Proapoptotic Bax, antiapoptotic Bcl-2 and Bcl-XL, Neurotrophic factors; Brain derived neurotrophic factor (BDNF) , Neurotrophin-3 (NT-3) and Fibroblast growth factor-2 (FGF-2) were semiquantitatively analyzed by using Western Blot and NIH image with macros and compared with those of control DDY.

RESULTS

Developmental changes of anti and proapototic factors (Bcl-2, BclXL, Bax)

In the parietal cortex (Seizure initiation site) (Fig. 1a). Pro-apoptotic Bax has shown over expression from 8-20 weeks old during development which covers the periods of ictogenesis and epileptogenesis. Anti-apoptotic Bcl-2 has also shown over expression corresponding to that of Bax. Anti-apoptotic Bcl-XL has shown the linearly increasing expression and reached maximum level around 20 weeks of age (periods of late epileptogenesis). In the hippocampus (Seizure generalization site) (Fig. 1b). Pro-apoptotic Bax has shown over-expression from 8 to 19 weeks old (which covers the periods of epileptogenesis). Anti-apoptotic Bcl-2 has shown over-expression from 8 to 19 weeks of age (which covers the periods of epileptogenesis). Anti-apoptotic Bcl-XL has shown the maximum expression at 5 and 24 weeks of age (which covered the early periods of ictogenesis and the late periods of epileptogenesis).

Developmental changes of neurotrophic factors (BDNF, NT-3, FGF-2)

In the parietal cortex (Seizure initiation site), BDNF has shown over expression during the early developmental period (8-12 weeks old, which covers the periods of ictogenesis). NT-3 has shown over expression after 15 weeks of age (which covers the periods of epileptogenesis). FGF-2 has been over expressed from 5 to 10 weeks of age (which covered the early periods of ictogenesis) (Fig. 1c).

In the hippocampus (Seizure generalization site), BDNF has shown the double phasic over-expression, from 8 to 12 and around 20 weeks old (which covered the periods of epileptogenesis). NT-3 has shown peak expression from 8 to 12 weeks old (which covered the early periods of epileptogenesis). FGF-2 has shown linearly increasing expression and reached maximum level at 24 weeks of age (which covered the late periods of epileptogenesis) (Fig. 1d).

Conclusions

DNA fragmentation without cell loss in EL mice, the susceptibility of hippocampal neurons to DNA fragmentation increases after experiencing repetitive seizures during the late period of development probably due to a change in the balance between protective mechanism and proapoptotic pathway's inactivation. Neurotrophic factors may also play a role in ictogenesis and

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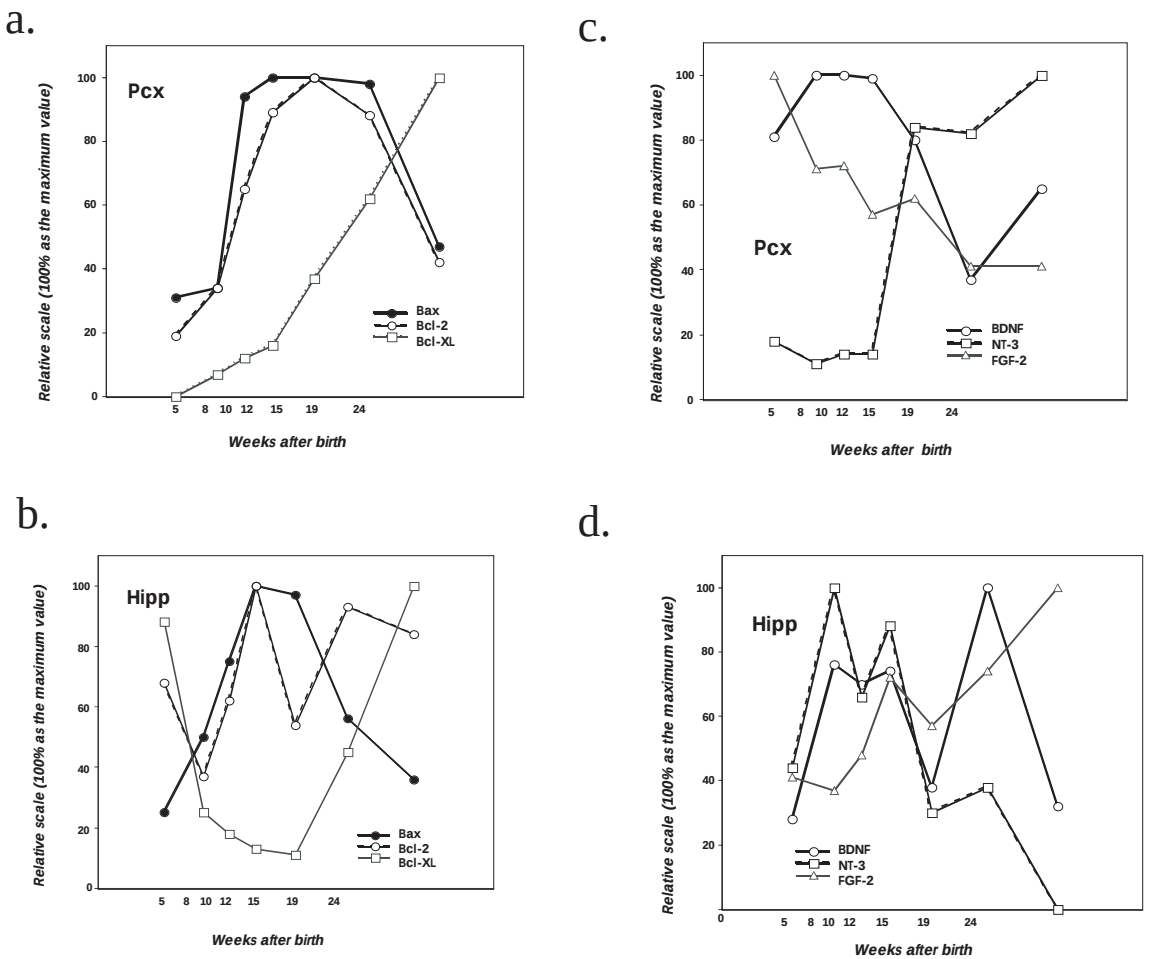

Fig.1. a,b: Relative expression of Bax, Bcl-2 and Bcl-XL in El mice brains during development. Pcx: Parietal cortex, Hipp: Hippocampus.
Fig.1. c,d: Relative expression of BDNF, NT-3 and FGF-2 in EL mice brains during development.

epileptogenesis through the developmental stages by promoting abnormal synaptic plasticity in the early period of development.

DISCUSSION

Recently the molecular mechanisms of the relationship between abnormal plasticity and, Bcl family and neurotrophic factor genes has been partially analyzed. In human samples obtained from temporal lobectomy, Bcl-2, Bax and caspase-3 immunoreactivity was increased predominantly in neurons, whereas Bcl-XL immunoreactivity was increased in glial cells. DNA fragmentation was detected in some but not all sections from epileptic brain samples.

Bcl-2, Bcl-XL and Bax are regulatory proteins which are variably expressed in brain tissue and are known to be involved in the regulation of apoptosis; Bcl-2 and Bcl-XL inhibit apoptosis and Bax generally promotes apoptosis. Western blot analysis demonstrated significant increases in levels of Bcl-2 and Bcl-XL protein in human seizure brain compared to control.

Application of BDNF increased the amplitude and frequency of spontaneous excitatory postsynaptic currents and increased the amplitude of evoked excitatory postsynaptic currents. BDNF had no effect on spontaneous inhibitory postsynaptic currents but produced a decrease in amplitude of evoked inhibitory postsynaptic currents. BDNF's effects were abolished by coapplication of the tyrosine kinase inhibitor K252a. Therefore, BDNF enhances fast excitatory transmission in the epileptic human dentate gyrus and may play an important role in epileptogenesis in temporal lobe epilepsy.

BDNF are hypothesized that the expression is related to pathological alteration after seizures, We reported that the expression of BDNF reached maximum during the period of epileptogenesis, not after the frequent seizures, because aster 20 weeks of age, the signal decreased and returned to basal level at the age of 24 weeks of age , when EL mice have experienced most frequent seizures.

In EL the parietal cortex plays the major role in the seizure initiation and the hippocampus plays the major role in the seizure propagation and generalization and BDNF may play an important role in ictogenesis and epileptogenesis. This raises the possibility of designing therapies for this disorder that may be both anticonvulsant and antiepileptics.

Interestingly, the expression of nerve growth factor and brain-derived neurotrophic factor is rapidly up-regulated following seizures, while NT-3 mRNA remains unchanged or undergoes a delayed down-regulation, suggesting that NT-3 might have a different function in epileptogenesis.

Chronic, but not acute, exposure to minimal electroconvulsive shock (ECS) has been shown to decrease vulnerability to neuronal cell death, without itself causing neuronal damage. One potential mechanism for the neuroprotective effect of ECS is the increase in fibroblast growth factor-2 (FGF-2) which occurs after chronic, but not acute, minimal electroconvulsive shock (ECS) exposure. In EL, FGF-2 expression increased linearly with the development. The sustained increased expression of FGF-2 will be protective to the seizures of EL. And it is compatible that although hippocampus and parietal cortex constitute the focus complex, no cell loss was found in the brain.

It is interesting that each neurotrophic factors (BDNF, NT-3 and FGF-2) showed the specific patterns in the hippocampus and parietal cortex, respectively in EL mice brain. The EL mouse is an inbred mutant strain which has been used as an animal model of secondarily generalized seizures. Several lines of evidence indicate that seizure discharges are initiated in the parietal cortex, and then generalized through the hippocampus.[1] These findings have been substantiated by the histochemical and biochemical analyses of glucose utilization and inhibitory neuro-transmissions by gamma-aminobutyric acid (GABA).[2] The developmental formation of the focus complex, which mainly consists of the parietal cortex and the hippocampus, has been hypothesized to be key to epileptogenesis in EL mice.

In our recent studies, excess free radicals mitgt produce DNA fragmentation.[3] The possible candidates as free radicals are superoxide and superoxynitrite caused by decreased Cu,Zn-superoxyde dysmutase (SOD) and increased inducible Nitric oxide synthetase (iNOS) in the brain of EL mice.[4,5] Ordinarily DNA fragmentation leads to apoptosis in the tissue. But no cell loss were found probably due to the imbalance between protective mechanism and inactivation of proapoptotic pathways.

Immediate early gene (IEG) expression is nonspecific. However, the expression of localized and continuous IEG expression means that some protein cascades occur in situ.[2] Bcl family proteins and neurotrophic factors which is closely related to cell loss and cell survival and further to epileptogenesis might be the candidate proteins.

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