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Hippocampal astrogliosis and neuronal cell loss in an experimental P10 neonatal model of mesial temporal lobe epilepsy

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Background

Mesial temporal lobe epilepsy (MTLE) is the most common form of intractable seizure disorder in adults. It is associated with an asymmetrical pattern of neuron loss within the hilus and CA1 hippocampal subfields, with relative sparing of the dentate granule neurons and the CA2 subfields. The amygdalar nuclei and the cortical neurons are other areas that are rarely involved in MTLE.¹

The neuropathological changes that bring about focally evoked seizures in the adult are thought to begin in the early stages of neonatal life. A wide range of factors have been implicated in the causation of MTLE, such as febrile seizures and traumatic brain injury.² Some studies indicate that up to 74% of those who experience early life status epilepticus develop epilepsy at a later stage.² The objective of this study is to investigate the neuropathological changes induced by intra-amygdalar kainic acid (KA) microinjection in a postnatal day 10 (P10) rat model of MTLE.³

Methods

Seizure induction by intra-amygdalar microinjection of KA was modified from experimental models developed by Henshall *et al.*⁴ Pups received either 2µl of vehicle (phosphate buffer, pH 7.4; n=4) or 2µl KA (n=4).

EEG was recorded for up to 90 minutes, during which behaviour associated with inter-ictal events were monitored.

Immunofluorescence microscopy was performed on fresh frozen

rat brains for glial fibrillary acidic protein (GFAP) (1:400), a marker of astrogliosis, and neuronal nuclei (NeuN) (1:400), a marker of neuronal nuclei. Fluoro-jade B staining (FJB) was also performed to assay for neuronal cell death. Statistical analysis was carried out using non-paired student t-tests.

Results

Following injection of 2µg of KA, initial low amplitude, high frequency EEG developed to high amplitude, high frequency epileptiform EEG seizures consistent with status epilepticus. All KA-treated pups (n=4) displayed seizure-like behaviour, including initial masticatory movements and salivation, developing periods of forelimb myoclonus and progressing to wild swimming behaviours.

Histological analysis of the rat brains treated with KA showed a reduction of the overall size of the ipsilateral hippocampus as well as atrophy of the pyramidal cell layers. Hydrocephalus *ex vacuo* of the ipsilateral lateral ventricle was also noted in one of four rat brains.

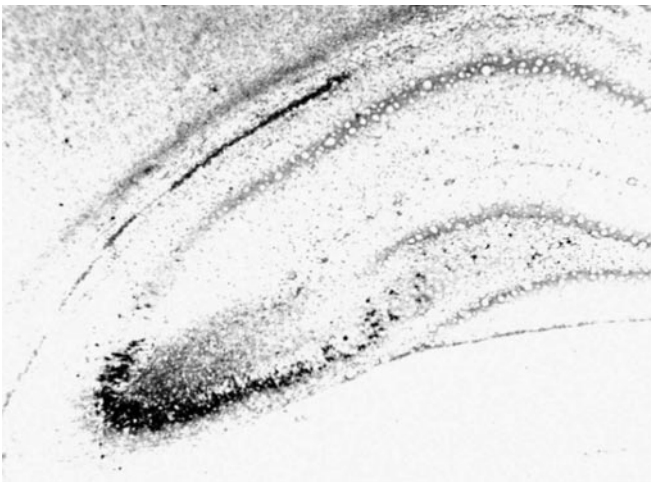
Ipsilateral P10 CA3 NeuN counts in KA-treated rats were approximately 75% of that in the control group (n=4, p<0.01), demonstrating preferential CA3 subfield-specific neuronal cell death in KA-treated pups.

Ipsilateral FJB-positive cell counts in treated animals were approximately 20 times greater than in the control animals (n=4, p<0.05). In addition, there was diffuse astrogliosis in the ipsilateral hippocampus of treated animals.

Conclusion

In summary, intra-amygdalar KA microinjection in neonatal rats causes status epilepticus and extensive acute unilateral hippocampal neurodegeneration. The ipsilateral hippocampus displayed neuropathological changes similar to the pattern reported previously by Dunleavy *et al.*³ The neurometabolic role

of extensive astrogliosis after cell death within the hippocampus requires further study. It is hoped that understanding of the mechanisms of neonatal status epilepticus and its role in the aetiology of MTLE will aid in the development of novel anti-epileptogenic treatments.



(a) (i) Ipsilateral hippocampus: 2µg KA.

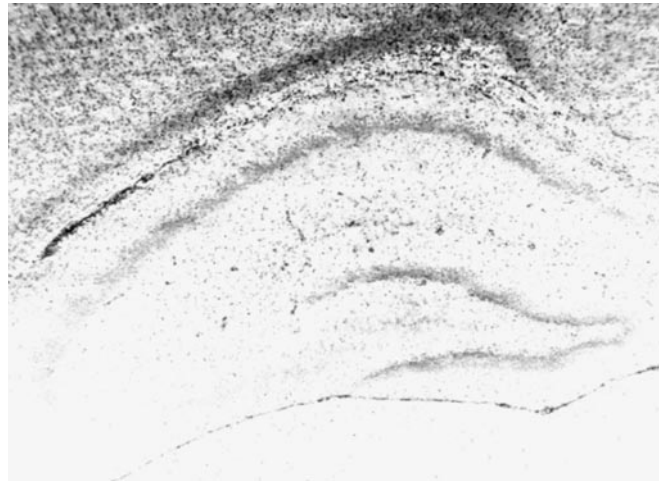
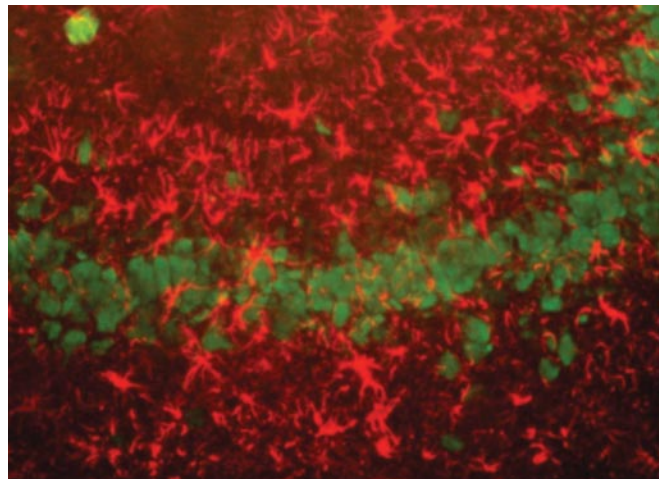


FIGURE 1: (a) Hippocampal cell death at P10 following neonatal status epilepticus; representative inverted pictomicrographs of the hippocampus of rats treated with intra-amygdalar microinjection of either 2µg (i) KA (n=4), or (ii) vehicle (n=4). Cell death occurs preferentially in the CA3/hilus of the hippocampus. The decrease in neuronal cell layer depth in the CA1 region in the KA-treated animal relative to control is indicative of cell death/apoptosis. (b) Diffuse astrogliosis (GFAP) and pyramidal cell layer narrowing (NeuN) within the ipsilateral CA3 region of a KA-treated animal.



(b) Ipsilateral CA3 NeuN/GFAP 2µg KA.

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