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Iron-induced experimental cortical seizures: Electroencephalographic mapping of seizure spread in the subcortical brain areas

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Summary The iron-induced model of post-traumatic chronic focal epilepsy in rats was studied by depth-electrode mapping to investigate the spread of epileptiform activity into subcortical brain structures after its onset in the cortical epileptic focus. Electrical seizure activity was recorded in the hippocampal CA1 and CA3 areas, amygdala and caudate-putamen, in rats with iron-induced chronic cortical focal epilepsy. These experiments showed that the epileptiform activity with its onset in the cortical focus synchronously propagated into the studied subcortical brain areas. Seizure behaviours seemed to increase in correspondence with the spread of the epileptic electrographic activity in subcortical areas. Comparison of the cortical focus electroencephalographic and associated multiple-unit action potential recordings with those from the subcortical structures showed that the occurrence and evolution of the epileptiform activity in the subcortical structures were in parallel with that in the cortical focus. The intracerebral anatomic progression and delineation of seizure spread (mapped by field potential (EEG) and multiple-unit action potentials (MUA) recordings) indicated participation of these regions in the generalization of seizure activity in this model of epilepsy. The seizure-induced activation of the hippocampus appeared to evolve into an epileptic focus independent of the cortical focus. The present study demonstrates the propagation of epileptic activity from the cortical focus into the limbic and basal ganglia regions. Treatment of iron-induced epileptic rats with ethosuximide, an anti-absence drug, resulted in suppression of the epileptiform activity in the cortical focus as well as in the subcortical brain areas.

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Introduction

Iron, in the form of $FeCl_2$ or $FeCl_3$, injected focally in the cerebral cortex of rats produces a spontaneously discharging chronic epileptogenic focus.[1] This experimental epileptogenic focus constitutes an appropriate model of human post-traumatic epilepsy (complex partial seizures resulting from traumatic brain injury),[2] and has often been investigated to understand the mechanism of clinical post-traumatic epilepsy.[1,3,4–7] The epileptiform electrographic activity in the iron-induced focus is thought to result from the reaction of cortical neurons to iron-induced oxidative stress, i.e. the neuronal membrane lipid-peroxidation caused by reactive oxygen species.[3,8,9] In humans, traumatic brain injury (resulting from closed head injury) is known to be a risk factor for subsequent development of clinical post-traumatic epilepsy. In the injured brain tissue, hemolysis of extravasated blood cells results in the deposition of iron within the brain tissue. The iron is thought to induce oxidative stress that may be responsible for post-traumatic epileptogenesis.[4] In an iron-induced epileptic focus, the astrocytal uptake of glutamic acid was found to be disrupted. This disruption appeared to be due to an oxidative stress-induced decrease in glial glutamate transporter protein.[2] Decreased levels of the transporter protein would lead to increased levels of extracellular glutamate which is likely to contribute to epileptogenesis. Increased levels of excitatory amino acids were found to be associated with traumatic brain injury.[10,11] Activated astrocytes themselves were also found to release glutamate in experimental seizures.[12]

In $FeCl_3$-induced epilepsy, the focal epileptiform activity spreads from its site of origin into the entire cerebral cortex of both the cerebral hemispheres.[13,14] In the process of this generalization of seizure activity, multiple subcortical areas are likely to operate as a network in the elaboration and exacerbation of spike—wave seizures after their initiation in the cortical focus.[15] Identification of subcortical brain regions involved is necessary for understanding the process of seizure generalization in the iron-model of epilepsy.[16—18] Stereo-encephalographic [depth electroencephalographic (EEG)] studies will be needed to determine the subcortical brain circuits or structures likely to be involved.[14,17,19] Our initial study of iron epilepsy has shown that the cortical focal epileptic activity is propagated to the thalamus, locus coeruleus and substantia nigra.[20,21] The purpose of the present study was to determine whether the subcortical spread of the epileptiform activity involves some other brain regions.

In this paper, with a view to map the seizure progression to various subcortical brain areas and to further characterize the iron-model of focal epilepsy, we have examined the hippocampus (CA1 and CA3 subfields), amygdala and striatum (caudate-putamen) by simultaneously recording the electroencephalographic epileptic activity and multiple-unit action potentials (MUA) from these structures. We also tested the effect of an antiepileptic drug ethosuximide on the epileptic electrographic activity in this model. Ethosuximide has been reported[22] to desynchronize the hypersynchronizing electrophysiological activity in the reticulo-thalmocortical circuit. This action of the drug may be particularly responsible for its anti-absence effect in humans.[22] Since in the iron-induced cortical focal epilepsy, synchronization of cortical and thalamic activity is involved,[20] it would be of interest to see if ethosuximide is effective against the iron-induced epileptiform electrophysiological seizure activity. Recently, the hypothesis of the subcortical origin of absence seizures has been challenged,[23,24] and it appears that a focal seizure initiation site for the absence seizures is present in the cerebral cortex rather than in the thalamus. Furthermore, ethosuximide microinfused into the perioral region of the primary somatosensory cortex of rats with genetically determined absence seizures has been found to abolish absence seizures.[23] Ethosuximide's effect on iron-induced seizures would also be of further interest because the iron induction of epilepsy is mediated by oxidative stress-induced lipid-peroxidation, and the drug ethosuximide being a calcium channel antagonist may have an anti-lipidperoxidative effect.[25]

Animals and drugs

Male Wistar rats (60) weighing 250—300 g were housed individually in plastic cages in an air-conditioned room in the University animal house and maintained on a 12-h light:12-h dark cycle, with food and water available *ad libitum*.

$FeCl_3$, urethane, ethosuximide and all other chemicals needed were purchased from Sigma Chemical Company (USA).

Surgical procedures

All experimental protocols were approved by the Jawaharlal Nehru University, Institutional Animal Ethics Committee (IAEC).

Rats were anaesthetized with ketamine (50 mg/kg) for the duration of the surgery and placed in a rat