Biochemical Markers of Excitability in Human Neocortex

A.L. Sherwin, O. Vernet, F. Dubeau and A. Olivier

ABSTRACT: We measured biochemical markers of excitability in brain excised for neurosurgical therapy of epilepsy. Intraoperative electrocorticography was used to identify and compare samples from regions of persistent interictal spike discharges and areas of the cerebral convexity which were free of interictal spiking. We found that interictal spiking was associated with elevated tissue levels of the excitatory amino acids glutamic acid (26%, p < 0.001) and aspartic acid (25%, p < 0.05). There was also a significant increase in the activity of the enzymes glutamic acid dehydrogenase (20%, p < 0.01) and aspartate acid aminotransferase (18%, p < 0.01) which are involved in their formation. There was no change in the levels of the inhibitory neurotransmitters GABA or taurine. We also found a significant increase in the activity of tyrosine hydroxylase (52%, p < 0.001), the rate controlling enzyme in catecholamine biosynthesis. There was a reduction in the density (Bmax) of cortical alpha-1 adrenoceptors (26%, p < 0.01) and a concomitant diminution of receptor coupled phosphatidylinositide metabolism (21%, p < 0.01). This blunting of inhibitory noradrenergic transmembrane signaling may contribute to a relative imbalance between excitatory and inhibitory mechanisms in epileptogenic neocortex.

RÉSUMÉ: Marqueurs biochimiques de l'excitabilité dans le néocortex humain. Certains marqueurs biochimiques de l'excitabilité ont été mesurés dans du tissu cérébral humain obtenu lors de la chirurgie de l'épilepsie. Un électrocorticogramme per-opératoire a permis d'identifier puis de comparer des échantillons de néocortex provenant d'une part de régions produisant continuellement des pointes en période inter-critique et d'autre part de zones totalement dépourvues de ce type d'anomalie électrique. La présence de pointes a pu être associée à une élévation des taux tissulaires des acides aminés excitateurs glutamate (26%, p < 0.001) et aspartate (25%, p < 0.05) ainsi que de l'activité des enzymes responsables de leur formation: la glutamate deshydrogénase (20%, p < 0.01) et l'aspartate aminotransférase (18%, p < 0.01). Par contre, aucune altération des taux des neurotransmetteurs inhibiteurs GABA et taurine n’a été objectivée. Nous avons également démontré une augmentation significative de l’activité de la tyrosine hydroxylase (52%, p < 0.001), l’enzyme modulant la biosynthèse des catécholamines. La présence de pointes a été de surcroît cor­rélée avec une diminution de la densité (Bmax) des récepteurs alpha-1-adrénergiques corticaux (26%, p < 0.01), ainsi que de l’hydrolyse du phosphatidylinositol (PI) qui leur est associée (21%, p < 0.01). Cette diminution du signal trans­membranaire inhibiteur noradrénérique pourrait contribuer à un déséquilibre relatif entre les influences excitatrices et inhibitrices dans le néocortex épileptique.


Location-related epilepsies have numerous etiologies but have in common a period of maturation, during which aberrant local neuronal networks are created and eventually develop a capacity for spontaneous synchronous discharges.1-3 Human tissue analysis is essential in this disorder because the prolonged latent period cannot be fully reproduced during the short life-span of laboratory animals.4 Epileptogenic lesions are subtle and require precise EEG localization; before operation all patients undergo extensive EEG-video seizure monitoring with various types of extracranial and in some cases intracranial electrodes.5,6 Antiepileptic drugs are partially or completely withdrawn to enhance the localization of epileptic activity. Sites of focal spiking as well as surrounding nonspiking regions are identified by means of electrocorticography (ECOg) with surface electrodes at operation under local anesthesia.7,8 Temporary depth electrodes are employed during surgery to detect epileptic activity in the hippocampus and amygdala. On the basis of the ECOb findings, we classify neocortical specimens into the following groups: (A) Spontaneous interictal spiking restricted to either the anterior or posterior portions of one of the first two temporal gyri. In this paradigm, a nonspiking control sample is obtained from the quiescent half of the same gyrus.9 (B) Widespread spontaneous interictal spiking recorded from the lateral surface of the temporal lobe. In this model, nonspiking control samples are obtained from patients in whom the epileptogenic lesion was confirmed to the hippocampus and/or amygdala sparing the cerebral convexity.10 (C) Neocortical specimens obtained from patients with no history of seizures are also used as controls.
These histologically normal tissues were excised during the course of deep seated tumor removal. After excision of samples for biochemical analysis, immediately adjacent regions of cortex and white matter are fixed or otherwise prepared for histopathology and immunochemistry. The extent of neuronal loss and astrocytosis are appraised separately and graded as described by Robitaille. Spiking and nonspiking areas sampled for biochemical determinations are always located outside regions of gross atrophy or other structural abnormalities.

Measurements of receptors, neurotransmitters, and regulatory enzymes in brain excised for surgical therapy of epilepsy have identified a number of biochemical markers of neocortical excitability. These metabolic abnormalities have been delineated further by examining signal transduction mechanisms and intracellular messengers in freshly prepared in vitro human neocortical slices. The electrographic hallmark of an epileptic focus is the ictal, or seizure, discharge accompanied by the patient's habitual attack. The interictal spike discharge remains however the earliest and simplest indicator of a region with a high epileptogenic potential. In appraising biochemical data derived from analysis of epileptogenic brain it is important to take into consideration whether the tissue sample was obtained: (a) during or following a seizure discharge accompanied by behavioral attack (ictal); (b) from a site of persistent interictal spike discharges (spiking); (c) or from a region of neocortex with a quiescent background activity (nonspiking).

Excitatory Amino Acids as Markers

The excitatory amino acid neurotransmitters (EAA) glutamic and aspartic acid have consistently been implicated in the pathophysiology of both experimental and human epileptic conditions. These observations have been highlighted by evidence that some EAA antagonists are potent anticonvulsants. We found a significant increase in the levels of glutamic acid (+26%, p < 0.001) and aspartic acid (+25%, p < 0.05) in actively spiking and white matter are fixed or otherwise prepared for biochemical analysis, immediately adjacent regions of cortex that are likely able to transiently increase glutamic and aspartic acid levels when stimulated by interictal spiking. There is a basal release of glutamic acid from brain surface which is augmented by interictal discharges. However, chronic epileptogenic cortex has a capacity to store and maintain higher total tissue glutamate concentrations, likely because of the relative increase in the size of the glial compartment. This may not be the case, however, if ictal epileptic foci could be examined. In this situation, it is most likely that the tissue levels of glutamic and aspartic acid would be diminished in keeping with the findings in cobalt-induced seizure foci in cats.

Noradrenergic Markers

The central noradrenergic system is another source of biochemical markers of excitability because it modulates synaptic activity by altering postsynaptic responsiveness to other neurotransmitters including GABA. Iontophoretic studies indicate that norepinephrine (NE) plays a mainly inhibitory role in the neocortex. Noradrenergic fibers arising in the locus coeruleus are widely distributed throughout the neocortex in a laminar fashion. Cortical noradrenergic afferents play a definitive role in the pathogenesis of several animal models of epilepsy. We found that the activity of tyrosine hydroxylase (TH), which is the rate-limiting enzyme in catecholamine synthesis, was significantly increased (mean +52%) in spiking versus nonspiking epileptogenic cortex (Table 1). The activity of the various TH enzymes reflect neocortical discharge rates; depolarizing stimuli including raised extracellular (K+) levels enhance total TH activity.

Amino Acid Enzymes Regulating Metabolism as Markers

Chronically epileptogenic human brain appears to have a greater capacity for the rapid formation of excitatory amino acids than the naive neocortex of animal models. This notion is supported by an examination of enzymes that regulate glutamic and aspartic acid metabolism (Figure 1). We found a modest but significant increase (+20.4%, p < 0.001) in the activity of glutamic acid dehydrogenase (GDH) in interictal spike foci versus nonspiking control neocortex. This enzyme plays an important role in the formation of glutamic acid by the reductiveamination of alpha-ketoglutarate derived from the tricarboxylic acid cycle. As GDH is localized to astrocytes, this reaction also serves to detoxify free ammonia which is a byproduct of epileptic activity. In collaboration with Dr. S. Kish, we also examined the behavior of aspartic acid aminotransferase (AAT), an astrogial enzyme involved in both aspartic and glutamic acid metabolism. This enzyme catalyzes the reversible reaction converting aspartic acid to glutamic acid and oxaloacetate to alpha ketoglutarate. AAT activity was significantly increased (+18%, p < 0.01) in spiking vs. nonspiking neocortex. A similar association between interictal epileptic discharges and the activity of enzymes involved in acetylcholine metabolism has been reported. A neurotoxic side effect of EAA excess would be neuronal loss as well as astrogliosis in both the gray matter and immediately subcortical white matter. Interestingly, there was a statistically significant correlation (p < 0.02, N = 16 patients) between the activity of AAT measured in the direction of aspartic acid formation and the graded histopathological severity of reactive astrocytosis. The abundance of mature metabolically-activated astrocytes likely contributes to enhanced EAA turnover in epileptogenic zones. In human neocortex, mature astrocytes with increased GDH and AAT activity are likely able to transiently increase glutamic and aspartic acid levels when stimulated by interictal spiking. There is a basal release of glutamic acid from brain surface which is augmented by interictal discharges. However, chronic epileptogenic cortex has a capacity to store and maintain higher total tissue glutamate concentrations, likely because of the relative increase in the size of the glial compartment. This may not be the case, however, if ictal epileptic foci could be examined. In this situation, it is most likely that the tissue levels of glutamic and aspartic acid would be diminished in keeping with the findings in cobalt-induced seizure foci in cats.

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Phosphatidylinositol Metabolites as Markers

Adrenergic mediated phosphatidylinositol (PI) metabolism was measured in vitro in neocortical tissue slices. We observed a significant reduction (mean, 21%, p < 0.01; N = 25 patients) in PI metabolism as measured by inositol phosphate (IP-1) accumulation (Table 1). There was a good correlation between the frequency of epileptic spike discharges recorded during intraoperative ECoG and the reduction in the PI response (Figure 2). The relative diminution in maximal epinephrine stimulated IP-1 accumulation (Emax) is to be expected in light of the decrease observed in alpha-1 adrenoceptor density (Bmax). In neuronal primary cultures, exposure of the cells to norepinephrine for 2 hours or more significantly downregulated the number of alpha-1 cell surface recognition sites and reduced alpha-1 receptor stimulated PI metabolism. Brief exposure (< 2 h) of neuronal primary cultures to NE however, results in a loss of responsiveness without a change in the density (Bmax).

Table 1: Comparison of Biochemical Markers of Catecholamine Metabolism in Epilepsy Surgery Specimens and Nonspiking Human Neocortex

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<tr>
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<th>Spiking</th>
<th>Nonspiking</th>
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<tr>
<td>Tyrosine hydroxylase activityb</td>
<td>18.7 ± 2.0*(10)</td>
<td>12.3 ± 2.0(10)</td>
</tr>
<tr>
<td>α-1 adrenoceptors density (Bmax)b</td>
<td>156.5 ± 13.1*(8)</td>
<td>227.0 ± 17.5(8)</td>
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<tr>
<td>Maximal PI response (% basal)d,e</td>
<td>526 ± 19*(19)</td>
<td>669 ± 35(9)</td>
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Values are mean ± SEM, * p < 0.01 (two-tailed t-test), parenthesis signify number of patients.

a - nmol/h/wet wt.; b - spiking and nonspiking samples from the same patient; c - fmol/mg protein; d - alpha-1 receptor coupled PI response expressed as a percentage of total (3H)IP1 accumulation; e - spiking and nonspiking samples from two separate groups of patients; f - non epileptic neurosurgical patients
The effect is mimicked by exposing the cells to phorbol ester which mechanism involves phosphorylation of various components of charges could contribute to the desensitization of alpha-1 adrenergic transmission may reflect a localized diminution in activity modulates PI transmembrane signalling either by receptor-effector coupling. We suggest that epileptic of alpha-1 receptor sites (desensitization). Interestingly, this effect is mimicked by exposing the cells to phorbol ester which activates protein kinase C, suggesting that the desensitization mechanism involves phosphorylation of various components of the system. In rat neocortical slices, NE stimulation of alpha-1 receptor mediated PI hydrolysis is inhibited by pretreatment with glutamic acid. This finding provides a mechanism whereby the excitatory amino acids released during epileptic discharges could contribute to the desensitization of alpha-1 adrenoceptor mediated responses. We suggest that epileptic activity modulates PI transmembrane signalling either by receptor downregulation or by other mechanisms of desensitization that modify receptor-effector coupling. This reduction of noradrenergic transmission may reflect a localized diminution in various neurotransmitter mediated inhibitory mechanisms in epileptic neocortex.

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