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Neuroglia: Gliosis and Focal Epilepsy

Abstract. *Normal neuroglial cells buffer the extracellular space around neurons and presynaptic terminals against increases in potassium ions. Epileptic foci resulting from brain injury are characterized by areas of intense fibrillary gliosis bordering neuronal tissue. The known pathological changes that occur in gliosis may impair glial control of extracellular potassium ions and lead to excessively excitable neuronal border regions.*

Penfield and co-workers (*1*) established that the glial scar (alone or as part of a meningocerebral cicatrix) is the irritative source for the focal seizures that develop after anoxic or traumatic brain damage. On the basis of our physiologic findings (*2*) and on known pathological observations (*1*), we now propose a pathophysiologic explanation of posttraumatic focal epilepsy.

During neuronal activity K^+ is released into the extracellular space. Increases in extracellular K^+ concentration lead to neuronal depolarization and to an increase in excitability of presynaptic terminals (*3*). In the normal brain the neuroglial K^+ spatial buffering system (*4*) transports enough K^+ away from sites of K^+ release to prevent marked (and undesirable) increases in neuronal excitability (*2*).

Intracerebral and intraventricular injections of potassium salts have long been known to induce seizures (*5*). Zuckermann and Glaser (*6*), who employed chronic intraventricular injection cannulas to enforce local K^+ accumulations to the normal feline dorsal hippocampus, produced seizures with K^+ concentrations as low as 8.1 meq/liter. Short trains of single shocks to the dorsal hippocampus reduced the K^+ concentration necessary for seizure induction to 5.4 meq/liter, and Zuckermann and Glaser noted that increased extracellular K^+ might similarly be a factor in precipitating human seizures.

In gliotic scar formation, protoplasmic astrocytes reorient, proliferate, fill with glial filaments, and become fibrous astrocytes (*1*). In "epileptogenic scars," fibrous glial cells, in various stages of proliferation and degeneration, border thinned-out gray matter or islands of relatively normal neurons (*1*). Neuroglial attachments to blood vessels, which are probably required to move K^+ into capillaries, disappear (*1*). The marked capillary decrease in the border zone (*1*) suggests a lowered glial metabolic activity. Intermittent ischemia in the glial border zone leads to patches of acute neuroglial degeneration (*1*). Brain slices of scar tissue from epileptic patients, unlike slices of normal brain, fail to extrude Na^+ and take up K^+ (*7*).

Structural, vascular, and metabolic defects likely to impair the "passive" K^+ spatial buffering system and the "active" K^+ uptake have thus been demonstrated or are strongly suggested for epileptogenic glial scars. Moreover, human fetal astrocytes raised under relatively anoxic conditions can proliferate but cannot maintain the high internal K^+ concentration needed to establish a high, resting membrane potential, and these cells move relatively little K^+ across their membrane in response to increases in extracellular K^+ (*8*). An abnormal handling of K^+ in severe gliosis may bear directly on neuronal hyperexcitability in focal epilepsy.

We therefore propose as a hypothesis that a decisive, but by no means the only, factor in the development of posttraumatic focal epilepsy is a degradation of neuroglial function, especially with respect to the spatial buffering of K^+ and active K^+ uptake. As the safety factor with respect to extracellular K^+ control decreases, ordinarily less critical factors (such as hyperthermia, alkalosis, and brain edema) can more easily initiate a given seizure. [If glial cells metabolize neurotransmitters (*9*), loss of such a function could be an additional factor altering neuronal excitability.] The known effects of anticonvulsants on Na^+ and K^+ balance (*10*) are consistent with our hypothesis. If deafferentation hypersensitivity is a factor in the development of focal epilepsy, as seems likely (*11*), it would appear to be a contributory rather than a sufficient factor because, although it would be expected to occur as frequently after clean surgical excision of brain tissue as after brain damage, the surgical procedure is rarely followed by the development of a seizure focus (*1*). The initiation of focal seizures that may follow surgical traction on a scar might result from interference with neuroglial metabolism secondary to vasospasm (*1*) and mechanically induced dendritic depolarization, or both, as suggested by Ward (*12*). Contraction of glial filaments might similarly produce scar traction.

Our hypothesis may offer new approaches to both treatment and prophylaxis of posttraumatic epilepsy. At present, therapy is generally directed against the convulsive activity associated with the scar rather than against the scarring process. Glial scarring is not a static process but may proceed relentlessly over decades (*1*). Adjacent neurons are victims of a chronic inflammatory process involving vascular elements and reactive gliosis. For this reason, the relevance of developing and testing antiinflammatory agents for selective effects against gliosis is apparent [see also (*13*)]. If the fibroblastic invasion and collagen deposition, which occur when the meninges are concomitantly injured, impair K^+ accessibility to glia, then efforts might also be directed against this mesenchymal aspect of scar formation.

Lastly, if development of posttraumatic focal epilepsy is dependent on genetic predisposition (*14*), we wonder whether the presence or absence of epileptogenicity in association with human glial scars follows genetically determined variations in the extent of scar formation. In any case, a biochemical understanding of the gliotic scar process might allow control of the deleterious extremes of scar formation that lead to focal epileptic discharge.

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Acetylcholine Sensitivity and Distribution on Mouse Neuroblastoma Cells

Abstract. *Action potentials resulted from iontophoretic application of acetylcholine to cloned mouse neuroblastoma cells maintained in culture. Cell bodies were sensitive to acetylcholine, but half the cells were insensitive along one of their processes, suggesting an analogy with normal axons and dendrites. Some cells were electrically coupled via their processes, but no chemically mediated synaptic transmission was seen.*

Sensitivity of innervated skeletal muscle fibers to acetylcholine (ACh) is confined to regions of the cell surface close to the synaptic terminals at the end plate. After denervation this sensitivity increases and covers the whole muscle fiber surface (*1*). Innervated parasympathetic neurons are most sensitive to ACh close to synaptic endings but develop a uniform high sensitivity after degeneration of the presynaptic nerve fibers (*2*). Embryonic muscle, like denervated muscle, is receptive to innervation and is sensitive to ACh over its whole surface (*3*), but it is not known whether the same is true of newly differentiated nerve cells before they receive synaptic contacts.

To study the sensitivity of newly formed nerve cells to transmitter, we have used a cloned line of mouse neuroblastoma cells (*4*). This line is capable of morphological "differentiation" in vitro, and thus provides a simple system for studying this question. These cells are propagated in suspension as round cells, but if they are plated on a surface of glass, collagen, or acid-treated plastic, many send out processes and take on some of the morphology of nerve cells. The plated cells show considerable variations in form, ranging from round cells that are distinguishable from the suspended cells only by their larger size, through cells with as many as a dozen short processes, to cells with one or two processes that are several millimeters in length.

Physiological properties of cells plated in plastic tissue-culture dishes and cultured for from 3 to 5 weeks were investigated by intracellular re-

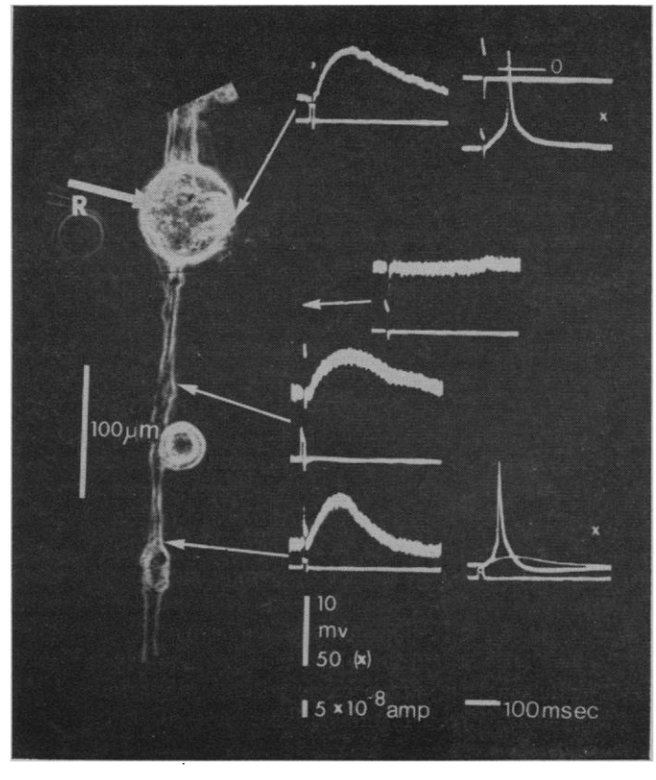

Fig. 1. Distribution of sensitivity to acetylcholine on the soma and process of a neuroblastoma cell 1 month after plating. The top of each pair of traces shows the potential change elicited by iontophoretic application of ACh to the point on the cell surface marked by the corresponding arrow. The current through the ACh electrode is monitored in each bottom trace (10-msec pulses were used in these and all subsequent records). The position of the recording electrode is marked by the heavy arrow (*R*). The top two sets of records show the response to ACh applied to the cell body; the left-hand set, at high gain (calibration, 10 mv), shows an ACh potential (responses of similar magnitudes and time course resulted no matter to which point on the soma the ACh electrode was applied); the right-hand set, at lower gain (calibration, 50 mv), shows the response to a larger pulse of ACh (the current trace is lifted; *O* marks the line of zero resting potential) where an action potential was triggered by the ACh potential. The sets of traces below show responses to ACh applied to the cell process, and as a control for diffusion of ACh, the response to ACh delivered into the bath. The set of records at bottom right, at lower gain, shows superimposed traces of a subthreshold ACh potential and a potential large enough to trigger a spike in the cell process. The 100-msec time scale applies to all records. Records at lower gain (calibration, 50 mv) are marked x.