

GAP JUNCTIONS IN EPILEPTOGENESIS: CHICKEN OR EGG?

Epileptiform Activity in Hippocampal Slice Cultures Exposed Chronically to Bicuculline: Increased Gap-Junctional Function and Expression

Samoilova M, Li J, Pelletier MR, Wentlandt K, Adamchik Y, Naus CC, Carlen PL

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Prolonged (18-hour) exposure of cultured hippocampal slices to the type-A γ -aminobutyric acid (GABA)-receptor blocker, bicuculline methiodide (BMI), 10 μM , increased the levels of connexin 43 (Cx43) and connexin 32 (Cx32) mRNAs, but not connexin 26 and connexin 36, as demonstrated by RNase protection assays. The levels of Cx43 and Cx32 proteins in membrane fractions detected by Western blotting also were significantly increased. Immunoblotting indicated that BMI also promoted a significant expression of the transcription protein c-fos. The rate of fluorescence recovery after photobleaching, an index of gap-junctional coupling, also was significantly increased, whereas it was blocked by the gap-junctional blocker, carbenoxolone (100 μM). Extracellular recordings in CA1 stratum pyramidale, performed in BMI-free solution, demonstrated that BMI-exposed cultures possessed synaptic responses characteristic of epileptiform discharges: (a) significantly greater frequency of spontaneous epileptiform discharges, (b) postsynaptic potentials with multiple population spikes, and (c) significantly longer duration of primary afterdischarges. Carbenoxolone (100 μM), but not its inactive analogue, oleanolic acid (100 μM), reversibly inhibited spontaneous and evoked epileptiform discharges. The findings of BMI-induced parallel increases in levels of gap-junction expression and function, and the increase in epileptiform discharges, which were sensitive to gap-junctional blockers, are consistent with the hypothesis that increased gap-junctional communication plays an intrinsic role in the epileptogenic process.

COMMENTARY

Gap-junctional coupling between neurons has reemerged as a critical property in the generation of epileptiform discharges. The present article by Samoilova et al. considers whether dynamic interplay might exist between the generation of additional gap junctions and paroxysmal discharge such that the formation of new gap junctions is responsible for epileptogenesis. The issue is complicated because, rather than considering an all-or-none event, the authors are studying whether the duration of a stimulus-induced epileptiform discharge is lengthened by a previous exposure to bicuculline. Thus *antiepileptogenic* here means that this persistent lengthening would be prevented, as opposed to *antiepileptogenic* meaning the prevention of *any* seizure development. Thus the investigators define *antiepileptogenic* as the prevention of persistent lengthening of the epileptiform discharge rather than the prevention of *any* seizure development. The experiments used hippocampal slice cultures to determine that primary afterdischarge (i.e., the epileptiform activity that was induced after 100-Hz stimulation of the Schaffer collaterals for 2 sec recorded in CA1) greatly increased after an 18-hour exposure to high-dose (10 μM), but not to low-dose (1 μM) bicuculline. The 10 μM exposure induced longer seizure discharge, presumably as a direct consequence of GABA_A-receptor blockade (induced by bicuculline), and this enhanced activity then persisted for a time, even after removal of the convulsant agent. It is not clear how long the abnormal activity persisted, but it is clear that inhibitory events recovered by the time the experiments were performed.

Associated with this persistent epileptiform activity was an increase in gene transcription of mRNAs that code for connexin proteins—the proteins that form the clusters of intercellular channels that make up gap junctions. In addition, increased connexin proteins were detected. The authors showed that uncoupling agents, including carbenoxolone and octanol, could stop epileptic activity during the exposure of slices to the agent and that this anticonvulsant action was reversible on washout of these drugs.

Further, Samoilova et al. performed an interesting experiment to demonstrate that additional gap junctions were present in bicuculline-exposed slices. This technique involved measuring the rate of fluorescence recovery after photobleaching. Thus

a portion of the slice in which coupled cells were filled with dye was photobleached (i.e., exposed to fluorescent light until the fluorescence is bleached out), so that the only way that fluorescence could be restored was by influx of unbleached dye from (unbleached) neighboring cells, via gap junctions. The faster the rate of recovery, the greater the number of gap junctions must be present. This experiment confirmed the presence of additional coupling in epileptic cultures.

This discovery is an interesting demonstration of the dynamic nature of gap junctions, which is reminiscent of other forms of use-dependent modifications of synaptic transmission (posttetanic and long-term potentiation of excitatory postsynaptic activity associated with chemical synaptic transmission). Clearly, additional intercellular channels were produced, which appear to be functional and participate in the heightened epileptic activity recorded. Nonetheless, the issue of whether gap-junctional transmission is involved in the genesis of this persistent enhancement of epileptiform activity remains unclear. A key experiment to perform would be to expose cultured slices to both bicuculline and an uncoupling agent for the entire 18 hours. If epileptic activity is not enhanced after slices are re-

turned to normal medium, then uncoupling may be antiepileptogenic.

What do uncouplers do under control conditions in this preparation? Do they prevent the persistent afterdischarge induced by repetitive stimulation in cultures? As noted in the text, uncoupling agents applied to slices desynchronize epileptic activity in a variety of preparations, both acute ones involving convulsants, such as 4-aminopyridine and low-magnesium solutions, and chronic ones. However, in these experiments, gap-junction blockers were not antiepileptogenic, and epileptic activity returned on removal of the agents.

Of course, the present findings are subject to the added vagaries of potential differences between slice cultures and intact brain, and we await corroboration of these results in more intact preparations. Although the authors suggest that increased coupling is the cause for the enhancement of afterdischarge, the experiments presented cannot rule out the possibility that these alterations in coupling are an epiphenomenon. Are they cause, or effect—the egg or the chicken?

by Larry S. Benardo, M.D., Ph.D.