

EXPLOITING THE OTHER INHIBITORY ION: KCNQ POTASSIUM CHANNELS AND REGULATION OF EXCITABILITY IN DEVELOPING AND MATURE BRAIN

Epileptiform Activity Induced by Pharmacologic Reduction of M-Current in the Developing Hippocampus In Vitro

Pena F, Alavez-Perez N

Epilepsia 2006;47(1):47–54

PURPOSE: Benign familial neonatal convulsions (BFNCs), an inheritable epilepsy that occurs in neonates but not in adults, is caused by hypofunctional mutations in genes codifying for the M-type K⁺ current. In an attempt to develop an in vitro model of this disease, we tested whether blocking M-current with linopirdine induces epileptiform activity in brain slices from animals of different ages.

METHODS: Horizontal hippocampus–entorhinal cortex slices were obtained from neonatal (1–2 weeks after birth) and adult (8–9 weeks after birth) rats. Extracellular field recordings of the CA1 region were performed. After recording control conditions, linopirdine was added to the bath, and field activity was recorded continuously for 3 hours. A drug 4-aminopyridine, commonly used to induce epileptiform activity in vitro, was used as a control for our experimental conditions.

RESULTS: Bath perfusion of linopirdine induced epileptiform activity only in slices from neonatal rats. Epileptiform activity consisted of interictal-like and ictal-like activity. In slices from adult rats, linopirdine induced erratic interictal-like activity. In contrast, 4-aminopyridine was able to induce epileptiform activity in slices from both neonatal and adult rats.

CONCLUSIONS: We demonstrated that blockade of M-current in vitro produces epileptiform activity with a developmental pattern similar to that observed in BNFCs. This could be an in vitro model that can be used to study the cellular mechanisms of epileptogenesis and the developmental features of BFNCs, as well as to develop some therapeutic strategies.

Conditional Transgenic Suppression of M Channels in Mouse Brain Reveals Functions in Neuronal Excitability, Resonance and Behavior

Peters HC, Hu H, Pongs O, Storm JF, Isbrandt D

Nat Neurosci 2005;8(1):51–60

In humans, mutations in the KCNQ2 or KCNQ3 potassium-channel genes are associated with an inherited epilepsy syndrome. We have studied the contribution of KCNQ/M-channels to the control of neuronal excitability by using transgenic mice that conditionally express dominant-negative KCNQ2 subunits in brain. We show that suppression of the neuronal M current in mice is associated with spontaneous seizures, behavioral hyperactivity and morphological changes in the hippocampus. Restriction of transgene expression to defined developmental periods revealed that M-channel activity is critical to the develop-

ment of normal hippocampal morphology during the first postnatal weeks. Suppression of the M current after this critical period resulted in mice with signs of increased neuronal excitability and deficits in hippocampus-dependent spatial memory. M-current-deficient hippocampal CA1 pyramidal neurons showed increased excitability, reduced spike-frequency adaptation, attenuated medium afterhyperpolarization and reduced intrinsic subthreshold theta resonance. M channels are thus critical determinants of cellular and neuronal network excitability, postnatal brain development and cognitive performance.

COMMENTARY

Drugs that potentiate the opening of GABA_A receptors are mainstays in the treatment of epilepsy and status epilepticus. GABA_A receptors are Cl⁻ channels whose openings drive the neuronal membrane potential toward the Cl⁻ reversal

potential, which is near the action potential threshold at birth and gradually becomes hyperpolarizing during the first weeks of life (1,2). Neurons also express a multitude of K^+ channels whose openings drive the membrane potential toward the K^+ reversal potential. Such K^+ channel openings are strongly hyperpolarizing both in neonates and adults. Although this functional profile makes K^+ channel openers an appealing category for antiepileptic drug development, pursuing this approach is not without challenges. The first challenge is the diversity of the K^+ channels themselves, which is markedly greater than other ion channel types (3). The second challenge is the relative difficulty of identifying potent, selective K^+ channel activator molecules. Whereas $GABA_A$ receptors have large extracellular domains containing neurotransmitter binding and modulatory sites that strongly regulate channel opening, K^+ channels have little extracellular domain and are regulated in complex, subtle, and overlapping ways by intracellular metabolism and membrane voltage. How can we identify, among the nattering crowd of highly related K^+ channels, potentially promising targets, that is, targets that are now commonly referred to as “druggable”?

Recent genetic and pharmacological studies point to the KCNQ subfamily of neuronal voltage-gated K^+ channels as candidate epilepsy drug targets (4). KCNQ2 and KCNQ3 subunit mutations cause benign neonatal familial seizures (BNFS), a dominantly inherited syndrome of recurrent seizures that begin in the first few days of life and remit after a few weeks. Selective KCNQ channel opener drugs have been shown to exhibit antiepileptic activity in animal and early stage human trials (including retigabine, which is currently in stage III clinical trials for adult partial epilepsy). Selective KCNQ channel blockers, including linopirdine and XE991, have also been identified, facilitating experimental studies. KCNQ channels mediate an extensively studied neuronal K^+ current, called M-current, which plays a central role in controlling the repetitive firing of neurons (5). So, KCNQ channels are functionally important and potentially druggable. But, we know very little about their specific functions in CNS neurons and circuits.

Seeking a model for analyzing KCNQ function during development, Peña and Alavez-Pérez prepared hippocampal-entorhinal brain slices from rats between 1 and 9 weeks of age. Using extracellular field recordings in hippocampal area CA1, they looked for changes in neuronal network synchronization during treatment with the KCNQ blocker linopirdine. In control slices, they found that slices taken from neonatal brain exhibit spontaneous bursting activity but that this activity is absent from slices cut from mature brain. Linopirdine caused the appearance of longer bursts of synchronized activity (which Peña and Alavez-Pérez termed “ictal-like activity”) in slices from 1 to 3 week old rats but not in older animals. By contrast, 4-

aminopyridine, which blocks several subtypes of K^+ channels but not KCNQ channels, produced enhanced bursting equally well in slices from immature and mature animals. These findings support the notion that KCNQ channels are particularly important for suppressing epileptic activity in neonatal brain and suggest that linopirdine-treated slices represent a simple model of the physiological state in which KCNQ channel activity is selectively diminished.

These results are of particular interest when viewed in the context of a recent study by Peters and colleagues, describing mice genetically engineered to block KCNQ channels *in vivo*. Peters et al. understood that KCNQ channels are assembled from four subunits that are arranged symmetrically around the water-filled transmembrane ion path or pore. Building on earlier work by others (6), they found a single amino acid position near the narrowest part of the pore, where mutation of only one subunit was sufficient to produce a channel tetramer that assembled normally but was completely blocked to ions. The investigators then engineered transgenic mice that overexpressed this channel-blocking mutant subunit, under the control of genes constituting a tetracycline responsive expression system. This combination of transgenes enabled the investigators to turn on and off KCNQ channel activity at will. Giving doxycycline (a tetracycline analogue) to these mice prevented expression of the blocking subunit and allowed normal KCNQ channels to form. Withholding doxycycline permitted blocked channels containing the mutant subunit to assemble *in vivo*.

Peters et al. found that blocking KCNQ channels throughout life resulted in adult mice with a severe phenotype. At the behavioral level, these mice showed recurrent spontaneous seizures, impaired spatial learning, and marked hyperactivity in open-field testing. Studies of hippocampal sections revealed laminar disorganization of area CA1. Pyramidal neurons exhibited diminished M-type K^+ current and were hyperexcitable, showing increased firing responses to depolarizing stimuli and diminished after-hyperpolarizations following action potentials. To separate the contributions of KCNQ channel blockade during development from the effects of blockade during the adult period, Peters et al. then studied additional mice, that had been administered doxycycline during the critical first 1–3 weeks of life. Such mice would be expected to have normal KCNQ channels during early development, but blocked channels thereafter. As adults, this group of mice exhibited normal hippocampal morphology, was not hyperactive, and did not show overt behavioral seizures. They did show impaired spatial learning, reduced M-type K^+ current, and neuronal hyperexcitability—effects that apparently were due to the KCNQ channel blockade in adulthood. A comparison of the results from the two groups of mice implicates KCNQ channel

blockade during early postnatal development in a severe behavioral phenotype that includes epilepsy, hyperactivity, and anatomical disorganization in the hippocampus.

Peters et al. do not describe the physiology or behavior of immature mice during transgenic KCNQ channel blockade. It is tempting to hypothesize that such mice experience ongoing “ictal-like” activity—paralleling the *in vitro* hyperexcitability Peña and Alavez-Pérez elicited by blocking channels pharmacologically with linopirdine. Further work will be required to test whether such *in vivo* neonatal hyperexcitability is present and whether it is such hyperexcitability per se that produces the disordered anatomy and behavioral abnormalities Peters et al. observe. Together, these papers further highlight the importance of KCNQ channels, especially in the neonatal period. It is likely that the onset of a more hyperpolarized Cl⁻ reversal potential (and, with that, more robust GABAergic inhibition) contributes to the diminishing effects of KCNQ channel blockade with age (1,2). However, understanding how and why nature uses its two inhibitory ions in such different ways during the early postnatal period is far from complete. The model systems introduced by

Peña and Alavez-Pérez and by Peters et al. provide new avenues for pursuing these questions.

by Edward C. Cooper, MD, PhD

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