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INTERNATIONAL SOCIETY FOR NEUROCHEMISTRY SYMPOSIUM: CHANNELS AND SYNAPSES: CHEMISTRY MEETS NEUROLOGICAL DISEASES?

THE ROLE OF T-TYPE CA^{2+} CHANNELS IN THE SENSORY GATING IN THE BRAIN

HEE-SUP SHIN

Korea Institute of Science & Technology, Seoul 136-791, Korea

Low threshold Ca^{2+} currents mediated by á1G T-type channels underlie burst spike activities of relay neurons in the thalamus. We have previously reported that knock-out mice for á1G T-type channels show an enhanced nociceptive response to visceral pain, accompanied by an increase in tonic spikes in the absence of burst spikes in thalamic relay neurons (Science, 2003). These results raised a possibility that á1G T-type channels are involved in thalamic sensory gating, blocking the relay of the pain signals to the cortex. We have tested this hypothesis by using several different sensory modalities: startle responses to auditory or tactile stimuli, and the response to chronic inflammatory pain. First, the mutant mice showed an enhanced startle response to auditory stimulations. The auditory brainstem recording (ABR) results indicated that the enhanced response was not due to increased input signals from the cochlear to the brainstem. Second, the mutant also displayed an enhanced startle response to tactile stimuli. Third, the mutant showed a selective increase in the late phase response to an intradermal injection of formalin into the hindpaw, which is known to be controlled by a supraspinal mechanism. Therefore, the mutant showed enhanced responses to sensory inputs of four different modalities, strongly supporting the idea that á1G T-type channels are required in thalamic sensory gating. We suggest that the burst spikes induced by the low threshold Ca^{2+} currents are the key element in this thalamic function. Furthermore, the mutant mice showed mania-like behaviors, an increased alcohol preference, and resistance to alcohol-induced hypnotic effect. These behavioral consequences will be discussed with regard to the sensory gating failure of the mutant.

MUTANT CALCIUM CHANNELS AND MIGRAINE

DANIELA PIETROBON

Department of Biomedical Sciences, University of Padova, Padova, Italy

Mutations in CACNA1A, the gene encoding the pore-forming subunit of $Ca_V2.1$ (P/Q-type) calcium channels, cause a group of dominantly inherited human neurologic disorders, including familial hemiplegic migraine type-1 (FHM-1), episodic ataxia type-2 (EA-2) and spinocerebellar ataxia type 6. P/Q-type calcium channels are located in presynaptic terminals and somatodendritic membranes throughout the brain, and play a prominent role in neurotransmitter release. Functional studies into FHM, a subtype of migraine with aura, may improve the insight into the pathophysiology of migraine, a debilitating illness that afflicts 10-15% of the population. We have investigated the functional consequences of FHM-1 mutations, at first, by expressing recombinant wild-type (wt) and mutant human $Ca_V2.1$ channel subunits in HEK293 cells and in cerebellar granule cells from *cacna1a*$^{-/-}$ mice, lacking $Ca_V2.1$ channels. These studies revealed two apparently contradictory functional effects common to all FHM-1 mutations analyzed: gain-of-function at the single channel level at V< -10 mV, due to channel activation at lower voltages and consequent increase in single channel Ca^{2+} influx, and loss-of-function at the whole-cell level at V > -20 mV due to reduced density of functional channels in the soma membrane (Tottene et al., Proc. Natl. Acad. Sci. 2002; 99:13284-89). Recently, we studied the consequences of FHM-1 mutations on $Ca_V2.1$ channels at their endogenous level of expression in neurons of a knock-in (KI) mouse model carrying the FHM-1 R192Q mutation (van den Maagdenberg et al., Neuron 2004;41:701-710). Patch-clamp recordings on cerebellar neurons in primary culture and dissociated cortical pyramidal cells revealed an increased $Ca_V2.1$ Ca^{2+} cur-

rent density in the KI mouse, as a consequence of mutant channels that open more readily and at lower voltages than wt channels. The density of functional $Ca_V2.1$ channels was similar in neurons of KI and wt mice. Gain-of-function of neuronal $Ca_V2.1$ channels resulted in a lowered threshold for induction and an increased velocity of propagation of cortical spreading depression (CSD: the likely mechanism for the migraine aura) in the intact animal. Our data show an important role of $Ca_V2.1$ channels in the initiation and spread of CSD, and point to cortical hyperexcitability as the basis for increased susceptibility to CSD in migraine. The R192Q FHM-1 mouse is a promising animal model to study migraine mechanisms and treatments.

FUNCTIONAL COMPENSATION OF CALCIUM CHANNELS AT SYNAPTIC TERMINALS FOLLOWING GENE KNOCKOUT

OSVALDO UCHITEL

Laboratorio de Fisiología y Biología Molecular, IFIBYNE CONICET, FCEyN, UBA, Ciudad Universitaria 1428 Buenos Aires.

Calcium channels of the P/Q subtype mediate transmitter release at the neuromuscular junction and at many central synapses, such as the calyx of Held. Genetic analyses have revealed an important association of the gene encoding the P/Q-type voltage dependent $Ca2^+$ channel $á_{1A}$ subunit with hereditary neurological disorders. The generation of $á_{1A}$-null mutant mice allows a critical examination of what features of neurotransmission depend on P/Q-type channels. The mutant mice showed a rapidly progressive neurological deficit with muscle weakness and ataxia, before dying 3-4 weeks after birth. Electrophysiological studies at the neuromuscular junction have shown that basic features of transmission are retained and mediated by N and R-type channels, but with significant changes in the relationship between Ca^{2+} entry and quantal release and short-term plasticity. At the calyx of Held of the knockout mice, we observed that N-type $Ca2^+$ channels functionally compensate for the absence of P/Q-type $Ca2^+$ channels and mediate transmitter release. However, while evoked paired-pulse facilitation of transitter release is prominent in wild type, this facilitation is greatly diminished in the KO. In addition, direct recording of presynaptic $Ca2^+$ currents at the P/Q knockout calyx revealed that one major functional difference was the absence of presynaptic $Ca2^+$ current facilitation. We conclude that one physiological function of P/Q channels is to provide additional facilitatory drive, so contributing to maintenance of transmission as vesicles are depleted during high through-put synaptic transmission.