SPECIFIC AND RAPID EFFECTS OF ACOUSTIC STIMULATION ON THE TONOTOPIC DISTRIBUTION OF Kv3.1b POTASSIUM CHANNELS IN THE ADULT RAT

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Abstract—Recent studies have demonstrated that total cellular levels of voltage-gated potassium channel subunits can change on a time scale of minutes in acute slices and cultured neurons, raising the possibility that rapid changes in the abundance of channel proteins contribute to experience-dependent plasticity in vivo. In order to investigate this possibility, we took advantage of the medial nucleus of the trapezoid body (MNTB) sound localization circuit, which contains neurons that precisely phase-lock their action potentials to rapid temporal fluctuations in the acoustic waveform. Previous work has demonstrated that the ability of these neurons to follow high-frequency stimuli depends critically upon whether they express adequate amounts of the potassium channel subunit Kv3.1. To test the hypothesis that net amounts of Kv3.1 protein would be rapidly upregulated when animals are exposed to sounds that require high-frequency firing for accurate encoding, we briefly exposed adult rats to acoustic environments that varied according to carrier frequency and amplitude modulation (AM) rate. Using an antibody directed at the cytoplasmic C-terminus of Kv3.1b (the adult splice isoform of Kv3.1), we found that total cellular levels of Kv3.1b protein—as well as the tonotopic distribution of Kv3.1b-labeled cells—was significantly altered following 30 min of exposure to rapidly modulated (400 Hz) sounds relative to slowly modulated (0–40 Hz, 60 Hz) sounds. These results provide direct evidence that net amounts of Kv3.1b protein can change on a time scale of minutes in response to stimulus-driven synaptic activity, permitting auditory neurons to actively adapt their complement of ion channels to changes in the acoustic environment. © 2010 IBRO. Published by Elsevier Ltd. All rights reserved.

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Kv3.1 is a voltage-gated potassium channel subunit that is critical for repetitive high-frequency action potential generation (Gan and Kaczmarek, 1998; Rudy and McBain, 2001). The Kv3.1 gene gives rise to two splice isoforms, Kv3.1a and Kv3.1b, which differ in the length of their C-terminal domains (Luneau et al., 1991). The longer splice variant, Kv3.1b, is regulated by protein kinase C and predominates in the mature nervous system (Kaczmarek et al., 2005). The principal neurons of the medial nucleus of the trapezoid body (MNTB) which require this channel to encode rapid temporal modulations in auditory stimuli, have proven to be a useful model for understanding how Kv3.1b is regulated. Previous work has demonstrated that Kv3.1b protein levels and current amplitudes vary systematically across the tonotopic axis of the MNTB, with the highest levels of the channel in the medial end, corresponding to neurons that respond selectively to high-frequency sounds (Li et al., 2001; Brew and Forsythe, 2005).

Chronic lack of sensory input results in the loss of this tonotopic gradient (von Hehn et al., 2004; Leao et al., 2006), however the extent to which the gradient can be modified by sensory experience is unknown. We now report that the tonotopic distribution of Kv3.1b changes on a time scale of minutes in response to specific features of the ambient sound environment.

EXPERIMENTAL PROCEDURES

Acoustic stimulation

Twenty-seven awake adult (8–12 week-old) Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, USA) were exposed to amplitude modulation (AM) stimuli for a 30 min period at 65 dB sound pressure level (SPL) in a small sound attenuating chamber. All experimental protocols involving animals were approved by the Yale University Animal Use and Care Committee. Protocols were carefully designed to minimize both the number of animals used and their suffering. A total of six stimuli were used in the study (Fig. 1). Stimuli were centered on either 4 kHz or 32 kHz carrier frequencies and one of three AM envelope conditions: a slow AM rate (0–40 Hz), intermediate AM rate (380–420 Hz), or high AM rate (380–420 Hz). Rats were randomly separated into six groups: group 1a (X1 = 4 kHz, X2 = 0–40 Hz, n = 4), group 2a (X1 = 4 kHz, X2 = 57–63 Hz, n = 4), group 3a (X1 = 4 kHz, X2 = 380–420 Hz, n = 5), group 1b (X1 = 32 kHz, X2 = 0–40 Hz, n = 4), group 2b (X1 = 32 kHz, X2 = 57–63 Hz, n = 5), and group 3b (X1 = 32 kHz, X2 = 380–420 Hz, n = 4), where X1 is the carrier frequency on which stimuli were centered, X2 is the envelope modulation range, and n is the number of rats. At the final minute of acoustic exposure, the exposure chamber was flooded with isoflurane (5% in oxygen) and rats were quickly and silently euthanized with a lethal dose of pentobarbital sodium followed by...
510 laser scanning confocal microscope system. Immunolabeling, MNTB sections were scanned using a Zeiss LSM 510 (Eugene, OR, USA) for 1–2 h at room temperature. Following secondary labeling, sections were incubated with Alexa Fluor 488 for 60 h at 4 °C (1:500 in blocking solution; antibody production and clonal antibody directed at the cytoplasmic C-terminus of Kv3.1b were pooled (81) and mean cell O.D. was computed (detailed methods in “Experimental Procedures”). At both 4 kHz and 32 kHz carrier frequencies, Kv3.1b immunoreactivity in the MNTB after 30 min of sound exposure. MNTB images from each stimulus group were examined by a blinded investigator to exclude artifacts. In calculation of the temporal envelope of the stimulus was amplitude modulated at either low rates (60–5% Hz; “400 Hz AM”) or high rates (4 kHz AM”) or high rates (400 Hz AM”). We tested the possibility that Kv3.1b expression was specifically related to rapid temporal modulation of the acoustic stimulus, and not its overall complexity, by exposing control rats to the DMR, a frequency-modulated stimulus (0–0.5 cycles/octave spectral contrast) with a broad range of low frequency temporal modulations (0–40 Hz) centered either on low (4±0.5 kHz octaves; “4 kHz”) or high (32±0.5 kHz octaves; “32 kHz”) carrier frequencies (Escabi and Schreiner, 2002). Animals were randomly divided into six groups corresponding to the six stimulation conditions, with four to five animals in each group. All tissue processing and digital image quantification was carried out by an investigator blind to the animal’s stimulation condition.

To address the first hypothesis—that Kv3.1b protein levels increase in vivo following exposure to rapid amplitude modulation—we compared the mean intensity of Kv3.1b immunoreactivity in the MNTB after 30 min of sound exposure. MNTB images from each stimulus group were pooled (n=81) and mean cell O.D. was computed (detailed methods in “Experimental Procedures”). At both the 4 kHz and 32 kHz carrier frequencies, Kv3.1b immunoreactivity was significantly enhanced when sounds were amplitude modulated at 400 Hz compared to either the 60 Hz or DMR conditions (Fig. 2a, b; one-way ANOVA with
Bonferroni post-hoc test, \(P<0.01\). We also observed a significant interaction between carrier frequency and modulation rate, such that maximal Kv3.1b labeling was achieved when the high frequency tone was modulated at the fastest rate. Data are presented as means ± SE. The number of cells (\(N\)) in each stimulus condition were as follows: \(N\) (4 kHz: DMR) = 1333; \(N\) (4 kHz: 60 Hz AM) = 1763; \(N\) (4 kHz: 400 Hz AM) = 1481; \(N\) (32 kHz: DMR) = 1970; \(N\) (32 kHz: 60 Hz AM) = 2430; \(N\) (32 kHz: 400 Hz AM) = 1535. Differences between groups were statistically evaluated using both a one-way and two-way ANOVA followed by Bonferroni post-hoc analyses. * indicates \(P<0.01\) compared to other 4 kHz stimuli; ** indicates \(P<0.001\) compared to other 32 kHz stimuli; ## indicates two-way interaction of \(P<0.001\) between carrier frequency and modulation rate.

We tested the second hypothesis—that changes in Kv3.1b levels are localized to specific regions of the MNTB—by quantifying the pattern of Kv3.1b immunoreactivity in animals exposed to 4 versus 32 kHz carrier frequencies. Each MNTB section (\(n = 99\)) was divided into 550 \(\mu\)m halves along the tonotopic axis and the number of Kv3.1b-immunoreactive cells in each half was quantified. A tonotopic ratio (medial immunoreactivity/lateral immunoreactivity) was calculated for each section, and all sections from each stimulus group were then pooled to compute group means. As stated above, low (4 kHz) carrier frequencies target the lateral region of the MNTB while high (32 kHz) carrier frequencies target the medial region. Consistent with our hypothesis, we found that in each of the 4 kHz conditions, the medial-to-lateral ratio of Kv3.1b-immunoreactive cells was lower than the medial-to-lateral ratios observed in rats exposed to 32 kHz tones (Fig. 3a: two-way ANOVA with Bonferroni post-hoc test, \(P<0.01\)). The number of Kv3.1b-immunoreactive cells per section did not vary significantly between stimulus conditions (\(\mu = 92, \sigma = 34\)).

To facilitate comparisons of Kv3.1b immunoreactivity patterns throughout the entire MNTB, we determined the precise locations of all Kv3.1b-immunoreactive cells along the tonotopic axis in each section, then pooled together all available cells from each stimulus condition (\(N=9688\)). We
The amount of Kv3.1b current in an MNTB principal neuron determines its ability to follow high rates of synaptic stimulation (Macica et al., 2003; Song et al., 2005). One established mechanism by which Kv3.1b currents become enhanced to permit high frequency firing is through dephosphorylation at Ser503 (Macica et al., 2003). Whereas Kv3.1b is basally phosphorylated at Ser503 under quiet/control conditions, it undergoes dephosphorylation following seconds to minutes of auditory stimulation in vivo or synaptic stimulation in vitro (Song et al., 2005; Song and Kaczmarek, 2006). It is not clear, however, whether changes in the phosphorylation state of the protein can provide a persistent enhancement of K⁺ currents nor whether post-translational modification alone provides neurons with an adequate dynamic range of Kv3.1 current. Our present results provide evidence that levels of Kv3.1b channel subunits, which serve to rapidly repolarize the membrane potential during trains of high-frequency firing, can become altered within 30 min of a change in the auditory environment. Although we are not able to determine the time course over which these newly synthesized channels are inserted into the plasma membrane in vivo, an increase in Kv3.1b membrane expression would serve to maintain the increase in Kv3.1b current that is triggered
by dephosphorylation of pre-existing Kv3.1b channels, and allow the neurons to maintain firing action potentials at high rates. Direct measurements of changes in Kv3.1b protein in the plasma membrane will require either electron microscopic techniques or in vitro brain slice models of this in vivo phenomenon to quantify expression by approaches such as surface biotinylation.

Previous in vivo studies examining the effects of afferent activity on Kv3.1 protein levels in central neurons have focused on chronic sensory deprivation. In the avian nucleus magnocellularis, Kv3.1 protein levels decline within 3 h of cochlear ablation and return to baseline within 24 h (Lu et al., 2004). Additionally, it has been shown that the normal tonotopic gradient in Kv3.1b levels within the MNTB is absent in both congenitally deaf mice (Leao et al., 2006) and in mice that gradually lose hearing throughout life (von Hahn et al., 2004). In the developing visual cortex, long-term sensory deprivation by dark rearing results in an upregulation in Kv3.1b and Kv3.2 protein levels in fast-spiking cortical basket cells that can be observed within 30 days (Grabert and Wahle, 2009). To the best of our knowledge, however, the present study is the first to report rapid alterations of the gross anatomical distribution of a voltage-gated ion channel in adult animals in response to specific features of sensory stimuli. The most striking aspect of these results is the exceedingly short time course of only 30 min over which auditory stimulation altered Kv3.1b immunoreactivity in the MNTB, which is comparable to amount of time required to observe stimulus-induced changes in immunoreactivity for proteins encoded by immediate early genes such as c-Fos (e.g. Graybiel et al., 1990).

Activity-dependent regulation of Kv3.1b mRNA has been shown to require 6 h in vitro (Liu and Kaczmarek, 1998), therefore it seems likely that transcriptional mechanisms can contribute to such rapid adjustment of Kv3.1b levels during brief periods of sound stimulation. Moreover, we performed all immunohistochemistry on membrane-permeabilized tissue using an antibody directed at the cytoplasmic C-terminus of the channel, which eliminates the possibility that the increase in Kv3.1b immunoreactivity resulted from activity-dependent protein trafficking (Misonou et al., 2004; Misonou and Trimmer, 2004; Kim et al., 2007). Our findings are most readily explained by a rapid increase in Kv3.1b protein synthesis and/or turnover in response to stimulus-evoked synaptic activity. This hypothesis is consistent with recent findings in hippocampal slices and cultured neurons indicating that levels of the voltage-gated potassium channel Kv1.1 in dendrites can be rapidly altered by activity-dependent regulation of local protein synthesis (Raab-Graham et al., 2006). Moreover, although Kv3.1 protein expression varies along the tonotopic axis, there appears to be no gradient of Kv3.1 mRNA in the MNTB, indicating that, as has been found for many other proteins, levels of Kv3.1b subunits do not directly reflect levels of its mRNA (Perney et al., 1992; Perney and Kaczmarek, 1997; Li et al., 2001). Intriguingly, Kv3.1 mRNA has been identified as a candidate binding partner for fragile X mental retardation protein (FMRP), a protein that regulates rapid activity-dependent translation from preexisting mRNAs (Darnell et al., 2001). Electron microscopy studies have shown that Kv3.1b is present on both pre- and post-synaptic membranes in the MNTB (Elezgarai et al., 2003), raising the possibility that FMRP could mediate local activity-dependent translation of Kv3.1b in synaptic terminals as well as in the postsynaptic somata. An increase in pre-synaptic Kv3.1b would result in action potential narrowing and a decrease in the amount of neurotransmitter released for each spike. These data suggest that rapid adjustments in intrinsic electrical excitability may compliment established contributions from ligand-gated receptor dynamics (von Gersdorff and Borst, 2002; Takahashi et al., 2003; Sarro et al., 2008) and local network plasticity (Dean et al., 2008; Polley et al., 2004) as a homeostatic mechanism to link cellular excitability to sensory experience.

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