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A concerted action of L- and T-type Ca^{2+} channels regulates locus coeruleus pacemaking

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ABSTRACT

Dysfunction of noradrenergic locus coeruleus (LC) neurons is involved in psychiatric and neurodegenerative diseases and is an early hallmark of Parkinson's disease (PD). The analysis of ion channels underlying the autonomous electrical activity of LC neurons, which is ultimately coupled to cell survival signaling pathways, can lead to a better understanding of the vulnerability of these neurons. In LC neurons somatodendritic Ca^{2+} oscillations, mediated by L-type Ca^{2+} channels, accompany spontaneous spiking and are linked to mitochondrial oxidant stress. However, the expression and functional implication of low-threshold activated T-type Ca^{2+} channels in LC neurons were not yet studied. To this end we performed RT-PCR expression analysis in LC neurons. In addition, we utilized slice patch clamp recordings of in vitro brainstem slices in combination with L-type and T-type Ca^{2+} channel blockers. We found the expression of a distinct set of L-type and T-type Ca^{2+} channel subtypes mediating a pronounced low-threshold activated Ca^{2+} current component. Analyzing spike trains, we revealed that neither L-type Ca^{2+} channel nor T-type Ca^{2+} channel blockade alone leads to a change in firing properties. In contrast, a combined application of antagonists significantly decreased the afterhyperpolarization amplitude, resulting in an increased firing frequency. Hence, we report the functional expression of T-type Ca^{2+} channels in LC neurons and demonstrate their role in increasing the robustness of LC pacemaking by working in concert with Cav1 channels.

1. Introduction

The spontaneously active noradrenergic locus coeruleus (LC) is involved in various adaptive behaviors and its projections innervate a large number of brain regions (Benarroch, 2009). Dysfunction of the LC noradrenergic system is implied to distinct psychiatric and neurodegenerative disorders, such as depression or Parkinson's disease (PD) (Berridge and Waterhouse, 2003; Gesi et al., 2000). Loss of LC neurons correlates with prodromal, i.e. premotor symptoms in PD, that precede up to several decades the onset of motor symptoms (Lang, 2011). The mechanism that renders these neurons vulnerable to the pathogenesis of PD during aging still remains unclear. At present it is proposed that activity-dependent oxidant stress is a common feature of LC and substantia nigra pars compacta (SNpc) neurons. In both LC and SNpc mitochondrial oxidant stress is attributed to Ca^{2+} entry through L-type Ca^{2+} channels (Chan et al., 2007; Sanchez-Padilla et al., 2014).

The existence of an intrinsic pacemaker mechanism in LC neurons has been shown in various studies both in vivo (Foote et al., 1980) and in brainstem slices (Alreja and Aghajanian, 1991; Williams et al., 1984). Firing rate of LC neurons is increased in response to noxious or stressful stimuli or during periods of arousal and increased wakefulness (Aston-Jones and Cohen, 2005; Gompf et al., 2010; Takahashi et al., 2010). On the other hand, in certain stages of sleep or in situations of low vigilance or drowsiness firing rate is decreased (Foote et al., 1980). On a cellular basis firing frequency is modulated by the action of neurotransmitters, the pH or the cAMP concentration (Alreja and Aghajanian, 1991; D'Adamo et al., 2011; Murai and Akaike, 2005).

Various ionic conductances, mediating inward and outward currents, have been characterized in LC neurons. However, it is noteworthy that most of these studies were performed in the rat, while in mice, despite the predominant role of these rodents for generating transgenic animal models, LC neurons are only poorly characterized. Outward potassium currents described in LC neurons comprise a transient K^+ (I_A),

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a persistent K^+ (I_K), a Ca^{2+} activated K^+ (I_{SK}/I_{BK}), an ATP dependent K^+ (I_{KATP}) and an inward rectifier K^+ (I_{Kir}) (Murai and Akaike, 2005; Murai et al., 1997; Nieber et al., 1995; Osmanović and Shefner, 1993; Zhang et al., 2010). Inward currents include a tetrodotoxin (TTX)-sensitive and -insensitive Na^+ current, an inward non-specific cation current carried primarily by Na^+ and L-, N-, P- and Q-type Ca^{2+} conductances (Chieng and Bekkers, 1999; Murai and Akaike, 2005; van den Pol et al., 2002; Zhang et al., 2010). The speed of the spontaneous depolarization during the interspike interval of mouse LC neurons was suggested to be primarily determined by a combination of a TTX-sensitive Na^+ and a TEA-sensitive K^+ current (de Oliveira et al., 2010). Although low-threshold L-type and T-type Ca^{2+} currents are known to regulate pacemaking in distinct central neurons (Deleuze et al., 2012; Guzman et al., 2009; Wolfart and Roeper, 2002), there is a lack of studies analyzing the functional role of these channels for the spontaneous activity of LC neurons. In a recent study by Sanchez-Padilla et al. the low-threshold activated L-type Ca^{2+} channel Cav1.3 was shown to be expressed in LC neurons and may therefore contribute to the Ca^{2+} currents phase-locked to spontaneous firing. Nevertheless, a direct influence on firing frequency was not found (Sanchez-Padilla et al., 2014).

In the present study we performed RT-PCR expression analysis of collected LC neurons in combination with slice patch clamp experiments to identify low-threshold activated L-type and T-type Ca^{2+} channels. We furthermore elucidated the potential role of these channels in the pacemaking mechanism of spontaneously active LC neurons. We therefore provide an important novel insight for the understanding of LC neuron activity which might be ultimately coupled to the vulnerability of these neurons during degenerative diseases.

2. Results

2.1. LC neurons are autonomous pacemakers

The region of the LC was first identified by immunohistochemistry, as an accumulation of tyrosine hydroxylase (TH) positive neurons at the border of the fourth ventricle (Fig. 1A). A typical LC neuron during a patch clamp recording is illustrated in Fig. 1B. To verify their noradrenergic nature, each neuron was filled with neurobiotin (NB) during recording and later co-stained with an anti-TH antibody (Fig. 1C). Autonomous activity of LC neurons was recorded in acute brainstem slices at room temperature in the whole-cell configuration with glutamatergic and GABAergic blockers added to the bath (Williams et al., 1984). The firing frequency was 3–4 Hz and action potentials were broad (1.8 ± 0.4 ms duration at half amplitude) with a mean overshoot of 32 ± 2.4 mV (n = 23) (Fig. 1D, E), which is consistent with previous studies (Sanchez-Padilla et al., 2014). LC neurons displayed a strong afterhyperpolarization (AHP) with a mean amplitude of 32 ± 2 mV (n = 23) (Fig. 1F) reaching hyperpolarized membrane voltages of −70 ± 2.5 mV (n = 23) (Fig. 1G).

2.2. LC neurons display a low-threshold activated Ca^{2+} conductance

To elucidate whether LC neurons express functional voltage dependent Ca^{2+} channels, we initially performed whole-cell voltage clamp recordings in acute brainstem slices perfused with ACSF. Voltage steps from −80 mV to +20 mV, for 500 ms, followed by repolarization to −40 mV elicited an outward current consisting of a fast inactivating and a persistent current component (Fig. 2A), mediated by voltage-gated potassium channels with so far unknown molecular identities. In addition, at membrane potentials of −60 mV to −30 mV an inward Ca^{2+} current with a peak amplitude of 375 ± 68 pA was evident (n = 7) (Fig. 2A, zoom-in). Plotting of the peak-current voltage relationship (I/V) (Fig. 2A, arrow) revealed that the Ca^{2+} inward current had a maximum at negative membrane voltages of −50 mV (Fig. 2B). Previous studies reported the functional expression of low-threshold activated Cav1.3 and Cav1.2 channels in LC neurons (Imber and Putnam, 2012; Sanchez-Padilla et al., 2014). However, these channels are known to have a maximal conductance at membrane potentials between −30 and −10 mV (Koschak et al., 2001; Xu and Lipscombe, 2001). Although these experiments were, due to the presence of K^+ conductances, not designed to isolate specific Ca^{2+} current components, they suggested the presence of low-threshold activated T-type Ca^{2+} channels in LC neurons. To provide further evidence for such a low-threshold activated Ca^{2+} conductance, we next applied voltage steps to −40 mV from more hyperpolarized potentials, as T-type Ca^{2+} channels might be already inactivated to a large fraction when holding at −80 mV. Using

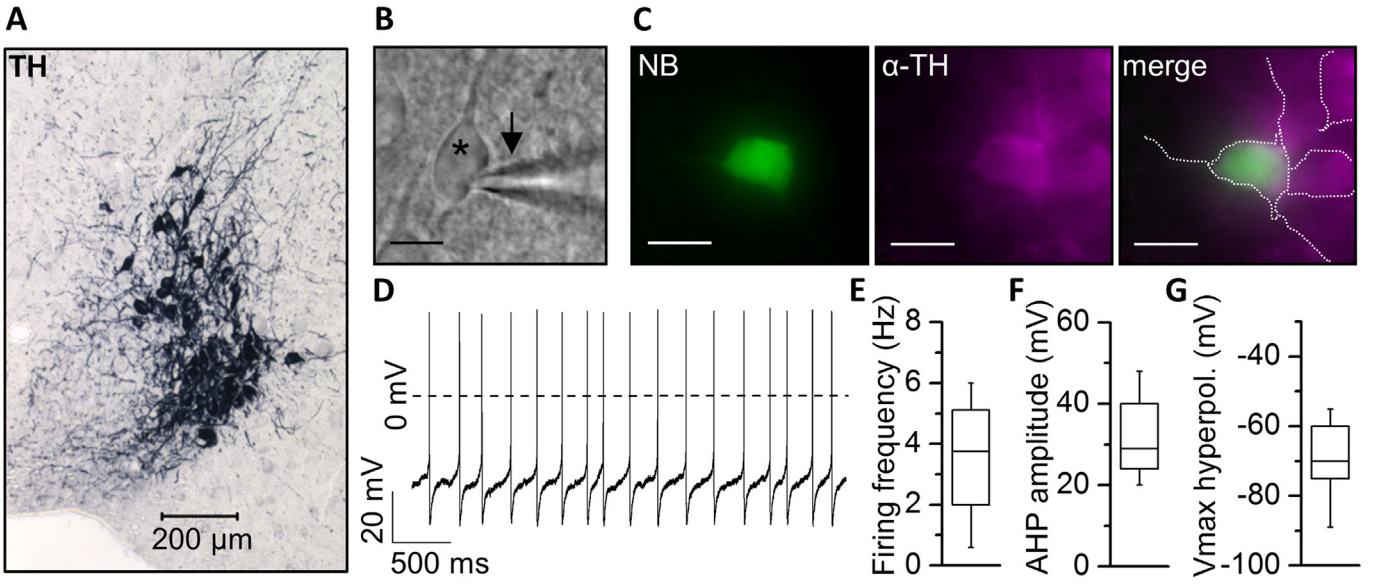

Fig. 1. Locus coeruleus neurons are autonomous pacemakers. (A) Brainstem section of a juvenile C57BL/6j mouse. LC neurons reactive for the monoaminergic marker enzyme tyrosin hydroxylase (TH) are stained black. (B) Patch pipette (black arrow) approaching a LC neuron (asterisk). Scale bar: 20 µm. (C) Neurobiotin (NB) filled neuron co-stained with Alexa488 conjugated streptavidin (green) and anti-TH/anti-rabbit Alexa568 (magenta). Dashed lines indicate the outlines of three TH-positive LC neurons. Scale bars: 20 µm. (D) Example recording of a spontaneously active LC neuron in the whole-cell current clamp configuration. To isolate autonomous spiking ACSF was complemented with GABAergic (CGP, gabazine) and glutamatergic (AP-5, NBQX) blockers. (E–G) Basic properties of LC spiking (n = 23 neurons): (E) firing frequency, (F) afterhyperpolarization (AHP) amplitude and (G) most negative potential reached during AHP (V_{max} hyperpol.).