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Research report

Kinetic properties and adrenergic control of TREK-2-like channels in rat medial prefrontal cortex (mPFC) pyramidal neurons

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ABSTRACT

TREK-2-like channels were identified on the basis of electrophysiological and pharmacological tests performed on freshly isolated and enzymatically/mechanically dispersed pyramidal neurons of the rat medial prefrontal cortex (mPFC). Single-channel currents were recorded in cell-attached configuration and the impact of adrenergic receptors (α_1, α_2, β) stimulation on spontaneously appearing TREK-2-like channel activity was tested. The obtained results indicate that noradrenaline decreases the mean open probability of TREK-2-like channel currents by activation of β_1 but not of α_1- and α_2-adrenergic receptors. Mean open time and channel conductance were not affected. The system of intracellular signaling pathways depends on the activation of protein kinase A. We also show that adrenergic control of TREK-2-like channel currents by adrenergic receptors was similar in pyramidal neurons isolated from young, adolescent, and adult rats. Immunofluorescent confocal scans of mPFC slices confirmed the presence of the TREK-2 protein, which was abundant in layer V pyramidal neurons. The role of TREK-2-like channel control by adrenergic receptors is discussed.

1. Introduction

Abnormal control of mPFC neuronal activity by noradrenaline has been implicated in several neuropsychiatric disorders, e.g., in depression (Lemogne et al., 2009), attention deficit hyperactivity disorder (Sheridan et al., 2010), and posttraumatic stress disorder (Koenigs and Grafman, 2009). These pathologies most likely reflect disordered activity of prefrontal cortical neurons; their activity depends, in turn, on the properties of the neuronal ion channels and the ways in which they are controlled by metabotropic receptors.

Noradrenaline activates metabotropic adrenergic receptors. There are α_1-, α_2-, and β-adrenergic receptors and all are expressed in the cortical neurons (Rainbow et al., 1984; Pieribone et al., 1994; Scheinin et al., 1994; Summers et al., 1995; Nicholas et al., 1996). These α_1-, α_2-, and β- adrenergic receptors are coupled to G_q, G_i, and G_s proteins, respectively, and control the cellular effectors, including ion channels, preferentially by either activation or inhibition of kinase A or C (Benovic et al., 1988; Cotecchia et al., 1990; Nishizuka, 1992; Simonds, 1999; Koshimizu et al., 2002; Hein, 2006; Ramos and Arnsten, 2007).

Leak K^+ channels (background or constitutively active K^+ channels) are responsible for setting the resting membrane potential and opposing any resting potential changes (Enyedi and Czirják, 2010). Leak channels have been divided into 6 subclasses based on their structures and functional properties. These are the TWIK family (TWIK-1, TWIK-2, KCNK7), whose members give rise to a weak, inward-rectifying K^+ current; the TREK family (TREK-1, TREK-2, TRAAK), whose members are activated by mechanical stimuli, lipids and intracellular acidification or alkalization; the TASK family (TASK-1, TASK-3, TASK-5), whose members are inhibited by extracellular acidification; the TALK family (TALK-1, TALK-2, TASK-2), whose members are sensitive to alkalinity; THIK channels (THIK-1 and THIK-2), which are inhibited by halothane; and the TRESK channel (see the review in Lotshaw, 2007; Enyedi and Czirják, 2010).

The TREK channel family is coupled to G_s (Patel et al., 1998; Lesage et al., 2000; Sandoz et al., 2006), G_i (Lesage et al., 2000;

Abbreviations: 8-Br-cAMP, 8-bromoadenosine 3′,5′-cyclic monophosphate; cAMP, adenosine 3′,5′-cyclic monophosphate; EGTA, ethylene glycol-bis(2-aminoethylether) – N,N,N′,N′-tetraacetic acid; H-89, N-[2-(p-bromocinnamylamino)ethyl]–5-isoquinolinesulfonamide dihydrochloride; HEPES, [N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulphonic acid)]; mPFC, medial prefrontal cortex; NMDG, N-methyl-D-glucamine; TASK, TWIK-related acid-sensitive potassium channel; THIK, TWIK-related halothane inhibited potassium channel; TRAAK, TWIK-related arachidonic acid stimulated potassium channel; TREK, TWIK-related potassium channel; TRESK, TWIK-related spinal cord potassium channel; TTX, tetrodotoxin; TWIK, weakly inward rectifying potassium channel.

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Cain et al., 2008), and G_q protein (Fink et al., 1996; Lesage et al., 2000; Chemin et al., 2003; Lopes et al., 2005; Murbartián et al., 2005; Kang et al., 2006; Sandoz et al., 2006) receptors. Therefore, it is possible that the increase in adrenergic input to the prefrontal cortex inhibits leak channel K^+ currents and thus depolarizes mPFC pyramidal neurons.

Control of non-inactivating ionic K^+ type TREK channel currents by adrenergic receptors in the pyramidal neurons of the mPFC, particularly at the level of single channel currents, has not been tested. The purpose of this study was to identify the kinetic properties of TREK channels and their expression in mPFC pyramidal neurons, and to verify their control by adrenergic receptors in young, adolescent, and adult rats.

2. Results

In this study, single leak channel K^+ currents were recorded in cell-attached configuration from dispersed mPFC pyramidal neurons. Na^+ and Ca^{2+} channel currents were eliminated by TTX and La^{3+} ions, respectively, which were added to the extracellular pipette solution. Because the channel currents were recorded in "symmetrical" K^+ solutions, the patch membrane potential was close to "0" mV.

2.1. Properties of large conductance leak channel currents

Two types of leak channel K^+ currents with different conductances, i.e., large and small, were recorded in young, adolescent, and adult rats. The recorded channel currents were arbitrarily divided into channels with "large" (>65 pS) and "small" (<65 pS) conductance (Fig. 1C). Both channels displayed irregular burst activity at both positive and negative patch membrane potentials (Fig. 1A,B). The large conductance channels had mean outward and inward conductance of 145.7 ± 3.9 pS and 168.2 ± 3.2 pS ($n = 64$, $P < 0.0001$, t-test), respectively. The mean outward and inward conductance of channels designed as "small" was 44.42 ± 3.86 pS and 28.82 ± 1.57 pS, respectively ($n = 20$, $P < 0.0001$ Mann-Whitney test).

Only constitutively active leak channel currents with large conductance were analyzed in this study. Their activity depended on the membrane potential such that P_o was larger and the mean open time was longer at positive than at negative membrane potentials. In turn, conductance was significantly larger in the inward than in the outward direction (see above, Fig. 1A, insets; Table 1). The large conductance leak channel currents were sensitive to mechanical stimuli (mechanical distortion of the patch membrane by suction increased the mean P_o from 0.07 ± 0.02 to 0.17 ± 0.06; P_o recovered to 0.08 ± 0.02 when suction was discontinued, $P < 0.05$, Tukey-Kramer test, $n = 5$, Fig. 2A) and heat (a rise in the bath temperature from room temperature to 29–30 °C increased the mean P_o by >4 times, $P < 0.05$, Wilcoxon test, $n = 6$, Fig. 2B).

The kinetic properties of the large conductance leak channel currents (outward conductance, inward conductance, mean open time and mean open probability) did not depend on the rats' age (Table 1).

The features of large conductance leak channel currents, as recorded in this study, resembled the properties of two-pore domain TREK-like family channel currents as described in mPFC pyramidal neurons (Ksiazek et al., 2013) and numerous other cells (Patel et al., 1998; Maingret et al., 1999, 2000; Han et al., 2002, 2003; Kang et al., 2005; Kang and Kim, 2006; Kang et al., 2007b; Yamamoto et al., 2009).

The TREK family includes TREK-1, TREK-2 and TRAAK leak channel currents which differ in their pharmacological and kinetic properties (see the review in Enyedi and Czirják, 2010); for example, in symmetrical K^+ solution in the presence of divalent cations, TREK-2 and TRAAK channels rectify the single-channel currents in the inward direction (Bang et al., 2000; Kim et al., 2001; Han et al., 2002, 2003; Kang and Kim, 2006), while TREK-1 channels rectify them in the outward direction (Patel et al., 1998; Kim et al., 2001; Maingret et al., 2002; Kang and Kim, 2006; Li et al., 2006). The large-conductance channels recorded in our study are inward rectifiers as currents measured in gap-free mode had larger inward than outward conductance (insets to Fig. 1A, Table 1). Also, current amplitudes were larger at hyperpolarizing than at depolarizing ramp membrane potentials (Fig. 3A,B). The presence of inward

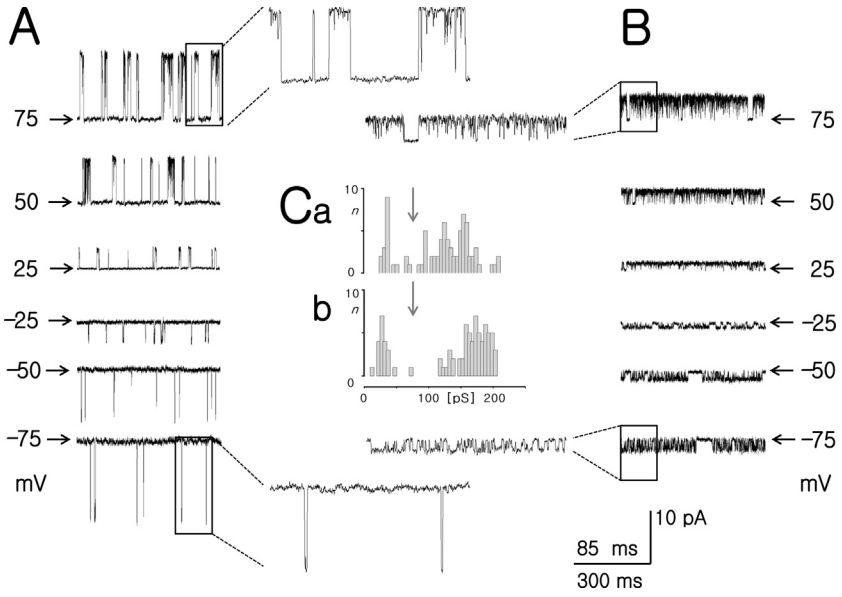

Fig. 1. Original recordings of single leak channel currents in mPFC pyramidal neurons at different patch membrane potentials. A. "Large amplitude" single leak channel currents. B. "Small amplitude" single leak channel currents. C. Distribution of single channel conductances: a) outward and b) inward; bin width – 10 pS. The channels were divided into those with "large" and "small" conductance (division marked by vertical arrows). The distribution shows that the predominant activity was that of the high conductance channel. Insets (A,B) – channel current shown with extended time base. Horizontal arrows indicate closed channel current level in this and the other figures. Patch membrane potential is indicated.