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BRAIN RESEARCH

Research Report

Amitriptyline inhibits currents and decreases the mRNA expression of voltage-gated sodium channels in cultured rat cortical neurons

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ABSTRACT

Amitriptyline (AMI) is widely used for migraine prophylaxis, but its mechanism is undetermined. Cortical spreading depression (CSD), reflected by alterations in calcium, sodium, and Na^+-K^+ATP channels, has been implicated in migraine and as a headache trigger. Evidences indicate that mutations in sodium and calcium channels are involved in migraine. Sodium channels are critical for the electrical excitability of sensory neurons and play a key role in pain sensation by controlling afferent impulse discharge. To investigate the mechanism underlying AMI effectiveness for migraine prophylaxis, we studied the effects of AMI on voltage-gated sodium channels in cultured rat cortical neurons by the whole-cell patch clamp recording and real-time reverse transcription polymerase chain reaction (RT-PCR). We found that AMI blocked sodium channel current (I_{Na}) in a concentration-dependent, not voltage-dependent manner. AMI also altered the activation and steady-state inactivation of I_{Na} toward hyperpolarization. Results of real-time RT-PCR indicated that AMI inhibited the expression for sodium channels in a concentration-dependent manner; inhibition of expression of the $Na_V1.1$ and $Na_V1.6$ sodium channels was more than that of $Na_V1.2$. From these results, we speculate that AMI may reduce CSD by inhibiting I_{Na} and mRNA expression of sodium channels. This may contribute to strategies for migraine prophylaxis.

1. Introduction

Tricyclic antidepressants were discovered in the early 1960s and are used as mood stabilizing agents (Barbui and Hotopf, 2001). Given their analgesic and sedative properties, these drugs have been used in the treatment of diabetic neuropathy, postherpetic neuralgia, chronic lower back pain and some types of cancer pain. AMI is a potent member of tricyclic antidepressants and has been widely used for migraine prophylaxis, it has mixed serotoninergic and noradrenergic reuptake inhibitor (SNRI) properties and has other pharmacological mechanisms including: adenosine-A1 agonism; effects on NMDA receptor; blocking

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Abbreviations: CSD, cortical spreading depression; FHM, familial hemiplegic migraine; TTX, tetrodotoxin; AMI, amitriptyline

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Na^+ channel and inhibiting the GABA transporter types 1 and 3 (GAT-1 and GAT-3)(Mico et al., 2006).

Migraine is one of the most common types of neurologic disorder associated with neuropathic pain. It is thought to originate from activation of the trigeminovascular system (i.e., the trigeminal innervation of cranial blood vessels and connections to the brainstem). Auras, which precede migraine pain, are considered to result from cortical spreading depression (CSD), described as an initial increase in neuronal activity followed immediately by a refractory and long-lasting depression of activity that slowly propagates across the cortex at 2–3 mm per minute (Goadsby, 2007). The pivotal event in the generation and propagation of CSD is a marked decrease in neuronal membrane resistance associated with a massive increase in extracellular K^+ and neurotransmitters, as well as an increase in intracellular Na^+ and Ca^{2+}.

It has been hypothesized that migraine may also be a result of channelopathy and that abnormalities in ionic channels may underlie abnormal interictal neuronal excitability predisposing to migraine, in particular familial hemiplegic migraine (FHM), which may involve mutation of sodium and calcium channels. Type 1 FHM (FHM1) is caused by mutation of the *CACNA1A* gene encoding the pore-forming α_1-subunit of the neuronal calcium channel $Ca_V2.1$ (Kors et al., 2004; Ophoff et al., 1996), whereas FHM2 is caused by mutations in the *ATP1A2* gene encoding the catalytic α_2-subunit of the glial Na^+/K^+-ATPase in adult brain (De Fusco et al., 2003; Vanmolkot et al., 2003). Two missense mutations (Q1489K and L1649Q) of the *SCN1A* gene encoding the pore-forming α-subunit of the voltage-gated sodium channel $Na_V1.1$ have been linked to FHM3 (Dichgans et al., 2005; Vanmolkot et al., 2007). The *SCN1A* gene is also involved in febrile seizures and epilepsy.

Some reports indicate a link between migraine and epilepsy (Rogawski, 2008). Haut et al. reported that epilepsy and migraine are comorbid disorders of hyperexcitability (Haut et al., 2006). As a result, some antiepileptic drugs are effective in the prevention of migraine (Rogawski and Loscher, 2004; Silberstein, 2006). The observation that spreading depression requires activation of a persistent Na^+ conductance (Somjen, 2001) accounts for the specific therapeutic effect of valproic acid and topiramate (TPM) in migraine. TPM has an inhibitory effect on the initiation and propagation of CSD (Ayata et al., 2006). Meanwhile, TPM inhibited the sodium channel in dissociated neurons of rat sensorimotor cortex (Curia et al., 2007). As with TPM, in experimental animals, AMI suppressed CSD (Ayata et al., 2006) and inhibited I_{Na} in rat gastric sensory neurons and dorsal root ganglion neurons as well as immature rat trigeminal ganglion neurons. However, whether AMI will affect the sodium channel activities in cortical neurons is unknown.

The $Na_V1.1$, $Na_V1.2$, and $Na_V1.6$ sodium channels are expressed in cortical tissue, however, $Na_V1.1$ and $Na_V1.6$ channels appeared to enter the gating mode more frequently and produce more persistent current than $Na_V1.2$ channels. (Klein et al., 2004; Maurice et al., 2001; Mechaly et al., 2005). Changes in properties of voltage-gate sodium channels would directly affect neuronal excitability and activities.

In this study, we examined the properties of Na^+ channels in cultured rat cortical cells in response to AMI treatment. To determine changes in Na^+ channel expression that may be associated with functional changes, we also assessed Na^+ channel mRNA expression by real time reverse-transcription polymerase chain reaction (RT-PCR).

2. Results

2.1. *Voltage-activated Na^+ channel currents in cultured rat cortical neurons*

The general characteristics of the voltage-gated sodium channel current (I_{Na}) described in this study are similar to those reported in previous patch clamp studies of cortical neurons (Liu et al., 2007; Peterson et al., 2006). In our present experiments, Ca^{2+} and K^+ currents were blocked pharmacologically by the extracellular application of tetraethylammonium (TEA-Cl) and Cd^{2+} as well as by the addition of Cs^+ and ethylene glycol tetraacetic acid (EGTA) in the recording electrode. Under this experimental condition, depolarizing voltage commands from a holding potential of –80 mV elicited a voltage-dependent inward current (Fig. 1A) sensitive to tetrodotoxin (TTX) (complete block with 0.5 μM) (Fig. 1B). The *I–V* relation obtained by averaging data from eight individual cells is shown in Fig. 1C. The threshold for inward current activation was approximately –60 mV, and the peak amplitude occurred approximately –40 mV.

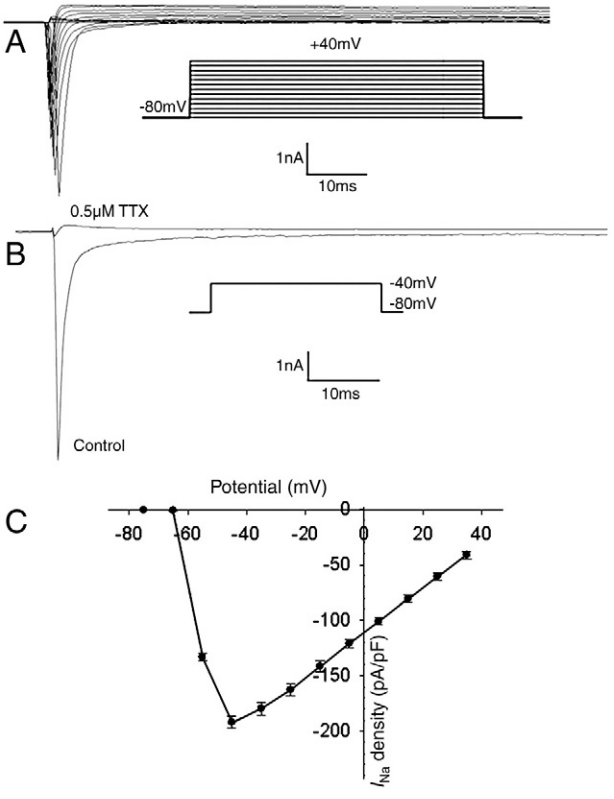

Fig. 1 – Whole-cell sodium channel currents (I_{Na}) in cultured cortical neurons. (A) Original recording of the currents evoked by a series of depolarizing pulses. (B) Inhibitory effect of 0.5 μM TTX on the I_{Na}. (C) Mean *I–V* relationship obtained by averaging results from eight individual cells. Error bars represent S.E.M.