Low Voltage-Activated Ca\textsuperscript{2+} Conductances in Thalamic Neurons


Low voltage-activated (LVA) Ca\textsuperscript{2+} conductances were characterized in the neurons of the associative laterodorsal (LD) thalamic nucleus in rat brain slices and in enzymatically isolated thalamic units using electrophysiological techniques. Voltage dependence, kinetics of inactivation, pharmacology, and selectivity of the LVA current in the thalamic neurons from animals older than 14 postnatal days were consistent with the existence of two, “fast” and “slow,” subtypes of LVA Ca\textsuperscript{2+} channels. “Slow” LVA current in enzymatically isolated thalamic neurons was much less prominent, compared with that in slice neurons, suggesting that respective channels are predominantly located on the distal dendrites. “Fast” Ca\textsuperscript{2+} channels were sensitive to nifedipine (K\textsubscript{D} - 2.6 \mu M) and La\textsuperscript{3+} (K\textsubscript{D} - 1.0 mM), whereas “slow” Ca\textsuperscript{2+} channels were sensitive to Ni\textsuperscript{2+} (25 \mu M). Selectivity of the “fast” Ca\textsuperscript{2+} channels was similar to that found for the LVA Ca\textsuperscript{2+} channels in other preparations (I\textsubscript{Ca} : I\textsubscript{Na} = 1.0 : 1.23 : 0.94), while selectivity of the “slow” Ca\textsuperscript{2+} channels more resembled selectivity of the HVA Ca\textsuperscript{2+} channels (I\textsubscript{Ca} : I\textsubscript{Na} = 1.0 : 2.5 : 3.4).

INTRODUCTION

Low voltage-activated (LVA) Ca\textsuperscript{2+} conductance is implicated to play a major role in generation of the bursting activity and low-threshold Ca\textsuperscript{2+}-dependent spikes. Such firing patterns can be found in many brain areas, but they are particularly prominent in thalamic neurons. In thalamic relay neurons rhythmic bursting can be correlated with many normal behavioral states, such as slow-wave sleep [1], as well as with some pathological phenomena [2, 3]. Consistent with the role of LVA Ca\textsuperscript{2+} conductance in such firing behavior, thalamic relay neurons possess a robust LVA Ca\textsuperscript{2+} current, which in a large proportion of these neurons represents the dominant or even the sole type of Ca\textsuperscript{2+} current. Moreover, in reticular thalamic neurons of rats in the model of human absence epilepsy LVA Ca\textsuperscript{2+} current is even largely overexpressed, compared with the seizure-free rat brain [4], suggesting the role of this current in generation of epileptic activity.

Although the first indirect evidence of the presence of LVA Ca\textsuperscript{2+} channels subsequently named T-type (“T” for “transient”) was obtained in the brain neurons [5], the respective current was first directly recorded in peripheral neurons [6]. Further exploration of the properties of this channel type was primarily conducted in peripheral neurons, cultured cell lines, and different types of muscle cells (for reviews see [7-9]). Systematic studies of LVA Ca\textsuperscript{2+} channels in central neurons began in the late 1980s, and it immediately became obvious that the properties of these channels are quite different from those in peripheral cells. Moreover, recent data suggest that LVA Ca\textsuperscript{2+} channels do not form a uniform group of channels, but their population comprises several subtypes that share the common feature of a low threshold for activation, but are dissimilar in other functional properties [10-12]. Further dissection of the subtypes of LVA Ca\textsuperscript{2+} channels based on the differences in their properties, in particular pharmacology, is extremely important, especially in such structures as the thalamus, where they determine normal as well as pathological firing patterns of the neurons.

METHODS

Experiments were performed on enzymatically isolated and intact (in brain slices) neurons from the...
**RESULTS**

**General Properties of the LVA Current.** For recording, neurons with medium to large somata (20-30 μm in the diameter) and radially arranged dendrites were selected. According to the neuronal type classification in the associative thalamic nuclei [14], such neurons mostly belong to the associative type. Measurements of the capacity in LD thalamic neurons in slices showed that its value is age-dependent, being 84 ± 4 pF, 107 ± 11.3 pF, and 156 ± 16 pF for 12-, 14-, and 17-day-old rats, respectively. For comparison, the membrane capacitance in enzymatically isolated thalamic neurons from 14-17-day-old rats was found to be more than 10 times lower, 11.4 ± 0.5 pF, on the average (𝑛 = 30), suggesting that after enzymatic and mechanical procedures the neurons became devoid of most of their dendritic tree.

Under experimental conditions that made possible adequate isolation of Ca²⁺ channel current of a patched neuron in slices, step depolarization (𝑉𝑡) from a very negative holding potential (𝑉ℎ = −100 mV) elicited an inward humped current-voltage (I–V) relation typical of the presence of two major current components, LVA and high voltage-activated (HVA) currents (Fig. 1B).

Such a general pattern of the I–V plot was similar for the neurons from all three animal age groups, of 12, 14, and 17 postnatal days. However, detailed examination of the I–V obtained from 14- and 17-, but not from 12-day-old animals (Fig. 1B) showed that the hump corresponding to activation of LVA current (𝑉cargo = −80 ... −40 mV) is not uniform, and it in turn consists of two minor humps located at the membrane potentials of about −65 mV and −50 mV. Such a shape of the I–V at low membrane potentials is consistent with the assumption that LVA Ca²⁺ current in the LD thalamic neurons from animals older than 14 days may be transferred through a non-homogeneous population of Ca²⁺ channels. In addition, overall LVA Ca²⁺ current can be represented as the superposition of two components (Fig. 1C, D), a dominant transient component demonstrating the fast time course of inactivation (𝑡ᵣ = 27 msec), and a smaller slowly decaying component showing very slow inactivation within several hundreds of milliseconds (𝑡ᵣ = 185 msec). Altogether, the data on the voltage dependence of the I–V and kinetics of the decay of LVA Ca²⁺ current in the LD thalamic neurons in slices from animals older than 14 postnatal days strongly suggest the presence of two, “fast” and “slow,” subtypes of LVA Ca²⁺ channels. At the 17th postnatal day, the mean amplitude of “slow” LVA current, 630 ± 58 pA, was found to be comparable with that of “fast” LVA current, 670 ± 74 pA. Qualitatively similar data were obtained in enzymatically isolated LD thalamic neurons from 14-17-day-old animals, although the amplitudes of overall LVA Ca²⁺ current, as well as of its “fast” and “slow” components, were much lower; this was consistent with a decreased surface area of the neurons due to removal of most of their dendritic tree.

The mean amplitudes of the “fast” and “slow” components of Ca²⁺ LVA current in isolated neurons were found to be 228 ± 21 pA (𝑛 = 22) and 20 ± 12 pA (𝑛 = 22). Respectively, considering the membrane capacitance of 11.4 ± 0.5 pF, this yields the densities of respective currents of about 20 pA/pF and 1.8 pA/pF. In addition, the voltage of the maximum net LVA current in isolated neurons was about 20 mV, i.e., more positive compared with a that in intact neurons. The “fast” component of this current also had a more rapid decay phase, compared with a similar component in the neurons in slices (𝑡ᵣ of 18 msec vs 27 msec).

**Pharmacology and Selectivity of LVA Ca²⁺ Current.** A dihydropyridine Ca²⁺ channel antagonist, nifedipine, which is known to be ineffective with respect to LVA Ca²⁺ current in peripheral cells, but capable of blocking this current in hypothalamic neurons [15], in a dose-dependent manner inhibited the “fast” component of the net LVA Ca²⁺ current in thalamic neurons without affecting the “slow” component (Fig. 2A, B). At the concentration of 100 μM nifedipine completely blocked the “fast” component of the current, thereby allowing one to obtain the “slow” component in a basically non-contaminated form. The 𝐾ᵅ value for the nifedipine block of the “fast” LVA current was 2.6 μM (Fig. 3B), which is very close to the value of 5 μM found in hypothalamic neurons [15].

We also found that two components of LVA Ca²⁺ current in the LD thalamic neurons in slices differentially reacted to such inorganic Ca²⁺ channel blockers, as La³⁺ and Ni²⁺. It appeared that La³⁺ preferentially blocks the “fast,” nifedipine-sensitive, component of the current, while a specific blocker of LVA Ca²⁺ current in peripheral cells, Ni²⁺, is much more effective with respect to the “slow,” nifedipine-insensitive, component. The concentration of La³⁺ required for complete inhibition of the “fast” component was found to be 1 μM (Fig. 2C), whereas the concentration of Ni²⁺ required for selective inhibition of the “slow” component was found to be 25 μM (Fig. 2D).

Thus, sensitivities of two components of LVA Ca²⁺ current in the LD thalamic neurons in slices to nifedipine, La³⁺, and Ni²⁺ confirm our initial suggestion that