Neuroscience 152 (2008) 469–476

AGE-RELATED FUNCTIONAL CHANGES OF HIGH-VOLTAGE-ACTIVATED CALCIUM CHANNELS IN DIFFERENT NEURONAL SUBTYPES OF MOUSE STRIATUM

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Abstract—By means of whole-cell patch-clamp recordings, we characterized the developmental profile of high-voltage-activated (HVA) calcium (Ca^{2+}) channel subtypes in distinct neuronal populations of mouse striatum. Acutely dissociated medium spiny neurons (MSNs) and cholinergic interneurons (ChIs) were recorded from mice at five developmental stages: postnatal-days (PD) 14, 23, 40, 150 and 270. During ageing, total HVA Ca^{2+} current recorded from both MSNs and ChIs was unchanged. However, the pharmacological analysis of the differential contribution of HVA Ca^{2+} channel subtypes showed a significant rearrangement of each component. In both neuronal subtypes, a large fraction of the total HVA current recorded from PD14 mice was inhibited by the L-type HVA channel blocker nifedipine. This dihydropyridine-sensitive component accounted for nearly 50%, in MSNs, and 35%, in ChIs, of total current at PD14, but its contribution was down-regulated up to 20–25% at 9 months. Likewise, the N-type, ω-conotoxin GVIA-sensitive component decreased from 35% to 40% to about 25% in MSNs and 15% in ChIs. The P-type, ω-agatoxin-sensitive fraction did not show significant changes in both neuronal subtypes, whereas the Q-type, ω-conotoxin MVIIC-sensitive channels did show a significant up-regulation at 9 months. As compared with striatal neurons, we recorded pyramidal neurons dissociated from cortical layers IV–V and found no significant developmental change in the different components of HVA Ca^{2+} currents.

In conclusion, our data demonstrate a functional reconfiguration of HVA Ca^{2+} channels in striatal but not cortical pyramidal neurons during mouse development. Such changes might have profound implications for physiological and pathophysiological processes of the striatum.

Key words: medium spiny neuron, cholinergic interneuron, cortical pyramidal neuron, development, patch-clamp, basal ganglia.

High-voltage-activated (HVA) Ca^{2+} channels are multisubunit complexes, containing the pore forming $\alpha 1$ subunit, and the auxiliary $\alpha 2\delta$, β, and γ subunits, each of them conferring unique gating properties. In neurons, five types of HVA Ca^{2+} channels have been described, based on their physiological and pharmacological properties, which are mediated by different $\alpha 1$ subunits. L-type channels are composed of Cav1 subunits; P/Q-, N-, and R-type channels are formed by the Cav2.1, Cav2.2 and Cav2.3 subunits, respectively ($\alpha 1$A, $\alpha 1$B and $\alpha 1$E) (Catterall et al., 2003). They have been classified into L-, N-, P-, Q- and R-type mainly on the basis of their sensitivity to different blocking agents: L-type channels are selectively blocked by dihydropyridines (i.e. nifedipine, NIFE), N-type channels by ω-conotoxin GVIA (CtxGVIA), P-type channels by ω-agatoxin IVA (AtxIVA) and Q-type channels by ω-conotoxin MVIIC (CtxMVIIC).

HVA Ca^{2+} currents are essential to neuronal function, playing a central role in neurotransmitter release, synaptogenesis, synaptic plasticity, neuronal excitability, gene regulation, neuronal survival and differentiation (Kennedy, 1989; Catterall, 1998; Pietrobon, 2005). Most of these processes undergo developmental changes that might be, at least to some extent, imputed to modifications in HVA Ca^{2+} currents (Salgado et al., 2005; Akopian and Walsh, 2006).

Both in morphological and electrophysiological terms, striatal neurons, and in particular medium spiny neurons (MSNs), undergo a long-lasting postnatal maturation, that is not fully complete up to some weeks after birth. In particular, intrinsic membrane properties of developing MSNs are mature only at the end of the third postnatal week (Tepper et al., 1998). In the present study, we analyzed the modifications in HVA Ca^{2+} current during development by performing whole-cell patch-clamp recordings from distinct neuronal populations of the striatum. MSNs and cholinergic interneurons (ChIs) were acutely dissociated from mice striatum in a broad range of ages, comprised between postnatal day (PD) 14 and 9 months. A systematic pharmacological analysis of the differential contribution of HVA channel subtypes was performed in these neuronal populations.

Characterizing the developmental profile of these channels appears of relevance not only to understand the physiological organization of the striatum, but may also help interpreting age-related motor disturbances.

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Abbreviations: AHP, afterhyperpolarization; ANOVA, analysis of variance; AtxIVA, ω-agatoxin IVA; ChI, cholinergic interneuron; CtxGVIA, ω-conotoxin GVIA; CtxMVIIC, ω-conotoxin MVIIC; HBSS, Hanks' balanced salt solution; HVA, high-voltage-activated; LTD, long-term depression; MSN, medium spiny neuron; NIFE, nifedipine; PD, postnatal day.

0306-4522/08$32.00+0.00
doi:10.1016/j.neuroscience.2007.12.040

EXPERIMENTAL PROCEDURES

Preparation and maintenance of acutely dissociated neurons

The experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC) and were approved by the animal subjects review board of the University of Rome "Tor Vergata." All efforts were made to minimize the number of animals used and their suffering. C57B/L6 mice were grouped by age: PD14, PD23, PD40, PD150, and PD270. Acutely dissociated striatal and cortical neurons were prepared from coronal slices of dorsal striatum and neocortex as described previously (Pisani et al., 2004; Martella et al., 2005). Briefly, mice were killed under ether anesthesia by cervical dislocation, the brain was rapidly removed and slices (400 μm thick) were cut from tissue blocks with a vibratome, in ice-cold Krebs' solution (see composition below). Afterward, slices were incubated in Hepes-buffered Hanks' balanced salt solution (HBSS), bubbled with pure oxygen. Temperature was kept at 35 °C. A single slice was then incubated in HBSS media plus 0.5-mg/ml protease XIV. After 30 min the tissue was repeatedly washed in HBSS and mechanically triturated with a graded series of fire-polished Pasteur pipettes. The obtained supernatant was placed in a Petri dish mounted on the stage of an inverted microscope (Nikon Diaphot, Japan). Healthy cells were allowed to settle for about 10 min.

Whole-cell patch-clamp recordings

MSNs and ChIs from mouse striatum (Fig. 1A) and layer IV–V cortical pyramidal neurons (Fig. 7A, inset) were identified by their peculiar shape and processes spared by the enzymatic dissociation (Bargas et al., 1994; Yan and Surmeier, 1997; Martella et al., 2005; Castelli and Magistretti, 2006). Patch-clamp recordings in the whole-cell configuration were performed utilizing fire-polished pipettes, whose resistance ranged between 6 and 9 MOhm. The internal solution consisted of (in mM): N-methyl-D-glucamine 185, Hepes 40, EGTA 11, $MgCl_2$ 4, finally added (in the daily arranged working solution) with phosphocreatine 20, ATP 2–3, GTP 0–0.2, leupeptin 0.2; the osmolarity was 275–280 mOsm/l. After obtaining the cell access, the neuron was usually bathed in a medium composed of (in mM): TEA–Cl 165, $CsCl_2$ 5, Hepes–Na 10, and $BaCl_2$ 5 as the charge carrier; pH was adjusted to 7.35 and the osmolarity to ~300 mOsm/l. Recordings were performed at room temperature. An Axopatch 1D amplifier (Axon Instrument, USA) was used. Series resistance compensation was routinely employed (70–80%). Data were low-pass filtered (corner frequency=5 kHz). For data acquisition and analysis pClamp9 software was used. I–V relationships for the different neuronal types analyzed were built by applying test pulse protocols consisting in progressive voltage pulses from −50 to 50 mV. In most of the experiments, Ca^{2+} currents were studied with both ramp test (from −70 mV to 40 mV, 0.3–0.6 mV/ms) and test pulse protocols (from −10 mV to 10 mV). Ramps were utilized in order to assess the quality of the voltage control and the effect of drugs over a broad voltage range; experiments implying current kinetics analysis and occlusion with antagonists were supported by step pulse protocols (Stefani et al., 2002; Song et al., 2000). Control and drug solutions were applied with a linear array of six, gravity-fed capillaries positioned within 500–600 μm from the patched neuron. To analyze the contribution of each type of HVA Ca^{2+} channel, the amount of Ca^{2+} current blocked by each antagonist was considered as the fraction of the whole cell Ca^{2+} current attributable to a given channel type. R-type Ca^{2+} current was calculated as the amount of residual current in the presence of all four blockers in the perfusing solution. Cd^{2+} was used to block this residual component (Foehring et al., 2000).

Data analysis was performed off-line using Microcal Origin and GraphPad Prism software. Values given in the text and in the figures are mean±S.D. of changes in the respective cell populations. The gaussian distribution of data was assessed before examining the differences among the groups by using one-way analysis of variance (ANOVA; $P<0.01$), with post hoc evaluation by Tukey's multiple comparison test (* $\alpha<0.01$; ** $\alpha<0.001$).

C57B/L6 mice were purchased from Charles River (Lecco, Italy). CtxGVIA, CtxMVIIC and AtxIVA were from Alomone Laboratories (Israel). NIFE and all other compounds utilized were from Sigma, Italy.

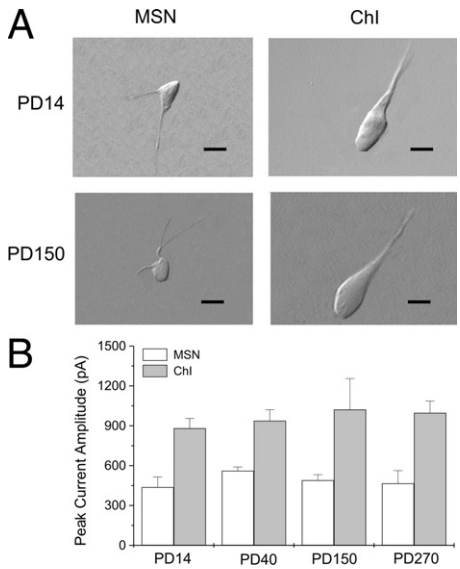

Fig. 1. Distinctive features of medium spiny and cholinergic neurons acutely dissociated from mouse striatum. (A) Representative images of MSNs (left) and ChIs (right) at two different developmental stages of the mouse. Scale bar=15 μM. (B) Summary plot comparing the peak amplitude of Ca^{2+} currents of MSNs and ChIs. Ca^{2+} currents were generated by voltage step protocols (from −10 to +10 mV) and measured at steady state. Peak amplitude of HVA Ca^{2+} current did not show significant changes during development either in MSNs or in ChIs. Note the significantly larger amplitude of HVA Ca^{2+} current in ChIs with respect to MSNs at each developmental stage considered. Data are presented as mean±S.D.

RESULTS

Distinctive morphological and electrophysiological features of MSNs and ChIs acutely dissociated from mouse striatum

MSNs and ChIs freshly isolated from mouse striatum could be distinguished for their peculiar morphological and electrophysiological characteristics. As previously described, the somatic size was larger in ChIs than in MSNs (Fig. 1A) (Hoehn et al., 1993; Bargas et al., 1994; Yan and Surmeier, 1997), and did not significantly change during the four developmental stages considered (Fig. 1A; $P>0.05$ one-way ANOVA); therefore, data were pooled for each neuronal population. The size of ChIs soma (along the widest diameter) was 28.1±3.0 μm ($n=23$), vs. 17.3±2.2 μm in MSNs ($n=28$; $P<0.0001$, Student's t-test). Another distinctive feature for neuronal identification was represented by the amplitude of HVA Ca^{2+} currents. A larger peak current amplitude was recorded from ChIs in comparison with MSNs (Surmeier et al., 1995; Yan and Surmeier, 1996) (Fig. 1B). HVA Ca^{2+}