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ALTERED PROFILE AND D2-DOPAMINE RECEPTOR MODULATION OF HIGH VOLTAGE-ACTIVATED CALCIUM CURRENT IN STRIATAL MEDIUM SPINY NEURONS FROM ANIMAL MODELS OF PARKINSON'S DISEASE

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Abstract—In the present work we analyzed the profile of high voltage-activated (HVA) calcium (Ca^{2+}) currents in freshly isolated striatal medium spiny neurons (MSNs) from rodent models of both idiopathic and familial forms of Parkinson's disease (PD). MSNs were recorded from reserpine-treated and 6-hydroxydopamine (6-OHDA)-lesioned rats, and from DJ-1 and PINK1 (PTEN induced kinase 1) knockout ($^{-/-}$) mice. Our analysis showed no significant changes in total HVA Ca^{2+} current. However, we recorded a net increase in the L-type fraction of HVA Ca^{2+} current in dopamine-depleted rats, and of both N- and P-type components in DJ-$1^{-/-}$ mice, whereas no significant change in Ca^{2+} current profile was observed in PINK$1^{-/-}$ mice. Dopamine modulates HVA Ca^{2+} channels in MSNs, thus we also analyzed the effect of D1 and D2 receptor activation. The effect of the D1 receptor agonist SKF 83822 on Ca^{2+} current was not significantly different among MSNs from control animals or PD models. However, in both dopamine-depleted rats and DJ-$1^{-/-}$ mice the D2 receptor agonist quinpirole inhibited a greater fraction of HVA Ca^{2+} current than in the respective controls. Conversely, in MSNs from PINK$1^{-/-}$ mice we did not observe alterations in the effect of D2 receptor activation. Additionally, in both reserpine-treated and 6-OHDA-lesioned rats, the effect of quinpirole was occluded by the selective L-type Ca^{2+} channel blocker nifedipine, while in DJ-$1^{-/-}$ mice it was mostly occluded by ω-conotoxin GVIA, blocker of N-type channels. These results demonstrate that both dopamine depletion and DJ-1 deletion induce a rearrangement in the HVA Ca^{2+} channel profile, specifically involving those channels that are selectively modulated by D2 receptors. © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: HVA calcium channels, medium spiny neuron, 6-hydroxydopamine, reserpine, DJ-1, PINK1.

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Abbreviations: CN, cell capacitance; HBSS, Hank's balanced salt solution; HVA, high voltage-activated; MSNs, medium spiny neurons; PD, Parkinson's disease; PINK1, PTEN induced kinase 1; SNpc, substantia nigra pars compacta; 6-OHDA, 6-hydroxydopamine.

Parkinson's disease (PD) is a neurodegenerative movement disorder characterized by bradykinesia, rigidity, resting tremor, and postural instability. Although the occurrence of PD is largely sporadic, an increasing number of monogenic mutations in distinct genes—such as the large exonic deletions or frame-shift truncations, suggestive of a "loss of function" mechanism, found in DJ-1 and PINK1 (PTEN induced kinase 1) genes (Bonifati et al., 2003; Valente et al., 2004; Kitada et al., 2007)—has been linked to familial forms of parkinsonism, which clinically resemble idiopathic PD. The clinical features of idiopathic and familial PD are thought to result from a reduced dopaminergic input to the striatum, inducing a complex rearrangement in the functional anatomy of the basal ganglia. In particular, dopamine depletion has been shown to cause the loss of spines in medium spiny neurons (MSNs) from both animal models of PD and parkinsonian patients (for review see: Deutch et al., 2007; Smith and Villalba, 2008). This morphological alteration of MSNs has been recently ascribed to the disinhibition of Cav1.3-containing L-type Ca^{2+} channels, as a consequence of the reduced striatal dopaminergic tone (Day et al., 2006). In fact, chronic administration of an L-type channel antagonist completely prevented the spine loss induced in rats by acute DA depletion. Conversely, spine density was found to be significantly increased in *Cacna1d*$^{-/-}$ mice, lacking the Cav1.3α1 subunits. L-type Ca^{2+} channels have already been implicated in the selective degeneration of dopaminergic neurons in PD (Chan et al., 2009); these experimental data suggest that high voltage-activated (HVA) Ca^{2+} channels might play a direct role in striatal dysfunction, as well.

EXPERIMENTAL PROCEDURES

All experiments were performed in accordance with both the EC and Italian guidelines (86/609/EEC; D.Lvo 116/1992, respectively) and approved by the University of Rome "Tor Vergata" (n. 153/2001A).

Animal models

6-hydroxydopamine (6-OHDA)-lesioned rats. Dopamine denervation was obtained by injecting the neurotoxin 6-OHDA (1 mg/kg) into the substantia nigra pars compacta (SNpc) of adult male Wistar rats (150–170 g). Animals were anaesthetized with xylazine (7.5 mg/kg, i.m.) and ketamine (50 mg/kg, i.m.) and secured in a stereotaxic apparatus (Stoelting, Woodale, IL, USA). The injection of 6-OHDA into the mesencephalon was performed according to the following coordinates (from bregma): AP, −4.4; L, +1.2; DV, −7.8. After 15 days, dopamine denervation was as-

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sessed by apomorphine-driven test (1 mg/ml/kg). Animals showing ≥400 rotations/h ($n=17$) were used for the electrophysiological experiments. Fifteen rats were injected with saline solution in the same surgical sessions (sham-operated group).

Reserpine model. Adult Wistar rats (135–170 g) were injected i.p. with reserpine (3–4 mg/kg in 0.5% glacial acetic acid in PBS). Either 20–24 h ($n=13$) or 48 h ($n=4$) after reserpine injection rats displaying typical parkinsonian-like symptoms (bradykinesia, posture stiffness, and tremor) were used for electrophysiological recordings. Vehicle-treated controls were prepared in the same sessions ($n=15$).

Preparation of acutely dissociated striatal neurons

Coronal corticostriatal slices (450 μm thick) were cut as previously described (Martella et al., 2008) from brain tissue blocks of either male Wistar rat (125–150 g) or wild-type, DJ-1$^{-/-}$, and PINK1$^{-/-}$ mice (aged 40–45 days) generated in J. Shen laboratories (Goldberg et al., 2005; Kitada et al., 2007). Data obtained from each animal group were compared to the respective controls. Vehicle-treated and sham-operated rats were utilized as controls for the reserpinized and 6-OHDA-lesioned animals, respectively, while wild-type littermates of the same mixed genetic background were utilized as controls for either DJ-1$^{-/-}$ or PINK1$^{-/-}$ mice. In brief, animals were killed under ether anaesthesia by cervical dislocation, the brains were rapidly removed, and coronal corticostriatal slices were cut with a vibratome in ice-cold Krebs' solution (in mM: 126 NaCl, 2.5 KCl, 1.3 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 10 glucose, and 18 $NaHCO_3$). Afterward, as previously described (Martella et al., 2008) the striatum was dissected from slices and incubated first in HEPES-buffered Hank's balanced salt solution (HBSS), bubbled with 100% O_2 at 35 °C, and then in HBSS containing 0.5 mg/ml protease XIV for 30 min. After repeated wash-out in HBSS, the tissue was mechanically triturated with a graded series of fire-polished Pasteur pipettes. The cell suspension 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 10–15 min.

Patch-clamp recordings

Freshly isolated MSNs were identified by their morphologic and electrophysiological properties (Martella et al., 2008). Patch-clamp recordings in the whole-cell configuration were performed by using glass pipettes (WPI PG52165-4, Germany) pulled with a Flaming-Brown puller (Sutter Instrument, Novato, CA, USA) and fire-polished before use. Pipette resistance ranged from 3 to 8 MΩ. The composition of the internal solution was (in mM): N-methyl-D-glucamine, 185; HEPES, 40; EGTA, 11; $MgCl_2$, 4; phosphocreatine, 20; ATP, 2 to 3; guanosine triphosphate (GTP), 0 to 0.2; leupeptin, 0.2; pH 7.36, 280 mOsm/L. After obtaining the cell access, cells were bathed in (mM): TEA-Cl, 155; $CsCl_2$, 5; HEPES, 10; and $BaCl_2$, 5, as the charge carrier; pH 7.35, 300 mOsm/L. Control and drug solutions were applied with a linear array of six gravity-fed capillaries positioned 500–600 μm close to the patched neuron. Recordings were made with an Axopatch 1D (Axon Instrument). Electrode resistances in bath were ~3–6 MΩ. After formation of a GΩ seal and subsequent cell rupture, series resistance was compensated (75–85%) and periodically monitored. Data were low-pass filtered (corner frequency, 5 kHz). For data acquisition and analysis, pClamp 9.2 software (Axon Instruments) was used.

Total HVA Ca^{2+} current was examined by utilizing either ramp test (from −70 mV to +40 mV) or test pulse protocols (a single step from −60 mV to +10 mV; or incremental 10 mV steps from −70 to +40 mV, Fig. 1A). In order to pharmacologically identify the distinct components of HVA Ca^{2+} current, selective Ca^{2+} channel blockers were applied sequentially: L-type channel blocker nifedipine (NIFE, 1–5 μM), N-type channel blocker ω-Conotoxin GVIA (Ctx-GVIA, 1 μM), P-type channels blocker ω-agatoxin IVA (Atx-IVA, 20 nM), and Q-type channel blocker ω-Conotoxin MVIIC (Ctx-MVIIC, 100 nM). The voltage dependence of activation was determined from measurements of tail current amplitude, which was expected to reflect the fraction of Ca^{2+} channels opened during the preceding depolarization.

Data analysis

Voltage-activated currents were leak subtracted. Cells exhibiting leak currents >10 pA were not included in this analysis. Cell capacitance (C_m) was monitored using the automated function of the Axopatch amplifier. A stable C_m-value over time was an important criterion for the evaluation of the quality of experiments. Statistical tests were performed using Microcal Origin (OriginLab, Northampton, MA, USA) and GraphPad Prism (GraphPad Software, San Diego, CA, USA) softwares. Values given in the text and in the figures are mean±SD of changes in the respective cell populations, unless otherwise stated. The *t*-test or Mann–Whitney test were used for assessing statistical significance where appropriate. Multiple groups were compared using one-way or two-way ANOVA. The Tukey HSD test was used for post hoc comparison of the ANOVA. Values were considered statistically significant when $P<0.05$ with $\alpha=0.001$.

Drug source

Nifedipine, ω-conotoxin GVIA, and ω-agatoxin IVA were from Tocris-Cookson, UK. All other compounds used were purchased from Sigma-Aldrich, Italy.

RESULTS

Increased L-type Ca^{2+} current fraction in striatal MSNs from dopamine-depleted rats

Two different experimental approaches were utilized to obtain striatal dopamine depletion modelling idiopathic PD (for review see: Bonsi et al., 2006; Gubellini et al., 2010). In a group of male Wistar rats ($n=17$) the neurotoxin 6-OHDA (1 mg/kg; Baunez et al., 1995) was delivered into the SNpc by sterotactic injection. In the same surgical sessions, saline was injected into the SNpc of another group of 15 rats (sham-operated group). Furthermore, in a different group of animals endogenous amines were depleted by 3–4 mg/kg, i.p. reserpine treatment (Harrison et al., 2001) and after 24 ($n=13$) or 48 h ($n=4$) rats manifesting typical parkinsonian-like symptoms were utilized for the experiments (Baunez et al., 1995; Spadoni et al., 2004). Vehicle-treated controls were prepared in the same sessions ($n=15$).

Total HVA Ca^{2+} current was recorded from 173 MSNs acutely isolated from the striata of either vehicle- and reserpine-treated rats, or 6-OHDA-lesioned and sham-operated rats (Fig. 1A). Peak amplitude of the current was not statistically different either between vehicle- (625.7±23.6 pA; $n=43$), and reserpine-treated rats (627.6±33.2 pA; $n=45$; $P>0.05$ Mann–Whitney test; not shown), or between sham-operated (671.8±35.4 pA; $n=41$) and 6-OHDA-lesioned rats (611.2±21.2 pA; $n=44$; $P>0.05$ Mann–Whitney test; not shown). Moreover, the amplitude of total HVA Ca^{2+} current was not statistically different between the groups ($P>0.05$ ANOVA followed by Tukey HSD test). In order to rule out changes in cell surface area