Low-Threshold L-type Calcium Channels in Rat Dopamine Neurons

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Durante, P., C. G. Cardenas, J. A. Whittaker, S. T. Kitai, and R. S. Scroggs. Low-threshold L-type calcium channels in rat dopamine neurons. J Neurophysiol 91: 1450–1454, 2004. First published November 26, 2003; 10.1152/jn.01015.2003. Ca2+ channel subtypes expressed by dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc) were studied using whole cell patch-clamp recordings and blockers selective for different channel types (L, N, and P/Q). Nimodipine (Nim, 2 μM), ω-conotoxin GVIA (Ctx, 1 μM), or ω-agatoxin IVA (Atx, 50 nM) blocked 27, 36, and 37% of peak whole cell Ca2+ channel current, respectively, indicating the presence of L-, N-, and P-type channels. Nim blocked approximately twice as much Ca2+ channel current near activation threshold compared with Ctx or Atx, suggesting that small depolarizations preferentially opened L-type versus N- or P-type Ca2+ channels. N- and L-channels in DA neurons opened over a significantly more negative voltage range than those in rat dorsal root ganglion cells, recorded from using identical conditions. These data provide an explanation as to why Ca2+-dependent spontaneous oscillatory potentials and rhythmic firing in DA neurons are blocked by L-channel but not N-channel antagonists and suggest that pharmacologically similar Ca2+ channels may exhibit different thresholds for activation in different types of neurons.

INTRODUCTION

Numerous studies have indicated that Ca2+ entry is important in regulating the firing patterns of dopaminergic (DA) neurons in the substantia nigra. The pacemaker-like slow depolarizations (PLSD), spontaneous oscillatory potentials (SOPs), and slow afterhyperpolarizations involved in regulation of DA neuron firing activity have all been shown to be Ca2+-dependent (Fujimura and Matsuda 1989; Grace and Onn 1989; Harris et al. 1989; Kang and Kitai 1993a,b; Mercuri et al. 1994; Nedergaard et al. 1993). A previous study suggests that DA neurons express L- and N-type Ca2+ channels. N- and L-channels in DA neurons opened over a significantly more negative voltage range than those in rat dorsal root ganglion cells, recorded from using identical conditions. These data provide an explanation as to why Ca2+-dependent spontaneous oscillatory potentials and rhythmic firing in DA neurons are blocked by L-channel but not N-channel antagonists and suggest that pharmacologically similar Ca2+ channels may exhibit different thresholds for activation in different types of neurons.

METHODS

Isolation of neurons

Brain slices containing the substantia nigra pars compacta (SNc) were prepared from young Sprague–Dawley rats (Harlan) 13–17 days of age as described previously (Scroggs et al. 2001). For isolation of DA neurons, pieces of SNc were cut from the brain slices and incubated for 30 min at 35°C in Tyrode’s (continuously bubbled with 100% O2) containing 2 mg/ml pronase E (Sigma). After incubation the pieces of SNc were washed with Tyrode’s solution containing (in mM): 140 NaCl, 4 KCl, 2 MgCl2, 2 CaCl2, 10 glucose, and 10 HEPES, pH 7.4 with NaOH. The pieces of SNc were then triturated in Tyrode’s solution with a fine-bore Pasteur pipette and the resulting suspension was plated onto the bottom of a 35-mm plastic petri dish (Falcon) that served as a recording chamber. DRG cells were isolated from male Sprague–Dawley rats (50–100 g), as previously described (d’Alcantara et al. 2002). All procedures involving animals were approved by The University of Tennessee Health Science Center Animal Care and Use Committee.

DA neuron identification

An analysis of acutely isolated neurons from the SNc (Fig. 1A) using antibodies against tyrosine hydroxylase (TH) showed that 76% of the neurons that were ≥30 μm along one axis were TH positive (TH+), while only 18% of cells < 30 μm were TH+. Thus neurons ≥30 μm were chosen for recording. In addition, we characterized neurons regarding their expression of IcaL and IcaT, which are selectively expressed in DA neurons versus other neurons in the SNc (Grace and Onn 1989; Kang and Kitai 1993a,b; Yung et al. 1991). IcaL was tested for with a family of voltage commands from a holding potential of −50 to −110 through −140 mV (Fig. 1B). IcaT was tested for by a

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Ca\(^{2+}\) channel currents were isolated with a solution containing (in mM): 160 tetraethylammonium chloride, 2 BaCl\(_2\), and 10 mM HEPES, pH 7.4 with tetraethylammonium hydroxide.

Data analysis

The effect of Ca\(^{2+}\) channel blockers Ctx, \(\omega\)-agatoxin IV (Bachem), and nimodipine, (Sigma-Aldrich) on the whole cell Ca\(^{2+}\) channel current amplitude was estimated from plots of current versus time. Ca\(^{2+}\) channel current rundown was taken into account as described previously (Scroggs and Fox 1992). Best fit values for \(V_{1/2}\) and the slope factor \(k\) for conductance-voltage relationships for activation were estimated by fitting the Boltzmann relationship: 

\[
g = g_{\text{max}}/[1 + \exp(V_{1/2} - V_g/k)]
\]

to the conductance observed at different test potentials. The data are presented as the mean \(\pm\) SE.

Results

The effects of 2 \(\mu\)M Nim, 1 \(\mu\)M \(\omega\)-agatoxin GVIA (Atx), and 50 nM Ctx on peak Ca\(^{2+}\) channel current amplitude was assessed in 33 putative DA neurons identified as described under METHODS (see Fig. 1). The blockers were studied in pairs (Nim vs. Ctx, \(n = 10\); Nim vs. Atx, \(n = 11\); Ctx vs. Atx, \(n = 12\)), and their order of addition was varied to address specificity. On average Nim (\(n = 21\)), Atx (\(n = 23\)), and Ctx (\(n = 22\)) blocked 27 \(\pm\) 1.9, 34 \(\pm\) 1.3, and 36 \(\pm\) 1.2\% of whole cell current, respectively (Fig. 2). The average effects of the blockers were changed by 5\% or less when added before versus after another blocker (not significant). Addition of all three blockers simultaneously reduced whole cell Ca\(^{2+}\) channel current by an average of 85 \(\pm\) 1.2\% (\(n = 5\)).

The fractions of current sensitive to blockade by Atx, Ctx, and Nim at different voltages were quantified by subtraction of current-voltage (I-V) data acquired before and after treatment with each blocker (Fig. 2, B, D, and F). Nim blocked significantly more current (45–50\%) that was evoked by test potentials to \(-50\) through \(-35\) mV compared with Atx and Ctx (18–25\%; two-way ANOVA; group \(P = 1.02 \times 10^{-6}\), test potential versus group \(P = 9.95 \times 10^{-12}\)). There was little variation in the estimate for total current blocked by the three agents versus voltage (Fig. 3A). The \(V_{1/2}\) for activation of the Nim-sensitive current was \(-31.1 \pm 0.7\) mV, which was significantly more negative than the average of \(-26.1 \pm 0.8\) mV for the Atx-sensitive current and \(-24.8 \pm 0.9\) mV for the Ctx-sensitive current (Fig. 3B, Tukey’s t-test, \(P = 0.0001\) for Nim vs. Atx and \(P = 0.0002\) for Nim vs. Ctx). In the five DA neurons where all three blockers were added simultaneously, the \(\approx 15\%\) unblocked current had a \(V_{1/2}\) of \(-25.7 \pm 0.5\) mV, similar to the Ctx- and Atx-sensitive fractions.

Nim (2 \(\mu\)M) and Ctx (1 \(\mu\)M) were also tested on acutely isolated DRG cells for comparison to DA neurons. The \(V_{1/2}\) of the conductance-voltage (g-V) relationships for Ctx- and Nim-sensitive currents in DA neurons were about 10 mV more negative than their counterparts in small diameter DRG neurons (\(n = 5\)). In the DRG cells included 2 \(\mu\)M Nim blocked 39 \(\pm\) 6.1\%, and 1 \(\mu\)M Ctx blocked 39 \(\pm\) 6.4\% of the whole cell Ca\(^{2+}\) channel current (Fig. 3B). Notice that Nim-sensitive current activated over significantly more negative voltages than Ctx-sensitive current in the DRG cells, similar to the situation in DA neurons.

![Figure 1](http://jn.physiology.org/)

**FIG. 1.** Identification of acutely isolated SNC-DA neurons. A: digital image of a tyrosine hydroxylase positive (TH\(^{+}\)) neuron adhered to the bottom of a 33-mm petri dish. The isolated neurons were plated onto a poly-L-lysine-coated coverslip and tested for expression of tyrosine hydroxylase (TH) using primary antibodies followed by diaminobenzene-conjugated secondary antibodies. The dark staining of the neuron in the center of the photograph indicates a positive reaction for TH. B: family of H-currents evoked in a putative acutely isolated SNC-DA neuron. C: blockade of \(I_h\) by 4-aminopyridine in a putative SNC-DA neuron. The current was inward because there was no K\(^{+}\) added to the pipette solution and there was 4 mM K\(^{+}\) in the external Tyrode’s solution.

Depolarizing voltage command to \(-10\) from a holding potential of \(-70\) mV (Fig. 1C). Only neurons that exhibited \(I_h\) and \(I_A\) were included in the study.

Recording

All experiments were done at room temperature (23\°C). Currents and voltages were recorded in the whole cell patch configuration using an Axopatch 200A, a Digidata 1200, and P-clamp 8.2 (Axon Instruments). Leak subtraction was carried out using the P/4 technique. Electrodes were coated with Sylgard to about 200 \(\mu\)m from the tip and fire polished to a final resistance of 2–4 M\(\Omega\). Whole cell capacitance was not nulled so that series resistance could be estimated from capacity transients after compensation, as described previously (Scroggs and Fox 1992).

Solutions

Solutions superfusing the neuron under study were changed using a series of glass capillary tubes mounted on a motorized micromanipulator as described previously (d’Alcantara et al. 2002). The patch electrodes were filled with a solution containing (in mM): 190 N-methylglucamine, 5 2Na-ATP, 0.48 2Li-GTP, 0.1 mM BAPTA, and 40 HEPES, adjusted to pH 7.4 with H\(_2\)SO\(_4\). \(I_h\) and \(I_R\) were tested for in external Tyrode’s solution containing 500 \(\mu\)M Cu\(^{2+}\) and 400 nM tetrodotoxin (citrate salt, Sigma). Ca\(^{2+}\) channel currents were isolated with a solution containing (in mM): 160 tetraethylammonium chloride, 2 BaCl\(_2\), and 10 mM HEPES, pH 7.4 with tetraethylammonium hydroxide.
FIG. 2. Effects of nimodipine (Nim) \(\omega\)-agatoxin IVA (Atx), and \(\omega\)-conotoxin GVIA (Ctx) on whole cell Ca\(^{2+}\) channel current amplitude in acutely isolated DA neurons. A, C, and E: plots of Ca\(^{2+}\) channel current versus time, showing the reduction of current amplitude produced by application of 2 \(\mu\)M Nim, 50 nM Atx, or 1 \(\mu\)M Ctx (A, C, and E, respectively) and then a further reduction produced by subsequent addition of 1 \(\mu\)M Ctx, 2 \(\mu\)M Nim, or 50 nM Atx (A, C, and E, respectively) in the continued presence of the first blocker. B, D, and F: plots of current-voltage (I-V) relationships for activation of Ca\(^{2+}\) channel current in the experiments depicted in A, C, and E, under initial control conditions, after treatment with the first and second blockers. Each data point represents the average of several identical experiments (A, \(n = 4\); C, \(n = 6\); and E, \(n = 8\)). The amplitudes of the data points have been corrected for rundown based on the estimated percent reduction of peak current produced by the various blockers in each case.

**DISCUSSION**

The major finding of this study is that, in DA neurons, current conducted through L-type Ca\(^{2+}\) channels makes up the bulk of whole cell Ca\(^{2+}\) channel current evoked by small depolarizations near the activation threshold. These data support previous studies showing that SOPs and spontaneous firing are abolished by L-channel blockers (Mercuri et al. 1994; Nedergaard et al. 1993). SOPs and the PLSD, which underlie regular spontaneous firing, generally involve fluctuations of the membrane potential between around –50 and –40 mV, where L-channel current may comprise the bulk of the whole cell Ca\(^{2+}\) current in DA neurons. Conversely, N-channel current appears to account for a much smaller fraction of whole cell Ca\(^{2+}\) current over this same voltage range, which could explain why the N-channel antagonist Ctx did not abolish SOPs or spontaneous firing in DA neurons in two studies (Fujimura and Matsuda 1989; Nedergaard et al. 1993).

Our estimates of current block by Nim, Ctx, and Atx are probably not highly accurate representations of the contribution of L-, P-, and N-channels to whole cell Ca\(^{2+}\) channel current at different membrane potentials under physiological conditions. The permeability of the different channels to Ba\(^{2+}\) is different from that for Ca\(^{2+}\). Also, the presence of rundown likely generates errors in the quantification of the block. Finally, 2 mM Ba\(^{2+}\) externally and the N-methyl-d-glucamine-based internal solution both produce negative shifts in the voltage-dependency of Ca\(^{2+}\) channels (Bargas et al. 1994; Hille 1992; Malecot et al. 1988). Although, regarding this last point, a previous study using Ca\(^{2+}\) imaging suggests that the threshold for Ca\(^{2+}\) entry in DA neurons is around –50 mV (Wilson and Callaway 2000), similar to what we observed for Ba\(^{2+}\) in this study.

Despite the above caveats, our finding that L-channel current predominates over N- and P-current during small depolarizations is likely qualitatively accurate. Nim, Ctx, and Atx each appeared to have a robust effect, and there was little overlap in the fractions estimated targeted by each agent, which should have been detected by changing the order of addition. The lack of overlap is not surprising since Nim, Ctx, and Atx have been shown to be selective for L-, N-, and P/Q-channels, respectively, at the concentrations we used (Boland et al. 1994; Mintz et al. 1992; Randall and Tsien 1995; Sather et al. 1993). Thus relatively pure and robust Nim-, Ctx-, and Atx-sensitive current fractions could be identified by subtraction and compared regarding their respective g-V relationships.

The relative nature of the g-V relationships for Nim-, Ctx-, and Atx-sensitive current would not likely be affected by a lack of saturation by one or more blockers, unless DA neurons express subpopulations of channels that have a differential sensitivity to a given blocker. For example, we did not prove that 2 \(\mu\)M Nim is a saturating concentration regarding L-channels in DA neurons, and different types of L-channels may vary in their sensitivity to Nim. Thus higher concentrations of Nim could block a second population of L-channels that have a g-V relationship closer to that of N- or P-channels. However, this possibility does not preclude the observation that \(\approx 45\text{--}50\%\) of the current evoked by test potentials ranging from –50 to –35 mV was blocked by 2 \(\mu\)M Nim. Another issue is the possibility that the 50 nM concentration of Atx used could have blocked a small fraction of Q-channels in addition to P-chan-
addition, this phenomenon has been observed in striatal and Ctx-sensitive current in small diameter DRG cells. In a previous study (Cardozo and Bean 1995), percent blockade and $g-V$ relationships versus test potential for Atx-, Ctx-, and Nim-sensitive current in small diameter dorsal root ganglion cells (open symbols, dashed lines). The current sensitive to the different toxins was quantified by measurement of $I-V$ curves fitted with splines in Systat (SPSS) at points corresponding to $-50$ through $0$ mV in $5$-mV increments. The lines were fitted to the control and treatment data points using a Boltzmann function as described under METHODS. The error bars represent the SE.

defined by the SOPS and spontaneous oscillations in DA neuron dendrites as well as soma.

In addition to providing a likely explanation for why selective L-channel blockers abolish SOPS and spontaneous firing in DA neurons, our study suggests that pharmacologically similar Ca$^{2+}$ channels may activate over significantly different voltage ranges in different types of neurons. In the present study N- and L-type Ca$^{2+}$ channel current activated over a significantly more negative voltage range in DA neurons versus DRG neurons. A similar difference in voltage of activation of whole cell Ca$^{2+}$ channel current (recorded using the same solutions) is apparent regarding striatal medium spiny neurons and neocortical neurons, which are similar to each other regarding the magnitude of L-, N-, P/Q-, and R-current fractions (Bargas et al. 1994; Lorenzon and Foehring 1995).

In summary, we conclude that L-channels play a critical role in the firing behavior of DA neurons. In DA neurons, current conducted through L-channels predominates over that conducted through other types of Ca$^{2+}$ channels at voltages ranging from around $-50$ to $-40$ mV, where Ca$^{2+}$ entry helps drive DA neurons to action potential threshold.

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**References**


