Neuropharmacology and analgesia

Interactions between calcium channels and SK channels in midbrain dopamine neurons and their impact on pacemaker regularity: Contrasting roles of N- and L-type channels

Veronne de Vrind, Jacqueline Scuvée-Moreau, Guillaume Drion, Cyrine Hmaied, Fabian Philippart, Dominique Engel, Vincent Seutin

Keywords: SK channel, Ca\textsuperscript{2+} channels, Dopaminergic neurons

A B S T R A C T

Although small-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} (SK) channels and various types of voltage-gated Ca\textsuperscript{2+} (Ca\textsubscript{v}) channels have been described in midbrain dopaminergic neurons, the nature of their interactions is unclear. More particularly, the role of various Ca\textsubscript{v} channel types in either promoting irregularity of firing (by generating an inward current during SK channel blockade) or promoting regularity of firing (by providing the source of Ca\textsuperscript{2+} for the activation of SK channels) has not been systematically explored. We addressed this question using intracellular and extracellular recordings from substantia nigra, pars compacta (SNc), dopaminergic neurons in rat midbrain slices. Neurons were pharmacologically isolated from their differences. When examining the ability of various Ca\textsubscript{v} channel blockers to inhibit the SK-mediated afterhyperpolarization (AHP), we found that only the N-type Ca\textsubscript{v} channel blocker ω-conotoxin-GVIA was able to reduce the apamin-sensitive AHP, but only partially (~40%). Specific blockers of L, P/Q, T or R channels had no effect on this AHP. Combining ω-conotoxin-GVIA and other specific blockers did not yield greater block and even the broad Ca\textsubscript{v} blocker Cd\textsuperscript{2+} induced a submaximal (~75%) effect. Extracellular recordings examining firing regularity yielded congruent results: none of the specific blockers was able to increase firing irregularity to the extent that the specific SK blocker apamin did. The irregularity of firing observed with apamin could only be reversed by blocking L-type Ca\textsuperscript{2+} channels. Thus various sources of Ca\textsuperscript{2+} appear to be required for SK channel activation in SNc neurons (some of them still unidentified), ensuring robustness of pacemaking regularity.

1. Introduction

Dopaminergic neurons of the substantia nigra, pars compacta (SNc), sustain important physiological functions such as the control of motricity, motivation and cognition. In vivo, these neurons exhibit a variety of firing patterns, switching from a regular "pacemaker" pattern to irregular firing and/or bursting. This pattern is absent in ex vivo recordings. Indeed, only a highly regular pacemaker-like pattern occurs spontaneously in mature dopaminergic neurons recorded ex vivo, suggesting that afferent inputs play an important role in the control of bursting (Grace and Onn, 1989).

The regularity of the slow pacemaking is disrupted to a variable extent by bath application of apamin, a selective blocker of small-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} (KCa\textsubscript{2+} and SK) channels (Shepard and Stump, 1999; Wolfart and Roeper, 2002; Johnson and Wu, 2004). SK channels mediate a medium duration AHP which is very prominent in dopaminergic neurons recorded ex vivo. Because SK channels are solely gated by the intracellular Ca\textsuperscript{2+} concentration in their vicinity, the source of Ca\textsuperscript{2+} which activates these channels is of utmost importance to the pacemaking. Generally speaking, this source appears to be quite variable depending on neuronal types. Indeed, SK channels have been reported to be selectively coupled to P/Q-type Ca\textsubscript{v} channels in rat cerebellar Purkinje neurons (Womack et al., 2004) while they are coupled to N-type Ca\textsubscript{v} channels in rat vestibular nucleus neurons (Smith et al., 2002), rat striatal cholinoergic interneurons (Goldberg and Wilson, 2005) and rat superior cervical ganglion (Davies et al., 1996). In this last preparation, a contribution of Ca\textsuperscript{2+} derived from intracellular
stores was also suggested. A recent study by our group also showed that N-type Ca\(^{2+}\) channels play a major role in the activation of SK channels in rat serotonergic neurons (Alix et al., 2014). In the case of SNC dopaminergic neurons, a previous study has suggested that T-type channels are the main source of Ca\(^{2+}\) for SK channel activation in juvenile mice but N-type Ca\(^{2+}\) channels also appeared to be involved (Wolfart and Roeper, 2002). Another study in dissociated rat dopaminergic neurons suggests the involvement of N- and P-type Ca\(_{\alpha}\) channels in the activation of calcium-activated K\(^+\) currents (Cardozo and Bear, 1995).

The aim of this study was to further explore the nature of the interaction between SK channels and various types of Ca\(_{\alpha}\) channels in SNC dopaminergic neurons. Theoretically this interaction could have two opposite consequences on the spontaneous firing pattern: on the one hand, an increase in intracellular Ca\(^{2+}\) through Ca\(_{\alpha}\) activation could induce the activation of SK channels and lead to a slowing of firing activity but, on the other hand, an increase of Ca\(^{2+}\) entry could also result in membrane depolarization and increase in firing rate when SK channels are not activated. Using intracellular recording techniques, we quantified the effect of specific antagonists of the different types of Ca\(_{\alpha}\) channels on the amplitude of the amphetamine-sensitive AHP. Using extracellular recordings, the influence of Ca\(_{\alpha}\) channel blockers on the regularity of firing was investigated and compared with the effect of apamin. Finally we investigated the ability of various Ca\(_{\alpha}\) channel blockers to reverse apamin-induced irregularity.

2. Material and methods

2.1. Animals

Adult male Wistar rats (6–8 week-old) were housed at room temperature in groups of three or four with a 12:12 h light-dark cycle. All animals had access to ad libitum food and water. All procedures were carried out in accordance with guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EE) and were accepted by the Ethics Committee for Animal Use of the University of Liège (protocols 86 and 1210). All efforts were made to minimize animal suffering.

2.2. Brain slice preparation and recording procedures

The methods used were as previously described (Seutin et al., 1990; Scuvené-Moreau et al., 2002). Briefly, rats were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and placed under a cap with oxygenated air (95% O\(_2\), 5% CO\(_2\)) two min prior to decapitation. After decapitation, the brain was rapidly removed and placed in ice cold (−2 °C) oxygenated artificial cerebrospinal fluid (aCSF) of the following composition (in mM): NaCl 130; KCl 3.5; NaH\(_2\)PO\(_4\) 1.25; NaHCO\(_3\) 24; Glucose 10; CaCl\(_2\) 2; MgSO\(_4\) 1.25. A block of tissue containing the midbrain was placed in a vibrating blade microtome (Vibratome 1000 Plus, Sectioning System) and a slice containing the SNC was cut horizontally (400 µm thick). The slice was placed on a nylon mesh in a recording chamber in which oxygenated aCSF (34.0 ± 0.5 °C) continuously flowed at a rate of 2–3 ml/min. All recorded neurons were in the antero-medial part of the SNC as defined by being rostral (and not medial) to the medial terminal nucleus of the accessory optic tract (MT) (Pennados and Watson, 1998). All experiments were performed in oxygenated aCSF with synaptic blockers consisting of 10 µM CNQX, 10 µM SR95531, 1 µM MK801, 1 µM sulpiride and 1 µM CGP55845, which block AMPA/Kainate, GABA\(_{\alpha}\), NMDA, D2/D3 and GABA\(_{\beta}\)-receptors, respectively.

Intracellular recordings of SNC dopaminergic neurons were performed with glass microelectrodes filled with 2 M KCl (impedance: 90–150 MΩ). All recordings were made in the bridge balance mode, using an NPI BA-1S amplifier (NPI Electronic GmbH, Tamm, Germany). Membrane potentials and injected currents were digitized by a Powerlab 4/30 converter and recorded using LabChart7 (AD Instruments, Spechbach, Germany). The accuracy of the voltage measurement was checked by withdrawing the electrode from the neuron at the end of the recording. SNC dopaminergic cells were characterized by a slow spontaneous action potential firing, broad action potentials, a prominent SK-mediated AHP peaking 45–55 ms after the peak of the action potential (see Fig. 1A) and a prominent sag during hyperpolarizing current pulses due to the activation of the I\(_{h}\) current. Taken together, these characteristics identify the neurons as dopaminergic (Seutin et al., 1997). In all experiments, care was taken to maintain the firing rate slow (−0.5 Hz) and constant (because the amplitude of the AHP is very sensitive to the firing rate). For this purpose, a small amount of hyperpolarizing current was injected (−30 to −150 pA) and sometimes needed very small adjustments (10–20 pA). The following protocol was used: after superfusion of synaptic blockers during 10 min, the drug under study (Ca\(_{\alpha}\) blocker or CPA) was added and superfused during 10 min; apamin was then superfused in order to completely block the AHP. Drug effects were measured during the last minute of their superfusion. Effects on the amphetamine-sensitive AHP were quantified as the percentage of reduction of the surface area of the AHP (in mV s) which was blocked by a supramaximal (300 nM) concentration of apamin. In order to measure this percentage, the total surface of the amphetamine-sensitive AHP (in mV s) was measured at the end of the experiment, by subtracting the area in apamin from the area in control conditions. Drug effects on the AHP were then quantified by using the following formula: (total surface − surface in the presence of the blocker)/ total surface.

Extracellular single cell recordings of SNC dopaminergic neurons were performed with glass microelectrodes filled with aCSF (resistance 5–10 M Ωm). Signals were passed through an impedance adapter and were amplified 1000X using a home-made amplifier. They were displayed on a Fluke Combiscope oscilloscope and fed to an analog–digital interface (CED 1401, Cambridge Electronic Design, Cambridge, UK) connected to a computer. Data were collected and analysed with the Spike 2 software (same Company). SNC dopaminergic cells were characterized by spontaneous and regular action potential firing (variation of less than 10% over a 5 min period), low firing frequencies (0.5–5.0 Hz), long action potential durations (>2 ms) and a decrease in firing during application of the D2 receptor agonist quinpirole (1 µM) ten min after wash-out of the synaptic blockers. This technique was chosen for studying the effect of drugs on firing regularity because it is more suitable for long duration recordings.

2.3. Compounds

The following drugs were used (all bath applied at a supramaximal concentration for the block of their target): apamin (300 nM), ω-agatoxin IVa (1 µM), ω-conotoxin-GVIA (1 µM), nifedipine (5 µM), SNX-482 (100 nM) and TTA-P2 (3,5-dichloro-N-[1-(2,2-dimethyl-tetrahydro-pyran-4-ylmethyl)-4-fluoro-piperidin-4-ylmethyl]-benzamide) (3 µM), which block SK-channels, P/Q-, N-, L- and T-type Ca\(^{2+}\) channels, respectively. Mibefradil (10 µM) was also used as an additional T- type blocker. Co\(^{2+}\) (1 mM), Cd\(^{2+}\) (100 µM) and Ni\(^{2+}\) (100 µM) were used as broad blockers of voltage-gated Ca\(^{2+}\) channels. Cyclopiazonic acid (CPA) (10 µM), an inhibitor of the sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\)-ATPase was used to block Ca\(^{2+}\) accumulation in intracellular stores. The sources of the compounds were as follows: CGP55845, MK801, CNQX, SR95531, CPA, quinpirole and mibefradil were obtained from Tocris Bioscience (Bristol, UK). Apamin, Co\(^{2+}\), Cd\(^{2+}\),
Ni²⁺, sulpiride and nifedipine were purchased from Sigma (St Louis, MO, USA), ω-conotoxin-GVIA and ω-agatoxin from Bachem (Bubendorf, Switzerland) and SNX-482 from Peptide International. TTA-P2 was generously provided by Merck and Co., Inc. All pharmacological agents were bath-applied during 10 min and drug effects reported are those measured during the last min of their superfusion.

2.4. Data analysis

The irregularity of firing during extracellular recordings was assessed by the coefficient of variation (CV) of the interspike intervals (ISIs) = standard deviation of ISIs/Mean of ISIs. Statistical analysis was performed using the Friedman test, Wilcoxon Signed-Rank test and Student’s t test. Data are reported as means ± S.E.M.

3. Results

3.1. No single Caᵥ channel blocker is able to completely block the apamin-sensitive AHP of dopaminergic neurons

As shown in Fig. 1 (A and B), our intracellular experiments yielded surprising results: thus, the T-type Ca²⁺ channel blockers mibefradil and TTA-P2 had almost no effect on the apamin-sensitive AHP. In contrast, ω-conotoxin-GVIA yielded a reproducible, but largely submaximal block of the AHP (41 ± 5%, n = 7). Nifedipine, SNX-482 and ω-agatoxin were devoid of any effect. Combining ω-conotoxin-GVIA with TTA-P2 and SNX-482 yielded a block which was similar to the one produced by the N-type blocker alone (39 ± 2%, n = 3). Even the broad Ca²⁺ blockers Co²⁺ and Cd²⁺ yielded submaximal effects (67 ± 5%, n = 8 and 76 ± 2%, n = 4). Nickel, which preferentially blocks T and P/Q Ca²⁺ channels, had a weak effect (10 ± 6%, n = 4). Finally, we did not see any effect of CPA (n = 4) on the AHP, although it has previously been found to affect SK channel opening under different circumstances (Fiorillo and Williams, 1998; Seutin et al., 2000). Taken together these data suggest that the source of Ca²⁺ which activates SK channels during the AHP is heterogenous. We next turned to extracellular recordings to check the significance of this finding, since apamin was found to induce firing irregularities in a majority of dopaminergic neurons (Shepard and Stump, 1999; Johnson and Wu, 2004).

3.2. No single Caᵥ channel blocker mimics the “irregularity-inducing” effect of apamin

We first tested the ability of apamin to induce firing irregularities in an extensive set of neurons. Although the amplitude of the effect was highly variable (the firing pattern of some neurons being marginally affected), the overall effect was highly significant: the CV rose from 0.056 ± 0.006 to 0.521 ± 0.072 (Wilcoxon Signed-Rank test, P < 0.001, n = 49) (Fig. 2A). We next investigated whether individual blockers could mimic the deregularizing effect of apamin. In these experiments, the protocol consisted of a control period followed by the superfusion of one Caᵥ channel blocker alone, a wash-out period and finally superfusion of apamin. We found that none of the specific Caᵥ channel blockers was able to deregularize the firing to the extent of apamin (Fig. 2B and Table 1). Even ω-conotoxin-GVIA, the most effective blocker of the AHP, had a largely smaller effect than apamin as it increased the CV from 0.04 ± 0.01 to 0.08 ± 0.03, whereas apamin increased it to 1.32 ± 0.72 in the same cells (n = 8). In agreement with the results on the block of the AHP, other blockers were also much less effective than apamin (Table 1). A similar observation was made when ω-conotoxin-GVIA was co-applied with other blockers (TTA-P2 and SNX-482) (Fig. 2C).

The influence of the various Caᵥ blockers on the firing rate of dopaminergic neurons was also investigated as significant effects had been reported in the literature in dissociated nigral neurons (Puopolo et al., 2007). No significant effect was observed for any of them on this parameter (Table 2). Nifedipine was not tested in this protocol as we have previously shown that this compound has a weak inhibitory effect on the frequency of pacemaking and have suggested that subtle differences between ion channel densities in dopaminergic neurons recorded in different laboratories may

---

Fig. 1. No specific Caᵥ channel blocker is able to completely block the apamin-sensitive AHP. A: Example of an intracellular recording of a dopaminergic neuron of the SNc in an adult rat brain slice. Synaptic blockers (see methods) were present throughout the experiment. The T-type Caᵥ blocker TTA-P2 had no effect on the apamin-sensitive AHP while the N-type blocker ω-conotoxin-GVIA induced a partial block. B: Summary of intracellular experiments performed on dopaminergic neurons of the SNc, exploring the potential inhibitory effect of various Caᵥ blockers on the apamin (Apa) sensitive AHP. The broad type Caᵥ channel blockers cobalt and cadmium induced a submaximal block of about 75%. Among selective Caᵥ blockers, only the N-type channel blocker ω-conotoxin-GVIA induced a significant inhibition of the AHP of about 40%. Drugs used were cobalt (Co²⁺ 1 mM, n = 8), cadmium (Cd²⁺ 100 µM, n = 4), nickel (Ni²⁺ 100 µM, n = 4), mibefradil (Mibe 10 µM, n = 7), TTA-P2 (TTA 3 µM, n = 4), ω-conotoxin-GVIA (Cono 1 µM, n = 7), SNX-482 (SNX 100 nM, n = 2), nifedipine (Nife 5 µM, n = 4), ω-agatoxin (Aga 1 µM, n = 3) and cyclopiazonic acid (CPA 10 µM, n = 4).
explain the highly variable effect of dihydropyridines on dopaminergic neuron firing rates (see Drion et al., 2011 for a full discussion on this issue).

3.3. Apamin-induced increase in firing irregularity is only reversed by nifedipine

We next tested the ability of Ca\(^{2+}\) channel blockers to affect the apamin-induced increase in CV. For these experiments, apamin was first superfused alone and then together with a specific Ca\(^{2+}\) channel blocker. As reported previously (Shepard and Stump, 1999), only nifedipine was able to reverse the apamin-induced irregularity (Fig. 2 D). None of the other specific Ca\(^{2+}\) channel blockers had a similar effect (Table 3). Thus the CV increase produced by apamin (from 0.07 ± 0.02 to 0.77 ± 0.19) was markedly reduced by nifedipine (to 0.27 ± 0.11, P=0.021 for nifedipine vs apamin, n=9). Note that ω-conotoxin-GVIA significantly (but weakly) increased the effect of apamin. The reason for this is unclear.

4. Discussion

Our results can be summarized as follows: two lines of evidence suggest that, in mature rat SNc dopaminergic neurons, the
The firing pattern of dopaminergic neurons was recorded extracellularly in adult rat brain slices. The coefficients of variation (CVs) of the interspike intervals are shown. They are much less modified by any of the specific Ca\textsubscript{v} blockers than by apamin. Statistical analysis was performed with the Friedman test (global comparison between groups) and the Wilcoxon Signed-Rank test (comparison between “apamin” and Ca\textsubscript{v} channel blocker “blocker(s)”).

Table 1

<table>
<thead>
<tr>
<th>Blocker</th>
<th>Control</th>
<th>Blocker(s)</th>
<th>Wash out</th>
<th>Apamin</th>
<th>P value Global test</th>
<th>P value Apa vs Blocker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apamin</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>1.10 ± 0.52</td>
<td>0.010</td>
<td>0.028</td>
</tr>
<tr>
<td>Conotoxin (n-6)</td>
<td>0.04 ± 0.01</td>
<td>0.08 ± 0.03</td>
<td>0.07 ± 0.02</td>
<td>1.32 ± 0.72</td>
<td>0.000</td>
<td>0.017</td>
</tr>
<tr>
<td>SNX (n-6)</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.38 ± 0.12</td>
<td>0.007</td>
<td>0.028</td>
</tr>
<tr>
<td>TTA (n-6)</td>
<td>0.04 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.84 ± 0.40</td>
<td>0.001</td>
<td>0.028</td>
</tr>
<tr>
<td>Conotoxin + SNX + TTA (n-8)</td>
<td>0.05 ± 0.01</td>
<td>0.10 ± 0.02</td>
<td>0.11 ± 0.03</td>
<td>0.73 ± 0.22</td>
<td>0.000</td>
<td>0.012</td>
</tr>
</tbody>
</table>

The firing rate of dopaminergic neurons, expressed as the number of spikes/second, was recorded extracellularly in adult rat brain slices. Statistical analysis was performed with a Student’s t-test.

Table 2

<table>
<thead>
<tr>
<th>Blocker</th>
<th>Control</th>
<th>Blocker(s)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apamin</td>
<td>2.56 ± 0.16</td>
<td>2.56 ± 0.17</td>
<td>0.97</td>
</tr>
<tr>
<td>Conotoxin (n-6)</td>
<td>2.99 ± 0.18</td>
<td>3.40 ± 0.32</td>
<td>0.28</td>
</tr>
<tr>
<td>SNX (n-6)</td>
<td>2.62 ± 0.15</td>
<td>2.53 ± 0.13</td>
<td>0.83</td>
</tr>
<tr>
<td>TTA (n-6)</td>
<td>2.63 ± 0.27</td>
<td>2.74 ± 0.26</td>
<td>0.89</td>
</tr>
<tr>
<td>Conotoxin + SNX + TTA (n-8)</td>
<td>2.52 ± 0.31</td>
<td>2.83 ± 0.35</td>
<td>0.53</td>
</tr>
</tbody>
</table>

The firing rate of dopaminergic neurons was recorded extracellularly in adult rat brain slices. The coefficients of variation of the interspike intervals are shown. Statistical analysis was performed with the Friedman test (global comparison between groups) and the Wilcoxon Signed-Rank test (comparison of “apamin” and “apamin + (Ca\textsubscript{v} channel blocker)”).

Table 3

<table>
<thead>
<tr>
<th>Blocker</th>
<th>Control</th>
<th>Apamin</th>
<th>Apamin + Blocker</th>
<th>P value Global test</th>
<th>P value Apa vs Blocker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apamin</td>
<td>0.05 ± 0.01</td>
<td>0.34 ± 0.11</td>
<td>0.34 ± 0.11</td>
<td>0.002</td>
<td>0.674</td>
</tr>
<tr>
<td>Conotoxin (n-6)</td>
<td>0.07 ± 0.03</td>
<td>0.74 ± 0.27</td>
<td>0.76 ± 0.26</td>
<td>0.001</td>
<td>0.161</td>
</tr>
<tr>
<td>SNX (n-6)</td>
<td>0.05 ± 0.01</td>
<td>0.40 ± 0.15</td>
<td>0.49 ± 0.15</td>
<td>0.001</td>
<td>0.017</td>
</tr>
<tr>
<td>Nifedipine (n-7)</td>
<td>0.07 ± 0.02</td>
<td>0.77 ± 0.19</td>
<td>0.27 ± 0.11</td>
<td>0.000</td>
<td>0.021</td>
</tr>
<tr>
<td>TTA (n-6)</td>
<td>0.05 ± 0.01</td>
<td>0.34 ± 0.11</td>
<td>0.39 ± 0.11</td>
<td>0.002</td>
<td>0.063</td>
</tr>
<tr>
<td>Apamin (n-8)</td>
<td>0.05 ± 0.01</td>
<td>0.51 ± 0.15</td>
<td>0.57 ± 0.14</td>
<td>0.001</td>
<td>0.069</td>
</tr>
</tbody>
</table>

The firing pattern of dopaminergic neurons was recorded extracellularly in adult rat brain slices. The coefficients of variation of the interspike intervals are shown. Statistical analysis was performed with the Friedman test (global comparison between groups) and the Wilcoxon Signed-Rank test (comparison of “apamin” and “apamin + (Ca\textsubscript{v} channel blocker)”).
channels such as transient receptor potential (TRP) channels. Some of these channels are activated by depolarizations in the range of voltages achieved during the action potential (http://www.guidopharmacology.org/) and are sensitive to divalent cation blockers. In this respect, there is evidence for the presence of some TRP subtypes in dopaminergic neurons (Gualtieri et al., 2005; Chung et al., 2011). The submaximal effect of Cd²⁺ and Co²⁺ also requires further investigation as it suggests that, besides Ca₉ channels, other sources of Ca²⁺ play a role in SK channel activation. Other ways for Ca₂⁺ to enter cells are second-messenger operated channels and receptor-operated channels. Several synaptic blockers were used in this study in order to minimize the contribution of such channels but it is not possible to claim that a complete blockade was obtained (some of them could still have been operational). The lack of effect of CPA in this study suggests that Ca²⁺ released from intracellular stores does not play a role in the activation of SK channels. However this conclusion is perhaps premature as depletion of intracellular Ca²⁺ stores can induce influx of Ca²⁺ from the extracellular space through store-operated channels in the plasma membrane, thereby counterbalancing a possible inhibitory effect of the drug on SK channel activation (Parekh and Putney, 2005).

Note that in many cases, superfusion of apamin converted the AHP to a small ADP (Fig. 1A), the nature of which is unclear but does not seem to involve Ca₉ channels, since it was observed in the presence of Cd²⁺ /Co²⁺.

Interestingly, in a recent study by our group, N-type channels also appear to be the source of Ca²⁺ required for the activation of SK channels in another type of monoaminergic neurons, dorsal raphe serotonergic neurons (Alix et al., 2014). However, the contribution of the N channels in these neurons is much more important than in dopaminergic neurons as they contribute to more than 80% of the Ca²⁺ which activates SK channels during the AHP.

An important question raised by our experiments is why there would be a need for various Ca²⁺ sources to activate SK channels in SNc dopaminergic neurons. What are the consequences of such an organization? A complete lack of SK channel activation after the action potential produces a massive deregularizing effect in many dopaminergic neurons (see results). This is not the case in serotonergic neurons, where the irregularity-promoting effect of SK blockade is much more modest (Rouchet et al., 2008), perhaps because of the presence of a robust apamin-insensitive fast AHP (Alix et al., 2014). Therefore, a variety of Ca²⁺ sources in dopaminergic neurons would provide a means of ensuring a regular firing pattern of these neurons in basal conditions, even when some of these sources are downregulated by synaptic inputs.

5. Conclusions

Our results emphasize the contrasting role of N-type and L-type channels in the regulation of the firing pattern of SNc dopaminergic neurons, activation of N-type channels promoting regularity by SK activation and activation of L-type channels promoting irregularity when SK channels are not activated. N-type channels however are far from being the only source of Ca²⁺ required for SK channel activation in these neurons. Because other types of Caν channels do not appear to provide Ca²⁺ for SK channel activation in our experimental conditions, other sources of Ca²⁺ remain to be explored.

Acknowledgements

The technical assistance of L. Massotte is gratefully acknowledged. This work was supported by grant T0015.13 from the "Fonds National de la Recherche Scientifique" (FNSR) (Belgium) (to VS and DE) and a grant from the Belgian Science Policy (Inter-university Attraction Poles program grant P7/10) (to VS).

References


