Regulation of Action Potential Size and Excitability in Substantia Nigra Compacta Neurons: Sensitivity to 4-Aminopyridine

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Nedergaard, S. Regulation of action potential size and excitability in substantia nigra compacta neurons: sensitivity to 4-aminopyridine. J. Neurophysiol. 82: 2903–2913, 1999. Slow, pacemaker-like firing is due to intrinsic membrane properties in substantia nigra compacta (SNc) neurons in vitro. How these properties interact with afferent synaptic inputs is not fully understood. In this study, intracellular recordings from SNc neurons in brain slices showed that spontaneous action potentials (APs) were attenuated when generated from lower than normal threshold. Such APs were blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and could be related to non-N-methyl-D-aspartate (NMDA) receptor–mediated spontaneous excitatory postsynaptic potentials (EPSPs). The AP attenuation was reproduced by stimulus-evoked EPSPs and by current injections to the soma. APs evoked from holding potentials between −40 and −60 mV were reduced in width by Cd2+ (0.2 mM). Tetraethylammonium chloride (TEA, 10 mM) or 4-aminopyridine (4-AP, 5 mM) increased the AP width. However, at more negative holding potentials, Cd2+ and TEA were ineffective, whereas 4-AP enlarged the AP, partly via induction of a Cd2+-sensitive component. A monophasic afterhyperpolarization (AHP), following attenuated APs, was little affected by either Cd2+ or TEA, but inhibited by 4-AP, which induced an additional, slow component, sensitive to Cd2+ or apamin (100 nM). The AP delay showed a discontinuous relation to the amplitude or slope of the injected current (delay shift), which was sensitive to low doses of 4-AP (0.05 mM). The initial time window before the delay shift was longer than the rise time of EPSPs. It is suggested that a 4-AP-sensitive current prevents or postpones discharge during slow depolarization’s, but allows direct excitation by fast EPSPs. Fast excitation leads to AP attenuation, primarily due to strong activation of 4-AP-sensitive current. This seems to cause inhibition of the Ca2+-dependent K+ currents. Together, these properties are likely to influence the excitability and the local, somatodendritic effects of the AP, in a manner that discriminates between firing induced by the intrinsic pacemaker mechanism and fast synaptic potentials.

INTRODUCTION

Neuronal responses to afferent inputs depend on nonlinear properties provided by voltage-dependent membrane conductances (Llinás 1988). In cells where the conductances interact to generate spontaneous spikes, the diversity of possible outcomes of a given stimulus could be particularly high. The mesencephalic dopaminergic (DA) neuron is an example of such a cell type. When recorded in vivo these neurons show a slow irregular firing or a burst firing pattern (Clark and Chiody 1988; Grace and Bunney 1984a,b; Nissbrandt et al. 1994; Tepper et al. 1995). These firing patterns are believed to depend on afferent synaptic inputs, and evidence is accumulating that excitatory pathways, using glutamate as neurotransmitter (Smith et al. 1996), are involved in the control of firing in DA substantia nigra pars compacta (SNc) cells (Charlety et al. 1991; Chergui et al. 1994; Christoffersen and Meltzer 1995; Overton and Clark 1992). In the in vitro slice preparation, however, the spontaneous activity is characterized by a regular, pacemaker-like discharge, which has been attributed entirely to intrinsic membrane properties (Grace and Onn 1989; Harris et al. 1989; Nedergaard et al. 1993; Yung et al. 1991). This discrepancy is explained if the synaptic drive on the in vitro cells is low (due to the truncation of afferent fibers during slice preparation), and implies that the in vivo activity could represent the integration of both an intrinsic and an extrinsic influence on the discharge. Electrophysiological studies from DA neurons in vitro have shown that synaptic stimulation (Johnson and North 1992; Merer et al. 1991) or exogenous application of glutamate agonists (Seutin et al. 1990; Wang and French 1993) elicits responses composed of both N-methyl-D-aspartate (NMDA)- and non-NMDA receptor–mediated events. However, it is not yet clear how, or to which extent, discrete excitatory inputs interfere with the background spontaneous activity and vice versa.

Unpublished observations in this laboratory have indicated that pacemaker discharge involves a large variability in the size of individual action potentials (APs) in the cell soma. The reason for this variability is not obvious from the assumption that spike generation is at a fixed location and entirely related to an underlying slow oscillatory potential (Nedergaard et al. 1993). The present study was undertaken to describe more closely the spontaneous AP variability and to investigate the possible contribution from afferent synaptic inputs and intrinsic properties to the regulation of the AP.

METHODS

Brain slice preparation and intracellular recording

Mesencephalic brain slices (coronal, 400 μm thick) were prepared from albino guinea pigs of either sex (250–350 g) as described previously (Nedergaard et al. 1993). The animals were deeply anesthetized with chloroform in an airtight container and killed by decapitation. The brain was removed, and a block of tissue containing the midbrain was isolated and used for slice preparation. Slices were transferred to a HEPES solution (see Drugs and solutions), bubbled with 95% O2-5% CO2 and stored at room temperature for at least 1 h before use. In the recording chamber the slice surface was at the interface between a humidified atmosphere of 95% O2-5% CO2 at 32–33°C and a standard perfusion medium (see below). Flow rate was 1.5 ml/min.
Intracellular recordings were made via glass microelectrodes, filled with 3 M potassium acetate (resistance 50–90 MΩ), and connected to an AXOCLAMP 2A bridge amplifier (Axon Instruments, Foster City, CA). Signals were digitized and stored on videotape. At the end of each experiment the electrode was retracted a few micrometers from the cell and the extracellular potential recorded. Focal stimulation was performed by means of a bipolar, insulated platinum wire electrode, placed on the slice surface ventral to the recording site. Current (100–500 µs, variable strength) was delivered by a stimulus isolation unit (ISOLATOR 10, Axon Instruments).

**Drugs and solutions**

The HEPES storage solution contained (in mM) 120 NaCl, 2.0 KCl, 1.25 KH₂PO₄, 2.0 CaCl₂, 20 NaHCO₃, 6.7 HEPES acid, 2.6 HEPES salt, and 10 glucose. The standard perfusion medium contained (in mM) 132 NaCl, 1.8 KCl, 1.25 KH₂PO₄, 1.3 MgSO₄, 2.4 CaCl₂, 20 NaHCO₃, and 10 glucose. The following drugs were kept in stock solutions and dissolved in the perfusion medium to the final concentration immediately before use: bicuculline [10 mM], 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 µM), d,l-2-amino-5-phosphonovaleric acid (APV, 50 µM), 4-aminopyridine (4-AP, 0.05–10 mM), CdCl₂ (200 µM), tetraethylammonium chloride (TEA, 10 mM), and apamin (100 nM).

**Data analyses**

Signals were analyzed off-line on a PC computer, using SIGAVG software (CED, Cambridge, UK). AP thresholds were determined as the membrane potential at the point where the rate of depolarization started to increase above the baseline rate. The AP threshold and height were expressed in absolute voltage (extracellularly recorded voltage set to 0 mV). The threshold variation of spontaneous APs was calculated by subtracting the mean threshold from the most negative threshold, recorded in a period of 60–120 s. The AP duration was measured as the width at half-amplitude between threshold and peak. The rates of rise and fall were calculated as the average slope between threshold, recorded in a period of 60–120 s. The AP duration was calculated by subtracting the mean threshold from the most negative threshold, recorded in a period of 60–120 s. The AP duration was measured as the width at half-amplitude between threshold and peak. The rates of rise and fall were calculated as the average slope between threshold and peak.

**Characteristics of spontaneous APs**

Spontaneous AP properties were analyzed in 49 neurons. The majority of APs had similar threshold and shape and were followed by a biphasic AHP, with a fast (fAHP) and a slow (sAHP) component. In most cells, however, some of the APs did not conform to the normal characteristics. These (referred to as variable APs) were generated at low and variable thresholds (ranging from a few millivolts to >15 mV negative to the average threshold), reached less positive overshoot potentials, and had shorter duration than the average AP (Fig. 1, A and B). The AHP following the variable APs had an early peak and fast decay with no distinct sAHP (Fig. 1, C).

**RESULTS**

**Cell identification**

Intracellular recordings were made from SNc neurons, located lateral to the accessory optic tract. A total of 112 neurons were selected for investigation, based on an AP width of >1.5 ms at threshold, a slow, regular firing pattern, and a prominent sag during hyperpolarizing current pulses, which are distinguishing criteria for DA neurons in this preparation (Grace and Omn 1989; Yung et al. 1991). Neurons with AP amplitudes of <45 mV beyond threshold, or inability to fire repetitively in response to a 1-s long depolarizing current pulse, were not included. Most neurons (n = 95) fired in a slow (1.6 ± 0.9 Hz; mean ± SD), pacemaker-like rhythm during passive recording. The remaining 17 neurons were silent at rest.

**FIG. 1. Normal and variable action potentials (APs) in a substantia nigra compacta (SNc) neuron, A: record of spontaneous discharge. One AP (b) is generated from a hyperpolarized membrane potential at a short interval from the preceding normal AP (a). B: the APs marked in A shown enlarged. The differentiated records are shown in inset. Note similar maximum rate of rise, but faster rate of fall of b compared with a. C: plots from the same cell of overshoot and half-width vs. threshold of all APs generated in 120 s (n = 115). Bicuculline 10 µM was present.**
TABLE 1. Characteristics of spontaneous APs

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<thead>
<tr>
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<th>Control APs</th>
<th>Variable APs</th>
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<tr>
<td>Threshold, mV</td>
<td>−39.7 ± 0.8</td>
<td>−52.2 ± 1.0*</td>
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<tr>
<td>Overshoot, mV</td>
<td>33.6 ± 0.9</td>
<td>30.1 ± 1.7†</td>
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<tr>
<td>Half width, ms</td>
<td>1.22 ± 0.05</td>
<td>0.77 ± 0.05*</td>
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<tr>
<td>Rate of rise, V · s⁻¹</td>
<td>193 ± 8</td>
<td>218 ± 12</td>
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<tr>
<td>Rate of fall, V · s⁻¹</td>
<td>44 ± 2</td>
<td>66 ± 5*</td>
</tr>
<tr>
<td>Ratio of rate of rise to rate of fall</td>
<td>4.6 ± 0.2</td>
<td>3.5 ± 0.3†</td>
</tr>
<tr>
<td>fAHP peak, mV</td>
<td>−67.4 ± 0.9</td>
<td>−69.7 ± 1.1</td>
</tr>
<tr>
<td>sAHP peak, mV</td>
<td>−71.0 ± 1.1</td>
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Values are means ± SE. Measurements were made on individual spontaneous APs generated at low frequency (0.2–1.0 Hz). The APs were selected from each cell on the basis of the threshold, being either close to the average (control APs; n = 35), or >8.0 mV negative to the average (variable APs; n = 18). AP, action potential; fAHP and sAHP, fast and slow afterhyperpolarization, respectively. *P < 0.001. †P < 0.05. ‡P < 0.01.

APs was reflected in a faster rate of fall and a reduced ratio of rate of rise to rate of fall. Their mean rate of rise was slightly higher than the control APs, but this difference was not statistically significant. The fAHP following the variable APs reached a similar peak potential as control APs.

Addition of the GABA_A receptor blocker bicuculline (10 μM) had no detectable effect on the discharge properties. The glutamate receptor antagonist CNQX (10 μM) applied alone, or together with APV (50 μM), caused a marked reduction of the AP variability (Fig. 2A). The threshold variation of spontaneous APs (see METHODS) in 46 cells in the absence of CNQX ranged between 1.0 and 16.7 mV (mean, 7.7 ± 0.5 mV). This value was reduced from 8.9 ± 1.3 mV to 2.7 ± 0.5 mV in seven cells exposed to CNQX (Fig. 2B). Application of APV alone (n = 6) had little and inconsistent effects on the variation in AP threshold and size (Fig. 2B).

Spontaneous excitatory postsynaptic potentials (EPSPs)

These observations indicate that the AP shape is influenced by spontaneous EPSPs. To verify the possible existence of such potentials, voltage records sampled over several minutes were examined in each cell. Isolated transient depolarization’s, with a rising slope fast enough to compare with the initial phase of full spikes, were found in 46 of the 49 cells (Fig. 3). These putative EPSPs occurred at irregular frequencies (ranging from <1 to >10 per min), had a time-to-peak between 1.0 and 3.0 ms, and varied in height in individual cells and between cells from 2 to 20 mV. They persisted during hyperpolarization below firing threshold (n = 20). The transients were not found in records of similar length in the presence of 10 μM CNQX (n = 7). A quantitative comparison between the threshold variation of APs and the transient depolarization’s was not attempted, due to the large variability in frequency and size.

Comparison of EPSP-evoked and current-pulse-evoked APs

In the presence of 10 μM bicuculline, EPSPs were produced by focal stimulation. Discharge induced by individual EPSPs consisted of a single AP, generated at or near the EPSP peak. The EPSP rise time (from beginning of depolarization to peak) was on average 2.8 ± 0.2 ms in 22 neurons held at membrane potentials between −60 and −70 mV (mean: −62 ± 1 mV; bicuculline 10 μM present). The
Effects of ion channel blockers on the AP and AHP

Ion channel blockers were used to examine the possible contribution of intrinsic conductances to the AP variability. To minimize the interference from spontaneous synaptic activity, these experiments were done in the presence of CNQX (10 μM), APV (50 μM), and bicuculline (10 μM). Injection of constant current (positive or negative, depending on the resting state of the cell), combined with brief depolarizing current pulses (2–4 ms), were used to generate APs from membrane potentials between −40 and −75 mV.

The Ca\(^{2+}\) channel blocker Cd\(^{2+}\) (200 μM) significantly reduced the half-width of APs generated at −40 mV (n = 5). This effect was associated with an increased rate of fall and disappearance of the shoulder on the falling phase of the AP (Fig. 5Aa). Hyperpolarization between −40 and −60 mV led to a progressive decrease in the effect of Cd\(^{2+}\). From −60 mV there was no detectable effect on the AP duration (Fig. 5, Aa and Ba). The AP height was not altered significantly by Cd\(^{2+}\) at any potential, although a slight increase in the mean overshoot was noted at holding potentials negative to −60 mV (Fig. 5Ba).

In separate experiments, TEA (10 mM) caused a broadening of the AP at −40 mV (half-width increased by >100%; n = 7), and induced a plateau-like delay during the repolarizing phase (Fig. 5Ab). The AP height was not consistently affected. The TEA effect decreased gradually with holding potentials between −40 and −60 mV, and became undetectable negative to −60 mV (Fig. 5, Ab and Bb, n = 7). Addition of 200 μM Cd\(^{2+}\) in the presence of TEA blocked the plateau induced from −40 mV, and reduced the AP half-width to less than control (Fig. 5Ab). Coapplication of 200 μM Cd\(^{2+}\) and 10 mM TEA had no effect on the half-width of APs generated from holding potentials negative to −60 mV (Fig. 5Ba).

The response to 5 mM 4-AP was tested in 11 neurons. Here, a lowering of the spontaneous firing threshold (3–5 mV) was noted in four of seven active neurons after exposure to 4-AP. Compared to control APs, evoked from equal potentials, the half-width increased in all neurons exposed to 4-AP. This effect involved a decreased rate of fall, starting from the beginning of the repolarization, near the AP peak, and without a distinct plateau phase as observed with TEA (Fig. 5Ac). The effect of 4-AP persisted during hyperpolarization below −60 mV (Fig. 5, Ac and Bc). The relative increase in half-width was between 70 and 90% at all membrane potentials (n = 5). The AP height was largely unaltered by 4-AP at the more depolarized potentials. However, from −60 mV the height increased significantly in response to 4-AP, an effect that became larger with further hyperpolarization. Thus the strong voltage dependency of the AP overshoot, seen under control conditions, was clearly diminished in the presence of 4-AP (Fig. 5Bc). In three experiments where 4-AP was already present, addition of Cd\(^{2+}\) caused a reduction of the AP width. The latter effect of Cd\(^{2+}\) persisted at hyperpolarized holding potentials (Fig. 5Ac).

Figure 6 shows the effects of ion channel blockers on the AHP of individual spikes. The biphasic AHP generated around −40 mV was highly sensitive to 200 μM Cd\(^{2+}\), which reduced the amplitude of the early peak (fAHP) and blocked the sAHP component (Fig. 6Aa). In 10 mM TEA alone, the fAHP decreased in amplitude, whereas the sAHP persisted (Fig. 6Ab). On the other hand, 4-AP (5 mM) had little and variable effects

![Figure 4](http://jn.physiology.org/)

**Fig. 4.** Comparison of EPSP- and current pulse-evoked APs. A, left: spontaneous AP (s) and AP generated by evoked EPSP (e) from a hyperpolarized potential (stimulus artifact denoted by star) in the presence of bicuculline (10 μM) and APV (50 μM). Record after addition of 10 μM CNQX is included. A, right: APs evoked by current pulses of 1–10 ms duration. Note similar threshold and shape of EPSP- and current-evoked APs. B: responses in the same cell to longer depolarizing current pulses (50–200 ms), adjusted to the minimal strength that gave an AP. The APs are aligned to the right. Note decreased overshoot and duration with decreasing latency from the pulse onset, and the lower threshold of the early AP on the 50-ms pulse.

EPSP-evoked APs were attenuated and had fast decaying AHPs, both when the membrane potential was kept stable by hyperpolarizing holding current (Fig. 4A) and during free firing. In the latter case, an unattenuated AP with normal AHP was evoked in a few trials, where the stimulation coincided with a potential close to the normal AP threshold. The degree of attenuation (reduced overshoot and half-width) varied with the prestimulus voltage similar to the variable APs. At −60 mV, or below, the apparent firing threshold was typically 5–10 mV negative to normal. In the presence of 50 μM APV the EPSP rise time was unaltered, although the amplitude was sometimes slightly reduced (n = 6). The evoked APs failed, together with a complete block of the EPSP, after application of 10 μM CNQX (Fig. 4A; n = 8).

In the same experiments depolarizing current pulses were adjusted to mimic EPSPs. Single APs, evoked by short (1–10 ms) pulses, resembled EPSP-induced APs, when compared in the same cell, at similar membrane potential (Fig. 4A). Weaker pulses, which failed to fire the cell within ~10 ms, needed to be sustained for much longer periods (~80 ms) to evoke an AP. These long-delay APs were normal-sized and were generated near the threshold of spontaneous APs (Fig. 4B).
on this AHP; of 10 cells examined, the fAHP did not change to any detectable degree (n = 4, example in Fig. 6a), or showed a small increase or decrease (by 2–4 mV, n = 6). The variation in the differences was not statistically significant when tested by the Wilcoxon test for pair differences (2α > 0.05). The sAHP was not consistently altered by 4-AP. When evoked

FIG. 5. Voltage-dependent effects of ion channel blockers on the AP. A: APs generated from −40 mV (top traces) and from −65 mV holding potential following a brief (3–4 ms) current pulse (bottom traces). Records from 3 different cells are shown before (Con.) and during application of either 200 μM Cd²⁺ (a), 10 mM tetraethylammonium (TEA; b), or 5 mM 4-aminopyridine (4-AP; c), or combined as indicated. Ba–Bc: plots of AP overshoot and half-width vs. holding potential in control periods (●) and during application of blockers (○); Cd²⁺ (200 μM, n = 4), TEA (10 mM, n = 5), 4-AP (5 mM, n = 5). Error bars = SE.

FIG. 6. Voltage-dependent effects of ion channel blockers on the spike AHP. A: AHPs following APs elicited from −40 mV (top traces) and by brief current pulses from −65 mV (bottom traces). Superimposed sweeps are taken before (Con.) and during application of blockers as indicated. Note the Cd²⁺-sensitive, slow component, generated in the presence of 4-AP. Records (a–c) are from 3 different experiments. B: AHPs (at −58 mV holding V_m) recorded in control medium (Con.), with 5 mM 4-AP present (4-AP), and after subsequent addition of either 100 nM apamin (4-AP + Apamin, top record), or, in another neuron, 10 mM TEA (4-AP + TEA, bottom record). Note block of the slow component in the presence of apamin, and reduction of the fast component by TEA.
The presence of 4-AP, was inhibited by subsequent application of the AHP (10 of 11 cells). The entire AHP complex, in the which was reflected in a delayed decay phase and prolongation, late component developed in the presence of 4-AP, Cd$^{2+}$ effects on the amplitude (unaltered in 4 of 7 cells; decreased by $\text{Ab}^5$; Fig. 6$^6$). Similarly, TEA did not alter the overall shape of this AHP (Fig. 6$^A$) and had inconsistent and nonsignificant $\text{Ab}^5$. First, the peak amplitude was markedly reduced in all cells tested (range: 5–18 mV; $n = 11$). The effect was not complete, as an early AHP was still discernible in most cells held between 60 mV, or below, the monophasic AHP, evoked in normal medium, showed little sensitivity to Cd$^{2+}$ alone (Fig. 6$^Aa$; $n = 5$). Similarly, TEA did not alter the overall shape of this AHP (Fig. 6$^Ab$) and had inconsistent and nonsignificant $\text{Ab}^5$. As indicated above (Fig. 4), the AP attenuation seems to rely on the depolarizing current being strong enough to give a short delay. The possibility that the delay itself is influenced by 4-AP–sensitive processes was next examined. AP delays, monitored from the onset of positive current steps from a holding potential between $-60$ and $-70$ mV, were inversely related to the current intensity. However, with high intensity, the delay shifted abruptly from an average of $82 \pm 8$ ms to $7 \pm 1$ ms ($n = 18$; mean holding $V_m = -65 \pm 1$ mV; Fig. 7$^A$). Logarithmic plots of delay versus current intensity showed an almost linear relationship on either side of the delay shift; the slope on the left being consistently smaller than on the right side of the shift (Fig. 7$^C$, top graph). In the presence of 4-AP (0.05–10 mM, $n = 15$), AP delays were generally reduced (Fig. 7$^A$) and accompanied by a lowered firing threshold (Fig. 7$^B$). These effects were dose dependent and were already marked at the lowest 4-AP concentration used (0.05 mM; Fig. 7$B$). Furthermore, with doses between 0.05 and 0.5 mM, the length of the delay shift decreased dramatically, and the delay–current relationship approximated a uniform slope (Fig. 7$C$). A quantitative estimate of the delay shift at different 4-AP concentrations was obtained in six cells (calculations made from linear regression lines; see Fig. 7 legend). On average, the control value was reduced by 60% in the presence of 0.05 mM, and by 93% in 0.5 mM 4-AP (Fig. 7$D$, holding $V_m = -65 \pm 1$ mV, $n = 6$). A further increase in dose gave a small additional effect (99% reduction obtained in 10 mM 4-AP). For comparison, the dose dependency of the 4-AP effect on the AP half-width was examined in the same cells at similar holding potentials. Here, the broadening of the AP became detectable at 0.2 mM 4-AP and increased progressively.
Fig. 8. Effects of current ramps on AP delay and threshold. A: action potentials generated by current ramp injections with different slope before (control) and during application of 5 mM 4-AP. Holding potential, −65 mV. B: from the same experiment the AP delay (top graph) and AP threshold (bottom graph) are plotted against the ramp slope in control medium (●) and in 5 mM 4-AP (○). Lines in bottom graph indicate the AP threshold during spontaneous firing at rest in control recording (-----) and in the presence of 4-AP (...).

with the concentration (Fig. 7D). The response showed no sign of saturation at high doses (415% increase in half-width in 10 mM 4-AP, n = 6).

The possible dependency of the AP delay and threshold on the rate of depolarization was tested by varying the slope of the injected current. Under these conditions the AP delay shifted between 45 ± 1 ms and 12 ± 1 ms at an average current slope of 102 ± 15 nA/s, (range: 58–159 nA/s; holding \( V_{\text{ref}} \): −65 ± 2 mV; n = 7). This delay shift was inhibited or blocked by 4-AP, depending on the dose (0.05–10 mM; n = 3; Fig. 8). In control medium, the threshold of the first AP decreased gradually with increasing current slope in the range below the critical value for inducing the delay shift. Higher slopes gave less variation in threshold (Fig. 8B). In the presence of 4-AP, the threshold obtained during slow currents were markedly lowered. However, with increased rate of depolarization the threshold approximated the control values (Fig. 8B). Similar effects were seen in three cells tested with ramp depolarization.

The threshold of spontaneous APs, generated at rest, was much less affected by 4-AP than the first AP during the ramp (see Fig. 8B). In neurons activated with square depolarizations, the average threshold of APs evoked at 5 ms delay in control medium (−45.7 ± 1.5 mV) was not different from those found in the presence of 5 mM 4-AP (−47.0 ± 0.7 mV, n = 6, P > 0.05). However, at 100 ms delay, the threshold was lowered by −13 mV in the presence of 5 mM 4-AP (from −40.3 ± 1.3 to −53.5 ± 1.3 mV, P < 0.01).

Discussion

EPSP-induced APs

In this study, SNC neurons with membrane properties characteristic of DA cells were examined. Spontaneous APs displayed large variabilities, which decreased in the presence of CNQX. Putative non-NMDA receptor–mediated EPSPs were found in the same cells. Both observations are in line with previous demonstration of a spontaneous excitatory input in this preparation (Mereu et al. 1991). It is conceivable that the observed effects were due to activity of glutamatergic interneurons located within the confines of the slice; however, evidence for the existence of such neurons is lacking. Midbrain DA cells receive afferent glutamatergic inputs from several external sources, including the cerebral cortex (Sesack et al. 1989), the subthalamic nucleus (Hammond et al. 1978; Kita and Kitai 1987), and the pedunculopontine nucleus (Charara et al. 1996; Scarnati et al. 1986). The effects seen here could reflect some form of spontaneous release of glutamate from terminals of cut fibers from any of these structures. Assuming that a spontaneous EPSP results from transmitter release from a single terminal or terminals of a single fiber, and that the variable APs were generated directly by such EPSPs, it seems possible that activity in one afferent axon can be sufficient to activate the postsynaptic cell. In the light of the constraints on spontaneous firing exerted by intrinsic membrane conductances (Grace and Onn 1989; Nedergaard et al. 1993; Shepard and Bunney 1991), such high excitability state seems surprising. One explanation could be that these terminals were localized close to the AP initiation site at a distance from the soma. Indeed, the efficacy of an input has been shown to depend on its location relative to the initial segment of the axon, which often emerges from a dendrite (Hausser et al. 1995). Furthermore, dendritic APs are facilitated by EPSPs in this preparation (Nedergaard and Hounsgaard 1996). Whether such effects play a significant role here is unsettled. Stimulus-evoked EPSPs had similar, voltage-dependent effects on the AP shape and were found to fire the cell from a lower apparent threshold than spontaneous APs. Both effects were also seen with current pulses in the soma. This indicates that the place of origin of the depolarization is not the only critical factor. Alternatively, as discussed below, the excitability could be variable, or conditional, depending on the dynamics of certain membrane conductances.

The finding that CNQX alone blocked discharge related to both spontaneous and stimulus-evoked EPSPs indicates that non-NMDA receptors are involved in either type of stimulation.

Voltage-dependent contribution of ionic currents to the AP

Current-pulse–evoked attenuated APs has been noted previously (Grace 1990; Grace and Onn 1989; Nedergaard and Greenfield 1992), but not systematically characterized. The contribution of different ionic currents to the AP shape was found here to be highly dependent on the membrane potential. Near the normal threshold of −40 mV, 200 μM Cd²⁺ caused an accelerated repolarization and a reduced AHP, which suggests that voltage-dependent Ca²⁺ current underlies a depolarizing component in the late phase of the AP and mediates activation of outward currents involved in the AHP. The prolonged AP and reduced fAHP in the presence of 10 mM TEA indicates that the Ca²⁺-dependent K⁺ current \( I_{\text{f}} \), and possibly a delayed rectifier (Silva et al. 1990) contribute significantly to the AP repolarization and to the fAHP. At −40 mV threshold the response to 5 mM 4-AP involved
a slowed repolarization with insignificant change in the AHP. A 4-AP–sensitive, A-type \( K^+ \) current has previously been demonstrated in acutely dissociated (Silva et al. 1990) and cultured (Liu et al. 1994) DA neurons from the rat, and, recently, in slices from the mouse (Bruns et al. 1998). This current activates at potentials positive to \(-55 \text{ mV}\), and steady-state inactivation begins at \(-80 \text{ mV}\) (half-maximal at \(-65 \text{ mV}\)). The broadening effect found here at \(-40 \text{ mV}\) threshold could therefore indicate the presence of a 4-AP–sensitive outward current, distinct from the A-type current. Depolarization-activated \( K^+ \) channels are composed of \( \alpha \)-subunits encoded by four gene families, Kv1–Kv4. Heteromorphic channels formed by Kv1.4, 3.4, 4.1, 4.2, or 4.3 subunits display rapid inactivation and are sensitive to 4-AP, properties similar to native A-type currents (Baldwin et al. 1991; Schröter et al. 1991; Serôdio et al. 1994, 1996; Stühmer et al. 1989). The Kv2.1, 2.2, 3.1, and 3.2 channels are slowly inactivating (delayed rectifier type), as are most members of the Kv1 group [Kv1.1, 1.2, and 1.5 acquire rapid inactivation when co-expressed with \( \beta 1 \) subunits (Heinemann et al. 1996; Rettig et al. 1994)]. The Kv3 channels are highly sensitive to both TEA and 4-AP (Rettig et al. 1992), whereas Kv2 channels show intermediate sensitivity to TEA, and Kv2.1 is also sensitive to 4-AP (at least in the rat) (Pak et al. 1991). The involvement of Kv3 channels here is questionable, because an in situ hybridization study failed to demonstrate mRNA encoding any of the known Kv3 related proteins in rat SNc (Weiser et al. 1994). The lacking effects of 4-AP on the AHP could reflect a fast deactivation rate of the 4-AP–sensitive current. This would not compare to the relatively long deactivation of the A-type current. It is likely that A-type channels underlie an increasing portion of the 4-AP–sensitive current at hyperpolarized potentials, and hence play a major role in the AP attenuation. Evidence from other studies indicate that Kv4 channels may contribute to this current: first, the rate of inactivation is shown to be voltage independent (Silva et al. 1990), a distinguishing characteristic of Kv4 channels (Serôdio et al. 1994), and, second, mRNA transcripts encoding Kv4.3 subunits has recently been identified in the SNC region (Serôdio and Rudy 1998). The Kv1.4 protein is abundant in the substantia nigra, but mRNA for Kv1.4 was not localized in this region and could originate from projecting neurons within the striatum (Sheng et al. 1992).

**Regulation of \( Ca^{2+} \)-dependent currents during the AP**

At hyperpolarized holding potentials the broadening effect of 4-AP involved a recruitment of \( Ca^{2+} \) current, because a partial reversal of the effect was obtained after \( Cd^{2+} \) application (Fig. 5). Furthermore, the AHP evoked in the presence of 4-AP had both an early component, sensitive to \( Cd^{2+} \) or TEA, and a slow component sensitive to \( Cd^{2+} \) or apamin (Fig. 6). The latter finding corresponds to the pharmacological profile of the biphasic AHP at normal threshold (Nedergaard et al. 1993). Hence the lack of \( Cd^{2+} \) sensitivity of the AP and AHP could not be attributed to a direct effect of the hyperpolarization on the \( Ca^{2+} \) current. Instead, the most likely explanation for these findings is that the additional 4-AP–sensitive current, activated from hyperpolarized potentials, is strong enough to inhibit the \( Ca^{2+} \) current during the AP, and thereby prevent activation of the \( Ca^{2+} \)-dependent \( K^+ \) currents underlying the AHP. A simple shunting effect, imposed by the 4-AP–sensitive current could also mask a \( Ca^{2+} \)-dependent component of the early AHP. However, it is difficult to see how this effect alone could account for the abolishment of the sAHP.

**4-AP–sensitive delay shift**

The delay shift constitutes a temporal separation between normal and attenuated APs, because the latter were confined to the initial time window (Fig. 4). The sensitivity to 4-AP of AP delays as long as 1 s indicates that a slowly inactivating current counteracts depolarization in a voltage range below firing threshold. This current is responsible for the delay shift. The higher sensitivity to 4-AP of the delay shift compared with the AP width (Fig. 7D) could indicate that these two parameters show a large difference in their dependency of the amount of 4-AP–sensitive current available. Alternatively, separate currents may coexist, with different sensitivity to 4-AP. Transient currents, distinguished from the \( I_h \) by a slow inactivation and high sensitivity to 4-AP, has been shown in different neurons to mediate delayed excitation (McCormick 1991; Nisenbaum et al. 1994; Spain et al. 1991; Storm 1988).
Role of 4-AP–sensitive current in regulation of AP threshold

The threshold of the first AP was lowered by 4-AP depending on the rate of depolarization. This suggests that the effect of the 4-AP–sensitive current on the AP delay can at least partly be attributed to an increased threshold of APs generated at long delays. Conversely, the threshold of short-latency APs, generated within the initial time window before the delay shift, was unaffected by 4-AP. The latter observation can be explained if the activation kinetics of the 4-AP–sensitive current is slow at subthreshold potentials, and therefore has little influence on the initiation of an early AP. Considering that the early APs were precisely the ones that were maximally attenuated by the 4-AP–sensitive current, it would seem that the rate of activation increases markedly during the spike. In fact, the activation time constant of the A-current in these neurons is reported to increase from 4.5 to 0.9 ms in the voltage range between −55 and −15 mV (Bruns et al. 1998). This finding seems, at least qualitatively, to be in accordance with the above interpretation.

With 4-AP present, the AP threshold decreased with increasing delay (decreasing rate of depolarization). The reason for this was not pursued here. However, such a relationship would be expected in a cell where the site of spike initiation is at an electrotonic distance from the recording site, due to the lower degree of electrotonic filtering of slow potentials. As reported by Grace (1990), the threshold of the initial segment (IS) component of the AP, as seen from the soma, is particularly sensitive to 4-AP, possibly due to the location of a 4-AP–sensitive conductance in the segment of dendrite between the IS and the soma. The present results seem compatible with this interpretation and indicate further that activation of this conductance may obscure and oppose the frequency-dependent variation of passive filtering.

Functional implications

The delay shift is likely to be exploited under physiological conditions, because the EPSP rise time (2.8 ms) was well below the lower limit of the shift (7–12 ms). The high efficacy of EPSPs at hyperpolarized potentials could be due to their ability to depolarize the membrane rapidly and thereby “escape” the delay shift. Judged from the effect of ramp injections, a somewhat slower depolarization would fail to cause direct excitation, unless sustained for several tens of milliseconds. It seems that this nonlinear response property would function as a filter discriminating between fast and slow events. An obvious consequence of such a mechanism is that fast EPSPs are allowed to mediate precisely timed outputs (in millisecond scale) with respect to presynaptic discharge. Because direct AP generation could occur over a range of membrane potentials (−40 to −65 mV) normally experienced by the cell during spontaneous firing, such activation might be more or less independent on the background activity. The pacemaker potential is rising slowly compared with an EPSP, which might be more or less independent on the background activity. The pacemaker potential is rising slowly compared with an EPSP, which might be more or less independent on the background activity.

In conclusion, this study suggests that the excitability and action potential size are regulated during excitatory synaptic transmission, depending on activation of 4-AP–sensitive current in the postsynaptic cell. This current may be composed of both delayed rectifier-type and A-type current, the latter of which becomes dominant at subthreshold membrane potentials. A regulatory role of 4-AP on the membrane response properties has previously been shown in cerebellar Purkinje cells and hippocampal pyramidal neurons (Andreasen and Lambert 1995; Hoffman et al. 1997; Midtgaard 1994), where its damped effect on dendritic excitability appears to be a means by which synaptic integration can be regulated locally. The present study adds to these findings and suggests that another important function of such current is to provide spontaneously active neurons with the ability to discriminate between different afferent inputs and their own intrinsic activity.

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