Calcium-Independent Afterdepolarization Regulated by Serotonin in Anterior Thalamus

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Chapin, Esther M. and Rodrigo Andrade. Calcium-independent afterdepolarization regulated by serotonin in anterior thalamus. J. Neurophysiol. 83: 3173–3176, 2000. Previous studies have identified an afterdepolarization (ADP) in thalamocortical neurons that is mediated by an upregulation of the hyperpolarization-activated current $I_h$. This ADP has been suggested to play a key role in the generation of spindle oscillations. In the lateral geniculate nucleus, upregulation of $I_h$ has been shown to be signaled by a rise in intracellular calcium leading to the activation of adenylate cyclase and formation of cAMP. However, it is unclear how generalizable this mechanism is to other thalamic nuclei. We have used whole cell recording to examine the electrophysiological properties of neurons of the anterodorsal thalamic nucleus, a nucleus thought not to undergo spindle oscillations. We now report that cells in this nucleus also display an ADP mediated by serotonin. Serotonin produced a robust enhancement in the amplitude of the ADP even after strong buffering of intracellular calcium and blockade of calcium influx. These results indicate that, in neurons of the anterodorsal thalamic nucleus, an $I_h$-mediated ADP can occur through a mechanism that does not involve a rise in intracellular calcium. We next examined the possibility that this calcium-independent ADP might be modulated by serotonin. Serotonin produced a robust enhancement in the amplitude of the ADP even after strong buffering of intracellular calcium and blockade of calcium channels. These results indicate that neurons of the anterodorsal thalamic nucleus display a calcium-independent, $I_h$-mediated ADP and that this ADP is a target for regulation by serotonin. These findings identify a novel mechanism by which serotonin can regulate neuronal excitability.

METHODS

Brain slices were prepared using standard protocols. Whole cell recordings were obtained from the ADN using the blind-tight-seal patch-clamp recording technique. The intracellular solutions are described in Table 1.

Recordings were obtained using an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA). Electrical signals were filtered at 10 kHz and recorded with a paper chart recorder (Model 3300, Gould Instruments, Valley View, OH) or were digitized using a Digidata 1200 under the control of Axoclamp 7 (Axon Instruments). Voltage-clamp experiments were conducted using the continuous voltage-clamp mode of the amplifier. Recordings were considered acceptable if the series resistance was $<30$ MΩ and could be compensated by $>70\%$. Magnitude of $I_h$ was determined by subtracting the instantaneous current from the steady-state current. Drugs were administered in the Ringer perfusing the bath. Most drugs were obtained from Sigma (St. Louis, MO). Data was analyzed using Origin (Microcal Software, Northampton, MA).

RESULTS

In our slices, neurons of the ADN are quiescent and exhibit a resting membrane potential near $-65$ mV. When transiently hyperpolarized with current injection, these cells exhibit a slow rebound afterdepolarization or ADP (Fig. 1A1). This is similar to results obtained in other nuclei of the thalamus (Bal and McCormick 1996), using the same stimulation protocol. In the geniculate, the ADP has been attributed to calcium influx secondary to rebound calcium spikes. Surprisingly, in the ADN, a significant ADP remains ($\geq50\%$, $n = 3$ cells, Fig. 1) even after blocking the calcium spikes with cadmium and nickel. If at least one component of the ADP in the ADN was calcium independent, then a strong ADP would be detectable after a single long hyperpolarization (Luthi and McCormick 1998). As can be seen in Fig. 1B, ADN cells when stimulated using this protocol, still express a robust ADP or, in voltage clamp, a slow inward current after current of similar time course ($I_{ADP}$).

Because these results suggested the presence of a calcium-independent ADP in the ADN, we next examined the ability of hyperpolarizing pulses to elicit an ADP (or $I_{ADP}$) after buffering intracellular calcium with EGTA and blocking calcium influx. Even under these conditions, a strong $I_{ADP}$ was observed ($n = 6$ cells, Fig. 1C). Of course, one possible inter-
interpretation for these results may be that residual calcium influx could be responsible for the ADP. However, administration of cadmium and nickel completely blocked the rebound calcium spike (Fig. 1C1), and 10 mM EGTA should be adequate to clamp calcium transients (Neher 1988). Nevertheless, because the kinetics of calcium chelation by EGTA is slow, it remained possible that a small residual influx of calcium might have produced a transient local calcium rise. Therefore we repeated the previous experiment using 25 mM bis-(o-aminophenoxy)-N,N,N,N'-tetraacetic acid (BAPTA) in the intracellular solution, a manipulation that should allow for faster calcium chelation and produce supramaximal calcium buffering capacity (Neher 1988). In seven cells tested using this procedure, we consistently observed a robust transient local calcium rise. Therefore we repeated this experiment using 25 mM BAPTA 80 25 BAPTA 7.5 100 0.5 10 2 2 5

TABLE 1. Description of intracellular solutions

<table>
<thead>
<tr>
<th>Intracellular Solution</th>
<th>Potassium Gluconate</th>
<th>Buffer</th>
<th>CaCl₂</th>
<th>Estimated [Ca²⁺]i, nM</th>
<th>GTP</th>
<th>HEPES</th>
<th>ATP</th>
<th>MgCl₂</th>
<th>NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>No buffer</td>
<td>115</td>
<td>0.20 EGTA</td>
<td>—</td>
<td>—</td>
<td>0.5</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>10 mM EGTA</td>
<td>115</td>
<td>10 EGTA</td>
<td>1</td>
<td>100</td>
<td>0.5</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>25 mM BAPTA</td>
<td>80</td>
<td>25 BAPTA</td>
<td>7.5</td>
<td>100</td>
<td>0.5</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

All concentrations are in mM. BAPTA, bis-(o-aminophenoxy)-N,N,N,N'-tetraacetic acid; EGTA, Bis (b-aminophenoxy)-N,N,N,N'-tetraacetic acid; GTP guanosine triphosphate; ATP, adenosine triphosphate.

In other areas of the thalamus, the ADP triggered by hyperpolarizing current injections is mediated by Iₜ (Bal and McCormick 1996; Lüthi and McCormick 1998). Therefore we tested whether this calcium-independent ADP is similarly due to a facilitation of Iₜ. As illustrated in Fig. 1C3, administration of 25–100 μM of the selective Iₜ inhibitor ZD7288 (BoSmith et al. 1993) blocked the ADP and Iₜ in four cells. In the same cells, ZD7288 also blocked Iₜ measured directly. These results suggest that Iₜ is mediated by Iₜ. If this was the case, it should be possible to directly observe an upregulation of Iₜ after the hyperpolarizing pulse. As shown in Fig. 2A, Iₜ activated by a small hyperpolarizing step given every 20 s was stable, displaying no detectable facilitation with pulse number. However, if a large hyperpolarization (to −100 mV) was substituted for the test step, the magnitude of Iₜ activated on the subsequent test steps was significantly increased (n = 3 cells, P < 0.01). This facilitation decayed during 1–2 min with a time course that is well fitted by a single exponential (τ = 27.34 ± 4.00 s).

These experiments were conducted using 10 mM EGTA. Therefore to ascertain that this was not due to any residual calcium transient, we repeated this experiment using 25 mM BAPTA in the intracellular solution while bathing the cells with 3 mM nickel and 200 μM cadmium. As shown in Fig. 2B,
Cadmium. Cells were held at 

hyperpolarization observed using 10 mM EGTA to buffer intracellular calcium. Lines reflect best fit using a single exponential. The 3 baseline measurements was used to normalize the currents for each cell.

**Effect of serotonin on the hyperpolarization-induced I_h facilitation**

Serotonin is known to modulate I_h (Bobker and Williams 1990). Therefore we asked whether serotonin would also facilitate the ADP. As illustrated in Fig. 3, serotonin (10 μM) significantly increased the I_{ADP} (n = 10 cells, Fig. 3A). This increase reflected a genuine increase in the upregulation of I_h because ZD7288 blocked I_{ADP} in control and in the presence of serotonin (n = 4 cells, data not shown). To determine if this effect reflects an increase in the calcium-independent form of the I_{ADP}, we repeated these experiments while perfusing the slices with the calcium channel blockers cadmium (200 μM) and nickel (2 mM) and buffering intracellular calcium with 10 mM EGTA (n = 2 cells) or 25 mM BAPTA (n = 2 cells). As illustrated in Fig. 3B, even under these conditions, serotonin was still capable of inducing a strong facilitation of the I_{ADP}.

**DISCUSSION**

In the present study we have found that cells in the ADn generate an I_h-mediated ADP and I_{ADP} in response to hyperpolarization and that there is a significant portion of the ADP that does not depend on calcium influx. Whereas previous studies found that intracellular calcium chelation could block the ADP (Luthi and McCormick 1998), we found that a strong ADP survived even after buffering intracellular calcium and blocking calcium channels. Further, we found that serotonin could increase this calcium independent form of the I_{ADP}.

How can a hyperpolarization induce this ADP? Luthi and McCormick (1999) have proposed a key role for cAMP in the generation of the ADP. In the geniculate, calcium entering during the rebound calcium spike indirectly facilitates I_h by activating adenylyl cyclase. Because cAMP shifts the voltage dependence of I_h toward more depolarized voltages, the net effect of the calcium influx is to upregulate I_h, an effect that is seen as an ADP at resting membrane voltages. A similar mechanism could account for the calcium-independent ADP we describe herein. It is known that the transition from the closed to the open state of the I_h channel is associated with increased affinity for cAMP (DiFrancesco 1999; Luthi and McCormick 1998). Thus hyperpolarization, by opening I_h channels and increasing their affinity for cAMP, could produce the calcium-independent I_{ADP} if sufficiently high levels of cAMP were available inside ADn neurons. Because biophysical studies suggest that the cAMP affinity for the open I_h channel is in the nanomolar range (≈70 nM) (DiFrancesco 1999), this possibility does not seem out of the question.

Serotonin can shift the voltage dependence of I_h by a cAMP-dependent mechanism (Bobker and Williams 1990). A similar effect occurs in the ADn (unpublished data). If the ADP in the ADn occurred through the mechanism outlined above, then one could expect significant facilitation of the ADP by serotonin. This is precisely what we observed. In fact, calcium spikes also can potentiate the ADP through a calcium-induced increase in cAMP (Luthi and McCormick 1999). Serotonin and an increase in intracellular calcium, therefore could work in similar ways to facilitate the ADP. Of course, further studies will be required to determine whether this, or another mechanism, accounts for the ability of serotonin to facilitate the ADP.

What is the function of the ADP in the ADn? The ADP in thalamic neurons plays an important role in generating the interval between spindle oscillations. However, cells of the anterior thalamus, including the ADn, are thought not to undergo spindle oscillations in the cat or the rat. Although this initially was thought to result from the lack of a GABAergic input from the reticular nucleus (Paré et al. 1987), recent evidence suggests that...
studies have documented a significant input from this nucleus to the anterior thalamus (Pinault and Deschenes 1998). This raises the possibility that differences in the electrophysiological properties of neurons in the anterior thalamus might contribute to the unique network properties of this region. Here we have shown that the cells in the ADn express a prominent ADP that can be triggered solely by membrane hyperpolarization and greatly enhanced by the neuromodulator serotonin. Further studies will be required to determine how generally applicable these results are to other nuclei of the thalamus and how these features contribute to the firing of ADn neurons.

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