Potassium channel blockade induces rhythmic activity in a conditional burster neuron

Ronald M. Harris-Warrick and Bruce R. Johnson
Section of Neurobiology and Behavior, Seeley G. Mudd Hall, Cornell University, Ithaca, NY 14853 (U.S.A.)
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In the lobster stomatogastric ganglion the Anterior Burster (AB) neuron loses its rhythmic bursting capabilities when isolated from all synaptic input. Here we report that compounds which reduce the current through several different types of potassium channels induce bursting in isolated AB neurons. These results suggest that when quiescent, this neuron has all the conductances necessary to support bursting, but bursting is actively inhibited by tonic potassium conductances.

Neurons with endogenous bursting characteristics have been described in a number of species\textsuperscript{8,10,41,42}. These neurons can be subdivided into two broad classes: (1) endogenous bursters, which continue to generate rhythmic bursts of action potentials even when totally isolated from all synaptic input\textsuperscript{1,2,3,17,18,29,30,41}; and (2) conditional bursters, which lose their ability to burst when isolated, but whose bursting mechanisms can be activated by unpatterned modulatory or hormonal inputs\textsuperscript{7,14,16,20,24,40}. In several systems, conditional bursters are components of neural circuits that generate rhythmic motor patterns. The activation of bursting capability in these cells would be important in the initiation of these motor patterns\textsuperscript{20-22}.

Although the ionic mechanisms underlying neuronal bursting have been studied in some detail\textsuperscript{1,2,3,8,9,10,12,22,24,29,30,40,46}, the mechanisms that lead to the initiation of bursting in a quiescent conditional burster are not well understood. It is generally thought that modulatory inputs induce long-term changes in membrane conductance that allow bursting to be expressed\textsuperscript{10}. This could occur by two general classes of mechanisms. First, the modulatory inputs could activate the conductances that support bursting. These conductances would thus be inactive in the quiescent cell. Second, modulatory inputs could inactivate inhibitory conductances that prevent bursting. By this mechanism, the quiescent cell has all the conductances to support bursting, but bursting is prevented by active inhibitory or shunting mechanisms. We have studied the initiation of bursting in a conditional burster neuron in the stomatogastric ganglion of the spiny lobster, \textit{Panulirus interruptus}; we show that reduction of potassium currents uncovers bursting activity in a quiescent neuron, supporting this second mechanism.

The Anterior Burster (AB) neuron in the stomatogastric ganglion (STG) is the primary pacemaker neuron in the central pattern generator circuit for the pyloric rhythm in the lobster stomach\textsuperscript{33,34}. With intact modulatory inputs from other ganglia, this cell generates large amplitude membrane potential oscillations called bursting pacemaker potentials (BPPs) that underly rhythmic bursts of action potentials. However, when these modulatory inputs are removed, the AB neuron slowly loses its bursting capability and falls silent\textsuperscript{12,22,34,36}. Our experiments were performed on AB neurons which had been isolated from all detectable synaptic
input of situ. The STG was dissected from the animal as described by Mulloney and Selverston, and superfused at 3 ml/min with cold (14–15 °C) oxygenated saline (composition in mM: NaCl 479, KCl 12.8, CaCl₂ 13.7, Na₂SO₄ 3.9, MgSO₄ 10.0, glucose 2.0, Tris base 11.1, maleic acid 5.1, pH 7.4). Two steps were used to eliminate all detectable input to the AB cell. First, synaptic inputs from other ganglia which send axons to the STG in the stomatogastric nerve were eliminated by a sucrose/tetrodotoxin (TTX) block, made by forming a vaseline ring around the nerve and replacing the saline in the ring with isotonic sucrose containing 1 μM TTX. Second, the only neurons within the STG that directly synapse on the AB cell (the two PD and the VD neurons) were eliminated by photoinactivation, using intracellular iontophoresis of 5,6-carboxyfluorescein and illumination with blue light. After these treatments, no synaptic input was detectable in the AB neuron for the remainder of the experiment. Although potential sources of input remain (discussed in detail in ref. 14), these were never in fact observed. The synaptically isolated AB neuron was impaled with a bevelled microelectrode (filled with 4 M potassium acetate, resistance 20–25 MΩ). In most experiments, current injection was performed using a switched single electrode current injection instrument (Almost Perfect Electronics, Basel).

When isolated from all synaptic input, the AB neuron typically showed no rhythmic activity or spontaneous action potentials, and displayed a stable resting potential (for example, Control of Fig. 1). Occasional isolated cells did, however, show weak bursting ability, with single bursts separated by long silences (for example, top Control of Fig. 3). Rhythmic bursting could not be induced in any isolated AB neuron by either brief or tonic depolarization or hyperpolarizing current injection. We verified the good condition of the isolated cell by eliciting bursting with a brief application of 10⁻⁴ M dopamine at the beginning of each experiment.

Extracellular TEA (5–10 mM) induced rhythmic endogenous bursting in 4 of 6 synaptically isolated AB cells (Fig. 1). These bursts were large, with peak depolarizations (peaks) and post-burst hyperpolarizations (troughs) more depolarized and hyperpolarized, respectively, from the control bursts observed before synaptic isolation. The onset of burst activity was rapid, occurring within 30 s–1 min after arrival of TEA in the bath. The effect of TEA was reversible, with bursting ceasing within 10–20 min after beginning the washout of TEA from the bath. In 2 cells where rhythmic bursting was not obtained, occasional spontaneous bursts were observed after addition of TEA, and single bursts could be reproducibly obtained by short (50 ms) depolarizing pulses or upon rebound from hyperpolarizing pulses.

Bath application of 1–2 mM 4-aminopyridine (4-AP), which blocks a transient A-type potassium channel in somata of molluscan neurons and stomatogastric neurons, also induced rhythmic bursting in 4 of 4 quiescent AB cells (Fig. 2). The onset of bursting activity occurred within 2 min of 4-AP entry into the bath. The reversibility of 4-AP induced bursting was, however, variable. In two experiments bursting ceased within 30 min of 4-AP washout but in two others 4-AP induced bursting appeared irreversible, even after 8 h washing in normal saline. Prolonged effects of 4-AP also have been noted in other preparations. Increasing the 4-AP concentration to 5 mM caused disruption of bursting with tonic or irregular action potential activity, presumably because potassium conductances play essential roles in normal bursting. Bursting induced by 4-AP was sensitive to TTX in experiments where the effects of 4-AP were prolonged, the addition of 10⁻⁷ M TTX completely eliminated bursting activity and returned the
AB cell to its initial quiescent state. Rhythmic bursting can also be induced by a reduction of calcium entry into quiescent AB cells. This was demonstrated with 3 different protocols (Fig. 3):

![Figure 3](image)

Fig. 3. Reduction in calcium entry induces bursting in a quiescent AB neuron. Results from 3 different cells are shown, each with its own control. The isolated AB cell was either silent (Ca^{2+} and Co^{2+} treatments) or showed weak bursting activity with single bursts separated by long silent periods (0Ca^{2+}, High-Mg^{2+} treatment). 0Ca^{2+}, High-Mg^{2+}: replacement of all the extracellular calcium by magnesium induces rhythmic bursting after 2 min. 0.1 mM Co^{2+}: addition of cadmium in the presence of normal calcium saline induces rhythmic bursting after about 2 min. 10 mM Co^{2+}: addition of cobalt to normal saline induces rhythmic bursting after 2.5 min. The differences in burst shape between the different treatments are not significant, but demonstrate variability in the normal burst shape of the AB cell in different preparations. Bars: 5 mV, 1 s.

(1) superfusion with saline in which all the calcium was replaced by magnesium; (2) addition of 0.1–0.5 mM cadmium ion to the normal saline (with 13.7 mM CaCl_2); and (3) addition of 10 mM cobalt ion to the normal saline. All 3 salines elicited bursting within 2 min. This bursting was initially very regular for several minutes. However, the bursting mechanism itself appears to depend absolutely on calcium entry with consequent increases in intracellular calcium^{16,22}, and as calcium entry declined with time in the altered salines, the bursts disappeared. The time course of this disappearance is shown in Fig. 4. The burst amplitude slowly declined, specifically due to a reduction in the amplitude of the hyperpolarizing trough separating the bursts. Eventually the cell ceased bursting and remained depolarized at a membrane potential near the initial peak amplitude of the burst^{16,22}. For several minutes after bursting ceased, rhythmic BPPs without action potentials could be obtained by tonic hyperpolarizing current injection. On the other hand, stable bursting lasting at least 20 min could be obtained by the addition of 0.1 mM Cd^{2+}, which apparently reduced calcium-dependent processes sufficiently to induce bursting but not so much

![Figure 4](image)

Fig. 4. Time course of bursting induced by addition of 10 mM Cd^{2+} to normal saline. Rhythmic bursting is observed at 2.5 min (same cell as in the bottom line of Fig. 3). With time, the bursting becomes shallower, due primarily to a reduction in the hyperpolarizing trough separating bursts. At 5 min, the cell stops bursting and remains depolarized near the original peak amplitude of the bursting potential. For a few minutes, rhythmic BPPs can be restored by hyperpolarizing current injection (5.5 min), but later the cell shows only passive repolarization upon current injection (not shown). Upon removal of Co^{2+}, the cell returns to its pre-Co^{2+} resting state (not shown). Similar results are seen with 0Ca^{2+}, High Mg^{2+} saline and saline containing 0.5 mM Cd^{2+}. Bars: voltage (V), 5 (top) or 10 (bottom) mV, current (I), 5 nA; time, 1 s.
as to disrupt the burst process itself.

One possible explanation for this result is that bursting is actively inhibited in quiescent AB cells at least in part by a tonic calcium-dependent potassium conductance. Apamin, a polypeptide toxin from bee venom, has been shown to block one class of calcium-activated potassium channel in mammalian neurons \(^{28,41}\). When apamin was bath-applied, the isolated AB neuron responded by slow, rhythmic bursting (2/4 preparations; Fig. 5). This required rather high concentrations of apamin (10^-6 M) and prolonged incubation times (10 min), possibly due either to species differences in the apamin-binding site of the calcium-dependent potassium channel between crustacean and mammalian neurons, or to altered affinity of apamin for receptors in marine salines with high salt concentrations. The effect of apamin was reversible after 20–30 min (Fig. 5). In 2 cells, apamin did not elicit bursting alone, but bursts could be induced by tonic depolarization (not shown).

These results suggest that during the quiescent phase of the AB cell, the conductances that support bursting are present, but their expression is actively blocked by the simultaneous presence of inhibitory conductances. Two lines of evidence suggest that this inhibition is mediated, at least in part, by a tonically active calcium-dependent potassium conductance. First, apamin and TEA, both of which are known to block calcium-dependent potassium channels in this \(^{32}\) and other systems \(^{26}\), can induce rhythmic burst activity. Second, reduction of calcium entry elicits rhythmic activity. Our results with zero-calcium, high-magnesium saline could have an alternative explanation: the removal of calcium could cause changes in extracellular surface charge density, activating voltage-sensitive conductances \(^{15}\). However, we do not believe that this explanation is correct, since addition of low concentrations of cadmium (0.1 mM) or high concentrations of cobalt (10 mM) in the presence of normal calcium concentrations (13.7 mM) have the same effect. Thus, low-calcium/high-magnesium, cadmium, and cobalt-containing salines probably all act directly to block resting calcium conductances, which in turn could inhibit a tonically activated calcium-dependent potassium conductance.

In several isolated AB cells, addition of TEA or apamin did not elicit rhythmic bursting, but only reduced the threshold for burst generation by current injection. Presumably, in these cells other conductances in addition to the calcium-dependent outward conductances were active to block bursting. One of these conductances could be related to a transient potassium current, known as the A current in molluscs and other cells \(^{12,23}\). This TEA-resistant current has been detected in isolated somata of stomatogastric neurons, and is the only known potassium current in these cells that is sensitive to low concentrations of 4-AP \(^{19,23}\). In addition, low concentrations of TEA block another potassium conductance in stomatogastric neurons related to the delayed outward current, I_K, in molluscs \(^{19}\). Thus, a number of tonic potassium conductances might normally act in concert to prevent bursting during the quiescent phase of this cell.

A number of identified neuromodulators can initiate bursting in quiescent AB cells, including the amines dopamine, serotonin and octopamine \(^{5,15,19,22,31,41}\), the peptides proctolin and FMRFamide \(^{36,37}\), and muscarinic agonists \(^{5,31,36,39}\). Nagy et al. \(^{39}\) showed that intracellular injection of TEA into non-isolated AB and PD neurons induces plateau potentials and bursting. They suggested that muscarinic agonists may activate bursting in AB and PD cells by reducing a tonic TEA-sensitive potassium conductance, thus uncovering the currents that allow bursting to occur. While this hypothesis is attractive and is supported by our data, there is little direct evidence that any modulator initiates rhythmic
activity simply by a conductance decrease mechanism. In other work, we have shown that the amines dopamine, octopamine and serotonin all induce bursting in the AB cell by different mechanisms, involving changes in several currents. Like bursting induced by 4-AP, octopamine-, serotonin- and procotolin-induced bursting are blocked by the addition of TTX; in contrast, dopamine-induced bursting is not affected by TTX. Thus, even if many modulators might initiate bursting by a conductance decrease

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