EVIDENCE FOR A PERSISTENT Na⁺ CONDUCTANCE IN NEURONS OF THE GASTRIC MILL RHYTHM GENERATOR OF SPINY LOBSTERS

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Summary

Evidence for a persistent Na⁺ conductance was obtained in identified motor neurons of the gastric mill network in the stomatogastric ganglion of the spiny lobster Panulirus interruptus. The cells studied were the lateral gastric and lateral posterior gastric motor neurons, which in vivo control chewing movements of the lateral teeth of the gastric mill. We examined basic cellular properties in the quiescent network of the isolated stomatogastric ganglion.

In current-clamp recordings, we found two types of evidence for a persistent Na⁺ conductance. First, tetrodotoxin-sensitive inward rectification occurred during depolarization from rest to spike threshold. Second, 5 mmol l⁻¹ tetraethylammonium (a K⁺ channel blocker) induced plateau potentials that persisted in the presence of Mn²⁺ or a low [Ca²⁺]o but were blocked by a low [Na⁺]o or 100 nmol l⁻¹ tetrodotoxin. The plateau potentials could drive trains of fast spikes in the motor axon and strong transmitter release at central output synapses within the ganglion.

This conductance probably corresponds to the persistent Na⁺ current, I_{NaP}, described in cultured stomatogastric neurons and in neurons from several other preparations. During normal neuronal activity, it may contribute to the prolonged plateau depolarizations and long spike trains typical of motor neuronal activity during gastric rhythm generation. Persistent inward currents of this type are likely to be important in neurons that must fire prolonged bursts in cycle after cycle of rhythmic activity.

Key words: Na⁺ conductance, gastric mill, rhythm generator, spiny lobster, Panulirus interruptus, plateau potential, burst, stomatogastric.

Introduction

Currents sustaining repetitive spiking are likely to be important in neurons that fire throughout prolonged depolarizations. Examples include the neurons participating in the motor rhythms that underlie walking, breathing, chewing, etc. These rhythms arise in circuits called central pattern generators (CPGs), in which neurons generate slow oscillations of membrane potential (many times the duration of fast Na⁺ spikes), as a result of endogenous currents and synaptic interactions, and fire repetitively during each depolarized phase (Getting, 1988, 1989; Grillner, 1985; Grillner et al. 1995). In the present paper, we present evidence for the role of a persistent inward current in the repetitive firing activity of neurons in a chewing CPG.

Chewing of ingested food in decapod crustaceans is completed by the action of three internal teeth of the gastric mill, a skeletomuscular structure in the foregut (Johnson and Hooper, 1992; Turrigiano and Heinzel, 1992). Coordinated, rhythmic movements of the teeth are produced by a set of striated muscles under the control of the gastric mill CPG, which is located in the stomatogastric ganglion (STG). The gastric CPG can continue to produce fictive motor rhythms when the STG and its associated ganglia are isolated from the foregut and placed in a dish. Typically, these rhythms are rather slow (cycle period duration 5–10 s) and consist of long, coordinated bursts of spikes in gastric motor neurons (burst durations 3–5 s; intraburst spike frequencies peaking in the range 20–60 Hz) (Russell, 1985a; Russell and Hartline, 1984). Still slower rhythms with longer, more intense bursts can occur when modulatory conditions are varied (Elson and Selverston, 1992; Heinzel and Selverston, 1988; Russell, 1985a). What cellular and synaptic properties shape the characteristic, slow voltage oscillations and long bursts of gastric neurons? Recently we reported the presence of slow inhibitory postsynaptic potentials (IPSPs) at gastric CPG connections (Elson and Selverston, 1995). Here we present evidence for a cellular property – a persistent Na⁺ conductance – that could sustain prolonged depolarization and repetitive firing.

Persistent Na⁺ current (I_{NaP}) is a component of the tetrodotoxin (TTX)-sensitive Na⁺ current of many excitable cells (Crill, 1996; Llinás, 1988; Stafstrom et al. 1982; Taylor, 1993). Like the more familiar, transient current (I_{Na}) which drives the upstroke of action potentials, I_{NaP} activates rapidly in a voltage-dependent manner. However, activation occurs at more negative potentials, and inactivation is much slower, than

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for \( I_{Na} \) (French et al. 1990; Stafstrom et al. 1982, 1985).

Although it may amount to only a small fraction of the peak transient current, \( I_{NaP} \) can influence voltage behavior at potentials below and near the spike threshold because few other voltage-dependent conductances are active in this range (Crill, 1996). In several classes of neuron from the mammalian brain there is now evidence that \( I_{NaP} \) can regulate spike threshold, influence the occurrence and rate of repetitive firing (French et al. 1990; Llinas and Alonso, 1992; Stafstrom et al. 1982, 1984) and generate subthreshold voltage oscillations (Alonso and Llinas, 1989; Klink and Alonso, 1993; Llinas and Alonso, 1992). Among neurons from rhythm-generating circuits, there is evidence that \( I_{NaP} \) occurs in premotor interneurons of the heartbeat CPG of the leech (Opdyke and Calabrese, 1994) and in unidentified, cultured neurons from the crustacean STG (Turrigiano et al. 1995).

We studied the single lateral gastric (LG) and the paired lateral posterior gastric (LPG) motor neurons of the intact STG of the spiny lobster *Panulirus interruptus*. In the periphery, these neurons innervate antagonistic muscles that open and close the lateral teeth during chewing. When the mill is at rest, tonic firing of the LPG neurons plays a postural role, holding the teeth open and supporting the apparatus within the thoracic cavity (Hartline and Maynard, 1975; Heinzel and Selverston, 1988; Mulloney, 1987; Mulloney and Selverston, 1974). Within the neuropil of the STG, the LG and LPG neurons make central input and output synapses, interacting with each other and with other gastric neurons and participating in pattern generation (Elson and Selverston, 1992, 1995; Mulloney and Selverston, 1974; Russell, 1985a,b; Selverston, 1987).

We present current-clamp evidence for a persistent Na\(^{+}\) conductance in these neurons. Because the cells have an extended electrotonic structure (Edwards and Mulloney, 1984; Graubard and Hartline, 1991; Hartline and Graubard, 1992), we did not attempt a voltage-clamp analysis. Nevertheless, the results are consistent with the presence of a current resembling \( I_{NaP} \). We discuss the integrative importance of the conductance in motor neurons that fire in prolonged, rhythmical bursts or tonic, postural patterns.

Materials and methods

Data were obtained from 25 preparations of the stomatogastric nervous system of the adult spiny lobster *Panulirus interruptus* Randall. Animals were caught locally and kept in running sea water until use. The stomatogastric nervous system, consisting of the STG, the anterior (commissural and oesophageal) ganglia, connecting nerves and motor nerves (Fig. 1A), was removed from the foregut (Mulloney and Selverston, 1974) and pinned out in a dish lined with silicone elastomer (Sylgard) and filled with normal lobster saline (in mmol l\(^{-1}\): NaCl, 479; KCl, 13; CaCl\(_2\), 14; MgSO\(_4\), 6; Na\(_2\)SO\(_4\), 4; Heps, 5; Tes, 5; pH 7.4). The STG and the dorsal ventricular nerve (dvn) were surrounded by a wall of petroleum jelly (Vaseline) and received separate superfusion at 14–17°C. During data collection, the temperature of the

Fig. 1. Stomatogastric nervous system: functional morphology and spontaneous activity of the lateral gastric motor neuron (LG) and the lateral posterior gastric motor neuron (LPG). (A) Schematic diagram of the stomatogastric nervous system *in vitro*. Vaseline wells (stippled) are used to allow selective superfusion of the stomatogastric ganglion (STG) and sucrose blockade of the stomatogastric nerve (stn). ‘Anterior ganglia’ refers to the unpaired oesophageal and paired commissural ganglia. Motor axons project bilaterally, but for clarity we show only unilateral projections. LGn, lateral gastric nerve; LPGn, lateral posterior gastric nerve, which is recorded in the dorsal lateral ventricular nerve; dvn, dorsal ventricular nerve; lvn, lateral ventricular nerve. (B) Diagram showing the functional morphology of motor neurons (LG or LPG) within the STG: i, microelectrode for current injection; v, microelectrode for voltage recording. (C) Typical spontaneous activity of LG and LPG during rhythm generation (under modulatory influence from anterior ganglia, stn intact) and in the quiescent circuit (after removal of modulatory inputs: stn blocked).
superfusate usually varied by no more than 1 °C. A tap in the inflow line allowed us to switch the STG superfusion between normal saline and various modified salines (see below). Bipolar, stainless-steel pin electrodes were used to record from, or to stimulate, identified motor nerves. After desheathing the STG, we recorded intracellularly from somata using microelectrodes filled with 3 mmol l\(^{-1}\) KCl (resistance approximately 20 M\(\Omega\)) or 3 mmol l\(^{-1}\) potassium acetate. Gastric motor neurons were identified by correlating intracellular spikes with impulses recorded in nerve trunks and by observing their characteristic phase of bursting during rhythmical activity. After identifying the neurons, we blocked descending modulatory inputs to the STG by placing isotonic sucrose in another Vaseline well on the stomatogastric nerve (stn: Fig. 1A; cf. Russell, 1979).

We impaled neurons with separate electrodes for current injection and voltage recording. Microelectrodes were connected to the input stages of M4-A (World Precision Instruments) or Axoclamp 2A (Axon Instruments) electrometers. Membrane potential was monitored throughout the recording and measured upon withdrawal. For recordings of synaptic transmission from LG to LPG, the postsynaptic cell (LPG) was held hyperpolarized using a current electrode filled with 3 mmol l\(^{-1}\) potassium acetate (to minimize Cl\(^{-}\) leakage). Recordings were digitized and stored on video tape (Vetter PCM 3000A), and transcribed on a Gould electrostatic recorder (ES 1000).

Tetraethylammonium chloride (TEA\(^{+}\), 5–10 mmol l\(^{-1}\), to reduce K\(^{+}\) currents), tetrodotoxin (TTX, 100–150 mmol l\(^{-1}\), to block Na\(^{+}\) current) or MnCl\(_2\) (Mn\(^{2+}\) ≤10 mmol l\(^{-1}\), to reduce Ca\(^{2+}\) currents) was added to saline without compensation. In most experiments, we added CsCl (Cs\(^{+}\), 3–5 mmol l\(^{-1}\)) to block hyperpolarization-activated inward current (I\(_{h}\); Golowasch and Marder, 1992a). To make reduced [Na\(^{+}\)]\(_{o}\) saline, we decreased [NaCl] to 20–50 % of normal by substitution with Tris–HCl.

Reduced [Ca\(^{2+}\)]\(_{o}\) saline was usually made by decreasing [CaCl\(_2\)] to 5 % of its normal value by equimolar substitution with MgCl\(_2\) and adding 10 mmol l\(^{-1}\) Mn\(^{2+}\) (low-Ca\(^{2+}\)/high-Mg\(^{2+}\)/Mn\(^{2+}\) saline). In a few cases, we reduced [CaCl\(_2\)] to 10 % of normal by substitution with equimolar MnCl\(_2\). Similar ionic conditions produce a large decrease in the detectable Ca\(^{2+}\) and Ca\(^{2+}\)-mediated currents of stomatogastric neurons (Golowasch and Marder, 1992a; Graubard and Hartline, 1991).

All chemicals were obtained from Sigma, except TTX (Calbiochem).

**Results**

*Lateral gastric (LG) and lateral posterior gastric (LPG) neurons*

As typical stomatogastric motor neurons, LG and the two LPGs have the functional morphology summarized in Fig. 1B (cf. Hartline and Graubard, 1992). The spike initiation zone is thought to lie at the origin of the axon near the margin of the neuropil. Spikes propagate actively in the axon (where they can be detected by extracellular electrodes placed on motor nerves), but spread to the neuropil and soma by passive conduction. Intracellular recordings made in the soma register spikes and neuropilar voltage events with various amounts of electrotonic decrement (Edwards and Mulloney, 1984; Miller, 1980).

LG and LPG generate alternating bursts of spikes during gastric rhythms, such as those produced when the gastric circuit within the STG receives modulatory input from anterior ganglia (Fig. 1C, stn intact). The oscillatory and synaptic activity seen under these conditions complicate the analysis of cellular properties. We therefore removed the modulatory inputs (by blocking the stomatogastric nerve, stn: Fig. 1A), thereby stopping the gastric rhythm (Russell, 1979). In this quiescent circuit, LG is silent at a resting potential usually in the range –55 to –65 mV, while the LPGs can fire tonically at a membrane potential in the range –40 to –50 mV (or, if silent, can be driven to spike tonically by slight depolarization) (Fig. 1C, stn blocked).

**Effects of TTX on slow, depolarizing potentials**

Fig. 2 shows results from two experiments in which an LPG neuron was stimulated by equivalent, depolarizing and hyperpolarizing current pulses (inset, +/-1 nA, 1–2 s duration) while the ganglion was exposed to different concentrations of TTX. In control conditions, the depolarizing pulse drove repetitive spiking (Fig. 2Ai). As expected, repetitive spiking was reduced in low concentrations of TTX (10 mmol l\(^{-1}\); Fig. 2Aii) and abolished at higher doses (100 mmol l\(^{-1}\); Fig. 2Aiii); partial recovery occurred upon washing (Fig. 2Aiv). However, TTX also exerted an effect on slow potentials. Whereas the electrotonic response to the hyperpolarizing pulse was little changed, the steady voltage response to depolarizing current underwent a small, reversible depression (Fig. 2Aii–iv).

This effect is clearer in Fig. 2B, which shows responses in TTX (Fig. 2Bi) and then during washout (Fig. 2Bii,iii). Again, hyperpolarizing potentials were unchanged by the removal of TTX, whereas steady, depolarizing responses were increased (see overlay, Fig. 2Biv). During the washout, we also saw a slow, depolarizing wave following the initial spikes (Fig. 2Bii, filled circle). As washing continued, this wave triggered a further spike (Fig. 2Biii,iv), suggesting the presence of a TTX-sensitive, pacemaker potential (see Barrio et al. 1991). Similar potentials were seen in low doses of TTX or low [Na\(^{+}\)]\(_{o}\) (data not shown).

The effects occurred in saline where 90 % of [Ca\(^{2+}\)]\(_{o}\) was substituted by Mn\(^{2+}\) (Fig. 2B), which should greatly reduce synaptic input. This suggests that TTX affected intrinsic membrane properties.

**TTX-sensitive inward rectification**

A more detailed study of TTX-sensitive, inward rectification in an LPG neuron is shown in Fig. 3. TTX (100 mmol l\(^{-1}\)) depressed the voltage responses to steps of depolarizing current, but had little effect on responses to equivalent steps of hyperpolarizing current (Fig. 3A). Slope resistance, a sensitive indicator of rectification (see Llinas and Alonso, 1992), was
monitored by measuring the voltage responses to small, negative current pulses at different membrane potentials (Fig. 3B). Before TTX, the apparent input resistance increased with depolarization (Fig. 3Bi); after TTX blockade, it decreased (Fig. 3Bii). A plot of input resistance against membrane potential showed inward rectification occurring with depolarization beyond approximately −55 mV (Fig. 3C, control); in the presence of TTX, the inward rectification was blocked and a competing outward rectification was revealed (Fig. 3C, TTX). Similar results were obtained in two other experiments.

The ionic conditions (low [Ca2+]o and Mn2+ to block...
Persistent Na⁺ conductance in gastric neurones (synaptic and Ca²⁺ currents, Cs⁺ to block Ih) and pharmacology (resistance to low [TEA⁺], blockade by TTX) suggest that the inward rectification was produced by a persistent Na⁺ current. In current-clamp recordings, the activation of this inward conductance can boost depolarizing swings of the membrane potential, resulting in an apparent increase in input resistance (see Connors et al. 1982; Llinas and Alonso, 1992; Stafstrom et al. 1982, 1985).

**TEA⁺-induced plateau potentials**

In a second type of experiment, we found that 5–10 mmol l⁻¹ TEA⁺, the same current pulse elicited prolonged (1–2 s) plateau potentials (Fig. 4Di). The plateau potentials could be triggered by brief depolarization (Fig. 4Dii) and terminated, in a voltage-dependent manner, by brief hyperpolarization (Fig. 4Diii). TEA⁺ induced plateau potentials in at least five other recordings of LPGs (see also Fig. 3A, filled circle).

Similar plateau potentials were induced in the LG cell (Fig. 5; N=6). Before TEA⁺, a pulse of depolarizing current (typically 2–3 s long) drove LG to fire an accelerating then adapting train of action potentials (Fig. 5Ai). In 5 mmol l⁻¹ TEA⁺, a similar depolarization evoked a series of plateau potentials, each of which could drive a high-frequency (approximately 50 Hz) burst of action potentials in the motor axon (Fig. 5Aii). A single plateau potential could be triggered by a brief, depolarizing current pulse (Fig. 5Bi) and showed all-or-none, threshold behavior (Fig. 5Bii). Neuronal input resistance was monitored by the voltage responses to brief, negative current pulses (Fig. 5Biii). During the plateau, the

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**Fig. 4.** TEA⁺ induces plateau potentials in LPG. (A,B) Transformation of spontaneous, repetitive spiking by 5 mmol l⁻¹ TEA⁺. (A) Before TEA⁺. (B) Progressive induction of plateau potentials at increasing times after adding 5 mmol l⁻¹ TEA⁺ to the superfusate. (C,D) Induction and triggering of prolonged plateau potentials. (C) Before TEA⁺, this LPG was not spontaneously active but fired tonically upon slight depolarization. (D) After the addition of 5 mmol l⁻¹ TEA⁺, the same depolarization drives plateau potentials. (Dii) Triggering by a brief depolarizing current pulse. (Diii) Voltage-dependent termination of a triggered plateau by brief hyperpolarization: overlay of responses to current pulses of two different amplitudes (1, 2). In this and subsequent figures, LPGi monitors current injected into LPG; LPGn is an extracellular recording of the motor nerve. Cs⁺ was present in both experiments to block Ih: A,B, 6 mmol l⁻¹; C,D, 3 mmol l⁻¹. Vertical current calibration: 6 nA (C,Di) or 3 nA (Dii,iii).
voltage responses decreased in both amplitude and (more markedly) time constant, indicating a fall in input resistance. The pulses used were too brief to charge the membrane completely at ‘rest’, thus underestimating the measurable change in resistance. Nevertheless, the results suggest a net increase in membrane conductance (Fig. 5Biii).

Typically, the motor axon fired at 30–50 Hz throughout the plateau potential, but these spikes were highly attenuated in somatic recordings (Figs 4B, 5). In some instances, the axon spiked only at the beginning, or at the beginning and end, of the plateau potential (Fig. 4B, see also Fig. 6). These examples of discharge failure show that continuous axon firing was not needed to sustain the plateau. The relationship between plateau potentials and axon spikes is treated further in the Discussion.

Ionic conductances underlying TEA+ -induced plateau potentials

TEA+ -induced plateau potentials were not blocked by saline containing 5% of normal [Ca2+]o (compensated by Mg2+) and 10 mmol l−1 Mn2+ (low-Ca2+/high-Mg2+/Mn2+) (Fig. 6; N=5). In LG, for example, plateau potentials driven by depolarizing current steps in 5 mmol l−1 TEA+ (Fig. 6Aii) were not suppressed in low-Ca2+/high-Mg2+/Mn2+ saline (+TEA+) (Fig. 6Aii). In the same saline, TEA+ -induced plateau potentials persisted in LPG (Fig. 6B).

In contrast, low [Na+]o or TTX abolished TEA+ -induced plateaus (Figs 7, 8). In Fig. 7, for example, a triggered plateau potential of LPG (Fig. 7A) was reversibly blocked by reducing [Na+]o to 20% of normal (Fig. 7B,C) (note the persistence of fast spikes, however: Fig. 7B). Reducing [Na+]o to 20–50% of normal abolished plateaus in all LG and LPG neurons tested (N=6). The block developed or washed out within 5–10 min of a change in [Na+]o in the STG superfusate. This time is consistent with diffusion and ion exchange in the neuropil (Johnson et al. 1992) and implies a rapid effect.

Plateau potentials were also blocked by TTX (Fig. 8; N=6). In the example shown, a TEA+ -treated LPG cell responded to depolarization with plateau and spike-like potentials (Fig. 8A). Addition of 100 nmol l−1 TTX caused, initially, a block of the axon spikes (Fig. 7B, 2 min), then a shortening of the plateau potentials (3 min) and finally the loss of all regenerative activity (6 min).

Plateau potentials with this pharmacology are another indicator of persistent Na+ conductance (e.g. Barrio et al. 1991; Davis and Stuart, 1988; Llinas and Alonso, 1992; Stafstrom et al. 1985).

Fig. 5. TEA+ induces plateau potentials in LG. (A) Effect of TEA+ on spike potentials. A long pulse of outward current (LG) evokes repetitive, single spikes before (Ai), and repetitive plateau-like potentials after (Aii), the addition of 5 mmol l−1 TEA+.* (B) Properties of single plateau potentials. (Bi) A single plateau potential is triggered by a brief positive current pulse. (Bii) Responses to pulses of increasing amplitude (overlay: apparent spike doublets and triplets result from superimposing slightly staggered trains). (Biii) Apparent increase in conductance during a triggered single plateau potential. Same neuron as in A (resting potential approximately −64 mV; more depolarized membrane potentials maintained by bias current); 3 mmol l−1 Cs+ is present throughout.
Persistent \( \text{Na}^+ \) conductance in gastric neurones

Integrative aspects of \( \text{TEA}^+ \)-induced plateau potentials

**Site of generation**

Several observations suggested that \( \text{TEA}^+ \)-induced plateau potentials were generated closer to the site of axonal spike initiation than to the site of intracellular recording in the soma (Fig. 9). First, depolarizing the soma affected the generation of plateau potentials but also activated outward rectification, which distorted the potentials seen in the somatic recording (Fig. 9A,B). Thus, in Fig. 9A, depolarizing the LG neuron to \(-45 \text{ mV}\) evoked a single plateau potential, while depolarization to \(-32 \text{ mV}\) elicited a train of repetitive, shortening plateaus. However, the potentials driven by the larger stimulus were reduced in amplitude, particularly during the ramp-like rise of membrane potential at the onset of the current step (Fig. 9A). Similar depolarizations in low \([\text{Na}^+]_o\) evoked transient and sustained decreases in membrane resistance (Fig. 9B). Presumably, this time- and voltage-dependent outward rectification resulted from the activation of residual \( \text{K}^+ \) conductances in or near the soma; the increased membrane conductance shunted voltage signals from the plateau potentials, which were generated elsewhere in the neuron, at some electrotonic distance from the cell body.

Second, regardless of the degree of shunting seen at the soma, each plateau potential drove the axon to fire at the same frequency (Fig. 9A; corresponding somatic and axon recordings are matched by the open or filled circles).

Third, antidromic action potentials could trigger plateau potentials (Fig. 9Ci). Single shocks to the LG motor nerve could evoke either a normal antidromic spike (response 1) or a plateau potential with an accompanying burst of spikes in the contralateral axon (response 2). Fig. 9Cii shows the plateau and axon burst evoked by a threshold depolarization of the soma. These observations suggested that the plateau potentials had good electrotonic access to spike-generating sites in the axon.

**Synaptic output**

Finally, \( \text{TEA}^+ \)-induced plateau potentials were strong drivers of transmission at central synapses (Fig. 10). We studied synaptic transmission from LG by recording the inhibitory postsynaptic potentials (IPSPs) which it evokes in LPG. The LPG was held hyperpolarized to shut off regenerative events and to reduce the slow component of the postsynaptic response. In this configuration, the remaining fast
IPSP was reversed and provided a relatively straightforward indication of transmitter release from LG (Elson and Selverston, 1995).

A triggered plateau potential (in TEA+) evoked an IPSP (Fig. 10Ai) that was considerably larger than the IPSP driven by a single spike, seen after blocking the plateau with low [Na+]o (Fig. 10Aii); both the plateau and the IPSP recovered with washing in normal [Na+]o (Fig. 10Aiii). In contrast, adding Mn²⁺ (6 mmol l⁻¹) spared the plateau potential (although raising its voltage threshold) while greatly reducing

![Fig. 8. Progressive blockade of plateau potentials by TTX.](image)

![Fig. 9. Integrative properties of plateau potentials in LG.](image)
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Fig. 10. TEA$^+$-induced plateau potentials drive large postsynaptic potentials at a central synaptic connection. Plateau potentials and spikes are evoked in the presynaptic cell, LG, and the resultant IPSPs recorded in the postsynaptic cell, LPG, which is held hyperpolarized below the synaptic reversal potential (IPSPs appear as depolarizing potentials). Throughout, the saline contains TEA$^+$ (5 mmol l$^{-1}$ in A, 10 mmol l$^{-1}$ in B) and 3 mmol l$^{-1}$ Cs$^+$. (A) $\text{Na}^+$-dependent plateau potentials drive Ca$^{2+}$-dependent synaptic transmission. (Ai) Control response: a short, positive current pulse triggers a plateau potential in LG, which evokes an IPSP in LPG. (Aii) The same stimulation in low [Na$^+$_o] (25% of normal; estimated exposure time 2–4 min). (Aiii) Recovery of responses after washing (4–6 min) in normal [Na$^+$_o]. (Aiv) Responses after addition of 6 mmol l$^{-1}$ Mn$^{2+}$ (a higher-amplitude pulse is needed to evoke a plateau potential). (B) A prolonged current pulse (Depol. LG) evokes a slow depolarization of LG with superimposed plateau potentials (arrows) and single spikes. The postsynaptic response of LPG reflects resultant transmitter release. lvn carries the LG axon spike. The diagram at the bottom of the figure shows the connection studied: the filled circle indicates synaptic inhibition.

sustained postsynaptic response to steady presynaptic depolarization, as in Fig. 10B; see Elson and Selverston, 1995; Graubard et al. 1983).

Discussion

The main findings of this study – the presence of TTX-sensitive inward rectification and $\text{Na}^+$-dependent, TTX-sensitive plateau potentials – argue for the presence of a persistent $\text{Na}^+$ conductance in the identified gastric motor neurons, LG and LPG, of the intact stomatogastric ganglion. As discussed below, this type of conductance is appropriate for the typical activity of these cells, long bursts or tonic sequences of spikes.

Evidence for a persistent $\text{Na}^+$ conductance in intact neurons

The evidence from current-clamp studies (more complete for LPG than for LG) indicates a conductance that is (1) activated by depolarizations above approximately $-55 \text{ mV}$ but below spike threshold, (2) persistent (sustaining plateau potentials which could reach up to $-30 \text{ mV}$ and continue long after spiking had ceased) and (3) $\text{Na}^+$-dependent and blocked by TTX. What membrane current(s) could account for these observations?

The features listed are consistent with the properties of the persistent $\text{Na}^+$ current, $I_{\text{NaP}}$ (Barrio et al. 1991; Davis and Stuart, 1988; French et al. 1990; Gestrelius et al. 1983; Johansen and Kleinhans, 1987; Llinas and Alonso, 1992; Saint et al. 1992; Stafstrom et al. 1982, 1985). In crustacean nervous systems, $I_{\text{NaP}}$ has been voltage-clamped in a putative neurosecretory cell of the barnacle (Davis and Stuart, 1988), in stretch receptor neurons of the lobster (Gestrelius et al. 1983) and in unidentified neurons cultured from the lobster STG (Turrigiano et al. 1995). These studies reported activation at membrane potentials ranging from $-75 \text{ mV}$ (Gestrelius et al. 1981, 1983) to $-50 \text{ mV}$ (Turrigiano et al. 1995), with maximal

the postsynaptic response (Fig. 10Aiv). Conceivably, the high-frequency burst of axon spikes driven by the plateau might have evoked a large postsynaptic response by summation of unitary, spike-mediated IPSPs. However, similar large IPSPs were elicited by plateau potentials in which the axon spiked only once or twice (Fig. 10B, arrows). Instead, we suggest that at least part of the postsynaptic potential driven by the plateau resulted from graded release of transmitter (which underlies the
current occurring at approximately −15 mV (Davis and Stuart, 1988; Turrigiano et al. 1995). In the stretch receptors, $I_{\text{NaP}}$ inactivated with a time constant of approximately 1.7 s at −50 mV (Gestrelius et al. 1983). Clearly, a current with very similar properties could account for the voltage behavior described in this paper.

Other, low-threshold and slowly inactivating Na$^+$ currents have been described in arthropod motor neurons, including a proctolin-activated current in the crab STG (Golowasch and Marder, 1992b) and a muscarinic current in moth larvae (Trimmer, 1994). However, these currents are resistant to TTX, in contrast to the conductance studied here.

Obviously, firm identification of the underlying current is not possible in the absence of voltage-clamp data. We did not attempt to clamp the conductance because these neurons show extended electrotonic structures and non-isopotential behavior (Edwards and Mulloney, 1984; Graubard and Hartline, 1991; Hartline and Graubard, 1992). Modeling studies caution that spurious, persistent currents can appear as voltage-clamp artifacts when a regenerative conductance is under inadequate spatial control (White et al. 1995).

**Plateau potentials: mechanism and integration**

When K$^+$ currents are decreased by TEA$^+$, the persistent Na$^+$ conductance can sustain a plateau potential. The potential is initiated when the neuron produces a spike. This spike propagates in the motor axon, but within the ganglion the neuron remains depolarized in a plateau phase. In many TEA$^+$-treated neurons, a spike ‘shoulder’ results from the action of Ca$^{2+}$ current (Horn and Miller, 1978; Klein and Kandel, 1978). Here, however, a plateau potential is formed by persistent Na$^+$ current. In response to the plateau depolarization, the axon can fire repetitively or fall silent, perhaps as a result of spike inactivation. The plateau phase ends with a sudden repolarization, presumably a regenerative event (see Hartline and Graubard, 1992; Hartline et al. 1988) occurring as residual K$^+$ currents overcome the slowly inactivating $I_{\text{NaP}}$ (Gestrelius et al. 1983; Yawo et al. 1985). Often the repolarization seemed to be triggered by the downstroke of a fast axon spike (Figs 4B, 6B).

The actual site of plateau generation is unknown. The plateau potential drives spiking in the axon but attenuates these spikes in the soma recording; it can be triggered by an antidromic axon impulse; and it drives a constant frequency of axon firing in the face of changes in depolarization and membrane conductance nearer to the soma. These observations suggest a site of generation near the origin of the axon and the spike initiation zone (see Fig. 1B).

Axon spikes that occur during the plateau are highly attenuated in somatic recordings. This suggests that the plateau-generating region lies between the region of spike generation in the axon and the recording site in the soma, and occludes the passive spread of spike potentials. At least two mechanisms could produce this occlusion. First, the spike mechanism of the proximal axon may be inactivated during the plateau. (The actual depolarization reached by the plateau potential is presumably underestimated in the soma recording.) Second, a high-conductance state during the plateau (see Fig. 5B) may shunt spike potentials. Similar plateau potentials, patterns of axon spiking and spike occlusion have been ascribed to the effects of a persistent Na$^+$ conductance in sensory and motor neurons of crayfish (Barrio et al. 1991; Cattaert et al. 1994), a putative neurosecretory cell of the barnacle (Davis and Stuart, 1988) and giant axons of the cockroach (Yawo et al. 1985).

At central synapses, plateau potentials drive much larger IPSPs than do single spikes, implying greater release of transmitter. At first sight, this might suggest that plateau potentials arise close to release sites in the fine synaptic neuropil (see Fig. 1B). However, the plateau potentials might release more transmitter simply because their depolarized phase is much longer and thus less attenuated by low-pass filtering during passive spread into the presynaptic neurites (from a site of generation closer to the axon; Edwards and Mulloney, 1984; Hartline and Graubard, 1992).

Although the plateau potentials are artifacts of K$^+$ channel blockade, they indicate the presence and possible localization of persistent Na$^+$ conductance within the neuron. A concentration of this conductance at or near spike-initiating regions would not be surprising if $I_{\text{NaP}}$ is mediated by ‘normal’, transient Na$^+$ channels operating in a non-inactivating mode (Alzheimer et al. 1993).

**Functional significance**

There are at least three important roles that a persistent Na$^+$ conductance might play in the normal function of gastric motor neurons.

First, it may contribute to pacemaking processes during repetitive spiking. Both LG and LPG can fire repetitively when depolarized by injected current, and the LPGs often show spontaneous, tonic firing. The conductance activates during subthreshold depolarization and the range of voltages traversed during the interspike interval (Figs 2–4). Results such as those in Fig. 2B suggest, but do not prove, that a persistent Na$^+$ conductance underlies slow, depolarizing prepotentials that may sustain repetitive firing.

Careful studies in pyramidal cells of sensorimotor cortex have shown that $I_{\text{NaP}}$ regulates spike threshold and the occurrence and rate of repetitive firing during sustained depolarization (Stafstrom et al. 1982, 1984a,b, 1985). In hippocampal CA1 neurons, $I_{\text{NaP}}$ is thought to generate slow prepotentials and depolarizing waves that regulate the rate of repetitive spiking during prolonged depolarizations (Lanthorn et al. 1984; Macvicar, 1985). Persistent inward currents can also oppose spike accommodation and the saturation of spike frequency (Schwindt and Crill, 1980, 1982; Stafstrom et al. 1984b). $I_{\text{NaP}}$ is prominent in several types of neuron that tend to fire tonically (Davis and Stuart, 1988; Llinas and Alonso, 1992).

If they apply, these features are relevant for gastric motor neurons, which must spike throughout the depolarized phase of each cycle of a slow voltage oscillation (during rhythm
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References


Second, persistent Na\textsuperscript{+} current could enhance excitatory synaptic inputs. In vertebrate neurons, I\textsubscript{NaP} can increase the amplitude and duration of EPSPs and shorten the electrotonic length constant of dendrites (Crill, 1996; Schwindt and Crill, 1995; Stafstrom et al. 1984b; Taylor, 1993). Although inhibition predominates at synapses within the gastric circuit (Selverston, 1987), some of the neurons (including LG and LPG) receive phasic bursts of EPSPs from extrinsic interneurons originating in anterior ganglia. These important interneurons help to drive spike bursts during gastric rhythms (Robertson and Moulins, 1984; Russell, 1976) and integrate mechanosensory inputs (Elson et al. 1994; Simmers and Moulins, 1988).

Third, persistent Na\textsuperscript{+} conductance may contribute to plateau potentials that generate bursts of spikes during gastric rhythms (Elson and Selverston, 1992; Russell and Hartline, 1984). These plateau potentials differ from the artifactual potentials that generate bursts of spikes during gastric rhythms (Nadim et al. 1995; Odyke and Calabrese, 1994). In STG neurons, I\textsubscript{NaP} may help to initiate and sustain these slow active depolarizations, too. A similar current helps to sustain long plateau potentials and trains of spikes during leech heartbeat rhythms (Nadim et al. 1995; Odyke and Calabrese, 1994). In STG neurons, I\textsubscript{NaP} may also participate in faster, pyloric-time oscillations (Gola and Selverston, 1981; Harris-Warrick and Flamm, 1987; Turrigiano et al. 1995). Another persistent, inward conductance – the proctolin current – is known to enhance excitability and burst generation in this system (Golowasch et al. 1992; Golowasch and Marder, 1992b; Hooper and Marder, 1987).

Bursting neurons from both vertebrates and invertebrates also display low-threshold, slowly inactivating Ca\textsuperscript{2+} currents (Brown and Griffith, 1983; Eckert and Lux, 1976; Huguenard, 1996; Johnston et al. 1980; Kramer and Zucker, 1985; Llinas and Yarom, 1981). Indeed, inward currents with these voltage and kinetic properties may be a common feature of neurons that fire repeated, long bursts of spikes during motor rhythms (as in chewing, walking, breathing, etc.). As further examples, we note a persistent Na\textsuperscript{+} current in jaw and oral motor neurons of guinea pig (Chandler et al. 1994; Mosfeldt-Laursen and Rekling, 1989), a persistent Ca\textsuperscript{2+} conductance in spinal motor neurons of cat and turtle (Hounsgaard and Kiehn, 1989; Schwindt and Crill, 1980, 1982) and a possible, persistent inward current in mammalian respiratory neurons (Richter et al. 1992). Modulation of I\textsubscript{NaP} by neurotransmitters (Cepeda et al. 1995; Yang and Seamans, 1996) could provide a powerful means of regulating excitability within motor pattern generators.

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