Ionic Basis for Serotonin-Induced Bistable Membrane Properties in Guinea Pig Trigeminal Motoneurons

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INTRODUCTION

The ability of motoneurons to produce plateau potentials (long-lasting periods of depolarization outlasting the transient stimuli that trigger them) and conditional burst oscillations has forced a reevaluation of the concept that motoneurons behave as “passive relays” in a motor system, because these nonlinear membrane properties can dramatically modify motoneuronal output in response to synaptic potentials (Hounsgaard et al. 1988; Rekling and Feldman 1997). A region of negative slope resistance (NSR) in the steady-state current-voltage (I-V) relationship endows motoneurons with the potential for bistability, which is required to produce plateau potentials and certain classes of bursting oscillations. The NSR is commonly generated by voltage-dependent Ca2+ or Na+ channels that exhibit sustained, or slowly inactivating kinetics (Benson and Adams 1989; Hounsgaard and Kiehn 1985; Li and Hatton 1996; Schwindt and Crill 1977; Smith 1975; Stafstrom et al. 1982), or in other neurons by activation of the N-methyl-D-aspartate (NMDA) glutamate receptors (Hochman et al. 1994; Hu and Bourque 1992; Kiehn et al. 1996b; Kim and Chandler 1995; Sigvardt et al. 1985; Tell and Jean 1993).

Bistable membrane behaviors, such as plateau potentials and burst oscillations, in motoneurons are associated with postural and locomotor activity in rat, cat, and lamprey, among other vertebrates (Eken and Kiehn 1989; Kiehn 1991; Kiehn et al. 1996a; Wallen and Grillner 1987) and may be present in humans (Kiehn and Eken 1997). Although synaptic integration is critical for the formation of an appropriate motoneuronal output pattern, plateau potentials and burst oscillations do not result from the temporal summation of excitatory postsynaptic potentials during a maintained barrage of synaptic events (Hounsgaard et al. 1988; Schwindt and Crill 1980). Rather, these behaviors result from membrane bistability, endowed by intrinsic membrane properties, and can be initiated or terminated by brief synaptic input (Hounsgaard et al. 1984, 1988; Rekling and Feldman 1997). Modulation of intrinsic membrane properties by endogenous neuromessengers provides neurons with the flexibility to recruit bistable properties selectively.

Mammalian trigeminal motoneurons (TMNs), which control movements of the jaw, can be influenced by serotonin (5-HT) (Kurasawa et al. 1990; Ribeiro-do-Valle et al. 1991). The trigeminal motor nucleus receives serotonergic projections from the raphe nuclei (Li et al. 1993; Saha et al. 1991), and raphe neurons increase their activity during oral-motor behaviors (Fornal et al. 1996; Veasey et al. 1993) enhances spike discharge for many minutes during cortically induced rhythmic jaw movements (Katakura and Chandler 1990). 5-HT enhances TMN excitability (Hsiao et al. 1997; Trueblood et al. 1996) in part by inducing a region of NSR in the steady-state I-V relationship (Chan-
dler and Trueblood 1995). The ionic basis for the inward current underlying the NSR and the potential for expression of bistable membrane properties under these conditions has not been examined.

Therefore this study was initiated to investigate the expression of bistable membrane behaviors in the presence of 5-HT and to determine the inward currents underlying these behaviors.

METHODS

The preparation of brain stem slices for intracellular recording in TMNs has been detailed in Chandler et al. (1994). Forty-two guinea pigs (150–250 g) were anesthetized with ketamine hydrochloride (150 mg/kg). Brains were dissected in cold modified artificial cerebrospinal fluid (M-ACSF; described below). Isolated brain stems were sliced into 450-μm coronal sections containing the trigeminal motor nucleus. Incubation of slices proceeded for 2 h during which time the tissue was transferred to normal artificial cerebrospinal fluid (ACSF).

ACSF contained (in mM) 130 NaCl, 3 KCl, 2.4 CaCl2, 1.3 MgSO4, 20 NaHCO3, 1.25 KH2PO4, and 10 d-glucose. M-ACSF contained 247 sucrose, 5 KCl, 0.2 CaCl2, 4 MgSO4, 20 NaHCO3, 1.25 KH2PO4, and 10 d-glucose. These solutions were bubbled with 95% O2-5% CO2 to maintain pH 7.35–7.4. In some experiments Ca2+ concentration was lowered to 0.4 mM and substituted isosmotically with Mn2+. Two or 3 mM Cs+ and 300 μM Ni2+ were added directly to the bath in some experiments. Drugs were bath applied at the following concentrations: 10 μM 5-HT (Sigma Chemical, St. Louis, MO), 5–10 μM nifedipine (RBI, Noytlick, MA), 10 μM Bay K 8644 (RBI), 10 μM veratridine (RBI), 0.5 μM tetrodotoxin (TTX; Sigma), and 0.2 μM apamin (Sigma).

Electrical recordings were obtained from TMNs in slices secured to a gas interface chamber held at 33–34°C, perfused by 95% O2-5% CO2 and ACSF (2 ml/min). Intracellular microelectrodes were fabricated from thin-walled glass capillary tubes (Sutter Instruments, San Francisco, CA) and filled with 3 M KCl (15–20 MΩ). Electrode tips were coated with 734 RTV sealant (Dow Corning, Midland, MI) to reduce capacitance and improve sampling rate. Experiments were performed with the use of an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA) in bridge, discontinuous current-clamp (DCC), or single-electrode voltage-clamp mode (SEVC). During DCC or SEVC mode, a sampling rate of 5–10 kHz was employed (30% duty cycle). Headstage voltage was monitored to ensure proper capacity adjustment and response settling. Voltage-clamp data were analyzed cautiously due to inherent limitations of SEVC and space clamp of large motoneurons.

Trigeminal motoneurons were identified by the criteria of Chandler et al. (1994). Viability of cells was confirmed by resting potentials less than −50 mV, input resistance of 6–18 MΩ, and action-potential amplitudes >60 mV. The effects of bath applied drugs were recognized in TMNs after ~3.5 min. A complete solution change generally required 20 min. No data were collected before this period had elapsed.

Data analysis employed pCLAMP software (Axon Instruments), Datapac III (Run Technologies, Irvine, CA), Sigmaplot 4.0 (Jandel Scientific, San Rafael, CA), and Excel (Microsoft, Redmond, WA).

RESULTS

Ionic currents underlying the region of negative slope resistance

5-HT has multiple effects on TMN membrane properties (Hsiao et al. 1997; Trueblood et al. 1996), including generation of a region of NSR in the steady-state I-V relationship (Chandler and Trueblood 1995) (Fig. 1A, ○ and ● – ●). To investigate the ionic basis for the NSR induced by 5-HT, voltage-clamp experiments were performed under different pharmacological conditions. Slow voltage ramps (6 mV/s) were applied to assess the steady-state I-V relationship. The NSR induced by 5-HT in the range from −52 to −42 mV (Fig. 1A, ○) was blocked by the specific L-type Ca2+ channel antagonist nifedipine in all cells tested (Fig. 1A, ●), suggesting the participation of L channels (n = 4). Low Ca2+ solution also eliminated the region of NSR (n = 4; not shown). Under nifedipine conditions, although the region of NSR was eliminated (note the extrapolated dashed line), a region of inward rectification was nevertheless prominent in an overlapping voltage range of the I-V relationship (−52 to −42 mV, Fig. 1A, ●) suggesting the presence of an additional inactivation-resistant inward current. To determine whether this was due to persistent Na+ current, we applied TTX in the presence of nifedipine. TTX linearized the I-V relationship (Fig. 1A, △; n = 4), suggesting the presence of a persistent Na+ current previously identified in TMNs by Chandler et al. (1994).
To investigate whether a persistent Na$^+$ current could generate a region of NSR independently if its contribution were enhanced, the following experiment was performed. In another cell, the NSR induced by 5-HT was blocked by low Ca$^{2+}$ solution (Fig. 1B, ○). Inward rectification was still present beginning near −55 mV. Veratridine was then added to enhance Na$^+$ currents (Leibowitz et al. 1986; Sutro 1986; Tian et al. 1995). Under these conditions the NSR was restored (Fig. 1B, □; n = 4). These data demonstrate that, although it was the L-type Ca$^{2+}$ channels that were normally responsible for generating the region of NSR under 5-HT conditions, the persistent Na$^+$ current was available in an overlapping voltage range and could be enhanced to generate a region of NSR of its own accord.

Bistable membrane behaviors

The presence of the NSR during 5-HT application, and in the presence of veratridine following Ca$^{2+}$ channel blockade, predicts that TMNs possess the potential for bistability. Therefore, under either of these pharmacological conditions, TMNs should be able to express plateau potentials and conditional bursting oscillations. These predictions were tested in specific current-clamp experiments (Figs. 2–5).

During 5-HT application, brief depolarizing stimuli elicited sustained periods of action-potential discharge that greatly outlasted the stimuli (n = 11), suggesting the presence of underlying plateau potentials (Fig. 2A, top). The underlying plateau potential was identified in the presence of TTX in other experiments: a 1-nA step current elicited a rising change in membrane potential culminating in a plateau response that outlasted the stimulus (Fig. 2B, dark trace). Because nifedipine blocked the 5-HT–induced NSR (Fig. 1A), it was reapplied during current-clamp experiments to test whether L-type Ca$^{2+}$ channels were involved in forming plateau potentials. In all cells tested, nifedipine application blocked the expression of plateau responses with superimposed spikes (Fig. 2A, bottom; n = 3) or plateau potentials in the presence of TTX (Fig. 2B, light trace; n = 4), demonstrating a role for L-type channels. Perfusion in low Ca$^{2+}$ solution also blocked the expression of plateau properties (not shown; n = 2).

Plateau responses could also be evoked after the termination of a hyperpolarizing current stimulus (anode break; Fig. 3, A and E; n = 11). Two intrinsic currents could be responsible for generating the rebound spike responses following the release from hyperpolarization sufficient to elicit a plateau potential: the mixed cationic, inwardly rectifying current $i_h$ or the low-threshold T-type Ca$^{2+}$ current $i_T$. To test the role of $i_h$, Cs$^+$ was applied [which blocks $i_h$ in TMNs (Chandler et al. 1994)] and the protocol repeated. The typical ‘‘sag’’ response due to recruitment of $i_h$ was prevented during the period of hyperpolarization, but the anode-break plateau response persisted (Fig. 3, B and F; n = 8). To evaluate the role of $i_T$, Ni$^{2+}$ was applied at concentrations previously shown in some cells to specifically block $i_T$ (Crunelli et al. 1989; Hess et al. 1986; Russo and Hougsaard 1996). Figure 3C shows that Ni$^{2+}$ completely eliminated the rebound response following anode break in TMNs (n = 3). However, bistability was not prevented by Cs$^+$ + Ni$^{2+}$ application because a transient depolarizing stimulus was nevertheless able to evoke a plateau potential (Fig. 3D).

To confirm that nifedipine-sensitive Ca$^{2+}$ channels were responsible for maintaining the plateau potential and that T-type Ca$^{2+}$ channels served only to generate a rebound spike-like response that initiated the plateau potential, the experiment of Fig. 3 (A–D) was repeated, and nifedipine was substituted for Ni$^{2+}$ (Fig. 3, E–H; n = 4). In response to a hyperpolarizing stimulus in the presence of 5-HT, a rebound response of several spikes was observed under Cs$^+$ + nifedipine conditions, but the plateau was eliminated (Fig. 3G). The rebound response in Cs$^+$ + nifedipine conditions represents the contribution of T-type Ca$^{2+}$ channels. To confirm that plateau potentials were blocked by nifedipine in this cell, a long-duration depolarizing stimulus was delivered, which failed to elicit a plateau response (Fig. 3H).
**Conditional bursting behavior**

The 5-HT–induced NSR predicts that TMNs should exhibit burst oscillations under certain theoretical conditions (Bertram et al. 1995; Hindmarsh and Rose 1984). In addition to plateau potentials evoked by depolarizing and hyperpolarizing stimuli, TMNs exhibited bursting activity after 5-HT application, when the membrane potential was adjusted to between −61 and −50 mV by DC bias. These responses, therefore, represent conditional bursting (Fig. 4; n = 37). When the membrane potential was adjusted to more hyperpolarized levels (−70 mV), no rhythmic synaptic activity was revealed, and cells remained quiescent (not shown), suggesting that the bursting activity at more positive levels could be attributed to intrinsic membrane properties. Therefore, the ionic basis of this rhythmical conditional bursting was...
FIG. 4. Electrophysiological and pharmacological properties of conditional bursting induced by 5-HT (10 μM). A: spontaneous bursting in 5-HT (left) was blocked by 10 μM nifedipine (right). Adjustment of DC bias (∇) failed to elicit plateau potentials or autonomous bursting activity. Action potentials have been truncated to expand membrane potential trajectory near threshold. Time and voltage calibrations apply to both traces. Holding current was +0.3 and +0.4 nA in control and nifedipine, respectively. B: application of Bay K 8644 (10 μM) reversibly enhanced burst duration (top traces). Time and voltage calibrations shown in wash out (top) apply to all top traces. Bottom traces are amplified to show burst onset and termination. Bay K 8644 induced a depolarizing afterpotential (DAP) at burst termination (bottom middle). Voltage calibration shown in wash out (bottom) applies to all bottom traces. The amplified traces of burst onset and termination have separate time calibrations as shown. Holding current in control was +0.3 nA Bay K 8644, and wash out conditions were at 0 nA.

was investigated. The role of L-type Ca\(^{2+}\) current was examined with the use of specific antagonists and agonists. The L-channel antagonist nifedipine (5–10 μM) blocked bursting (Fig. 4A; n = 6). To confirm that bistability was eliminated by nifedipine, the membrane potential was manually depolarized with bias current, but bursts could not be evoked (Fig. 4A, right, ∇). Perfusion in low Ca\(^{2+}\) solution similarly eliminated 5-HT–induced bursting (Fig. 5, A and B; n = 5). In addition, the application of the L-channel agonist Bay K 8644 reversibly enhanced both the duration of individual bursts (Fig. 4B, top) and the magnitude of the depolarizing afterpotential (DAP) following burst termination (Fig. 4B, bottom; n = 4). During Bay K 8644 application, bias current readjustment was required to obtain bursts. Both the enhancement of burst duration and of the DAP would be expected as a result of Bay K 8644 prolongation of L-type Ca\(^{2+}\) current deactivation kinetics (Bargas et al. 1994; Hess et al. 1986).

The restoration of NSR by veratridine application (Fig. 1B) predicts that the persistent Na\(^{+}\) current could generate bistable behavior independently if its contribution were enhanced, and could potentially generate conditional bursting under certain conditions (Bertram et al. 1995; Hindmarsh and Rose 1984). To test this, 5-HT–induced bursting was first blocked by perfusion in low Ca\(^{2+}\) solution (Fig. 5, A and B). Under these conditions, dramatically shortened action-potential volleys could be elicited by adjustment of bias current (Fig. 5B). However, maintained bursts were precluded. These data suggest that the TMN was no longer bistable under low Ca\(^{2+}\) conditions, corresponding to the elimination of NSR under these conditions (Fig. 1B, ○). The TMN illustrated in Fig. 5 generated the I-V curves displayed in Fig. 1B. Veratridine was subsequently applied to enhance Na\(^{+}\) currents and restore the NSR. This reinitiated bursting in all cells tested in low Ca\(^{2+}\) solution (Fig. 5C; n = 2) and nifedipine conditions (5 μM, n = 4; not shown). These data indicate that restoration of bursting activity by veratridine is correlated with restoration of the NSR in the steady-state I-V relationship. Interestingly, veratridine application could not induce bursting activity in the absence of...
5-HT \((n = 2)\). This suggested that several effects of 5-HT on TMN intrinsic conductances (Hsiao et al. 1997) were involved in generating bistability, in addition to enhancement of inward current.

During 5-HT application, persistent Na\(^+\) and L-type Ca\(^{2+}\) currents exhibit overlapping voltage dependence in the steady-state \(I-V\) relationship (Fig. 1A). Therefore both currents must contribute to the generation of plateau potentials and bursting oscillations in this voltage range under 5-HT conditions. To test whether TTX-sensitive Na\(^+\) currents (including persistent and transient Na\(^+\) currents) were essential for the expression of conditional bursting, TTX was applied to TMNs exhibiting bursting in the presence of 5-HT (Fig. 6A). Oscillating plateau potentials continued in seven of nine TMNs after TTX application (Fig. 6B), which were subsequently blocked by nifedipine in all seven cells (Fig. 6C). The other two TMNs exhibited no rhythmic activity after TTX application (not shown). These data suggest that, although a persistent Na\(^+\) current contributes to bursting activity by virtue of its voltage dependence, in the majority of TMNs the L-type Ca\(^{2+}\) current is the major contributor to 5-HT-induced burst oscillations and can produce bistable behavior independently.

**Effects of 5-HT**

Bistable membrane behaviors induced by 5-HT can be caused by enhancement of Ca\(^{2+}\) current, a reduction in Ca\(^{2+}\)-dependent K\(^+\) current, or a combination of both factors (Booth et al. 1997; Hounsgaard and Kiehn 1989; Hounsgaard and Mintz 1988). In addition, the depression of leakage K\(^+\) current may also facilitate the expression of bistability by helping to uncover, and negatively shift, a region of NSR in the steady-state \(I-V\) relationship. We previously showed in TMNs that 5-HT reversibly depresses Ca\(^{2+}\)-dependent and leakage K\(^+\) currents (Hsiao et al. 1997). To test whether these effects alone could induce bistable membrane behaviors, we coapplied apamin (0.2 \(\mu\)M) to suppress Ca\(^{2+}\)-dependent K\(^+\) currents and Cs\(^+\) (3 mM) to decrease leakage currents. The effectiveness of apamin at 0.2 \(\mu\)M was monitored by high-resolution recordings of the medium-duration afterhyperpolarization (mAHP) in TMNs (Chandler et al. 1994). Coapplication of apamin and Cs\(^+\) failed to elicit sustained, rhythmic bistable membrane behaviors in four of five cells tested, although spike trains could be evoked in response to depolarizing current injection (Fig. 7A). Similar data were obtained in response to apamin applied alone (not shown, \(n = 2\)). However, in one cell, apamin + Cs\(^+\) elicited conditional bursting activity (Fig. 7C), but the spiking activity was erratic (Fig. 7D), and the bursts were less robust than normally expressed in the presence of 5-HT. These data suggest that selective reduction of Ca\(^{2+}\)-dependent and leakage K\(^+\) currents does not induce the robust bistable membrane behaviors normally exhibited in the presence of 5-HT and that 5-HT may have additionally enhanced L-type Ca\(^{2+}\) currents in TMNs.

**DISCUSSION**

We show that TMNs of mature guinea pigs express bistable membrane behaviors in the presence of 5-HT. The inward
currents underlying the voltage plateaus include a nifedipine-sensitive L-type Ca\(^{2+}\) current and a TTX-sensitive persistent Na\(^+\) current. We address three questions regarding the effects of 5-HT. 1) How does 5-HT affect TMN membrane properties to induce bistability? 2) What are the relative contributions of inward Ca\(^{2+}\) and sustained Na\(^+\) currents, and how do they interact to produce these membrane behaviors? 3) What is the physiological significance of these properties for TMNs?

Effects of 5-HT

The presence of 5-HT was essential for the expression of bistable properties in TMNs. Previously, we showed that 5-HT has multiple effects on TMNs including the following:

1) reduction of a leakage K\(^+\) current, 2) reduction in a Ca\(^{2+}\)-dependent K\(^+\) current underlying mAHP, 3) enhancement of an inwardly rectifying mixed cationic current \(I_h\), 4) enhancement of excitatory amino acid receptor-mediated currents, and 5) induction of a voltage-independent Na\(^+\) current (distinct from the persistent Na\(^+\) current described here) (Hsiao et al. 1997; Trueblood et al. 1996). Chandler and Trueblood (1995) showed that 5-HT induced a region of NSR in the steady-state \(I-V\) relationship of TMNs. Here we add that the ionic current underlying the NSR is primarily a nifedipine-sensitive L-type Ca\(^{2+}\) current with a contribution from a persistent Na\(^+\) current.

Bistability can occur when the \(I-V\) relationship possesses a region of NSR and crosses the zero-current axis three times: two crossings with positive slope (stable) and one

![FIG. 6. Burst oscillations continue after blockade of Na\(^+\) channels in 5-HT (10 \(\mu\)M). A: autonomous burst oscillations. B: TTX application to block Na\(^+\) currents revealed oscillating plateau potentials. Time and voltage calibrations in B apply to all traces (A–C). Membrane potential (−54 mV) is shown as dotted lines in A and B. Although burst frequency was altered by TTX, burst duration and threshold voltage were unchanged. C: oscillating plateau potentials in TTX conditions were blocked by nifedipine (10 \(\mu\)M). Holding current in A–C was +0.2, +0.3, and +0.4 nA, respectively.]

![FIG. 7. Effects of 0.2 \(\mu\)M apamin and 3 mM Cs\(^+\) on TMN membrane properties in the absence of 5-HT. A: apamin + Cs\(^+\) conditions does not induce bistability. Accommodating spike trains were observed in response to depolarizing current injection. Voltage calibration in A applies to A, C, and D. Current and time calibrations apply to A only. B, same cell as A, action potentials elicited by rheobase current pulses under control and apamin + Cs\(^+\) conditions show that apamin blocks the medium-duration afterhyperpolarization. Time and voltage calibration are shown. C: in another cell apamin + Cs\(^+\) application causes conditional bursting. Time and current calibrations are shown. D: the time scale of the middle burst in C is enlarged to illustrate the erratic spiking during the burst.]

Control

Apamin + Cs\(^+\)

Apamin + Cs\(^+\) + Nifedipine
with negative slope (unstable). In some cases a neuron at rest could possess a region of NSR that lies in the area of net outward current endowing the cell with one stable state, its resting potential. Therefore, to produce intrinsic bistability, the membrane must also support voltage- and time-dependent processes that can negatively shift the I-V relationship so that, in the region of NSR, the zero-current axis is crossed three times. In this case, transient stimuli can move the membrane potential from the resting stable state to the depolarized stable state of the plateau.

Bursting behavior is possible when either intrinsic factors or bias current shift the NSR to lie in the region of net inward current. In this case, the I-V curve intersects the zero-current axis once with positive slope at potentials more positive than spike threshold, enabling a cell to initiate the plateau phase without need of a stimulus.

Given the above requirements, bistability in TMNs is most likely related to several concurrent effects of 5-HT. The reduction in a Ca²⁺-dependent K⁺ current by 5-HT could account for some of the inward current underlying bistability, similar to turtle motoneurons (Hounsgaard and Kiehn 1989). However, the selective reduction in Ca²⁺-dependent K⁺ current alone (n = 2), or the reduction of this current in combination with decreased leakage current (n = 4), did not induce the robust bistability normally observed in TMNs in the presence of 5-HT. Therefore we propose that a direct enhancement of L-type Ca²⁺ channels by 5-HT underlies the sustained conditional bursting and plateau potentials. Additionally, the reduction in leakage K⁺ current and induction of a voltage-independent Na⁺ current can negatively shift the I-V curve and are therefore able to position the region of NSR as required for either plateau potentials or bursting. Which membrane behavior is expressed depends on the extent to which the I-V relationship is shifted.

The veratridine experiments further support the idea that the 5-HT—induced bistability in TMNs depends on both creation of a region of NSR (the potential for bistability) as well as appropriate positioning of the NSR with respect to the zero-current axis. These two effects of 5-HT were uncoupled using 5-HT primarily to reduce leakage K⁺ current and induce voltage-independent Na⁺ current in low Ca²⁺ solutions, and veratridine to selectively enhance inward current (persistent Na⁺ current in this case). Veratridine induced bursting only in the presence of 5-HT, showing that the augmentation of inward current is effective only when coupled to the effects of 5-HT, which shift the position of the I-V curve.

**Interaction of Ca²⁺ (L and T type) and persistent Na⁺ currents**

Anode break plateau potentials were initiated by low-threshold Ca²⁺ spikelike responses. The Ni²⁺-sensitive T-type Ca²⁺ channels involved in these responses deactivate at hyperpolarized potentials and subsequently activate on repolarization near resting levels, Typically producing the low-threshold Ca²⁺ spikes (LTS) as reported in many systems (Crunelli et al. 1989; Jahnsen and Llinás 1984; Llinás and Yarom 1981; Morisset and Nagy 1996; Russo and Hounsgaard 1996; Viana et al. 1993). A typical LTS was not observed in TMNs under 5-HT conditions because plateau potentials invariably resulted on release from hyperpolarization. However, nifedipine application did reveal the presence of a low-threshold Ca²⁺ spikelike response by preventing the longer-lasting nifedipine-sensitive plateau (Fig. 3G).

The maintenance of plateau potentials does not depend on activation of T-type Ca²⁺ channels because under Ni²⁺ conditions the plateaux evoked by depolarizing stimuli were unaffected (Fig. 3D). Furthermore, at potentials near −60 mV (when bursting occurs) the T current is normally inactivated.

Both L-type Ca²⁺ currents and persistent Na⁺ currents contribute to plateau potentials and conditional bursting activity by virtue of their voltage dependence. However, because bistable membrane behavior continues in TTX conditions in the majority of TMNs (Figs. 2B and 6), the persistent Na⁺ current is of lesser importance for inducing bistability. Nevertheless, in some TMNs (n = 2 of 9), TTX application eliminated bistable membrane behavior (in contrast to Fig. 6), suggesting that the contribution of persistent Na⁺ current was essential in these cells and its elimination precluded bistability.

Divalent ion substitution was employed in some experiments (Figs. 1 and 5), and this has been shown to modify the voltage dependence of the persistent Na⁺ current and induce bistable behavior (Li and Hatton 1996). This type of effect in TMNs cannot account for veratridine-induced restoration of bistable properties because of the following. 1) Voltage-dependent inward rectification in the steady-state I-V curve due to persistent Na⁺ current was not affected by perfusion in low Ca²⁺ solution (Fig. 1). 2) Perfusion in low Ca²⁺ solution alone did not restore bursting; veratridine application was necessary. 3) Bursting activity was also restored by veratridine following nifedipine block in other experiments (which does not employ divalent substitution).

Persistent Na⁺ currents mediate bistability in several cell types associated with motor control including respiratory rhythm-related neurons of the neonatal rat preBötzinger Complex (Butera et al. 1997; Johnson et al. 1994), swallowing-related motoneurons of neonatal mouse ambiguous nucleus (Rekling and Feldman 1997), and guinea pig prepositus hypoglossal motoneurons (after pharmacological manipulation) (Rekling and Mosfeldt Laursen 1989).

Bistable properties mediated by both persistent Na⁺ and high-threshold Ca²⁺ currents were reported in guinea pig cerebellar Purkinje cells (Llinás and Sugimori 1980). Similar to TMNs, under specific pharmacological conditions, each Purkinje cell current (Na⁺ or Ca²⁺) could generate bistable behaviors independently; whereas normally the currents produce these bistable membrane behaviors in conjunction.

**Physiological significance**

Rhythmic oral-motor behaviors are undoubtedly produced by central pattern generating circuits of the brain stem that create spatiotemporally appropriate synaptic drive to TMNs (Goldberg and Chandler 1990; Lund 1991; Nakamura and Katakura 1995). However, this by no means suggests that TMNs to a role as passive follower neurons (Chandler et al. 1994; Chandler and Trueblood 1995; Curtis and Appenteng 1993; Kobayashi et al. 1997). 5-HT potentially regulates TMN
activity during oral-motor activity because endogenously active serotonergic neurons of the brain stem raphe nuclei are associated with oral-motor behaviors (Fornal et al. 1996; Veasey et al. 1995) and TMN activity is enhanced by 5-HT during jaw movements (Katakura and Chandler 1990; Kurasawa et al. 1990; Ribeiro-do-Valle et al. 1991). A cellular mechanism for this enhancement should include the induction of bistable properties.

The interaction of T- and L-type Ca\(^{2+}\) currents may be especially important for shaping the discharge properties of jaw closer TMNs during rhythmic masticatory activity. Closer motoneurons are synthetically inhibited during jaw opening (Goldberg and Chandler 1990; Nakamura and Kudo 1978). The motoneurons are then disinhibited in the transition to the closing phase, which could facilitate postinhibitory rebound-induced plateau potentials. Therefore an intrinsic mechanism could contribute to the burst discharge of TMNs during the jaw closing phase of mastication without requiring sustained excitatory premotoneuronal drive. The importance of postinhibitory rebound for initiation and timing of rhythmic motor activity has been demonstrated in molluscan motor systems for feeding (Siegler et al. 1974) and swimming (Marder and Calabrese 1996; Panchin et al. 1995) as well as neonatal rat lumbar locomotion (Bertrand and Cazalets 1998).

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REFERENCES


