Voltage-Dependent Calcium Currents in Trigeminal Motoneurons of Early Postnatal Rats: Modulation by 5-HT Receptors

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Hsiao, Chie-Fang, Nanping Wu, and Scott H. Chandler. Voltage-dependent calcium currents in trigeminal motoneurons of early postnatal rats: modulation by 5-HT receptors. J Neurophysiol 94: 2063–2072, 2005. First published June 22, 2005; doi:10.1152/jn.00178.2005. Trigeminal motoneurons relay the final output signals generated within the oral-motor pattern generating circuit(s) to muscles for execution of various motor patterns. In recent years, these motoneurons were shown to possess voltage dependent nonlinear membrane properties that allow them to actively participate in sculpting their final output. A complete understanding of the factors controlling trigeminal motoneuronal (TMN) discharge during oral-motor activity requires, at a minimum, a detailed understanding of the palette of ion channels responsible for membrane excitability and a determination of whether these ion channels are targets for modulation. Toward that end, we studied in detail the properties of calcium channels in TMNs and their susceptibility to modulation by 5-HT in rat brain slices. We found that based on pharmacological and voltage-dependent properties, high-voltage-activated (HVA) N-type [α-conotoxin GVIA (α-CgTX)]-sensitive, and to a lesser extent P/Q-type [α-agatoxin IVA (α-Aga IVA)]-sensitive, calcium channels make up the majority of the whole cell calcium current. 5-HT (5.0 μM) decreased HVA current amplitude in TMNs. HVA calcium current inhibition was mimicked by 1-CT, a 5-HT1A receptor agonist, and by R(+)-8-hydroxydipropylaminitetralin hydrobromide (8-OH-DPAT), a specific 5-HT1A agonist. The effects of 5-HT were blocked by the 5-HT1A antagonist 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazinotetralin hydrobromide (8-OH-DPAT), a specific 5-HT1A agonist. The effects of 5-HT were blocked by the 5-HT1A antagonist 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazinotetralin hydrobromide (8-OH-DPAT), a specific 5-HT1A agonist. Under current clamp, α-CgTX and 5-HT were most effective in suppressing the mAHP and both increased the spike frequency and input/output gain in response to current injection. Calcium current modulation by 5-HT1A receptors likely is an important mechanism to fine tune the input/output gain of TMNs in response to small incoming synaptic inputs and accounts for some of the previously reported effects of 5-HT on TMN excitability during tonic and burst activity during oral-motor behavior.

INTRODUCTION

Oral-motor activity underlies ingestion of food, swallowing, speech, and respiration, among other behaviors, and abnormalities in this system occurring at birth or over time can produce mild to severe (debilitating) disorders (De Laat 1998). Compared with what is known for respiratory and locomotor systems, our understanding of the cellular/molecular properties of the neurons within oral-motor circuits is lagging. A complete understanding of how these neurons function within pattern generating circuits to produce the appropriate discharge patterns during varied behaviors requires, at a minimum, characterization of the ion channels and their potential for modulation by synaptic inputs (Grillner 1999; Kiehn and Butt 2003; Kiehn et al. 2000). The ion channels underlying oral-motor activity in trigeminal neurons are now being characterized (Del Negro et al. 1999; Hsiao et al. 1997; Kang et al. 2004; Kobayashi et al. 1997; Oh et al. 2003). A number of lines of study indicate that serotonergic systems are important in control of trigeminal motoneuronal (TMN) output. The trigeminal motor nucleus receives a dense serotonergic input (Kolta et al. 1993; Saha et al. 1991) and contains serotonergic receptors (Kolta et al. 1993). Importantly, some serotonergic raphe cells increase their discharge specifically during oral-motor activity (Fornal et al. 1996; Veasey et al. 1995), and intact serotonergic systems are necessary for sucking behavior in neonatal rats (Ristine and Spear 1984). Furthermore, in the guinea pig, iontophoretic application of serotonin onto individual motoneurons exhibiting either I) rhythmic burst discharge during cortically induced rhythmic jaw movements (RJMs) (Katakura and Chandler 1990) or 2) during glutamate evoke discharge (Katakura and Chandler 1990; Kurasawa et al. 1990) potently facilitates their discharge over many minutes. At the cellular level, previously we showed in guinea pig trigeminal motoneurons that 5-HT facilitates plateau potential generation and induces rhythmic bursting that is critically dependent on L-type calcium channel activity (Hsiao et al. 1998). Moreover, 5-HT increases TMN excitability through actions on a number of intrinsic ionic conductances, such as Ih and Ihk (Hsiao et al. 1997), and reduces the mAHPr after an action potential in these neurons (Hsiao et al. 1997; Inoue et al. 1999). Considering the important role of the medium duration after hyper polarization (mAHPr) in spike discharge and calcium in the production of the mAHPr (Chandler et al. 1994; Hsiao et al. 1997; Inoue et al. 1999), it is of interest to determine the effects of 5-HT on calcium channel currents in trigeminal motoneurons. Presently, this has been examined in only one study that, in contrast to this one, used dissociated TMN where dendrites are in most instances absent (Oh et al. 2003). In that study, the high-voltage-activated (HVA) calcium channel components were established, and the effect of 5-HT on HVA channels was examined. Although informative, that study was performed on a subpopulation of dissociated jaw closer TMNs and the underlying 5-HT receptor subtype mediating those effects was not determined nor were the effects of 5-HT examined on the mAHPr or correlated with alterations in spike discharge.

Calcium channels play an important role in regulating many neuronal functions, including electrical excitability, transmitter...
release, and contraction (Anwyl 1991; Hille 1994). In trigeminal motoneurons, calcium influx is required for the mAHP and subsequent spike discharge (Chandler et al. 1994; Kobayashi et al. 1997). Currents through these channels have been classified into low-voltage-activated (LVA) and HVA components (Carbon and Swandulla 1989; Umemiya and Berger 1994), including an \( \omega \)-conotoxin GVIA (\( \omega \)-CgTX)-sensitive N-type component, \( \omega \)-agatoxin IVA (\( \omega \)-AgA IVA)-sensitive P/Q-type component, 1,4-dihydropyridine (DHP)-sensitive L-type component, and a residual component (R-type) for its resistance to \( \omega \)-CgTX, \( \omega \)-AgA IVA, and DHPs (Dunlap et al. 1995). Although the identities of calcium channels have been established, either directly or indirectly, based on some combination of voltage, time, pharmacology, and molecular profiles in many types of neurons (reviewed in Catterall 1995; Trimmer and Rhodes 2004), including motoneurons (Bayliss et al. 1995; Berger and Takahashi 1990; Miles et al. 2004; Plant et al. 1998; Umemiya and Berger 1994), for trigeminal motoneurons there are far fewer studies (Chandler et al. 1994; Hsiao et al. 1998; Inoue et al. 1999; Kohike et al. 1994), but the effects can be varied depending on neuron type and species examined. In guinea pig trigeminal motoneurons, in vitro, 5-HT produces a negative slope conductance (NSC) in and species examined. In guinea pig trigeminal motoneurons, 5-HT by itself does not induce a NSC in the steady-state I-V relationship that is mediated by L-type calcium channel activation and forms the basis for 5-HT rhythmic bursting (Hsiao et al. 1998). Concomitantly, 5-HT suppresses the mAHP and increases the frequency of spike discharge most likely through suppression of calcium channels (Hsiao et al. 1997; Inoue et al. 1999; Kohike et al. 1994), although direct effects on calcium-dependent potassium channels cannot be excluded (Andrade and Nicoll 1987). However, in rat trigeminal motoneurons, 5-HT by itself does not induce a NSC in the steady-state I-V or induce burst discharge (Hsiao et al. 2002). Moreover, a previous study on hypoglossal motoneurons reported that 5-HT suppressed HVA current without producing effects on LVA calcium currents (Bayliss et al. 1995) and also showed that the mAHP is mediated by P- and N-type calcium channels. However, in postnatal spinal motoneurons, 5-HT enhanced LVA currents without effecting HVA currents (Berger and Takahashi 1990). Clearly, one cannot assume an understanding of the role of calcium channel subunits and their potential for modulation based on knowledge of the presence or absence of these channels and the effects of neuromessengers on those channels in a “typical” spinal or brain stem motoneuron.

Therefore to more precisely understand the role that calcium currents, and in particular the different calcium channel subtypes, play in control of rat trigeminal motoneuronal subthreshold and suprathreshold excitability, we extended the previous study on calcium channels and their modulation by 5-HT (Oh et al. 2003). We show that HVA N- and P/Q-type currents, as opposed to LVA calcium currents, are suppressed by 5-HT through activation of 5-HT\(_1A\) receptor subtype, and this is paralleled by a decrease in mAHP and an increase in repetitive discharge behavior. Contrary to that previously reported (Oh et al. 2003), we found that calcium currents recorded from all TMNs sampled within the nucleus were suppressed by 5-HT. These observations could explain, partly, the previously described facilitation of trigeminal motoneuronal discharge by 5-HT in vivo (Katakura and Chandler 1990) and in vitro (Hsiao et al. 1997).

**METHODS**

**Preparation for whole cell recording**

Whole cell patch-clamp experiments were performed on transverse slices of a neonatal rat brain stem (1–4 days). Rats were anesthetized by halothane inhalation (Halocarbon Laboratories, River Edge, NJ). The brain was removed and placed in oxygenated ice-cold cutting solution. Coronal sections (250 \( \mu \)m) through the trigeminal motor nucleus were obtained and transferred into a holding chamber in incubation solution at 37°C for 40 min (see Hsiao et al. 2002).

**Solutions**

Solutions were bubbled with 95% \( \text{O}_2 \)-5% \( \text{CO}_2 \) and maintained at pH 7.25–7.35 (22–24°C). Cutting solution was composed of the following (in mM): 126 NaCl, 3 KCl, 1.25 Na\(_2\)H\(_4\)PO\(_4\), 26 NaHCO\(_3\), 10 glucose, 1 CaCl\(_2\), 5 MgCl\(_2\), and 4 lactic acid. The recording solution consisted of the following (in mM): 124 NaCl, 3 KCl, 1.25 Na\(_2\)H\(_4\)PO\(_4\), 26 NaHCO\(_3\), 10 glucose, 2 CaCl\(_2\), and 2 MgCl\(_2\). The incubation solution was identical to the recording solution except for the addition of 4 mM lactic acid. The external solution used for recording barium currents through calcium channels contained (in mM) 121 NaCl, 3 KCl, 10 HEPES (base), 10 glucose, 1 BaCl\(_2\), 2 MgCl\(_2\), 30 TEA-Cl, and 0.001 TTX. Normal internal solution contained (in mM) 115 K-glucuronate, 9 NaCl, 25 KCl, 1 MgCl\(_2\), 10 Heps buffer, 0.2 EGTA, 3 K\(_2\)-ATP, and 1 Na-GTP, with a pH of 7.25–7.30 and osmolarity of 280–290 mM. The internal solution used for recording calcium current contained (in mM) 100 CsMeSO\(_4\), 4 NaCl, 10 HEPES (base), 10 EGTA, 30 TEA-Cl, 0.5 CaCl\(_2\), 1 MgCl\(_2\), 3 Mg-ATP, and 1 GTP-tris salt. Lucifer yellow (0.1%, Sigma Chemical, St. Louis, MO) was added to small volumes of electrode solution for fluorescent viewing in initial experiments.

**Drug application**

Stock solutions of the calcium channel antagonists \( \omega \)-conotoxin-GVIA (\( \omega \)-CgTX: 50 \( \mu \)M; Bachem, Torrance, CA), and \( \omega \)-agatoxin-IVA (\( \omega \)-AgA IVA: 50 \( \mu \)M; Pfizer, Groton, CT) were dissolved in water and frozen. Each of the stock solutions were diluted to the appropriate concentration in the external recording solution containing 0.1% cytochrome C immediately before the experiment. Final toxin concentrations (0.5 and 0.3–0.5 \( \mu \)M for \( \omega \)-CgTX and \( \omega \)-AgA IVA, respectively) were chosen that completely blocked nontoverlapping components of calcium current (Bayliss et al. 1997; Mintz et al. 1995). This was verified in these experiments because we found that the percentage of current sensitive to each toxin was independent of the order of toxin application. As is well known from calcium channel experiments using whole cell recordings, run-down occurs variably. Therefore to assess the effects of drug on calcium currents in the face of any run-down rather than using the data before drug administration as a control, we used the “predicted” calcium current obtained from linear regression of data points before drug administration as our control value and compared that with the actual data obtained at the time of drug test measurement, as described by others (Miles et al. 2004; Plant et al. 1998).

Serpentoxin (5-HT), nimodipine, 5-carboxanidrotamine nulate (5-CT), \( R^+ \)-8-hydroxydipropylaminotetralin hydrobromide (8-OH-DPAT), 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperezine hydrobromide (NAN-190), \( R^- \)-1-(2,5-dimethoxy-4-iiodophenyl)-2-aminopropane hydro-
Whole cell recording

Slices were perfused with oxygenated recording solution (2 ml/min) while secured in a recording well mounted on a Zeiss fixed-stage Axioskop microscope equipped with bright-field, fluorescence, and Nomarski optics in combination with infrared video microscopy for enhanced resolution of individual neurons. Patch recordings were obtained with the use of an Axopatch 1D (Axon Instruments, Foster City, CA) for voltage-clamp experiments or an Axoclamp 2A (Axon Instruments) in bridge mode for current-clamp experiments in concert with pCLAMP acquisition software (Version 8.0.2, Axon Instruments) operating on a Pentium-based personal computer. Signals were digitized on-line, and filtered at 2 (voltage clamp) or 5 kHz (current clamp). Patch pipettes were fabricated from conventional thin-wall glass (1.5 mm OD, 0.86 mm ID; Warner Instrument, Hamden, CT), pulled on a Brown/Flaming P-97 micropipette puller (Sutter Instruments, Novato, CA) and had bath resistances of 3–5 MΩ. Signals were grounded by a 3 M KCl agar bridge electrode (Ag/AgCl wire) mounted in the recording well. Liquid junction potentials were measured between the pipette and bath solutions and varied between 9 (normal pipette solution) and 7 mV (modified pipette solutions) and were corrected off-line. Whole cell capacitance (C_{inw}) for each trigeminal motoneuron recorded in voltage clamp was determined from the integral of capacity current in response to 15-ms hyperpolarizing voltage commands or directly obtained by the pClamp software during the experiment. Uncompensated series resistance (R_s) was calculated from the decay time constant (τ) of the transient and was usually < 20 MΩ. Sixty percent to 80% compensation was routinely employed. Trigeminal motoneurons were identified by the criteria of Chandler et al. (1994).

Data collection began 10 min after gaining whole cell access to allow equilibration between pipette and cytosol. Calcium currents were elicited by applying voltage step commands to varying potentials, and from this, I-V relationships were obtained. The inactivation of the LVA calcium component was studied using a test pulse to −40 mV preceded by a 500-ms conditioning pulse to potentials between −120 and −40 mV. The inactivation of HVA Ca^{2+} currents were examined from a test pulse to −10 mV preceded by a 15-s conditioning pulse to potentials between −100 and 0 mV. To test effects of 5-HT, HVA currents were elicited during voltage steps to −10 from −70 mV at 21-s intervals. The peak current during each voltage step was measured under control conditions and throughout the experiment (Penington et al. 1992). To test whether current inhibition by 5-HT was relieved by strong depolarizations, we used a protocol in which a test pulse to potentials between −120 and −40 mV preceded by a 15-s conditioning depolarization to potentials between −100 mV and 0 mV was repeated. Whole cell calcium channel currents were recorded using Ba^{2+} as a charge carrier. Sodium currents were eliminated by application of TTX (0.5 μM) except during current-clamp experiments. K^{+} channel currents were minimized by the combination of external TEA and internal Cs^{+}.

General properties of calcium currents in trigeminal motoneurons

Ca^{2+} currents were activated by steps of membrane potential from a holding potential of either −80 or −50 mV to various test potentials (Fig. 1A). A fast transient component of current followed by more slowly inactivating component over the time period measured was activated from a holding potential of −80 mV, while a more sustained, slowly inactivating component was activated from a holding potentials of −50 mV. Figure 1B shows the summary I-V relationship plotted for a subset of neurons (n=11) that were initiated from the two different holding potentials and spanned the full range of voltages. When data from all neurons were examined, the maximal current in the I-V relationship evoked from −80 mV was clearly larger compared with the maximal current evoked from −50-mV holding potential (−2,631.1 ± 263.5 vs. −1,555.2 ± 195.0 pA; n=11; P<0.001).

**Fig. 1.** Calcium currents in trigeminal motoneurons. **A**: currents elicited by depolarizing steps (300 ms) from holding potentials of −80 (left) and −50 mV (right). B: summary I-V relationship for peak currents taken from holding potentials of −80 and −50 mV (n=11 neurons, mean ± SE). **C**: currents elicited by depolarizing steps (300 ms) from holding potentials of −80 and −50 to −40 mV (n=11 neurons, mean ± SE). **D**: voltage dependence of steady-state activation and inactivation for high-voltage-activated (HVA) and LVA calcium currents. Voltage dependence of activation was determined from analysis of tail currents obtained after returning from each test potential (C). Inactivation curves of HVA Ca^{2+} currents was measured using a test pulse to −10 mV from different holding potentials after a 15-s conditioning depolarization to potentials between −100 mV and 0 mV (○). Inactivation curves for LVA Ca^{2+} currents were measured using a test pulse to −40 mV after a 500-ms conditioning pulse to potentials between −120 and −40 mV (△). Current amplitude was normalized to peak current. Data were fitted with a Boltzmann relationship. In this and subsequent figures, bars indicate SE.

Data analysis

Current- and voltage-clamp data were analyzed in Clampfit 8.0 (Axon Instruments), SigmaPlot 5.0 (Jandel Scientific, San Rafael, CA), Excel (Microsoft, Redmond, WA), and StatView 5.0 (SAS Institute, Cary, NC). Values are expressed as mean ± SE. Significant differences were tested with Student’s paired and unpaired t-test or ANOVA with or without repeated measures. ANOVAs were followed by Bonferroni tests for group differences. A significance level of P<0.05 was used in all tests unless otherwise stated.

**RESULTS**

The database consists of TMNs that exhibited a resting potential greater than −50 mV, action potential amplitude >80 mV, input resistance >100 MΩ, and a series resistance <10 MΩ after compensation. Whole cell calcium channel currents were recorded using Ba^{2+} as a charge carrier. Sodium currents were eliminated by application of TTX (0.5 μM) except during current-clamp experiments. K^{+} channel currents were minimized by the combination of external TEA and internal Cs^{+}.
Figure 1C1 shows that Ca\(^{2+}\) currents elicited at −40 mV from a holding potential of −80 mV consisted of transient (inactivating) and steady-state components with a decay time constant of 37.7 ± 2.9 ms (n = 7), typical of LVA calcium currents (Plant et al. 1998; Umemiya and Berger 1994), whereas the current from a holding potential of −50 mV to the same test potential showed no transient component and a small steady-state component. Figure 1C2 shows that the Ca\(^{2+}\) current elicited at −10 mV from a holding potential of −80 mV exhibited a slowly inactivating transient component, whereas the current elicited from a holding potential of −50 mV showed very little inactivation. The time constant for inactivation from a holding potential of −80 to −10 mV was 166.7 ± 16.7 ms (n = 7) compared with 361.2 ± 33.7 ms (n = 7) obtained from a holding potential of −50 mV. This indicates that the Ca\(^{2+}\) currents elicited at −10 mV have at least two components.

To further define Ca\(^{2+}\) currents in TMNs, we studied the voltage dependence of activation and steady-state inactivation (Fig. 1D). The summary activation curve from seven neurons was constructed from the tail currents at the end of depolarizing steps to various potentials from a holding potential of −80 mV (Fig. 1D, ▲). The Ca\(^{2+}\) current was mostly HVA and began to activate at −50 mV. The data were fit to a Boltzmann function that yielded a half-activation voltage of −34.5 ± 1.0 mV (n = 7), with a slope factor (k) of 2.0 ± 0.5. To determine the voltage dependence of inactivation of HVA current, Ca\(^{2+}\) currents were activated to a test potential of −10 mV preceded by a 15-s conditioning pulse to potentials between −100 and 0 mV. The resulting inactivation curve is shown in Fig. 1D (●). Despite the long conditioning prepulses, inactivation was not complete. A fit of a Boltzmann distribution to the data gave a potential for half-inactivation of −43.8 ± 3.4 mV (n = 8) and a slope factor of 13.4 ± 1.3, very similar to that reported in facial motoneurons (Plant et al. 1998). The inactivation of the LVA component was studied using a test pulse to −40 mV that was preceded by a 500-ms conditioning pulse to potentials between −120 and −40 mV (Fig. 1D, ○). Currents were reduced at conditioning potentials more positive than −100 mV, and little further reduction of the current was observed at potentials positive to −60 mV. Half-inactivation of the LVA transient current was obtained at −76.6 ± 2.8 mV (n = 12), with a slope factor of 8.5 ± 0.6, similar to that reported in facial motoneurons (Plant et al. 1998). As shown, HVA current inactivation was essentially complete at −20 mV and was nearly fully removed at −80 mV, whereas LVA current inactivation is almost complete at −60 mV and is fully removed near −100 mV.

**Pharmacology of HVA current in TMNs**

The presence of four types of HVA (L-, N-, P/Q-, and R-type) Ca\(^{2+}\) channels has been previously shown in other motoneurons (Bayliss et al. 1997; Plant et al. 1998; Scamps et al. 1998) but in only one study in rat neonatal (p5-10) dissociated trigeminal motoneurons. Therefore to characterize the different HVA Ca\(^{2+}\) current subtypes in TMNs (p1-4) from slice preparations where dendrites are more intact, we applied specific antagonists of HVA calcium currents. HVA current was evoked at −10 from −70 mV, and the peak amplitude of the current was plotted as a function of time. Figure 2A shows that, for one cell, bath application of 0.5 μM ω-CTx-GVIA (ω-CgTX), an N-type channel antagonist, inhibited 33.7% of HVA current. Subsequent application of 0.5 μM ω-Aga IVA, a P/Q-type channel antagonist, produced a further 26.7% decrease in the current. The inhibition was maintained after termination of application of both toxins, consistent with an irreversible block of N- and P/Q-type channels by these toxins commonly observed by others (Bayliss et al. 1995; Plant et al. 1998; Umemiya and Berger 1994). After ω-CgTX and ω-Aga IVA, application of the L-type channel antagonist nimodipine (10 μM) produced an additional 18.1% inhibition of the current. Application of the HVA current antagonist Cd\(^{2+}\) (100 μM) reduced the remaining calcium current to 7% of the total, similar to that reported, previously, in facial motoneurons (Plant et al. 1998). Figure 2B is a summary from a subset of experiments in which all drugs were tested in the same cell and shows that ω-CgTX- and ω-Aga IVA-sensitive currents are the predominant HVA calcium currents in TMNs, similar to that describe previously (Oh et al. 2003).

**5-HT has differential effects on LVA and HVA currents in TMNs**

Other studies showed that the effects of 5-HT on motoneuron Ca\(^{2+}\) currents are variable. In neonatal rat hypoglossal motoneurons, 5-HT inhibited HVA current but did not affect LVA current (Bayliss et al. 1995), whereas in neonatal rat spinal motoneurons, 5-HT enhanced LVA Ca\(^{2+}\) currents, but did not appreciably affect the amplitude of the HVA current (Berger and Takahashi 1990). Furthermore, in guinea pig trigeminal motoneurons, 5-HT facilitates L-type calcium cur-
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5-HT predominately suppresses N- and P/Q-type calcium currents

To determine the sensitivity of these components to 5-HT, we isolated HVA currents using channel blockers and tested the effect of 5-HT on these current components. As shown in Fig. 4A, peak currents were measured during voltage steps from −70 to −10 mV and plotted as a function of time. 5-HT (5.0 μM) caused 33.7% inhibition of calcium channel current under control conditions. The inhibition by 5-HT on HVA currents in the presence of ω-CgTX, ω-Aga IVA/ω-CgTX, and nimodipine/ω-Aga IVA/ω-CgTX were 21.2, 12.5, and 7.7%, respectively. Therefore 37.1% of 5-HT–sensitive current in this neuron were N-type, 25.8% were P/Q-type, 14.2% were L-type, and 23.0% were R-type currents. Figure 4B shows that the mean inhibition on N-, P/Q-, L-, and R-type currents by 5-HT for all neurons examined. These data indicate that >70% of the 5-HT–sensitive HVA calcium currents were N- and P/Q-type.

Voltage dependence of calcium current inhibition by 5-HT

The inhibition of HVA Ca²⁺ currents by 5-HT was reduced by a depolarizing prepulse, a phenomenon that is characteristic of G protein–mediated modulation of voltage-dependent ion channels (Hille 1994). To study the voltage dependence of the 5-HT effect on TMNs, we used a double-pulse voltage protocol in which currents were evoked from −70 mV, before and after a strong depolarizing prepulse to +70 mV. Figure 5A shows that, under control conditions, calcium current induced by the first (prepulse) and second (postpulse) test pulses were similar in amplitude. However, in the presence of 5-HT, the current evoked during the first test pulse was reduced to a greater extent compared with that evoked by the second test pulse after the strong depolarization. The time-course for the effect of 5-HT is shown in Fig. 5B, which plots the current amplitude of both the first test pulse (○)
and the second test pulse (○) in the top plot and the ratio of the two in the bottom plot (post/pre). In six TMNs tested, 5-HT increased the ratio from 1.00 ± 0.01 to 1.17 ± 0.03 (P = 0.002). These data suggest that the inhibition of calcium currents and the slowing of activation kinetics by 5-HT were partially relieved after strong depolarizations, as shown previously (Oh et al. 2003).

Pharmacology of the 5-HT receptor mediating calcium current inhibition

Previous studies in other types of neurons showed that the inhibition of HVA calcium currents is mediated by 5-HT₁A receptors (Bayliss et al. 1995, 1997; Lin et al. 2001; Rhee et al. 1996; Williams et al. 1998). To determine the 5-HT receptor subtype that mediates calcium current inhibition in TMNs, we performed the following pharmacological experiments. For the first experiment, we determined the 5-HT receptor subtype(s) that mediate the inhibition by application of various 5-HT receptor subtype agonists. As shown in Fig. 6, A and B, HVA current inhibition was mimicked by the 5-HT₁A agonist 8-OH-DPAT (5 μM) and 5-CT (5 μM), a 5-HT₁ receptor agonist. DOI (5 μM), a 5-HT₂/₁C agonist, was without effect after the effects of calcium current run-down were accounted for (see METHODS). Figure 6C shows a summary histogram of the effects of 5-HT and the various agonists on HVA inhibition. As shown, 5-HT, 5-CT, and DPAT were similar, whereas DOI produced the smallest effect (ANOVA, F = 12.02; Bonferroni, P < 0.001 vs. 5-HT).

For the second experiment, we tested the efficacy of 5-HT on calcium current inhibition in the presence of specific 5-HT receptor antagonists. As shown in Fig. 7A, in the presence of the 5-HT₁A antagonist NAN-190 (10 μM), the inhibition of calcium current by 5-HT was progressively attenuated, and was effectively abolished after ~20 min antagonist treatment. In contrast, the inhibition by 5-HT was only modestly reduced by the 5-HT₂/₁C receptor antagonist 3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl][ethyl]-2,4(1H,3H)-quinazolinedione tartrate (ketalserin; 10 μM; Fig. 7B). Figure 7C summarizes the mean inhibition of HVA currents by 5-HT before and during bath application of NAN-190 and ketalserin. Group data indicate that pretreatment with NAN-190 differed from pretreatment...
with ketanserin (repeated measures ANOVA, \( F = 39.7, \) Bonferroni, \( P < 0.014 \)), showing that 5-HT\(_{1A}\) receptors mediate HVA calcium current inhibition.

**Effects of 5-HT and HVA calcium channel blockers on spike afterhyperpolarization**

To define the role of Ca\(^{2+}\) conductances in the production of afterpotentials, we studied the effects of various agents shown previously to block HVA Ca\(^{2+}\) currents. Action potentials were elicited by brief (3 ms) current pulses. For the TMNs examined for these experiments, 59% (17/29) exhibited a biphasic AHP pattern after an individual action potential. This consisted of a rapid spike repolarization and subsequent development of a fast, short-duration AHP (fAHP) followed by a mAHP. In these cases, an afterdepolarization (ADP) typically took the form of a depolarizing hump in the membrane potential that separated the fAHP and mAHP. In the remaining 41% of the neurons, an ADP was not observed. Figure 8 shows superimposed traces taken before and during bath application of \(\omega\)-CgTX (0.5 \(\mu\)M, \( n = 6 \)), \(\omega\)-Aga IVA (0.5 \(\mu\)M, \( n = 8 \)), nimodipine (10 \(\mu\)M, \( n = 6 \)), and 5-HT (5 \(\mu\)M, \( n = 9 \)). Table 1 summarizes the effects of the drugs on the mAHP amplitude. The most consistent and largest effect on mAHP amplitude was suppression after either \(\omega\)-CgTX (~42%; 6/6 neurons) or 5-HT (~65%; 9/9 neurons) application. Although \(\omega\)-Aga IVA produced an ~27% reduction in six of eight neurons, this was not significant (\( P > 0.05 \)). The data suggest that the mAHP is mediated, in part, by \(\omega\)-CgTX-sensitive, and to a lesser extent \(\omega\)-Aga IVA-sensitive, calcium currents that are both targets for modulation by 5-HT.

**Effect of calcium current inhibition on repetitive discharge behavior**

To study the role of P/Q- and N-type calcium channels and their modulation by 5-HT in control of TMN repetitive discharge in neonatal rats, we examined the effects of \(\omega\)-Aga IVA, \(\omega\)-CgTX, and 5-HT on discharge characteristics, and this is shown in Fig. 9, A–C. Maintained repetitive discharge was observed in most neurons tested with 1-s constant current pulses of varying intensities. As reported previously, increasing the current intensity produced a steady-state frequency-current relationship with multiple phases consisting of an initial steep rise of maximal slope (Chandler et al. 1994). The relationship between discharge frequency and current intensity (f-I relationship) is graphically represented in Fig. 9D for steady-state discharge (calculated from the mean number of spikes in the last 500 ms of the current pulse). Table 1 summarizes the main findings of toxin or 5-HT application on steady-state discharge (measured from the midpoint of the f-I relationship determined after drug application) and primary slope of the f-I relationship (Chandler et al. 1994; Hsiao et al. 1997). Consistent with the small effects on mAHP evoked by a single pulse, \(\omega\)-Aga IVA produced small changes in the steady-state frequency-current relationship mean discharge and slope (Fig. 9D; Table 1) compared with \(\omega\)-CgTX and 5-HT. These results suggest that inhibition of N-type and to a less extent P/Q-type calcium currents in TMNs, whether by application of toxin or 5-HT, caused a decrease in the spike AHP and increased the spike frequency response to current injection.
DISCUSSION

Our results indicate that, in the slice preparation, TMN HVA calcium currents are suppressed by 5-HT, similar to that shown previously in dissociated TMNs (Oh et al. 2003). However, we found that this suppression is directed to N- and to a lesser extent P/Q-type calcium current components, whereas LVA currents were not significantly altered by 5-HT. Moreover, the 5-HT induced inhibition of calcium currents is mediated by activation of 5-HT1A receptors, similar to that found in hypoglossal motoneurons (Bayliss et al. 1995) and likely accounts for at least part of the 5-HT–induced suppression of the mAHP and increased membrane excitability. In contrast to that reported previously (Oh et al. 2003), where only a small subpopulation of jaw closer TMN calcium currents were suppressed, we found that this inhibition was observed in all TMNs tested throughout the nucleus. This difference could be related to the different preparations used; in dissociated neurons, dendrites are severely comprised. Alternatively, calcium current components and 5-HT1A are developmentally regulated (Miles et al. 2004; Talley et al. 1997); expression of 5-HT1A goes down during development rendering those neurons recorded in the previous study (Oh et al. 2003) less susceptible to inhibition. In our study we recorded uniformly throughout the trigeminal motor nucleus; presumably sampling all populations of TMN. However, the previous study recorded selectively from jaw closer motoneurons and that subpopulation of TMN could be less susceptible to 5-HT inhibition.

Calcium currents in TMNs

Any computational model of trigeminal neuronal activity requires a detailed characterization of the ion channels involved (Wu et al. 2005), and one should not assume these properties from an “idealized motoneuron” even in motoneurons from similar regions of CNS. Calcium current components in various neuron types, including motoneurons, are varied and, in particular, when TMNs are compared with hypoglossal and facial motoneurons, there are similarities and some differences. LVA currents with similar properties to those shown here have been reported (Miles et al. 2004; Plant et al. 1998; Umemiya and Berger 1994). However, most of the variability is in the HVA calcium currents reported. In TMNs, HVA current activates with a threshold at approximately −50 mV and shows peak conductance near −20 mV, which is about 10 mV more negative than that reported previously in other brain stem motoneurons (Miles et al. 2004; Plant et al. 1998; Umemiya and Berger 1994). Furthermore, compared with neonatal hypoglossal motoneurons (Umemiya and Berger 1994), the \( V_{1/2 \text{max}} \) reported for activation in TMNs was −34 mV compared with −17 mV, and the slope factor was 2 versus 13, suggesting more rapid maximal activation of HVA currents in TMNs. Compared with facial motoneurons, \( V_{1/2 \text{max}} \) of −25.9 mV and a slope factor of −8.9 were reported (Plant et al. 1998).

Moreover, when HVA calcium currents are separated into N- (−33%), P/Q- (−29%), L- (−12%), and R-type (−26%) components according to their sensitivity to calcium channel antagonists, further similarities and differences are noted. For instance, in the only previous study, which used dissociated TMN, 38% were N-type, 27% were P/Q, 16% were L-type, and 19% were R-type, similar to that observed in our study. However, in hypoglossal motoneurons, one study reported 50% P/Q-, 29% N-, and 7% L-type currents (Umemiya and Berger 1994), in contrast to a study on those neurons that reported 43% P/Q- and only 11% N-type, with 0% L-type. In rat facial motoneurons, P/Q- was absent and N- and L- were 30 and 5%, respectively (Plant et al. 1998). In all three brain stem motoneuron types, L-type channels are very low and differ in the percentages of P/Q type currents. For facial motoneurons, a unique current exists that is resistant to toxin and dihydropyridine block and distinct from traditionally characterized R-type calcium channels (Plant et al. 1998). It is unlikely that these differences result from developmental changes (Miles et al. 2004) because most of the studies were done in neonates between 1 and 7 days.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>mAHP Amplitude, %</th>
<th>Frequency, %</th>
<th>Slope, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ω-CgTx</td>
<td>↓ 41.8 ± 9.9 (6/6)*</td>
<td>↑ 51.9 ± 12.5 (5/5)*</td>
<td>↑ 39.20 ± 14.89 (4/4)*</td>
</tr>
<tr>
<td>ω-Aga IVA</td>
<td>↓ 27.0 ± 10.2 (6/8)</td>
<td>↑ 16.0 ± 6.4 (6/6)</td>
<td>↑ 6.59 ± 4.06 (4/4)</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>↓ 7.0 ± 4.0 (4/6)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5-HT</td>
<td>↓ 64.6 ± 8.1 (9/9)*</td>
<td>↑ 56.3 ± 13.1 (8/8)*</td>
<td>↑ 44.24 ± 10.12 (7/7)*</td>
</tr>
</tbody>
</table>

Values are means ± SD. Numbers in parentheses indicate numbers of cells showing either an increase (↑) or decrease (↓) compared with the total numbers of cells tested. *Statistically significant at \( P < 0.05 \).
5-HT inhibition of HVA currents in TMNs

A recent study found that 5-HT suppressed calcium currents in a small population of TMNs (Oh et al. 2003). However, the calcium channel subtypes that are targets for this modulation and the 5-HT receptor subtype mediating the suppression were not examined. The results of this study show that 5-HT inhibited 31.3 ± 2.2% of TMN HVA currents in a concentration-dependent manner without producing significant effects on LVA calcium currents, similar to that previously reported for hypoglossal motoneurons (Bayliss et al. 1995) but in contrast to that reported in spinal motoneurons (Berger and Takahashi 1990). Although the reasons for these differences between TMNs and spinal motoneurons are not clear, this study used lower concentrations of 5-HT and, as suggested previously (Bayliss et al. 1995), the possibility that the facilitation of LVA by 5-HT in spinal motoneurons is mediated by a low affinity 5-HT receptor cannot be excluded.

In this study, we found that 5-HT mainly inhibited N- and P/Q-type HVA calcium currents with a small reduction of L- and R-type currents. In hypoglossal motoneurons, all of the inhibition by 5-HT was attributed to suppression of N- and P-type channels (Bayliss et al. 1995). Interestingly, in juvenile TMNs, based on application of antagonists of calcium channel subtypes, the mAHP is mediated predominately by N-type calcium channels (Kobayashi et al. 1997). However, it was subsequently shown that, in normal external calcium, 5-HT is without effects on the mAHP, suggesting that N-type channels are not targets for 5-HT modulation (Inoue et al. 1999). As proposed previously (Talley et al. 1997), this difference is likely caused by developmental changes in expression of 5-HT receptors and is supported by the observation that their study (Inoue et al. 1999) was performed in juvenile rats compared with this study that used rats 1–4 days old.

Based on application of 5-HT agonists and antagonists, this study showed that 5-HT_{1A} receptors mediate the inhibition of N- and P/Q-type calcium currents. This finding is also supported by the presence of 5-HT_{1A} binding sites in the trigeminal motor nucleus (Manaker and Zucchi 1998; Talley and Bayliss 2000; Wright et al. 1995). Similar results were reported in hypoglossal motoneurons (Bayliss et al. 1995), but in those neurons, DOI (5-HT_{2A/C} agonist) weakly mimicked the 5-HT inhibition. However, this was attributed to a weak affinity of DOI for the 5-HT_{1A} receptor because this was not observed in the presence of NAN-190.

Calcium conductances and TMN afterpotentials

This study found that the mAHP was strongly suppressed by application of N-type, and to a lesser extent P/Q-type, calcium channel toxins, consistent with the shown effect on those calcium currents. Furthermore, 5-HT produced similar results, suggesting that at least part of the 5-HT–induced suppression of the mAHP reported previously (Oh et al. 2003) is mediated by block of N- and P/Q-type calcium conductances. This is similar to that previously reported for hypoglossal motoneurons (Umemiya and Berger 1994), but in contrast to that reported in juvenile TMNs (Inoue et al. 1999). In that study, the mAHP was suppressed by 5-HT only in the presence of high concentrations of external calcium (6 mM) and in response to high 5-HT concentrations (50 µM). Furthermore, they were not able to associate a particular 5-HT receptor subtype with the mAHP suppression. They concluded that the 5-HT–induced suppression of the mAHP was mediated by a second messenger cascade that modulated the calcium-dependent K^+ conductance, as opposed to a direct suppression of N-type channels through a membrane-delimited mechanism suggested in this study and shown by others (Anwyl 1991; Bayliss et al. 1995; Hille 1994; Oh et al. 2003). These differences are most likely related to the changes in both calcium channel currents and 5-HT receptor subtype expression during maturation (Miles et al. 2004; Talley et al. 1997).

Functional consequences

Previously, we showed that 5-HT increases TMN excitability in juvenile guinea pigs (Katakura and Chandler 1990) through multiple effects on ion channels (Hsiao et al. 1997). In that study, we showed that an increase in discharge frequency was associated with a suppression of a leak potassium conductance, which subsequently resulted in an increase in membrane resistance and membrane depolarization. However, the specific role of calcium conductances in control of membrane excitability was not examined, and additional effects of 5-HT on mAHP through reduction in calcium conductances could not be excluded. This study lends support to the hypothesis that the 5-HT increase in membrane excitability and spike frequency is mediated by suppression of calcium conductances that are responsible for the mAHP; a significant factor controlling spike frequency. In guinea pig motoneurons, we found that 5-HT reduced the mAHP, shifted the f-I relationship to the left, and increased the slope of the relationship (Hsiao et al. 1997). This study used neonatal rats, and similar to that observed in guinea pigs, the f-I slope was increased and the curve was shifted to the left after application of the N-type calcium channel toxin or 5-HT. Because there is a strong serotonergic input from raphe nuclei to the trigeminal motor nucleus in the rat (Li et al. 1993), and subpopulations of raphe neurons are specifically active during rhythmic jaw movements (Fornal et al. 1996; Veasey et al. 1995), raphe neurons would be expected to lower the threshold for spike discharge and increase the input/output gain in response to small synaptic inputs. Because AHP amplitude and duration are important factors in control of discharge frequency (Kernell 1965), functionally, the 5-HT effects are likely to modulate TMN discharge frequency during behaviors that activate serotonergic systems during oral-motor behaviors (Fornal et al. 1996; Veasey et al. 1995). Our data provide additional mechanisms for our previous observation that 5-HT enhances trigeminal motoneuron excitability and discharge during rhythmic jaw movements in vivo (Katakura and Chandler 1990), as well as increases membrane excitability through effects on multiple ion channels in vitro (Hsiao et al. 1997). Whether or not serotonergic raphe input produces significant modulation of spike discharge in trigeminal motoneurons during oral-motor activities will require additional whole animal experiments.

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