R-type calcium channels in myenteric neurons of guinea pig small intestine

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Bian, Xiaochun, Xiaoping Zhou, and James J. Galligan. R-type calcium channels in myenteric neurons of guinea pig small intestine. Am J Physiol Gastrointest Liver Physiol 287: G134–G142, 2004; 10.1152/ajpgi.00532.2003.—Currents carried by L-, N-, and P/Q-type calcium channels do not account for the total calcium current in myenteric neurons. This study identified all calcium channels expressed by guinea pig small intestinal myenteric neurons maintained in primary culture. Calcium currents were recorded using whole cell techniques. Depolarizations (holding potential = −70 mV) elicited inward currents that were blocked by CdCl2 (100 μM). Combined application of nifedipine (blocks L-type channels), Ω-conotoxin GVIA (blocks N-type channels), and Ω-agatoxin IVA (blocks P/Q-type channels) inhibited calcium currents by 56%. Subsequent addition of the R-type calcium channel antagonists, NiCl2 (50 μM) and SNX-482 (0.1 μM), abolished the residual calcium current. NiCl2 or SNX-482 alone inhibited calcium currents by 46%. The activation threshold for R-type calcium currents was −30 mV, the half-activation voltage was −5.2 ± 5 mV, and the voltage sensitivity was 17 ± 3 mV. R-type currents activated fully in 10 ms at 10 mV. R-type calcium currents inactivated in 1 s at 10 mV, and they inactivated (voltage sensitivity of 16 ± 1 mV) with a half-inactivation voltage of −76 ± 5 mV. These studies have accounted for all of the calcium channels in myenteric neurons. The data indicate that R-type calcium channels make the largest contribution to the total calcium current in myenteric neurons. The relatively positive half-activation voltage and rapid activation kinetics suggest that R-type channels could contribute to calcium entry during somal action potentials or during action potential-induced neurotransmitter release.

ion channels; enteric nervous system; electrophysiology

THE ENTERIC NERVOUS SYSTEM (ENS) controls gastrointestinal function (12) and the myenteric plexus, a division of the ENS, is particularly important for control of gastrointestinal motility (3, 24). Studies of the myenteric plexus of the guinea pig ileum have provided most information on neuronal types and pathways in the myenteric plexus. With the use of electrophysiological criteria, the following two types of myenteric neuron have been identified: S- and AH-type neurons. AH neurons have a calcium contribution to the action potential (12, 16, 33), and calcium entering AH neurons during the action potential activates a calcium-dependent potassium channel, causing a long-lasting action potential afterhyperpolarization (17, 34, 51). AH neurons may be enteric sensory neurons (12, 22, 25). The action potential in S neurons is blocked by TTX; however, action potentials in S neurons are associated with increases in intracellular calcium (43). S neurons are interneurons and motoneurons (3, 11, 24).

On the basis of molecular, electrophysiological, and pharmacological properties, six different calcium channel subtypes (L, N, P, Q, R, and T) can be distinguished (4, 7, 31). Neuronal L-type calcium channels are composed of CaV1.2 (α1D), CaV1.3 (α1C), CaV1.4 (α1B), CaV1.5 (α1E), and CaV1.6 (α1F) subunits. These channels carry a current that is resistant to the toxins described above (36, 37).

Myenteric neurons express N-type (40, 47, 49, 52), P/Q-type (40, 45, 49, 52), and L-type calcium channels (13, 40). However, summation of currents carried by N-, P/Q-, and L-type channels doesn’t account for the total calcium current in myenteric neurons. Therefore, the goal of this study was to identify all of the calcium channels expressed by guinea pig myenteric neurons.

METHODS AND MATERIALS

Animals

The All University Committee on Animal Use and Care at Michigan State University approved all animal use protocols.

Primary Culture of Myenteric Neurons

Newborn (1–2 days old) guinea pigs were killed by severing the major neck blood vessels after deep halothane anesthesia. The entire length of small intestine was placed in cold (4°C) sterile-filtered Krebs solution of the following composition (mM): 120 NaCl, 5 KCl, 2.5 CaCl2, 1.2 MgCl2, 1.3 NaH2PO4, 25 NaHCO3, and 11 glucose. The longitudinal muscle myenteric plexus was stripped free using a cotton swab and cut into 5-mm pieces. Tissues were divided into four aliquots, and each aliquot was transferred to 1 ml Krebs solution containing 1 mg/ml trypsin for 15–20 min at 37°C. Tissues were then triturated 30 times and centrifuged at 900 g for 5 min using a bench-top centrifuge. The supernatant was discarded, and the pellet was resuspended and incubated (30 min, 37°C) in Krebs solution containing 1 mg/ml collagenase. The suspension was triturated again 30 times and centrifuged at 900 g for 5 min. Cells were plated on glass coverslips coated with poly-L-lysine (50 μg/ml for 2 h) and maintained in an incubator at 37°C with 5% CO2 from 1 day up to 2 wk. After 2 days in culture, cytosine arabinoside (10 μM) was added to MEM to limit smooth muscle and fibroblast proliferation. The medium was changed two times weekly.
Electrophysiological Methods

Whole cell patch-clamp recordings were carried out at room temperature. Fire-polished patch pipettes with tip resistances of 3–6 MΩ were used for whole cell recordings. Seal resistances for all recordings were >5 GΩ. The extracellular solution contained (in mM) 97 NaCl, 20 tetraethylammonium, 4.7 CsCl, 1 M MgCl₂, 1.2 NaH₂PO₄, 25 NaHCO₃, 11 glucose, and 0.0003 TTX. The pipette solution contained (mM): 160 CsCl, 2 MgCl₂, 10 EGTA, 10 HEPES, 1 ATP, and 0.25 GTP. The pH and osmolality for the extracellular solutions were 7.4 (using CsOH or KOH) and 310–320 mOsm/kgH₂O (using CsCl). Series resistance (up to 80%) and capacitative currents were electronically compensated. Calcium currents were recorded by using depolarizations in 10-mV increments to +40 mV from a holding potential of −80 mV. All recordings were made using an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). Data were acquired using pClamp 6.0 (Axon Instruments). In most experiments, currents were sampled at 5 kHz and were filtered at 2 kHz (4-pole Bessel filter). When making measurements of tail currents, the sampling rate was 50 kHz.

Drug Application

Drugs were applied using a quartz, gravity-fed flow tube (320 μm ID and 450 μm OD; Polymicro Technologies, Phoenix, AZ). The distance from the mouth of the tubes to the cell examined was ∼200 μm, and the position of the tubes was controlled manually using a micromanipulator.

Data Analysis

Concentration-response curves for inhibition of currents by calcium channel toxins were constructed by fitting the data to the following logistic equation:

\[
y(x) = y_{\text{min}} + \frac{y_{\text{max}} - y_{\text{min}}}{1 + \left( \frac{x}{EC_{50}} \right)^n}
\]

where \(x\) is the toxin concentration, \(EC_{50}\) is the half-maximum effective concentration, and \(n\) is the slope factor. Activation and inactivation curves for calcium currents were fitted to the following Boltzmann distribution:

\[
y = y_{\text{max}} + \frac{y_{\text{min}} - y_{\text{max}}}{1 + e^{\frac{V - V_{50}}{p}}}
\]

where \(V_{50}\) is the membrane potential for half-activation or -inactivation, and \(p\) is the slope factor describing the steepness of the voltage dependence of activation or inactivation. The effects of different treatments on calcium currents were compared using Student’s t-test (paired or unpaired) or ANOVA when applicable. \(P < 0.05\) was considered statistically significant. Curve fitting and statistical analysis were accomplished using Origin (6.1) software (Northampton, MA). Data are expressed as the means ± SE, and “n” values refer to the number of neurons from which the data were obtained.

Drugs

The following drugs were used: ATP (disodium salt, A-3377; Sigma), CdCl₂ (C-3141; Sigma), GTP (sodium salt, G-8752; Sigma), NiCl₂ (N-5756; Sigma), nifedipine (N-7634; Sigma), SNX-482 (S-500; Alomone), tetraethylammonium chloride (TEA, T-2265; Sigma), TTX (T-5651; Sigma), ATX (A-500; Alomone), Ω-CTX (C-300; Alomone), collagenase (234200; Calbiochem), collagenase type 1A (C-9891; Sigma), cytosine-D-arabinoside (Ara-C, C-6645; Sigma), Eagle’s MEM (M-0268; Sigma), penicillin/streptomycin (P-0781; Sigma), poly-l-lysine (P-2636; Sigma), and trypsin (T-5266; Sigma).

RESULTS

Calcium Current in Myenteric Neurons

Data were obtained from 102 neurons. Inward currents were activated by depolarizing commands from a holding potential of −80 to −10 mV in all neurons (Fig. 1A). CdCl₂ (100 μM), a nonselective calcium channel antagonist, blocked the voltage-gated inward current, indicating that it was a calcium current (Fig. 1A). The current-voltage relationship of the calcium current was constructed by depolarizing the membrane potential from a holding potential of −80 to +80 mV in 10-mV increments (Fig. 1B). The activation threshold was −30 mV, the peak current was near 0 mV, and the peak current amplitude was −2.4 ± 0.5 nA (n = 10). The calcium current reversed polarity at +40 mV (Fig. 1B).

Before characterizing the pharmacological and functional properties of the calcium channels expressed by myenteric neurons, the stability of calcium currents over time was first established. This was accomplished by applying depolarizing steps to −10 mV every 5 min for 60 min. The average calcium current amplitudes during the first and last trials were −1.3 ± 0.3 and −1.2 ± 0.2 nA (n = 5; P > 0.05).

Fig. 1. Calcium current in myenteric neurons. A: tracings of inward currents recorded after depolarizing from a holding potential (\(V_h\)) of −80 mV to a test potential (\(V_t\)) of −10 mV. Inward currents were blocked by CdCl₂ (100 μM) and restored after washing with CdCl₂-free solution, indicating that the inward current was a calcium current. B: current-voltage relationship of calcium currents recorded from myenteric neurons. The threshold for activation of the calcium current was at −30 mV, and the peak current was recorded at a test potential of 0 mV. The peak amplitude was −2.4 ± 0.5 nA.
Effects of CTX, ATX, and Nifedipine on the Calcium Current

The P/Q-type channel blocker ATX (0.3–100 nM) caused a concentration-dependent inhibition of the calcium current ($n = 6$; Fig. 2, A and B). ATX (100 nM) inhibited calcium currents by a maximum of 25 ± 3%, and the concentration causing 50% inhibition (IC$_{50}$) was 3 ± 1 nM. CTX caused a concentration-dependent (0.3–100 nM) inhibition of the calcium current (Fig. 2C). The IC$_{50}$ for CTX was 12 ± 6 nM ($n = 8$), and the maximum inhibition caused by CTX (100 nM) was 34 ± 4%. Nifedipine (10–1,000 nM) also concentration-dependently inhibited the calcium current ($n = 6$; Fig. 2D). The IC$_{50}$ and maximum calcium current inhibition caused by nifedipine (1 μM) were 109 ± 15 nM and 20 ± 6%, respectively.

The data described above confirm previous studies that had established that myenteric neurons express L-, N-, and P/Q-type channels; however, it has not been established whether these channels accounted for the total calcium current in myenteric neurons. Therefore, we tested the combined application of maximum concentrations of nifedipine (1 μM), ATX (100 nM), and CTX (100 nM) on myenteric neuronal calcium currents. It was found that combined application of the three calcium channel blockers caused only a 56 ± 6% inhibition of the total calcium current (Fig. 3). Therefore, another calcium channel subtype must be expressed by myenteric neurons.

R-type Calcium Channel Blockers Inhibit the Calcium Current

A low concentration of NiCl$_2$ (50 μM) can block selectively R-type calcium channels in the nervous system (14, 38, 48). We found that NiCl$_2$ (1–50 μM) caused a concentration-dependent calcium current inhibition ($n = 5$). In two of five cells, this inhibition was partly reversed after washing the cells with NiCl$_2$-free solution (Fig. 4B). The NiCl$_2$ concentration-response curve did not have a clear maximum effect, so an IC$_{50}$ value could not be calculated; however, at 50 μM, NiCl$_2$ inhibited the calcium current by 46 ± 4%. At concentrations >50 μM, NiCl$_2$ blocks multiple subtypes of calcium channels. Therefore, we did not test higher concentrations of this calcium channel blocker.

SNX-482 is a toxin that exhibits high selectivity for R-type calcium channels (32). SNX-482 caused a concentration-dependent (1–100 nM) inhibition of the calcium current in myenteric neurons (Fig. 5, A and B). Concentrations of SNX-482 >100 nM inhibit N-type calcium channels (31). Therefore, we did not test higher concentrations of this toxin. The IC$_{50}$ for SNX-482 was 12 ± 4 nM ($n = 5$), and the inhibition of the calcium current caused by 100 nM SNX-482 was 46 ± 1% ($n = 5$). In the presence of a maximum concentration of SNX-482 (100 nM), NiCl$_2$ (50 μM) did not further reduce the calcium current (Fig. 5C, $P > 0.05$).

To determine the relative percentage of neurons expressing R-type calcium currents, the effect of maximum concentrations of NiCl$_2$ (50 μM) or SNX-482 (100 nM) on the total calcium current was examined. SNX-482 reduced the calcium current by up to 50% in all five neurons tested, whereas NiCl$_2$ reduced the calcium current by up to 50% in 32 of 37 neurons tested. These data indicate that R-type calcium channels are expressed by ~88% of myenteric neurons maintained in primary culture.

In the next set of experiments, we used combined blockade of P/Q-, N-, and L-type calcium channels to determine whether subsequent addition of R-type antagonists would block the residual calcium current. In this series of experiments, combined application of maximum concentrations of nifedipine, CTX, and ATX inhibited calcium currents by 42 ± 3% (Fig.

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Fig. 2. P/Q-, N-, and L-type calcium channel antagonists inhibited the calcium current. A: original tracings of calcium currents activated by depolarization from −70 to −10 mV. ω-Agatoxin IVA (ATX; P/Q-type calcium channel toxin) caused a concentration-dependent inhibition of the calcium current. B, C; and D: summary data from experiments similar to that shown in A. Concentration-response curves for inhibition of the total calcium currents by ATX (B), the N-type antagonist ω-conotoxin GVIA (CTX; C), and the L-type channel antagonist nifedipine (D) are shown. Data are presented as current amplitude recorded in the presence of antagonist ($I$) expressed as a fraction of the current recorded before antagonist application ($I_{control}$). Data are expressed as means ± SE; and "n" indicates the number of neurons from which the data were obtained.
Subsequent addition of NiCl₂ (50 μM) to the extracellular solution abolished the calcium current (Fig. 6A). Calcium currents largely recovered after washing the cell with drug-free solution (Fig. 6A). Altering the sequence of drug application did not change the relative inhibition of the calcium current. When NiCl₂ (50 μM) was applied first, it inhibited the calcium current by 45.0 ± 3.9%, and subsequent combined application of nifedipine, CTX, and ATX blocked the remaining calcium current (Fig. 6B).

Kinetic Properties of R-type Calcium Channels in Myenteric Neurons

Because these were the first studies to identify R-type calcium currents in myenteric neurons, we characterized the kinetic properties of this current. R-type calcium currents were isolated by adding maximum concentrations of nifedipine, CTX, and ATX to the extracellular solution. The current-voltage relationship of R-type calcium current was constructed.

Fig. 3. Combined application of P/Q-, N-, and L-type calcium channel antagonists does not block the total calcium current. A: original recordings of calcium currents obtained in the absence of any drug (Control) and in the presence of sequential cumulative application of ATX, CTX, and nifedipine. Approximately 50% of the calcium current remained in the combined presence of maximum concentrations of the channel blockers. Depolarizing the membrane potential from −70 to −10 mV activated calcium currents. B: mean data from experiments in A showing that combined application of ATX (100 nM), CTX (100 nM), and nifedipine (1 μM) did not abolish the calcium current. Data are means ± SE of measurements made on 4 neurons.

Fig. 4. R-type calcium channel blockers inhibit calcium currents. A: original tracings of calcium currents in the absence of presence of NiCl₂ (50 μM). NiCl₂ caused a large and partly reversible inhibition of the total calcium current. Depolarizing the membrane potential from −70 to −10 mV activated the calcium current. B: concentration curve for inhibition of calcium currents by NiCl₂. Data are expressed as currents obtained in the presence of NiCl₂ normalized against the control current obtained before NiCl₂ treatment. Data are expressed as means ± SE. In 5 cells, NiCl₂ concentration dependently inhibited the calcium current, and in 2 of the 5 cells, the calcium current partly recovered on NiCl₂ washout.
by depolarizing the membrane potential from a holding potential of $-70 \text{ mV}$ to $+40 \text{ mV}$ using step-voltage increments of 10 mV (Fig. 7B). In six cells, the threshold of activation for the R-type calcium current was $-30 \text{ mV}$, the peak current was recorded at $+10 \text{ mV}$ (Fig. 7B), and the peak amplitude was $-1.1 \pm 0.4 \text{ nA}$ ($n = 6$). R-type calcium currents had an extrapolated reversal potential of 40 mV (Fig. 7B). The R-type calcium current activated rapidly and reached a steady-state plateau within 10 ms after each step command (Fig. 7A). The activation curve for R-type currents was determined from Fig. 5. SNX-482 inhibits the calcium current, and the inhibition of the calcium current caused by SNX-482 and NiCl$_2$ is mutually occlusive. A: SNX-482 (100 nM) and NiCl$_2$ (50 μM) produced a reversible inhibition of the calcium current. Depolarizing the membrane potential from $-70$ to $-10 \text{ mV}$ activated the calcium current. B: concentration-response curve for inhibition of calcium currents by SNX-482. Data are expressed as currents obtained in the presence of SNX-482 normalized against the control current obtained before SNX-482 treatment. Data are means ± SE. C: maximum concentration of SNX-482 (100 nM) inhibited the calcium current, and subsequent addition of NiCl$_2$ (50 μM) did not produce any further inhibition of this current. Data are expressed as currents obtained in the presence of SNX-482 or SNX-482 plus NiCl$_2$ normalized against the control current obtained before SNX-482 treatment. Data are means ± SE.

Fig. 6. Combined application of ATX, CTX, nifedipine, and NiCl$_2$ completely blocked calcium currents. A: combined application of ATX, CTX, and nifedipine reduced the total calcium current by 50%. Further application of NiCl$_2$ (50 μM; R-type calcium channel toxin) abolished the calcium current. B: partial inhibition of the calcium current by NiCl$_2$ (50 μM); subsequent addition of ATX, CTX, and nifedipine completely blocked the remaining calcium current. C: partial inhibition of the calcium current by ATX, CTX and nifedipine; subsequent addition of NiCl$_2$ completely blocked the remaining calcium current.
measurements of tail current amplitudes recorded at −70 mV after depolarizations to between −60 and +40 mV (Fig. 7C). Tail current amplitudes were fitted by a single exponential Boltzmann distribution with slope factor of 17.3 mV (n = 6, Fig. 7D). The maximum current flowing through the R-type calcium channels was −2.4 ± 0.5 nA, n = 6) at +40 mV. The maximum conductance was −19.4 ± 3.4 nS. The half-activation voltage was −5 ± 5 mV.

Inactivation of R-type calcium current reached steady state 1 s after step depolarization (Fig. 8A). The time to half-inactivation at −20 mV was 389 ± 149 ms (n = 4). The voltage dependence of R-type channel inactivation was studied using the following protocol. From an initial holding potential of −70 mV, the R-type calcium current was evoked using a test potential to −10 mV after conditioning pulses (1 s duration) to potentials between −110 and −10 mV (Fig. 8B). The amplitude of currents evoked at −10 mV after the 1-s duration conditioning pulses followed the Boltzmann distribution with a slope factor of 16 ± 1 (n = 4, Fig. 8C) and a voltage at half-inactivation of −76 ± 5 mV.

DISCUSSION

The major new finding presented here is that the R-type calcium channel is the predominant calcium channel expressed on the somatodendritic region of myenteric neurons. Furthermore, we show that R-, N-, P/Q-, and L-type channels account for the total calcium current recorded from these cells.

Calcium Channels in Myenteric Neurons

The present study identified the relative contribution of different calcium channel subtypes to the total calcium current. This was accomplished by constructing concentration-response curves for calcium channel antagonists to determine the concentration at which antagonists reached a plateau for calcium current inhibition. This approach allowed us to identify the relative contribution of N-, L-, P/Q-, and R-type channels to the total calcium current and to characterize the properties of R-type currents in myenteric neurons. We were not able to classify the neurons as S- or AH-type neurons using electrophysiological criteria. TTX and potassium channel blockers
TEA and Cs⁺ were present in the recording solutions throughout these studies. TTX will block synaptic transmission; therefore, we were unable to record the fast excitatory postsynaptic potentials that are a property of S neurons (11, 12, 16, 24). The potassium channel blockers we used would block the slow action potential afterhyperpolarization that is characteristic of AH-type neurons (11, 12, 16, 24). We did record calcium currents from every neuron studied, and R-type calcium currents were identified in almost 90% of neurons tested with an R-type channel antagonist. This suggests that R-type calcium channels are probably not restricted to one subset of myenteric neuron in primary culture.

L-type channels. L-type calcium channels contribute <20% to the total calcium current recorded from guinea pig small intestinal myenteric neurons. These functional data are supported by immunohistochemical studies showing that the L-type channel subunits, Caᵥ1.2 and Caᵥ1.3, are expressed by guinea pig myenteric neurons (19). Blockade of L-type channels does not alter the active or passive properties of enteric neurons (23, 34), and L-type blockers do not alter neurotransmitter release from enteric neurons in the ileum or cecum (6, 26, 39, 40). Nifedipine produces a modest inhibition of electrically evoked ACh release from myenteric neurons in guinea pig proximal colon (30). Therefore, L-type calcium channels do not play a major role in controlling excitability or synaptic transmission on a moment-to-moment basis in the ENS. However, calcium entry through L-type channels may mediate changes in calcium channel expression during periods of prolonged stimulation (10).

N-type channels. There is a CTX-sensitive component to calcium current recorded from guinea pig, rat, and mouse enteric neurons (2, 5, 10, 20, 42, 46). In addition, Caᵥ2.2 is expressed by enteric neurons from guinea pigs and rats (21, 52). Our data confirm that guinea pig myenteric neurons express functional N-type channels, and we also show that the current carried by this channel subtype comprises 25% of the total calcium current. Although this appears to be a relatively small fraction of the total calcium current, N-type channels play a prominent role in enteric neuronal function. N-type channels contribute to the calcium shoulder on the action potential in AH neurons (12, 52), and these channels mediate calcium entry into S neurons during trains of action potentials (43). N-type channels also contribute to calcium entry required for neurotransmitter release from myenteric (39, 40, 47) and submucosal neurons (6).

P/Q-type calcium channels. A previous study of calcium channels in myenteric neurons used ω-conotoxin MVIIC to inhibit the calcium current (45). Because this toxin blocks N-type and P/Q-type channels, it was concluded that both channels were expressed by myenteric neurons. We also conclude that myenteric neurons express P/Q-type channels, which contribute 20% to the total calcium current. These functional data are supported by immunohistochemical data showing that Caᵥ2.1 subunits are present in guinea pig and rat myenteric neurons (21).

Our data show that P/Q-type channels are expressed in the somatodendritic region of myenteric neurons, but calcium entry through these channels does not contribute to the action
potential, at least in AH-type neurons (42). Therefore, a function for somatodendritic P/Q-type channels in enteric neurons has not been established. However, P/Q-type channels do contribute a portion of the calcium needed for neurotransmitter release from enteric neurons (6, 39, 40).

**R-type currents.** We showed that combined application of L-, N-, and P/Q-type calcium channel antagonists reduced the total calcium current by ~50%. We also showed that SNX-482, a selective R-type calcium channel toxin (32, 44), blocked the residual calcium current. Similar data were obtained when we used a low concentration of NiCl₂ to block R-type calcium channels (14, 38, 48). SNX-482 or NiCl₂ alone reduced calcium current by ~50%, the amount of calcium current remaining in the presence of L-, N-, and P/Q-type blockers. Finally, the inhibitory effects of NiCl₂ and SNX-482 on the calcium current were occlusive, indicating that they acted on the same channels. These data demonstrate that myenteric neurons express R-type calcium channels.

**Properties of R-type Calcium Currents**

R-type calcium channels are gated by strong depolarizations in the central nervous system (CNS; see Refs. 8 and 54). This study showed that the R-type calcium channels in myenteric neurons also require strong depolarizations, since the half-activation voltage for these channels was ~5 mV. At 0 mV, R-type currents activate fully in ~10 ms, a value similar to that for R-type calcium currents in CNS neurons (8, 28). The requirement for strong depolarizations and the fast activation kinetics of R-type channels would allow them to contribute to calcium entry during somatodendritic action potentials. Our studies of the steady-state activation and inactivation of R-type calcium channels indicate that less than one-half of the R-type channels are available for activation during depolarization from ~60 mV. Recruitment of additional R-channel availability would require membrane hyperpolarization as occurs during action potential afterhyperpolarization, which last >1 s in AH neurons (12).

N-type channels contribute to the calcium component of action potentials in AH-type neurons from the guinea pig myenteric plexus (12), and the currents carried by N- and R-type channels have similar kinetic properties. Both channels have an activation threshold of ~30 mV and a half-activation voltage near 0 mV (9). These data indicate that R- and N-type channels would gate calcium entry only during the strong depolarization that occurs during action potentials. Weaker depolarizations that would occur during subthreshold fast or slow excitatory postsynaptic potentials would activate very few R- or N-type calcium channels. Our data show that the voltage dependence of activation of the R channel is somewhat steeper (slope factor = 17 mV) than that for the N channel (slope factor = 6 mV). However, both channels activate fully is <20 ms but require several hundred milliseconds for full inactivation at 0 mV (9), a membrane potential that would be reached during a somatodendritic action potential. Fast activation kinetics make the N and R channels suitable for gating calcium entry during action potentials; however, the slow inactivation of both channel types requires that mechanisms other than channel inactivation are responsible for action potential repolarization.

There is no overlap of the activation and inactivation curves for R channels in myenteric neurons; therefore, R-type calcium channels are not likely to be a major contributor to the persistent calcium current active at resting potentials in myenteric AH-type neurons (34).

These studies have established that myenteric neurons from the guinea pig small intestine express N-, L-, P/Q-, and R-type calcium channels. R-type calcium channels alone contribute ~50% of the total calcium current. The specific function of R-type channels in myenteric neurons is not yet clear. However, R-type calcium currents activate rapidly at potentials that would be reached during somatodendritic or nerve terminal action potentials. R-type channels could contribute to calcium entry during somatodendritic action potentials and therefore participate in regulation of cell excitability. Alternatively, R-type calcium channels could contribute to action potential-induced calcium entry required for neurotransmitter release from myenteric nerve endings.

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