Ryanodine-Sensitive Stores Regulate the Excitability of AH Neurons in the Myenteric Plexus of Guinea-Pig Ileum

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Hillsley, K., J. L. Kenyon, and T. K. Smith. Ryanodine-sensitive stores regulate the excitability of AH neurons in the myenteric plexus of guinea-pig ileum. J Neurophysiol 84: 2777–2785, 2000. Myenteric afterhyperpolarizing (AH) neurons are primary afferent neurons within the gastrointestinal tract. Stimulation of the intestinal mucosa evokes action potentials (AP) that are followed by a slow afterhyperpolarization (AHPslow) in the soma. The role of intracellular Ca2+ ([Ca2+]i) and ryanodine-sensitive Ca2+ stores in modulating the electrical activity of myenteric AH neurons was investigated by recording membrane potential and bis-fura-2 fluorescence from 34 AH neurons. Mean resting [Ca2+]i was ~200 nM. Depolarizing current pulses that elicited APs evoked AHPslow and an increase in [Ca2+]i, with similar time courses. The amplitudes and durations of AHPslow and the Ca2+ transient were proportional to the number of evoked APs, with each AP increasing [Ca2+]i by ~50 nM. Ryanodine (10 μM) significantly reduced both the amplitude and duration (by 60%) of the evoked Ca2+ transient and AHPslow over the range of APs tested (1–15). Calcium-induced calcium release (CICR) was graded and proportional to the number of APs, with each AP triggering a rise in [Ca2+]i of ~30 nM Ca2+ via CICR. This indicates that CICR amplifies Ca2+ influx. Similar changes in [Ca2+]i, and AHPslow were evoked by two APs in control and six APs in ryadenone. Thus, the magnitude of the change in bulk [Ca2+]i and not the source of the Ca2+ is the determinant of the magnitude of AHPslow. Furthermore, lowering of free [Ca2+]i, either by reducing extracellular Ca2+ or injecting high concentrations of Ca2+ buffer, induced depolarization, increased excitability, and abolition of AHPslow. In addition, activation of synaptic input to AH neurons elicited a slow excitatory postsynaptic potential (sEPSP) that was completely blocked in ryanodine. These results demonstrate the importance of [Ca2+]i, and CICR in sensory processing in AH neurons. Activity-dependent CICR may be a mechanism to grade the output of AH neurons according to the intensity of sensory input.

INTRODUCTION

The enteric nervous system is comprised of the myenteric and submucosal plexi, which are interconnected and control complex reflexes within the gastrointestinal tract. Intracellular microelectrode recordings have revealed two broad electrophysiological classes of myenteric neurons, S/Type I and AH/Type II neurons (Bornstein et al. 1994; Hirst et al. 1974; North 1973; Wood 1994). S neurons lack a slow afterhyperpolarization (AHPslow), have a prominent fast synaptic input (Furukawa et al. 1986; Hirst and McKirdy 1974), and comprise both interneurons and motoneurons (Bornstein et al. 1991; Smith et al. 1992). Afterhyperpolarizing (AH) neurons are named for the characteristic AHPslow (4–20 s), which follows action potential (AP) firing in these neurons (Hirst et al. 1974; Nishi and North 1973). AH neurons are primary afferent neurons in the guinea-pig intestine (Furness et al. 1990; Kunze et al. 1995; Regina et al. 1993; Song et al. 1994) and are found in both the myenteric and submucosal plexi. They are multipolar and have projections to the mucosa. Stimulation of the mucosal villi results in the activation of AP firing in these mucosal processes, which results in an AP that can be detected in the cell soma, that is followed by a prominent AHPslow in the soma (Kunze et al. 1995; Smith 1994). This gating of the soma by the AHPslow thereby reduces neurotransmission from these neurons to second-order neurons in the reflex pathways that regulate motility.

In AH neurons, addition of tetrodotoxin (TTX) only partially ablates APs, and residual events are blocked by the removal of extracellular Ca2+ ions (Hirst et al. 1985; North 1973). The Ca2+ current contributing to AH neuron APs has been characterized (Hirst et al. 1985a), but the identity of the channel is unclear (Vogalis et al. 2000b). Ca2+ influx that occurs during AP firing ultimately leads to activation of a Ca2+-activated K+ conductance, resulting in the AHPslow (Hirst et al. 1985).

Preliminary studies using fura-2 to image Ca2+ transients in AH neurons have demonstrated that AP firing transiently increases intracellular free calcium ([Ca2+]i), with a time course that closely matches the time course of AHPslow (Tatsumi et al. 1988; Vogalis et al. 2000a). This suggests that intracellular Ca2+ is an important mediator of AHPslow.

However, the mechanism by which Ca2+ influx leads to AHPslow is not understood in AH neurons. Prolonged Ca2+ influx across the neuronal membrane may contribute to AHPslow (Hirst et al. 1985a), although activation of internal stores has also been proposed (North and Tokimasa 1987). In several other types of neurons, an analogous AHPslow is also observed. Activation of the AHPslow is at least partially dependent on intracellular Ca2+ stores in vagal afferent (Moore et al. 1998), vagal dorsal motor (Sah and McIlachlan 1991), sympathetic (Kawai and Watanabe 1989), and parasympathetic neurons (Yoshizaki et al. 1995). In particular, Ca2+ influx associated with APs triggers Ca2+ release from ryanodine-sensitive stores, and it is this calcium-induced calcium release (CICR) that underlies AHPslow.

The aim of this study was to perform an analysis of changes in [Ca2+]i with changes in electrical activity in AH neurons.
and in particular, to investigate the role of ryanodine-sensitive intracellular Ca\(^{2+}\) stores in the generation of AHP\(_{\text{slow}}\) in myenteric AH neurons.

**METHODS**

**General**

Guinea pigs of either sex (250–350 g) were killed by CO\(_2\) asphyxiation followed by severing of the carotid arteries and exsanguination. This procedure was authorized by the Institutional Animal Care and Use Committee at the University of Nevada, Reno. A 15-cm length of ileum was taken 10–12 cm above the caecum and the luminal contents were flushed away with modified Krebs-Ringer Buffer (KRB) injected into the lumen with a syringe inserted into the oral end of the segment. A segment of ileum (~40-mm-long) was then opened along its mesenteric border and pinned mucosa uppermost to a Sylgard\® dish and the mucosa, submucosa, and circular muscle layer were removed to expose the ganglia of the myenteric plexus. After dissection, the preparation was transferred to the Sylgard floor of an electrophysiological chamber (diameter 3 cm). In the central region of the chamber, the sylgard was removed and the preparation stretched over a thin glass coverslip window and pinned at either end and held firm and flat against the glass coverslip by drawing it under two elastic bands. The recording chamber was then mounted on the stage of an inverted microscope (Nikon Diaphot) and perfused with warmed KRB (recording chamber was then mounted on the stage of an inverted microscope against the glass coverslip by drawing it under two elastic bands. The

**Recording methods**

Standard microelectrode techniques (Shuttleworth and Smith 1999) were used to impale neurons within the myenteric plexus. Microelectrodes were fabricated from borosilicate glass capillary (1.0 mm od, 0.7 mm id 1B100F-4; WPI Corp., Sarasota, FL) on a Brown and Flaming Microelectrode Puller (Sutter Instruments, San Francisco, CA) and filled with a solution consisting of bis-fura-2 (1–5 mM) in 1 M KCl. These electrodes had resistances ranging from 150 to 240 MF. Electrical activity was sampled using an Axoprobe 2B dual-channel high-impedance preamplifier (Axon Instruments, Foster City, CA). Depolarizing or hyperpolarizing current pulses were injected via a bridge circuit, and the pulse duration and frequency regulated using an output from a programmable eight-channel pulse generator (Master-8, AMPI, Jerusalem, Israel). Current pulses varied from 1–500 ms in duration and 0.05–0.95 nA in intensity. The intracellular voltage and current were displayed on a two-channel digital storage oscilloscope (Tektronix 468R, Beaverton, OR). Data were digitized and analyzed using Ionoptix software (IonWizard software from the ratio of E\(_{500}\) following excitation at 340 and 380 nm. Calibration constants were determined using in vitro calibration solutions.

**Statistics**

Data are expressed in means ± standard error (SE). Linear regression and exponential decay analysis were performed using Graphpad Prism software. Student’s paired t-tests were used to assess significant differences between calculated means; P < 0.05. Unless specified, n is the number of neurons, where one neuron was sampled from each animal.

**Drugs and solutions**

Atropine, caffeine, nicardipine, and ryanodine were all from Sigma Chemical Co (St. Louis, MO). bis-Fura-2-pentapotassium salt was from Molecular Probes (Eugene, OR).

**RESULTS**

Simultaneous intracellular recordings and calcium imaging measurements were obtained from 34 myenteric AH/Type II neurons, which were characterized according to previously established criteria (Bornstein et al. 1994; Hirst et al. 1974; Nishi and North 1973). The size and shape of the neuronal cell body was revealed with bis-fura-2 filling, although neuronal process emanating from the cell body were rarely discerned. AH neurons were elliptical with a maximum diameter of 31.7 ± 2.1 μm and a minimum diameter of 19.4 ± 1.8 μm (n = 14), a size consistent with other studies of AH neurons (Bornstein et al. 1991; Furness et al. 1988; Hanani et al. 1998; Smith et al. 1999; Vogalis et al. 2000).

**Evoked and spontaneous Ca\(^{2+}\) transients**

Myenteric AH neurons had a mean resting membrane potential (RMP) of −65 ± 2.2 mV (n = 21) and a mean resting [Ca\(^{2+}\)]\(_i\) of 203.6 ± 8.4 nM (n = 12). Depolarizing current pulses stimulated APs (1–15), which were followed by an afterhyperpolarization (AHP\(_{\text{slow}}\)) with a mean amplitude of 8.6 ± 0.5 mV and a mean duration of 12.6 ± 1 s (n = 60 trial in 14 neurons). The voltage and [Ca\(^{2+}\)]\(_i\) responses of a typical neuron to the injection of a depolarizing current pulse (0.25, 400 ms) are shown in Fig. 1.

Phasic increases in [Ca\(^{2+}\)]\(_i\) were also detected following depolarizing current pulses, as shown in Fig. 1A. In 73 trials...
Ca\(^{2+}\) and slow afterhyperpolarization (AHP slow) should be noted. The change in [Ca\(^{2+}\)] associated with AP firing is necessary for the Ca\(^{2+}\) transient profile. [Ca\(^{2+}\)] began to rise during or immediately after the first AP and rose to its peak in 299 ± 16 ms after the onset of the depolarizing current pulse (n = 70 trials in 14 neurons). In 11 neurons, there was a linear correlation between the timing of the peak [Ca\(^{2+}\)], and the maximum AHP\(_{\text{slow}}\) (R\(^2\) = 0.73, P < 0.001), although the Ca\(^{2+}\) peak always preceded the maximum AHP\(_{\text{slow}}\). The peak [Ca\(^{2+}\)], was only sustained briefly, as the [Ca\(^{2+}\)], was within 10% of the peak value for 281 ± 68 ms (range = 30–670 ms, n = 12 neurons) during maximal stimulation. The decay of the Ca\(^{2+}\) transient was well fit by a single-phase exponential curve (mean R\(^2\) = 0.78 ± 0.04), with a mean time constant of decay (\(\tau\)) of 1.93 ± 0.34 s (n = 17 trials from 10 neurons). An example of a single exponential decay fit is shown in Fig. 1.

**Spontaneous APs**

Spontaneous APs in AH neurons were occasionally observed in this study (6/34 neurons) typically when the cell became depolarized. Spontaneous AP firing was followed by small brief afterhyperpolarizations (2.5 ± 0.7 mV amplitude, 2.2 ± 0.2 s duration). In 3/6 neurons, small and brief Ca\(^{2+}\) transients were also evoked by spontaneous APs with a mean increase in [Ca\(^{2+}\)], of 38.3 ± 18.1 nM. Examples of spontaneous events are shown in Fig. 4.
Inexcitable cells

Several studies have previously reported data from inexcitable cells that are of unknown cell type. Recordings were made from six large inexcitable cells with relatively negative RMPs (−275 to −285 mV, n = 6). Depolarizing current pulses (0.01 to 5.0 nA, 100–500 ms) could not evoke an AP or any change in \([\text{Ca}^{2+}]_i\) and all six of these cells remained inexcitable for up to 60 min following impalement. In three of these cells, focal electrical stimulation (0.2 ms, 10 Hz, for 0.5 s) produced a burst of antidromic APs that was accompanied by a robust \(\text{Ca}^{2+}\) transient (see Fig. 5) and a small sEPSP, suggesting that these cells were neurons. Repetitive synaptic stimulation for up to 30 min converted all inexcitable cells to “typical” AH neurons. After the conversion, the cells had RMPs in the range of −265 to −275 mV and APs could be elicited by a stimuli of ≤0.25 nA.

Cytoplasmic \([\text{Ca}^{2+}]_i\) changes

In seven preliminary experiments, electrodes were filled with 5 mM bis-fura-2 in a 1 M KCl solution. Using this higher concentration of bis-fura-2, relatively inexcitable AH neurons, with a resting membrane potential of approximately −70 mV, were rapidly converted (within 5–10 min) into excitable AH neurons (RMP ≈ −50 mV) and \(\text{AHP}_{\text{slow}}\) characteristic of these neurons was virtually abolished, as shown in Fig. 6A. As changes in electrical properties were never observed with electrodes filled with 1 mM bis-fura-2, which was used in all other experiments in this study, it is probable that high concentrations of bis-fura-2 acted as a buffer of \([\text{Ca}^{2+}]_i\). As prominent
AHP slow is virtually abolished, even though a 0.15-nA, 400-ms stimulus with 5 mM bis-fura-2 for 8 min. The neuron is depolarized to demonstrate the changes in cellular properties after the cell has been injected. Pairs of hyperpolarizing current pulses were injected every 30 s and the changes in electrical properties and [Ca\(^{2+}\)] were monitored. In 4/6 experiments, the impalement of the AH neuron was lost within 5 min due to vigorous contractions of the smooth muscle evoked by caffeine. However, within this limited time frame, a modest but significant hyperpolarization of AH neurons was detected from -63.3 ± 3.8 to -69.1 ± 2 mV (P < 0.05, n = 6). This change in the RMP induced by caffeine was coincident with an increase in the resting [Ca\(^{2+}\)], of 21.8 ± 6.2 nM, and an increase in the amplitude of evoked Ca\(^{2+}\) transients of 136.7 ± 27.2 nM. These observations suggest that the release of Ca\(^{2+}\) from intracellular stores is enhanced by caffeine which is reflected in the elevation of resting [Ca\(^{2+}\)], and an increase in Ca\(^{2+}\) transient amplitude.

The effect of ryanodine perfusion (10 μM) was tested in nine AH neurons. Ryanodine had no significant effect on the resting [Ca\(^{2+}\)], the RMP, or on input resistance in four neurons where hyperpolarizing current pulses were applied throughout the drug perfusion (0.15 nA, 200 ms, 0.2 Hz). Depolarizing current pulses, to elicit APs and Ca\(^{2+}\) transients, were applied in control conditions and in the presence of ryanodine (Fig. 7).

Evoked Ca\(^{2+}\) transients were decreased by ryanodine, with the amplitude changing from a Δ ratio of 0.3 to 0.12 (P < 0.05, n = 9), a change of 91.1 ± 23.1 nM. The duration of the Ca\(^{2+}\) transients decreased from 11.82 ± 2.03 to 5.21 ± 1.17 s (P < 0.05, n = 9). AHP\(_{\text{slow}}\) was also reduced from 9.17 ± 0.85 to 3.73 ± 0.63 mV (P < 0.01, n = 9) in the presence of ryanodine. The duration of AHP\(_{\text{slow}}\) decreased from 16.39 ± 2.7 to 5.61 ± 1.6 s (P < 0.01, n = 9) in ryanodine. An example of all of these effects of ryanodine (10 μM) on an AH neuron is illustrated in Fig. 7. These data imply that the release of Ca\(^{2+}\) from internal stores by CICR via ryanodine receptors is responsible for part of the Ca\(^{2+}\) transient and AHP\(_{\text{slow}}\).

As described earlier, a relationship exists between the number of action potentials and the amplitude of the Ca\(^{2+}\) transient. The effect of ryanodine on this relationship was tested in five AH neurons, with the results summarized in Fig. 8. In control conditions, the slope of the linear relationship was 47 nM/AP over the range of 1–4 APs. Ryanodine significantly reduced the amplitude of evoked Ca\(^{2+}\) transients to 16 nM/AP (34% of control). Thus, this suggests that CICR is activated by a single AP. However, the change in the slope implies that CICR is not fully activated by a single AP, i.e., successive APs trigger additional CICR. The difference between the two slopes, 29 nM/AP, provides an estimate of the contribution of CICR through ryanodine receptors to the Ca\(^{2+}\) transient evoked by each AP.

In the presence of ryanodine, more APs were necessary to evoke a given change in [Ca\(^{2+}\)]. In 11 experiments in seven different neurons, the amplitudes of a control and ryanodine Ca\(^{2+}\) transient were matched and the amplitude of the resultant AHP\(_{\text{slow}}\) was recorded. The Ca\(^{2+}\) transient amplitudes were well matched in control (90.2 ± 14.4 nM) and ryanodine (90 ± 13 nM, P = 0.95) conditions. The amplitude of AHP\(_{\text{slow}}\) evoked by these changes in [Ca\(^{2+}\)], were not significantly different in control (4.9 ± 0.6 mV) and in ryanodine (5.0 ± 1.7 mV, P = 0.61) conditions. However, the number of APs necessary to evoke the same changes in control and ryanodine conditions was significantly different (control median = 2 APs, ryanodine median = 6 APs, P < 0.001). That is, the same changes in Ca\(^{2+}\) and AHP\(_{\text{slow}}\) were elicited by two APs in control and six APs in ryanodine. Therefore, these data dem-


onstrate that membrane potential is controlled by \([Ca^{2+}]_i\), regardless of the source of the change in \([Ca^{2+}]_i\). This conclusion is supported by data in Fig. 8B, which shows that the relationship between the durations of AHP慢 and the \(Ca^{2+}\) transient is not significantly different in the presence of ryanodine.

**Ryanodine blocks slow synaptic transmission**

Electrical stimulation of internodal strands with high-frequency trains (20 Hz, 1 s) evoked sEPSPs, which gave rise to \(Ca^{2+}\) transients during AP firing. Evoked sEPSPs were completely abolished in the presence of ryanodine in 8/8 AH neurons tested. An example of the complete blockade of sEPSPs by ryanodine is shown in Fig. 9. This blockade typically took 10–20 min after ryanodine application to become effective. The sEPSP blockade was not dependent on any changes in RMP.

**Discussion**

There are two principal results of this study. 1) Myenteric AH neurons have a mechanism for amplifying \([Ca^{2+}]_i\), following an AP. \(Ca^{2+}\) influx associated with an AP triggers ryanodine-sensitive CICR, which elevates \([Ca^{2+}]_i\), that underlies the AHP慢. CICR also plays some role in the generation of slow synaptic events. 2) AHP慢 in myenteric AH neurons is regulated by bulk changes in \([Ca^{2+}]_i\), regardless of whether CICR is functional or not.

Initial experiments, where high concentrations of bis-fura-2 were used in the electrode, caused AH neurons to depolarize and become very excitable. bis-Fura-2 is a calcium buffer related to bis-(o-aminophenoxy)-N,N,N’N’-tetraacetic acid (BAPTA), and so high [bis-fura-2], buffers all of the free \([Ca^{2+}]_i\). Depolarization and blockade of AHP慢 by high concentrations of calcium buffers has previously been reported in nodose (Moore et al. 1998) and hippocampal neurons (Lancaster and Zucker 1994). Thus, it is clear that cytoplasmic-free \([Ca^{2+}]_i\) is important in regulating the low RMP and relative inexcitability of myenteric AH neurons.

Electrodes filled with 1 mM bis-fura-2 did not alter any of the characteristics of AH neurons, and the RMP of AH neurons reported in this study fall within the normal range of these cells (Bornstein et al. 1994). At −65 mV, the mean resting \([Ca^{2+}]_i\) in AH neurons was approximately 200 nM. Shuttleworth and Smith (1999) used an almost identical experimental approach and found that highly excitable S neurons had a mean resting \([Ca^{2+}]_i\) of approximately 100 nM, half that of AH neurons. It is of interest to note that when \([Ca^{2+}]_i\) is depleted to levels recorded in S neurons using zero \(Ca^{2+}\) KRB, AH neurons assume many of the electrical characteristics of S neurons. Thus, the high resting \([Ca^{2+}]_i\) is proposed to be an important factor in determining the differences in RMP and excitability between AH and S neurons. However, the mechanism behind establishing and maintaining the high \(Ca^{2+}\) levels in AH neurons is unclear.

Ryanodine perfusion had no effect on the RMP, input resistance, or on the resting \([Ca^{2+}]_i\) of cells. This lack of effect of ryanodine on the resting properties of neurons has been reported in superior cervical ganglia (Davies et al. 1996) and in cultured myenteric neurons (Kimball et al. 1996). This indicates that the high levels of \([Ca^{2+}]_i\) are independent of CICR. Further comparisons between the present study and that of Kimball et al. (1996) are limited by differences in the nature of the stimuli used and the populations of cells studied. It is of interest to note however that virtually all cultured myenteric neurons responded to 10 mM caffeine (Kimball et al. 1996), indicating that virtually all cultured myenteric neurons have ryanodine-sensitive stores. In the present study, caffeine evoked an increase in the resting level of \([Ca^{2+}]_i\), which was accompanied by a hyperpolarization of the RMP. These results suggest that although there is no measurable spontaneous CICR, the RMP can be modulated by elevations in \([Ca^{2+}]_i\), induced by CICR.

The RMP was not altered by ryanodine at a time when
AHP slow was attenuated. Davies et al. (1996) similarly found in superior cervical ganglia that AHP slow was inhibited by ryanodine with no effect on RMP. It has been proposed previously, based on the differential effects of barium, that the gKCa underlying the AHP slow and the RMP in AH neurons is the same channel but operated by different Ca\textsuperscript{2+} pools (Hirst et al. 1985; North and Tokimasa 1987). The simplest explanation of our data is that gKCa is activated by the high resting levels of [Ca\textsuperscript{2+}]\text{cyt} (200 nM) to keep the RMP low. During AP firing, CICR further elevates the global Ca\textsuperscript{2+} leading to activation of these same channels to evoke AHP slow. Therefore, gKCa appears to be regulated solely by [Ca\textsuperscript{2+}]\text{cyt}, rather than being regulated by separate Ca\textsuperscript{2+} pools.

Ca\textsuperscript{2+} transients were evoked by APs, as has previously been reported in myenteric AH (Tatsumi et al. 1988; Vogalis et al. 2000a), myenteric S (Shuttleworth and Smith 1999), nodose (Cohen et al. 1997), sympathetic (Hua et al. 1993), and hippocampal neurons (Lancaster and Zucker 1994). There was a linear relationship found between the number of APs evoked and the amplitude of the Ca\textsuperscript{2+} transient. The maximum stimulus in this study elicited 15 APs and no saturation of the amplitude of the Ca\textsuperscript{2+} transient was recorded. Cohen et al. (1997) reported a quasi-linear relationship between the number of action potentials and Ca\textsuperscript{2+} transient amplitude in nodose neurons up to a 20 AP stimulus, at which point the Ca\textsuperscript{2+} transient amplitude/CICR became saturated.

There is a low activation threshold for induction of CICR in AH neurons, as compared with the activation threshold that has been reported in Purkinje (Llano et al. 1994), sympathetic ganglia (Hua et al. 1993), and dorsal root ganglion (DRG) neurons (Shmigol et al. 1995). Subthreshold stimuli do not evoke AHP\textsubscript{slow} and hence CICR, as has previously been reported (North 1973) in AH neurons. However, slow AHPs in celiac neurons, which were subsequently shown to be inhibited by ryanodine (Jobling et al. 1993), are activated by subthreshold depolarizing pulses (Cassell and McLachlan 1987) and thus have an even lower activation threshold than AH neurons. In myenteric AH neurons, a single AP is a sufficient stimulus to trigger CICR, but does not maximally activate CICR. This is quite different from CICR in cardiac myocytes, where each single AP triggers a maximal global Ca\textsuperscript{2+} release event (Niggli 1999) and may reflect a greater degree of processing control in AH sensory neurons.

As in nodose neurons (Cohen et al. 1997), in myenteric AH neurons there is a decrease in the slope of the linear relationship between the number of APs and the amplitude of the Ca\textsuperscript{2+} transient in the presence of ryanodine. Hence, as the number of spikes is increased, CICR is increased. This may be due to either an increase in the activity of ryanodine receptors or a decrease in the effector activity of ryanodine.
recruitment of more ryanodine receptors. Estimates from this study suggest that CICR from ryanodine receptors contributes ~30 nM of the ~50 nM change in [Ca\(^{2+}\)], triggered by each AP. Thus, CICR amplifies the change in [Ca\(^{2+}\)] by two- to threefold, whereas in nodose neurons a 10–20 fold amplification of Ca\(^{2+}\) transients induced by CICR was found (Cohen et al. 1997).

As reported previously in myenteric AH (Tatsumi et al. 1988; Vogalis et al. 2000a) and nodose neurons (Cohen et al. 1994), there was a correlation between the durations of the Ca\(^{2+}\) transient and AHP\(_{slow}\). The duration of the Ca\(^{2+}\) transient was consistently shorter than the duration of AHP\(_{slow}\), as has previously been reported (Hanani and Lasser-Ross 1997). The relationship between the durations was not significantly altered by ryanodine. The tonic channel that is responsible for AHP\(_{slow}\) in myenteric AH neurons was identified as a Ca\(^{2+}\)-activated potassium channel (Hirst et al. 1974, 1985b; North 1973). Thus, a prolonged elevation in [Ca\(^{2+}\)], provides the sustained activation of g\(_{K_{Ca}}\) which produces AHP\(_{slow}\).

The contribution of ryanodine-sensitive intracellular Ca\(^{2+}\) stores to AHP\(_{slow}\) was assessed using ryanodine and caffeine. The size of both the evoked Ca\(^{2+}\) transients and the afterhyperpolarization were significantly attenuated to similar degrees by ryanodine. Ryanodine reduced the amplitude (40% of control) and duration (44% of control) of the Ca\(^{2+}\) transients and reduced the amplitude (41% of control) and duration (34% of control) of the afterhyperpolarization. In addition, caffeine (1 mM) increased the amplitude of the evoked Ca\(^{2+}\) transients by 50%. Therefore, at least 60% of the afterhyperpolarization in AH neurons is mediated through CICR via ryanodine receptors. Ryanodine has been reported to have a variety of effects in different neurons. A partial block of AHP\(_{slow}\) was reported in CA pyramidal neurons (Pineda et al. 1999; Tanabe et al. 1998) and celiac ganglia (Jobling et al. 1993), while a complete block was reported in nodose neurons (Moore et al. 1998), dorsal motor nuclei (Sah and McLachlan 1991), and otic ganglia (Yoshizaki et al. 1995). No ryanodine block was reported in hippocampal CA1 neurons (Zhang et al. 1995) or sympathetic neurons (Goh et al. 1992). The mechanism through which the remainder of AHP\(_{slow}\) is mediated in myenteric AH neurons remains a source of speculation. This remnant of AHP\(_{slow}\) may be mediated by Ca\(^{2+}\)-influx associated with spike firing, Ca\(^{2+}\) release from different intracellular Ca\(^{2+}\) stores, or a signal transduction cascade.

Ryanodine completely abolished sEPSPs in myenteric AH neurons, indicating that CICR is critical in either the synaptic transmission or transduction of sEPSPs in these cells. Although previous studies in bullfrog sympathetic ganglia have indicated that ryanodine-sensitive stores can have only a modulatory effect on slow synaptic transmission (Cao and Peng 1999), this is the first report to our knowledge of a blockade by ryanodine of synaptic transmission. The mechanism responsible for this blockade may be a mix of both presynaptic and postsynaptic effects and clearly requires further investigation.

The characteristic properties of AH neurons are dramatically influenced by the levels of free [Ca\(^{2+}\)]. The results of this study indicate that AH neurons have a high resting level of [Ca\(^{2+}\)], compared with myenteric S neurons. Experimental perturbations that effectively diminish the amounts of free [Ca\(^{2+}\)], depolarize AH neurons and alter their electrical properties, making them virtually indistinguishable from S neurons. [Ca\(^{2+}\)] rises in response to AP firing and activates a Ca\(^{2+}\)-activated potassium conductance to “gate” sensory neuron output. Furthermore, the results of this study indicate that the source of any changes in [Ca\(^{2+}\)], do not influence the close relationship between [Ca\(^{2+}\)], and RMP. In the presence of ryanodine, if the amplitude of a Ca\(^{2+}\) transient is matched to one in control conditions, the amplitude of AHP\(_{slow}\) evoked is the same. In addition, the strong correlation found between AHP\(_{slow}\) and Ca\(^{2+}\) transient durations is not affected by ryanodine. Therefore, although other intracellular messengers undoubtedly influence their behavior (Bertrand and Galligan 1995; Zafirov et al. 1985), the level of free [Ca\(^{2+}\)], clearly is an important modulator of the activity of AH neurons.

In summary, CICR modulation of [Ca\(^{2+}\)], plays a crucial role in regulating the excitability of myenteric AH neurons. These neurons appear to be most similar to nodose ganglia neurons, as CICR is activated by every action potential. Blockade of CICR by ryanodine has two major effects, an inhibition of AHP\(_{slow}\) and blockade of sEPSPs. Hence, CICR modulation of [Ca\(^{2+}\)], plays a critical role in gating the transmission of sensory information to the output processes of myenteric AH neurons.

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