Localized IP$_3$-evoked Ca$^{2+}$ Release Activates a K$^+$ Current in Primary Vagal Sensory Neurons

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Abstract

Electrophysiological and microfluorimetric techniques were used to determine whether intracellular photorelease of caged IP$_3$, and the consequent release of Ca$^{2+}$, could trigger a Ca$^{2+}$-activated K$^+$ current (I$_{IP3}$). Photorelease of caged IP$_3$ evoked an I$_{IP3}$ that averaged 2.36 $\pm$ 0.35 pA/pF in 24 of 28 rabbit primary vagal sensory neurons (nodose ganglion neurons, NGNs) voltage-clamped at $-50$ mV. I$_{IP3}$ was abolished by intracellular BAPTA (2 mM), a Ca$^{2+}$ chelator. Changing the K$^+$ equilibrium potential by increasing extracellular K$^+$ ion concentration caused a predicted Nernstian shift in the reversal potential of I$_{IP3}$. These results indicated that I$_{IP3}$ was a Ca$^{2+}$-dependent K$^+$ current. I$_{IP3}$ was unaffected by three common antagonists of Ca$^{2+}$-activated K$^+$ currents: bath-applied iberiotoxin (50 nM) or apamin (100 nM), and intracellular 8-Br-cAMP (100 $\mu$M) included in the patch pipette. We have previously demonstrated that both IP$_3$-evoked Ca$^{2+}$ release and Ca$^{2+}$-induced Ca$^{2+}$ release (CICR) are co-expressed in NGNs, and that CICR can trigger a Ca$^{2+}$-activated K$^+$ current. In the present study, using caffeine, a CICR agonist, to selectively attenuate intracellular Ca$^{2+}$ stores, we showed that IP$_3$-evoked Ca$^{2+}$ release occurs independently of CICR, but interestingly, that a component of I$_{IP3}$ requires CICR. These data suggest that IP$_3$-evoked Ca$^{2+}$ release activates a K$^+$ current that is pharmacologically distinct from other Ca$^{2+}$-activated K$^+$ currents in NGNs. We describe several models that explain our results based on Ca$^{2+}$ signaling microdomains in NGNs.
Introduction

Cytosolic Ca\(^{2+}\) ions are ubiquitous second messengers that regulate a broad spectrum of cellular phenomena, including membrane excitability, ion channel gating (Hille 2001), gene expression (Bito et al. 1996), neurotransmitter release (Katz and Miledi 1968), muscle contraction (Fabiato and Fabiato 1975), and secretion of hormones (Curry et al. 1968) and digestive juices (Petersen 1992). Increases in cytosolic free Ca\(^{2+}\) ion concentration ([Ca\(^{2+}\)]\(_i\)) can result from Ca\(^{2+}\) influx through the plasma membrane, Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores through intracellular Ca\(^{2+}\) release channels, or alterations in Ca\(^{2+}\) ATPase activity or Na\(^+\)/Ca\(^{2+}\) exchange.

Two types of intracellular Ca\(^{2+}\) release channels exist: ryanodine receptor (RyR) channels, and d-myo-inositol 1,4,5-trisphosphate receptor (IP\(_3\)R) channels (Berridge 1998). While these channels both mediate Ca\(^{2+}\) release from intracellular stores, they differ in their mechanisms of activation. Ca\(^{2+}\) release via RyRs is activated by increases in [Ca\(^{2+}\)]\(_i\) (Ca\(^{2+}\)-induced Ca\(^{2+}\) release, CICR). Typically, Ca\(^{2+}\) ions that trigger CICR arise from Ca\(^{2+}\) influx through plasma membrane voltage- or ligand-gated channels (for review of CICR in neurons, see Verkhratsky and Shmigol 1996). In contrast, Ca\(^{2+}\) release through IP\(_3\)Rs is activated by increases in [IP\(_3\)] (IP\(_3\)-induced Ca\(^{2+}\) release). IP\(_3\) is normally generated through cleavage of phosphoinositide lipids by phospholipase C (PLC) coupled to cell-surface receptors (Berridge 1993).

One important role of intracellular Ca\(^{2+}\) ions is the regulation of Ca\(^{2+}\)-activated K\(^+\) currents (I\(_{K(Ca)}\)) that can influence both action potential shape and the pattern of action potential firing. Such I\(_{K(Ca)}\), which are triggered as a consequence of action potentials, fall into at least three temporally distinct classes: I\(_C\), a fast current lasting 5 – 20 msec; I\(_{AHP}\), a current with intermediate kinetics, lasting hundreds of msec; and I\(_{sAHP}\), a slow...
current lasting many hundreds to thousands of msec. These currents can be further distinguished based on their disparate physiological, pharmacological, and biophysical properties (Sah 1996). $I_C$ underlies the fast after-hyperpolarization (fAHP) following an action potential, is activated by both membrane depolarizations and increases in $[Ca^{2+}]_i$, and is inhibited by tetraethylammonium (TEA) ions (<1 mM), iberiotoxin, and charybdotoxin (Adams et al. 1982; Shao et al. 1999). $I_C$ is believed to be mediated by large-conductance (100 – 200 pS) $Ca^{2+}$-activated $K^+$ channels (BK channels) (Shao et al. 1999; Sah and Louise Faber 2002). $I_{AHP}$ underlies the medium AHP (mAHP), is activated by increases in $[Ca^{2+}]_i$, is voltage-independent, and is inhibited by TEA ions (~10 – 20 mM) and apamin (50 – 200 nM; Pennefather et al. 1985). Small conductance (5 – 15 pS) $Ca^{2+}$-activated $K^+$ channels (SK channels), which are voltage-insensitive, and some of which are apamin-sensitive, are believed to mediate $I_{AHP}$ (Sah and Louise Faber 2002). $I_{sAHP}$ underlies the slow afterhyperpolarization evoked by action potentials in primary vagal afferents. It is activated by increases in $[Ca^{2+}]_i$, is voltage-independent, and is inhibited by elevations in intracellular [cAMP] (Cordoba-Rodriguez et al. 1999). The molecular identity of the channels that mediate $I_{sAHP}$ remains undefined (Sah and Louise Faber 2002).

In a population of primary vagal sensory neurons (nodose ganglion neurons, NGNs), action potential-triggered CICR activates an $I_{sAHP}$ that controls spike frequency adaptation (Weinreich and Wonderlin 1987; Moore et al. 1998). We recently reported that photoreleased IP$_3$ evokes intracellular $Ca^{2+}$ release in NGNs (Hoesch et al. 2002). In the present study, we ask whether IP$_3$-induced $Ca^{2+}$ release, like CICR, could also activate membrane currents in NGNs. Our results show that IP$_3$-evoked intracellular
\( \text{Ca}^{2+} \) release can activate a \( \text{K}^{+} \) current, \( I_{\text{IP3}} \), with properties distinct from those of the \( I_{\text{sAHP}} \).

**Methods**

**Cell Dissociation**

Male New Zealand White rabbits, weighing 3 – 4 kg, were purchased from Robinson Services (Clemmons, NC) and killed by sodium pentobarbital overdose (100 mg/kg), as approved by the Institutional Animal Care and Use Committee of the University of Maryland Biotechnology Institute. Dissociated NGNs were prepared as described previously (Leal-Cardoso et al. 1993) with the exception that sterile technique was used and the final neuronal pellet was resuspended in Leibovitz L-15 medium (Gibco-BRL, Grand Island, NY) containing 10% fetal bovine serum (FBS, JRH Bioscience, Lenexa, KS), and 0.1% by volume penicillin-streptomycin (10,000 units penicillin G and 10,000 \( \mu \)g/ml streptomycin sulfate; Gibco-BRL, Grand Island, NY). The resulting cell suspension was then plated in 0.2-mL aliquots onto 25-mm round No. 1 glass coverslips (Fisher Scientific, Newark, DE) coated with poly-D-lysine (0.1 mg/mL; M.W. 30 – 70 kD, Sigma, St. Louis, MO). NGNs were incubated at 37°C for 12 h, then maintained at room temperature to prevent neurite outgrowth, and used for experiments for up to 48 h.

**\([\text{Ca}^{2+}]_i\) Measurements and Calibration**

Fluo-3 indicator was used to measure \([\text{Ca}^{2+}]_i\). NGNs were loaded with 1 \( \mu \)M fluo-3/AM (Teflabs, Inc., Austin, TX) in L-15 medium for 1 – 3 hr. Single-cell microfluorimetry was performed as previously described (Cohen et al. 1997), except that excitation was
at 490 nm, and that the fluorescence emission was passed through a 530-nm bandpass filter before photometric quantitation. Fluo-3 fluorescence intensity records were corrected by subtracting the background fluorescence intensity, measured after cell lysis with digitonin (20 µM). Ca²⁺ transient amplitudes are reported as changes in fluorescence intensity (ΔF), or changes in fluorescence intensity normalized to baseline fluorescence intensity immediately preceding the transient (ΔF/F₀).

**Extracellular Solutions**

Neurons were superfused with physiological saline solution (20-24°C) that contained (mM): 120 NaCl, 3.0 KCl, 1.0 NaH₂PO₄, 25.0 NaHCO₃, 1.5 MgCl₂, 2.2 CaCl₂, and 10.0 dextrose, equilibrated with 95%O₂-5%CO₂; pH adjusted to 7.4. For photorelease experiments where it was important to eliminate any potential Ca²⁺ influx, nominally Ca²⁺-free medium, from which CaCl₂ was omitted, was used. For experiments that examined the effects of shifting E_K, NGNs were superfused with saline solution with elevated [K⁺] (High-K⁺ medium) that contained (mM): 100 NaCl, 23.0 KCl, 1.0 NaH₂PO₄, 25.0 NaHCO₃, 1.5 MgCl₂, 2.2 CaCl₂, and 10.0 dextrose, equilibrated with 95%O₂-5%CO₂; pH adjusted to 7.4. In High-K⁺ medium, [Cl⁻]₀ and E_Cl are unchanged.

A recording chamber with a narrow rectangular flow path allowed superfusion of NGNs on a glass coverslip at 7 ml/min via a gravity flow system. The chamber was mounted on an inverted microscope (Diaphot, Nikon, Melville, NY) equipped with a X40 phase-contrast oil-immersion objective (Fluor, N.A. 1.3, Nikon) to allow fluorescence measurements and direct visualization of NGNs for positioning patch pipettes. In experiments where Ca²⁺-free solution was used, nominally Ca²⁺-free physiological saline was superfused for at least 30 sec before and after drug application. Solution
changes were complete in 14 sec, as determined with fluorescent tracers.

**Electrophysiological Measurements**

**Intracellular Solutions**

Control patch-pipette solutions contained (mM): 152 KCH$_3$SO$_3$, 10.0 HEPES, 2.0 MgCl$_2$, 1.0 Na$_3$ATP, 1.0 Na$_3$GTP, and 1.0 KCl; pH adjusted with KOH to 7.2. KCH$_3$SO$_3$ was used to avoid excess intracellular Cl$^-$, which is known to inhibit G proteins (Lenz et al. 1997). Aliquots of stock pipette solution were stored frozen at 0°C. Each aliquot of pipette solution was thawed, stored on ice, and used for only one day. K$_5$Fluo-3 was added to the pipette solution to a final concentration of 50 µM; sufficient CaCl$_2$ was added to set free [Ca$^{2+}$] = 100 nM (taking the Ca$^{2+}$ dissociation constant of fluo-3 under physiological conditions to be 400 nM (Minta et al. 1989)). For photolysis experiments, 0.5 mM of the trisodium salt of d-myo-inositol 1,4,5-trisphosphate P$_{4(5)}$-1-(2-nitrophenyl)ethyl ester (caged IP$_3$) was included in the pipette solution, which was loaded only into the tip of the pipette. The shaft of the pipette was filled with pipette solution containing no caged IP$_3$. For BAPTA experiments, NGNs were loaded with 20 µM BAPTA/AM (Molecular Probes, Eugene, OR) in L-15 medium containing 10% v/v FBS and 0.0075% w/v of the surfactant, Pluronic F-127 (BASF Wyandotte, Wandotte, WI) for 1-2 hours. The patch-pipette solutions contained (mM): 146.4 KCH$_3$SO$_3$, 10.0 HEPES, 2.0 MgCl$_2$, 1.0 Na$_3$ATP, 1.0 Na$_3$GTP, 1.0 KCl, 2.0 K$_4$BAPTA; pH adjusted with KOH to 7.2. Sufficient CaCl$_2$ was added to set free [Ca$^{2+}$] ≈ 75 nM (taking the Ca$^{2+}$ dissociation constant of BAPTA under physiological conditions to be approximately 190 nM; Tsien 1980). Caged IP$_3$, 8-Br-cAMP, K$_4$BAPTA, and K$_5$Fluo-3 were delivered via patch pipette and allowed to equilibrate for at least 5 min before start of experiments.
Patch-Clamp Recording

The whole-cell configuration of the patch clamp technique (Hamill et al. 1981) was used to measure membrane currents. Patch pipettes (2 – 3 MΩ), fabricated from borosilicate glass stock (O.D. 1.5 mm, I.D. 1.12 mM, World Precision Instruments, Sarasota, FL) on a Flaming-Brown P97 micropipette puller (Sutter Instruments, Novato, CA) were connected to an Axopatch 200B amplifier (Axon Instruments, Union City, CA). Data acquisition through the Digidata 1200 interface was controlled with pClamp 8 software (Axon Instruments). NGNs were first loaded with fluo-3/AM to allow fluorimetric measurement of [Ca^{2+}] in parallel with electrophysiological recording. After a gigaohm seal (>1.0 GΩ) was formed, the whole-cell configuration was established, with neurons voltage-clamped to -50 mV. Membrane input resistance and capacitance were determined from current transients elicited by 5 mV depolarizing voltage steps from the holding potential. NGNs were considered suitable for study if membrane input resistance was >150 MΩ and holding current was <200 pA.

Ramp Protocols

To determine I-V relations, the following voltage command protocol was applied to voltage-clamped NGNs: 1) From a holding potential of –50 mV, the membrane potential was stepped to +50 mV for 100 msec to inactivate voltage-gated Na^+ channels; 2) An I-V relation was then generated by a voltage ramp that decreased from +50 mV to –110 mV at 1 mV/msec; 3) At the end of the ramp, the cell was returned to a holding potential of –50 mV. This protocol was applied twice to each NGN tested: first immediately before IP_3 photorelease, and again two seconds after IP_3 photorelease, when the IP_3-evoked current had developed substantially (a representative time course...
of the current is shown in Fig. 1). Taking the difference between these two I-V relations yielded the I-V relation for the IP$_3$-evoked current.

**Flash Photolysis of Caged IP$_3$ (IP$_3$ Photorelease)**

To photolyze caged IP$_3$, we delivered 500-msec flashes of ultraviolet (UV) light to NGNs loaded with 0.5 mM caged IP$_3$. The multiline UV output (333.6 – 363.8 nm) of an argon ion laser (BeamLok 2065-7S, Spectra-Physics, Mountain View, CA) was used for photolysis. The output beam of the laser was directed through an objective lens (U-27X, Newport Corp., Irvine, CA) and focused onto the 50-µm diameter silica core of a step-index multimode optical fiber (CeramOptec, East Longmeadow, MA) that was cleaved orthogonally to expose an optically flat surface. For adjusting alignment, the objective lens and the chuck holding the optical fiber were both mounted on a multimode fiber coupler assembly (F-915T, Newport Corp. Irvine, CA). The output end of the optical fiber was ensheathed in a glass pipette, which was mounted on a hydraulic micromanipulator to allow the output of the fiber to be directed onto cells being viewed under the microscope. The duration of UV flashes was regulated by a laser shutter (LS200F, NM Laser Products, Sunnyvale, CA) interposed between the laser head and the UV objective lens of the coupler. Shutter gating was controlled by TTL signals through pClamp 8 software (Axon Instruments, Union City, CA).

**Data Analysis**

Unless otherwise stated, the following conventions apply: 1) numerical results are reported as a mean ± standard error of the mean (SEM); 2) when multiple responses were elicited from a NGN, the response amplitude under a given
experimental condition was normalized to the control response amplitude; 3) Student's t-test (two-tailed) was used to assess significant differences between calculated means and p < 0.05 was considered significant. Origin software (Microcal Software, Inc., Northampton, MA) was used for all data analysis and least-squares curve fitting.

Reagents

Reagents were obtained from the following sources: caged IP$_3$, 8-Br-cAMP, heparin sulfate (13.5 – 15 kD), and ryanodine from Calbiochem (La Jolla, CA); acetoxymethyl ester and pentapotassium salt of fluo-3 (fluo-3/AM and K$_5$Fluo-3) from Teflabs, Inc. (Austin, TX); caffeine and forskolin from Sigma (St. Louis, MO); iberiotoxin and apamin from Tocris (Ellisville, MO); acetoxymethyl ester and tetrapotassium salt of BAPTA (BAPTA/AM and K$_4$BAPTA) from Molecular Probes (Eugene, OR). Inorganic salts were from VWR (Piscataway, NJ).

Reagent solutions were prepared daily from concentrated stock solutions in dimethylsulfoxide (Fisher Biotech, Fair Lawn, NJ) or water that were stored frozen. Unless otherwise noted, drugs were delivered via the superfusate by switching a three-way valve to a reservoir containing a known concentration of the drug in the extracellular solution.

Results

We have previously reported (Hoesch et al. 2002) that intracellular photorelease of IP$_3$ evokes Ca$^{2+}$ release from intracellular stores in primary vagal sensory neurons (nodose ganglion neurons, NGNs). Because these neurons are known to express a variety of Ca$^{2+}$-activated currents (Higashi et al. 1984; Fowler et al. 1985; Weinreich and
Wonderlin 1987; Christian et al. 1994; Schild et al. 1994; Hay and Kunze 1994b; Lancaster et al. 2002), we asked whether IP$_3$-evoked Ca$^{2+}$ release could also activate Ca$^{2+}$-dependent membrane currents. To address this question, we loaded NGNs with caged IP$_3$ and fluo-3 via patch pipette, and measured whole-cell current and [Ca$^{2+}$], simultaneously at a holding potential of -50 mV. As shown in Fig. 1, IP$_3$ photorelease triggered both a transient rise in [Ca$^{2+}$], i.e. a Ca$^{2+}$ transient (trace 1) and an outward current (trace 2). During photorelease episodes, NGNs were superfused with nominally Ca$^{2+}$-free medium to prevent Ca$^{2+}$ influx. In 24 out of 28 NGNs (86%) tested, IP$_3$ photorelease evoked an outward current (I$_{IP3}$; Fig. 1, trace 1) that averaged 108.1 ± 16.2 pA, or 2.36 ± 0.35 pA/pF when normalized to membrane capacitance. I$_{IP3}$ decayed with a $t_{1/2}$ of 13.9 ± 3.0 sec ($n = 14$). In four control NGNs loaded via patch pipette with fluo-3, but no caged IP$_3$, UV pulses did not evoke detectable currents or Ca$^{2+}$ transients. Similarly, in ten control NGNs that were loaded with fluo-3/AM only and not patched, UV pulses never evoked Ca$^{2+}$ transients. These control experiments indicate that the photorelease-evoked Ca$^{2+}$ transients and currents were activated by IP$_3$, and not by UV light alone. Intracellular application of heparin (13.5 – 15 kD, 1 mg/ml), a selective IP$_3$R antagonist (Ehrlich et al. 1994), completely inhibited both I$_{IP3}$ and the Ca$^{2+}$ transient evoked by IP$_3$ photorelease ($n = 3$; data not shown). Thus, functional IP$_3$Rs are required for both I$_{IP3}$ and the IP$_3$-evoked Ca$^{2+}$ transient (see also Hoesch et al. 2002).

Because IP$_3$ photorelease activated a Ca$^{2+}$ transient and an outward I$_{IP3}$ in parallel, I$_{IP3}$ could be a Ca$^{2+}$-activated K$^+$ current (I$_{K(Ca)}$). To determine if I$_{IP3}$ was a K$^+$ current, we tested whether its reversal potential ($E_{rev}$) showed a Nernstian dependence on extracellular [K$^+$]. Ramp voltage commands were used to generate I-V relations, as described in Methods. Trace A in Fig. 2 is the averaged I-V relation of I$_{IP3}$ determined in
24 NGNs in normal physiological saline containing 3 mM K\(^+\). The I-V relation showed slight outward rectification and \(E_{\text{rev}} = -90.4 \pm 2.3 \text{ mV}\). The proximity of \(E_{\text{rev}}\) to \(E_K\) (-100.0 mV) suggested that \(I_{IP3}\) may be principally a K\(^+\) current. To further test the role of K\(^+\) ions as the predominant charge carrier of \(I_{IP3}\), we examined the effects of changing \(E_K\) on the \(E_{\text{rev}}\) of \(I_{IP3}\). Superfusing NGNs with High-K\(^+\) medium containing 23 mM K\(^+\) shifted \(E_K\) to -48.5 mV without changing \(E_{Cl}\) (see Methods). Trace B in Fig. 2 is the averaged I-V relation for \(I_{IP3}\) in High-K\(^+\) medium for four NGNs, with \(E_{\text{rev}} = -40.8 \pm 1.6 \text{ mV}\). Thus, raising [K\(^+\)]\(_o\) from 3 to 23 mM shifted the \(E_{\text{rev}}\) of \(I_{IP3}\) by 49.6 mV, close to the value expected for ideal Nernstian behavior (51.5 mV). Under the ionic conditions used, Cl\(^-\) was the only other ion that might have carried an outward current at the holding potential of -50 mV (\(E_{Cl} = -82.5 \text{ mV}\)). This possibility was ruled out because, while \(E_{Cl}\) was kept constant, a large positive shift in \(E_K\) caused a corresponding shift in \(E_{\text{rev}}\) (Fig. 2, trace B). Therefore, our results indicate that \(I_{IP3}\) is principally a K\(^+\) current. Most ion channels have imperfect selectivity, and K\(^+\) channels typically exhibit detectable Na\(^+\) permeability. From the values of \(E_K\), \(E_{Na}\), and \(E_{\text{rev}}\) in normal and High-K\(^+\) media, the relative permeability of Na\(^+\) to K\(^+\) for \(I_{IP3}\) was estimated, using the GHK equation, to be \(P_{Na}/P_K = 0.037 \pm 0.028\).

To test the Ca\(^{2+}\)-dependence of \(I_{IP3}\), we examined the effect of intracellular BAPTA, a high-affinity Ca\(^{2+}\) chelator. NGNs were loaded with caged IP\(_3\), BAPTA, and fluo-3 via patch pipette for these experiments. Trace C in Fig. 2 shows the averaged I-V relation for \(I_{IP3}\) in the presence of intracellular BAPTA for four NGNs. The flat I-V relation for \(I_{IP3}\) indicates that BAPTA precluded \(I_{IP3}\). As expected, in these same NGNs, IP\(_3\)-evoked Ca\(^{2+}\) transients were also completely prevented by BAPTA (data not shown). Because BAPTA blocked both \(I_{IP3}\) and IP\(_3\)-evoked Ca\(^{2+}\) transients, we infer that \(I_{IP3}\) is
Ca^{2+}-dependent, rather than being directly activated by photoreleased IP_{3}. Furthermore, because all IP_{3} photorelease experiments were performed in Ca^{2+}-free medium, which eliminated Ca^{2+} influx, IP_{3}-evoked Ca^{2+} transients are attributable to intracellular Ca^{2+} release. Therefore, I_{IP3} requires IP_{3}-evoked intracellular Ca^{2+} release. These results, together with the finding that I_{IP3} is primarily a K^{+} current, suggest that I_{IP3} is an I_{K(Ca)}.

Because I_{IP3} is dependent on an elevation of [Ca^{2+}]_i, we asked if there was a correlation between the amplitudes of I_{IP3} and the activating Ca^{2+} transient. Fig. 3 is a plot of I_{IP3} (normalized to membrane capacitance) versus peak Ca^{2+} transient amplitude (normalized to baseline fluorescence intensity measured immediately preceding photorelease), for 24 NGNs that expressed I_{IP3}. Linear regression of the data yielded a correlation coefficient of R = 0.0267, indicating essentially no correlation between the amplitude of the IP_{3}-evoked Ca^{2+} release and the amplitude of I_{IP3}. Thus, I_{IP3} appears insensitive to the magnitude of the global, whole-cell Ca^{2+} signal. One possible interpretation of this result is that while all IP_{3}Rs can contribute to the global Ca^{2+} transient, only relatively few IP_{3}Rs are closely apposed to the plasma membrane, and only Ca^{2+} release from such closely-apposed IP_{3}Rs can trigger I_{IP3}. Such a spatial arrangement constitutes a Ca^{2+} signaling "microdomain" (e.g. Delmas et al. 2002). Because of this arrangement, the K^{+} channels mediating I_{IP3} are optimally positioned for activation by localized Ca^{2+} release within the microdomain, even though such localized release may not contribute significantly to the measured *global* (whole-cell) Ca^{2+} transients.

Several types of I_{K(Ca)} exist that can be distinguished pharmacologically (for review, see Sah and Louise Faber 2002). We examined the sensitivity of I_{IP3} to three
common $I_{K(Ca)}$ antagonists: bath-applied apamin (100 nM; $I_{AHP}$ antagonist) and iberiotoxin (50 nM; IbTx; $I_{C}$ antagonist); and intracellular 8-Br-cAMP (100 $\mu$M in the patch pipette; $I_{sAHP}$ antagonist). Because apamin and iberiotoxin were bath-applied, we were able to assess inhibition by comparing $I_{IP3}$s evoked in the same neuron in the presence and absence of toxin. In the case of 8-Br-cAMP, which was included in the patch pipette solution, same-cell control was not possible. Therefore we assessed inhibition by comparing the average current amplitude in the treated population against that from the control population. Table 1 summarizes the results from this series of experiments. None of the antagonists tested significantly inhibited $I_{IP3}$ compared to control. Additionally, bath-applied forskolin (10 $\mu$M), which elevates intracellular [cAMP] by activating adenylyl cyclase (Seamon et al. 1981), also did not inhibit $I_{IP3}$ significantly ($n = 3$, data not shown).

We also examined the kinetics of $I_{IP3}$. Fig. 4A shows the time course of $I_{IP3}$ evoked by IP$_3$ photorelease resulting from a 500-msec UV pulse (record shown is the average of 12 individual traces). There appears to be little latency between the start of photorelease and current onset. To allow closer examination of the kinetics of current onset, the portion of the trace corresponding to the start of photorelease is shown at higher resolution in Fig. 4B. Although noise in the data precluded precise quantitation of latency, visual inspection suggests that any latency between the start of photolysis and current onset can be no more than 50 msec.$^2$

We have previously reported that NGNs exhibit robust CICR (Cohen et al. 1997; Cordoba-Rodriguez et al. 1999; Hoesch et al. 2001). Because IP$_3$–evoked Ca$^{2+}$ release may, in turn, trigger CICR, we asked if CICR contributes to the activation of $I_{IP3}$. To address this question, caffeine (Caf), a CICR agonist, was used to selectively deplete
Caf-releasable intracellular $\text{Ca}^{2+}$ stores on which CICR depends (Trafford et al. 1998; Trafford et al. 2001). The trace in Fig. 5A shows a $[\text{Ca}^{2+}]_i$ record from a NGN, to which six 10-sec pulses of 10 mM Caf were applied at 15-sec intervals. This "Caf-pulse series" was followed by a 10-sec "Test" Caf pulse, which was delivered 75 sec after completion of the Caf-pulse series. The six Caf pulses evoked progressively smaller $\text{Ca}^{2+}$ responses, and the test Caf pulse was markedly diminished relative to the first Caf-evoked response. Similar profiles of responses to caffeine were observed in four other NGNs. The results from all five NGNs are summarized in Fig. 5B, which shows the average response to each Caf pulse. In each NGN, all responses were normalized to the first response. Each point in Fig. 5B is the average of responses evoked in two intact NGNs and in three NGNs patch-clamped at $-50 \text{ mV}$; the responses from these two groups were not significantly different. These results show that the Caf-pulse series effectively diminishes the CICR store, and thus attenuates subsequent CICR to $49 \pm 5.5\%$ of control.

To probe the possible role of CICR in activating $I_{\text{IP3}}$, we first asked if CICR contributes significantly to IP$_3$-evoked $\text{Ca}^{2+}$ release. To test this, we examined the effects of the Caf-pulse series shown in Fig. 5A on IP$_3$-evoked $\text{Ca}^{2+}$ release. For these experiments, NGNs were loaded with fluo-3 and caged IP$_3$ via patch pipette. Shown in Fig. 6A are two pairs of $\text{Ca}^{2+}$ transients evoked by photoreleasing IP$_3$ 300 sec apart in two separate NGNs (corresponding $I_{\text{IP3}}$s are shown in Fig. 6B). In the first NGN (left panel of Fig. 6A), there was no pharmacological manipulation during the 300-sec interval between the two photorelease events (labeled 1 and 2). In the second NGN (right panel of Fig. 6A), after the first IP$_3$ photorelease, the Caf-pulse series was applied to attenuate the CICR $\text{Ca}^{2+}$ stores before the second IP$_3$ photorelease. Comparing the
amplitudes of the second (test) response to the first (control) response shows that the
Caf-pulse series did not attenuate the second IP$_3$-evoked response relative to the first.
In these two neurons, the test-to-control amplitude ratios were 1.02 and 1.16,
respectively. In seven NGNs, the average ratio of the test response to the control
response was 1.21 ± 0.06, which was not significantly different from the ratio
determined in NGNs not treated with the Caf-pulse series protocol (ratio = 1.25 ± 0.06, n
= 10). Three of the Caf-treated NGNs were also treated with 10 µM ryanodine (Ry), a
CICR antagonist. In these three experiments, Ry was present throughout the Caf-pulse
series and during the test response, but had no additional effect (data traces not
shown); therefore, data from all NGNs treated with the Caf-pulse series were combined
for analysis. Because the Caf-pulse series attenuates CICR, but did not significantly
attenuate IP$_3$-evoked Ca$^{2+}$ transients, we infer that CICR does not contribute
significantly to the global IP$_3$-evoked Ca$^{2+}$ transient.

Although CICR did not contribute significantly to global IP$_3$-evoked Ca$^{2+}$
transients, it was conceivable that Ca$^{2+}$ release via IP$_3$Rs could trigger CICR via nearby
RyRs within a Ca$^{2+}$ signaling microdomain. Such localized CICR would not contribute
significantly to global Ca$^{2+}$ transients, but it could participate in the activation of I$_{IP3}$. To
test this possibility, we examined the effects of a Caf-pulse series (see Fig. 5) on the
magnitude of I$_{IP3}$. Fig. 6B shows the two pairs of photorelease-evoked I$_{IP3}$s recorded
simultaneously with the Ca$^{2+}$ transients shown in Fig. 6A. The left panel of Fig. 6B
shows two I$_{IP3}$s evoked 300 sec apart (labeled 1 and 2) in the untreated NGN, while the
right panel shows I$_{IP3}$s triggered in the second NGN before (1) and after (2) the Caf-
pulse series to reduce CICR Ca$^{2+}$ stores. Comparing the amplitudes of the second (test)
I$_{IP3}$ to the first (control) I$_{IP3}$ shows that the Caf-pulse series markedly attenuated the
second IP$_3$-evoked current response relative to the first. In these two neurons, the test-
to-control $I_{IP3}$ amplitude ratios were 1.09 and 0.70, respectively. In six NGNs treated with
the Caf-pulse series, the ratio of the test to control $I_{IP3}$ amplitudes averaged 0.77 ± 0.09,
which was significantly smaller (by 35%) than the average ratio (1.18 ± 0.13) recorded
in 10 control NGNs that were not subjected to the Caf-pulse series. These results
suggest that CICR can contribute to $I_{IP3}$ activation. We further tested whether random
fluctuation might have accounted for the difference in the test-to-control ratios for the
IP$_3$-evoked Ca$^{2+}$ and current responses (1.21 ± 0.06 and 0.77 ± 0.09, respectively). The
difference proved statistically significant ($p = 0.00152$) and therefore not attributable to
random fluctuation. The observation that CICR participates in activating $I_{IP3}$, and yet
does not contribute significantly to the global IP$_3$-evoked Ca$^{2+}$ transient (Fig. 6), further
suggests the existence of Ca$^{2+}$ signaling microdomains. That is, Ca$^{2+}$ release via IP$_3$Rs
could trigger CICR via nearby RyRs; together, Ca$^{2+}$ released from neighboring IP$_3$Rs
and RyRs could activate K$^+$ channels residing within the same Ca$^{2+}$ signaling
microdomain.

**Discussion**

Our major findings in rabbit primary vagal sensory neurons (nodose ganglion
neurons, NGNs) are: 1) IP$_3$-evoked Ca$^{2+}$ release activates a K$^+$ current ($I_{IP3}$); 2) $I_{IP3}$ is
insensitive to apamin, iberiotoxin and 8-Br-cAMP, three common antagonists of $I_{K(Ca)}$S;
and 3) Ca$^{2+}$-induced Ca$^{2+}$ release (CICR) consequent to IP$_3$-evoked Ca$^{2+}$ release
makes a small, but significant, contribution to the activation of $I_{IP3}$.

In the present study, we found that $I_{IP3}$ is activated by IP$_3$-evoked Ca$^{2+}$ release in
86% of rabbit NGNs, develops with little or no latency following IP$_3$ photorelease (<50
msec), has a minor dependence on CICR, and is insensitive to apamin, iberiotoxin and 8-Br-cAMP. In our previous studies, we have found that a subpopulation of rabbit NGNs (~35%) express a Ca\(^{2+}\)-activated K\(^+\) current that underlies the slow afterhyperpolarization following action potentials (I\(_{sAHP}\); Cohen et al. 1997; Moore et al. 1998; Cordoba-Rodriguez et al. 1999). Activation of the I\(_{sAHP}\) is absolutely dependent on CICR consequent to action-potential-induced Ca\(^{2+}\) influx through N-type channels, and exhibits post-spike latency to onset of ≥100 msec (Cohen et al. 1997; Moore et al. 1998; Cordoba-Rodriguez et al. 1999). We have shown that I\(_{sAHP}\) is insensitive to apamin and iberiotoxin, but is completely blocked by treatments that elevate intracellular cAMP (Cordoba-Rodriguez et al. 1999). Contrasting the above observations suggests that I\(_{IP3}\) is distinct from I\(_{sAHP}\). We have no evidence that different channels underlie these two macroscopic currents. Therefore, it is possible that a single channel protein mediates both currents, and that observed differences in pharmacology and kinetics reflect differences in regulation. Interestingly, Ca\(^{2+}\)-induced Ca\(^{2+}\) release and IP\(_3\)-induced Ca\(^{2+}\) release both give rise to global (whole-cell) Ca\(^{2+}\) transients in NGNs, and yet activate functionally distinguishable Ca\(^{2+}\)-dependent K\(^+\) currents. This suggests the existence of Ca\(^{2+}\) signaling microdomains in NGNs (see also Cordoba-Rodriguez et al. 1999).

Our data suggest several possible models for the activation of I\(_{IP3}\), some of which are illustrated in Figure 7. In the first model (Fig. 7A), Ca\(^{2+}\) is released via IP\(_3\)Rs and RyRs from separate intracellular Ca\(^{2+}\) stores. Ca\(^{2+}\) released via IP\(_3\)Rs induces consequent CICR via closely-neighboring RyRs. Ca\(^{2+}\) released through both of these channels activates closely-apposed I\(_{IP3}\) channels in the plasma membrane. Such a tight spatial organization of channels would constitute a Ca\(^{2+}\) signaling microdomain. This model fits well with two key observations. First, that global IP\(_3\)-evoked Ca\(^{2+}\) release was
not measurably affected by depletion of CICR stores (Fig. 6) implies that IP₃Rs and RyRs likely mediate Ca²⁺ release from separate intracellular Ca²⁺ stores. Second, that depletion of CICR stores attenuated Iₛ₃ without affecting global IP₃-evoked Ca²⁺ release suggests that IP₃-evoked Ca²⁺ release can trigger localized CICR to augment Iₛ₃ activation.

Other models are also conceivable where Ca²⁺ is released via IP₃Rs and RyRs from a shared intracellular Ca²⁺ store (Figs. 7B and 7C). In the model in Fig. 7B, as in the model of Fig. 7A, both CICR and IP₃-evoked Ca²⁺ release trigger Iₛ₃ within a Ca²⁺ signaling microdomain; however, Ca²⁺ is released via IP₃Rs and RyRs from a common intracellular Ca²⁺ store. Because depletion of CICR stores did not diminish global IP₃-evoked Ca²⁺ transients (Fig. 6A), shared intracellular Ca²⁺ stores must occur principally within Ca²⁺ signaling microdomains, but not to any significant extent elsewhere in the cell. In this model, depletion of CICR stores by caffeine would necessarily deplete the shared IP₃-releasable Ca²⁺ stores within microdomains, leading to diminished IP₃-evoked Ca²⁺ release, and attenuated Iₛ₃ (Fig. 6B), but would not affect global IP₃-evoked Ca²⁺ transients (Fig. 6A). An alternative common-pool model is also possible, as illustrated in Fig. 7C, where Ca²⁺ is released via IP₃Rs and RyRs from a shared intracellular Ca²⁺ store, but only Ca²⁺ released via IP₃Rs within Ca²⁺ signaling microdomains activates I₃ channels. In this model, Ca²⁺ release via RyRs would not occur within sufficiently close proximity to the plasma membrane I₃ channel to activate it. As in the model of Fig. 7B, RyRs and IP₃Rs release Ca²⁺ from a common Ca²⁺ store, depletion of CICR stores would be equivalent to depletion of IP₃-releasable Ca²⁺ stores, resulting in attenuated I₃. The models of Figs. 7B and 7C require greater structural complexity: IP₃Rs and RyRs mediate Ca²⁺ release from a common Ca²⁺ store within
Ca$^{2+}$ signaling microdomains, but from separate stores elsewhere in the cell. At present, we have no evidence to support the greater structural complexity of the latter two models. Therefore, we prefer the more parsimonious model of Fig. 7A.

Intracellular Ca$^{2+}$ release evoked by photoreleased IP$_3$ has also been reported to activate K$^+$ currents in cerebellar Purkinje neurons (Khodakhah and Armstrong 1997) and in midbrain dopamine neurons (Morikawa et al. 2000). The expression of I$_{IP3}$ by central and peripheral neurons raises the question of the nature of the physiological stimuli that trigger I$_{IP3}$. ATP, through P2Y receptor activation, can trigger IP$_3$ signaling in NGNs (Hoesch et al. 2002), as well as many other cell types (Dubyak and el-Moatassim 1993). Therefore, ATP is a probable physiological stimulus to trigger I$_{IP3}$ in NGNs. Other signaling molecules are also likely to employ IP$_3$ signaling in NGNs, and thus could activate I$_{IP3}$. In midbrain dopamine neurons, activation of both metabotropic glutamate and muscarinic acetylcholine receptors, which are known to act through IP$_3$-evoked Ca$^{2+}$ signaling, both evoked outward currents (Fiorillo and Williams 1998; Fiorillo and Williams 2000), suggesting physiological roles for IP$_3$-evoked outward currents in those neurons (Morikawa et al. 2000). Metabotropic glutamate receptors in NGNs have been physiologically characterized (Hay and Kunze 1994a) and recently cloned (Hoang and Hay 2001). Therefore, if activation of metabotropic glutamate receptors could trigger IP$_3$-evoked Ca$^{2+}$ signaling, then glutamate might evoke I$_{IP3}$ in NGNs. The prevalence of metabotropic receptors and the ubiquity of the IP$_3$ signaling pathway favor the hypothesis that I$_{IP3}$ is a common and robust mechanism through which metabotropic receptor activation could control membrane excitability.

Acknowledgements
We thank Dr. Michael Gold for helpful discussions and for reading an earlier draft of this manuscript.

 Grants

This work was supported by NIH grants GM-56481 to J.P.Y. Kao and NS-22069 to D. Weinreich.
1. The abbreviations used are: $[Ca^{2+}]_i$, cytosolic free $Ca^{2+}$ concentration; Caf, caffeine; caged IP$_3$, d-"myo"-inositol 1,4,5-trisphosphate $P_4^{1(5)}$-1-(2-nitrophenyl)ethyl ester; CICR, $Ca^{2+}$-induced $Ca^{2+}$ release; $\Delta F$, change in fluo-3 fluorescence intensity; IP$_3$, d-"myo"-inositol 1,4,5-trisphosphate; $I_{IP3}$, outward current activated by IP$_3$ photorelease; IP$_3$R, d-"myo"-inositol 1,4,5-trisphosphate receptor; NGNs, nodose ganglion neurons; Ry, ryanodine; RyR, ryanodine receptor.

2. The time interval from the start of photolysis to the release of $Ca^{2+}$ via IP$_3$Rs was reported to be of the order of 8 – 14 msec (Carter and Ogden 1997). Therefore, in our experiments, the minimum expected latency for $I_{IP3}$ onset would be 8 – 14 msec.

3. In all NGNs, the second IP$_3$-evoked $Ca^{2+}$ response was always somewhat larger than the first. The cause of this increase is not clear.
**Figure Legends**

Figure 1. IP$_3$ photorelease evokes a Ca$^{2+}$ transient and an outward current simultaneously in the same NGN. Trace 1: Ca$^{2+}$ transient evoked by IP$_3$ photorelease.

Trace 2: Outward current recorded simultaneously in the same NGN. The NGN was loaded with 500 µM caged IP$_3$ and 50 µM fluo-3 via whole-cell patch pipette and voltage-clamped at –50 mV. The filled triangle marks the start of the 500-msec UV laser pulse. The interval during which the UV flash precluded photometry is indicated by the dashed line. Changes in fluo-3 fluorescence intensity relative to baseline ($\Delta F/F_0$) are plotted. Fluorimetric and electrophysiological data were acquired at 4 Hz.

Figure 2. I-V relations for I$_{IP3}$.

Trace A: Averaged, control I-V relation for IP$_3$-evoked current (I$_{IP3}$) recorded from 24 NGNs in normal medium ([K$^+$]$_o$ = 3 mM); the trace shows slight outward rectification. Mean reversal potential, $E_{rev} = -90.4 \pm 2.3$ mV.

Trace B: Averaged I-V relation for I$_{IP3}$ recorded in High-K$^+$ medium ([K$^+$]$_o$ = 23 mM), with $E_{rev} = -40.8 \pm 1.6$ mV ($n = 4$). Compared to control, $E_{rev}$ for I$_{IP3}$ in High-K$^+$ medium was more positive by 49.6 mV, close to the 51.5 mV expected for ideal Nernstian behavior.

Trace C: Averaged I-V relation for I$_{IP3}$ recorded in the presence of intracellular BAPTA. Four NGNs were loaded via patch pipette with 2 mM BAPTA to buffer [Ca$^{2+}$]$_i$. The flat I-V relation indicates that I$_{IP3}$ was blocked by BAPTA and thus dependent on an elevation in [Ca$^{2+}$]$_i$. 

NGNs were loaded with 500 µM caged IP$_3$ and 50 µM fluo-3 via patch pipette for >5 min. Photorelease experiments were performed in nominally Ca$^{2+}$-free external medium. The horizontal dotted line marks the zero-current level. Vertical dotted lines mark the reversal potentials of I$_{IP3}$ in control (E$_{rev}$) and in High-K$^+$ medium (E$_{rev, Hi-K^+}$). I-V relations were generated by ramp protocols as described in the Methods section. For clarity, I-V relations are shown from -30 to -100 mV.

Figure 3. I$_{IP3}$ and Ca$^{2+}$ transient amplitudes are poorly correlated. For each NGN, the amplitude of I$_{IP3}$ was normalized to membrane capacitance, and then plotted against the amplitude of the IP$_3$-evoked Ca$^{2+}$ transient. Linear regression (solid line) yielded a correlation coefficient of $R = 0.027$, indicating that the two variables are poorly correlated.

Figure 4. Time course of I$_{IP3}$ onset. A: Averaged I$_{IP3}$ for 12 NGNs evoked by a 500-msec UV flash. UV flash duration is indicated by the bar below the trace. The part of the trace enclosed in the rectangular box corresponds to the start of photorelease and is shown at higher time resolution in B. B: Expanded portion of the trace in A corresponding to the start of photorelease. Solid curve is an exponential fit to the data points. The latency between IP$_3$ photorelease and onset of I$_{IP3}$ is <50 msec. The horizontal dashed line marks the zero-current level. All NGNs were loaded with 500 µM caged IP$_3$ and 50 µM fluo-3 via patch pipette for >5 min. Data were acquired at 4 kHz.

Figure 5. Repetitive caffeine pulses diminish caffeine-releasable intracellular Ca$^{2+}$ stores.
A: Six 10-sec pulses of 10 mM caffeine (Caf) were delivered at 15-sec intervals (each triangle marks the start of a Caf pulse). After 75 sec, a 10-sec "Test" pulse of 10 mM Caf was applied. Compared with the response to the first Caf pulse, the response to the "Test" Caf pulse was significantly attenuated. Fluo-3 was used to monitor changes in $[\text{Ca}^{2+}]_i$.

B: Average attenuation of the caffeine-releasable $\text{Ca}^{2+}$ store by a series of Caf pulses (following the protocol shown in A). For each of five NGNs, every response to Caf was normalized to the first response. The normalized responses from individual cells to each Caf pulse were then averaged. These data show that the Caf pulse series attenuates the Test Caf response by ~50% relative to the first (control) response. Data recorded from intact ($n = 2$) and patch-clamped ($n = 3$) NGNs were not significantly different and were grouped in this study. Error bars are $\pm$ SEM.

Figure 6. Depletion of CICR $\text{Ca}^{2+}$ stores by repetitive caffeine pulses does not attenuate $\text{Ca}^{2+}$ transients evoked by IP$_3$ photorelease, but does attenuate I$_{IP3}$.

A: Two pairs of $\text{Ca}^{2+}$ transients evoked by photoreleasing IP$_3$ 300 sec apart in two separate NGNs. In the first NGN (left panel), there was no pharmacological manipulation during the 300-sec interval between the two photorelease events (labeled 1 and 2; time of photorelease marked by filled triangle). In the second NGN (right panel), 90 sec after the first IP$_3$ photorelease (labeled 1), a "Caf-pulse series" was used to attenuate the CICR $\text{Ca}^{2+}$ stores before the second IP$_3$ photorelease. A Caf-pulse series consisted of six 10-sec pulses of 10 mM caffeine delivered at 15-sec intervals. Seventy-five seconds after the last Caf pulse, a second $\text{Ca}^{2+}$ transient (labeled 2) was
evoked by photoreleasing IP$_3$. In these two experiments, the ratio of Ca$^{2+}$ transient amplitude 2 to amplitude 1 was 1.02 in the untreated NGN and 1.16 in the NGN that received the Caf-pulse series. Therefore, diminishing the CICR Ca$^{2+}$ stores does not attenuate the IP$_3$-evoked Ca$^{2+}$ transients. NGNs were loaded with 500 µM caged IP$_3$ and 50 µM fluo-3 via whole-cell patch pipette >5 min before the first photorelease and voltage-clamped at −50 mV. Each filled triangle marks the start of a 500-msec UV laser pulse. The interval during which the UV flash precluded photometry is indicated by a dashed line in the trace. IP$_3$ photorelease was performed during superfusion of nominally Ca$^{2+}$-free medium. Changes in fluo-3 fluorescence intensity (ΔF) are plotted.

B: Two pairs of photorelease-evoked I$_{IP3}$s recorded simultaneously with the Ca$^{2+}$ transients shown in Fig. 6A. The left panel of Fig. 6B shows two I$_{IP3}$s evoked 300 sec apart (labeled 1 and 2) in the untreated NGN, while the right panel shows I$_{IP3}$s triggered in the second NGN before (1) and after (2) the Caf-pulse series to reduce CICR Ca$^{2+}$ stores. In these two experiments, the ratio of I$_{IP3}$ amplitude 2 to amplitude 1 was 1.09 in the untreated NGN and 0.70 in the NGN that received Caf-pulse series. Therefore, diminishing the CICR Ca$^{2+}$ stores by applying the Caf-pulse series markedly attenuated the second IP$_3$-evoked current response relative to the first.

Figure 7. Microdomain models of I$_{IP3}$ activation by intracellular Ca$^{2+}$ release.

A: Microdomain containing I$_{IP3}$ channels, and IP$_3$Rs and RyRs on separate Ca$^{2+}$ stores. In microdomains (delimited by dashed line) near the plasma membrane, IP$_3$-evoked Ca$^{2+}$ release can trigger CICR via closely-neighboring RyRs. IP$_3$Rs and RyRs mediate
Ca\(^{2+}\) release from separate Ca\(^{2+}\) stores. Ca\(^{2+}\) release through both IP\(_3\)Rs and RyRs in
the microdomain activates I\(_{IP3}\) channels in the plasma membrane.

**B:** Microdomain containing I\(_{IP3}\) channels, and IP\(_3\)Rs and RyRs on a *common* Ca\(^{2+}\) store.

In microdomains (delimited by dashed line), IP\(_3\)-evoked Ca\(^{2+}\) release triggers CICR via
closely-neighboring RyRs, and Ca\(^{2+}\) release through both IP\(_3\)Rs and RyRs activates I\(_{IP3}\)
channels in the plasma membrane. IP\(_3\)Rs and RyRs mediate Ca\(^{2+}\) release from a
common (shared) store in microdomains, but from separate stores elsewhere in the cell.

**C:** Microdomain containing I\(_{IP3}\) channels and IP\(_3\)Rs.

Only Ca\(^{2+}\) released from IP\(_3\)Rs in the microdomain (delimited by dashed line) can
activate I\(_{IP3}\) channels in the plasma membrane. Because RyRs are not contained within
the microdomain, Ca\(^{2+}\) release through IP\(_3\)Rs cannot trigger CICR through RyRs; nor
can Ca\(^{2+}\) release via RyRs activate I\(_{IP3}\) channels. IP\(_3\)Rs and RyRs share a common
Ca\(^{2+}\) store near the plasma membrane, but mediate Ca\(^{2+}\) release from separate stores
elsewhere in the cell.
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Table 1. Effects of antagonists of Ca\textsuperscript{2+}-activated K\textsuperscript{+} currents on I\textsubscript{IP3}.*

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Concentration</th>
<th>Site of Action</th>
<th>I\textsubscript{IP3} (pA/pF)</th>
<th>Drug/Control</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>2.36 ± 0.35</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>8-Br-cAMP</td>
<td>100 µM</td>
<td>I\textsubscript{sAHP}</td>
<td>2.05 ± 0.61</td>
<td>0.87 ± 0.29</td>
<td>6</td>
</tr>
<tr>
<td>Apamin</td>
<td>100 nM</td>
<td>I\textsubscript{AHP}</td>
<td>2.25 ± 0.75</td>
<td>1.03 ± 0.05</td>
<td>3</td>
</tr>
<tr>
<td>Iberiotoxin</td>
<td>50 nM</td>
<td>I\textsubscript{C}</td>
<td>2.30 ± 0.62</td>
<td>1.16 ± 0.17</td>
<td>4</td>
</tr>
</tbody>
</table>

*This table compares the effects of various antagonists of Ca\textsuperscript{2+}-activated K\textsuperscript{+} currents (I\textsubscript{K(Ca)s}) on an I\textsubscript{K(Ca)} evoked by IP\textsubscript{3} photorelease (I\textsubscript{IP3}). All I\textsubscript{IP3}s reported were evoked by photolysis in nodose ganglion neurons loaded with 500 µM intracellular caged IP\textsubscript{3} and bathed in Ca\textsuperscript{2+}-free medium. The peak amplitude of each I\textsubscript{IP3} was normalized to membrane capacitance for each NGN, and all of the peak amplitudes for a particular treatment were averaged and reported in the column labeled “I\textsubscript{IP3}”. Control I\textsubscript{IP3}s were evoked in the absence of any pharmacological agent. The ratio of I\textsubscript{IP3} amplitudes evoked in the presence and absence of each antagonist is reported in the “Drug/Control” column. Apamin and iberiotoxin were both applied by bath superfusion, which allowed same-cell controls; therefore, the Drug/Control ratios reported are the average of individual ratios determined in each neuron. Because the effect of 8-Br-cAMP was tested by its inclusion in the patch pipette solution, same-cell controls were not possible. Therefore, the Drug/Control ratio reported for 8-Br-cAMP is the ratio of population averages, with the S.E. derived by error propagation. All reagents were applied for ≥ 5 min before evoking I\textsubscript{IP3}. All means were not significantly different from control. I\textsubscript{C}, I\textsubscript{AHP}, and I\textsubscript{sAHP} refer to fast, intermediate, and slow I\textsubscript{K(Ca)s} that can be
distinguished on the basis of their kinetics and pharmacology (see Introduction for details).
Fig. 1 (Hoesch et al.)

\[ I_{IP3} = 150 \text{ pA} \]
\[ \Delta F/F_0 = 1.0 \]
Fig. 2 (Hoesch et al.)
Fig. 3 (Hoesch et al.)

Peak $I_{p3}$ (pA/pF) vs. Peak $\Delta F/F_0$
Fig. 4A (Hoesch et al.)
Fig. 4B (Hoesch et al.)

$\text{Time (msec)}$

$\text{I}_{IP_3} \text{ (pA)}$

$-50 \quad 0 \quad 50 \quad 100 \quad 150 \quad 200$
Fig. 5A (Hoesch et al.)
Fig. 5B (Hoesch et al.)

![Graph showing the relative response to caffeine pulse.](image-url)
Figs. 6A & 6B (Hoesch et al.)

A

\[ \frac{2}{1} = 1.02 \]

No Caf Pulses

\[ \frac{2}{1} = 1.16 \]

+Caf Pulses

B

\[ \frac{2}{1} = 1.09 \]

No Caf Pulses

\[ \frac{2}{1} = 0.70 \]

+Caf Pulses
Fig. 7 (Hoesch et al.)

A

B

C