Inositol 1,4,5-Triphosphate-Evoked Responses in Midbrain Dopamine Neurons

Hitoshi Morikawa,[1] Farzin Imani,[2] Kamran Khodakhah,[2] and John T. Williams[1]

[1]*Vollum Institute, Oregon Health Sciences University, Portland, Oregon 97201, and* [2]*Department of Physiology and Biophysics, University of Colorado Health Sciences Center, Denver, Colorado 80262*

Synaptically released glutamate evokes slow IPSPs mediated by metabotropic glutamate receptors (mGluRs) in midbrain dopamine neurons. These mGluR IPSPs are caused by release of Ca^{2+} from intracellular stores and subsequent activation of small-conductance Ca^{2+}-activated K^+ channels (SK channels). To further investigate the intracellular mechanisms involved, the effect of photolyzing intracellular caged inositol 1,4,5-triphosphate ($InsP_3$) on membrane conductance and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was examined in rat midbrain slices. Photolytic release of $InsP_3$ elicited a transient outward current and a sharp rise in $[Ca^{2+}]_i$ that lasted for ~5 sec. Apamin, a blocker of SK channels, abolished the $InsP_3$-induced outward current without affecting the rise in $[Ca^{2+}]_i$. Depleting intracellular Ca^{2+} stores with cyclopiazonic acid completely blocked both the outward current and the Ca^{2+} transient elicited by $InsP_3$. $InsP_3$-evoked Ca^{2+} mobilization was not affected by blockade of ryanodine receptors with ruthenium red, whereas depleting ryanodine-sensitive Ca^{2+} stores with ryanodine almost eliminated $InsP_3$-induced Ca^{2+} release. Increasing the size of intracellular Ca^{2+} stores by means of prolonged depolarization added a late component to the outward current and a slow component to the rising phase of $[Ca^{2+}]_i$. These effects of depolarization were blocked by ruthenium red. These results show that $InsP_3$ activates SK channels by releasing Ca^{2+} from $InsP_3$-sensitive stores that also contain ryanodine receptors. Increasing intracellular Ca^{2+} stores boosts $InsP_3$-evoked responses by invoking Ca^{2+}-induced Ca^{2+} release through ryanodine receptors. This intracellular signaling pathway may play a significant role in regulating the excitability of midbrain dopamine neurons.

Key words: intracellular Ca^{2+}; inositol 1,4,5-triphosphate; inositol 1,4,5-triphosphate receptors; ryanodine receptors; SK channels; midbrain dopamine neurons; flash photolysis

Intracellular Ca^{2+} plays a pivotal role in controlling the excitability of neurons by activating various Ca^{2+}-sensitive ion channels on the plasma membrane (Vergara et al., 1998). One major pathway to elevate intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is mobilization of Ca^{2+} from intracellular stores. This is achieved by activating inositol 1,4,5-triphosphate ($InsP_3$) receptors or ryanodine receptors located on the membranes of these stores. Firing of presynaptic fibers is known to evoke a rise in $[Ca^{2+}]_i$ in postsynaptic neurons, mainly via Ca^{2+} influx (Denk et al., 1996). Recent evidence has demonstrated that synaptically released glutamate, acting at metabotropic glutamate receptors (mGluRs), results in the generation of $InsP_3$ and subsequent mobilization of Ca^{2+} in cerebellar Purkinje neurons (Finch and Augustine, 1998; Takechi et al., 1998) and hippocampal pyramidal neurons (Nakamura et al., 1999).

Dopaminergic neurons in the ventral midbrain (ventral tegmental area and substantia nigra pars compacta) are involved in the perception of reward, motivational behavior, and the reinforcing actions of addictive drugs. In addition, impaired functioning of dopamine neurons is associated with the etiology of human disorders such as Parkinson's disease and schizophrenia (for review, see Schultz, 1998). It has recently been shown that glutamate released from presynaptic terminals elicits slow IPSPs mediated by activation of mGluRs in midbrain dopamine neurons (Fiorillo and Williams, 1998). This mGluR-mediated hyperpolarization results from mobilization of Ca^{2+} from intracellular stores, probably through production of $InsP_3$, and subsequent activation of small-conductance Ca^{2+}-activated K^+ channels (SK channels). Furthermore, spontaneous release of Ca^{2+} from internal stores has been implicated in the generation of spontaneous hyperpolarizations observed in dopamine neurons from neonatal rats (Seutin et al., 2000). The involvement of ryanodine-sensitive Ca^{2+} stores has been suggested in both cases on the basis of sensitivity to caffeine and ryanodine.

In the present study, the intracellular signaling pathway ensuing from the generation of $InsP_3$ was investigated in midbrain dopamine neurons using flash photolysis of caged $InsP_3$ loaded into the cell (Walker et al., 1989). The results demonstrate that $InsP_3$ elicits activation of SK channels through mobilization of Ca^{2+} from intracellular stores and that these $InsP_3$-sensitive stores are invariably equipped with ryanodine receptors. In addition, Ca^{2+}-induced Ca^{2+} release (CICR) through ryanodine receptors was found to boost $InsP_3$-evoked release of Ca^{2+} when internal Ca^{2+} stores were filled by depolarization-induced Ca^{2+} influx.

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Correspondence should be addressed to John T. Williams, Vollum Institute, Oregon Health Sciences University, 3181 SW Sam Jackson Park Road, Portland, OR 97201. E-mail: williamj@ohsu.edu.

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MATERIALS AND METHODS

Whole-cell recordings were made from dopamine neurons in horizontal midbrain slices (250 μm) from Wistar rats (10–21 d). Preparation of slices has been described previously (Cameron and Williams, 1994). Slices were placed in a recording chamber and superfused with warmed (35°C) physiological saline containing (in mM): 125 NaCl, 2.5 KCl, 1 $MgCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 11 glucose, 2.4 $CaCl_2$, bubbled with 95% O_2/5% CO_2; pH 7.4, 295 mOsm/kg. Cells were visualized using an upright microscope with infrared optics, and recordings were made with patch pipettes containing (in mM): 145 $KMeSO_4$, 8 KCl, 10 HEPES, 2 MgATP, and 0.2 GTP; pH 7.2, 285 mOsm/kg. The pipette solution also contained Fura-6F (500 μM; Molecular Probes, Eugene, OR) and caged $InsP_3$ [100 μM; made in house; Womack et al. (2000)]. Ruthenium red (20 μM; Calbiochem, San Diego, CA) or ryanodine (10 μM; Sigma, St. Louis, MO) was added to the pipette solution when indicated. The membrane potential was clamped at −60 mV unless stated otherwise. Recordings were started at least 15 min after whole-cell access was gained to ensure equilibrium of the pipette solution with the cytosol. Dopamine cells were identified by the presence of a large I_H current (>200 pA at −120 mV) (Johnson and North, 1992).

In experiments in which mGluR-mediated IPSCs were measured, EGTA (100 μM) was added to the pipette solution instead of Fura-6F and caged $InsP_3$. The superfusion medium contained 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]-quinoxaline (5 μM), picrotoxin (100 μM), and CGP35348 (100 μM) to block AMPA-, $GABA_A$-, and $GABA_B$-mediated synaptic currents, and slices were pretreated with MK-801 (50 μM) to block the NMDA-mediated synaptic current. A train of 10 stimuli (500 μsec at 70 Hz) was delivered every 60 sec, using a bipolar tungsten stimulating electrode placed close (30–100 μm) to the soma. The stimulus intensity was adjusted to obtain a maximal IPSC in each cell. The slow IPSC thus recorded was inhibited by the mGluR antagonist (*S*)-α-methyl-4-carboxyphenylglycine (1 mM).

Fluorescence measurements were made from an area just covering the soma, defined by a rectangular diaphragm in a conjugate image plane of the microscope. The Ca^{2+} indicator dye Fura-6F (K_d = 5.3 μM), introduced into the cell via the whole-cell patch pipette, was excited at a single wavelength of 425 ± 15 nm, and the emitted light was collected at 510 ± 15 nm. Fura indicators, when fully saturated with Ca^{2+}, emit negligible fluorescence when excited at ~420 nm (Ogden et al., 1995). $[Ca^{2+}]_i$ was calculated from the formula $[Ca^{2+}] = K_d(F - F_{min})/(F_{max} - F)$, where F is the background-corrected fluorescence, F_{min} is fluorescence of the indicator at 0 $[Ca^{2+}]$, and F_{max} is fluorescence of the indicator at saturating $[Ca^{2+}]$, which was assumed to be zero as stated above. F_{min} was obtained in each cell by assuming that the resting fluorescence emitted by the indicator, after background correction, reflects fluorescence at 50 nM $[Ca^{2+}]_i$.

A xenon arc lamp (Carin Research, Faversham, UK) produced UV pulses of ~1 msec in duration to photolyze a known fraction of caged $InsP_3$ loaded into the cytosol. The interval between two successive pulses was >4 min to allow reequilibration of caged $InsP_3$ with the cytosol. The extent of photolysis was calibrated using a fluorescent pH indicator, taking advantage of the stoichiometric release of a proton with ATP during photolysis of caged MgATP, which has the same photolytic efficiency as caged $InsP_3$ (Walker et al., 1988).

Data are expressed as means ± SEM. Statistical significance was determined with Student's *t* test (unpaired or paired) or ANOVA. The difference was considered significant if $p < 0.05$.

RESULTS

Intracellular $InsP_3$ induces SK channel activation in dopamine neurons

Whole-cell recordings (holding potential, −60 mV) were made from dopamine neurons dialyzed with the calcium indicator Fura-6F (500 μM) and caged $InsP_3$ (100 μM) in ventral midbrain slices. Pulses of UV light (1 msec) were applied to rapidly release (~3 msec) (Walker et al., 1989) a known concentration of $InsP_3$, and the resulting changes in membrane current and fluorescence were measured. Photolytic release of $InsP_3$ (30 μM) in the cytosol elicited a transient outward current and a rapid rise in $[Ca^{2+}]_i$, which decayed over a period of ~5 sec (Fig. 1*A*). The outward current invariably decayed faster than $[Ca^{2+}]_i$, which may reflect the actual subplasmalemmal $[Ca^{2+}]_i$ and also the steep Ca^{2+} concentration-dependence of apamin-sensitive SK channels (Köhler et al., 1996). On average, the amplitude of the peak outward current and the magnitude of the increase in $[Ca^{2+}]_i$ produced by $InsP_3$ (30 μM) was 104 ± 18 pA and 1.1 ± 0.1 μM, respectively (n = 18). These $InsP_3$-evoked responses were reproducible throughout the duration of recordings (~3 hr).

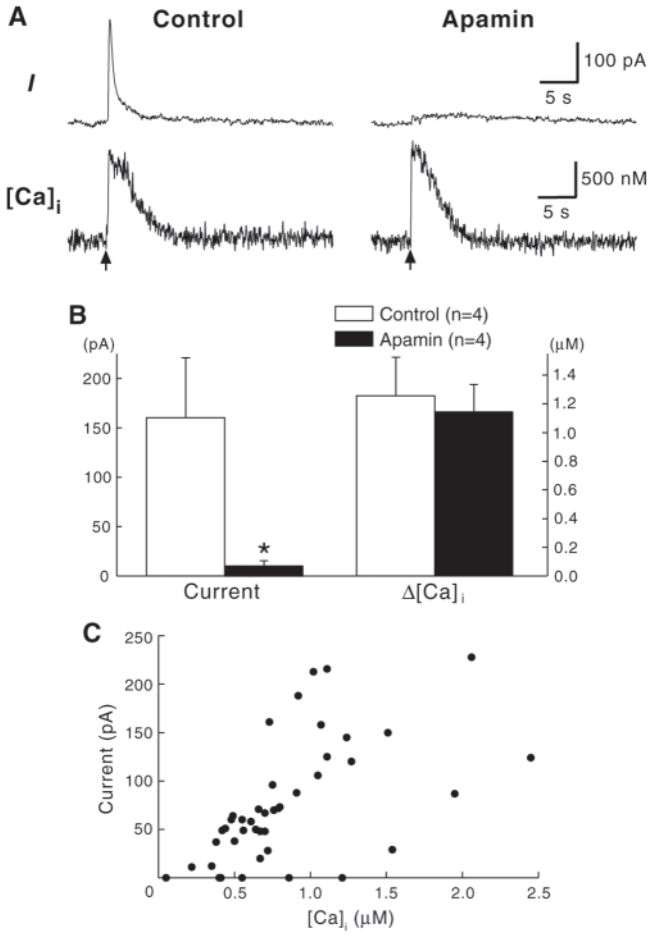

Figure 1. Photolytic release of $InsP_3$ induced apamin-sensitive SK channel activation in dopamine neurons. *A*, A pulse of UV light (1 msec) was applied at the time indicated by the *arrow* to rapidly release $InsP_3$ (30 μM) in the cytosol. The resulting changes in membrane current (*top traces*) and $[Ca^{2+}]_i$ (*bottom traces*) are shown before (*left*) and after (*right*) bath application of apamin (100 nM). *B*, Pooled data from four cells tested for the effect of apamin (100 nM). $*p < 0.05$. *C*, Scatter plot of the amplitude of outward current versus the peak $[Ca^{2+}]_i$ evoked by photolytic release of $InsP_3$ (0.75–30 μM). The data are from 18 cells. Up to five different concentrations of $InsP_3$ were tested in each cell.

Extracellular application of apamin (100 nM), a blocker of SK channels (Köhler et al., 1996), irreversibly blocked the $InsP_3$-induced outward current (10 ± 4% of control, n = 4) without significantly affecting the $[Ca^{2+}]_i$ elevation (94 ± 5% of control, n = 4) (Fig. 1*A*,*B*). The amplitude of the outward current produced by various concentrations of $InsP_3$ (0.75–30 μM) correlated with the peak $[Ca^{2+}]_i$ (r = 0.70) (Fig. 1*C*).

The effects of cyclopiazonic acid (CPA) on the $InsP_3$-evoked responses were examined next. CPA depletes intracellular Ca^{2+} stores by blocking the endoplasmic reticulum Ca^{2+}-ATPase (Seidler et al., 1989). The experiment in Figure 2*A* illustrates that bath application of CPA (10 μM) completely abolished both the outward current and the rise in $[Ca^{2+}]_i$ evoked by $InsP_3$. In five cells tested, CPA (10 μM) reduced the $InsP_3$-induced increase in $[Ca^{2+}]_i$ to 9 ± 4% of control and completely inhibited the outward current (Fig. 2*B*). In contrast, CPA had no effect on the rise in $[Ca^{2+}]_i$ caused by a depolarizing step to 0 mV (1 sec), although it slowed the decay time course of $[Ca^{2+}]_i$ after depolarization (Fig. 2*A*).

Taken together, these results indicate that $InsP_3$ released in the cytosol mobilizes Ca^{2+} from intracellular stores and activates apamin-sensitive SK channels.

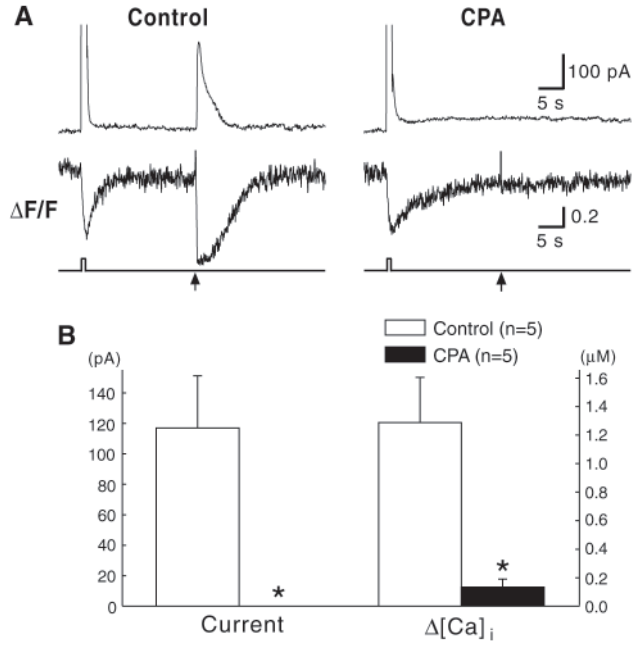

Figure 2. Depletion of intracellular Ca^{2+} stores abolished $InsP_3$-evoked responses. *A*, A depolarizing step to 0 mV (1 sec; voltage protocol illustrated *below*) followed 20 sec later by photolytic release of $InsP_3$ (30 μM; *arrow*) was applied before (*left*) and after (*right*) perfusion of CPA (10 μM). The resulting changes in membrane current (*top traces*) and fluorescence (*bottom traces*) are shown. In this cell, a high-affinity Ca^{2+} indicator Fura-2 (200 μM; k_d = 140 nM) was used to enhance detection of small changes in $[Ca^{2+}]_i$. The fluorescence was therefore not converted to $[Ca^{2+}]_i$. *B*, Pooled data from five cells tested for the effect of CPA (10 μM). *$p < 0.05$.

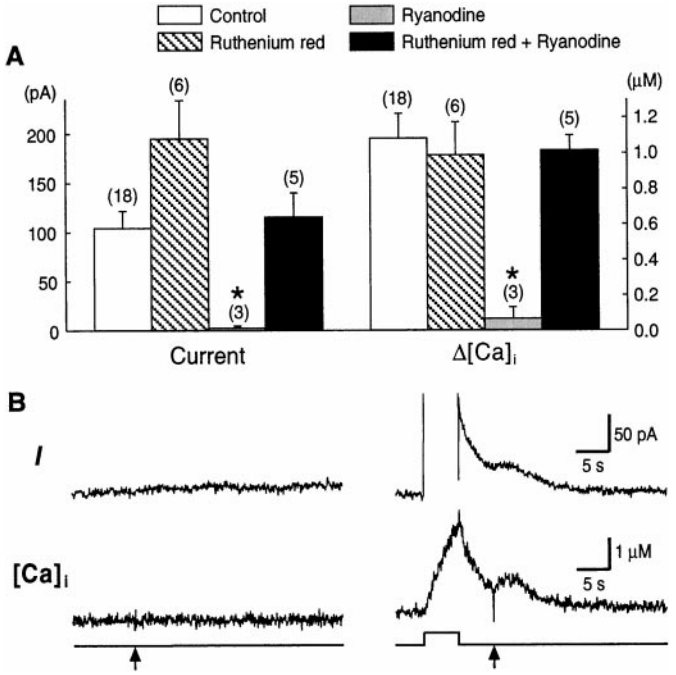

Figure 3. The effects of ruthenium red and ryanodine on $InsP_3$-induced responses. *A*, Summary histogram showing $InsP_3$-evoked responses in cells dialyzed with control pipette solution, ruthenium red (20 μM), ryanodine (10 μM), and both ruthenium red (20 μM) and ryanodine (10 μM). The number of cells tested is indicated in *parentheses.* *$p < 0.05$. *B*, $InsP_3$ (30 μM) was released at the time indicated by the *arrow* without (*left*) or with (*right*) a preceding depolarizing step to 0 mV (5 sec) in a cell dialyzed with ryanodine (10 μM). The voltage protocol is shown *below*. The interval between the depolarizing step and release of $InsP_3$ was 5 sec. *Top traces* and *bottom traces* depict membrane current and $[Ca^{2+}]_i$, respectively.

$InsP_3$ receptors and ryanodine receptors are colocalized on the same intracellular Ca^{2+} stores

It has been suggested that activation of mGluRs triggers CICR through ryanodine receptors in midbrain dopamine neurons (Fiorillo and Williams, 1998). To investigate whether photolytic release of $InsP_3$ evokes CICR, the contribution of ryanodine receptors was assessed with ruthenium red and ryanodine. Ruthenium red is a blocker of ryanodine receptors, whereas ryanodine locks ryanodine receptor channels in a subconductance open state and depletes ryanodine-sensitive Ca^{2+} stores (Smith et al., 1988). Ruthenium red (20 μM in the patch pipette) had no significant effect on the outward current or the rise in $[Ca^{2+}]_i$ caused by release of $InsP_3$ (30 μM) (Fig. 3*A*), indicating that CICR through ryanodine receptors does not play a major role in the $InsP_3$-induced mobilization of Ca^{2+}. Furthermore, the amplitude of mGluR IPSCs elicited by extracellular stimuli was not significantly different between control and ruthenium red-containing internal solutions (121 ± 26 pA, $n = 5$ vs 106 ± 25 pA, $n = 6$, $p > 0.6$). In contrast, ryanodine (10 μM in the patch pipette) almost completely blocked $InsP_3$-induced responses (Fig. 3*A*). However, after a depolarizing step (5 sec) to 0 mV, $InsP_3$ elicited a clear increase in $[Ca^{2+}]_i$ and a small outward current even in the presence of ryanodine. The effect of depolarization lasted for at least 30 sec ($n = 3$). This observation suggests that Ca^{2+} entry during depolarization partially refilled the $InsP_3$-sensitive stores that had been depleted by ryanodine. The inhibitory action of ryanodine (10 μM) was fully blocked by co-addition of ruthenium red (20 μM) to the internal solution (Fig. 3*A*).

Taken together, these data strongly suggest that $InsP_3$ elicits Ca^{2+} mobilization from the $InsP_3$-sensitive stores that also express ryanodine receptors but does not trigger CICR through these ryanodine receptors.

Increasing Ca^{2+} stores invokes CICR

We next asked under what conditions ryanodine receptors could be activated in dopamine neurons. Repetitive firing of dopamine neurons for 20–50 sec has been shown to induce transient facilitation of mGluR IPSPs that lasts for several minutes, an effect that may be caused by loading of intracellular Ca^{2+} stores (Fiorillo and Williams, 1998). Thus, filling internal Ca^{2+} stores by prolonged depolarization may invoke a component of the $InsP_3$-evoked Ca^{2+} release that is attributable to CICR. A prolonged depolarization (20 sec) was applied 1 min before photolytic release of $InsP_3$ to examine this possibility. This depolarization induced a slow component in the rising phase of $[Ca^{2+}]_i$, a marked prolongation of the overall duration of the Ca^{2+} transient, and a late component in the outward current (Fig. 4*A*). These effects of depolarization persisted for >5 min ($n = 3$). When cells were dialyzed with ruthenium red (20 μM), depolarization produced no significant change in the profile of the $InsP_3$-elicited Ca^{2+} transient, and no late component was observed in the outward current ($n = 3$) (Fig. 4*B*). These results suggest that CICR through ryanodine receptors augments the $InsP_3$-evoked Ca^{2+} release when intracellular stores are enlarged by prolonged depolarization. A transient reduction in the peak amplitude of the $InsP_3$-induced current was also observed after the prolonged depolarization with both control and ruthenium red-containing internal solutions (Fig. 4*A*,*B*). The reason for this decrease in $InsP_3$-induced current is not known.

DISCUSSION

The present study demonstrates that intracellularly released $InsP_3$ induces activation of apamin-sensitive SK channels through mobilization of Ca^{2+} from intracellular stores in midbrain dopa-

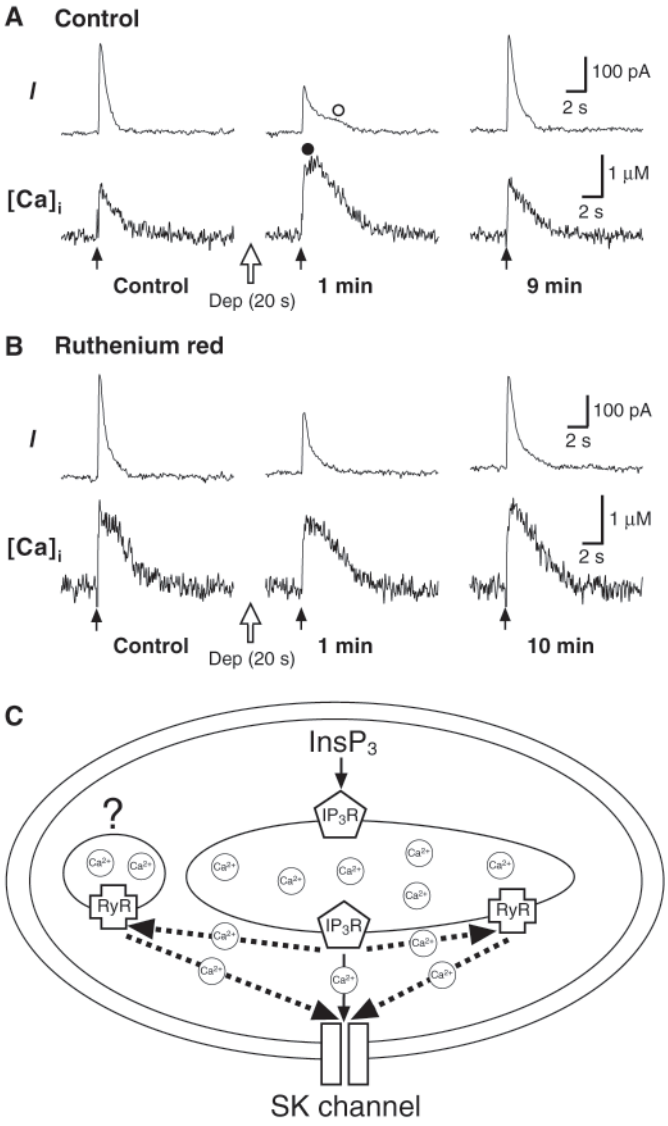

Figure 4. Prolonged depolarization added a CICR-mediated component to $InsP_3$-induced responses. *A*, $InsP_3$ (30 μM)-evoked responses before (*left traces*) and after (*middle* and *right traces*) application of a prolonged depolarizing step to 0 mV (20 sec; *open arrow*), which caused a large increase in $[Ca^{2+}]_i$ (>2 μM). The interval between depolarization and release of $InsP_3$, which is indicated *below*, was at least 1 min to allow the membrane current and $[Ca^{2+}]_i$ to recover to control levels after depolarization. The late component in the outward current and the slow component in the rising phase of $[Ca^{2+}]_i$ are marked with *open* and *closed circles*, respectively, which were observed when $InsP_3$ was released 1 min after depolarization. *B*, Same experiment as in *A* with ruthenium red (20 μM) included in the pipette solution. *C*, Intracellular signaling cascade elicited by $InsP_3$ in midbrain dopamine neurons. *Solid arrows* represent the pathway in control conditions, and *dashed arrows* represent the possible pathways invoked when intracellular Ca^{2+} stores are enlarged with prolonged depolarization. *IP_3R*, $InsP_3$ receptor; *RyR*, ryanodine receptor.

mine neurons. It is shown that $InsP_3$-sensitive stores are functionally connected to ryanodine-sensitive stores. Furthermore, evidence is provided suggesting that increasing Ca^{2+} stores facilitates $InsP_3$-induced mobilization of Ca^{2+} by bringing ryanodine receptors into play.

The amplitude of the outward current was correlated with the peak $[Ca^{2+}]_i$ after release of $InsP_3$. Thus, the increase in membrane conductance can be a reasonable measure of the increase in free cytosolic Ca^{2+} concentration. Furthermore, the relationship between the peak $[Ca^{2+}]_i$ and the current amplitude illustrated in Figure 1*C* is in good agreement with the known EC_{50} of Ca^{2+} for activation of cloned SK channels determined from inside-out patches (630–700 nM) (Köhler et al., 1996).

Depleting intracellular Ca^{2+} stores with CPA almost completely blocked the $InsP_3$-evoked Ca^{2+} transient, even when the high-affinity Ca^{2+} indicator Fura-2 was used to enhance detection of a small change in $[Ca^{2+}]_i$. Furthermore, $InsP_3$ elicited no change in the holding current after treatment with CPA, indicating that the outward current results entirely from mobilization of Ca^{2+} from intracellular stores. The time course of decay of $[Ca^{2+}]_i$ after Ca^{2+} influx attributable to membrane depolarization was slowed in the presence of CPA, possibly reflecting the blockade of Ca^{2+} sequestration into intracellular stores. Thus, Ca^{2+} entering the cell during depolarization can indeed be pumped into intracellular stores and charge them with Ca^{2+}. It should be noted, however, that internal Ca^{2+} stores remained stable in dopamine cells clamped at −60 mV, because release of $InsP_3$ could elicit reproducible responses without membrane depolarization throughout the duration of the recording.

Ruthenium red, which blocks ryanodine receptors, failed to affect the $InsP_3$-induced release of Ca^{2+} and the amplitude of mGluR IPSCs. Hence, ryanodine receptors do not appear to make a significant contribution to the $InsP_3$-mediated responses. On the other hand, depletion of ryanodine-sensitive Ca^{2+} stores with ryanodine abolished $InsP_3$-induced Ca^{2+} mobilization as well as the outward current. Ryanodine also blocked mGluR IPSPs (Fiorillo and Williams, 1998) and an outward current induced by iontophoresis of acetylcholine (Fiorillo and Williams, 2000) in dopamine neurons. Ruthenium red reversed the inhibitory effects of ryanodine on the $InsP_3$-evoked responses (Fig. 3*A*) and the acetylcholine-induced outward current (Fiorillo and Williams, 2000), providing a positive control for the effectiveness of ruthenium red. These data, together with the lack of effect of ruthenium red on $InsP_3$-induced Ca^{2+} mobilization, suggest that $InsP_3$-sensitive stores also possess ryanodine receptors and can thus be depleted by ryanodine. Interestingly, the $InsP_3$-evoked responses abolished by ryanodine partially recovered after depolarization of the cells. This observation is consistent with the idea that depolarization-induced Ca^{2+} influx refilled the $InsP_3$-sensitive stores that had been depleted by ryanodine. This effect of depolarization could not be ascribed to CICR triggered by an increase in $[Ca^{2+}]_i$ after depolarization, because CICR is blocked in the presence of ryanodine. Furthermore, it is unlikely that this could be caused by Ca^{2+}-induced activation of $InsP_3$ receptors, because when $InsP_3$ was released 5 sec after the depolarizing step, $[Ca^{2+}]_i$ was still considerably high, i.e., ~800 nM (Fig. 3*B*), which is in a concentration range where Ca^{2+} inhibits $InsP_3$ receptors (Bezprozvanny et al., 1991). It has also been reported that $InsP_3$-sensitive stores are equipped with ryanodine receptors in cerebellar Purkinje neurons (Khodakhah and Armstrong, 1997) and hippocampal pyramidal neurons (Nakamura et al., 1999). However, the possibility remains that there are separate stores, in addition to these $InsP_3$-sensitive stores equipped with ryanodine receptors, that are regulated exclusively by ryanodine receptors.

Increasing the size of intracellular Ca^{2+} stores with prolonged depolarization potentiated $InsP_3$-evoked responses by inducing CICR through ryanodine receptors. Regenerative release of Ca^{2+} via ryanodine receptors produced a slow component in the rising phase of $[Ca^{2+}]_i$ and an overall prolongation of the duration of Ca^{2+} transient, which was reflected in a late component of the outward current. It should be noted that increasing the size of internal stores with prolonged depolarization did not cause an increase in the magnitude of $[Ca^{2+}]_i$ elevation when ryanodine receptors were blocked with ruthenium red. It is possible that filling the $InsP_3$-sensitive stores did not result in an elevated peak $[Ca^{2+}]_i$ attained by Ca^{2+} mobilization through $InsP_3$ receptors themselves, because enhanced initial release of Ca^{2+} can in turn

cause Ca^{2+}-induced inactivation of $InsP_3$ receptors to terminate $InsP_3$ receptor-mediated Ca^{2+} release (Ogden and Capiod, 1997). Alternatively, Ca^{2+} entering the cell during depolarization may have preferentially filled separate ryanodine-sensitive stores that are devoid of $InsP_3$ receptors.

The schematic illustration depicted in Figure 4*C* summarizes how generation of $InsP_3$ inside the cell leads to activation of SK channels on the plasma membrane in midbrain dopamine neurons. Intracellular $InsP_3$ activates $InsP_3$ receptors on the internal stores that also contain ryanodine receptors. Under control conditions in which the membrane potential is clamped at −60 mV, $InsP_3$ receptor-mediated release of Ca^{2+} leads to activation of SK channels with no significant contribution from ryanodine receptors. When intracellular stores are enlarged by depolarization-induced Ca^{2+} influx, Ca^{2+} released from the $InsP_3$-sensitive stores can now elicit ryanodine receptor-mediated release of Ca^{2+}, i.e., CICR, from the same stores and/or from separate stores that possess only ryanodine receptors. This will result in an augmentation of Ca^{2+} mobilization and a prolongation of SK channel activation. Dopamine neurons are spontaneously active and fire continuously both *in vivo* and *in vitro* (Sanghera et al., 1984). It is therefore possible that this spontaneous firing keeps internal Ca^{2+} stores filled and thus enables $InsP_3$ to induce CICR under physiological conditions.

SK channels are known to participate in controlling the firing pattern of midbrain dopamine neurons (Shepard and Bunney, 1991). Thus, it is very likely that mobilization of Ca^{2+} from intracellular stores plays an important role in regulating the activity of dopamine neurons. Activation of dopamine neurons is critically involved in the reinforcing actions of drugs of abuse, such as opioids and psychostimulants (Koob et al., 1998). Future investigation on how these drugs interact with the intracellular signaling pathway described here may lead to a better understanding of the cellular mechanisms underlying drug addiction.

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