A rise in intracellular Ca\(^{2+}\) (Ca\(^{2+}\)) mediates various cellular functions ranging from fertilization to gene expression. A ubiquitous Ca\(^{2+}\) influx pathway that contributes significantly to the generation of Ca\(^{2+}\) signals, especially in non-excitable cells, is store-operated Ca\(^{2+}\) entry (SOCE). Consequently, the modulation of SOCE current affects Ca\(^{2+}\) entry (SOCE). Consequently, the modulation of SOCE current affects Ca\(^{2+}\) entry (SOCE). Consequently, the modulation of SOCE current affects Ca\(^{2+}\) entry (SOCE).

In this paper, we show that both Ca\(^{2+}\) and Ca\(^{2+}\)-calmodulin-dependent protein kinase II (CaMKII) positively modulate SOCE current (I\(_{\text{SOCE}}\)).

Both intracellular and extracellular Ca\(^{2+}\) have been shown to modulate SOCE activity in a complex fashion. This is best characterized for the Ca\(^{2+}\)-release-activated Ca\(^{2+}\) current (I\(_{\text{CRAC}}\)) (7), which is the first described SOCE current. Hallmarks of I\(_{\text{CRAC}}\) include inward rectification, an ionic selectivity sequence of Ca\(^{2+}\) > Ba\(^{2+}\) > Mn\(^{2+}\) > Na\(^{+}\), and a small unitary Ca\(^{2+}\) conductance estimated to be \(\sim 20\) pmSiemens (8, 9). Intracellular Ca\(^{2+}\) has been shown to negatively regulate I\(_{\text{SOCE}}\) by two mechanisms. 1) Fast Ca\(^{2+}\)-dependent inactivation is due to inhibition of I\(_{\text{SOCE}}\), arguably following Ca\(^{2+}\) binding to an intracellular site (8, 9). 2) Slow Ca\(^{2+}\)-dependent inactivation is due in part to store refilling but also has a store-independent component that is poorly understood (11). In contrast, extracellular Ca\(^{2+}\) (Ca\(^{2+}\)o) potentiates I\(_{\text{SOCE}}\) through a process termed Ca\(^{2+}\)-dependent potentiation (CDP). CDP is attributed to a positive effect of extracellular Ca\(^{2+}\) on I\(_{\text{SOCE}}\) (12, 13) and probably results from Ca\(^{2+}\) binding to an extracellular site on the channel because it can be replicated by Ni\(^{2+}\) (12). Ni\(^{2+}\) does not permeate SOCE channels but potentiates the Ca\(^{2+}\) current through SOCE channels (12). Furthermore, CDP alters channel gating and not permeation (12), because current inactivation (which is dependent on permeation (8)) remains unchanged during CDP.

In this paper, we describe a novel positive regulation of I\(_{\text{SOCE}}\) by intracellular Ca\(^{2+}\) through CaMKII in Xenopus oocytes. Xenopus oocyte SOCE current has similar characteristics to I\(_{\text{CRAC}}\) including high Ca\(^{2+}\) selectivity, inward rectification (14), and as shown here extracellular Ca\(^{2+}\)-dependent potentiation. Allowing a Ca\(^{2+}\), rise during SOCE activation results in larger I\(_{\text{SOCE}}\). Furthermore, expression of a constitutively active CaMKII (CaMKII\(^{\text{ca}}\)) also leads to enhancement of I\(_{\text{SOCE}}\). CaMKII potentiates I\(_{\text{SOCE}}\) by dramatically increasing the levels of CDP. Because CDP affects SOCE channel gating, our data argue that CaMKII potentiates SOCE by altering gating. CaMKII-mediated potentiation of I\(_{\text{SOCE}}\) has important physiological consequences because store depletion is physiologically accompanied by a rise in Ca\(^{2+}\). Because CaMKII is able to decode Ca\(^{2+}\) signals, dynamics into different levels of enzyme activity (15), CaMKII-mediated potentiation of I\(_{\text{SOCE}}\) provides a mechanism for Ca\(^{2+}\) to regulate its own entry into the cell depending on the levels and kinetics of Ca\(^{2+}\) release following receptor activation.
**EXPERIMENTAL PROCEDURES**

**Oocyte and Electrophysiological Methods—*Xenopus laevis* oocytes were prepared as described previously (16). SOCE was activated by the depletion of intracellular Ca\(^{2+}\) stores with either ionomycin (10 \(\mu\)M) or thapsigargin (1 \(\mu\)M for \(\geq 3 \text{ h}\) unless otherwise indicated). Oocytes were injected with 7 nmol of BAPTA to buffer the Ca\(^{2+}\), rise and block the endogenous Ca\(^{2+}\)-activated Cl\(^{-}\) current (I\(_{\text{SOCE}}\)) that would otherwise mask the SOCE current. Assuming an oocyte volume of 1 \(\mu\)l, this would result in a final concentration of \(-7 \text{ mM BAPTA}\) that completely blocks I\(_{\text{SOCE}}\). Oocytes were voltage-clamped with two microelectrodes by the use of a GeneMachines 500 (Axon Instruments). Electrodes were filled with 3 M KCl and had resistances of 0.5–2 megohms. Voltage stimulation and data acquisition were controlled using a pClamp8 (Axon Instruments). Current data were filtered at 10 kHz, digitized, and analyzed using Clampfit8.0 (Axon Instruments) and Origin\(^\text{\textregistered}\) software (Microcal Software Inc.). I\(_{\text{SOCE}}\) was typically measured in 30 Ca\(^{2+}\) solution (in mM: 55 NaCl, 30 CaCl\(_2\), 10 Hepes, pH 7.4), and Ca\(^{2+}\)-free solution in I\(_{\text{SOCE}}\)-recording experiments was 70 Mg\(^{2+}\) (in mM: 70 MgCl\(_2\), 10 Hepes, pH 7.4). I\(_{\text{SOCE}}\) time course is plotted as the mean current 30–35 ms after the voltage step, and the leak current at the beginning of the experiment was subtracted.

Ca\(^{2+}\)-activated Cl\(^{-}\) currents were recorded in Ringer solution (in mM: 123 NaCl, 2.5 KCl, 1.8 CaCl\(_2\), 18 MgCl\(_2\), 10 Hepes, pH 7.4) or Ca\(^{2+}\)-free Ringer solution that had the same composition as Ringer solution with the exception that CaCl\(_2\) was omitted, MgCl\(_2\) increased to 5 mM, and 0.1 mM EGTA was added.

cRNA for injection into oocytes was transcribed in vitro using the mMessage mMachine SP6 transcription kit (Ambion). Both pGEM3–5HT1c and pSP-CaMKII(T286D) were linearized with EcoRI and trancribed with SP6 polymerase.

**Ca\(^{2+}\)** Imaging—*Xenopus oocytes were injected with \(-7.6 \mu\text{M Ca}^{2+}\). Green-1 coupled to 70 kDa of dextran and voltage-clamped as described above. The dye was allowed at least 30 min to equilibrate within the oocyte. Imaging experiments were performed in Ringer and Ca\(^{2+}\)-free Ringer solutions. Confocal Ca\(^{2+}\) imaging was performed using an Olympus Fluoview confocal scanning system fitted to IX70 microscope using a \(\times 10\) (0.3 numerical aperture) objective. Images (256 \(\times\) 256 pixels) were collected and analyzed using Olympus Fluoview software.

**CaMKII Assay—CaMKII kinase activity was measured by lysing oocytes (20 \(\mu\text{l/oocyte}) in CaMKII extraction buffer (80 mM beta-glycerophosphate, 20 mM Hepes, pH 7.5, 15 mM MgCl\(_2\), 1 mM sodium vanadate, 50 mM NaF, 1 mM dithiothreitol, 1 \(\times\) protease inhibitor mixture (Calbiochem)). Lysates were centrifuged at 500 \(\times\) g for 15 min, and the supernatant was stored at \(-70^\circ\text{C}\) until use in the kinase assay. The CaMKII kinase was performed using the SignaTECT\(\text{TM}\) kinase kit (Promega) according to manufacturer’s instructions.

**RESULTS**

**Intracellular Ca\(^{2+}\)** Potentiates SOCE—Physiological activation of SOCE is invariably preceded by a rise in Ca\(^{2+}\), because of Ca\(^{2+}\) release from stores. Although it is clear that Ca\(^{2+}\) is not required for SOCE activation, it is not known whether this Ca\(^{2+}\) rise is due to intracellular Ca\(^{2+}\) rise or not. To activate SOCE in the absence of a Ca\(^{2+}\) rise, we injected cells with BAPTA and then depleted Ca\(^{2+}\) stores with ionomycin (BAPTA-Ion). This results in the activation of a characteristic SOCE current as previously described (14, 17). To allow a Ca\(^{2+}\) rise during SOCE activation, Ca\(^{2+}\) stores were depleted with ionomycin followed by repetitive hyperpolarization to induce Ca\(^{2+}\) influx (Ion-BAPTA). The cell was then injected with BAPTA, and I\(_{\text{SOCE}}\) was recorded (Fig. 1B, open circles). Treating cells according to the Ion-BAPTA protocol resulted in a significant \((\text{p} = 1.6 \times 10^{-7})\) potentiation of I\(_{\text{SOCE}}\) levels compared with control oocyte (BAPTA-Ion) (Fig. 1, B and C). Therefore, allowing a Ca\(^{2+}\) rise during SOCE activation leads to current potentiation.

The Ca\(^{2+}\)-mediated potentiation of SOCE described above was obtained under conditions designed to maximize Ca\(^{2+}\) rise by hyperpolarization-induced Ca\(^{2+}\) influx. To determine whether such Ca\(^{2+}\) influx is required for I\(_{\text{SOCE}}\) potentiation, a similar experiment was performed without hyperpolarization using the ion-BAPTA protocol, that is Ca\(^{2+}\) stores were simply depleted in the absence of BAPTA injection. In this case, I\(_{\text{SOCE}}\) was also potentiated (Fig. 1D), showing that hyperpolarization per se is not required for the observed Ca\(^{2+}\)-mediated I\(_{\text{SOCE}}\) potentiation.

As discussed above, SOCE has been shown to be potentiated by extracellular Ca\(^{2+}\), which affects SOCE channel gating in a process referred to as CDP (12). To avoid confusion with CDP, we will refer to the intracellular Ca\(^{2+}\) effect on I\(_{\text{SOCE}}\) as Ca\(^{2+}\)-mediated potentiation (CMP). CMP provides a fitting feedback model for Ca\(^{2+}\)-mediated SOCE potentiation.
Correlation between Receptor-induced Ca\(^{2+}\) Mobilization and SOCE—To assess the physiological relevance of CMP, we sought to determine whether it could be observed following IP\(_3\)-linked receptor stimulation. We were interested in inducing different levels of Ca\(^{2+}\) rise while minimizing experimental manipulation of Ca\(^{2+}\)\(_i\) (such as BAPTA injection or ionomycin treatment). Our approach was to express the G-protein-coupled serotonin receptor (5HT1c) and monitor changes in the endogenous Ca\(^{2+}\)-activated Cl\(^-\) currents as markers of Ca\(^{2+}\)\(_i\) (19). 5HT1c stimulation with serotonin (5HT) results in IP\(_3\) production through phospholipase-\(\beta\) activation (20). We have previously shown that Ca\(^{2+}\)-activated Cl\(^-\) currents (I\(_{\text{Cl1}}\) and I\(_{\text{Cl1T}}\)) faithfully report Ca\(^{2+}\)\(_i\) changes below the plasma membrane in terms of amplitude and kinetics (19). I\(_{\text{Cl1T}}\) activates in response to Ca\(^{2+}\) release from internal stores, whereas I\(_{\text{Cl1}}\) responds to Ca\(^{2+}\) influx from the extracellular space. During Ca\(^{2+}\) release, I\(_{\text{Cl1T}}\) activates as a sustained outward current upon depolarization (+40 mV, Fig. 3A, upper trace). Ca\(^{2+}\) release results in store depletion and SOCE activation. Ca\(^{2+}\) flowing through SOCE channels activates I\(_{\text{Cl1T}}\) as a transient current, only when the depolarization pulse is preceded by a hyperpolarization step, which induces Ca\(^{2+}\) influx (Fig. 3A, lower trace). I\(_{\text{Cl1T}}\) is transient because the Ca\(^{2+}\) that enters through SOCE channels during the −140-mV pulse dissipates rapidly during the subsequent 40-mV pulse, leading to I\(_{\text{Cl1T}}\) current decay (Fig. 3A, lower trace) (19) for a more detailed description of the relationship between Ca\(^{2+}\) signals and Ca\(^{2+}\)-activated Cl\(^-\) currents, see Refs. 19, 21, and 22). Thus, monitoring I\(_{\text{Cl1}}\) and I\(_{\text{Cl1T}}\) allows the real-time determination of Ca\(^{2+}\)\(_i\) (I\(_{\text{Cl1}}\)) and SOCE (I\(_{\text{Cl1T}}\)) levels following 5HT1c activation.

To generate graded Ca\(^{2+}\) release responses and determine the effect on SOCE, we injected cells with different amounts of 5HT1c cRNA (10 or 30 ng) and allowed them to express for 2 days. Cells were then stimulated with 5HT (10 μM) (Fig. 3, B and C), leading to Ca\(^{2+}\) release, which activates I\(_{\text{Cl1}}\) (Fig. 3, B and C, squares) followed by I\(_{\text{Cl1T}}\) due to SOCE activation (Fig. 3, B and C, circles). I\(_{\text{Cl1T}}\) activates to a certain threshold and gradually returns to base line as a result of Ca\(^{2+}\) store refilling and SOCE inactivation (Fig. 3, B and C, circles). Cells injected with 10 ng of 5HT1c cRNA produced a smaller I\(_{\text{Cl1}}\) (Fig. 3B) than those injected with 30 ng of the receptor (Fig. 3C). Furthermore, I\(_{\text{Cl1T}}\) activated to a lower threshold and inactivated more rapidly in cells injected with 10 ng of 5HT1c receptor cRNA (Fig. 3, compare B with C, circles). The responses of the Cl\(^-\) currents summarized in Fig. 3D show that both Ca\(^{2+}\)\(_i\) (as indicated by I\(_{\text{Cl1}}\)) and SOCE (as indicated by I\(_{\text{Cl1T}}\)) were significantly enhanced at high 5HT1c (30 ng) expression. Therefore, enhanced Ca\(^{2+}\) release correlates with larger SOCE. A simple explanation for these data is that receptor stimulation in cells expressing high levels of 5HT1c leads to a more dramatic Ca\(^{2+}\) store depletion and larger SOCE. However, as shown in Fig. 2, the extent of Ca\(^{2+}\) store depletion does not linearly correlate with SOCE magnitude. Therefore, SOCE potentiation as reported by I\(_{\text{Cl1T}}\) in cells expressing more 5HT1c receptors is probably because of some mechanism other than the extent of store depletion. CMP of I\(_{\text{SOCE}}\) provides such a mechanism. High 5HT1c expression produces increased Ca\(^{2+}\)\(_i\), as indicated by I\(_{\text{Cl1}}\), which would be expected to potentiate I\(_{\text{SOCE}}\) (I\(_{\text{Cl1T}}\)) through CMP. Note that store depletion provides a required signal for SOCE activation, but the extent of store depletion per se does not modulate SOCE magnitude. Rather, the data in Figs. 2 and 3 argue that it is the magnitude of the Ca\(^{2+}\)\(_i\) rise that modulates SOCE through CMP. Clearly the magnitude of Ca\(^{2+}\)\(_i\) rise can correlate with the extent of store depletion. These data reveal a subtle but important distinction in the mechanism of SOCE modulation and are consistent with
CaMKII Potentiates SOCE—Ca²⁺-mediated potentiation of ISOC could be either direct or indirect through the activation of Ca²⁺-dependent downstream effectors. A direct Ca²⁺-mediated potentiation of ISOC is unlikely because Ca²⁺ has been shown to inactivate ISOC, probably through a direct effect on SOCE channels (fast Ca²⁺-dependent inactivation) (8, 10). This finding suggests that CMP is the result of activation of Ca²⁺-dependent effectors, which in turn act on SOCE. A primary candidate for such an effector pathway is the Ca²⁺-CaM-activated protein kinase pathway. The most widespread Ca²⁺-CaM-dependent kinase is CaMKII, which is expressed in Xenopus oocytes (24). If Ca²⁺ potentiates ISOC through CaMKII, it is expected that ectopic activation of CaMKII would lead to increased ISOC levels.

We used a constitutively active CaMKII mutant (CaMKIIca) to determine whether CaMKII activation potentiates ISOC. The CaMKII holoenzyme is a multisubunit complex that is kept inactive by an autoinhibitory domain. The binding of Ca²⁺-CaM relieves autoinhibition and stimulates autophosphorylation at Thr-286, rendering the enzyme Ca²⁺-CaM-independent (25). CaMKIIca is a T286D mutation that mimics the effects of autophosphorylation by replacing Thr-286 with Asp, resulting in a Ca²⁺-CaM-independent and thus constitutively active CaMKII (26). We injected oocytes with CaMKIIca and measured ISOC (Fig. 4A). Expression of CaMKIIca results in an ~3-fold increase in ISOC (p = 1.1 × 10⁻⁷) (Fig. 4, A and B), consistent with CMP acting through CaMKII. In addition, CaMKIIca-mediated ISOC potentiation is expected to be independent of Ca²⁺, because CaMKIIca is Ca²⁺-CaM-independent. This prediction is confirmed in CaMKIIca-expressing cells, as ISOC is potentiated whether store depletion is preceded by a rise in Ca²⁺, (Ion-BAPTA) or not (BAPTA-Ion) (Fig. 4C). This finding further suggests that Ca²⁺-mediated potentiation is primarily through CaMKII activation, because no additive effect on ISOC is observed in CaMKIIca-expressing cells subjected to the Ion-BAPTA protocol (Fig. 4C).

To directly confirm CaMKIIca expression, we measured CaMKII-specific activity in lysates from control and CaMKIIca-injected cells. Cells expressing CaMKIIca had higher levels of CaMKII activity (Fig. 4D), showing that CaMKIIca was expressed and functional in these cells. Because specific activity was measured in the absence of Ca²⁺-CaM (Fig. 4D), CaMKII activity data confirm the Ca²⁺-CaM independence of CaMKIIca.

It is important to note that the expression of CaMKIIca by itself is not sufficient to activate ISOC (Fig. 4A, circles). In CaMKIIca-expressing cells, store depletion is still required to
activate SOCE because no $I_{\text{SOC}}$ is detected before ionomycin treatment (Fig. 4A, circles). However, $I_{\text{SOC}}$ levels reach a significantly larger maximal amplitude in CaMKII-expressing cells compared with control cells (Fig. 4A and B). Therefore, CaMKII is not a component of the SOCE activation pathway induced in response to store depletion but rather positively modulates SOCE activity following SOCE activation.

If CMP is acting through CaMKII, CaMKII should be activated in cells where a Ca$^{2+}$ rise is induced (Ion-BAPTA). We were unable to detect such an increase in CaMKII activity in oocytes treated according to the Ion-BAPTA protocol. This result argues that either Ca$^{2+}$ and CaMKII potentiate $I_{\text{SOC}}$ by separate mechanisms or that CaMKII activation is transient and/or spatially localized, resulting in small changes in CaMKII activity that are difficult to detect in whole cell lysates. Furthermore, basal CaMKII activity was quite variable in oocytes donated from different females, making it difficult to reliably measure a small increase in CaMKII activity in different batches of cells. However, a Ca$^{2+}$ rise has been shown to activate endogenous CaMKII in Xenopus oocytes using an in viv o CaMKII-specific kinase assay (27).

Nonetheless, if CMP is mediated by CaMKII, blocking endogenous CaMKII should inhibit CMP. Therefore, we blocked endogenous CaMKII and determined the effect on SOCE. Oocytes were injected with AIP, a specific inhibitory peptide of CaMKII that mimics the autoinhibitory domain and blocks CaMKII activity (28). Allowing a Ca$^{2+}$ rise in control oocytes (Ion-BAPTA) potentiates $I_{\text{SOC}}$ by ~2-fold but not in cells injected with the CaMKII inhibitor AIP (Fig. 5A). Furthermore, AIP added to the CaMKII assay blocks kinase activity in a dose-dependent manner (Fig. 5B). These data show that inhibiting endogenous CaMKII activity blocks CMP, supporting the conclusion that Ca$^{2+}$ potentiates SOCE through CaMKII activation.

**Mechanism of CaMKII Action on SOCE**—Because SOCE is activated in response to store depletion, CaMKII could potentiate $I_{\text{SOC}}$ by targeting either the coupling mechanism between Ca$^{2+}$ stores and SOCE or the SOCE channel. To differentiate between these possibilities and obtain a better understanding of the mechanism of action of CaMKII, we wanted to study the effects of CaMKII independently of store depletion. This was accomplished by irreversibly depleting Ca$^{2+}$ stores with thapsigargin before CaMKII expression (Fig. 6B). Oocytes were incubated in thapsigargin (1 μM) for 3 h to fully deplete Ca$^{2+}$ stores followed by CaMKII cRNA injection and incubation in nominally Ca$^{2+}$-free (50 μM free Ca$^{2+}$) solution. Control cells were treated with thapsigargin alone. Under these conditions, store depletion is complete before CaMKII expression and store Ca$^{2+}$ load remains low throughout the experiment because thapsigargin irreversibly inhibits the ER Ca$^{2+}$-ATPase. This allows us to study the effects of CaMKII on $I_{\text{SOC}}$ after store depletion and determine whether CaMKII is affecting the coupling mechanism or SOCE channel gating or permeation. In this experiment, SOCE was activated in the absence of the conducting ion (Ca$^{2+}$) and therefore no SOCE current was observed at the beginning of the experiment (Fig. 6A). Switching control (thapsigargin-treated) oocytes to a Ca$^{2+}$-containing solution produced an initial $I_{\text{SOC}}$ that was further enhanced over time, eventually saturating (Max $I_{\text{SOC}}$) within ~6 min (Fig. 6A, squares). This behavior is the result of classical CDP where extracellular Ca$^{2+}$ exerts a positive effect on SOCE channel gating (12, 13). CDP can be estimated as the ratio of

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**Fig. 4. CaMKII Potentiates SOCE**

Oocytes were injected with a constitutively active CaMKII (CaMKIIca, 1 ng/oocyte) and allowed to express for 12–16 h. SOCE was measured using voltage protocol number 1 in Fig. 1A with the exception that the voltage was stepped to −120 mV instead of −140 mV. A, time course of $I_{\text{SOC}}$ activation in control and CaMKIIca-injected cells treated according to the BAPTA-Ion or Ion-BAPTA protocols as described in Fig. 1. The asterisks above the bars indicate the significantly different groups (n as indicated; p < 0.0164). B, basal CaMKII kinase activity from control and CaMKIIca-injected oocytes measured using an in vitro kinase assay without the addition of Ca$^{2+}$—CaM (n = 5; p = 0.0469). C, normalized $I_{\text{SOC}}$ levels from control and CaMKIIca-injected oocytes treated according to the Ion-BAPTA protocol. This result argues that either Ca$^{2+}$ and CaMKII potentiate $I_{\text{SOC}}$ by separate mechanisms or that CaMKII activation is transient and/or spatially localized, resulting in small changes in CaMKII activity that are difficult to detect in whole cell lysates. Furthermore, basal CaMKII activity was quite variable in oocytes donated from different females, making it difficult to reliably measure a small increase in CaMKII activity in different batches of cells. However, a Ca$^{2+}$ rise has been shown to activate endogenous CaMKII in Xenopus oocytes using an in vitro CaMKII-specific kinase assay (27).
FIG. 5. Inhibition of endogenous CaMKII blocks Ca²⁺-mediated SOCE potentiation. Control and AIP-injected (Inj.) (10 μM) oocytes were treated according to the BAPTA-Ion and Ion-BAPTA protocols as indicated. A, normalized $I_{SOCE}$ levels in the different treatment groups. The asterisk indicates the only significantly different group ($p < 2.1 \times 10^{-4}$). B, basal CaMKII activity in oocyte lysate without AIP (Con) and with 10 and 40 μM AIP as indicated ($n = 6$).

FIG. 6. CaMKII potentiates $I_{SOCE}$ by increasing the levels of CDP. Cells were incubated with thapsigargin (Thaps) (1 μM) in nominally Ca²⁺-free medium (50 μM) for 3 h to fully deplete Ca²⁺ stores. A subset of cells was then injected with 1 ng of CaMKII⁺⁺ RNA (Thaps-CaMK⁺⁺) and incubated in nominally Ca²⁺-free medium for 12–16 h. A, $I_{SOCE}$ recorded from a representative control (Thaps) and CaMKII⁺⁺-injected oocyte (Thaps-CaMK⁺⁺). Cells were incubated in Ca²⁺-free solution (70 Mg²⁺) before switching to Ca²⁺-containing solution (30 Ca²⁺) as indicated by the line. SOCE was measured using voltage protocol number 1 in Fig. 1A with the exception that the voltage was stepped to −120 mV instead of −140 mV. The addition of La³⁺ to block ISOC is also indicated. B, normalized $I_{SOCE}$ levels showing initial $I_{SOCE}$ (indicated by the asterisk in A) and maximal $I_{SOCE}$ at the end of the experiment (indicated by the open square and filled circle in the Thaps and Thaps-CaMK⁺⁺ groups, respectively). The average levels of CDP calculated as maximal $I_{SOCE}$/initial $I_{SOCE}$ are also shown. Maximal $I_{SOCE}$ and CDP are significantly different between the two groups ($p < 0.00132$). C and D, current traces in response to a step voltage (−20 to −120 mV for 500 ms) (E and F, superimposed current traces of maximal $I_{SOCE}$ from Thaps and Thaps-CaMK⁺⁺-treated cells in response to a step voltage pulse (E) and a voltage ramp (F).

maximal $I_{SOCE}$/initial $I_{SOCE}$ (12) and is 3.11 ± 0.23 in thapsigargin-treated cells (Fig. 6B).

Surprisingly, exposing CaMKII⁺⁺-expressing cells to Ca²⁺-containing solution results in an initial $I_{SOCE}$ with a similar amplitude to initial $I_{SOCE}$ in control cells (Fig. 6A, open circles). However, $I_{SOCE}$ in CaMKII⁺⁺-expressing cells gradually increased to significantly higher levels than in control cells (Fig. 6A, open circles). This shows that CaMKII⁺⁺ expression does
not affect initial ISOC levels but results in a significantly \( (p = 1.46 \times 10^{-5}) \) larger maximal ISOC (Fig. 6B). It follows that CDP, calculated as the ratio of maximal/initial ISOC, was also augmented \((5.72 \pm 0.61)\) in CaMKII$$^\text{a}$$-expressing cells (Fig. 6B). Therefore, CaMKII potentiates ISOC by increasing the levels of CDP without affecting initial ISOC levels, even after prolonged expression of CaMKII$$^\text{a}$$.

These data provide important insights into the mechanism of action of CaMKII. The whole cell SOCE current is defined by the following equation: \( I_{\text{SOCE}} = N P_i \), where \( N \) is the number of active channels, \( P_i \) is the probability of opening, and \( i \) is the single channel conductance. Therefore, CaMKII can potentiate ISOC by increasing either \( N \), \( i \), or \( P_i \). If CaMKII was affecting the coupling mechanism, an increase in the number of active channels \((N)\) is expected. The fact that CaMKII$$^\text{a}$$ expression does not enhance initial ISOC provides evidence against an increase in channel number \((N)\) or single channel conductance \((i)\) (Fig. 6, A and B). This is because initial ISOC is due to current flowing through all of the open channels after Ca$$^{2+}$$ addition. If either \( N \) or \( i \) was augmented by CaMKII, initial ISOC would be larger in CaMKII$$^\text{a}$$-expressing cells. This hypothesis argues that CaMKII potentiates ISOC by enhancing the probability of opening \((P_i)\) of SOCE channels.

The observed increase in CDP levels in CaMKII$$^\text{a}$$-expressing cells strongly supports the conclusion that CaMKII potentiates ISOC by increasing \( P_i \). This is because CDP has been shown to be the result of extracellular Ca$$^{2+}$$ exerting a positive effect on SOCE channel gating \((P_i)\) (12, 13).

Representative current traces in response to a step pulse to \(-120\) mV (Fig. 6C) and a voltage ramp from \(-140\) to \(50\) mV (Fig. 6D) obtained from control (Thaps) and CaMKII$$^\text{a}$$-injected cells (Thaps-CaMK$$^\text{a}$$) are shown. The time points during the experiments at which the traces were obtained are indicated in Fig. 6A (Thaps, open symbols; Thaps-CaMK$$^\text{a}$$, filled symbols). Note the similar levels of initial ISOC in both treatments (Fig. 6C, open and filled stars). Initial ISOC traces from CaMKII$$^\text{a}$$-injected cells show a time-dependent increase in current amplitude (Fig. 6C, filled star) that is due to CDP occurring during the 500-ms duration of the voltage pulse. Zweifach and Lewis (8) have shown that the extent of Ca$$^{2+}$$-dependent inactivation of \( I_{\text{RIP}} \) following a brief hyperpolarization depends on the single channel current \((i)\) (8). That is, increased unitary current \((i)\) results in a faster rate of \( I_{\text{RIP}} \) inactivation. Assuming that the same relationship holds for Xenopus oocyte ISOC, if CaMKII increases unitary conductance \((i)\), we expect a faster ISOC inactivation rate shortly after the hyperpolarization pulse. However, the rate of ISOC inactivation shortly after \(100\) ms hyperpolarization is similar between control and CaMKII$$^\text{a}$$-expressing cells (Fig. 6E). In fact, at steady-state, control cells exhibit a more marked ISOC inactivation (Fig. 6E). These inactivation kinetics argue that CaMKII does not enhance ISOC unitary conductance. Superimposed current-voltage relationships from control and CaMKII$$^\text{a}$$-injected cells were similar (Fig. 6F), indicating that CaMKII does not affect the voltage dependence of ISOC.

These results argue that CaMKII$$^\text{a}$$ potentiates ISOC by targeting SOCE channel gating and not the coupling mechanism between Ca$$^{2+}$$ stores and SOCE. Three pieces of evidence support the conclusion that CaMKII potentiates ISOC by affecting channel gating \((P_i)\) and not channel number \((N)\) or unitary conductance \((i)\): 1) similar levels of initial ISOC in control and CaMKII$$^\text{a}$$-expressing cells (Fig. 6, A and B) arguing against an effect of CaMKII on \(N\) or \(i\), 2) enhanced CDP in CaMKII$$^\text{a}$$-expressing cells (Fig. 6B), suggesting that CaMKII enhances channel \(P_i\), and 3) similar ISOC inactivation rates shortly after hyperpolarization in control and CaMKII$$^\text{a}$$-expressing cells (Fig. 6E) arguing against an increase in unitary conductance \((i)\).

**DISCUSSION**

Ca$$^{2+}$$ has been shown to negatively regulate ISOC by inducing channel inactivation either directly or through store refilling (8, 10, 11). Here we show that Ca$$^{2+}$$, can in addition have a potentiating effect on SOCE. The Ca$$^{2+}$$ effect on SOCE is probably the result of CaMKII activation, because Ca$$^{2+}$$, because inhibition of endogenous CaMKII activity blocks CMP and expression of CaMKII$$^\text{a}$$ is sufficient to potentiate ISOC independently of Ca$$^{2+}$$, Although CaMKII potentiates ISOC, it is not sufficient by itself to activate SOCE independently of store depletion (Fig. 4A). This observation is consistent with the fact that a Ca$$^{2+}$$ rise is not required for SOCE activation (1). Therefore, the CaMKII pathway modulates SOCE activity but is not an essential component of the SOCE activation pathway in response to store depletion. For CaMKII to exert its effects, SOCE has to be activated by store depletion. Using pharmacological inhibitors, a role for CaMKII in skeletal muscle SOCE (29) and myosin light chain kinase (a Ca$$^{2+}$$, Ca-M-dependent kinase related to CaMKII (25)) in endothelial SOCE (30) have been postulated. This finding argues that CaMKII modulation of ISOC is a widespread mechanism that is not cell type-specific. Nonetheless, a previous report by Matifat et al. (27) suggests a negative effect of CaMKII on SOCE in Xenopus oocytes. However, it is not clear from this study that the reported CaMKII effect was due to SOCE modulation because the Ca$$^{2+}$$-activated Cl$$^-$$ current was used as an indicator of SOCE, making it impossible to differentiate between an effect of CaMKII on the Cl$$^-$$ current or SOCE. In contrast, we have directly measured the SOCE current while modulating CaMKII activity and show that CaMKII activity potentiates ISOC. This finding argues that the effects of CaMKII reported by Matifat et al. (27) are due to modulation of the Cl$$^-$$ currents or other Ca$$^{2+}$$ influx pathways in the oocyte. CaMKII provides an excellent modulator of SOCE activity because of its ability to decode spatial and temporal information encoded in Ca$$^{2+}$$ signals into different levels of kinase activity. This capacity is attributable to spatial and structural features of CaMKII. CaMKII localizes to specific subcellular compartments such as the nucleus and cytoskeleton (31, 32), and its activity increases exponentially based on the number and frequency of Ca$$^{2+}$$, oscillation (15, 33). These exceptional attributes allow CaMKII to provide specificity to Ca$$^{2+}$$, signals by differentially activating effectors based on the kinetics of Ca$$^{2+}$$, signals. CaMKII plays important roles in regulating various cellular functions such as gene expression, cell cycle progression, and learning and memory (25, 34). The data presented here show that SOCE can now be added to the list of cellular functions modulated by CaMKII.

In a similar fashion to the CaMKII effect on SOCE described here, CaMKII has been shown to augment both L-type (Ca$$^{2+}$$, T-type (Ca$$^{2+}$$, and voltage-gated and store-operated Ca$$^{2+}$$ channels argue for a common cross-talk between Ca$$^{2+}$$, kinetics and Ca$$^{2+}$$, influx pathways. It is attractive to postulate that CaMKII provides a mechanism for phospholipase-linked receptors to differentially modulate SOCE activity. That is, the spatiotemporal features...
of the Ca\(^{2+}\) signals downstream of receptor activation differentially activate CaMKII and thus SOCE.

In addition to the CaMKII-mediated regulation of SOCE described here, SOCE has been shown to be modulated by protein kinase C (38, 39). Both protein kinase C and CaMKII are downstream kinases that can be induced following activation of phospholipase-linked receptors. Therefore, the interplay between various modulatory mechanisms in a specific cellular context combines to generate different levels of Ca\(^{2+}\) influx through SOCE. This Ca\(^{2+}\) influx affects Ca\(^{2+}\) kinetics and thus Ca\(^{2+}\)-dependent cellular responses. Consequently, CaMKII regulation of SOCE is likely to have broad and important physiological consequences.

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