Permeation of Monovalent Cations through the Non-capacitative Arachidonate-regulated Ca\(^{2+}\) Channels in HEK293 Cells

COMPARISON WITH ENDOGENOUS STORE-OPERATED CHANNELS*

Received for publication, March 14, 2001, and in revised form, March 30, 2001
Published, JBC Papers in Press, April 2, 2001, DOI 10.1074/jbc.M102311200

Olivier Mignen and Trevor J. Shuttleworth‡

From the Department of Pharmacology and Physiology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

In a manner similar to voltage-gated Ca\(^{2+}\) channels and Ca\(^{2+}\) release-activated Ca\(^{2+}\) (CRAC) channels, the recently identified arachidonate-regulated Ca\(^{2+}\) (ARC) channels display a large monovalent conductance upon removal of external divalent cations. Using whole-cell patch-clamp recording, we have characterized the properties of these monovalent currents in HEK293 cells stably transfected with the m3 muscarinic receptor and compared them with the corresponding currents through the endogenous store-operated Ca\(^{2+}\) (SOC) channels in the same cells. Although the monovalent currents seen through these two channels displayed certain similarities, several marked differences were also apparent, including the magnitude of the monovalent current/Ca\(^{2+}\) current ratio, the rate and nature of the spontaneous decline in the currents, and the effects of external monovalent cation substitutions and removal of internal Mg\(^{2+}\). Moreover, monovalent ARC currents could be activated after the complete spontaneous inactivation of the corresponding SOC current in the same cell. We conclude that the non-capacitative ARC channels share, with voltage-gated Ca\(^{2+}\) channels and store-operated Ca\(^{2+}\) channels (e.g. SOC and CRAC) the general property of monovalent ion permeation in the nominal absence of extracellular divalent ions. However, the clear differences between the properties of these currents through ARC and SOC channels in the same cell confirm that these represent distinct conductances.

Receptor-stimulated increases in Ca\(^{2+}\) entry in non-excitable cells are known to play a pivotal role in the generation and maintenance of the intracellular Ca\(^{2+}\) signals responsible for the control of such diverse functions as secretion, motility, growth, proliferation, and gene expression. However, despite extensive study, the mechanisms underlying such receptor-stimulated Ca\(^{2+}\) entry are currently far from clear. To date, most studies have focused on the so-called capacitative or store-operated channel. Although store-operated Ca\(^{2+}\) entry appears to be an almost ubiquitous feature of cells, the biophysical characterization of SOC channels from other cell types is rather limited, and the mechanism of activation of the channels is still unknown. Moreover, it seems unlikely that such channels are the exclusive route for the receptor-stimulated entry of Ca\(^{2+}\) in non-excitable cells (9). Recently, we have identified a novel receptor-activated Ca\(^{2+}\) entry pathway that appears to be specifically responsible for the Ca\(^{2+}\) entry associated with agonist stimulation at physiologically relevant concentrations (10–12). This pathway is independent of store depletion (i.e. non-capacitative) and is instead regulated by the receptor-mediated generation of arachidonic acid (AA). Similar AA-dependent non-capacitative Ca\(^{2+}\) entry pathways have now been identified in a variety of different cell types, including Balb/c 3T3 fibroblasts (13), A7r5 smooth muscle cells (14), and mouse parotid cells. Subsequent characterization of the Ca\(^{2+}\)-selective conductance associated with this pathway (named \(I_{\text{ARC}}\), for arachidonate-regulated Ca\(^{2+}\) current) in HEK293 cells stably transfected with the m3 muscarinic receptor (m3-HEK cells) revealed that it showed certain features that clearly distinguished it from the store-operated current (\(I_{\text{SOC}}\)) in the same cells (12).

Both ARC and CRAC channels, as well as the endogenous SOC channels of m3-HEK cells (HEK-SOC channels), share the properties of being highly Ca\(^{2+}\)-selective with relatively small magnitude currents (~0.5–1 pA/pF at ~80 mV), voltage-independent gating, inward rectification, and very positive reversal potentials (4, 5, 7, 12). The maintenance of the high selectivity for Ca\(^{2+}\) shown by these channels (as well as by voltage-gated Ca\(^{2+}\) channels) is problematic given that, under normal conditions, Na\(^+\) ions vastly outnumber Ca\(^{2+}\) ions in the extracellular medium. Despite this high selectivity for Ca\(^{2+}\) over Na\(^+\) under normal conditions, a characteristic feature of both CRAC channels and voltage-gated Ca\(^{2+}\) channels is that lowering external divalent cation concentrations to the micromolar range results in the appearance of large monovalent currents through these channels (7, 15, 16). Evidence from voltage-gated Ca\(^{2+}\) channels suggests that the high selectivity for Ca\(^{2+}\) over monovalent ions seen under normal conditions does not result from a

* This work was supported by NIGMS Grant GM 40457 from the National Institutes of Health (to T. J. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ To whom correspondence should be addressed: Dept. of Pharmacology and Physiology, University of Rochester Medical Center, P. O. Box 711, 601 Elmwood Ave., Rochester, NY 14642. Tel.: 716-275-2076; Fax: 716-273-2652; E-mail: trevor.shuttleworth@urmc.rochester.edu.
1 The abbreviations used are: SOC, store-operated Ca\(^{2+}\); CRAC, Ca\(^{2+}\) release-activated Ca\(^{2+}\); ARC, arachidonate-regulated Ca\(^{2+}\); AA, arachidonic acid; pF, picofarad; NMDG, N-methyl-D-glucamine; IV, current/voltage.
2 O. Mignen, J. Bruce, and T. J. Shuttleworth, unpublished data.
process of rejection as from a molecular “sieve,” but rather from the binding of Ca$^{2+}$ ions to sites within the channel. In other words, the Ca$^{2+}$ selectivity of these channels is Ca$^{2+}$-depend-
ent. A similar conclusion was reached in an analysis of the monovalent permeability of CRAC channels (15, 16). Interest-
ingly, we observed a similar increase in monovalent ion perme-
ability in the nominal absence of extracellular divalent cations in our earlier studies on the ARC channels (12). This raises the possibility that, despite the diverse mechanisms of gating shown by these different Ca$^{2+}$-selective channels (depolarization versus store depletion versus arachidonic acid), they all appear to share similar mechanisms for Ca$^{2+}$ selectivity.

In this study, we examine this feature in more detail with the overall aim of attempting to further develop a unique “finger-
print” for the ARC channels to provide the means of distin-
guishing them from the store-operated channels in the same cells.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Cells from the human embryonic kidney cell line HEK293 that had been stably transfected with the human m3 musca-
rinic receptor were a generous gift from Dr. Craig Logsdon (University of Michigan). The cells were cultured in Dulbecco’s modified Eagle’s medium with 10% calf serum and antibiotics in a 5% CO$_2$ incubator at 37 °C as previously reported (11). Cells were plated on glass coverslips that formed the bottom of a patch-clamp chamber (Warner Instrument Corp., Hamden, CT) at least 24 h before experimentation.

**Whole-cell Patch-clamp Recordings**—Patch-clamp recordings using the standard whole-cell mode (17) were performed at room temperature (20–22 °C) using an Axopatch-1C patch-clamp amplifier (Axon Instruments, Inc., Foster City, CA). Patch pipettes were pulled from borosili-
cate glass (GC150-F, Warner Instrument Corp.) and fire-polished to a resistance of 3–6 MΩ when filled with internal solution. Whole-
cell currents were recorded using 250-ms voltage steps from a holding potential of 0 to −80 mV delivered every 2 s. Alternatively, current-
voltage relationships were recorded using 150-ms voltage ramps from −100 to +30 mV. Ramps were terminated at +30 mV to avoid any contribution from a depolarization-activated Cl$^-$ current. Data were sampled at 20 kHz during the voltage steps and at 5.5 kHz during the voltage ramps and digitally filtered off-line at 1 kHz. Initial traces obtained upon going whole cell (i.e. before activation of I$_{\text{NaC}}$ or I$_{\text{isoC}}$) were averaged and used for leak subtraction of subsequent current recordings. The external (bath) solution was bathed in normal divalent cation-free internal solutions, respectively. Where indicated, the Cs$^+$ concentration in this nominally diva-
lent ion-containing external medium. The observed current was entirely dependent on the presence of AA, as no significant currents were seen to develop upon nominal removal of external divalent cations without prior addition of AA. In the con-
tinued absence of extracellular divalent ions, the AA-dependent current slowly declined in magnitude (50% reduction in 229 ± 23 s) (Fig. 1A). In contrast, replacing the divalent ion-
free bath solution with normal divalent ion-containing medium after the current had reached its maximum value resulted in a rapid inhibition of the current to levels consistent with those seen in normal medium (i.e. −0.5 pA/pF) (data not shown). We used voltage ramps from −100 to +30 mV to examine the current/voltage (I/V) relationship of the AA-dependent current observed in the nominally absence of divalent cations. As shown in Fig. 1B, the I/V relationship showed a reversal potential of 0 mV with significant inward and outward currents (mean current at +30 mV equals 22.6 ± 3.4 pA/pF, n = 5). The I/V curve showed a characteristic tild (−) shape, with marked inward rectification at negative voltages and outward rectification at positive voltages. The obvious nonlinear nature of the I/V curve strongly suggested that the observed macroscopic current was not a simple nonspecific “leak” current caused by removal of extracellular divalent ions. This was further confirmed by the demonstration that substitution of extracellular Na$^+$ with NMDG$^+$ completely inhibited the inward AA-dependent current, but did not affect the outward current, whereas substitution of Cs$^+$ in the pipette solution with NMDG$^+$ resulted in the complete absence of any outward current, without affecting the magnitude of the observed inward current (see Fig. 4B). Moreover, AA-dependent inward currents were completely inhibited (mean inhibition of 95.6 ± 3.5%, n = 5) by extracellular La$^{3+}$ (50 μM) and partially inhibited (50.8 ± 2.2%, n = 4) by extracellular Ca$^{2+}$ (50 μM). Both of these inhibitions were reversible. This further supports the contention that the AA-dependent current observed was not a nonspecific leak and indicates that the large current seen upon nominal removal of extracellular divalent cations represents the activity of an AA-dependent monovalent cation-permeable conductance. Comparison of the magnitudes of the inward current (at −80 mV) and the corre-
sponding outward current (at +30 mV) showed a direct linear relationship (r$^2$ = 0.89) (Fig. 1C), consistent with both currents being carried through the same channels. As both inward and outward currents were observed under these divalent ion-free conditions, it is clear that the channels responsible are appreciably permeable to both Na$^+$ and Ca$^{2+}$ ions (see below); however, they show negligible conductance to NMDG$^+$.  

As already noted, both voltage-gated Ca$^{2+}$ channels and the CRAC channel of non-excitable cells have been shown to dis-

**RESULTS**

**Ca$^{2+}$-selective ARC Channels Become Permeable to Monova-
lent Cations upon Removal of External Divalent Cations**—In normal external medium containing Ca$^{2+}$ and Mg$^{2+}$ in milli-
molar concentrations, addition of exogenous arachidonate acid (8 μM) to the bath solution resulted in the activation of a small inward current at −80 mV. Consistent with our previous report (12), this current displayed a marked inward rectification, a positive (greater than +30 mV) reversal potential, and an absence of any fast inactivation and was inhibited by La$^{3+}$ (50 μM). Substitution of external sodium with NMDG$^+$ had negligible effects on the current, confirming a minimal permeability to Na$^+$ under these conditions. All these features are consistent with the characteristic of this arachidonate-induced current as a Ca$^{2+}$-selective current (I$_{\text{CaC}}$).

Upon nominal removal of external divalent cations, a much larger inward current (measured at −80 mV) was seen to develop (Fig. 1A). Under normal conditions (i.e. Na$^+$ in the bath solution and Ca$^{2+}$ in the pipette solution), this current increased to reach a maximum amplitude of 28.3 ± 2.7 pA/pF (n = 5) or −50 times the Ca$^{2+}$-selective current seen in normal divalent ion-containing external medium. The observed current was entirely dependent on the presence of AA, as no significant currents were seen to develop upon nominal removal of external divalent cations without prior addition of AA. In the con-
tinued absence of extracellular divalent ions, the AA-dependent current slowly declined in magnitude (50% reduction in 229 ± 23 s) (Fig. 1A). In contrast, replacing the divalent ion-
free bath solution with normal divalent ion-containing medium after the current had reached its maximum value resulted in a rapid inhibition of the current to levels consistent with those seen in normal medium (i.e. −0.5 pA/pF) (data not shown). We used voltage ramps from −100 to +30 mV to examine the current/voltage (I/V) relationship of the AA-dependent current seen in the nominal absence of divalent cations. As shown in Fig. 1B, the I/V relationship showed a reversal potential of 0 mV with significant inward and outward currents (mean current at +30 mV equals 22.6 ± 3.4 pA/pF, n = 5). The I/V curve showed a characteristic tild (−) shape, with marked inward rectification at negative voltages and outward rectification at positive voltages. The obvious nonlinear nature of the I/V curve strongly suggested that the observed macroscopic current was not a simple nonspecific “leak” current caused by removal of extracellular divalent ions. This was further confirmed by the demonstration that substitution of extracellular Na$^+$ with NMDG$^+$ completely inhibited the inward AA-dependent current, but did not affect the outward current, whereas substitution of Cs$^+$ in the pipette solution with NMDG$^+$ resulted in the complete absence of any outward current, without affecting the magnitude of the observed inward current (see Fig. 4B). Moreover, AA-dependent inward currents were completely inhibited (mean inhibition of 95.6 ± 3.5%, n = 5) by extracellular La$^{3+}$ (50 μM) and partially inhibited (50.8 ± 2.2%, n = 4) by extracellular Ca$^{2+}$ (50 μM). Both of these inhibitions were reversible. This further supports the contention that the AA-dependent current observed was not a nonspecific leak and indicates that the large current seen upon nominal removal of extracellular divalent cations represents the activity of an AA-dependent monovalent cation-permeable conductance. Comparison of the magnitudes of the inward current (at −80 mV) and the corre-
sponding outward current (at +30 mV) showed a direct linear relationship (r$^2$ = 0.89) (Fig. 1C), consistent with both currents being carried through the same channels. As both inward and outward currents were observed under these divalent ion-free conditions, it is clear that the channels responsible are appreciably permeable to both Na$^+$ and Ca$^{2+}$ ions (see below); however, they show negligible conductance to NMDG$^+$.
play a large permeability to monovalent ions upon removal of extracellular divalent cations (7, 15, 16). The features of the AA-dependent monovalent current described above are consistent with the existence of a similar phenomenon in the ARC channels we have previously identified. To examine this, we compared the magnitude of the observed AA-dependent monovalent current measured at −80 mV with that of the corresponding AA-dependent Ca^{2+}-selective current measured in the same cell immediately prior to removal of extracellular divalent cations. The data obtained show a direct linear relationship between the magnitudes of the two AA-dependent currents ($r^2 = 0.88$) (Fig. 1D), supporting the contention that they reflect the behavior of a common set of channels (i.e. ARC channels).

Endogenous Store-operated Channels of HEK293 Cells (HEK-SOC Channels) Also Show a Permeability to Monovalent Cations upon Removal of Extracellular Divalent Cations—As noted, the aim of this study was to further define the features of the non-capacitative arachidonate-regulated conductance that distinguish it from store-operated or capacitative conductances. The features of the macroscopic monovalent conductance that develops upon reduction of external divalent cations in the store-operated conductance ($I_{\text{CRAC}}$) of Jurkat lymphocytes have been extensively characterized (15, 16). However, it appears likely that store-operated channels represent a family of conductances of which $I_{\text{CRAC}}$ is only one member. Therefore, it was important to be able to compare the AA-activated conductance with the endogenous SOC conductance in the same cell type. We therefore examined the effect of removal of divalent cations from the extracellular medium in cells in which the internal Ca^{2+} stores had been passively depleted by dialyzing with a highly buffered Ca^{2+}-free pipette solution. In the normal bath solution containing millimolar Ca^{2+} and Mg^{2+}, a small inward current measured at −80 mV slowly developed upon achieving whole-cell conditions. As previously reported, this current displayed all the features consistent with its characterization as a Ca^{2+}-selective store-operated current ($I_{\text{SOC}}$), including marked inward rectification, very positive reversal potential, fast inactivation, and inhibition by La^{3+} (50 μM) (12). Essentially identical currents were observed in cells in which intracellular Ca^{2+} stores were maximally depleted by preincubation in thapsigargin (1 μM for 15 min) (12) or by inclusion of the non-metabolizable inositol 1,4,5-trisphosphate analog adenosphostin A (2 μM) in the pipette solution (data not shown). Subsequent exposure to nominally divalent ion-free external solution evoked an increase in the inward current measured at −80 mV to reach a maximum amplitude of 11.7 ± 1.6 pA/pF (n = 8) or some 20 times the current observed in normal divalent ion-containing external medium (Fig. 2A). This store-operated current subsequently declined fairly rapidly in the continued absence of extracellular divalent ions, showing a 50% reduction in magnitude in a mean time of 17.6 ± 4.2 s (n = 5).

IV curves for the store-operated current in the nominal absence of divalent cations were obtained by application of voltage ramps from −100 to +30 mV (Fig. 2B). Under standard conditions (Na^{+} in the bath solution and Cs^{+} in the pipette solution), these showed a current that reversed at −0 mV with significant inward and outward currents (mean current at +30 mV of 10.8 ± 1.7 pA/pF, n = 6). Inward rectification at negative voltages and outward rectification at positive voltages were also apparent, giving the IV curve a “tilde” shape. The magnitudes of the inward (at −80 mV) and outward (at +30 mV) currents in individual cells were linearly related ($r^2 = 0.72$) (Fig. 2C). Moreover, the inward store-operated currents were reversibly inhibited by extracellular La^{3+} (50 μM), and both inward and outward currents were rapidly inhibited upon re-exposure to normal divalent ion-containing medium in the bath (data not shown). Substitution of extracellular Na^{+} with NMDG^{+} completely inhibited the inward currents, but did not affect the outward currents. Similarly, substitution of cesium in the pipette solution with NMDG^{+} resulted in the complete absence of any outward current, without affecting the magnitude of the observed inward current (see Fig. 4D). Based on these data, we conclude that the observed macroscopic currents are not the result of a simple nonspecific leak and that the currents seen upon nominal removal of extracellular divalent cations after depletion of the intracellular Ca^{2+} stores repre-
sent the activity of the endogenous store-operated conductance ($I_{\text{SOC}}$). This was further confirmed by comparison of the magnitude of the macroscopic SOC current in its Ca$^{2+}$-selective mode seen in the presence of divalent ions in the bath solution with that of the corresponding current seen in the same cell upon removal of external divalent ions. This showed that the magnitudes of the two currents were linearly related to each other ($r^2 = 0.64$) (Fig. 2D).

From the above results, it is clear that both the endogenous store-operated or capacitative conductance ($I_{\text{SOC}}$) and the non-capacitative AA-regulated Ca$^{2+}$-selective conductance ($I_{\text{ARC}}$) display a marked permeability to monovalent cations upon nominal removal of extracellular divalent ions, a property shared with voltage-gated Ca$^{2+}$ channels and the classic store-operated Ca$^{2+}$ conductance ($I_{\text{CRAC}}$). The most immediately obvious difference between the macroscopic currents displayed by ARC and SOC channels is the much larger monovalent conductance shown by $I_{\text{ARC}}$ relative to $I_{\text{SOC}}$ (Fig. 3A). Thus, although the whole-cell currents observed for the two conductances when in their Ca$^{2+}$-selective mode are similar in magnitude (~0.5–0.6 pA/pF at ~80 mV), the monovalent ARC currents seen upon removal of extracellular divalent ions are ~2.5 times the corresponding monovalent SOC currents (28.3 versus 11.7 pA/pF at ~80 mV). Such a difference may reflect a different sensitivity of ARC and SOC channels to the very low (submicromolar) Ca$^{2+}$ concentrations in our nominally divalent ion-free external medium. However, such sensitivity would have to be much higher than that previously reported for CRAC channels ($IC_{50}$ for Ca$^{2+}$ block of Na$^+$ currents = 4 μM) (13).

Moreover, as noted above, the monovalent SOC currents displayed a fairly rapid spontaneous decline, whereas the decline in the monovalent ARC current was much slower (Fig. 3, B and C). As discussed further below, the decline in the monovalent SOC current appears to reflect some kind of "inactivation" process of unknown origin. Given this, it is possible that the inactivation process shown by the SOC channels is occurring simultaneously with the activation of the monovalent permeability upon removal of extracellular divalent ions and could therefore contribute to the relatively smaller monovalent current seen with these channels. Despite these reservations, it is clear that, like the store-operated CRAC channels (7, 15, 16), both the endogenous SOC channels and the ARC channels of HEK293 cells are capable of conducting monovalent ions much more readily than Ca$^{2+}$ ions, although there may be some relative difference in this ability between the ARC and SOC channels.

**Monovalent Selectivity**—As noted above, both $I_{\text{SOC}}$ and $I_{\text{ARC}}$ in the nominal absence of extracellular divalent ions displayed characteristic tilde-shaped I/V curves with significant inward and outward monovalent currents under the standard conditions (Na$^+$ and Cs$^+$ as the principal cations in the bath and pipette solutions, respectively). Large inward currents developed upon nominal removal of external divalent cations and then spontaneously declined back to resting levels. $B$, current-voltage relationship of the store-operated current in the nominal absence of extracellular divalent cations. Currents were recorded during 150-ms voltage ramps from −100 to +30 mV at the peak of the monovalent store-operated current. $C$, relationship between the magnitude of the inward and the simultaneous outward store-operated currents (measured at −80 and +30 mV, respectively). Each point represents the value from a different cell. $D$, relationship between the magnitude of the inward Ca$^{2+}$-selective store-operated current measured at −80 mV immediately prior to removal of extracellular divalent ions and the corresponding peak inward monovalent current (also measured at −80 mV) in the same cell.

Substitution of Na$^+$ in the bath solution with Cs$^+$ had a statistically significant (p = 0.07) effect on the inward current density of the ARC channels measured at −80 mV (Fig. 4, A and E). When external Na$^+$ ions were replaced by Li$^+$, the inward current density at −80 mV was markedly reduced (from 28.3 ± 2.7 pA/pF (n = 5) to 5.0 ± 2.1 pA/pF (n = 6)) (Fig. 4, A and E). Outward (Cs$^+$) currents were also affected by substitutions of the external cations (Fig. 4A). Substitution of external Na$^+$ with Cs$^+$ reduced the outward (Cs$^+$) current measured at +30 mV by ~50% to 11.9 ± 3.0 pA/pF (n = 6; p = 0.02), whereas substitution with Li$^+$ further reduced the outward current to 8.0 ± 3.4 pA/pF (n = 6). Clearly then, the identity of...
the external ion affects the ability of the internal ion to carry outward current through the ARC channels. We have not analyzed the basis for these effects in detail, but they are indicative of an interaction of the different ions within the channel, consistent with the channel possessing a multi-ion conductance pathway (19). Based on these values of relative inward current density, a selectivity sequence for the monovalent conductance of the ARC channels of Cs$^+$ $\gg$ Na$^+$ $\gg$ Li$^+$ $\gg$ NMDG$^-$ is indicated. True relative permeabilities are normally analyzed from changes in reversal potentials. However, in the experiments reported here, the small magnitudes of the overall currents and the fact that the I/V curves obtained were generally rather flattened around the reversal potential made such analysis quantitatively difficult. Nevertheless, examination of the changes in reversal potentials observed indicated a similar relative permeability sequence of Cs$^+$-Na$^+$-Li$^+$-NMDG$^-$.

A small but statistically significant ($p = 0.05$) increase in the inward current density at $-80$ mV upon substitution of external Na$^+$ with Cs$^+$ was seen with the SOC current (Fig. 4, C and E). However, as noted above, the magnitude of the observed currents may be significantly influenced by the simultaneous rate of the inactivation process. In this case, the rate of the spontaneous inactivation of the SOC current was noticeably reduced in the presence of external Cs$^+$ (current magnitude reduced to $50\%$ in a mean time of $49.3 \pm 2.4 \text{ s} (n = 3)$ versus $17.6 \pm 4.2 \text{ s} (n = 5)$). Thus, the apparent increased inward SOC current density in the presence of external Cs$^+$ may result, at least in part, from the slower rate of inactivation seen under these conditions. Replacement of external Na$^+$ with Li$^+$ resulted in an approximate $50\%$ reduction of the inward current density to $6.3 \pm 3.0 \text{ pA/pF} (n = 4)$ (Fig. 4C), a value very similar to that seen with ARC currents (Fig. 4E). In marked contrast to the response of ARC currents, outward (Cs$^+$) currents through SOC channels were not significantly affected by replacement of external Na$^+$ with either Cs$^+$ or Li$^+$. The data obtained indicated a monovalent selectivity sequence for inward currents in the HEK-SOC channels of Cs$^+$ $\gg$ Na$^+$ $>$ Li$^+ \gg$ NMDG$^-$. Once again, despite the difficulties in obtaining any precise quantitative assessment, examination of the changes in reversal potential observed indicated an identical relative permeability sequence of Cs$^+$-Na$^+$-Li$^+$-NMDG$^-$.

Substitutions were also made to the monovalent cations in the internal (pipette) solutions. Substitution of internal Cs$^+$ with Na$^+$ markedly reduced the outward current density measured at $+30$ mV through the ARC channels (from $22.6 \pm 3.4$ to $8.1 \pm 1.0 \text{ pA/pF}, n = 5$) (Fig. 4, B and E). A similar, although much less dramatic decrease in outward current density at $+30$ mV was seen with SOC channels when Na$^+$ replaced internal Cs$^+$ (from $10.8 \pm 1.7 \text{ pA/pF} (n = 8)$ to $5.2 \pm 0.8 \text{ pA/pF} (n = 6)$) (Fig. 4, D and F). Importantly, this latter response could not be caused by an effect on the rate of spontaneous inactivation, as this was much slower when Na$^+$ replaced Cs$^+$ in the pipette solution (time to attain $50\%$ inactivation of $54.0 \pm 11.4 \text{ versus } 17.6 \pm 4.2 \text{ s}$). As has already been noted, replacing internal Cs$^+$ with NMDG$^-$ resulted in the complete absence of any significant outward currents through either ARC or SOC channels (Fig. 4, B and D). Based on these data for outward current densities, a monovalent cation selectivity for both ARC and SOC channels of Cs$^+$ $>$ Na$^+$ $\gg$ NMDG$^-$ is indicated. Again, despite the caveats already mentioned, examination of the
The principal external cation was Na$^+$, and SOC currents were induced by passive depletion using a Ca$^{2+}$-free pipette solution. A–D, shown are mean current-voltage curves illustrating the effects of substitution with different cations compared with the currents seen under standard conditions with Na$^+$ as the principal cation in the bath solution and Cs$^+$ as the principal cation in the pipette solution (Δ). The effects of substitution of Na$^+$ in the external (bath) solution with either Cs$^+$ (black bars) or Li$^+$ (white bars) are illustrated for the monovalent currents through ARC (A) and SOC (C) channels. Similarly, the effects of substitution of Cs$^+$ in the internal (pipette) solution with either Na$^+$ (black bars) or NMDG$^+$ (white bars) are illustrated for monovalent currents through ARC (B) and SOC (D) channels. E, summary data illustrating the effects of different external cations on inward ARC and SOC currents measured at −80 mV (mean ± S.E., n = 4–11). The principal external cation was Na$^+$ (white bars), Cs$^+$ (black bars), or Li$^+$ (shaded bars). F, summary data illustrating the effects of different internal cations on outward ARC and SOC currents measured at +30 mV (mean ± S.E., n = 4–8). The principal internal cation was Cs$^+$ (black bars) or Na$^+$ (white bars). As can be seen in the corresponding I/V traces (B and D), there was no detectable outward current when the principal internal cation was NMDG$^+$.

Changes in reversal potential observed indicated an identical relative permeability sequence of Cs$^+$-Na$^+$-NMDG$^+$. 

**Effects of Internal Mg$^{2+}$**—The similarity of the inward currents carried by Na$^+$ and Cs$^+$ reported here for both ARC and HEK-SOC channels (Fig. 4E) is not seen in the CRAC channels of Jurkat cells measured under the same conditions (15, 16). However, it was reported that magnesium-free internal solutions increase the magnitude of the inward Na$^+$ currents and, more particularly, dramatically increase the Cs$^+$ permeability of the Jurkat CRAC channels such that the Na$^+$ and Cs$^+$ currents are essentially identical (16). It was also noted that large outward currents develop in the absence of internal Mg$^{2+}$ that are not observed in the presence of internal Mg$^{2+}$ (16). We therefore examined the effects of removal of internal Mg$^{2+}$ on the monovalent currents recorded for ARC and HEK-SOC channels.

Under the standard conditions of Na$^+$ in the bath solution and Cs$^+$ in the pipette solution, removal of internal (pipette) Mg$^{2+}$ had no significant effect on the magnitude of the inward (Na$^+$) currents through ARC channels (Fig. 5A). Similarly, no significant change in the magnitude of the inward current was seen when the external cation was Cs$^+$ (data not shown). However, examination of the I/V relationship clearly indicated that removal of internal Mg$^{2+}$ reduced the degree of rectification in the inward (Na$^+$) current (Fig. 5B). Mg$^{2+}$-free internal solutions also markedly reduced the outward current (measured at +30 mV) carried by Cs$^+$ from 22.6 ± 3.4 to 13.4 ± 2.2 pA/pF (n = 5 and 4, respectively; p = 0.04) (Fig. 5, A and B).

When monovalent SOC currents were examined, Mg$^{2+}$-free internal solutions again had no significant effect on inward currents, whether these were carried by Na$^+$ (Fig. 5C) or Cs$^+$ (data not shown). Unlike the ARC currents, however, removal of internal Mg$^{2+}$ did not significantly influence the degree of rectification of the inward current (Fig. 5D). Moreover, in marked contrast to the response seen in ARC currents, removal of internal Mg$^{2+}$ significantly increased the outward (Cs$^+$) current measured at +30 mV from 10.8 ± 1.7 to 16.6 ± 2.1 pA/pF (n = 6 and 8, respectively; p = 0.03) (Fig. 5, C and D). Finally, Kerschbaum and Cahalan (16) reported that removal of internal Mg$^{2+}$ eliminates the spontaneous inactivation of the monovalent current through the CRAC channels in Jurkat cells. However, no such effect was observed in the endogenous SOC channels of HEK293 cells measured under the standard conditions (Na$^+$ in the bath solution and Cs$^+$ in the pipette solution). 

**FIG. 4. Selectivity of ARC and SOC conductances for monovalent cations**. ARC currents were activated by addition of exogenous arachidonic acid (8 μM), and SOC currents were induced by passive depletion using a Ca$^{2+}$-free pipette solution. A–D, shown are mean current-voltage curves illustrating the effects of substitution with different cations compared with the currents seen under standard conditions with Na$^+$ as the principal cation in the bath solution and Cs$^+$ as the principal cation in the pipette solution (Δ). The effects of substitution of Na$^+$ in the external (bath) solution with either Cs$^+$ (black bars) or Li$^+$ (white bars) are illustrated for the monovalent currents through ARC (A) and SOC (C) channels. Similarly, the effects of substitution of Cs$^+$ in the internal (pipette) solution with either Na$^+$ (black bars) or NMDG$^+$ (white bars) are illustrated for monovalent currents through ARC (B) and SOC (D) channels. E, summary data illustrating the effects of different external cations on inward ARC and SOC currents measured at −80 mV (mean ± S.E., n = 4–11). The principal external cation was Na$^+$ (white bars), Cs$^+$ (black bars), or Li$^+$ (shaded bars). F, summary data illustrating the effects of different internal cations on outward ARC and SOC currents measured at +30 mV (mean ± S.E., n = 4–8). The principal internal cation was Cs$^+$ (black bars) or Na$^+$ (white bars). As can be seen in the corresponding I/V traces (B and D), there was no detectable outward current when the principal internal cation was NMDG$^+$. 

changes in reversal potential observed indicated an identical relative permeability sequence of Cs$^+$-Na$^+$-NMDG$^+$. 

**Effects of Internal Mg$^{2+}$**—The similarity of the inward currents carried by Na$^+$ and Cs$^+$ reported here for both ARC and HEK-SOC channels (Fig. 4E) is not seen in the CRAC channels of Jurkat cells measured under the same conditions (15, 16). However, it was reported that magnesium-free internal solutions increase the magnitude of the inward Na$^+$ currents and, more particularly, dramatically increase the Cs$^+$ permeability of the Jurkat CRAC channels such that the Na$^+$ and Cs$^+$ currents are essentially identical (16). It was also noted that large outward currents develop in the absence of internal Mg$^{2+}$ that are not observed in the presence of internal Mg$^{2+}$ (16). We therefore examined the effects of removal of internal Mg$^{2+}$ on the monovalent currents recorded for ARC and HEK-SOC channels.

Under the standard conditions of Na$^+$ in the bath solution and Cs$^+$ in the pipette solution, removal of internal (pipette) Mg$^{2+}$ had no significant effect on the magnitude of the inward (Na$^+$) currents through ARC channels (Fig. 5A). Similarly, no significant change in the magnitude of the inward current was seen when the external cation was Cs$^+$ (data not shown). However, examination of the I/V relationship clearly indicated that removal of internal Mg$^{2+}$ reduced the degree of rectification in the inward (Na$^+$) current (Fig. 5B). Mg$^{2+}$-free internal solutions also markedly reduced the outward current (measured at +30 mV) carried by Cs$^+$ from 22.6 ± 3.4 to 13.4 ± 2.2 pA/pF (n = 5 and 4, respectively; p = 0.04) (Fig. 5, A and B).

When monovalent SOC currents were examined, Mg$^{2+}$-free internal solutions again had no significant effect on inward currents, whether these were carried by Na$^+$ (Fig. 5C) or Cs$^+$ (data not shown). Unlike the ARC currents, however, removal of internal Mg$^{2+}$ did not significantly influence the degree of rectification of the inward current (Fig. 5D). Moreover, in marked contrast to the response seen in ARC currents, removal of internal Mg$^{2+}$ significantly increased the outward (Cs$^+$) current measured at +30 mV from 10.8 ± 1.7 to 16.6 ± 2.1 pA/pF (n = 6 and 8, respectively; p = 0.03) (Fig. 5, C and D). Finally, Kerschbaum and Cahalan (16) reported that removal of internal Mg$^{2+}$ eliminates the spontaneous inactivation of the monovalent current through the CRAC channels in Jurkat cells. However, no such effect was observed in the endogenous SOC channels of HEK293 cells measured under the standard conditions (Na$^+$ in the bath solution and Cs$^+$ in the pipette solution).
In these cells, the times required to achieve a 50% decline in the measured current at 280 mV were 17.6 ± 4.2 s in the presence of internal Mg\(^{2+}\) and 23.6 ± 4.9 s in its absence (n = 5).

**Activation of ARC Currents after SOC Current Inactivation**—As already noted, the monovalent HEK-SOC current showed a rather rapid spontaneous decline in magnitude after activation upon removal of extracellular divalent cations (see Fig. 3B). Following such inactivation, re-exposure of the cells to normal divalent ion-containing medium (i.e. with Ca\(^{2+}\)) demonstrated that the Ca\(^{2+}\)-selective current was also now absent (Fig. 6A). However, continued exposure of the cells to external divalent ions induced a gradual reactivation of the normal Ca\(^{2+}\)-selective current over a 1–2-min period (Fig. 6A, inset). Subsequent exposure to divalent ion-free medium showed that this reappearance of the Ca\(^{2+}\)-selective current was also associated with a reactivation of the monovalent current. The data show that the sizes of these two currents (monovalent and Ca\(^{2+}\)-selective) were directly related to each other during this reactivation process (r\(^2 = 0.94\)) (Fig. 6B), confirming that the two currents reflect the activity of the same family of channels. An essentially identical phenomenon was observed in the CRAC channels of Jurkat cells (15). Without implying any specific molecular mechanism for this spontaneous decline and in accordance with the similar decline reported for monovalent CRAC currents (15, 16), we have described this phenomenon as a process of inactivation. Use of such a term is consistent with the demonstrated ability to reactivate the current by exposure to extracellular divalent ions.

The rate of decline in the corresponding monovalent ARC current after initial activation was always very much slower (typically 10–15 times) than those seen with the HEK-SOC currents under identical conditions (see Fig. 3B). In addition, after the decline in the monovalent ARC current was complete, re-exposing the cells to external divalent cations produced only a minimal subsequent reactivation of the monovalent ARC current (equal to 5.6 ± 0.9% of the original monovalent current, n = 5). These data suggest that the decline in the monovalent ARC current likely involves a rather different process from that seen with HEK-SOC currents. To distinguish between these two apparently rather different phenomena and consistent with the failure to reactivate ARC currents upon re-exposure to extracellular divalent ions, we will refer to this decline in the monovalent ARC current as a "run-down" rather than an inactivation. Again, the use of this term is not meant to signify the involvement of any specific molecular mechanism.

As noted above, the inactivated HEK-SOC current was absolutely dependent on exposure to normal divalent ion-containing solutions in the bath for its re-activation, and no "spontaneous" reactivation of the monovalent HEK-SOC current was ever seen without exposure of the cell to divalent ion-containing external medium. However, addition of exogenous AA (8 \(\mu\)M) to a cell in which the monovalent SOC current had been completely inactivated resulted in the development of a large inward monovalent current (Fig. 6C). The appearance of this AA-dependent current was not due to the activation of any residual SOC current resulting from any failure to completely
deplete the internal Ca\(^{2+}\) stores, as an identical AA-dependent monovalent current could be activated after spontaneous inactivation of SOC currents that had been stimulated by incubation in thapsigargin or by inclusion of adenophostin A (2 \(\mu M\)) in the pipette solution (data not shown). The magnitude of the AA-activated inward current in cells whose stores had been completely depleted by either thapsigargin or adenophostin A was not significantly different from that of the normal monovalent ARC current (26.2 ± 1.1 pA/pF \((n = 6)\) versus 28.3 ± 2.7 pA/pF \((n = 5)\)) and was consistently larger than the magnitude of the previously recorded monovalent SOC current in the same cell (7.5 ± 0.7 pA/pF, \(n = 6\)). Moreover, it displayed the characteristic slow spontaneous decline previously described for the monovalent ARC current. These data demonstrate that the ARC currents can be activated in a cell whose SOC currents have been completely inactivated and that the two currents therefore represent the activities of distinct conductances.

**DISCUSSION**

The data reported here show that ARC channels in HEK293 cells along with the endogenous SOC channels share, with CRAC channels and voltage-gated Ca\(^{2+}\) channels, the general property of monovalent cation permeation in the presence of low external divalent ion concentrations. This transition from high Ca\(^{2+}\) selectivity to monovalent cation permeation has been most extensively analyzed in voltage-gated Ca\(^{2+}\) channels (20–23). In these channels, the normally high selectivity for Ca\(^{2+}\) is thought to involve the binding of Ca\(^{2+}\) with high affinity to a site (or sites) in the pore of the channel. This binding enables Ca\(^{2+}\) to act as a blocking ion to reduce nonselective monovalent currents. A similar analysis has more recently been applied to the store-operated CRAC channels of Jurkat lymphocytes (15, 16). The proposed binding of Ca\(^{2+}\) also plays a role in Ca\(^{2+}\) permeation through these channels, as it explains the saturation of the Ca\(^{2+}\) current with increasing external Ca\(^{2+}\) concentrations. Specific models based on these principles have been developed that can simulate the selectivity, permeation, and block properties of voltage-gated Ca\(^{2+}\) channels (20, 21). However, it has recently been argued that such binding models can take several different possible forms, so the precise details are currently far from fully understood (24). Nevertheless, the general principles are reasonably clear. Selectivity in these channels is not a result of any molecular sieving process, but instead relies on the binding of ions to sites within the channel. These sites show a high affinity for Ca\(^{2+}\) compared with Na\(^{+}\). In the presence of external divalent cations, occupation of the sites by Ca\(^{2+}\) precludes the permeation of Na\(^{+}\) and other monovalent cations. At the same time, Ca\(^{2+}\) permeation is favored, despite the intrinsic high affinity of the sites, possibly by repulsive effects (strong ion-ion interactions) of ions in two closely positioned binding sites of similar high affinity (20, 22) or by a “stepwise” series of sites with graded binding affinities (24). At low concentrations of external divalent cations, Ca\(^{2+}\) is lost from the binding sites and thus permits the permeation of Na\(^{+}\) (and other monovalent cations), whose high permeation reflects their low affinity for the channel binding sites.

As such, it would seem that the overall behavior of both the ARC channels and the endogenous SOC channels of HEK293 cells is consistent with these models. Both ARC and HEK-SOC channels are highly Ca\(^{2+}\)-selective in normal divalent cation-
containing external medium and display a saturating current magnitude with increasing external Ca\textsuperscript{2+} concentrations. Despite this high selectivity for Ca\textsuperscript{2+}, these channels reveal relatively large monovalent currents in the nominal absence of extracellular divalent cations. These monovalent currents are rapidly and markedly inhibited by extracellular Ca\textsuperscript{2+} and are blocked by the same ions that block the Ca\textsuperscript{2+} conductance. Moreover, in both cases, the magnitude of the measured monovalent current is directly related to the magnitude of the corresponding Ca\textsuperscript{2+} current. All these features argue that the Ca\textsuperscript{2+} currents measured in the presence of extracellular divalent ions and the much larger monovalent currents measured in the nominal absence of divalent ions reflect the behavior of the same channels.

The characteristic small Ca\textsuperscript{2+} current and yet relatively large monovalent current of CRAC channels is therefore a feature shared by both HEK-SOC and ARC channels. Nevertheless, certain striking differences are apparent in the details of this phenomenon between these different channels. We have summarized the key features of the three channels (CRAC, HEK-SOC, and ARC) in Table I. First, although both ARC and HEK-SOC channels have broadly similar Ca\textsuperscript{2+}-selective current densities compared with the CRAC channels of Jurkat cells (~0.5–0.6 pA/pF for ARC and HEK-SOC channels and 0.8 pA/pF for CRAC channels) (8, 12), HEK-SOC and, more particularly, ARC channels have much higher monovalent current densities than Jurkat CRAC channels. Monovalent (Na\textsuperscript{+})/Ca\textsuperscript{2+} current density ratios for CRAC channels range from 5 to 10 (mean = 7.5 ± 2.7) (14). The corresponding value for HEK-SOC channels was ~20 and was even larger for ARC channels (~50). In CRAC channels, this ratio was profoundly influenced by removal of internal Mg\textsuperscript{2+}, which markedly increased inward Na\textsuperscript{+} currents. Under these conditions, the Na\textsuperscript{+}/Ca\textsuperscript{2+} current ratio for the CRAC channels becomes equal to 24.6 ± 4.9 (16), a value similar to that observed for HEK-SOC channels in the presence of internal Mg\textsuperscript{2+}, but still considerably less than that observed for ARC channels. Moreover, unlike Jurkat CRAC currents, removal of internal Mg\textsuperscript{2+} had no significant effect on the magnitude of the inward Na\textsuperscript{+} currents for either HEK-SOC or ARC channels. The underlying basis for the observed differences in the relative magnitudes of the Ca\textsuperscript{2+} and Na\textsuperscript{+} currents between the different channels remains unclear. As explained, estimates of the monovalent (Na\textsuperscript{+}) currents for both HEK-SOC and CRAC channels may be underestimated due to the spontaneous inactivation phenomenon seen in both these conduccances. However, it seems unlikely that this can account for all the differences seen, especially those between HEK-SOC and CRAC channels, both of which show broadly similar inactivaion kinetics. Until these properties have been examined in detail at the single channel level, we can only speculate on the basis for the observed differences. They may reflect, for example, distinct permeation properties of the individual channels or differences in the respective channel kinetics (e.g. open probability) induced by divalent ion-free external medium.

With regard to the monovalent selectivity, the data on inward current densities indicate a broadly similar conductance sequence for both ARC and HEK-SOC channels of Cs\textsuperscript{+} ≈ Na\textsuperscript{+} > Li\textsuperscript{+} >> NMDG\textsuperscript{+}, although, compared with HEK-SOC channels, ARC channels did show a much lower conductance for Li\textsuperscript{+} relative to that for Na\textsuperscript{+} or Cs\textsuperscript{+}. The corresponding sequence for the outward current densities for both channels is Cs\textsuperscript{+} > Na\textsuperscript{+} >> NMDG\textsuperscript{+}. It should be noted that these apparent conductance sequences are based on the measured macroscopic current densities and therefore do not allow for any possible effects of ion substitution on the open probability of the channels. Such analysis would require information on the behavior of single channels, which is currently unavailable. The observed monovalent selectivity sequence for both ARC and HEK-SOC channels stands in marked contrast to that reported for the CRAC channels of Jurkat cells under the same conditions (15, 16). In these cells, the measured Cs\textsuperscript{+} conductance through the CRAC channels is much smaller than the corresponding Na\textsuperscript{+} conductance, with a reported ratio of inward Na\textsuperscript{+} to Cs\textsuperscript{+} currents (measured at ~80 mV) of 26 ± 3. The corresponding ratios for ARC and HEK-SOC channels are 0.80 and 0.74, respectively (Fig. 4). Lepple-Wienhues and Cahalan (15) noted that they sometimes observed a nonspecific leak conductance in Jurkat cells that showed a Cs\textsuperscript{+} permeability similar to that of Na\textsuperscript{+}. However, this leak showed a linear I/V relationship and lacked the characteristic inactivation seen with either CRAC or HEK-SOC currents. Given these features, it seems unlikely that the high apparent Cs\textsuperscript{+} permeability we have observed in the HEK293 cells results from such a similar leak current. Clearly, CRAC channels are highly selective for Na\textsuperscript{+} over Cs\textsuperscript{+} in the nominal absence of extracellular divalent cations, a feature that is not shared by either the ARC channels or the endogenous HEK-SOC channels. Importantly, Kirschbaum and Cahalan (16) reported that the very low conductance to Cs\textsuperscript{+} shown by CRAC channels (relative to Na\textsuperscript{+}) is another feature that is dramatically affected by internal Mg\textsuperscript{2+}. Although removal of internal Mg\textsuperscript{2+} did significantly increase outward Cs\textsuperscript{+} currents through HEK-SOC channels (Fig. 5C), the effect was quantitatively much smaller than that reported for CRAC channels (16). Moreover, in marked contrast to the data from both CRAC and HEK-SOC channels, removal of internal Mg\textsuperscript{2+} significantly decreased outward Cs\textsuperscript{+} currents through the ARC channels (Fig. 5A). Obviously, the conclusion reached by Kershbaum and Cahalan (16) that internal Mg\textsuperscript{2+} normally blocks outward current through CRAC channels clearly does not apply to ARC channels.

Another reported effect of internal Mg\textsuperscript{2+} removal on CRAC channels is the loss of the spontaneous inactivation of the

### Table I

Comparison of the key features of monovalent currents through CRAC channels of Jurkat lymphocytes, HEK-SOC channels, and ARC channels

| Feature | CRAC | HEK-SOC | ARC |
|---------|------|---------|-----|
| Na\textsuperscript{+}/Ca\textsuperscript{2+} current ratio | ~5–10 | ~20 | ~50 |
| Internal Mg\textsuperscript{2+}-free effect | Increase to ~20 | No effect | Run-down (~230 s) |
| Spontaneous decline (time for 50% reduction) | Inactivation (~10 s) | No effect | |
| Internal Mg\textsuperscript{2+}-free effect | Inactivation abolished | No effect | |
| Reactivation | Ca\textsuperscript{2+}-dependent | Ca\textsuperscript{2+}-dependent | Ca\textsuperscript{2+} = Na\textsuperscript{+} >> Li\textsuperscript{+} >> NMDG\textsuperscript{+} |
| Monovalent selectivity (inward currents) | Na\textsuperscript{+} = Li\textsuperscript{+} = Cs\textsuperscript{+} | Na\textsuperscript{+} = Na\textsuperscript{+} >> Li\textsuperscript{+} >> NMDG\textsuperscript{+} | Ca\textsuperscript{2+} = Na\textsuperscript{+} >> Li\textsuperscript{+} >> NMDG\textsuperscript{+} |
| Internal Mg\textsuperscript{2+}-free effect | Large increase in Cs\textsuperscript{+} currents (Cs\textsuperscript{+} ≈ Na\textsuperscript{+}) | Small increase in outward (Cs\textsuperscript{+}) currents | Decrease in outward (Cs\textsuperscript{+}) currents |
| Internal Mg\textsuperscript{2+} and rectification | ? (increases inward rectification for Ca\textsuperscript{2+} current) | No significant effect | Increases inward rectification |
monovalent current (16). Although this was not seen in the HEK-SOC currents, many of the other features of the inactivation of monovalent CRAC currents were seen in the HEK-SOC currents, including the ability to reactivate the monovalent currents only after a brief period of re-exposure to external Ca$^{2+}$. This reactivation process involved both Ca$^{2+}$ currents (measured in normal divalent ion-containing medium) and the monovalent currents (measured in divalent ion-free medium), which reactivated in synchrony, as was previously reported for CRAC channels (15). A rather different kind of decay was observed in the monovalent currents through the ARC channels. This decay was much slower than the inactivation seen in the HEK-SOC currents, and after decaying to resting levels, re-exposure to external Ca$^{2+}$ was able to induce only a minimal reactivation of the monovalent ARC currents. Although we have no direct information on the basis for this phenomenon, such behavior is suggestive of a run-down of the currents rather than an inactivation. Precise characterization of these two processes and the mechanisms involved must await further studies. Nevertheless, we were able to utilize the absolute requirement of exposure to extracellular Ca$^{2+}$ for the reactivation of the HEK-SOC currents after their spontaneous inactivation to demonstrate that ARC currents could be activated (by addition of extracellular AA) in cells in which the monovalent SOC currents had been previously fully inactivated. The subsequently activated AA-dependent monovalent currents displayed all the properties characteristic of ARC currents. Clearly then, ARC channels can be activated in a cell whose SOC channels have been completely inactivated, consistent with our earlier findings that the two conductances are entirely separate and distinct entities (12, 25).

Together, the data presented indicate that there are both similarities and marked differences in the behavior of the CRAC channels of Jurkat cells, the endogenous SOC channels of HEK293 cells, and non-capacitative ARC channels. We have already described the similarities and differences between the HEK-SOC and ARC channels in their normal Ca$^{2+}$-conducting state (12). In this study, we have shown that, as highly Ca$^{2+}$-selective conductances, these channels share the property of conversion to a monovalent cation-permeable mode upon removal of extracellular divalent cations. Comparing first the two store-operated conductances CRAC and HEK-SOC, it is clear that, although they are very similar in several respects, they also appear to display some marked differences. Examination of these differences reveals that they particularly revolve around the effects of internal Mg$^{2+}$ ions on the channel properties. For example, two of the most obvious differences between the CRAC and HEK-SOC conductances are their respective Na$^{+}$/Ca$^{2+}$ current density ratios and their monovalent selectivity sequence. However, these differences largely disappear when CRAC currents are measured in the absence of internal Mg$^{2+}$ (16). In other words, HEK-SOC channels under normal conditions behave much like CRAC channels in the absence of internal Mg$^{2+}$, suggesting that the principal difference between these two store-operated conductances lies simply in the presence or absence of some Mg$^{2+}$-binding site(s) or in its affinity for Mg$^{2+}$. Whether this reflects the influence of differences in the cellular environment (membrane composition, presence of modulatory factors, etc.) or actual differences in the molecular identity of the channel proteins involved must await their characterization at the molecular level. However, it seems that these two store-operated conductances are probably closely related members of what is likely to be a family of similar channels.

More importantly, our ability to measure the endogenous SOC channels and ARC channels in the same cells allows a much more direct comparison between these two conductances. Here, several marked differences are apparent. These include differences in the magnitude of the ratio of monovalent to Ca$^{2+}$ currents, in the rate and nature of the spontaneous decline in monovalent current, and in the effects of external monovalent cation substitutions and of internal Mg$^{2+}$ removal on outward currents. All these features indicate that ARC and SOC currents reflect the activity of distinct conductances. Moreover, we have demonstrated that monovalent ARC currents can be activated after the complete spontaneous inactivation of SOC currents in the same cell. As such, this confirms our previous findings based on the characteristics and additive nature of the Ca$^{2+}$-selective currents for these two conductances (12) and on their distinct relative abilities to activate Ca$^{2+}$-sensitive adenyl cyclases (25). Of course, the most important and fundamental distinction between these two conductances is that, unlike SOC channels, ARC channels are not activated by store depletion (12). Importantly, the individually unique characteristics displayed by these two conductances in their monovalent cation-permeable modes described here, together with the much larger macroscopic currents observed, are features that undoubtedly prove useful in the identification of the specific activities of the respective channels under different experimental conditions and, in addition, raise the possibility of further more detailed analysis at the single channel level, as has already been demonstrated for CRAC channels (26, 27).

Acknowledgments—We thank Jill Thompson for excellent technical assistance and Drs. Ted Begenisich and Bob Dirksen for helpful discussions and suggestions and comments on an earlier version of the manuscript.

REFERENCES

1. Putney, J. W., Jr. (1986) Cell Calcium 7, 1–12
2. Putney, J. W., Jr. (1990) Cell Calcium 11, 611–624
3. Berridge, M. J. (1995) Biochem. J. 312, 1–11
4. Farquhar, G. B., and Penner, R. (1997) Annu. Rev. Physiol. 77, 961–930
5. Lewis, R. S. (1999) Adv. Second Messenger Phosphoprotein Res. 33, 279–307
6. Hoth, M., and Penner, R. (1992) Nature 355, 353–356
7. Hoth, M., and Penner, R. (1993) J. Physiol. (Lond.) 465, 359–386
8. Zweifach, B., and Lewis, R. S. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6259–6299
9. Shuttleworth, T. J. (1999) Cell Calcium 25, 237–246
10. Shuttleworth, T. J. (1996) J. Biol. Chem. 271, 31720–21725
11. Shuttleworth, T. J., and Thompson, J. L. (1998) J. Biol. Chem. 273, 32636–32643
12. Mignen, O., and Shuttleworth, T. J. (2000) J. Biol. Chem. 275, 9114–9119
13. Munaron, L., Antoiotti S., Distasi C., and Lovisolo D. (1997) Cell Calcium 22, 179–188
14. Broad, L. M., Armstrong, D. L., and Putney, J. W., Jr. (1999) J. Biol. Chem. 274, 32881–32888
15. Lepple-Wienhues, A., and Cahalan, M. D. (1996) Biophys. J. 7, 787–794
16. Kerschbaum, H. H., and Cahalan, M. D. (1998) J. Gen. Physiol. 111, 521–537
17. Hille, B. (1992) Ionic Channels in Excitable Membranes, 2nd Ed., Sinauer Associates, Inc., Sunderland, MA
18. Hess, P., and Tsien, R. W. (1984) Nature 309, 453–456
19. Almers, W., McCleskey, E. W., and Palade, P. T. (1984) J. Physiol. (Lond.) 353, 565–583
20. Almers, W., and McCleskey, E. W. (1984) J. Physiol. (Lond.) 353, 565–580
21. Hess, P., Lansman, J. B., and Tsien, R. W. (1980) J. Gen. Physiol. 86, 295–319
22. McCleskey, E. W. (1999) J. Gen. Physiol. 113, 765–772
23. Shuttleworth, T. J., and Thompson, J. L. (1999) J. Biol. Chem. 274, 31374–31378
24. Kerschbaum, H. H., and Cahalan, M. D. (1999) Science 283, 836–839
25. Braun, F.-J., Broad, L. M., Armstrong, D. L., and Putney, J. W., Jr. (2001) J. Biol. Chem. 276, 1063–1070
Permeation of Monovalent Cations through the Non-capacitative
Arachidonate-regulated Ca$^{2+}$ Channels in HEK293 Cells: COMPARISON WITH
ENDOGENOUS STORE-OPERATED CHANNELS
Olivier Mignen and Trevor J. Shuttleworth

J. Biol. Chem. 2001, 276:21365-21374.
doi: 10.1074/jbc.M102311200 originally published online April 2, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M102311200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 25 references, 11 of which can be accessed free at
http://www.jbc.org/content/276/24/21365.full.html#ref-list-1