Depletion of Intracellular Calcium Stores Activates a Calcium Conducting Nonselective Cation Current in Mouse Pancreatic Acinar Cells*

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Receptor-mediated Ca\(^{2+}\) release from inositol (1,4,5)-trisphosphate (IP\(_3\))-sensitive Ca\(^{2+}\) stores causes “capacitative calcium entry” in many cell types (Putney, J. W., Jr. (1986) Cell Calcium 7, 1–12; Putney, J. W., Jr. (1990) Cell Calcium 11, 611–624). We used patch-clamp and fluorescence techniques in isolated mouse pancreatic acinar cells to identify ion currents and cytosolic calcium concentrations under conditions in which intracellular Ca\(^{2+}\) stores were emptied. We found that depletion of Ca\(^{2+}\) stores activated a calcium-release-activated nonselective cation current (I\(_{\text{CRAN}}\)) which did not discriminate between monovalent cations. I\(_{\text{CRAN}}\) possessed a significant conductance for Ca\(^{2+}\) and Ba\(^{2+}\). It was not inhibited by La\(^{3+}\), Gd\(^{3+}\), or Cd\(^{2+}\) but was completely abolished by flufenamic acid or genistein. In whole cell and cell-attached recordings, a 40–45 pS nonselective cation channel was identified which was activated by Ca\(^{2+}\) store depletion. Calcium entry as detected by single-cell fluorescence measurements with fluo-3 or fura-2, showed the same pharmacological properties as I\(_{\text{CRAN}}\). We conclude that in mouse pancreatic acinar cells 40–45 pS nonselective cation channels serve as a pathway for capacitative Ca\(^{2+}\) entry. This entry pathway differs from the previously described I\(_{\text{CRAC}}\) (Hoth, M., and Penner, R. (1992) Nature 355, 353–356) in its ion-selectivity, pharmacological profile, and single-channel conductance.

In several nonexcitable cell types activation of cell membrane receptors by hormones or neurotransmitters results in a biphasic calcium signal. An initial Ca\(^{2+}\) peak produced by calcium release from intracellular inositol (1,4,5)-trisphosphate (IP\(_3\))-sensitive Ca\(^{2+}\) stores is followed by a sustained Ca\(^{2+}\) plateau due to Ca\(^{2+}\) entry from the extracellular space (1–5). The hypothesis that it is the decrease in the Ca\(^{2+}\) concentration in the internal store which causes Ca\(^{2+}\) influx into the cell was first proposed in 1986 by Putney and has been termed “capacitative calcium influx” (1, 2). Accordingly, not only hormonal stimulation, but also Ca\(^{2+}\) pool depletion following treatment with inhibitors of the Ca\(^{2+}\)-ATPase such as di-tert-butyldihydroquinone (t-BHQ) or thapsigargin (6) or with Ca\(^{2+}\) ionophores (7) leads to activation of Ca\(^{2+}\) entry.

We describe here that calcium store depletion by the agonist acetylcholine (ACh), IP\(_3\), the Ca\(^{2+}\)-ATPase inhibitor t-BHQ, or the Ca\(^{2+}\) ionophore ionomycin activates a calcium conducting nonselective cation channel which can also be blocked by genistein but not by La\(^{3+}\). Capacitative Ca\(^{2+}\) entry as measured by fluorescence methods was also completely inhibited by genistein, whereas La\(^{3+}\) had no effect on Ca\(^{2+}\) influx in mouse but completely inhibited Ca\(^{2+}\) influx in rat pancreatic acini. This indicates the presence of different Ca\(^{2+}\) influx pathways in different animal species. We conclude from our data that in mouse pancreatic acinar cells the “calcium release-activated nonselective cation current” (I\(_{\text{CRAN}}\)) is responsible for capacitative calcium entry.

**EXPERIMENTAL PROCEDURES**

**Cell Preparation**—Mouse and rat pancreatic acinar cells were prepared from male CD-1 mice and male Wistar rats, respectively, as described previously (14). Acinar cells from male Wistar rats were prepared in the same way (14).

**Electrophysiology**—Patch-clamp experiments were performed in the tight-seal, whole cell, and cell-attached configuration (15) at room temperature (24 ± 2 °C) in a standard bath solution containing in mM: 140 NaCl, 4.7 KCl, 1.3 CaCl\(_2\), 1 MgCl\(_2\), 10 HEPES, 10 glucose, pH 7.4. Patch pipettes were manufactured from borosilicate glass capillaries and had resistances of 2 to 4 M\(\Omega\) when filled with a standard buffer containing in mM: 125 K\(^{+}\)-Asp, 10 HEPES, 10 EGTA, 30 KOH, 2.5 mM Mg-ATP, 70 mM free Ca\(^{2+}\), pH 7.2. In some experiments BAPTA (10 mM) was used instead of EGTA, which did not influence the results. Patch-clamp experiments were recorded with a computer controlled EPC9 patch-clamp amplifier (HEKA; Lambrecht, Germany). Cell capacitance and series resistance were calculated with the software-supported internal routines of the EPC9 and compensated before each experiment. Data were sampled at 1 kHz on the computer hard disk after low pass filtering at 400 Hz. In whole cell experiments voltage ramps were applied every 2 s to the cells (−140 to 100 mV, slope 1 V/s) near the reversal. Either the resulting IV curve is shown or current values at −60 mV (reversal potential of Cl\(^−\) currents) were extracted from every ramp and presented as time courses. Single-channel measurements were done in the whole cell mode with the same pipette solution as described above. In the cell-attached configuration pipettes were filled with the standard bath solution. Single-channel data were collected on a video tape by use of a modified pulse code modulator (Sony, PCM 501).
and were later resampled (2 kHz) on a “80486” personal computer hard disk after low pass filtering at 600 Hz and were analyzed with laboratory written software.

The permeability ratios of \( I_{\text{CRANCl}} \) were calculated as,

\[
E_{\text{rev}} = \frac{RT}{2F} \ln \frac{4P_{\text{Ca}}(\text{Ca})}{P_{\text{Cl}}(\text{Cl})} \tag{Eq. 1}
\]

\((E_{\text{rev}}) = \text{reversal potential (V)}, \ T = \text{temperature (K)}, \ P_{\text{Ca}} = \text{relative permeability for calcium, } P_{\text{Cl}} = \text{relative permeability for cations, } [\text{Ca}]_{\text{c}} = \text{calcium concentration outside the cell, } [\text{cat}] = \text{concentration of monovalent cations inside the cell)}\). \( E_{\text{rev}} \) was measured, \( P_{\text{Ca}} \) was set to 1, and \( P_{\text{Cl}} \) was calculated.

**Fluorescence Techniques**—Isolated single cells and acini were loaded with 3–4 \( \mu \text{M} \) fluo-3/AM or fura-2/AM for 30 min at room temperature. After dye loading the cell suspension was stored at 4 °C and used for experiments within 5 h. Measurements with fluo-3 were done with a confocal laser scanning microscope (Zeiss Axiovert 35 microscope equipped with the confocal laser scanning and imaging system Bio-Rad MRC-600) on single cells from multiple cell clusters (up to 5 cells) as described in greater detail elsewhere (13). The internal \([\text{Ca}^{2+}]\) is given as the mean fluorescence of fluo-3.

Fura-2 ratiometric measurements were made with an imaging system (Zeiss Axiovert 135 equipped with an apparatus from T.I.L.L. Photonics, Munich) on single cells from multiple cell clusters. Cells were excited alternately at 345 and 380 nm wavelength and the emission was collected for 50–200 ms above 510 nm wavelength. The results are given as the ratio of the fluorescence intensities at the different wavelengths (345/380).

**Materials**—Genistein and lavendustin A were purchased from Calbiochem-Novabiochem (Bad Soden/Germany). Furo-3/AM and fura-2/AM were obtained from Molecular Probes (Eugene, OR). t-BHQ was from Aldrich (Steinheim, Germany) and all other chemicals from Sigma (Deisenhofen, Germany).

**RESULTS**

**Depletion of Intracellular Ca\(^{2+}\) Pools Activates a Nonselective Cation Current**—In order to activate capacitative Ca\(^{2+}\) entry we depleted intracellular Ca\(^{2+}\) stores of isolated mouse pancreatic acinar cells by extracellular addition of Ca\(^{2+}\)-ATPase inhibitor t-BHQ (1 \( \mu \text{M} \)), ACh (1 \( \mu \text{M} \)), ionomycin (10 \( \mu \text{M} \)) or by intracellular addition of IP\(_3\) (10 \( \mu \text{M} \)). Cells were dialyzed in the whole cell mode with the Ca\(^{2+}\)-free chelators EGTA (10 \( \text{mM} \)) or BAPTA (10 \( \text{mM} \)) through the patch pipette (free Ca\(^{2+}\) clamped to 70 \( \text{mM} \)) to prevent increases in free Ca\(^{2+}\) leading to activation of previously described Ca\(^{2+}\)-dependent Cl\(^-\) and nonselective cation currents (16).

Under activating conditions, currents began to develop, but with different time courses depending on the test substances used for store depletion (Fig. 1, a and b). A Ca\(^{2+}\)-free pipette solution induced a slow response (\( t_{90} = 484 \pm 137 \) s), most likely by depletion of stores through Ca\(^{2+}\) leaks. ACh, t-BHQ, or intracellular application of IP\(_3\) accelerated the current development significantly (\( t_{90} = 240 \pm 90 \) s, mean of the pooled data from all three conditions) and ionomycin induced the fastest response (\( t_{90} = 90 \pm 17 \) s). To obtain current-voltage curves (I/V curves) we applied voltage ramps (−140 to 100 \( \text{mV} \), 1 V/s). The linearity of the I/V curve (Fig. 1c) indicates voltage independence. The maximal conductance of the current was variable from cell to cell. The mean was \( 253 \pm 111 \text{ pS/} \mu \text{F} \) (\( n = 17 \)) in the case of t-BHQ activation. The conductance did not differ significantly when currents were activated by ACh (220 ± 99 pS/\( \mu \text{F} \), \( n = 5 \)), IP\(_3\) (228 ± 111 pS/\( \mu \text{F} \), \( n = 6 \)), or ionomycin (195 ± 109 pS/\( \mu \text{F} \), \( n = 13 \)).

**Ion Selectivity of \( I_{\text{CRANCl}} \)**—Ion exchange experiments revealed a permeability sequence for the current of \( \text{Rb}^+ > K^+ > \text{Na}^+ \gg N\)-methyl-d-glucamine (NMDG\(^+\)) as determined by changes in the reversal potentials (Fig. 1d, \( n = 5 \)). A Cl\(^-\) component could be excluded since no changes in current were observed at the reversal potential of monovalent cations (0 \( \text{mV} \), Fig. 1c). Furthermore, substitution of bath NMDG-Cl with NMDG-aspartate had no effect on outward currents (Fig. 1e, 1f).

**Fig. 1. Activation of a nonselective cation current by depletion of intracellular Ca\(^{2+}\) pools.** a, time course of activation of nonselective cation currents (\( I_{\text{CRANCl}} \)) in response to Ca\(^{2+}\) store depletion. Control: standard pipette solution contained 2.5 \( \text{mM} \) ATP and 70 \( \text{nM} \) free Ca\(^{2+}\) to maintain filling of the Ca\(^{2+}\) pool. No current was activated (\( n = 5 \)) under these conditions. Ca\(^{2+}\) free, 0 ATP: no Ca\(^{2+}\) and no ATP were added to the pipette solution (\( n = 5 \)). IP\(_3\): 10 \( \mu \text{M} \) IP\(_3\) was added to the standard pipette solution (\( n = 6 \)). ACh: ACh (1 \( \mu \text{M} \)) was added to the bath solution (\( n = 5 \)). t-BHQ: t-BHQ (1 \( \mu \text{M} \)) was added to the bath solution (\( n = 5 \)). Ionomycin: ionomycin (10 \( \mu \text{M} \)) was added to the cells from the bath side by a wide-tipped glass capillary (\( n = 13 \)). \( t_b \) (arrow) indicates the time of addition of test substances to the bath medium (t-BHQ, ACh, and ionomycin). In the case of IP\(_3\) activation, \( t_b \) is the beginning of the whole cell configuration. Current traces were generated by applying voltage ramps (−140 to 100 \( \text{mV} \), 1 V/s) every 2 s. The actual current values at \( V_{\text{hold}} = −60 \text{ mV} \) (reversal potential of Cl\(^-\) ions) were extracted and plotted against the time. The data shown are representative of a total given in b, b, columns present the latencies between addition of test substances and 90% activation of the depletion-activated current (\( t_{90} \)). Averaged data were presented as the mean ± S.D. Student’s t test was used to determine significant differences between the \( t_{90} \) values of currents activated with different substances. The latencies of t-BHQ, ACh, IP\(_3\) (\( p < 0.005 \)), and ionomycin (\( p < 0.001 \)) were significantly shorter in comparison to the 0 Ca\(^{2+}\)/0 ATP condition. While the effects for t-BHQ, ACh, and IP\(_3\) did not differ significantly from each other (\( p > 0.05 \)) the ionomycin effect was significantly faster (\( p < 0.005 \)). \( n \) gives the number of independent experiments with a single cell each. c, representative I/V curves taken before and following activation of \( I_{\text{CRANCl}} \) with t-BHQ (1 \( \mu \text{M} \)). d, permeabilities for different monovalent cations. Currents were activated by t-BHQ (1 \( \mu \text{M} \)). The standard bath solution was exchanged for solutions with equimolar concentrations of NMDG\(^+\), Rb\(^+\), or K\(^+\) instead of Na\(^+\) as indicated. The order of the reversal potentials were taken as indicators for the relative permeabilities (one representative out of five similar experiment is shown; for clarity the I/V curve for KCl is not shown, since it is identical to the NaCl curve). The determined permeability sequence is Rb\(^+\)>Na\(^+\)=K\(^+\)>NMDG\(^+\), permeability for Cl\(^-\) and aspartate. Currents were activated by t-BHQ (1 \( \mu \text{M} \)). First Na\(^+\) was exchanged for NMDG\(^+\) thereafter in the same experiment Cl\(^-\) was exchanged for aspartate (Asp) as indicated (one representative out of six similar experiments is shown).
FIG. 2. Permeabilities for divalent cations. The data shown were taken from representative experiments (a, n = 5; b, n = 3; c, n = 3). Currents were activated by application of t-BHQ (1 μM) to the bath. Patch-clamp pipettes were filled with the standard solution as described under “Experimental Procedures.” a, representative I-V curves were taken when I_{CRANC} had reached a maximum (control), following substitution of extracellular NaCl by equimolar NMDG-Cl (NMDG-CI) and subsequent substitution of all NMDG+ by 70 mM Ca2+ (CaCl2) or by 70 mM Ba2+ (Bacl2). Ca2+ and Ba2+ produced significant inward currents in comparison to the current in the presence of impermeant NMDG+.

In particular the lack of effect of La3+ on the Ca2+ component of I_{CRANC} after full activation of I_{CRANC} (control), substitution of bath NaCl with NMDG-Cl (NMDG-CI) and subsequent addition of genistein (50 μM, NMDG-Cl + gen) resulted in a complete reduction of inward and outward currents. In contrast to the finding shown in a, 70 mM Ca2+ in the bath did not produce any inward current in the presence of genistein (CaCl2 + gen). c, effect of La3+ on the calcium component of I_{CRANC}. After full activation of I_{CRANC} (control), bath NaCl was substituted by 70 mM CaCl2 (CaCl2), resulting in a reduction of the inward component of I_{CRANC}. Addition of La3+ (100 μM; CaCl2 + La3+) did not reduce this current, but subsequent addition of genistein (50 μM; CaCl2 + La3+ + gen) blocked the calcium current through I_{CRANC}.

n = 4). The whole cell current was also able to carry Ca2+ and Ba2+ ions (Fig. 2a). Referring to the more negative reversal potentials measured after Na+ substitution by Ca2+ (ΔV_{rev(Na)} = −23 ± 6.5 mV, n = 7), an apparent permeability ratio for the cations inside versus Ca2+ outside the cell of 13:1 was calculated. Because I_{CRANC} does not discriminate between Na+ and K+, this permeability ratio equals the ratio for both Na+: Ca2+ and K+:Ca2+.

Inhibitors of I_{CRANC} and Ca2+ Influx—To test if the described current is responsible for capacitative calcium influx in pancreatic acinar cells we compared pharmacological properties of I_{CRANC} and Ca2+ influx measured as fluo-3 fluorescence with a confocal microscope. In fluorescence experiments the plateau phase of the calcium signal is exclusively produced by extracellular calcium entering the cell (13, 17). Both I_{CRANC} and calcium influx were inhibited by genistein (Figs. 2, b and c, and 3 a and c). Therefore it seemed likely that Ca2+ influx occurred through the same genistein-inhibitable calcium pathway. Other tyrosine kinase inhibitors such as tyrphostin B56 (100 μM, n = 4, data not shown) showed 50% inhibition of both I_{CRANC} and Ca2+ influx measured with fluorescence while lavendustin A and tyrphostin B56 (n = 5, 100 μM each) had no effect. The dihydroxy analog of genistein (daidzein, 50 μM, n = 10, data not shown) which lacks the ability to regulate tyrosin kinase was also ineffective on capacitative Ca2+ influx.

The assumption that Ca2+ influx was inhibited by genistein (50 μM, gen) and the Ca2+ influx plateau (right, n = 10); c, La3+ (100 μM) neither blocked I_{CRANC} (left, n = 4) nor the Ca2+ release spike or the Ca2+ influx plateau (right, n = 10). Subsequent application of genistein (50 μM) blocked the influx plateau completely (n = 5), whereas wash-out of genistein resulted in immediate increase in [Ca2+]. Data were taken from representative experiments. n gives the number of independent experiments (one cell in each electrophysiological experiment, 20–30 cells in each fluorescence experiment).

FIG. 3. Effect of genistein (50 μM), flufenamic acid (100 μM), and La3+ (100 μM) on I_{CRANC} and Ca2+ fluorescence. In fluorescence experiments drugs were applied at the Ca2+ plateau phase of a calcium transient induced by influx of extracellular calcium. a, left, inward current at V_{hold} = −60 mV. During activation of I_{CRANC} in the presence of t-BHQ (1 μM) administration of genistein (50 μM, gen) inhibited the current completely (n = 20). Right, application of acetylcholine (ACh, 500 nM) induced an increase in fluorescence indicating an increase in [Ca2+]. The plateau of the calcium signal was completely inhibited by genistein (50 μM, gen), indicating that Ca2+ influx was inhibited (n = 20). b, experiment of the same type as in a. Flufenamic acid (flufe, 100 μM) blocked I_{CRANC} (left, n = 4) and the Ca2+ influx plateau (right, n = 10); c, La3+ (100 μM) neither blocked I_{CRANC} (left, n = 4) nor the Ca2+ release spike or the Ca2+ influx plateau (right, n = 10). Subsequent application of genistein (50 μM) blocked the influx plateau completely (n = 5), whereas wash-out of genistein resulted in immediate increase in [Ca2+]. Data were taken from representative experiments. n gives the number of independent experiments (one cell in each electrophysiological experiment, 20–30 cells in each fluorescence experiment).
the presence of sodium (Fig. 3c, left) or in the presence of calcium (Fig. 2c), and on the capacitative calcium entry measured with fluorescence techniques (Figs. 3c and 4a) is remarkable because in other systems La$$^{3+}$$ is known as an inhibitor of capacitative Ca$$^{2+}$$ influx and in higher concentration also of Ca$$^{2+}$$ efflux due to Ca$$^{2+}$$ pump inhibition (18). We therefore tested the effect of La$$^{3+}$$ in more detail. We took into consideration that the maintenance of a Ca$$^{2+}$$ plateau, which is usually interpreted to show Ca$$^{2+}$$ influx, should also occur if both Ca$$^{2+}$$ influx and Ca$$^{2+}$$ extrusion was inhibited by La$$^{3+}$$. In this case the effect of La$$^{3+}$$ could not be taken as indication for Ca$$^{2+}$$ influx following Ca$$^{2+}$$ release. We therefore tested a wide range of La$$^{3+}$$ concentrations (100 nM, 1 μM, 10 μM, 100 μM, and 250 μM) to inhibit agonist-stimulated Ca$$^{2+}$$ entry without Ca$$^{2+}$$ extrusion at low concentrations and to inhibit also Ca$$^{2+}$$ extrusion at higher concentrations (5, 17, 18). In no case did La$$^{3+}$$ reduce fluo-3 fluorescence whereas subsequent application of genistein in the presence of La$$^{3+}$$ ([La$$^{3+}$$], 100 or 250 μM) always abolished the calcium plateau (Fig. 3c). It therefore appears to be unlikely that La$$^{3+}$$ inhibited Cu$$^{2+}$$ entry in this concentration range. To further test the effect of La$$^{3+}$$ on Ca$$^{2+}$$ influx we performed the experiments shown in Fig. 4a, indicating that following hormonal depletion of Ca$$^{2+}$$ stores in the absence of Ca$$^{2+}$$, readdition of Ca$$^{2+}$$ caused Ca$$^{2+}$$ influx which was not inhibited by La$$^{3+}$$. Comparison of Ca$$^{2+}$$ Influx in Mouse and Rat Pancreatic Acinar Cells—Since La$$^{3+}$$ had been described to inhibit capacitative Ca$$^{2+}$$ influx in rat pancreatic acinar cells using fura-2 (17), we repeated the experiments with La$$^{3+}$$ in rat to compare the results with mouse pancreatic acinar cells. In mouse pancreatic acinar cells La$$^{3+}$$ had no effect on calcium entry as measured with fluo-3 (see Fig. 4a, left) or fura-2 (see Fig. 4a, right). However, in rat pancreatic acinar cells capacitative Ca$$^{2+}$$ entry was inhibited by 100 μM La$$^{3+}$$ (Fig. 4b). Our results confirm experiments described previously by Tsuonoda et al. (17) on rat pancreatic acinar cells and indicate different capacitative Ca$$^{2+}$$ influx pathways in mouse and rat. In contrast, genistein (50 μM) completely inhibited capacitative Ca$$^{2+}$$ influx in both mouse (see Fig. 3, a, right, and c, right) and rat as measured by fluorescence (data not shown). Resolution of Single Channel Events in I$$^{\text{CRANC}}$$. In some experiments (n = 6 out of n = 32 and 7 out of 52 cells) in which store depletion resulted in the activation of only a small current it was possible to recognize single channel events in the whole cell mode of the patch-clamp technique. The IV curves of these channels were linear and reversed at 0 mV (Fig. 5a). The conductance in the whole cell mode measured with standard solutions was in the range of 40–45 pS (43 ± 3.3 pS, n = 7). In
the cell-attached mode the 40–45 pS channel could be irreversibly activated with t-BHQ (Fig. 5b) or reversibly with ACh (Fig. 5c). Under these conditions the channel always coexisted with the previously described 27 pS channel (Fig. 5b) which is Ca\(^{2+}\) activated and therefore is observed under conditions at which cytoplasmic Ca\(^{2+}\) rises (19). Because the 27 pS channel has a high density (about 500 channels/cell (20)) compared to the 45 pS channel (about 70 channels/cell, calculated as the mean single channel conductance/single channel conductance assuming a P\(O\) of 0.6 for the single channel) it is not surprising that the 40–45 pS channel was seen in the cell-attached mode in only \(~10\%\) of the cells. The possibility that the two conductance levels which we found in cell-attached experiments are sublevels of one channel type can be ruled out by the finding that in all whole cell experiments only a single conductance level of 40–45 pS was observed. Excising patches with the 40–45 pS channel into a standard bath solution resulted in an immediate run-down of this channel type leaving only the 27 pS channel active (n = 4).

**DISCUSSION**

The results presented here indicate that capacitative Ca\(^{2+}\) entry in mouse pancreatic acinar cells is produced by a calcium release-activated Ca\(^{2+}\) conducting nonselective cation channel (I\(_{\text{ICRANC}}\)). The main characteristic of I\(_{\text{ICRANC}}\) is its insensitivity to La\(^{3+}\) which argues against the presence of the previously described ICRAC (7) in mouse pancreatic acinar cells. Flufenamic acid, an inhibitor of nonselective cation channels (21), and genistein, a tyrosine kinase inhibitor, also inhibited both I\(_{\text{ICRANC}}\) and capacitative Ca\(^{2+}\) entry. The genistein effect should be emphasized because it was shown before (13) that it inhibits calcium entry without affecting calcium release from IP\(_3\)-sensitive pools. We believe that these pharmacological similarities of I\(_{\text{ICRANC}}\) and capacitative calcium entry are consistent with the possibility that I\(_{\text{ICRANC}}\) is responsible for capacitative Ca\(^{2+}\) influx in mouse pancreatic acinar cells. The mechanism for the effect of genistein on calcium influx is not yet clear because genistein effects are diverse (22). The lack of effect of other tyrosine kinase inhibitors like lavendustin A or tyrphostin B56 on mouse pancreatic acinar cells led us to conclude that a more direct interaction between channel and genistein rather than the proposed inhibition of a tyrosine kinase (23, 24) is responsible for inhibition.

**Comparison of I\(_{\text{ICRANC}}\) to Other Capacitative Ca\(^{2+}\) Influx Currents**—The nonselective cation current, which is activated by Ca\(^{2+}\) store depletion (I\(_{\text{ICRANC}}\)) in mouse pancreatic acinar cells, differs from capacitative Ca\(^{2+}\) influx described in other systems. In mast cells and other cell types I\(_{\text{ICRANC}}\) seems to be the dominant influx pathway for Ca\(^{2+}\) (7–10). It is highly Ca\(^{2+}\) selective and can be inhibited over 90% with low concentrations of La\(^{3+}\) (10 \(\mu\)M) and in part by Cd\(^{2+}\) and Co\(^{2+}\) (25). Single channels producing I\(_{\text{ICRANC}}\) could not be identified so far and noise analysis of I\(_{\text{ICRANC}}\) made it likely that the single channel conductance is much below the resolution threshold of the patch-clamp technique (25). Comparing the characteristics of I\(_{\text{ICRANC}}\) as described here with those of I\(_{\text{ICRAC}}\) it appears that both currents are different although they have the same activation mechanism.

Single channels activated by Ca\(^{2+}\) store depletion were identified in vascular endothelium (26). These channels were relatively Ca\(^{2+}\) selective (permeability ratio Ca/Na = 10/1) and had a conductance of 11 pS (with 10 mM Ca\(^{2+}\)). Also in A431 cells single channels activated by store depletion were found which had a relative low conductance in the presence of high Ca\(^{2+}\) or Ba\(^{2+}\) concentrations (200 mM Ca\(^{2+}\), conductance 2 pS or 160 mM Ba\(^{2+}\), conductance 16 pS, respectively) (27). These channels clearly differ from the I\(_{\text{ICRANC}}\) channel in mouse described here.

Nonselective cation channels have been discussed as candidates for mediating Ca\(^{2+}\) influx in nonexcitable cells (28). While it had been described for several systems that agonist activated nonselective cation channels allow calcium influx into cells (29–31) our study demonstrates that depletion of Ca\(^{2+}\) stores can directly activate those channels.

**Comparison of I\(_{\text{ICRANC}}\) and I\(_{\text{ICRAC}}\)**—Whereas I\(_{\text{ICRAC}}\) is inhibited by several di- and trivalent ions such as Co\(^{2+}\), Cd\(^{2+}\), and most effectively by La\(^{3+}\) (25), these ions are ineffective in mouse pancreatic acinar cells. In particular the lack of effect of La\(^{3+}\) on I\(_{\text{ICRANC}}\) measured as electrical current and as calcium fluorescence with both fluo-3 and fura-2 should be emphasized. La\(^{3+}\) at low concentrations (micromolar) has been reported to inhibit capacitative calcium entry, while at higher concentrations (millimolar) it inhibited the calcium extrusion mechanism in larcimal acinar cells (18). Moreover it was shown in rat pancreatic acinar cells (17) and guinea pig pancreatic acinar cells (5) that La\(^{3+}\) (25–250 \(\mu\)M) inhibited capacitative calcium entry. In mouse pancreatic acinar cell 1 mM La\(^{3+}\) inhibited Ca\(^{2+}\) extrusion and the authors assumed that Ca\(^{2+}\) influx was inhibited, too (32). Direct evidence for this assumption was not given, however. In contrast to these results we did not find any inhibition of Ca\(^{2+}\) entry by La\(^{3+}\) up to 250 \(\mu\)M in mouse pancreatic acinar cells with different experimental protocols including different depletion methods (ACh and t-BHQ) and different detection methods (measurements of electrical current and of fluorescence with fluo-3 or fura-2). The reason for the discrepancy between the data in the literature and our data are mostly likely due to species differences which can also be concluded from our own results which compare the effects of 100 \(\mu\)M La\(^{3+}\) in rat and mouse (Fig. 4). These differences in the La\(^{3+}\) sensitivity between rat and mouse make it likely that rat but not mouse pancreatic acinar cells use I\(_{\text{ICRAC}}\) for capacitative Ca\(^{2+}\) influx as it had been assumed already by Bahnson et al. (33).

If in addition to I\(_{\text{ICRANC}}\) mouse pancreatic acinar cells would also contain I\(_{\text{ICRAC}}\) we would have expected at least, in part, inhibition of capacitative Ca\(^{2+}\) influx by La\(^{3+}\). It therefore appears that in mouse pancreatic acinar cells I\(_{\text{ICRAC}}\) is not present or in such small amounts that it is undetectable by our methods. In conclusion our data indicate that a Ca\(^{2+}\) conducting nonselective cation channel is activated following depletion of intracellular IP\(_3\)-sensitive Ca\(^{2+}\) stores in mouse pancreatic acinar cells.

The characteristics of I\(_{\text{ICRANC}}\) are different from I\(_{\text{ICRAC}}\) previously described in mast cells and other cell types (34) in that the latter is highly Ca\(^{2+}\) selective, completely inhibited by La\(^{3+}\), and in part by Cd\(^{2+}\) and Co\(^{2+}\). Evidence suggests that mammalian Ca\(^{2+}\) influx channels are homologues of insect trp and trpl channels (35–37). Whereas trp is selective for Ca\(^{2+}\), has a high La\(^{3+}\) sensitivity, and is activated by Ca\(^{2+}\) store depletion (and therefore seems to have similarities with I\(_{\text{ICRAC}}\), 38), trpl is a nonselective cation channel conducting also Ca\(^{2+}\) and Ba\(^{2+}\) ions. It has a lower La\(^{3+}\) sensitivity compared to trp but does not seem to be activated by Ca\(^{2+}\) store depletion (35, 38, 39). Whether I\(_{\text{ICRANC}}\) in pancreatic acinar cells shares genetic homologies with these insect channels remains to be determined in future studies.

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