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Distinct Ca^{2+}-permeable Cation Currents Are Activated by Internal Ca^{2+}-Store Depletion in RBL-2H3 Cells and Human Salivary Gland Cells, HSG and HSY

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Abstract. Store-operated Ca^{2+} influx, suggested to be mediated via store-operated cation channel (SOC), is present in all cells. The molecular basis of SOC, and possible heterogeneity of these channels, are still a matter of controversy. Here we have compared the properties of SOC currents (I_{SOC}) in human submandibular glands cells (HSG) and human parotid gland cells (HSY) with I_{CRAC} (Ca^{2+} release-activated Ca^{2+} current) in RBL cells. Internal Ca^{2+} store-depletion with IP_3 or thapsigargin activated cation channels in all three cell types. 1 μM Gd^{3+} blocked channel activity in all cells. Washout of Gd^{3+} induced partial recovery in HSY and HSG but not RBL cells. 2-APB reversibly inhibited the channels in all cells. I_{CRAC} in RBL cells displayed strong inward rectification with $E_{rev}(Ca) = > +90$ mV and E_{rev} (Na) = +60 mV. I_{SOC} in HSG cells showed weaker rectification with $E_{rev}(Ca) = +25$ mV and $E_{rev}(Na) = +10$ mV. HSY cells displayed a linear current with $E_{rev} = +5$ mV, which was similar in Ca^{2+}- or Na^{+}-containing medium. pCa/pNa was >500, 40, and 4.6 while pCs /pNa was 0.1,1, and 1.3, for RBL, HSG, and HSY cells, respectively. Evidence for anomalous mole fraction behavior of Ca^{2+}/Na^{+} permeation was obtained with RBL and HSG cells but not HSY cells. Additionally, channel inactivation with Ca^{2+} + Na^{+} or Na^{+} in the bath was different in the three cell types. In aggregate, these data demonstrate that distinct store-dependent cation currents are stimulated in RBL, HSG, and HSY cells. Importantly, these data suggest a molecular heterogeneity, and possibly cell-specific differences in the function, of these channels.

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Introduction

Store-operated Ca^{2+} entry (SOCE or CCE) is ubiquitously present in all non-excitable cells and probably in excitable cells as well and is proposed to be mediated via store-operated calcium channels (SOC). Calcium entry mediated through SOC is critical in the regulation of a wide spectrum of physiological processes in different cell types, such as exocytosis, smooth muscle contraction, gene expression, cell proliferation, and apoptosis (Berridge, Lipp & Bootman, 2000; Venkatachalam et al., 2002). The major criterion for identifying this type of Ca^{2+} entry is its activation by depletion of internal Ca^{2+} stores without activation of PIP_2 turnover by thapsigargin (which blocks SERCA activity) or ionomycin, or by introduction of Ca^{2+} buffers into the cytosol or ER (Hoth & Penner, 1992; Putney & Bird 1993; Parekh & Penner 1997; Putney & McKay 1999; Bakowski & Parekh, 2002a,b; Prakriya & Lewis, 2003). Electrophysiological and fura-2 fluorescence studies have demonstrated that agonist stimulation of IP_3 generation and internal Ca^{2+} release via IP_3R also leads to activation of the same type of calcium influx in some cases. Thus, it has been suggested that agonist-stimulated Ca^{2+} influx is mediated either completely or in part by the SOCE pathway, depending on the type of receptor and cell involved.

The molecular composition of SOC as well as the mechanism that links store depletion to channel activation are still not understood (Hoth & Penner 1992; Putney & McKay 1999; Bakowski & Parekh 2002b; Venkatachalam et al., 2002). Although it is

well recognized that agonist-induced Ca^{2+} entry involves a variety of different Ca^{2+}- entry channels, including receptor- and second messenger-regulated channels as well as SOCs, the concept of heterogeneity among SOCs has thus far been barely addressed. Recent studies have suggested that there can be at least two possible channels activated in response to PIP_2 hydrolysis (Venkatachalam et al., 2002). The first is activated by DAG and the second, by internal Ca^{2+}-store depletion per se. Other channels, such as arachidonate-regulated channels, have also been reported to be activated at lower concentrations of the agonist, which do not induce detectable internal Ca^{2+}-store depletion (Mignen et al., 2003). Ca^{2+} channels that meet the generally accepted criteria for being termed SOC have been measured in a number of cell types (Trepakova et al., 2000; Nilius & Droogmans, 2001; Rychkov et al., 2001; Albert & Large, 2003; Cioffi Wu & Sterens, 2003). The first SOC channels to be described were CRAC channels, which underlie the store-operated Ca^{2+} conductance measured in mast cells, lymphocytes, and megakaryocytes (I_{CRAC}) (Putney & Bird 1993; Parekh & Penner, 1997; Bakowski & Parekh 2002a; Parekh, 2003; Prakriya & Lewis 2003). Presently, CRAC is the best characterized SOC and its characteristics have been used as the basis to identify SOC in other cell types. These previous studies suggest that SOCs, distinct from CRAC, are present in several cell types. CRAC can be distinguished from these SOCs by its remarkably high selectivity for Ca^{2+} and extremely low unitary conductance. The question that arises from these previous studies is whether store-operated channels are truly different in different cell types or do variations in the experimental conditions used for measurement of the currents account for the differences in the characteristics of these currents as compared to those of CRAC.

We have previously shown that both carbachol or IP_3 (in the pipette) as well as thapsigargin strongly induce store-operated Ca^{2+} entry and the store-operated Ca^{2+} current (I_{SOC}) in the human submandibular gland cells (HSG) (Liu O' Connells & Ambudkar, 1998a). We have also measured this activity at the single-channel level (Liu & Ambudkar, 2001), following activation by carbachol, thapsigargin, and the permeant divalent cation chelator, TPEN. We have shown that Ca^{2+} oscillations in HSG cells are completely dependent on extracellular Ca^{2+} and that Ca^{2+} influx is required to refill the stores before each release event (Liu, Rojas & Ambudkar, 1998b). Human parotid gland cells, HSY, also display IP_3- and Tg- activated Ca^{2+} influx. Importantly, and in contrast to HSG cells, $[Ca^{2+}]_i$ oscillations in HSY cells are relatively independent of external Ca^{2+} (Liu, Liao & Ambudkar, 2001). Interestingly, we also found that HSY cells express relatively higher levels of the sarcoendoplasmic reticulum Ca^{2+} pump (SERCA) and inositol trisphosphate receptors (IP_3Rs) than HSG cells. Thus, HSY cells appear to have a different functional requirement for Ca^{2+} influx than HSG cells.

This study was designed to compare the properties of Ca^{2+}-influx channels that are activated by depletion of intracellular Ca^{2+} stores in HSG and HSY cells. CRAC activity in RBL-2H3 cells was also measured and used as a basis for comparing the channels in HSY and HSG cells, since it is considered to be a prototypical SOC. Our data demonstrate that store-operated channels in RBL, HSG and HSY cells have distinct properties, including cation selectivity and anomalous mole fraction behavior.

Materials and Methods

Cell Culture

All the cells were cultured on glass coverslips with Dulbecco modified Eagle's medium (DMEM) for rat basophilic leukemia cells (RBL-2H3), Earle's minimal essential medium (EMEM) for HSG and HSY cells. Both media were supplemented with 10 % fetal calf serum, 2 mM glutamine, 1 % penicillin/streptomycin at 37°C in 5 % CO_2.

Electrophysiological Measurements

For patch-clamp experiments, coverslips were transferred to the recording chamber and kept in a Ringer's solution of the following composition (mM): NaCl 145; KCl 5; $MgCl_2$ 1; $CaCl_2$ l; Hepes 10; Glucose 10; pH 7.4 (NaOH). The patch pipette had resistances between 3–5 MΩ after filling with the standard intracellular solution that contained (mM): Cs methane-sulfonate 145; NaCl 8; $MgCl_2$ 10; HEPES 10; EGTA 10; pH 7.2 (CsOH). For testing the dependence of the current on intracellular Na^+, 8 mM NaCl was replaced with 1 or 20 mM NaCl. 10 μM inositol 1,4,5-trisphosphate (Calbiochem) was added to the pipette solution for all the experiments except where indicated. External solutions were composed as follows (mM). *Ca^{2+} & Na^+ solution:* NaCl 145; CsCl 5; $MgCl_2$ 1; $CaCl_2$ 10; HEPES 10; glucose 10; pH 7.4 (NaOH). *Ca^{2+} solution:* NMDG 150; CsCl 5; $MgCl_2$ 1; $CaCl_2$ 10; HEPES 10; glucose 10; pH 7.4 (HCl). *DVF-Na^+ solution:* NaCl 165; CsCl 5; EDTA 10; HEPES 10; glucose 10; pH 7.4 (NaOH). *Na^+ solution:* NaCl 165; CsCl 5; HEPES 10; glucose 10; pH 7.4 (NaOH). *NMDG solution:* NMDG 170; CsCl 5; $MgCl_2$ 1; HEPES 10; glucose 10; pH 7.4 (HC1). *Ba^{2+} solution:* NaCl 145; CsCl 5; $MgCl_2$ 1; $BaCl_2$ 10; HEPES 10; glucose 10; pH 7.4 (NMDG). Osmolality for all solutions was adjusted with mannose to 300 to 315 mmol/kg using Vapor Pressure Osmometer (Wescor). $[Ca^{2+}]$ in nominally Ca^{2+}-free medium was about 10 μM, measured using a Ca^{2+} electrode.

Patch-clamp experiments were performed in the tight-seal whole-cell configuration at room temperature (22–25°C) using Axopatch 200A amplifier (Axon Instrument). The development of the current was assessed by measuring the current amplitudes at a potential of −80 mV, taken from high-resolution currents in response to voltage ramps ranging from −90 to 90 mV over a period of 1 s, which were imposed every 4 s from a holding potential of 0 mV, and digitized at a rate of 1 kHz. A liquid-junction potential of less than 8 mV was not corrected, capacitative currents and series resistance were determined and minimized. For analysis, the first ramp was used for leak subtraction

for the subsequent current records. For calculation of permeability ratios the equations published by Voets et al. were used (Voets et al., 2002).

Single-channel measurements were made as described previously (Liu & Ambudkar, 2001) using the cell-attached patch-clamp method.

Thapsigargin (Tg) was purchased from Calbiochem. 2-APB and other chemicals were obtained from Sigma-Aldrich.

Results

EGTA- and IP_3-induced Ca^{2+} and Na^+ Currents in RBL, HSG and HSY Cells

Whole-cell currents in all three cell types were measured using the experimental conditions previously used to measure I_{CRAC} (Hoth & Penner, 1992; Schindl et al., 2002). Note that 10 mM Mg was included in the pipette solution to prevent contaminating TRPM7-mediated currents (Prakriya & Lewis, 2002). Further, the pipette solution (details given in Materials and Methods) contained 10 mM EGTA in all the experiments described below.

In RBL cells perfused with normal Ringer's solution that contained Ca^{2+} + Na^{2+}, or a divalent cation-free medium (DVF-Na^+, Ca^{2+}-free medium $_+$10 mM EGTA, see Materials and Methods for details), dialysis with solution containing 10 mM EGTA, alone was sufficient to generate I_{CRAC} (*data not shown*). Peak current was typically reached within 120 ± 35 s (n = 12). When 10 μM IP_3 was included in the pipette solution with 10 mM EGTA, there was rapid development of the whole-cell current; peak current was seen after 65 ± 20 s (n = 25; Fig. 1*A* shows a typical response in $Ca^{2+}+Na^+$ or DVF medium measured at −80 mV). Notably, there was no difference in the maximum amplitude of the currents elicited by either EGTA alone or EGTA + IP_3. However, the current was significantly larger in the DVF-Na^+ medium than in the Ca^{2+} + Na^+ medium (*see* Fig. 1*A*). In all subsequent experiments shown below, IP_3 and EGTA were used together for current activation. *I-V* curves of the currents measured at the point indicated by arrow labelled *B* are shown in Fig. 1*B*. In normal Ringer's solution, a relatively small inward current was seen at negative membrane potentials, which showed very strong inward rectification and no reversal of the current was detectable up to > +90 mV. This current also displayed a relatively fast rundown with an average $t_{½}$ (time required for loss of 50 % of the peak current) = 194 (± 31 seconds (n = 13). In the DVF-Na^+ medium, the current was again inwardly rectifying and reversed at 60 ± 5 mV. This current also showed a gradual rundown with $t_{1/2}$ of 401 ± 44 seconds (n = 17). Although these data generally agree with the previously reported characteristics of I_{CRAC} (Parekh & Penner, 1997; Parekh, 1998, 2003; Prakriya & Lewis, 2003), the slow rundown of the Na^+ current we have noted in DVF-Na^+ containing high internal [Mg^{2+}] is more consistent with the studies reported by Parekh and coworkers.

Similar measurements were made with both HSG and HSY cells. In either cell type, intracellular dialyis with 10 mM EGTA alone either activated currents only after a remarkably long delay (4/17 cells) or failed to activate currents at all (13/17 cells). It has been suggested that the rate of SOC activation by EGTA might be determined by the relative level of SERCA activity in some cell types; i.e., higher SERCA activity will result in slower activation (Parekh, 2003). Consistent with this concept, it was necessary to include EGTA + IP_3 in the pipette solution to induce currents in these cells. Under these conditions, currents were activated in the majority of HSG cells (31/42, 74%) in both normal Ringer's as well as DVF-Na^+. The time required to achieve peak current was not different from that seen in RBL cells (Fig. 1*C* shows typical traces of the currents at −80 mV). Further, like RBL cells (i) both Ca^{2+} and Na^+ currents induced by EGTA + IP_3 in HSG cells showed marked inward rectification (Fig. 1*D*), and (ii) the Na^+ current seen in DVF-Na^+ was larger than the current in the Ca^{2+} + Na^+ medium. However, the reversal potentials for these currents were significantly less positive than those seen in RBL cells. Reversal potentials for the current in the DVF-Na^+ was +10 ± 2 mV (n = 16) and in the Na^+ and Ca^{2+} medium it was +25 (±3 mV; n = 19). Additionally, *I-V* curves of the current at earlier or later time points were similar to that shown in Fig. 1*D* (*data not shown*). Importantly, and consistent with our previous studies, the latter displayed a faster rundown, with $t_{1/2}$ of 197 ± 20 s (n = 19) as compared to the Na^+ current, which was relatively more sustained (more than 80 % of the current remained 8 min, n = 16, Fig. 1*C*). Thus, while the inactivation of the current in $Ca^{2+}+Na^+$ medium was similar to that in RBL cells, the inactivation in DVF-Na^+ was much slower.

EGTA + IP_3 also induced currents in the majority of HSY cells (19/27, 70%). However, significant differences were seen in the characteristics of the current (Fig.1*E* and *F*). In both divalent cation-free as well as $Ca^{2+}+Na^+$ Ringer's solution, HSY cells displayed currents that were relatively linear, with reversal potential near zero (+5 ± 2 mV, n = 13) for both currents. As was the case in RBL and HSG cells, similar currents were detected at earlier and later time points, suggesting that a single type of SOC is being activated. Another distinct characteristic of the currents in HSY cells was that both currents showed faster rundown than in RBL and HSG cells,

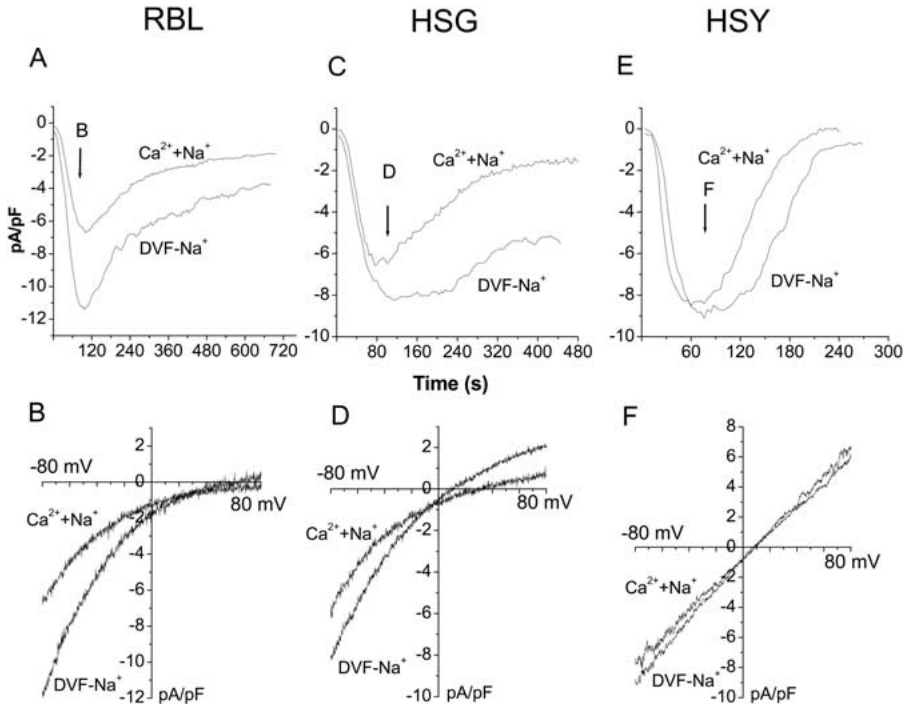

Fig. 1. IP_3- and EGTA-induced currents in RBL, HSG and HSY cells. 10 μM IP_3 and 10 mM EGTA were introduced through patch pipette into the cells. Voltage ramps ranging from −90 to +90 mV from a holding potential of 0 mV (duration 1 s, *see* Materials and Methods for details) were applied every 4 s. Leak-current correction was done by subtracting the currents measured in the first ramp. Inward currents measured from the ramps at −80 mV are shown in *A*, *C*, and *E*. Each trace is a representative recording from 5–15 cells. Currents were measured in Ca^{2+} + Na^+ solution or divalent cation-free solution (DVF-Na^+). *I-V* curves of the currents at arrows in *A*, *C*, and *E* are shown in *B*, *D*, and *F*, respectively.

with $t_{1/2}$ of 149 ± 12 for Na^+ (n = 6) and 115 ± 18 for Ca^{2+} (n = 7) currents (Fig. 1*E*, 1*F*).

Thapsigargin-induced Activation of Cation Currents in RBL, HSG and HSY Cells

Figure 2 shows that thapsigargin also stimulated currents in all three cell types. It is important to note that the characteristics (rectification, reversal potential, etc.) of the Na^+ (*not shown*) as well as Ca^{2+} current stimulated by inclusion of Tg in the bath solution (IP_3 was excluded from the pipette solution in these experiments) was similar to that induced by IP_3 + EGTA in all three cells. The only apparent difference was that the Tg-stimulated currents displayed a faster rundown than IP_3 + EGTA-stimulated currents. These data demonstrate that both HSY and HSG cells express channels that, like classical CRAC channels, are activated by depletion of intracellular Ca^{2+} stores and thus meet the criteria of being termed SOC. Interestingly, the data in Figs. 1 and 2 suggest that distinct currents can be stimulated by internal Ca^{2+}-store depletion.

Single-channel characteristics of the Tg-activated channels in HSG and HSY cells were determined using the cell-attached patch-clamp method. The data are shown in Figs. 2 *G–J*. Although burst-like activity was seen in both cases, the unitary current amplitude was less well defined in HSY cells and several overlapping levels were noted. Using the major current level, the calculated slope conductance for Ca^{2+} was about 20 pS for HSG cells, while it was about 28 pS for HSY cells. Mean open time (ms) and open probability were 4.3 ± 0.7 and 0.11 ± 0.02 compared to 6.5 ± 1.2 and 0.12 ± 0.01 in HSG cells. The HSG cell data are consistent with those we have previously reported (Liu & Ambudkar, 2001). These data strongly indicate the presence of different channels in HSY and HSG cells.

Inhibition of SOC Channels in RBL, HSG and HSY Cells by Gd^{3+} and 2-APB

1 μM Gd^{3+} has been reported to block store-operated Ca^{2+}-influx channels in several cell types, while higher concentrations of Gd^{3+} appear to block other types of channels as well (Trebak et al. 2003a; Vazquez et al. 2003). We therefore tested the effect of 1 μM Gd^{3+} on the currents induced by IP_3 and EGTA in RBL, HSG, and HSY cells. There was a rapid block of the current in all three cell types (Fig. 3*A*, *C*, *E*). An interesting finding was that wash-out of Gd^{3+} did not result in immediate recovery of the current in RBL cells (Fig. 3*A*), but did so in HSG (Fig. 3*C*) and HSY (Fig. 3*E*) cells. Similar results were also seen with the current generated in Na^+ + Ca^{2+} medium in these cells (*data not shown*). Importantly, Gd^{3+} did not alter the *I-V* characteristics of the current but only decreased its amplitude in all three cells. This can be clearly seen in the *I-V* curves of the current in HSY and HSG cells (Fig. 3*D* and *F*).

We also used 2-APB to further characterize the currents. 2-APB has also been widely used as a blocker of store-operated Ca^{2+} channels and it has been shown to induce a slowly reversible block

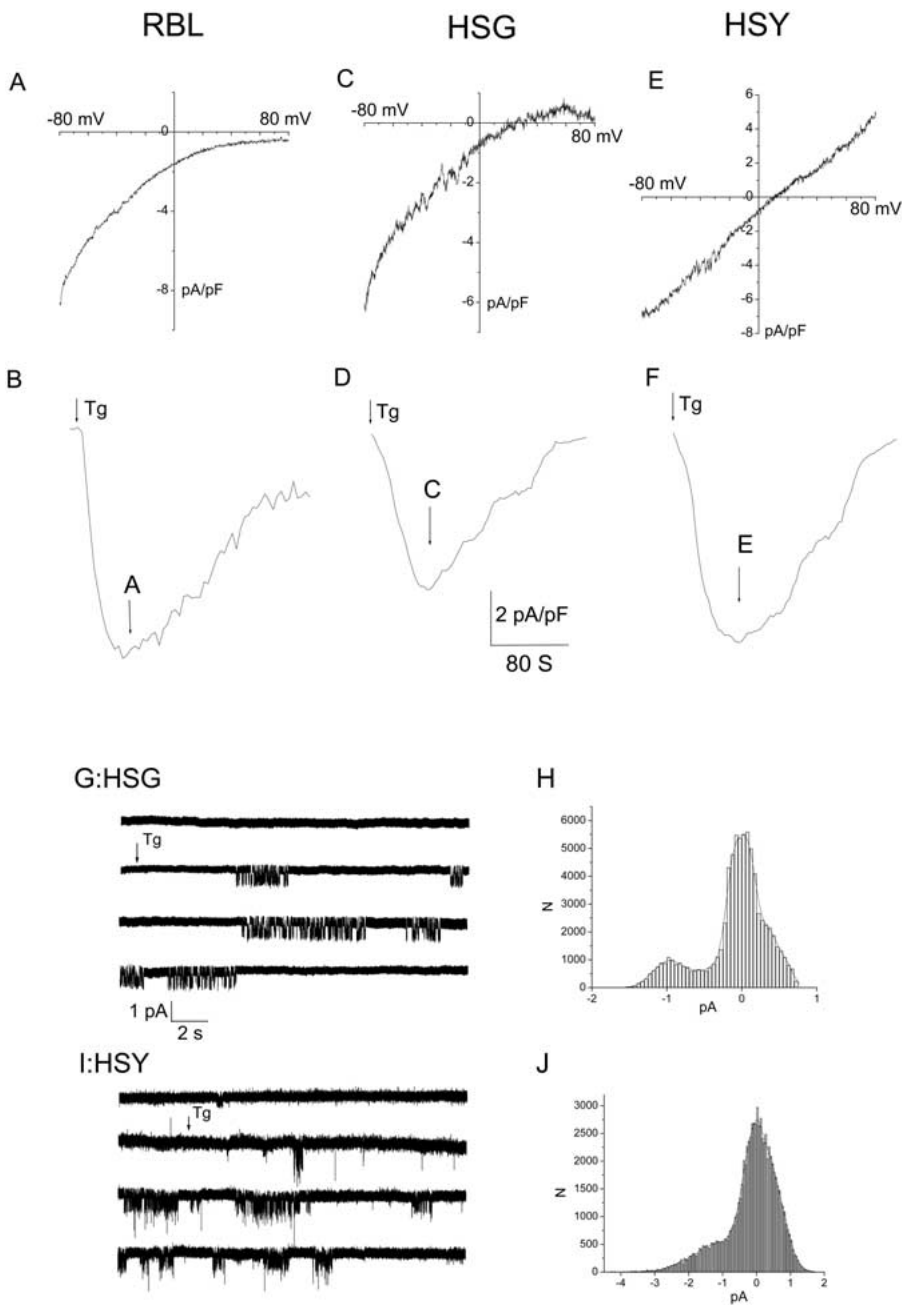

Fig. 2. Tg-induced currents in RBL, HSG and HSY cells. (*A*), (*C*) and (*E*) show traces of Tg-induced currents in RBL, HSG, and HSY cells, respectively, in Ca^{2+} + Na^{+} medium. *I-V* of ramps of the currents at arrows in (*A*), (*C*) and (*E*) are shown in (*B*), (*D*) and (*F*), respectively. Each trace is a representative recording from 4–6 cells. (*G*, *I*) Continuous recording of single-channel activity (30 s) in HSG (*G*) and HSY (*I*), respectively (holding potential −40 mV). Pipette solution contained 2 mM Ca^{2+} and 100 mM Na-HEPES. Perfusion with medium containing Tg was started where indicated in each trace. (*H*, *J*) All-point amplitude histograms corresponding to the currents at −40 mV for HSG (*H*) and HSY (*J*), respectively.

(Schindl et al., 2002; Liu et al., 2000). 75 μM 2-APB inhibited the currents induced by IP_3 + EGTA in RBL, HSG and HSY in DVF-Na^{+} medium (Fig 4 A–C, *I-V* curves not shown). The currents were restored after removal of 2-APB. Tg-stimulated currents were also similarly blocked by 1μM Gd^{3+} and 2-APB (*data not shown*). Current in $Na^{+}+Ca^{2+}$ was also blocked by 2-APB (*data not shown*). In aggregate, these data suggest that the currents induced by IP_3 and Tg in RBL, HSG, and HSY cells are store-operated. Notably, these currents appear to have distinct *I-V* curves, suggesting differences in their ion selectivity in different cell types.

Ca^{2+} and Na^{+} Permeability of the Store Depletion-activated Channels in RBL, HSG and HSY Cells

Figure 5 shows a continuous recording of current in an RBL cell perfused successively with three different external solutions. The cells were perfused with DVF-Na^{+} solution for at least 3 min before whole-cell configuration was established. Upon intracellular dialysis with IP_3 and EGTA an inward current developed (Fig. 5*A* shows the current at −80 mV). An *I-V* curve of the current at peak (point indicated by arrow in Fig. 5*A*) shows that it is inwardly

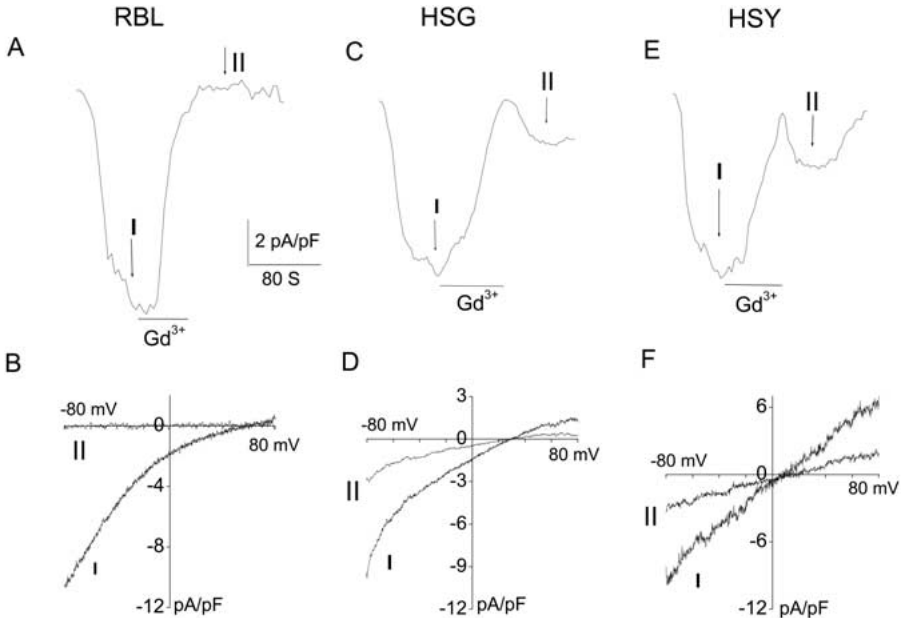

Fig. 3. Low [Gd^{3+}] inhibits IP_3- and EGTA-induced currents in RBL, HSG and HSY cells. Cells were first perfused with DVF-Na^+ solution, then with the same solution containing 1.0 μM Gd^{3+} (perfusion time shown by horizontal bar), and finally with DVF-Na^+ solution again. (*A*), (*C*) and (*E*) show the currents measured at −80 mV in RBL, HSG, and HSY cells. The *I-V* ramps of the currents measured at points (*I*) and (*II*) are shown in (*B*), (*D*) and (*F*), respectively. Each trace is a representative recording from 5–7 cells. Currents in Ca^{2+} + Na^+ medium were similarly blocked (*data not shown*).

rectifying and reverses at about +60 mV (Fig. 5*B*) similar to that shown in Fig. 1*B*. This Na^+ current decreased to baseline when the external medium was replaced with medium in which Na^+ was replaced by the impermeant cation NMDG (*see* Materials and Methods for details; note that since the Na^+ medium was replaced with NMDG medium before maximum current was reached the Na^+ current is not larger than the Ca^{2+} current in this trace). When the NMDG medium was replaced with medium containing Ca^{2+} + NMDG (*see* Materials and Methods for details), the current redeveloped. The *I-V* curve of this current (measured at point indicated by arrow *C* in Fig. 5*A* and shown in Fig. 5*C*) demonstrated strong inward rectification with no detectable reversal (up to +90 mV). Reperfusion with NMDG medium reduced the Ca^{2+} current to baseline. Notably, there was no significant difference in the pattern of the *I-V* curves of the currents seen with Ca^{2+} + Na^+ solution (Fig. 1) or Ca^{2+} + NMDG solution. Further, based on the current densities in Ca^{2+} + Na^+ medium and Ca^{2+} + $NMDG^+$ medium (Table 1), it can be suggested that Na^+ does not significantly contribute to I_{CRAC} in normal physiological medium (i.e., external medium containing Ca^{2+} + Na^+). E_{rev} for Ca^{2+} could not be determined (considered as >90 mV), while E_{rev} for Na^+ was +60 mV. We examined the effect of varying the Na^+ gradient on the current in the DVF medium (*data not shown*). Decreasing the external Na^+ concentration from 165 mM Na^+ to 16.5 mM Na^+ induced a left-shift of 27 ± 2.5 mV (n = 6) in the reversal potential. Similarly, when intracellular Na^+ concentration was changed from 8 to 20 or 1 mM, the E_{rev} shifted from 60 ± 5 mV to 35 ± 4 mV or 70 ± 6 mV for 20 mM and 1 mM, respectively (n = 4 or 5). In addition, the current with 1 mM internal Na^+ displayed a higher current density (Table 1). Based on these data, pCs^+/

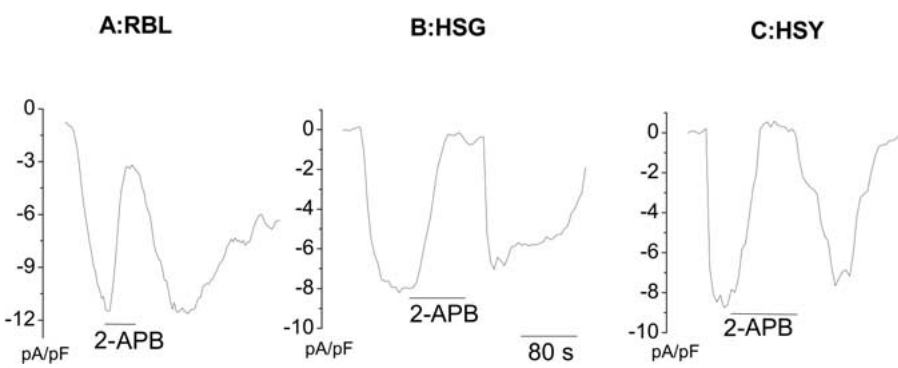

Fig. 4. 2-APB inhibits IP_3- and EGTA-induced currents in RBL, HSG and HSY cells. Cells were perfused with DVF-Na^+ solution and switched to 75 μM 2-APB in DVF-Na^+ solution for the time shown by the horizontal bar. (*A*) (*B*) and show the trace of the current measured at −80 mV in RBL, HSG and HSY cells, respectively. Each trace is a representative recording from 4–7 cells. Currents in Ca^{2+} + Na^+ medium were similarly blocked (*data not shown*).

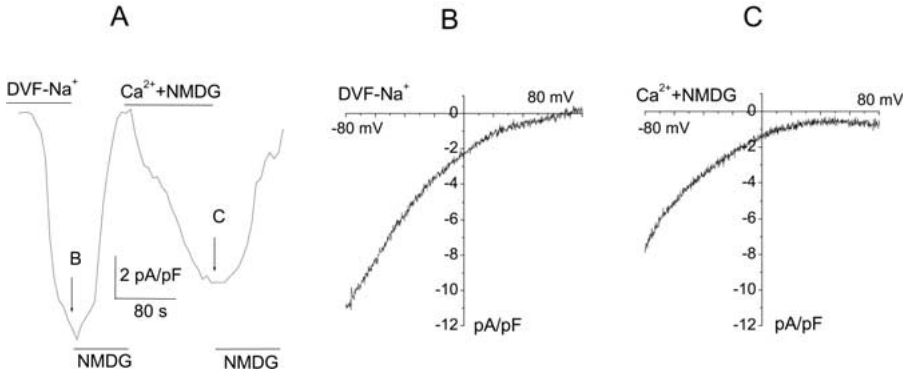

Fig. 5. CRAC-mediated Ca^{2+} and Na^+ currents in RBL cell. The cell was perfused with Na^+-DVF solution or NMDG solution containing Ca^{2+} (Ca^{2+} + NMDG solution) as shown in (*A*). *I-V* ramps of the currents corresponding to arrows *B* and *C* are shown in (*B*) and (*C*), respectively. These are representative of results obtained with 9 cells.

Table 1. Comparison of current density in various solutions.

	Current density (pA/pF)		
	RBL	HSG	HSY
Ca^{2+} + Na^+	6.6 ± 1.3	6.4 ± 0.9	8.1 ± 1.5
Ca^{2+} + $NMDG^+$	6.5 ± 1.1	6.3 ± 1.0	7.3 ± 1.2
DVF-Na^+	11.4 ± 1.9*	9.3 ± 1.3*	8.4 ± 2.1

Currents were measured following activation of the cells by inclusion of IP_3 in the pipette solution; external medium in each case is indicated. Other conditions are described in Materials and Methods. Values that are significantly different from others in the same group are indicated by *; n = 4–7 for each condition.

pNa^+ was calculated to be 0.1, while pCa^{2+}/pNa^+ was estimated to be >500. These data are consistent with previously reported characteristics of I_{CRAC} in RBL cells (Parekh & Penner 1997; Parekh, 1998, 2003).

In HSG cells, IP_3 + EGTA induced an inward current in DVF-Na^+ (Fig. 6*A*), which displayed relatively weak inward rectification and reversed at around +10 mV (*I-V* curve of current at arrow *B* is shown in Fig. 6*B*). This current was decreased to baseline when the cell was perfused with the NMDG medium. The current increased again when the NMDG medium was replaced with the Ca^{2+}-NMDG medium. *I-V* plot of current at arrow *C* in Fig. 6*A* is shown in Fig. 6*C*. The current displayed inward rectification with E_{rev} = +25 mV. As in the case of RBL cells, the *I-V* curve in Ca^{2+} + $NMDG^+$ medium was similar to that seen in the Ca^{2+} + Na^+ medium. Further, as seen in RBL cells, Na^+ did not appear to contribute to the currents in a normal physiological external solution, although the channels appear to be permeable to Na^+ in the absence of external Ca^{2+} (Table 1). Interestingly, while $NMDG^+$ was able to reduce both Na^+ and Ca^{2+} currents to the baseline in HSY cells (Fig. 7*A*), the characteristics of the currents were distinct from that seen in either RBL or HSG cells. Consistent with the data shown in Fig. 1, *I-V* curves were almost linear in the Na^+ medium or Ca^{2+} + NMDG medium and displayed reversal in the range between 0 and +10 mV (Fig. 7*B* and *C*, respectively).

Ability of Ca^{2+} to Block Na^+ Currents through SOCs in RBL, HSG and HSY Cells

A characteristic property of Ca^{2+}-selective channels is that external Ca^{2+} blocks Na^+ permeation via the channel (Bakowski & Parekh, 2002a). To examine the Na^+ permeability of the store-operated Ca^{2+} channels in RBL, HSY, and HSG cells in normal physiological medium, we examined the effect of external Ca^{2+} on store-operated Na^+ currents mediated via these channels. In DVF-Na^+ medium an inward current, carried by Na^+, developed in all three cell types (Fig. 8*A*, *C* and *E*. *I-V* curves from this experiment not shown). Switching the external medium to Na^+ medium (i.e., without EDTA, with nominal $[Ca^{2+}]$ = 10 μM), induced a decrease in the current to baseline in RBL and HSG cells, but not in HSY cells. Reperfusion with DVF-Na^+ medium caused an increase in the inward Na^+ current in RBL and HSG cells, but did not affect the current in HSY cells. These data are consistent with previous studies with RBL cells, which show that CRAC channels do not permit Na^+ entry in nominally Ca^{2+}-free medium unless a divalent cation chelator is added to it (Bakowski & Parekh, 2002b), indicative of an anomalous mole-fraction effect. Figure 8*C* shows that SOC in HSG cells exhibits this same behavior, while SOC in HSY cells (Fig. 8*E*) does not.

This was further confirmed by the experiments shown in Fig. 8*B*, *D*, and *F*. Again, perfusion of cells with DVF-Na^+ medium caused an inward Na^+ current, which was decreased to baseline when it was replaced with an NMDG medium. Perfusion of the cell with normal external solution (Ca^{2+} + Na^+) induced an increase in the current. *I-V* curves (*not shown*) demonstrated that it is carried by Ca^{2+} influx in RBL and HSG cells. In the case of HSY cells, the *I-V* curve is linear. Furthermore, when the Ca^{2+} + Na^+ solution was replaced with DVF-

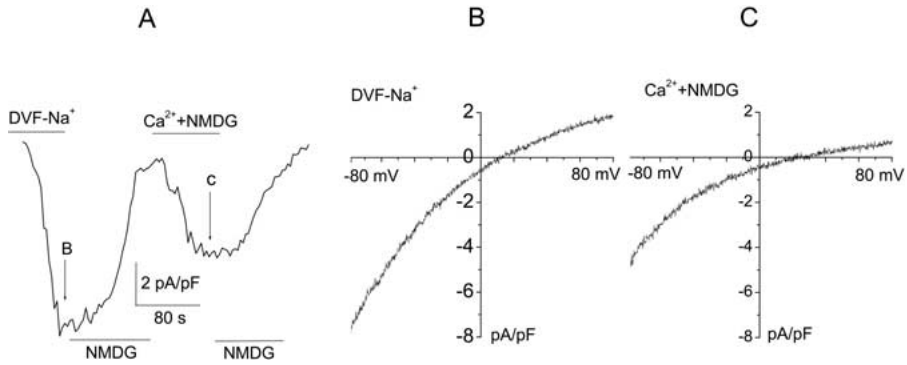

Fig. 6. SOC-mediated Ca^{2+} and Na^+ currents in HSG cells. Cells were perfused as described for Fig. 5 with either DVF-Na^+ solution or Ca^{2+} + NMDG solution. Current recorded at −80 mV is shown in (*A*). *I-V* ramps of the current at arrows *B* and *C* are shown in (*B*) and (*C*), respectively. These data are representative of results from 7 different cells.

Na^+ solution in RBL cells, the currents were first inhibited and then reactivated. The initial inhibition is due to a combination of lowering of external [Ca^{2+}] and Ca^{2+} block of the Na^+ current via the channel. As Ca^{2+} was washed off and EDTA chelated the residual Ca^{2+} and Mg^{2+}, the Na^+ current redeveloped. When the external medium contained 10 mM EGTA instead of EDTA, there was a rapid increase in the current (*see* inset in Fig. 8*B*). Similar results were obtained with HSG cells (*see* inset in Fig. 8*D*). Importantly, and consistent with the experiment shown in Fig. 8*E*, replacing the Ca^{2+} + Na^+ medium with DVF-Na^+ or Na^+ + EGTA medium did not alter the currents in HSY cells, which displayed a usual rundown (Fig. 8*F*). These data clearly demonstrate that under physiological conditions, I_{CRAC} in RBL cells and I_{SOC} in HSG cells only allow Ca^{2+} to permeate the cell. However, HSY cells do not appear to exhibit the anomalous mole fraction behavior and thus could potentially allow both Na^+ and Ca^{2+} to enter the cell.

Discussion

The data presented above describe store-operated channel current characteristics in three cells types. We have measured the well characterized I_{CRAC} in RBL cells, and I_{SOC} in two human salivary gland ductal cells, HSG and HSY, under exactly the same experimental conditions. This allowed accurate comparisons of the epithelial cell SOCs with the more "classical" SOC, CRAC. In all three cell types, currents were activated when the cytosol was dialyzed with solution containing 10 mM EGTA and 10 μM IP_3 or when 1 μM Tg was included in the bath solution (data are summarized in Table 2). The current in RBL cells measured in either Ca^{2+} + Na^+ or DVF-Na^+ medium was primarily inward. HSG cells displayed small outward currents in Ca^{2+} + Na^+ medium and slightly larger outward currents in DVF-Na^+ medium. In contrast, HSY cells displayed a linear current in either medium. Both inward and outward currents in all the cells were blocked by 2-APB and by 1 μM Gd^{3+}. In aggregate, these data demonstrate that the currents described above in RBL, HSG, and HSY cells are indeed store-operated.

Importantly, we have shown that the store-operated channels in these three cell types differ markedly in their characteristics, especially the cation selectivity, anomalous molar fraction behavior and rates of inactivation. In RBL cells, EGTA, IP_3 + EGTA, or Tg induced typical I_{CRAC}, which displayed strong inward rectification and high selectivity for Ca^{2+} over Na^+ ($pCa/pNa = >500$) and Na^+ over Cs^+ ($pCs/pNa = 0.1$). The typical block of Na^+ permeation by Ca^{2+}, consistent with strong anoma-

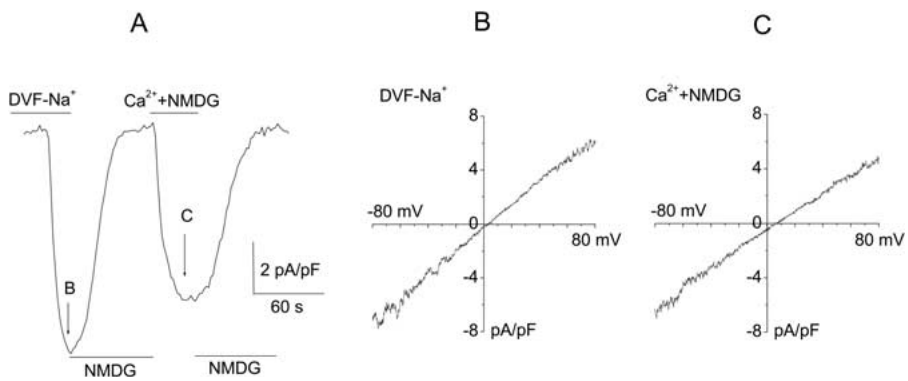

Fig. 7. SOC-mediated Ca^{2+} and Na^+ currents in HSY cells. Cells were perfused as described for Fig. 5 with either DVF-Na^+ solution or Ca^{2+} + NMDG solution. Current recorded at −80 mV is shown in (*A*). *I-V* ramps of the current at arrows *B* and *C* are shown in (*B*) and (*C*), respectively. These are representative traces from 6 different cells.

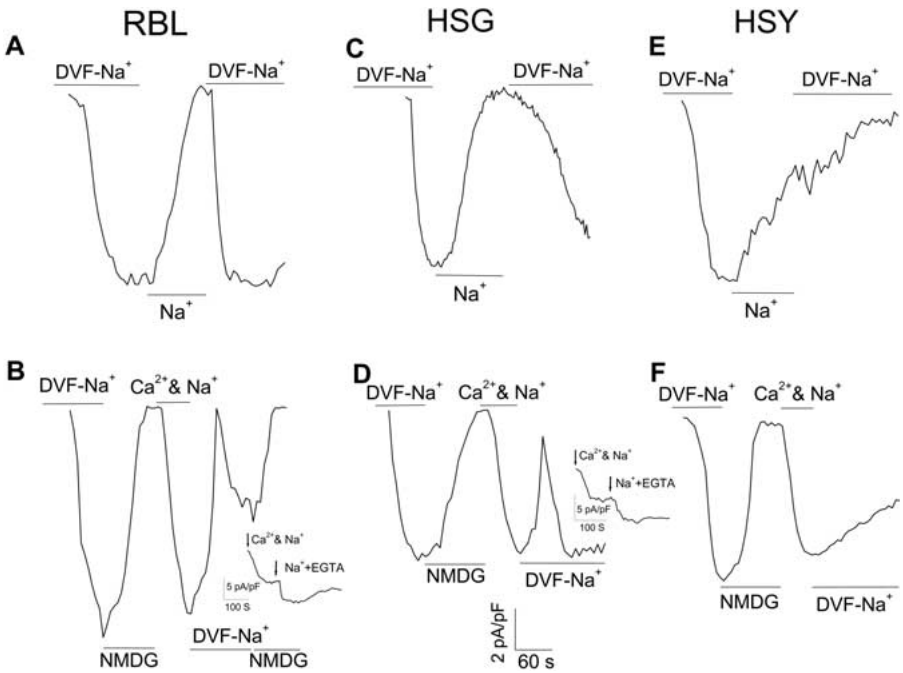

Fig. 8. Anomalous molar fraction (AMF) behavior of store-operated channels in RBL, HSG and HSY cells. All the currents were activated by IP_3 and EGTA as described for previous figures. (*A*), (*C*) and (*E*) show currents in cells perfused with Na^+ (DVF-Na^+, containing 10 mM EDTA), or Na^+-medium (without EDTA, shown by horizontal bar) in RBL, HSG, and HSY cells, respectively. In (*B*), (*D*) and (*F*), cells were perfused with DVF-Na^+, NMDG + Ca^{2+}, or NMDG medium as indicated (horizontal bars indicate period during which the solutions were introduced). Each trace is a representative recording from 6–9 cells. Insets show traces in which Ca^{2+} + Na^+ medium was replaced by DVF-Na^+ containing 10 mM EGTA instead of 10 mM EDTA.

lous molar fraction behavior, was observed in RBL cells, suggesting that under physiological conditions, the current is primarily carried by Ca^{2+}. I_{SOC} in HSG cells, while displaying some properties in common with I_{CRAC}, was also clearly distinct from it. I_{SOC} with Ca^{2+} as well as Na^+ exhibited weaker inward rectification and appeared to be less selective for Ca^{2+} over Na^+ ($pCa/pNa = 46$) and non-selective for Na^+ over Cs^+ ($pCs/pNa = 1$). Similar to CRAC channels of RBL cells, Na^+ permeation through the store-operated channels of HSG was blocked by micromolar Ca^{2+}. This finding suggests anomalous molar fraction behavior and implies that under physiological conditions, when both Na^+ and Ca^{2+} are present in the external medium, only Ca^{2+} permeates the channel. Our data suggest that Na^+ influx occurs only when external Ca^{2+} is decreased to levels < 1 μM (i.e., when EDTA or EGTA are added to the medium). The most interesting finding of this paper is that in HSY cells, store depletion activates a non-selective cation channel that is completely distinct from CRAC in RBL cells or SOC in HSG cells. This channel displayed minimal selectivity for Ca^{2+} over Na^+ ($pCa/pNa = 4$), and for Na over Cs ($pCs/pNa = 1.3$). More importantly, Na^+ permeation through this store-operated channel was not blocked by extracellular Ca^{2+}. Thus, under physiological conditions, the current mediated by this channel is carried by both Na^+ and Ca^{2+}. The current densities shown in Table 1 suggest that the bulk of the current might be carried by Na^+. However, we have previously demonstrated that the amount of Ca^{2+} entering HSY cells is sufficient to refill internal Ca^{2+} stores in cells stimulated with high concentrations of agonists or reversible SERCA inhibitors. Further, sustained increase in Ca^{2+}-activated K^+-channel current can also be regulated via this Ca^{2+} entry mechanism. Thus, although the store-operated cation channel in HSY cells appears to be a relatively non-selective channel (i.e., Ca^{2+} vs Na^+) it mediates influx of physiologically relevant amounts of Ca^{2+} into the cell. Store-operated non-selective cation channels have been previously reported in endothelial cells and smooth muscles (Trepakova et al., 2000; Nilius & Droogmans 2001; Albert & Large, 2003; Cioffi et al., 2000). In the latter cell type, this channel has been proposed to be involved in muscle contraction as well as proliferation.

The characteristics of the currents in RBL, HSG, and HSY cells suggest that they are mediated via different channels, which are likely to be composed of different proteins. Yet, all three channels appear to be stimulated via a common mechanism associated with internal Ca^{2+} store depletion. Several interesting questions arise when considering the present data. Although this mechanism is yet unknown, we can speculate that all three channels might have a common subunit or accessory protein that senses the internal pool depletion and induces gating of the channel. Alternatively, store depletion could trigger multiple signaling pathways, which could in turn activate different types of Ca^{2+} channels. Understanding the mechanisms of SOCE will ultimately depend on the identification of the components of the SOCs in various cell types. Presently, TRP proteins appear to be the most promising candidates of SOCs. TRPC1, TRPC4, and TRPV6 have been reported to generate store-operated Ca^{2+}-selective channels (Liu

Table 2. Summary of SOCC channel properties in RBL, HSG and HSY cells

	RBL	HSG	HSY
Rectification	Pronounced	Weak	No
$E_{rev\text{-}Na+}$ (mV)	60	10	5
$E_{rev\text{-}Ca2+}$ (mV)	> 90	25	5
pCs^+/pNa^+	0.1	1	1.3
pCa^{2+}/pNa^+	> 500	40	4.6
Single-channel Conductance (pS)	0.2*	20	28
Ca^{2+} selectivity	Highly selective	Selective	Not selective
AMF	Yes	Yes	No
Current divalent-free	Larger	Larger	Equal
Divalent conductance	$Ca^{2+} > Ba^{2+} = Sr^{2+}$*	$Ca^{2+} > Ba^{2+}$	$Ca^{2+} = Ba^{2+}$
Na^+-current inactivation	Slow	Very slow	Fast
Ca^{2+}-current inactivation	Fast	Fast	Very fast
2-APB inhibition	Yes	Yes	Yes
Gd^{3+} inhibition	Irreversible	Reversible	Reversible
Store dependence	Yes	Yes	Yes

*from Prakriya and Lewis, 2003

et al., 2000; Freichel et al., 2001; Schindl et al., 2002; Beech et al., 2003). On the other hand, TRPC3, which also forms store-operated Ca^{2+} channels under some conditions, appears to be relatively non-selective (Kamouchi et al., 1999; Trebak et al., 2003b). Additionally, several studies have demonstrated that different combinations of TRP proteins produce channels that display distinct properties (Lintschinger et al., 2000; Strubing et al., 2001). Our previous studies show that TRPC1 is critical for the SOC activity in HSG cells (Liu, Singh & Ambudkar, 2003; Liu et al., 2000), although it might do so in combination with another TRP or an as yet unidentified protein. Thus, further studies will be required to determine the contribution of TRPs to the SOC activity in RBL and HSY cells and determine whether the differences in SOC in RBL, HSG, and HSY cells are due to differences in the channel components.

Importantly, we have also shown that channel inactivation is different in the three cell types. For example, in HSY cells both Ca^{2+} and Na^+ currents demonstrated similar rapid inactivation, which was faster than the currents in either RBL or HSG cells. Na^+ currents in HSG cells displayed the slowest inactivation. Thus, while the activation of the channels appears to be via the same mechanism, their inactivation appears to be regulated differently. Store-operated Ca^{2+} influx in both RBL and HSG cells undergoes Ca^{2+}-dependent feedback inhibition (Parekh & Penner, 1997; Singh et al., 2002). This likely accounts for the faster inactivation of the Ca^{2+} current, as compared to inactivation of the Na^+ current in these cells. The exact mechanism involved in the inactivation of the Na^+ current is not clear. Although the mechanism of inactivation of the channel in HSY cells is not clear, since the Ca^{2+} and Na^+ currents inactivate at the same rate, we can suggest that it is likely not due to Ca^{2+}. However, it will be important to consider that the activity of Ca^{2+}-regulatory systems such as mitochondria, SERCA and PMCA pumps might also affect the function of SOC (Liu & Ambudkar, 2001; Parekh, 2003).

Different voltage-gated Ca^{2+} channels, which belong to the same protein family, have similar activation mechanisms but exhibit distinct characteristics, cellular localization, and physiological function (Arikkath & Campbell, 2003; Catterall, 2000). Analogous to these channels, it is possible that the differences seen in SOCs, described here and previously, reflect their specific physiological function. While store-operated Ca^{2+} influx appears to be primarily regulated by the status of Ca^{2+} in the internal Ca^{2+} store (Putney & Bird, 1993; Putney & McKay, 1999; Venkatachalam et al., 2002; Parekh, 2003), its function does not appear to be only to refill internal Ca^{2+} stores. SOC-mediated Ca^{2+} influx regulates a variety of critical functions in various cell types (Ambudkar, 2000; Berridge et al., 2000; Venkatachalam et al., 2002; Lewis, 2003). CRAC in RBL cells has been reported to support secretion (Parekh & Penner, 1997). In T-lymphocytes, CRAC has been associated with the activation process (Lewis, 2003). The exact physiological role for SOCs in salivary gland ductal cells is not yet known. Our previous studies have shown that sustained elevation of $[Ca^{2+}]_i$ depends on SOCE in HSY cells, although oscillatory $[Ca^{2+}]_i$ increases can occur for some time in the absence of external Ca^{2+}. Interestingly, in HSY cells stimulated with high concentrations of agonist, a sustained Ca^{2+} elevation is seen, which is converted to an oscillatory increase if external Ca^{2+} is removed. In HSG cells both types of $[Ca^{2+}]_i$ increases are completely dependent on external Ca^{2+} (Liu et al., 1998; 2001). In

the latter, Ca^{2+} influx-dependent refill of internal stores occurs during each oscillation, while in HSY cells, Ca^{2+} is re-accumulated from the cytosol during each oscillation. Consistent with this observation, we have reported that HSY cells had a larger Ca^{2+}-accumulating capacity, i.e., more SERCA pumps and IP_3Rs, as well as more Ca^{2+} within their intracellular stores. It is therefore interesting that the Ca^{2+} influx channels in these two cell types are so distinct.

In conclusion, the present study demonstrates heterogeneity in the type of SOCs in different cells. We have shown that Ca^{2+}-selective as well as non-selective Ca^{2+}-permeable cation currents are activated by internal Ca^{2+}-store depletion. Future studies should be directed towards elucidating the mechanism(s) involved in the activation of these diverse SOCs, as well as their molecular composition (e.g., channel subunits, accessory/regulatory proteins), in different cells in order to determine whether the inherent physiological Ca^{2+} requirement of the cell contributes towards the type of channel that is expressed.

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