J. Membrane Biol. 157, 231–236 (1997)

The Journal of
Membrane
Biology

Regulation of Cation-selective Channels in Liver Cells

S.D. Lidofsky[1], **A. Sostman**[2], **J.G. Fitz**[2]

[1]Department of Medicine and the Liver Center, University of California, San Francisco, CA 94143, USA
[2]Department of Medicine, Duke University Medical Center, Durham, NC, USA

Received: 8 October 1996

Abstract. In liver cells, cation-selective channels are permeable to Ca^{2+} and have been postulated to represent a pathway for receptor-mediated Ca^{2+} influx. This study examines the mechanisms involved in the regulation of these channels in a model liver cell line. Using patch-clamp recording techniques, it is shown that channel open probability is a saturable function of cytosolic [Ca^{2+}], with half-maximal opening at 660 nM. By contrast, channel opening is not affected by membrane voltage or cytosolic pH. In intact cells, reduction of cytosolic [Cl^-], a physiological response to Ca^{2+}-mobilizing hormones and cell swelling, is also associated with an increase in channel opening. Finally, channel opening is inhibited by intracellular ATP through a mechanism that does not involve ATP hydrolysis. These findings suggest that opening of cation-selective channels is coupled to the metabolic state of the cell and provides a positive feedback mechanism for regulation of receptor-mediated Na^+ and Ca^{2+} influx.

Key words: Calcium — Hepatocytes — Nonselective cation channels — Patch clamp — Signaling

Introduction

Stimulation of Ca^{2+} influx into hepatocytes by Ca^{2+}-mobilizing hormones and peptide growth factors is essential to sustain a broad spectrum of processes as diverse as glycogenolysis and cellular proliferation [16, 34]. Although considerable effort has been made to elucidate the underlying mechanisms involved, the characteristics of Ca^{2+} influx pathways in hepatocytes are incompletely understood. We have previously shown that Ca^{2+}-mobilizing hormones increase cytosolic [Na^+] and stimulate the opening of hepatocellular cation channels that exhibit nearly equal permeability to Na^+, K^+, and Ca^{2+} [7, 22]. Cation-selective channels are abundant in hepatocytes and liver cell lines, and channel opening is likely to mediate the influx of Na^+ and Ca^{2+} under normal conditions. Thus, such channels are positioned to play an important role in Ca^{2+} signaling in liver.

Cation-selective channels are widely distributed among different tissues but vary considerably with respect to selectivity, conductance, and regulatory mechanisms [4, 9, 13, 19, 23, 24, 27, 28, 30, 31]. In particular, changes in cytosolic [Ca^{2+}], membrane voltage (V_m), cytosolic pH, cytosolic [Cl^-], and intracellular nucleotide levels can have distinct effects on channel opening depending on the cell type under investigation. In hepatocytes, these variables can change significantly under physiological conditions, such as during hormonal signaling or nutrient uptake, as well as under conditions of cell injury [3, 10, 11, 33]. Regulation of cation-selective channels by these variables could therefore profoundly affect Na^+ and Ca^{2+} influx in response to physiological stimuli as well as metabolic stress.

Previous work in HTC cells, a model liver cell line, has shown that cation-selective channels are activated by thapsigargin, an endoplasmic reticulum Ca^{2+} pump inhibitor that increases cytosolic [Ca^{2+}] by mechanisms independent of receptor occupation, and that channel opening is prevented by chelation of cytosolic Ca^{2+} [7]. These findings raise the possibility that hepatocellular cation-selective channels are regulated in part by increases in cytosolic [Ca^{2+}] and could serve as positive feedback elements for receptor-regulated Ca^{2+} influx.

Based on these observations, we have examined the mechanisms of regulation of hepatocellular cation-selective channels in greater detail. Here we show that such channels are Ca^{2+}-activated, voltage and pH-independent, and that they are influenced by cytosolic [Cl^-] and inhibited by intracellular ATP. These data

Correspondence to: S.D. Lidofsky

suggest that channel opening may be modulated not only by receptor occupation by Ca^{2+}-mobilizing hormones but that it is closely coupled to the metabolic state of the cell as well.

Materials and Methods

Reagents

Adenosine, ADP, ATP, ATP-γ-S, and AMP-PNP were obtained from Fluka Biochemika. GTP-γ-S was from Calbiochem, and 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) was from Biomol. All other reagents were purchased from Sigma.

Cell Culture

HTC rat hepatoma cells were grown in minimal essential medium supplemented with 5% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified 5% CO_2 atmosphere as previously described [7]. Cells were studied approximately 16–24 hr after plating.

Measurement of Single-Channel Currents

Single-channel currents were measured using patch-clamp recording techniques in cell-attached or excised inside-out patches as previously described [7, 20]. Measurements were performed at room temperature in cells plated on 35 mm plastic tissue culture dishes or cells on glass coverslips placed in a perfusion chamber (Warner Instruments). Currents were continuously monitored on an oscilloscope and chart recorder. Cation-selective channels were recognized by their characteristic linear current-voltage relation and single-channel conductance of approximately 28 pS [7, 22]. Selected segments of data (10–30 sec) were digitized and directly stored on computer for subsequent analysis with pClamp software.

In cell-attached recordings, the standard bath and pipette solution contained (in mM): 140 NaCl, 4 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 glucose, and 10 HEPES-NaOH (pH 7.35). The pipette solution also contained NPPB (10 μM) and CsCl (2 mM) to minimize opening of Cl^- and K^+ channels, respectively. In selected studies, extracellular [Cl^-] was lowered to 15 mM by substitution with 135 mM Na-aspartate or Na-nitrate. In additional selected studies, pipette NaCl was replaced by isosmotic substitution by KCl, LiCl, CsCl, $CaCl_2$ and $BaCl_2$ or by high Ca^{2+} solutions (10 mM $CaCl_2$ and 130 mM NaCl, or 65 mM $CaCl_2$ and 65 mM NaCl).

In excised patches, the solution bathing the cytosolic face of the membrane contained (in mM): 140 NaCl, 2 $MgCl_2$, 1 EGTA, and 10 HEPES-NaOH (pH 7.30). Free Ca^{2+} was adjusted by titration with 0.5 M $CaCl_2$ according to published algorithms [8]. The pipette (extracellular) solution was identical to the standard solution above.

Data Analysis and Statistics

Open probability (P_o) was calculated in two ways using pClamp software. In patches with 3 or fewer channels, P_o was derived from analysis of channel density and open times as previously described [20]. In patches with greater than 3 channels, mean P_o was estimated using all-points amplitude histograms according to previously reported methods [28]. Briefly, in a patch with N active channels, each level representing the simultaneous opening of n channels was fitted with a Gaussian curve. The probability of n channels being open simultaneously (P_n) was calculated from the area under the peak corresponding to the level n divided by the total area under all peaks. These analyses yielded similar values of P_o under comparable experimental conditions, and the results have been combined. All results are presented as mean ± SEM.

Results

Channel Characteristics: Basal Conditions

The basic characteristics of hepatocellular cation-selective channels have been previously described [7, 22]. They are permeable to Na^+, K^+, Ca^{2+} and Mg^{2+}. The primary channel type, which has a linear conductance of approximately 28 pS, is the focus of the present studies. After formation of a high resistance seal, patches were excised into the NaCl bath containing 1 mM free Ca^{2+}. In 20 consecutive patches, where the pipette contained 65 mM $CaCl_2$ and 65 mM NaCl, the average number of open channels was 11 ± 1, and mean P_o was 0.55 ± 0.05 (V_m = +40 mV).

Regulation by Cytosolic Ca^{2+}

Cation-selective channels in intact hepatocytes are activated by maneuvers that would be expected to increase cytosolic [Ca^{2+}] [7, 22]. Therefore, the effect of varying cytosolic [Ca^{2+}] on P_o was assessed in order to evaluate whether Ca^{2+} directly affects channel opening. For these experiments, membrane patches were excised into bath (cytosolic) solutions with defined free [Ca^{2+}] values. As shown in Fig. 1, P_o was a saturable function of cytosolic [Ca^{2+}]. P_o was estimated to be half-maximal (EC_{50}) when cytosolic [Ca^{2+}] was 660 nM. These data suggest that cytosolic Ca^{2+} influences channel gating.

Effect of V_m

In hepatocytes, receptor-regulated Ca^{2+} influx does not appear to be voltage-gated [17]. If cation-selective channels participate in this process, it would be predicted that they are not voltage-gated as well. The influence of V_m on cation-selective channel opening was therefore examined. P_o was measured in individual membrane patches in response to varying V_m over a wide range of potentials (−60 to +40 mV) that included physiological values. V_m had no significant effect on P_o relative to its value at −40 mV (Fig. 2*A*). These data suggest that hepatocellular cation-selective channels are not voltage-gated.

Effect of Cytosolic pH

Intracellular acidosis inhibits cation-selective channels in certain cell types [4, 13]. In hepatocytes, intracellular

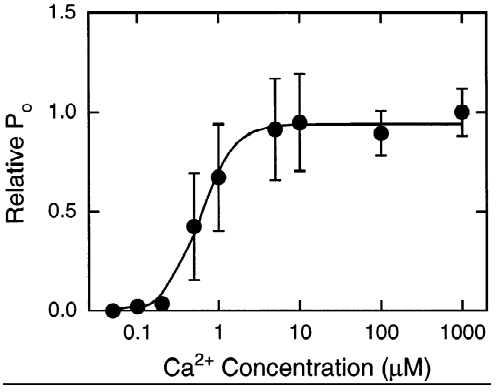

Fig. 1. Effect of cytosolic [Ca^{2+}] on channel opening. Single-channel currents were measured in membrane patches excised into bath (cytosolic) solutions of defined free [Ca^{2+}]. Membrane voltage (V_m) was held at −40 mV. Open probability (P_o) was calculated as described in Materials and Methods and plotted as a function of cytosolic [Ca^{2+}] relative to its mean value at 1 mM, where relative $P_o = 1$. Each point corresponds to the mean ± SEM of 3–10 patches.

acidosis accompanies significant metabolic stress [11] and might be expected to influence Na^+ and Ca^{2+} influx through effects on cation-selective channel opening. We therefore studied the effect of cytosolic pH on P_o. At a constant [Ca^{2+}] of 1 μM, varying the pH of the bath solution to values between 6.8 and 7.4 had no effect on channel opening (Fig. 2*B*). This indicates that hepatocellular cation-selective channels are not regulated by cytosolic pH.

Regulation by Cytosolic Cl^-

In certain epithelia, reduction of cytosolic [Cl^-] has been shown to stimulate opening of monovalent cation-selective channels [29, 31]. However, the possibility of Cl^--mediated regulation has not been investigated in the case of Ca^{2+}-permeable cation-selective channels such as those in hepatocytes, which have a high membrane Cl^- permeability [12]. To evaluate the influence of cytosolic [Cl^-] on channel opening, single-channel recordings were made in cell-attached patches before and after partial replacement of bath Cl^- by aspartate or nitrate, maneuvers that favor efflux of Cl^- from the cell. These substitutions would each be expected to decrease cytosolic [Cl^-] but to have opposite effects on V_m, based on the anion permeability of the hepatocellular membrane of nitrate > Cl^- and the low permeability of Cl^- channels to aspartate [21, 35]. For these experiments, Cs^+ (2 mM) and NPPB (10 μM) were present in the pipette to block K^+ and Cl^- channels, respectively.

Spontaneous channel openings were rare in the cell-attached configuration. Lowering bath [Cl^-] by substitution with aspartate for 2 min led to the opening of channels that carried inward current in 30/42 cells, where

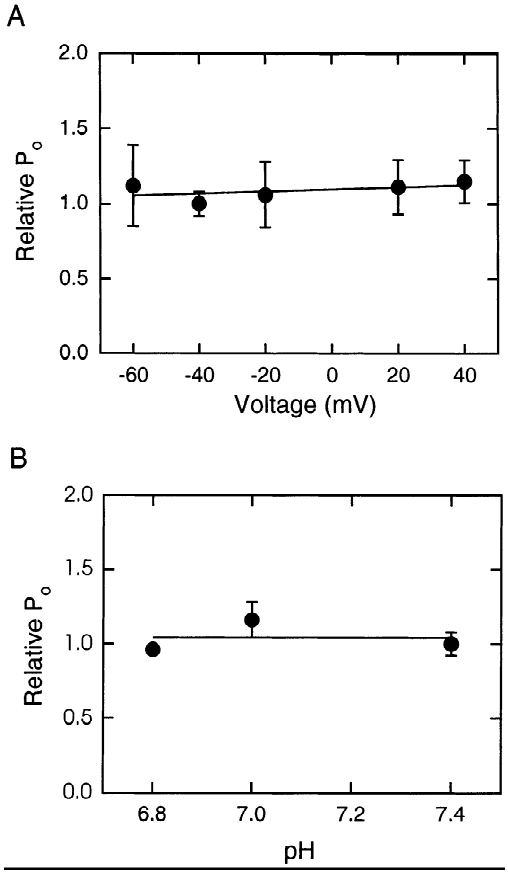

Fig. 2. Voltage and pH independence of cation-selective channel opening. (*A*) Effect of membrane voltage (V_m) on channel opening. Single-channel currents were measured in excised membrane patches, and V_m was varied for each patch. Bath (cytosolic) [Ca^{2+}] was 1 mM. P_o was plotted as a function of V_m relative to its value at −40 mV, where relative $P_o = 1$. Data correspond to mean ± SEM of 10 patches. (*B*) Effect of cytosolic pH on channel opening. Single-channel currents were measured in excised membrane patches, and pH was varied for each membrane patch. Bath (cytosolic) [Ca^{2+}] was 1 mM, and V_m was held at −30 mV. P_o was plotted as a function of pH relative to its value at 7.4, where relative $P_o = 1$. Data correspond to mean ± SEM of 6 patches.

the pipette solution contained 1 of 6 cations (Li^+, Na^+, K^+, Cs^+, Ca^{2+}, and Ba^{2+}) as the predominant charge carrier (Fig. 3). These effects were reversible, with closure of channels following restoration of basal [Cl^-]. The conductances of these channels ranged between 28 and 31 pS, and single-channel currents reversed between +20 and +60 mV relative to resting V_m (*data not shown*). These data are consistent with previously published values for hepatocellular cation-selective channels [7, 22]. Substitution of 135 mM nitrate for Cl^- had similar effects ($n = 7$, *data not shown).*

Regulation by Cytosolic Nucleotides

Although cytosolic adenine nucleotides block monovalent cation-selective channels in a number of cell types

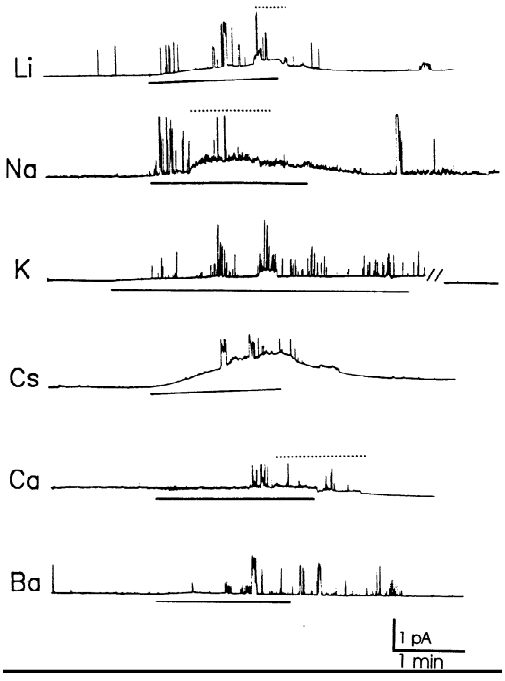

Fig. 3. Effect of $[Cl^-]$ on channel opening. Single-channel currents were measured in the cell-attached configuration under basal conditions and during partial substitution of bath (extracellular) $[Cl^-]$ by aspartate. Cs^+ (2 mM) and NPPB (10 μM) were present in the pipette to block K^+ and Cl^- channels, respectively. The decrease in extracellular $[Cl^-]$ from 150 to 15 mM, indicated by the solid line, created a gradient favoring Cl^- efflux. Lowering bath Cl^- (solid bar) caused a reversible increase in channel opening. The primary cation in the pipette (130 mM) is indicated at the left of the trace. V_m was hyperpolarized 40 mV relative to the resting potential except as indicated by the dashed lines, where it was transiently returned to the resting potential.

[4, 13, 28, 30], the role of nucleotides in regulation of Ca^{2+}-permeable cation-selective channels, such as those in hepatocytes, is uncertain. The effects of both purine and pyrimidine nucleotides on channel opening were therefore examined. In the presence of 1 μM free Ca^{2+}, application of ATP to the cytosolic face of excised membrane patches rapidly and reversibly reduced P_o (Fig. 4*A*). ATP concentrations above 10 μM were required to produce statistically significant reductions in P_o, with half-maximal inhibition between 50 and 100 μM, and maximal effects achieved at 250 μM (Fig. 4*B*). ADP, AMP, and the nonhydrolyzable ATP analogues ATP-γS and AMP-PNP, each had similar effects (3 or more patches each), decreasing relative P_o to ≤10% of basal values at concentrations of 250 μM (Fig. 4*B*). Exposure to adenosine, UTP and GTP, and the nonhydrolyzable GTP analogue GTP-γS had no effect on channel opening (250 μM, 3 or more patches each, *data not shown*). At a higher cytosolic $[Ca^{2+}]$ (2 μM), channel opening was only partially inhibited by 250 μM ATP (relative P_o 0.55 ± 0.13, $n = 5$). These data suggest that ATP modifies the sensitivity of the channels to cytosolic Ca^{2+}.

Discussion

Cation-selective channels are abundant in liver cells and are activated by Ca^{2+}-mobilizing agonists [7, 22]. The present studies build on these observations and examine the regulatory mechanisms involved in greater detail. The principal findings are that cytosolic $[Ca^{2+}]$, $[Cl^-]$, and nucleotide levels each influence channel opening. Since these channels are Ca^{2+} permeable, these results have potential implications for intracellular Ca^{2+} homeostasis and Ca^{2+} signaling in hepatocytes.

First, we have demonstrated that channel opening is positively regulated by cytosolic Ca^{2+}. The findings here shed light on previous work which indicated that the proportion of patches with measurable channel openings increased as cytosolic $[Ca^{2+}]$ was raised [7]. Here we have shown that P_o is a saturable function of cytosolic $[Ca^{2+}]$. This implies that cytosolic Ca^{2+} influences channel gating either directly or through a closely related Ca^{2+}-binding protein. The EC_{50} for channel opening reported here (660 nM) is within the range for values of hepatocellular $[Ca^{2+}]$ obtained following exposure to Ca^{2+}-mobilizing agonists [7, 18]. Therefore, activation of these channels would provide a positive feedback mechanism for receptor-regulated Ca^{2+} influx. It is notable that the concentration of Ca^{2+} required to open cation-selective channels in pancreatic acinar cells is higher in excised membrane patches than in cell-attached patches [30]. Thus, the apparent EC_{50} for cation-selective channel opening in intact liver cells may be lower than the values reported here.

Second, we have shown that P_o is not influenced by V_m, indicating that channel opening is voltage-independent. This is consistent with the viewpoint that receptor-regulated Ca^{2+} influx in hepatocytes is not intrinsically voltage-gated, in contrast with electrically excitable cells [5]. Hepatocellular cation-selective channels share the property of voltage independence with those in myelocytes and keratinocytes, in which the channels are Ca^{2+}-permeable and open in response to increases in cytosolic $[Ca^{2+}]$ [19, 24]. By contrast, their regulation is distinct from that in smooth muscle and vascular endothelium, where cation-selective channels are activated in response to membrane stretch or osmotic stress and channel opening is steeply voltage-dependent [9, 23].

Third, we have shown that changes in cytosolic pH that span the range of physiological values have no effect on P_o. The role of cytosolic pH in regulation of Ca^{2+}-permeable cation-selective channels has had limited study. Although Ca^{2+}-mobilizing agonists increase H^+ extrusion through activation of Na^+/H^+ antiport and increase cytosolic pH in many cell types [26], they do not affect intracellular pH in hepatocytes [1, 22]. Consequently, it would be expected that activation of hepatocellular cation-selective channels by such agonists would

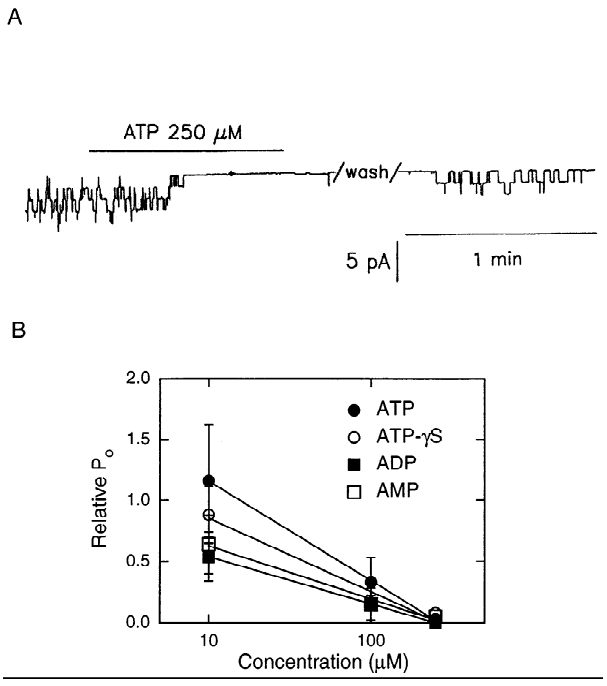

Fig. 4. Effect of adenine nucleotides on channel opening. Single-channel currents were measured in excised membrane patches before and after exposure to adenine nucleotides of defined concentrations. Bath (cytosolic) [Ca^{2+}] was 1 μM, and V_m was held at −30 mV. (*A*) Transient exposure of the cytosolic face of the membrane patch to 250 μM ATP was associated with reversible complete channel closure. (*B*) Relative P_o as a function of nucleotide concentration. P_o following exposure to nucleotides was calculated relative to its basal value, where relative $P_o = 1$. Each point corresponds to the mean ± SEM of 3–8 patches.

involve a mechanism other than intracellular alkalinization, and the pH-independence of channel opening is thus not surprising.

Fourth, our data suggest that channel opening is influenced by cytosolic [Cl^-]. In cell-attached patches, P_o increased following substitution of bath Cl^- with aspartate or nitrate. These maneuvers lower cytosolic [Cl^-] under conditions in which the pipette solution maintains [Cl^-] at a constant level at the extracellular face of the membrane patch. An inverse relation between P_o and cytosolic [Cl^-] has been reported for monovalent cation-selective channels in secretory epithelium such as fetal lung cells [31]. However, such a phenomenon has not been previously documented in Ca^{2+}-permeable cation-selective channels. In fetal lung epithelium, [Cl^-] appears to modulate the sensitivity of P_o to changes in cytosolic [Ca^{2+}]. Attempts to directly examine this in excised patches in HTC cells were hampered by the steep relation between P_o and cytosolic [Ca^{2+}] (Fig. 1). Thus, the responsible mechanisms involved remain uncertain.

Modulation of hepatocellular channel opening by Cl^- has two important implications. First, a variety of Ca^{2+}-mobilizing agonists promote Cl^- efflux from hepatocytes through opening of Cl^- channels [3]. The resultant reduction in cytosolic [Cl^-] would be expected to potentiate cation-selective channel opening and further increase Ca^{2+} influx. Second, cation-selective channels may play a role in cell volume regulation. Hepatocytes react to cell swelling from osmotic stress by opening of Cl^- channels [25, 32]. Subsequent activation of cation-selective channels by the reduction in cytosolic [Cl^-] would promote cation influx. This could prevent an overshoot in the cellular loss of fluid and electrolytes that occurs following cell swelling and would provide a servomechanism for control of cell volume. These hypotheses remain to be tested.

Finally, we have shown that channel opening is inhibited by intracellular adenine nucleotides. This mechanism does not appear to require channel phosphorylation or ATP hydrolysis, since ATP-γS was as effective as ATP in channel inhibition. Adenine nucleotides have been previously shown to reduce the opening of monovalent cation-selective channels in a number of cell types [4, 13, 28, 30], but to our knowledge, they have not been shown to affect Ca^{2+}-permeable cation channels. Channel inhibition by adenine nucleotides would tend to serve as a brake against Na^+ and Ca^{2+} influx under basal conditions. Our results contrast with those in myelocytes and mast cells, where guanine nucleotides activate Ca^{2+}-permeable cation-selective channels, and a role for G proteins in channel regulation has been suggested [19, 27]. Of interest, G proteins appear to regulate Ca^{2+} influx in hepatocytes [2, 14]. If this effect involves Ca^{2+} influx through cation-selective channels, our results suggest that the responsible mechanisms by which G proteins influence Ca^{2+} influx are indirect, involving G protein regulation of other intermediate signaling molecules.

Although it is clear that receptor-regulated Ca^{2+} influx plays a pivotal role in hepatocellular Ca^{2+}-signaling, the array of cellular processes that are controlled by Ca^{2+} influx through cation-selective channels remains to be defined, as there are likely alternative Ca^{2+} influx pathways. Recent information suggests the presence of other Ca^{2+}-selective channels in hepatocytes that are also activated by Ca^{2+}-mobilizing agonists [6]. These channels may be related to a class of channels (I_{CRAC}) present in mast cells and lymphocytes that appear to exhibit negative feedback regulation by cytosolic Ca^{2+} [15, 36]. By contrast, our data suggest that cation-selective channels act as positive feedback elements for receptor-regulated Ca^{2+} influx. The potential for both positive and negative feedback mechanisms for regulation of Ca^{2+} influx in a single cell provides a possible basis for spatial and temporal control of Ca^{2+}-mediated signal transduction.

This work was supported by National Institutes of Health grants DK01987 (S.D.L.), DK46082 (J.G.F.), and DK43278 (J.G.F.), as well as by the UCSF Huntingon Fund (S.D.L.). S.D.L. was the recipient of

a Fiterman Award in Basic Research from the American Gastroenterological Association. We thank Elisabeth Barfod, Bruce Scharschmidt, and Hal Yee for helpful discussions.

References

1. Anwer, M.S., Atkinson, J.M. 1992. Intracellular calcium-mediated activation of hepatic Na^+/H^+ exchange by arginine vasopressin and phenylephrine. *Hepatology* **15:**134–143
2. Berven, L.A., Crouch, M.F., Katsis, F., Kemp, B.E., Harland, L.M., Barritt, G.J. 1995. Evidence that the pertussis toxin-sensitive trimeric GTP-binding protein G_{i2} is required for agonist- and store-activated Ca^{2+} inflow in hepatocytes. *J. Biol. Chem.* **270:**25893–25897
3. Capiod, T., Ogden, D.C. 1989. Properties of membrane ion conductances evoked by hormonal stimulation of guinea-pig and rabbit hepatocytes. *Proc. R. Soc. Lond. B* **236:**187–201
4. Chraïbi, A., Guinamard, R., Teulon, J. 1995. Effects of internal pH on the nonselective cation channel from the mouse collecting tubule. *J. Membrane Biol.* **148:**83–90
5. Clapham, D.E. 1995. Calcium signaling. *Cell* **80:**259–268
6. Duszynski, J., Elensky, M., Cheung, J.Y., Tillotson, D.L., LaNoue, K.F. 1995. Hormone-regulated Ca^{2+} channel in rat hepatocytes revealed by whole cell patch clamp. *Cell Calcium* **18:**19–29
7. Fitz, J.G., Sostman, A., Middleton, J.P. 1994. Regulation of cation channels in liver cells by intracellular calcium and protein kinase C. *Am. J. Physiol.* **266:**G677–G684
8. Föhr, K.J., Warchol, W., Gratzl, M. 1993. Calculation and control of free divalent cations in solutions used for membrane fusion studies. *Methods Enzymol.* **221:**149–157
9. Franco, A., Lansman, J.B. 1990. Stretch-sensitive channels in developing muscle cells from a mouse cell line. *J. Physiol.* **427:**361–380
10. Gasbarrini, A., Borle, A.B., Farghali, H., Bender, C., Francavilla, A., Van Thiel, D. 1992. Effect of anoxia on intracellular ATP, Na^+, Ca^{2+}, Mg^{2+}, and cytotoxicity in rat hepatocytes. *J. Biol. Chem.* **267:**6654–6663
11. Gores, G.J., Nieminen, A.L., Wray, B.E., Herman, B., Lemasters, J.J. 1989. Intracellular pH during ''chemical hypoxia'' in cultured rat hepatocytes. *J. Clin. Invest.* **83:**386–396
12. Graf, J., Henderson, R.M., Krumpholz, B., Boyer, J.L. 1987. Cell membrane and transepithelial voltages in isolated rat hepatocyte couplets. *J. Membrane Biol.* **95:**241–254
13. Gray, M.A., Argent, B.E. 1990. Non-selective cation channel on pancreatic duct cells. *Biochim. Biophys. Acta* **1029:**33–42
14. Hansen, C.A., Yang, L., Williamson, J.R. 1991. Mechanisms of receptor-mediated Ca^{2+} signaling in rat hepatocytes. *J. Biol. Chem.* **266:**18573–18579
15. Hoth, M., Penner, R. 1992. Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature* **355:**353–356
16. Joseph, S.K., Coll, A.P., Thomas, K.E., Rubin, R., Williamson, J.R. 1985. The role of extracellular Ca^{2+} in the response of the hepatocyte to Ca^{2+}-dependent hormones. *J. Biol. Chem.* **260:**12508–12515
17. Kass, G.E.N., Llopis, J., Chow, S.C., Duddy, S.K., Orrenius, S. 1990. Receptor-operated calcium influx in rat hepatocytes. Identification and characterization using manganese. *J. Biol. Chem.* **265:**17486–17492
18. Kawanishi, T., Blank, L.M., Harootunian, A.T., Smith, M.T., Tsien, R.Y. 1989. Ca^{2+} oscillations induced by hormonal stimulation of individual hepatocytes. *J. Biol. Chem.* **264:**12859–12866
19. Krautwurst, D., Seifert, R., Hescheler, J., Schultz, G. 1992. Formyl peptides and ATP stimulate Ca^{2+} and Na^+ inward currents through non-selective cation channels via G-proteins in dibutyryl cyclic AMP-differentiated HL-60 cells. *Biochem. J.* **288:**1025–1035
20. Lidofsky, S.D. 1995. Convergent and parallel activation of low-conductance potassium channels by calcium and cAMP-dependent protein kinase. *Proc. Natl. Acad. Sci. USA* **92:**7115–7119
21. Lidofsky, S.D., Fitz, J.G., Weisiger, R.A., Scharschmidt, B.F. 1993. Hepatic taurocholate uptake is electrogenic and influenced by transmembrane potential difference. *Am. J. Physiol.* **264:**G4778–G485
22. Lidofsky, S.D., Xie, M.-H., Sostman, A., Scharschmidt, B.F., Fitz, J.G. 1993. Vasopressin increases cytosolic sodium concentration in hepatocytes and activates calcium influx through cation-selective channels. *J. Biol. Chem.* **268:**14632–14636
23. Ling, B.N., O'Neill, W.C. 1992. Ca^{2+}-dependent and Ca^{2+}-permeable ion channels in aortic endothelial cells. *Am. J. Physiol.* **263:**H1827–H1838
24. Mauro, T.M., Isseroff, R.R., Lasarow, R., Pappone, P.A. 1993. Ion channels are linked to differentiation in keratinocytes. *J. Membrane Biol.* **132:**201–209
25. Meng X.-J., Weinman, S.A. 1996. cAMP- and swelling-activated chloride conductance in rat hepatocytes. *Am. J. Physiol.* **271:**C112–C120
26. Noel, J., Pouyssegur, J. 1995. Hormonal regulation, pharmacology, and membrane sorting of vertebrate Na^+/H^+ exchanger isoforms. *Am. J. Physiol.* **268:**C283–C296
27. Penner, R., Matthews, G., Neher, E. 1988. Regulation of calcium influx by second messengers in rat mast cells. *Nature* **334:**499–504
28. Rae, J.L., Dewey, J., Cooper, K., Gates, P. 1990. A non-selective cation channel in rabbit corneal endothelium activated by internal calcium and inhibited by internal ATP. *Exp. Eye Res.* **50:**373–384
29. Robertson, M.A., Foskett, J.K. 1994. Na^+ transport pathways in secretory acinar cells: membrane cross talk mediated by $[Cl^-]_i$. *Am. J. Physiol.* **267:**C146–156
30. Thorn, P., Peterson, O.H. 1992. Activation of non-selective cation channels by physiological cholecystokinin concentrations in mouse pancreatic acinar cells. *J. Gen. Physiol.* **100:**11–25
31. Tohda, H., Foskett, J.K., O'Brodovich, H., Marunaka, Y. 1994. Cl^- regulation of a Ca^{2+}-activated nonselective cation channel in beta-agonist-treated fetal distal lung epithelium. *Am. J. Physiol.* **266:**C104–109
32. Wang, Y., Roman, R., Lidofsky, S.D., Fitz, J.G. 1996. Autocrine signaling through ATP release represents a novel mechanism for cell volume regulation. *Proc. Natl. Acad. Sci. USA* **93:**12020–12025
33. Wang, K., Wondergem, R. 1993. Redistribution of hepatocyte chloride during L-alanine uptake. *J. Membrane Biol.* **135:**237–244
34. Zhang, B.H., Farrell, G.C. 1995. Effects of extracellular Ca^{2+} and HCO_3^- on epidermal growth factor-induced DNA synthesis in cultured rat hepatocytes. *Gastroenterology* **108:**477–486
35. Zhang, J., Rasmusson, R.L., Hall, S.K., Lieberman, M. 1993. A chloride current associated with swelling of cultured chick heart cells. *J. Physiol.* **472:**801–820
36. Zweifach, A., Lewis, R.S. 1995. Slow calcium-dependent inactivation of depletion-activated calcium current. Store-dependent and -independent mechanisms. *J. Biol. Chem.* **270:**14445–14451