Extracellular pH Modifies Mitochondrial Control of Capacitative Calcium Entry in Jurkat Cells*

Krzysztof Zabłocki‡, Joanna Szczepanowska, and Jerzy Duszynski

From the Nencki Institute of Experimental Biology, Pasteura 3, 02-093 Warsaw, Poland

It was found that a collapse of the mitochondrial calcium buffering caused by the protonophoric uncoupler CCCP, antimycin A plus oligomycin, or the inhibitor of the mitochondrial Ca2+/Na+ exchanger led to a strong inhibition of thapsigargin-induced capacitative Ca2+ entry (CCE) into Jurkat cells suspended in a medium at pH 7.2. The effect of these inhibitors was markedly less significant at higher extracellular pH. Moreover, dysfunction of the mitochondrial calcium handling greatly decreased CCE sensitivity to extracellular Ca2+ when the pH of the extracellular solution was 7.2 (apparent Kd toward extracellular Ca2+ rose from 2.3 ± 0.6 mM in control cells to 11 ± 1.7 mM in CCCP-treated cells) as compared with pH 7.8 (apparent Kd toward extracellular Ca2+ increased from 1.3 ± 0.4 mM in control cells to 2.4 ± 0.4 mM in uncoupler-treated cells). Changes in intracellular pH triggered by methylamine did not influence Ca2+ influx. This suggests that, in Jurkat cells, store-operated calcium channels sense extracellular pH change as a parameter that modifies their sensitivity to intracellular Ca2+. In contrast, in human osteosarcoma cells, changes in extracellular pH as well as mitochondrial uncoupling did not exert any inhibitory effects on CCE.

Opening of the plasma membrane (PM)1 calcium channels and, in consequence, Ca2+ influx into electrically non-excitable cells is triggered by a depletion of intracellular calcium stores in the lumen of the endoplasmic reticulum (ER). This regulatory mechanism leading to the PM calcium permeability is known as capacitative calcium entry (CCE) (1, 2). This is the most common mechanism of Ca2+ entry into electrically non-excitable cells, e.g. in T lymphocytes, in which ER calcium stores are defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1728 solely to indicate this fact.‡ To whom correspondence should be addressed. Tel.: 48-22-6866073; Fax: 48-22-8225342; E-mail: k.zablocki@nencki.gov.pl.

1 The abbreviations used are: PM, plasma membrane; BCECF, 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein; BBS, buffered saline solution; [Ca2+]i, cytosolic calcium ion concentration; CCE, capacitative calcium entry; CCCP, carbonyl cyanide m-chlorophenylhydrazone; CGP 37157, 7-chloro-5-(2-chlorophenyl)-1,5-dihydro-4,1-benzoazole-2(3H)- one; ER, endoplasmic reticulum.

Received for publication, October 8, 2004
Published, JBC Papers in Press, November 29, 2004, DOI 10.1074/jbc.M411507200

Ca2+ influx into mitochondria is driven by the electrical potential across the inner mitochondrial membrane (6). The mitochondrial Ca2+ uniporter, which is a major route of calcium entry into mitochondrial matrix, exhibits a high Kd (>10 muM) value for calcium ions, whereas the bulk [Ca2+]i varies between 100 nm in resting cells and 1 μM in cells responding to a stimulus. Because of the discovery that [Ca2+]i, in a close proximity of ER or of PM calcium channels may reach higher levels than measured in the bulk phase, it has been shown that, in these cellular microdomains, mitochondrial Ca2+ accumulation occurs efficiently. Thereby mitochondria may play a role in the regulation of intracellular calcium signals (7, 8). Respiring mitochondria located in the close vicinity of ER can buffer Ca2+ released from these stores, and this may regulate the opening probability of Ca2+-sensitive ER calcium channels. Additionally, mitochondria compete with the store-loading activity of Ca2+-ATPase, counteracting the refilling of the calcium stores. This results in a more complete depletion of ER and eventually a more efficient activation of CCE (9, 10). Mitochondria can take up Ca2+ entering cells via plasma membrane channels. This may protect CCE from a feedback inhibition exerted by the excess of calcium accumulated in the subplasma membranous space, close to the mouths of calcium channels (7, 10–12). Moreover, not only the buffering but also the signaling roles of mitochondria in the regulation of calcium influxes have been postulated (13, 14).

The role of mitochondria in the regulation of calcium influx into electrically non-excitable cells has been intensively investigated (7, 9–11, 15). It was found that a decrease of the mitochondrial membrane potential inhibits Ca2+ influx into Jurkat cells activated by depletion of intracellular calcium stores (11, 12). This effect was attributed to the feedback inhibition of CCE by Ca2+ entering the cell. De-energized mitochondria could not buffer the excess of calcium and protect CCE against such inhibition.

Previously, we have documented that a decrease in the electrochemical potential of the mitochondrial inner membrane results in a decrease of the initial rate of calcium influx into Jurkat cells pretreated with thapsigargin and that this effect was strongly dependent on extracellular pH (16). We have proposed that PM calcium channels in Jurkat cells could act as pH sensors, which modify their own sensitivity to intracellular Ca2+ independently on extracellular pH. In this study, we describe further progress in the study of mitochondrially dependent CCE regulation in electrically non-excitable cells. We demonstrated that, in Jurkat cells, not only dissipation of the mitochondrial potential but also other disturbances in mitochondrial Ca2+ handling, e.g., inhibition of the Na+/Ca2+ exchanger, cause a decrease in the initial rate of calcium influx in an extracellular pH-dependent manner. At pH 7.2, the CCE is depressed after mitochondrial calcium buffer blocking due to a marked increase of CCE Kd for extra-
cellular Ca\(^{2+}\). In contrast, in human osteosarcoma cells, changes in extracellular pH as well as mitochondrial uncoupling do not exert any inhibitory effects on Ca\(^{2+}\) entry.

**MATERIALS AND METHODS**

**Chemicals**—High glucose Dulbecco’s modified Eagle’s medium, glutamine, and fetal bovine serum were purchased from Invitrogen. RPMI 1640 medium was from the Institute of Immunology and Experimental Therapy (Wrocław, Poland). Fura-2/AM and BCECF/AM were from Molecular Probes (Eugene, OR). Streptomycin plus penicillin for cell culture as well as thapsigargin, CCCP, oligomycin, antimycin A, and ionomycin were purchased from Sigma, and CGP 37157 was from Tocris Cookson Ltd. (Northpoint, Avonmouth, Bristol, UK). Other chemicals were of analytical grade.

**Cell Culture**—Human lymphoblastoid (Jurkat) T cells were grown in RPMI 1640 medium supplemented with 2 mM glutamine, 10% fetal bovine serum, penicillin (100 units/ml) plus streptomycin (50 \(\mu\)g/ml). Human osteosarcoma cells were grown on cover glass slides in high glucose Dulbecco’s modified Eagle’s medium supplemented with 2 mM glutamine, 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (50 \(\mu\)g/ml). All cell cultures were kept in a humidified atmosphere of 5% CO\(_2\)/95% air at 37 °C.

**[Ca\(^{2+}\)]\(_c\)** Measurements—The buffered saline solution (BSS) consisted of 132 mM NaCl, 5 mM KCl, 25 mM HEPES, 1 mM MgCl\(_2\), 0.5 mM NaHPO\(_4\), 1 mM pyruvate, and 5 mM glucose; the pH was adjusted to a desired value with NaOH. Where indicated, 0.12 \(\mu\)M oligomycin, 2 \(\mu\)M CCCP, 0.5 \(\mu\)g/ml antimycin A, 10 \(\mu\)M CGP 37157, and 100 \(\mu\)M thapsigargin (final concentrations) were added as MeSO solutions.

Cytosolic free Ca\(^{2+}\) was measured with Fura-2 (17). The cells were loaded with this probe in the culture medium supplemented with 1 \(\mu\)M Fura-2/AM at 37 °C for 15 min during incubation. After washing by centrifugation (Jurkat cells) in BSS supplemented with 0.1 mM CaCl\(_2\), the cells were suspended in nominally calcium-free BSS containing 0.05 mM EGTA, instead of CaCl\(_2\), and used for experiments in which [Ca\(^{2+}\)]\(_c\) was monitored. Osteosarcoma cells were rinsed twice with the same BSS as above. The cover glasses were installed in the fluorometer cuvettes. Where indicated, the mixtures were supplemented with uncouplers or inhibitors and an appropriate amount of CaCl\(_2\). The fluorescence was measured at 30 °C in a Shimadzu RF5000 fluorometer set in the ratio mode using 340 and 380 nm as excitation wavelengths and fluorescence was measured at 30 °C in a Shimadzu RF5000 fluorometer set in the ratio mode using 340 and 380 nm as excitation wavelengths and emission wavelengths of 500 nm.

RESULTS

Fig. 1 shows the effects of CCCP (Fig. 1A), antimycin A (Fig. 1B), and CGP 37157 (Fig. 1C) on calcium influx to Jurkat cells suspended in BSS at pH 7.2. The first part of each trace reflects [Ca\(^{2+}\)]\(_c\) response to thapsigargin; transient increase in [Ca\(^{2+}\)]\(_c\) represents calcium release from the ER followed by Ca\(^{2+}\) extrusion to the extracellular space. Subsequent addition of CaCl\(_2\) results in a potent increase in [Ca\(^{2+}\)]\(_c\), because of the activation of CCE. Decrease in the mitochondrial membrane potential caused by the action of CCCP or antimycin A plus oligomycin as well as the inhibition of Ca\(^{2+}\) efflux from mitochondria produced by the inhibitor of Na\(^+/Ca\(^{2+}\) exchanger CGP 37157 causes a significant reduction of the initial rates of calcium influx. On the other hand, in Jurkat cells suspended in BSS at pH 7.8, the inhibition of CCE because of disturbed mitochondrial Ca\(^{2+}\) buffering by CCCP, antimycin A plus oligomycin, or CGP 37157 was much less pronounced (Fig. 1, D–F). The plateau of [Ca\(^{2+}\)]\(_c\), following the addition of CaCl\(_2\) reflects a steady-state balance between two opposite processes: Ca\(^{2+}\) influx into the cytosol through the CCE system and Ca\(^{2+}\) efflux from the cells mediated by PM Ca\(^{2+}\)-ATPase and the Ca\(^{2+}\)/Na\(^+\) exchanger (this latter activity in Jurkat cells is rather negligible, if any) (19). As shown in Fig. 1, D–F, the mitochondrial uncouplers or inhibitors of the respiratory chain (antimycin A) or of the Na\(^+/Ca\(^{2+}\) exchanger (CGP 37157) have only small effects on the maximal value of [Ca\(^{2+}\)]\(_c\), in cells suspended in BSS at pH 7.8. Decrease in the extracellular pH from 7.8 to pH 7.2 due to the addition of HCl shifts the equilibrium between influx and efflux to lower steady-state levels of [Ca\(^{2+}\)]\(_c\). This results most probably from both pH-dependent inhibition of CCE (20) and pH-dependent stimulation of Ca\(^{2+}\) efflux due to increased activity of PM Ca\(^{2+}\)-ATPase (21, 22). However, a decrease of the final [Ca\(^{2+}\)]\(_c\), related to the reduction of BSS alkalinity is more pronounced in the cells with disturbed mitochondrial calcium buffering (Fig. 1, D–F). In other words, intracellular calcium homeostasis appeared to be more sensitive to the pH of the extracellular milieu in the cells pretreated with the mitochondrial uncouplers or inhibitors than in control cells. Because there is no reason to suspect that such modifications in the mitochondrial status might activate PM Ca\(^{2+}\)-ATPase, these findings clearly indicate that disturbances in mitochondrial Ca\(^{2+}\) handling inhibit the activity of CCE in a pH-dependent manner.

Statistical evaluation of the data described above is shown in Fig. 2. Note that the absolute values of the initial rate of calcium entry into Jurkat cells suspended in BSS at pH 7.8 are significantly higher than those in cells suspended in BSS at pH 7.2.

The relatively more potent effects of CCCP in comparison with those produced by antimycin A or CGP 37157 may be partially related to the CCCP-induced dissipation of PM electrical potential, which provides the driving force for Ca\(^{2+}\) influx. To test this possibility, the pH-dependent effect of CCCP on CCE was studied in the cells suspended in high potassium BSS (65 mM KCl and NaCl concentration lowered to 72 mM). Under such conditions, PM depolarization occurs, and this allows us to differentiate the effect of CCCP on the mitochondrial status. Despite depolarization of the PM, CCCP still much more strongly inhibited CCE in Jurkat cells suspended in BSS at pH 7.2 (from 934 ± 119 to 340 ± 40) than in BSS at pH 7.8 (from 1018 ± 136 to 784 ± 166). As expected, the absolute values of the initial rates of Ca\(^{2+}\) entry were significantly lower because of the depolarization of PM (compare with Fig. 2).

Alkalization of the extracellular milieu led to the alkalization of the cytosol but to a much smaller extent due to homeostasis of intracellular parameters (16, 23, 24). To support the notion that changes in extracellular pH are critical for the regulation of the CCE, Jurkat cells suspended in BSS at pH 7.2...
were exposed to methylamine to increase cytosolic pH without influencing extracellular pH (25). The amount of methylamine used increased cytosolic pH by 0.2–0.3 of a unit, which corresponded to cytosolic pH change after switching the extracellular pH from 7.2 to 7.8. As shown in Fig. 3, methylamine had only a small, if any, effect on the balance between Ca\(^{2+}\)/H\(^{+}\) influx and efflux in the cells treated with CCCP, whereas an increase in extracellular pH from 7.2 to 7.8 due to the addition of NaOH caused a fast and significant increase in [Ca\(^{2+}\)]\(_c\). Similar effects were obtained for both control and antimycin-treated cells (not shown).

Fig. 4 shows that the inhibitory effect of CCCP on the initial rate of calcium entry into cells suspended in BSS at pH 7.2 was inversely proportional to the concentration of extracellular CaCl\(_2\). On the other hand, in cells suspended in BSS at pH 7.8, the inhibition of calcium influx by CCCP was, as already described, smaller and virtually independent of the extracellular CaCl\(_2\) concentration in the range of 1–4 mM. This finding indicates that the kinetic parameters of CCE are modified by extracellular pH and dependent on the mitochondrial calcium-handling ability in Jurkat cells suspended in BSS at pH 7.2. Lineweaver-Burk analysis shown in Fig. 5 confirms this sup-
Only if the extracellular pH is rather low, it is necessary to keep Jurkat cells sensitive to extracellular calcium, because of the high complexity of the whole-cell system. However, one could conclude that respiring mitochondria are necessary in the presence of the mitochondrial uncoupler (Fig. 6).

Interestingly, thapsigargin-induced Ca^{2+} entry into human osteosarcoma cells suspended in BSS at pH 7.2 was not inhibited in the presence of the mitochondrial uncoupler (Fig. 6).

Moreover, in these cells, CCCP caused an enhancement of the amplitude of the Ca^{2+} transient. The increase in extracellular pH from 7.2 to 7.8 influenced neither the initial rate of Ca^{2+} entry nor the sensitivity of CCE to the mitochondrial uncoupler (data not shown).

**DISCUSSION**

Previously, we have shown that the uncoupling of oxidative phosphorylation by CCCP in Jurkat cells strongly decreases the rate of Ca^{2+} influx. This effect was postulated to be dependent on the extracellular pH (12, 16). In this study, we present convincing evidence indicating that not only mitochondrial uncoupling but also other disturbances in mitochondrial calcium handling may reduce the activity of CCE and that these inhibitory effects are evidently dependent on extracellular pH. Moreover, we performed quantitative estimation of the effect of the mitochondrial uncoupler on the kinetic properties of the CCE system in Jurkat cells and proposed a functional model explaining the described phenomenon.

In many cell types, especially in most electrically non-excitable ones, store-operated calcium channels require energized mitochondria for buffering the excess of Ca^{2+} and thereby for the protection of calcium influx against feedback inhibition (10–12, 15, 16). Inhibition of Ca^{2+} influx into Jurkat cells suspended in BSS at pH 7.2 results not only from the reduction of mitochondrial potential due to the addition of CCCP or antimycin plus oligomycin but may also be caused by inhibition of the mitochondrial Na^{+}/Ca^{2+} exchanger. This effect indicates...
that mitochondrial Ca\(^{2+}\) buffering capacity not only depends on the mitochondrial Ca\(^{2+}\) uptake driven by membrane potential but also on mitochondrial capability for Ca\(^{2+}\) release. This is in agreement with the data showing that mitochondrial Ca\(^{2+}\) uptake, followed by tunneling and release of Ca\(^{2+}\) far from PM calcium channels, is crucial for the efficient calcium buffering in close vicinity of the PM (15).

Inhibitory effects of CCCP, antimycin A plus oligomycin, and CGP 37157 on the initial rate of calcium entry into Jurkat cells were strong when the cells were suspended in BSS at pH 7.2. In cells suspended in BSS at pH 7.8, Ca\(^{2+}\) influx was only slightly reduced. This finding confirms our previous observation that a gradual increase in extracellular pH from 7.0 to 7.8 progressively diminishes the sensitivity of CCE to the mitochondrial uncoupler (16). A small (if any) effect of intracellular alkalinization by methylamine on [Ca\(^{2+}\)]\(_i\), in Jurkat cells suspended at pH 7.2 (see Fig. 3) indicates that the increase in intracellular pH could not explain potent effects of extracellular alkalinization on CCE activity. This is in agreement with our previous results with NH\(_4\)Cl used for transient alkalinization of the cellular interior (16).

On the other hand, a very small but reproducible methylamine-induced increase in [Ca\(^{2+}\)]\(_i\), in Jurkat cells might be in agreement with the observation on human platelets, in which an increase in cytosolic pH causes slight activation of calcium influx (26). Intracellular pH may, to some extent, regulate Ca\(^{2+}\) flux into the cell; however, these effects are small, and they could not be responsible for extracellular pH-dependent changes in the rate of CCE. This is also in agreement with observations on vascular endothelial cells (20). The finding that CCE needs functioning mitochondria for full activity in cells suspended at pH 7.2 and that this dependence is less obvious at pH 7.8 is further supported by the observation that the kinetic parameters of CCE in relation to inwardly transported Ca\(^{2+}\) are modified by extracellular pH. A large increase in the apparent K\(_v\) value for extracellular Ca\(^{2+}\) observed in the cells, suspended in BSS at pH 7.2 and exposed to CCCP in comparison to that in the cells in coupled mitochondria, suggests a competition-like relationship between extra- and intracellular calcium ions (Fig. 5). In fact, a decrease of mitochondrial potential has a negligible influence on calcium influx into Jurkat cells suspended in BSS at pH 7.2 and exposed to a very high (>6 mM) concentration of CaCl\(_2\) (data not shown).

On the basis of the data presented in this study, we want to support our previous model of pH-dependent regulation of the channels involved in the CCE in Jurkat cells (16). According to this concept, intracellular Ca\(^{2+}\) could efficiently bind to and inhibit the channel only if the pH of the extracellular milieu dropped below 7.4. Under such conditions, mitochondria that accumulate calcium are crucial for keeping the channel active. Under more alkaline conditions, intracellular calcium, and in consequence, the mitochondrial energy state have only a slight influence on the kinetic parameters of CCE in Jurkat cells. In other words, the calcium channel acts as an extracellular pH sensor that may switch calcium conductivity from being sensitive to the intracellular Ca\(^{2+}\) mode (extracellular pH 7.2) to non-sensitive (extracellular pH 7.8).

Recently, it has been demonstrated that a slight acidification of the extracellular milieu leads to protonation of the glutamate residue of the TRPV5 calcium channel protein and, in consequence, to the inhibition of calcium entry into the cell (27). It has been postulated that this pH-sensing mechanism is responsible for pH-dependent conformational changes in the channel protein. In cultured vascular A7r5 cells, external acidosis decreases CCE (28), and this effect is not mimicked by intracellular acidification (for review, see Ref. 29). Similar dependences between extracellular pH and Ca\(^{2+}\) entry were found in rabbit-resident alveolar macrophages, neurons, and pancreatic cells (23, 30, 31). The results presented in this study not only correspond well to these data but also indicate a novel mechanism connecting both extracellular pH and mitochondrial effects on CCE in Jurkat cells.

It seems likely that extracellular pH-dependent regulation of CCE and its sensitivity to the mitochondrial energy state might be partially responsible for the abnormal behavior of lymphocytes exposed to the acidic environment and the limited availability of oxygen under some pathological conditions (33–35).

The regulatory mechanism of calcium entry into Jurkat cells (and many other electrically non-excitable cells) described in this study is based on the feedback inhibition of PM Ca\(^{2+}\) channels by intracellular calcium and a counteracting action of mitochondria buffering the excess of Ca\(^{2+}\). Surprisingly, mitochondrial de-energization does not reduce the rate of calcium entry into human osteosarcoma cells. Moreover, Ca\(^{2+}\) transients in osteosarcoma cells preincubated with thapsigargin exhibit much higher amplitude when electrical potential across the inner mitochondrial membrane collapsed (Fig. 6). This resembles, to some extent, the effects of mitochondrial uncouplers on the calcium entry into electrically excitable cells, in which mitochondrial sequestration of Ca\(^{2+}\), entering the excited cells decreases the amplitude of Ca\(^{2+}\) spikes and modulates the intensity of calcium signals. The gradual release of Ca\(^{2+}\) accumulated in the mitochondrial matrix prolongs the Ca\(^{2+}\) signal after the initial stimulus has been turned off. Reduction of the calcium buffering capacity by mitochondria (e.g. by depolarization of the mitochondrial inner membrane) results in the increased amplitude and shortened duration of the cytosolic Ca\(^{2+}\) signals (36–38). The comparison of the regulatory mechanisms of calcium entry into Jurkat cells and osteosarcoma cells suggests that Ca\(^{2+}\) fluxes into these cells occur via different channel types.

Acknowledgment—We thank Professor Lech Wojtczak for critical reading of the manuscript.

REFERENCES

1. Putney, J. W., Jr. (1986) Cell Calcium 7, 1–12
2. Parekh, A. B., and Penner, R. (1997) Physiol. Rev. 77, 901–929
3. Putney, J. W. (1990) Cell Calcium 11, 611–624
4. Venkatraman, K., van Rossum, D. B., Patterson, R. L., Ma, H-T., and Gill, D. L. (2002) Nat. Cell Biol. 4, E263–E272
5. Thastrup, O., Dawson, A. P., Scharff, O., Foder, B., Cullen, P. J., Drobak, B. K., Bjerrum, P. J., Christensen, S. B., and Hanley, M. R. (1994) Agents Actions 35, 187–193
6. Nichols, D. G. (1978) Biochem. J. 176, 463–474
7. Malii, R., Frieden, M., Osibow, K., and Graier, W. F. (2003) J. Biol. Chem. 278, 10807–10815
8. Rizzuto, R., Brini, M., and Pozzan, T. (1993) Science 262, 744–747
9. Gilhert, J. A., Bakowski, D., and Parekh, A. B. (2001) EMBO J. 20, 2672–2679
10. Gilhert, J. A., and Parekh, A. B. (2000) EMBO J. 19, 6401–6407
11. Hoth, M., Fanger, C. M., and Lewis, R. S. (1997) J. Cell Biol. 137, 633–648
12. Makowska, A., Zablocki, K., and Duszynski, J. (2000) Eur. J. Biochem. 267, 877–884
13. Glitsch, M. D., Bakowski, D., and Parekh, A. B. (2002) EMBO J. 21, 6744–6754
14. Parekh, A. B. (2003) News Physiol. Sci. 18, 252–256
15. Malili, R., Frieden, M., Osibow, K., Zaratti, C., Mayer, M., Demaurex, N., and Graier, W. F. (2003) J. Biol. Chem. 278, 44769–44779
16. Zhlobicki, K., Makowska, A., and Duszynski, J. (2003) Cell Calcium 33, 81–99
17. Grynkiewicz, G., Poenie, M., and Tsien, R. Y. (1985) J. Biol. Chem. 260, 3440–3450
18. Rink, T. J., Tsien, R. Y., and Pozzan, T. (1982) J. Cell Biol. 95, 189–196
19. Bautista, D. M., Hoth, M., and Lewis, R. S. (2002) J. Physiol. (Lond.) 543, 877–894
20. Wakabayashi, J., and Groschner, K. (1996) Biochem. Biophys. Res. Commun. 231, 762–767
21. Carafoli, E. (1997) Basic Res. Cardiol. 92, 59–61
22. Grover, A. K., and Samson, S. E. (1986) Am. J. Physiol. 251, C529–C534
23. Heming, A. T., Bulayeva, N. N., and Bidani, A. (2003) Clin. Sci. (Lond.) 105, 81–88
24. Wakabayashi, J., and Groschner K. (1997) Biochem. J. 323, 567–573
25. Danthuluri, N. R., Kim, D., and Brock, T. A. (1990) J. Biol. Chem. 265, 19071–19076
26. Gende, O. A. (2003) Platelets (Edinb.) 14, 9–14

Downloaded from http://www.jbc.org/ by guest on July 26, 2018
27. Yeh, B-I., Sun, T-J., Lee, J. Z., Chen, H-H., and Huang, C-L. (2003) *J. Biol. Chem.* **278**, 51044–51052
28. Iwasawa, K., Nakajima, T., Hazama, H., Goto, A., Shin, W. S., Toyo-Oka, T., and Omata, M. (1997) *J. Physiol. (Lond.)* **503**, 237–251
29. Austin, C., and Wray, S. (2000) *Circ. Res.* **86**, 355–363
30. Hochstrate, P., Dierkes, P. W., and Schlue, W. R. (2001) *J. Membr. Biol.* **184**, 13–25
31. Muallem, S., Pandol, S. J., and Beeker, T. G. (1989) *Am. J. Physiol.* **257**, G917–G924

32. Deleted in proof
33. Fischer, B., Muller, B., Fischer, K. G., Baur, N., and Kreutz, W. (2000) *Clin. Immunol.* **96**, 252–263
34. Muller, B., Fischer, B., and Kreutz, W. (2000) *Immunology* **99**, 375–384
35. Bosticardo, M., Ariotti, S., Losana, G., Bernabei, P., Furni, G., and Novelli, F. (2001) *Eur. J. Immunol.* **31**, 2829–2838
36. Werth, J. L., and Thayer, S. A. (1994) *J. Neurosci.* **14**, 348–356
37. Friel, D. D., and Tsien, R. W. (1994) *J. Neurosci.* **14**, 4007–4024
38. Friel, D. D. (2000) *Cell Calcium* **28**, 307–316
