Phorbol 12-myristate 13-acetate activates an electrogenic H^+ -conducting pathway in the membrane of neutrophils

András KAPUS,* Katalin SZÁSZI and Erzsébet LIGETI

Department of Physiology, Semmelweis University Medical School, Puskin u. 9, P.O. Box 259, H-1444 Budapest 8, Hungary

The mode of activation of an H^+ -conducting pathway present in the membrane of neutrophils was investigated. (1) Resting neutrophils released protons through an electrogenic Cd^{2+} -inhibitable ($K_{0.5} \approx 20 \mu M$) route when a pH gradient and appropriate charge compensation was provided. (2) The rate of H^+ efflux was stimulated over 2.5-fold by 4β -phorbol 12-myristate 13-acetate (PMA; $K_{0.5} \approx 0.7 nM$) or by 4β -phorbol 12,13-dibutyrate ($K_{0.5} \approx 20 nM$) even when the NADPH oxidase was blocked by *p*-chloromercuribenzoate. (3) Staurosporine inhibited the effect of PMA. (4) The H^+ egress was not enhanced by 4α -phorbol 12,13-didecanoate. (5) Low concentrations of Cd^{2+} ($< 40 \mu M$) inhibited the H^+ flux without influencing the oxidase. The results raise the possibility that protein kinase C could be involved in the activation of an electrogenic H^+ -conducting pathway in the membrane of neutrophils. The activation of this route by phorbol esters seems to be independent of the stimulation of NADPH oxidase.

INTRODUCTION

The superoxide-generating NADPH oxidase plays a pivotal role in the microbicidal activity of neutrophil granulocytes [1,2]. This plasma-membrane-associated enzyme is dormant in resting cells and becomes assembled into an active form from different components after exposure of the cells to a large variety of physiological and artificial stimuli, including, e.g., complement components, bacterial peptides, arachidonic acid or phorbol esters [1–3]. Though the mechanism of activation has not yet been fully characterized, the translocation of at least two cytoplasmic factors to the membrane is a prerequisite for the process [4–7]. Once activated, the enzyme catalyses the transmembrane movement of electrons from cytosolic NADPH through a flavoprotein and a cytochrome *b*-containing chain to molecular oxygen, resulting in the appearance of $O_2^{\cdot -}$ in the exterior or in the phagosome [1,8,9]. Henderson *et al.* [10] have shown that the operation of the oxidase is electrogenic: its stimulation by PMA led to the depolarization of neutrophil cytoplasts, and this phenomenon was prevented by the oxidase inhibitor diphenyleneiodonium [11–13]. This finding suggests that the oxidase contributes to the depolarization generally observed upon stimulation of neutrophil granulocytes [14–17]. However, the membrane potential of the activated cytoplasts stabilized at a new steady state after about 1 min, whereas $O_2^{\cdot -}$ production proceeded linearly for several minutes, pointing to the existence of a charge-compensating mechanism [10]. Searching for this, the authors cited found a Cd^{2+} -inhibitable H^+ -conducting pathway (referred to as the ' H^+ -channel'), which was suggested to operate in parallel with the electron-conveying oxidase. According to their hypothesis, the oxidase first becomes activated, and after a lag phase the NADPH-derived H^+ ions are translocated through the putative channel [10,18,19]. The latter process would provide the necessary charge compensation for the continuous $O_2^{\cdot -}$ production and, in addition, would help to prevent the cytoplasmic acidification, and to promote the release of protons into the intraphagosomal space [20].

The nature of activation of the proposed channel remained to

be elucidated. The aim of the present work was to gain some insight into this mechanism by trying to answer the following questions. (1) Is an electrogenic, Cd^{2+} -sensitive, H^+ -conducting channel present in the membrane of resting neutrophils, i.e. when the NADPH oxidase and the signal-transducing pathways leading to its activation are non-functional? (2) Does the activation of the enzyme or the signal-transducing routes modify the H^+ flux through this putative channel?

The results suggest that the plasma membrane of resting neutrophils possesses a Cd^{2+} -inhibitable H^+ -channel, the conductance of which can be significantly enhanced, independently of the oxidase, by phorbol esters.

MATERIALS AND METHODS

Chemicals and solutions

BCECF/AM, nigericin and CCCP were purchased from Calbiochem, and DMSO, PMA, cytochrome *c*, DTPA and staurosporine were from Sigma; valinomycin was from Serva, *p*CMB from BDH, superoxide dismutase from Boehringer, and Percoll from Pharmacia. Iodonium biphenyl and the phorbol derivatives were generously given by Dr. M. C. Pilloud and Dr. G. Hajnóczky respectively.

The PBS medium contained 137 mM-NaCl, 8.1 mM- Na_2HPO_4 , 2.7 mM-KCl, 1.5 mM- KH_2PO_4 and 5 mM-glucose, pH 7.3. The KCl medium contained 140 mM-KCl, 5 mM-Tris/HCl and 5 mM-glucose, pH 7.4.

Cell preparation

Granulocytes were isolated from 2 litres of citrated pig blood basically as described in [21]. Briefly, erythrocytes were eliminated from the blood by hypo-osmotic lysis (4 vol. of distilled water, 30 s). After re-establishing iso-osmoticity by adding 9% (w/v) NaCl, cells were pelleted (20 °C, 200 g, 10 min), washed with PBS medium, centrifuged again and resuspended in 40 ml of PBS medium. Samples (10 ml) were placed on to 10 ml of 55% (v/v) Percoll in PBS. Granulocytes which sedimented at the bottom layer were collected and resuspended in 45 ml of PBS to wash out

Abbreviations used: PMA, phorbol 12-myristate 13-acetate; BCECF/AM, 2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester; CCCP, carbonyl cyanide *m*-chlorophenylhydrazide; DTPA, diethylenetriaminepenta-acetic acid; PKC, protein kinase C; *p*CMB, *p*-chloromercuribenzoate (K^+ salt); DMSO, dimethyl sulphoxide; DCCD, *NN'*-dicyclohexylcarbodi-imide; pH_i , intracellular pH; ΔpH , pH difference across the plasma membrane.

* To whom correspondence should be addressed.

Fig. 1. Effect of valinomycin and Cd^{2+} on the ΔpH -driven H^+ efflux from neutrophils

BCECF-loaded acidified cells (9.3×10^6) were suspended in 2.5 ml of KCl medium. (a) At the first arrow CdCl_2 was added at different concentrations (indicated in μM above the traces). Other additions: Val, $0.8 \mu\text{M}$ -valinomycin; Nig, $2.1 \mu\text{M}$ -nigericin. (b) Inhibition of the valinomycin-promoted H^+ release from neutrophils by different Cd^{2+} concentrations.

the Percoll. After centrifugation, their final concentration was adjusted to 10^9 cells/ml. The purity of the preparation was about 95 %.

Measurement and manipulation of intracellular pH (pH_i)

Cells (10^9) suspended in 1 ml of PBS were incubated with $4 \mu\text{M}$ -BCECF/AM at 37°C for 20 min. BCECF-loaded cells were then acidified by using the ammonium pre-pulse technique [22]: they were diluted with 40 ml of a medium containing 50 mM- NH_4Cl , 100 mM-choline chloride, 5 mM-Tris/HCl and 5 mM-glucose, pH 7.3, and kept at room temperature for 20 min. Thereafter cells were pelleted and resuspended in 40 ml of an NH_4^+ -free medium containing 150 mM-choline chloride, 5 mM-Tris/HCl and 5 mM-glucose, pH 7.4. After an additional 15 min the cells were sedimented again and suspended at $(6-9) \times 10^8/\text{ml}$ in the latter medium. These indicator-loaded acidified cells were kept at 4°C , otherwise they gradually lost their $\text{O}_2^{\cdot-}$ -producing capability. In cooled cells the PMA-induced $\text{O}_2^{\cdot-}$ formation did not decrease significantly up to 4 h. For measuring pH_i changes $(0.9-1.25) \times 10^7$ cells were suspended and magnetically stirred in 2.5 ml of KCl medium at $22-24^\circ\text{C}$. Fluorescence was monitored by a Perkin-Elmer 3000 spectrofluorimeter with wavelengths of 485 and 540 nm for excitation and emission respectively. These relatively less sensitive wavelengths were chosen because they were reported to minimize artifacts due to PMA-induced shape changes [23]. Calibration of the signal in terms of pH_i was carried out in the KCl medium by addition of small pulses of 0.125 M-HCl in the presence of the K^+ ionophore valinomycin and the K^+/H^+ exchanger nigericin, when both the H^+ and K^+ gradients across the membrane are completely abolished [24]. Changes in extracellular pH were measured under identical conditions with a Radiometer pH electrode. No significant change in the baseline fluorescence was observed up to 5 h from the time of loading cells with BCECF, and the nigericin-plus-valinomycin-induced rise in the signal decreased by less than 7 % during this period. These findings indicate that the leakage of the probe from the cooled cells must have been negligible. Control experiments were carried out to test whether the different agents used in this study induce any indicator release from neutrophils. We added PMA, valinomycin, or PMA plus valinomycin in the presence or absence of pCMB to the cells suspended in KCl medium. After 7 min of incubation at room temperature, the neutrophils were pelleted and the fluorescence of the supernatant was compared with that of untreated controls. In three separate experiments, none of the

above additions caused any detectable rise in the extracellular fluorescence.

Superoxide generation

This was determined photometrically as the superoxide dismutase-inhibitable reduction of cytochrome *c* [25]. The A_{550} was either monitored continuously in cell suspension or measured in the supernatant after pelleting the neutrophils. Further details are described in the Figure legends. Results are presented as representative traces of at least four experiments or as mean $\pm$ S.E.M. of the number of experiments indicated.

RESULTS AND DISCUSSION

ΔpH -driven H^+ translocation in resting neutrophils

In order to decide whether an electrogenic Cd^{2+} -sensitive H^+ -conducting pathway is present in the plasma membrane of resting neutrophils, we had to establish appropriate conditions under which the movement of protons reflects the operation of such a specific route. For this purpose pig neutrophils were loaded with the fluorescent pH indicator BCECF, and their cytoplasm was acidified to $\text{pH}_i = 6.501 \pm 0.12$ ($n = 22$) by the ammonium pre-pulse technique [22]. These cells were suspended in a KCl-based medium (140 mM) buffered at pH 7.4. Thus a pH gradient of approx. 1 unit served as a driving force for H^+ efflux. The high external K^+ concentration largely dissipated the internal negative membrane potential [14-17] that would have hindered the movement of protons down their chemical gradient. The lack of external Na^+ excluded the participation of the rapid Na^+/H^+ exchanger [23,26,27] in the pH_i changes.

Under these conditions a slow basal efflux of H^+ occurred from the cells (Fig. 1a), causing a cytoplasmic alkalization with an average rate of 0.021 ± 0.006 pH unit/min ($n = 20$). In order to abolish any remaining part of the membrane potential, and to ensure that H^+ fluxes are not limited by the rate of charge-compensating counter-ion movement (i.e. by the formation of an H^+ diffusion potential), we added the K^+ ionophore valinomycin to the cells. The ionophore increased the rate of H^+ efflux in most preparations (Fig. 1a), reaching an average of 0.043 ± 0.012 ($n = 20$) pH unit/min. This finding is in good agreement with previous observations showing that K^+ is the most permeant cation in neutrophils only at normal membrane potentials, whereas its permeability is substantially decreased in the depolarized state [28]. As expected, the K^+/H^+ exchanger nigericin rapidly dis-

sipated the pH gradient across the membrane. In further measurements we tested whether the detected H⁺ permeability was Cd²⁺-sensitive. Fig. 1(a) shows that Cd²⁺ inhibited both the basal and the valinomycin-promoted H⁺ release. Nigericin, added after Cd²⁺, elevated the fluorescence to the same level as in the absence of this cation, proving that Cd²⁺ did not quench the signal and that both the dye and the protons remained in the cytoplasmic compartment. The release of H⁺ could be restored after by-passing the Cd²⁺-inhibited channel with the protonophore CCCP, too. The effect of Cd²⁺ was immediate and reversible: it was promptly abolished by the heavy-metal chelator DTPA, suggesting that its site of action was extracellular (results not shown). Fig. 1(b) demonstrates the dependence of the rate of the valinomycin-triggered H⁺ release on the applied Cd²⁺ concentration. Half-maximal inhibition was attained between 15 and 35 μ M in four separate experiments.

No spontaneous or valinomycin-induced change was seen in the p*H*_i of the acidified cells for over 10 min if they were suspended in a choline chloride-based medium, i.e. when they kept a high membrane potential and the possibility of charge compensation was impaired.

Taking these findings together, we can conclude that a Cd²⁺-inhibitable electrogenic H⁺-conducting pathway is manifest in the membrane of resting neutrophils. Its basal activity does not require the switching-on of the oxidase (there was no detectable O₂^{-•} production under these conditions; see Fig. 2a). Membrane depolarization itself might be a channel-opening factor. However, at present it seems to be unlikely, because, when cells suspended in choline chloride were exposed to an inwardly directed pH gradient (extracellular pH = 6.2, p*H*_i = 7.2), the slow H⁺ influx was not stimulated by addition of 20 mM-KCl.

Effect of phorbol ester-induced cell activation on the H⁺ translocation through the Cd²⁺-sensitive channel

The first observations suggesting the existence of an electrogenic H⁺ channel were obtained in cytoplasts stimulated with the protein kinase C (PKC) activator PMA [10,18,19]. As we found a Cd²⁺-sensitive H⁺-conducting activity in unstimulated cells, the question arises whether the operation of this putative channel can be enhanced by PMA. Fig. 2(a) shows that after a lag phase, PMA induced O₂^{-•} production in these acid-loaded cells suspended in the KCl medium. Under identical conditions PMA elicited a triphasic response in p*H*_i (Fig. 2c, trace B). After its addition, first a lag phase (80–90 s) occurred. This was followed by a marked increase in p*H*_i, the initial rate of which (0.064 ± 0.023 , $n = 8$) was more than 3 times faster than the spontaneous change and exceeded the valinomycin-induced response as well (Fig. 2c, trace A, and Fig. 2e, trace A). After a further 3–4 min a plateau phase was reached, representing a new steady state. Control experiments confirmed the following. (1) The increase in fluorescence was not due to PMA-induced dye release (see the Materials and methods section). (2) The plateau was not caused by a O₂^{-•}-mediated bleaching of the probe: addition of nigericin immediately increased the fluorescence (results not shown). (3) The PMA-induced rise in p*H*_i was really due to H⁺ egress from the cells and not to a cytoplasmic H⁺-consuming process, as 40 μ M-Cd²⁺ added at the plateau induced re-acidification (Fig. 2c, trace B). Thus PMA caused a net cytoplasmic alkalinization, though NADPH oxidase is an H⁺-producing enzyme [27,29]. Consequently, under the present conditions the rate of H⁺ efflux must have exceeded that of H⁺ generation. PMA might increase the rate of H⁺ efflux by two different mechanisms. (1) First it may have an effect on the driving force: activation of the oxidase initiates the translocation of electrons through the membrane, which themselves might act as compensating charges for the H⁺ release. As two electrons are

Fig. 2. Demonstration of the separate effects of PMA on NADPH oxidase and on the H⁺-conducting pathway

O₂^{-•} production (a, b) and p*H*_i changes (c–f) were monitored simultaneously. Traces in panels (a), (c) and (e) were obtained in the absence of pCMB, whereas traces in panels (b), (d) and (f) were in the presence of 50 μ M-pCMB added before 1.25×10^7 cells. Where indicated by the arrows, 10 nM-PMA, 0.8 μ M-valinomycin (Val) or 40 μ M-Cd²⁺ was added. Other conditions were identical with those for Fig. 1, except that for (a) and (b) the medium was supplemented with 100 μ M-cytochrome c and absorbance was detected continuously at 550 nm. The initial p*H*_i was 6.47.

transferred per H⁺ generated [29], an electrical driving force component is given to Δ p*H*. (2) The other possibility is that PMA augments the conductance of the channel. The following findings argue for the contribution of this mechanism in the effect of PMA. Fig. 2(e, traces A and B) shows that in the presence of valinomycin, when H⁺ egress is not limited by the rate of charge compensation, PMA elicited a 3-fold increase in the rate of H⁺ egress [0.129 ± 0.046 , ($n = 12$) versus 0.043 ± 0.012 , ($n = 20$) pH unit/min]. The fact that valinomycin added at the end of the lag phase approximately doubled the rate of the PMA-induced increase in p*H*_i (cf. Fig. 2e, trace B, with Fig. 2c, trace B) indicates that in the absence of the ionophore the movement of protons still remained limited by the rate of translocation of the compensating charges.

Both the PMA- and the PMA-plus-valinomycin-stimulated H⁺ efflux remained Cd²⁺-sensitive (see below and Fig. 5), suggesting that these fluxes were mediated through the same pathway as the H⁺ movements observed in the absence of PMA.

In order to dissociate the effect of PMA on the oxidase (H⁺ generation plus electron translocation) from its possible effect on the channel, we blocked the enzyme with the thiol reagent pCMB [30]. As shown in Fig. 2(b), 50 μ M-pCMB completely abolished

Fig. 3. Effect of various phorbol esters and Cd^{2+} on the H^+ efflux from pCMB-treated granulocytes

The cells (1.25×10^7) were incubated for 1 min in the KCl medium supplemented with $50 \mu\text{M}$ -pCMB. After 1 min incubation the indicated (final) concentrations of PMA (●, ○), 4β -phorbol 12,13-dibutyrate (▲) or 4α -phorbol 12,13-didecanoate (■) were added in $5 \mu\text{l}$ of DMSO. At 2.5 min cells were treated with $0.8 \mu\text{M}$ -valinomycin. At '0' concentration only $5 \mu\text{l}$ of DMSO was added. Symbol ○ denotes that 0.5 mM - CdCl_2 was present in the medium.

Fig. 4. Inhibitory effect of staurosporine on the PMA-induced stimulation of H^+ release from neutrophils

When present, 215 nM -staurosporine (stauro) and 10 nM -PMA were added at arrows 1 and 2 respectively. Valinomycin (Val; $0.8 \mu\text{M}$) was added where indicated. Other conditions were as in Fig. 3.

the PMA-induced $\text{O}_2^{\cdot -}$ production. Fig. 2(d) (trace A) demonstrates that pCMB stopped the basal H^+ efflux from the cells. The fact that valinomycin immediately restored H^+ release (Fig. 2f, trace A) suggests that pCMB did not block the H^+ channel, but inhibited the movement of charge-compensating ions (presumably K^+) across the membrane. The valinomycin-induced H^+ efflux from pCMB-treated cells was only slightly less ($< 25\%$) than the ionophore-promoted release from untreated controls (cf. Fig. 2e, trace A and Fig. 2f, trace A). In the presence of pCMB, PMA alone failed to induce any change in pH_i (Fig. 2d, trace B). At the same time PMA markedly potentiated the valinomycin-triggered H^+ efflux (cf. Fig. 2f, trace B versus A). In six separate experiments 10 nM -PMA caused a 2.77 ± 0.64 -fold increase in the rate of H^+ efflux from the pCMB-treated cells. Half-maximal activation was attained at approx. 0.7 nM (Fig. 3). This concentration is similar to the dose necessary for half-saturation of different PKC-dependent processes [27,31]. Therefore the question arises whether the action of PMA could be

Fig. 5. Effect of different Cd^{2+} concentrations on superoxide formation (○) and pH_i changes (●) in PMA-plus-valinomycin-treated granulocytes

For measurement of superoxide production, 1 ml of KCl medium was supplemented with $100 \mu\text{M}$ -cytochrome *c*. When present, Cd^{2+} was added to the medium before the cells ($10^7/\text{ml}$). After 1 min 10 nM -PMA was added, and then 1.5 min later $0.8 \mu\text{M}$ -valinomycin. After 5 min the superoxide formation was stopped by superoxide dismutase ($50 \mu\text{g}$), cells were pelleted and the A_{550} of the supernatant was measured. Changes in pH_i were monitored simultaneously exactly as in Fig. 2(f).

mediated via PKC also in this case. In order to investigate this possibility, we tested the effect of other phorbol esters and the PKC inhibitor staurosporine [32] on the process of activation. 4β -Phorbol 12,13-dibutyrate, a more specific but lower-affinity PKC activator [31], stimulated H^+ efflux at higher concentrations ($K_{0.5} \approx 20 \text{ nM}$), whereas 4α -phorbol 12,13-didecanoate, which fails to activate PKC [33], had no effect on the rate of H^+ translocation (Fig. 3). Staurosporine, given at 215 nM 1 min before 10 nM -PMA, decreased the activation by $79 \pm 9\%$ ($n = 3$) (see Fig. 4). Although further investigation is needed to substantiate whether PKC is involved in the activation of the channel, our findings are in agreement with this possibility.

Cd^{2+} , added at 0.5 mM , completely prevented the valinomycin- and PMA-induced elevation of pH_i (Fig. 3).

Our observations show that the channel can be activated by phorbol esters even when the oxidase is blocked. Basically similar results (not shown) were obtained when iodonium biphenyl, a more specific inhibitor of the oxidase, was used.

Next we investigated whether the inverse of the above relationship holds true also: can the operation of the channel be inhibited without influencing the oxidase? We measured the $\text{O}_2^{\cdot -}$ production in parallel with the rate of pH_i changes at various Cd^{2+} concentrations in the presence of PMA plus valinomycin (Fig. 5): $40 \mu\text{M}$ - Cd^{2+} caused 90% decrease in the alkalinization, whereas it had no effect on $\text{O}_2^{\cdot -}$ production, in agreement with previous findings [34]. It should be emphasized that under these conditions the activity of the oxidase was not restricted by charge separation in itself, because valinomycin could maintain electroneutrality.

These observations suggest that, at least functionally, the oxidase and the channel are distinct entities.

Nature of the H^+ permeability pathway

It has been recently reported that the pH_i recovery of macrophages after an acid load is partly mediated by an electrogenic vacuolar-type H^+ -ATPase [20,22,35–37]. Although this enzyme has not been demonstrated in the membrane of neutrophils, the question arises whether the Cd^{2+} -sensitive

electrogenic proton channel can be identical with an analogous ATPase. This possibility cannot be excluded with certainty at present, but several strong arguments are against it. The rate of H⁺ extrusion by the macrophage ATPase is 15–30 times faster than the fluxes observed by us in neutrophils [37]. the ATPase operated also when the extracellular medium was composed of organic cations [22,35–37], whereas no H⁺ efflux occurred from neutrophils suspended in choline chloride. The most important differences are found in the inhibitor sensitivities of these pathways. The ATPase is blocked by DCCD and by pCMB [22]. Though both agents substantially decreased the basal H⁺ efflux from neutrophils, neither of them affected significantly the valinomycin-induced release (not shown for DCCD). This finding suggests that the above compounds acted on the counter-ion movements and not on the proton-conducting pathway, which remained functional and activatable by PMA.

Further studies should clarify whether the PMA-stimulatable H⁺ flux is mediated by one of the numerous PKC-dependent cation channels [38] or whether it reflects the activity of an independent, H⁺-specific, regulated pathway.

We are indebted to Professor Attila Fonyó for helpful discussions, to Ms. Edit Fedina and Ms. Erzsébet Seres-Horváth for excellent technical assistance, and to Mr. Tamás Schnaider for valuable help. Experimental work was financially supported by grants from the National Research Fund (OTKA) and the Ministry of Health.

REFERENCES

- Rossi, F. (1986) *Biochim. Biophys. Acta* **853**, 65–89
- Segal, A. W. (1989) *J. Clin. Invest.* **83**, 1785–1793
- Lu, D. J. & Grinstein, S. (1990) *J. Biol. Chem.* **265**, 13721–13729
- Volpp, B. D., Nauseef, W. M. & Clark, R. A. (1988) *Science* **242**, 1295–1297
- Nunoi, H., Rotrosen, D., Gallin, J. I. & Malech, H. L. (1988) *Science* **242**, 1298–1300
- Tanaka, T., Imajoh-Ohmi, S., Kanegasaki, S., Takagi, Y., Makino, R. & Ishimuru, Y. (1990) *J. Biol. Chem.* **265**, 18717–18720
- Clark, R. A., Volpp, B. D., Leidal, K. G. & Nauseef, W. M. (1990) *J. Clin. Invest.* **85**, 714–721
- Cross, A. R., Parkinson, J. F. & Jones, O. T. G. (1985) *Biochem. J.* **226**, 881–884
- Laporte, F., Doussiere, J. & Vignais, P. V. (1990) *Eur. J. Biochem.* **194**, 301–308
- Henderson, L. M., Chappell, J. B. & Jones, O. T. G. (1987) *Biochem. J.* **246**, 325–329
- Cross, A. R. & Jones, O. T. G. (1986) *Biochem. J.* **237**, 111–116
- Cross, A. R. (1987) *Biochem. Pharmacol.* **36**, 489–493
- Ellis, J. A., Mayer, S. J. & Jones, O. T. G. (1988) *Biochem. J.* **251**, 887–891
- Seligman, B. E. & Gallin, J. I. (1980) *J. Clin. Invest.* **66**, 493–503
- Kuroki, M., Kamo, N., Kobatake, Y., Okimasu, E. & Utsumi, K. (1982) *Biochim. Biophys. Acta* **693**, 326–334
- Di Virgilio, F., Lew, D. P., Andersson, T. & Pozzan, T. (1987) *J. Biol. Chem.* **262**, 4574–4579
- Myers, J. B., Cantiello, H. F., Schwartz, J. H. & Tauber, A. I. (1990) *Am. J. Physiol.* **259**, C531–C540
- Henderson, L. M., Chappell, J. B. & Jones, O. T. G. (1988) *Biochem. J.* **251**, 563–567
- Henderson, L. M., Chappell, J. B. & Jones, O. T. G. (1988) *Biochem. J.* **255**, 285–290
- Lukács, G. L., Rotstein, O. D. & Grinstein, S. (1990) *J. Biol. Chem.* **265**, 21099–21107
- Roos, D. & De Boer, M. (1986) *Methods Enzymol.* **132**, 225–243
- Swallow, C. J., Grinstein, S. & Rotstein, O. D. (1988) *J. Biol. Chem.* **263**, 19558–19563
- Grinstein, S., Elder, B. & Furuya, W. (1985) *Am. J. Physiol.* **248**, C379–C386
- Kapus, A., Ligeti, E. & Fonyó, A. (1989) *FEBS Lett.* **251**, 49–52
- Ligeti, E., Doussiere, J. & Vignais, P. V. (1988) *Biochemistry* **27**, 193–200
- Simchowitz, L. (1985) *J. Biol. Chem.* **260**, 13248–13255
- Grinstein, S. & Furuya, W. (1986) *Am. J. Physiol.* **251**, C55–C65
- Majander, A. & Wikström, M. (1989) *Biochim. Biophys. Acta* **980**, 139–145
- Gabig, T. G., Lefker, B. A., Ossanna, P. J. & Weiss, S. J. (1984) *J. Biol. Chem.* **259**, 13166–13171
- Bellavite, P., Serra, M. C., Davoli, A., Bannister, J. V. & Rossi, F. (1983) *Mol. Cell. Biochem.* **52**, 17–25
- Tauber, A. I., Brettler, D. B., Kennington, E. A. & Blumberg, P. M. (1982) *Blood* **60**, 333–339
- Dewald, B., Thelen, M., Wymann, M. P. & Baggiolini, M. (1989) *Biochem. J.* **264**, 879–884
- Kikkawa, U., Takai, Y., Tanaka, Y., Miyake, R. & Nishizuka, Y. (1983) *J. Biol. Chem.* **258**, 11442–11445
- Simchowitz, L., Foy, M. A. & Cragoe, E. J., Jr. (1990) *J. Biol. Chem.* **265**, 13449–13456
- Bidani, A., Brown, S. E. S., Heming, T. A., Gurich, R. & Dubose, T. D., Jr. (1989) *Am. J. Physiol.* **257**, C65–C76
- Bidani, A. & Brown, S. E. S. (1990) *Am. J. Physiol.* **259**, C586–C598
- Swallow, C. J., Grinstein, S. & Rothstein, O. D. (1990) *J. Biol. Chem.* **265**, 7645–7654
- Shearman, M. S., Sekiguchi, K. & Nishizuka, Y. (1989) *Pharmacol. Rev.* **41**, 211–237

Received 5 June 1991/27 August 1991; accepted 12 September 1991