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The plasma membrane potential of human neutrophils. Role of ion channels and the sodium/potassium pump

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Calcium-depleted human neutrophils are depolarised when suspended in calcium-free media containing sodium ions, and are repolarised by extracellular replenishment of Ca^{2+}. The depolarisation is due to a high inward sodium current, which is blocked by calcium and by several other divalent cations, but not by barium. Addition of calcium results in a rise in the cytosolic concentration from approx. 20 nM to the resting level of approx. 130 nM. Calcium influx is strongly accelerated by a voltage-gated calcium channel. This channel might be responsible for the depolarising Na^+ current in the absence of divalent cations. In the polarised state the neutrophil membrane has a high intrinsic permeability to K^+, which may be low or absent in the depolarised state. Generation of membrane potential from the depolarised state is mainly due to the electrogenic sodium/potassium pump. However, the resting potential of about −75 mV is maintained primarily by the K^+ conductance, and only to a small extent by the sodium/potassium pump.

Introduction

The activation of neutrophils in the inflammatory response consists of several chemical and physical events, such as production of superoxide, phagocytosis and degranulation [1,2]. These events are accompanied or preceded by changes in membrane potential [3–5] and in the cytosolic concentration of free calcium [6–10]. The mechanism underlying the extensive depolarisation of the plasma membrane in this situation has not been established unequivocally.

The membrane potential of resting neutrophils has been proposed to have a significant contribution from the electrogenic sodium/potassium pump [11,12]. Bashford and Pasternak [13] have gone further to suggest that the pump is alone responsible for the resting potential in human neutrophils. In contrast to previous work, they found very small or no depolarisation upon addition of extracellular potassium.

Here we report on an apparent cooperation between the sodium/potassium pump and a voltage-gated potassium conductance in generating and maintaining the resting potential of human neutrophils. We also present evidence for a voltage-gated calcium conductance that opens only at high (negative) plasma membrane potentials.

Materials and Methods

Neutrophils were isolated from human buffy coats given by the Finnish Red Cross Blood Transfusion Centre. The isolation was performed following a modification of standard methods [14,15]. Erythrocytes were sedimented for 25 min over a solution containing 1% Dextran T-500 and 0.9% NaCl. The supernatant was layered on 5.7% Ficoll/0.9% sodium diatrizoate and centrifuged at 250 × *g* for 15 min. Contaminating erythrocytes in the pellet were haemolysed with ice-cold distilled water for 25 s before isotonicity was restored with an equal volume of hypertonic NaCl. Neutrophils were sedimented by centrifugation at 250 × *g* for 5 min and then resuspended in saline medium containing 5 mM glucose/138 mM NaCl/2.7 mM KCl/8.1 mM sodium phosphate/1.5 mM potassium phosphate (pH 7.4). The stock of neutrophils containing $1.5 \cdot 10^8$ cells/ml was kept at room temperature under continuous shaking to avoid aggregation.

Absorbance measurements were made with a Shimadzu UV-3000 spectrophotometer. We used oxonol V to measure the electrical potential across the plasma

Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetatic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Tris, 2-amino-2-hydroxymethylpropane-1,3-diol

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membrane [16]. Changes in absorbance at 630 minus 590 nm were registered. Measurements were performed in a 2 ml volume cuvette containing $1.5 \cdot 10^7$ cells and 2.5 μM oxonol V. The cuvette was thermostatted at 37 °C and the contents stirred magnetically.

Fluorescence intensities of the calcium indicator fura-2 were measured with a Hitachi F-4000 fluorescence spectrophotometer. Neutrophils were loaded by incubating $5 \cdot 10^6$ cells/ml of saline medium containing 1 μg/ml fura-2 (corresponding to 0.24 nmol/10^6 cells) in a shaking water bath thermostatted to 37 °C for 30 min. Loaded cells were washed twice and a sample of $5 \cdot 10^6$ cells in a 2 ml volume of calcium-free saline medium was used in a measurement. Excitation was at 340 ± 10 nm and emission was recorded through a band-pass filter of 510 ± 10 nm. Free cytosolic calcium concentrations were calculated by the equation [17]:

$$(Ca^{2+}) = K_d((F - F_{min})/(F_{max} - F))$$

where fluorescence intensities (F) were measured in arbitrary units. A dissociation constant (K_d) of 135 nM was used in the calculations [17]. F_{max} and F_{min} correspond to the intensities of calcium-saturated and free fura-2 [17]. Calibration was done by making cells freely permeable to calcium with ionomycin in the presence of excess external calcium, yielding F_{max}. The same permeabilisation was done in the presence of excess EGTA to chelate all calcium, and the resulting fluorescence was taken as F_{min} [18].

Oxonol V was a gift from Dr. C.L. Bashford. Fura-2 was from Molecular Probes, Junction City, OR. Valinomycin, ionomycin, monensin and ouabain were from Sigma Chemical MO. Other chemicals were of the highest purity commercially available.

Results

Our initial observation was that suspending neutrophils in Ca^{2+}-free saline medium yielded depolarised cells. Addition of cells to Ca^{2+}-free saline containing oxonol V first caused an absorption increase due to equilibration of the dye with the cells. Subsequent addition of 0.5 mM Ca^{2+} induced complete polarisation within 5 min (Fig. 1).

Addition of K^+ to cells polarised in this way caused immediate depolarisation, which was independent of the K^+ ionophore valinomycin. Addition of valinomycin to the depolarised cells, in place of calcium, caused rapid polarisation (Fig. 1). Thus a potassium gradient is sustained across the membrane of the depolarised cells, but the intrinsic potassium conductance is apparently too low to polarise the cells significantly in these conditions. Calcium subsequently polarised the cells to the same extent as with calcium alone (Fig. 1).

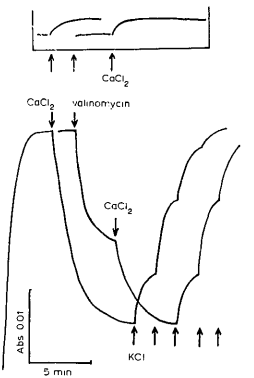

Fig. 1. Membrane potential and free cytosolic calcium. $1.5 \cdot 10^7$ calcium-depleted neutrophils were suspended in 2 ml of saline medium (see Materials and Methods) containing 2.5 μM oxonol V. Increase in the absorbance at 630–590 nm corresponds to depolarisation of membrane potential (lower panel). Fluorescence (upper panel) of $5 \cdot 10^6$ fura-2-loaded neutrophils in 2 ml of saline medium was measured on the same time-scale as the potential measurements. The lower level of fluorescence corresponds to 20 nM cytosolic free calcium, and the higher level to 130 nM 0.5 mM $CaCl_2$ and 2.5 μg/ml of valinomycin were added as indicated by the arrows. Each KCl addition corresponds to 15 mM.

Fig. 1 (inset) shows measurements of cytosolic free calcium under identical conditions. The cytosolic Ca^{2+} concentration falls to a level of approx. 20 nM in cells isolated and stored in the absence of added calcium, and suspended in calcium-free saline medium. Addition of 0.5 mM extracellular Ca^{2+} increases the intracellular level to approx. 130 nM. Higher concentrations of extracellular calcium did not enhance this level further, suggesting that a regulated resting calcium level was reached intracellularly. The resting cytosolic calcium level was reached with a half-time of approx. 40 s in these conditions. If membrane potential is generated with valinomycin prior to the addition of calcium, the half-time is shortened (see below).

We then attempted to determine the current responsible for depolarisation in calcium-free medium. Cells were suspended in Ca^{2+}-free buffer at decreasing concentrations of NaCl, which was replaced iso-osmotically with choline chloride. As shown in Fig. 2, depolarisation was less extensive the lower the extracellular concentration of Na^+. In the absence of added Na^+, there was no further polarisation of the cells on addition of 0.5 mM Ca^{2+}; the cells were fully polarised. Instead, a

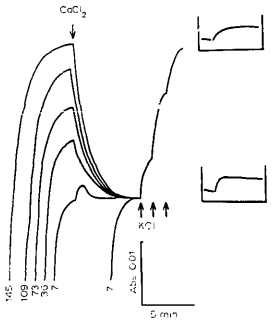

Fig. 2. Neutrophils were suspended in 10 mM Hepes-Tris buffer (pH 7.4) containing 5 mM glucose, 2.5 μM oxonol, 4.5 mM KCl and the indicated concentration of NaCl together with sufficient choline chloride to give a final concentration of 145 mM (NaCl plus choline chloride). $CaCl_2$ and KCl were added as in Fig. 1. The upper inset shows the increase in cytosolic free calcium as 0.5 mM calcium was added to cells suspended in 145 mM NaCl. The lower inset shows the corresponding effect for cells in 145 mM choline chloride.

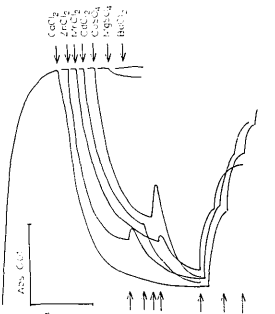

Fig. 3. Blocking of Na^+ conductance by divalent cations. Neutrophils were suspended in saline medium as in Fig. 1. 0.5 mM $CaCl_2$, 0.1 mM $ZnCl_2$, 0.3 mM $MnCl_2$, 0.5 mM $CdCl_2$, 0.1 mM $CoSO_4$, 0.5 $MgSO_4$ or 0.5 mM $BaCl_2$ was added as indicated by the arrows. Additional 0.5 mM $CaCl_2$ was added subsequently; later additions each of 15 mM KCl are also shown.

small transient depolarisation was observed, indicating that calcium entry is an electrogenic process. Subsequent additions of potassium again depolarised the cells, independently of added calcium.

Measurements of cytosolic free calcium (Fig. 2, inset) showed again that the velocity of calcium entry is accelerated when the membrane potential is maintained at a high level, in this case due to the absence of extracellular sodium ($t_{1/2} = 15$ s vs. 40 s).

These results indicate that an inward Na^+ current is responsible for the depolarisation of the cells in the absence of added calcium, and that calcium blocks this current. In support of this view the sodium ionophore monensin [18] effectively depolarised calcium-treated cells in saline medium (data not shown). Further support came from the finding that Cd^{2+}, Mn^{2+}, Co^{2+} and Zn^{2+} mimicked the effect of calcium, while Mg^{2+} and Ba^{2+} were ineffective (Fig. 3). La^{3+} was also effective but aggregation of the cells prevented quantitative evaluation in this case. Octanol is known to reduce sodium currents in frog nerve cells [20]. Interestingly, 0.5 mM octanol was found to mimick the calcium effect, causing rapid polarisation in Ca^{2+}-free saline medium (Fig. 4).

Next we attempted to elucidate the main mechanisms responsible for generating and sustaining the resting membrane potential in neutrophils.

Fig. 5 shows that membrane polarisation upon addition of calcium was largely (about 80%) blocked in the presence of 0.5 mM ouabain, or if potassium was omitted from the medium. Both conditions are well-known to block Na^+/K^+-ATPase (see Ref. 13). Addition of KCl to the K^+-depleted medium caused rapid polarisation in a ouabain-sensitive fashion (see Fig. 6).

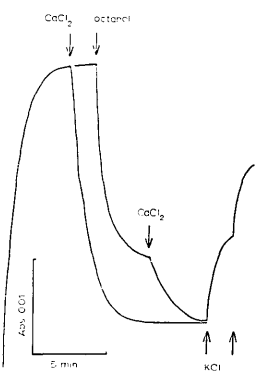

Fig. 4. Effect of octanol on membrane potential. 0.5 mM $CaCl_2$ or 0.5 mM octanol was added to cells suspended in saline medium as in Fig. 1. Potassium was added as described before (see legend to Fig. 3).

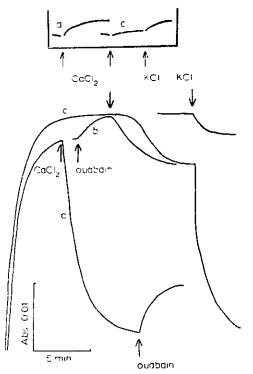

Fig. 5. Upper and lower panels correspond to fura-2 and oxonol experiments, respectively. (a) Addition of 0.5 mM calcium to neutrophils suspended in saline medium as in Fig. 1. 0.5 mM ouabain is added in the lower panel. (b) Same as (a), but ouabain added before calcium. (c) Neutrophils suspended in potassium-free medium, containing 10 mM Hepes-Tris buffer (pH 7.4)/145 mM NaCl/5 mM glucose. 0.5 mM calcium is added, followed by 4.5 mM KCl. Addition of 4.5 mM KCl before calcium is shown by the unmarked curve.

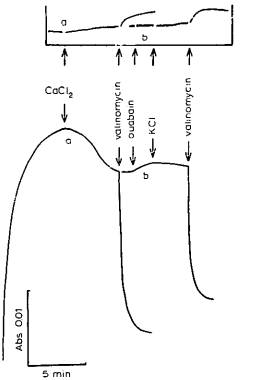

Fig. 6. Upper and lower panels correspond to fura-2 and oxonol signals, respectively. Neutrophils were suspended in potassium-free medium, as described in Fig. 5. 0.5 mM $CaCl_2$ and 2.5 μg/ml valinomycin were added (curves a). 0.5 mM ouabain was added after calcium as shown in curves (b). Subsequent addition of 4.5 mM KCl and 2.5 μg/ml valinomycin are shown.

In contrast to the report of Bashford and Pasternak [13], we found that the addition of 0.5 mM ouabain to fully polarised cells (in the presence of calcium) caused only partial (about 20%) depolarisation of the resting membrane potential (Fig. 5). Thus, whilst blocking the sodium/potassium pump largely prevents generation of membrane potential in the depolarised state, it has a much smaller effect on the already established resting potential.

These results suggest that the electrogenic ouabain-sensitive sodium/potassium pump is the main activity responsible for initial generation of membrane potential in depolarised human neutrophils. However, other ion fluxes dominate the membrane potential when it is maintained in the polarised steady-state.

Figs. 5 and 6 (insets) show that calcium entry into the cytosol is greatly retarded in the absence of extracellular potassium. Addition of K^+ strongly accelerates calcium entry (Fig. 5, inset) in an ouabain-sensitive fashion (Fig. 6, inset). Addition of valinomycin at low extracellular K^+ also strongly accelerates calcium entry, independently of ouabain (Fig. 6, inset).

From these data, and the corresponding effects on membrane potential, we conclude that the velocity of calcium entry into the cytosol is strongly accelerated by (inside negative) membrane polarisation (see Figs. 1 and 2).

Fig. 7 shows that the depolarising action of potassium is blocked by Ba^{2+}. High concentrations of potassium were required to release the block, as also found in rat skeletal muscle [21]. In Fig. 8, cells were suspended in 125 mM NaCl/35 mM KCl, in which case calcium

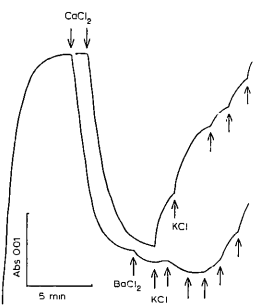

Fig. 7. Neutrophils were suspended in saline medium and calcium was added as in Fig. 1. In the upper curve 10 mM medium KCl was added by each addition. In the lower curve 0.25 mM $BaCl_2$ was added as indicated, followed by additions of 20 mM KCl each.

induces only partial polarisation due to the high concentration of extracellular potassium. Blocking the potassium channel by Ba^{2+} causes further polarisation in this case, which is abolished by valinomycin. Evidently, barium blocks the intrinsic potassium conductance, as also found in murine macrophages [22]. In contrast, barium had no effect on the depolarisation caused by Na^+ (Fig. 3). Hence, the potassium conductance is clearly distinct from the sodium conductance in the absence of calcium described above.

Fig. 9 shows titrations of the oxonol V signal with extracellular potassium. The data are plotted against the potassium equilibrium potential, assuming 50 mM intracellular K^+ in the present conditions [13]. In saline medium supplemented with 0.5 mM $CaCl_2$, the oxonol signal is very sensitive to added potassium. The titrations with or without valinomycin are similar, which shows that the neutrophil membrane has a high intrinsic potassium conductance in the resting polarised state, as also concluded above.

However, Fig. 9 also shows that at zero potassium equilibrium potential the membrane is still polarised to approx. 35% of the maximum resting potential in calcium-containing saline medium ($A = 0$ and 0.035 correspond to the fully polarised and depolarised states, respectively).

In choline chloride (without calcium) the potential is also very sensitive to potassium in the absence of valinomycin (Fig. 9; solid circles). Hence the high potassium conductance cannot be a result of the addition of extracellular calcium.

In Fig. 9 (open triangles), membrane potential is generated by adding valinomycin to depolarised cells in the absence of added calcium (see Fig. 1). Here the NaCl concentration is decreased iso-osmotically as the KCl concentration is increased. In this case the depolarised state is reached at approx. zero potassium equilibrium potential.

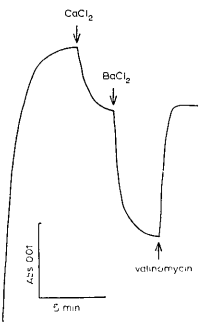

Fig. 8. Neutrophils were suspended in 10 mM Hepes-Tris buffer (pH 7.4)/125 mM NaCl/35 mM KCl/5 mM glucose/2.5 μM oxonol. 0.5 mM $CaCl_2$, 0.5 mM $BaCl_2$ and 2.5 μg/ml valinomycin were added as indicated by the arrows.

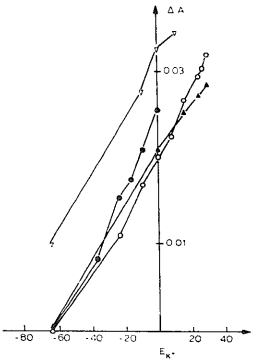

Fig. 9. Changes in the oxonol absorbance at 630–590 nm upon varying extracellular potassium is plotted against the potassium equilibrium potential. The equilibrium potential was calculated by the Nernst equation, assuming 50 mM intracellular potassium. $A = 0$ corresponds to the maximal polarisation level. (○) KCl additions to cells polarised with calcium in saline medium (see Fig. 1). (●) potassium additions to cells polarised with valinomycin and calcium (see Fig. 1). (▲) potassium additions to cells suspended in 145 mM choline chloride and thus polarised independently of extracellular calcium (see Fig. 2). (▽) neutrophils in Hepes/Tris buffer where the NaCl concentration was decreased iso-osmotically as the KCl concentration was increased. Polarisation was induced by adding valinomycin. Maximum polarisation ($A = 0$) was never reached without calcium (see Fig. 1).

Valinomycin has little or no effect on the membrane potential in polarised cells, which is consistent with a high intrinsic potassium conductance. Yet, valinomycin causes strong polarisation when added to depolarised cells at low extracellular potassium concentrations (Fig. 1 and 9), suggesting a low potassium conductance. This apparent paradox is discussed below.

Discussion

Sodium and calcium conductances

Neutrophils were depolarised when suspended in a calcium-free medium containing sodium. The reason for this was found to be an inward sodium current, which was blocked by calcium, cadmium, manganese, cobalt and lanthanum ions, and by octanol, but not by barium or magnesium ions.

A non-selective cation channel has been reported to account for Ca^{2+} uptake in activated neutrophils. However, opening of this channel required an increase in the cytosolic free Ca^{2+} concentration. No such channel activity was observed at cytosolic calcium concentrations corresponding to the resting level in neutrophils [23].

Voltage-gated calcium channels that are activated at relatively high (negative) threshold potentials have been described in mouse neuroblastoma cells [24,25], mouse oocytes [26], mollusc neurons [27] and in neoplastic mouse lymphocytes [28]. In human B-lymphocytes, a sizable depolarisation was observed if calcium was omitted from a Na^+-containing medium [29]. In neuroblastoma cells, these channels were maximally activated at -70 mV and inactivated beyond -30 mV. These channels were permeable to monovalent cations when the external concentration of divalent cations was reduced, or when the membrane potential was shifted to large positive values [24].

According to our data, the rate of calcium influx into the cytosol was strongly dependent on membrane potential, being slow in the fully depolarised state. In this respect, there is a resemblance to the voltage-gated calcium channels described above. It is possible that the high sodium conductance observed here in the absence of divalent cations can also be ascribed to this channel, but we cannot presently exclude that the two are unrelated.

Our finding of depolarisation of neutrophils in calcium-free medium provides a useful tool for studying the mechanisms of generation of the resting membrane potential.

In contrast to our finding, Bashford and Pasternak [13] have reported that human neutrophils were fully polarised in a medium without added Ca^{2+}. However, in their case, 1.35 mM Ca^{2+} was present in the stock cell suspension, which might have prevented or delayed depolarisation of cells subsequently suspended in calcium-free medium.

A gated potassium channel

In polarised neutrophils, the addition of potassium immediately and invariably caused a decrease in membrane potential without requirement of valinomycin. We conclude that the neutrophil membrane contains a potassium channel, the conductance of which may be blocked by Ba^{2+}. Clearly, this conductance is different from the sodium (calcium) conductance discussed above. It is also not dependent on calcium, since potassium caused effective depolarisation in choline chloride medium without added calcium (Figs. 2 and 9).

Classically, the efflux of potassium has been thought to be responsible for the resting potential in many cell types [30]. In neutrophils the potential has been proposed to have a contribution from the electrogenic sodium/potassium pump as well [11,12]. In contrast, Bashford and Pasternak [13] have found none or very small and variable depolarisation by increasing extracellular potassium. They proposed that the sodium/potassium pump alone is responsible for the resting potential.

Our results contrast to those of the latter workers, but are in agreement with Kuroki et al. [12]. We found virtually no effect of valinomycin on the dependence of potential on potassium (Fig. 9). Hence the 'null-point' method of determining the membrane potential (see, for example, Ref. 13) is not applicable in our case.

However, we also found that the intrinsic potassium conductance is small relative to the high Ca^{2+}-sensitive Na^+ conductance in depolarised cells, since addition of valinomycin to such cells caused a sizable polarisation at low extracellular potassium concentrations (Fig. 1). Hence, the potassium conductance may be voltage-dependent, and significant only at high (negative) membrane potentials. It would thus resemble K^+ channels with properties of 'inward rectifiers' described e.g., in murine macrophages [22], which are also blocked reversibly by external Ba^{2+}.

The resting potential of neutrophils

From the present data, we cannot deduce precisely the magnitude of the resting membrane potential of neutrophils [3–5]. However, a rough estimate can be made. The membrane potential ($\Delta\psi_m$) appears to consist of two components, $\Delta\psi_p$ (potential difference due to the electrogenic sodium/potassium pump) and $\Delta\psi_i$ (potential difference due to ion diffusion potentials), so that

$$\Delta\psi_m = \Delta\psi_p + \Delta\psi_i$$

(see Ref. 31).

Assuming that potassium flux is the major contributor to $\Delta\psi_i$, the latter is approx. -63 mV at 4.5 mM extracellular potassium (Fig. 9). Ouabain causes a 20% decrease in the resting $\Delta\psi_m$ (Fig. 6). From the equation it then follows that $\Delta\psi_m$ is -79 mV, which is in the range reported by Bashford and Pasternak [13]. It should be noticed that the data in Fig. 9 suggest that the relative contribution of the sodium/potassium pump increases at lower potentials.

We conclude that the resting membrane potential of neutrophils has a major contribution from an outward-directed potassium conductance. However, there is a significant contribution from the electrogenic sodium pump, as seen from the partial (about 20%) depolarisation upon addition of ouabain (Fig. 5; see Kuroki et al., Ref. 12).

When neutrophils generate membrane potential from an initially depolarised state, e.g., upon closure of the sodium conductance by divalent cations, the sodium/

potassium pump is primarily responsible for the electrogenic activity (Fig. 5) even though there is a sizable outward potassium gradient available.

The K^+ channel opens only at higher (negative) transmembranous potentials (see above). It does not seem to depend on cytosolic Ca^{2+} concentration. We suggest that the function of the voltage-gated potassium channel may be to cooperate with the primary sodium/potassium pump by maintaining a potential difference first built up by the pump. Obviously, the function of the pump is also primary with respect to the K^+ channel, in that the former generates the potassium gradient.

Acknowledgements

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References

1 Rossi, F. and Zatti, M. (1964) Br. J. Exp. Path. 45, 548–559.
2 Sbarra, A.J. and Karnovsky, M.L. (1959) J. Biol. Chem. 234, 1355–1362.
3 Korchak, H.M. and Weissmann, G. (1978) Proc. Natl. Acad. Sci. USA 75, 3813–3822.
4 Jones, G.S., VanDyke, K. and Castranova, V. (1981) J. Cell. Physiol. 106, 75–83.
5 Mottola, C. and Romeo, D. (1982) J. Cell Biol. 93, 129–134.
6 White, J.R., Naccache, P.H., Molski, T.F.P., Borgeat, P. and Sha'afi, R.I. (1983) Biochem. Biophys. Res. Commun. 113, 44–50.
7 Pozzan, T., Lew, D.P., Wollheim, C.B. and Tsien, R.Y. (1983) Science 221, 1413–1415.
8 Hamachi, T., Hirata, M. and Koga, T. (1986) Biochim. Biophys. Acta 889, 136–148.
9 Lazzari, K.G., Proto, P.J. and Simons, E.R. (1986) J. Biol. Chem. 261, 9710–9713.
10 Chandler, D.E. and Kazilek, C.J. (1987) Biochim. Biophys. Acta 931, 175–179.
11 Kuroki, M., Satoh, H., Kamo, N. and Kobatake, Y. (1981) FEBS Lett. 123, 177–180.
12 Kuroki, M., Kamo, N., Kobatake, Y., Okimasu, E. and Utsumi, K. (1982) Biochim. Biophys. Acta 693, 326–334.
13 Bashford, C.L. and Pasternak, C.A. (1985) Biochim. Biophys. Acta 817, 174–180.
14 Böyum, A. (1968) Scand. J. Clin. Lab. Invest. 21, Suppl. 97, 77–89.
15 Segal, A.W., Dorling, J. and Coade, S. (1980) J. Cell Biol. 85, 42–59.
16 Bashford, C.L. and Pasternak, C.A. (1984) J. Membr. Biol. 79, 275–284.
17 Grynkiewicz, G., Poenie, M. and Tsien, R.Y. (1985) J. Biol. Chem. 260, 3440–3450.
18 Von Tscharner, V., Deranleau, D.A. and Baggiolini, M. (1986) J. Biol. Chem. 261, 10163–10168.
19 DiVirgilio, F. and Gomperts, B.D. (1983) Biochim. Biophys. Acta 763, 292–298.
20 Hirche, G. (1985) Pflug. Arch. 405, 180–187.
21 Miller, C., Latorre, R. and Reisin, I. (1987) J. Gen. Physiol. 90, 427–449.
22 Randriamampita, C. and Trautmann, A. (1987) J. Cell Biol. 105, 761–769.
23 Von Tscharner, V., Prod'hom, B., Baggiolini, M. and Reuter, H. (1986) Nature 324, 369–372.
24 Mollenaar, W.H. and Spector, I. (1979) J. Physiol. 292, 307–323.
25 Tang, C-M., Presser, F. and Morad, M. (1988) Science 240, 213–215.
26 Okamoto, H., Takahashi, K. and Yamashita, N. (1977) J. Physiol. 267, 465–495.
27 Kostyuk, P.G. and Krishtal, O.A. (1977) J. Physiol. 270, 569–580.
28 Fukushima, Y. and Hagiwara, S. (1985) J. Physiol. 358, 255–284.
29 MacDougall, S.L., Grinstein, S. and Gelfand, E.W. (1988) J. Clin. Invest. 81, 449–454.
30 Williams, J.A. (1970) J. Theor. Biol. 28, 287–296.
31 Felber, S.M. and Brand, M.D. (1982) Biochem. J. 204, 577–585.