Inositol 1,4,5-trisphosphate may be a signal for f-Met-Leu-Phe-induced intracellular Ca mobilisation in human leucocytes (HL-60 cells)

Gillian M. Burgess, Jerry S. McKinney, Robin F. Irvine[+], Michael J. Berridge[†], Peter C. Hoyle and James W. Putney jr

Department of Pharmacology, Medical College of Virginia, Richmond, VA 23298-0001, USA,[+] Department of Biochemistry, AFRC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT and [†]AFRC Unit of Insect Neurophysiology and Pharmacology, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, England

Pemeabilised, dimethyl sulphoxide-differentiated HL-60 human myelomonocytic leukemia cells accumulate ^{45}Ca in an ATP-dependent manner. The ^{45}Ca is taken up by a pool thought to be a component of the endoplasmic reticulum. Inositol trisphosphate induced a rapid release of Ca from this pool, suggesting that this molecule which is formed in these cells in response to f-Met-Leu-Phe may play a role in agonist-induced Ca metabolism.

HL-60 cell   f-Met-Leu-Phe   Calcium   Inositol 1,4,5-trisphosphate   Intracellular messenger

1. INTRODUCTION

When HL-60 human myelomonocytic leukemia cells are treated with dimethyl sulphoxide (DMSO) they differentiate into granulocytes which perform many of the functions of neutrophils [1,2]. They are able to show chemotaxis and to degranulate in response to f-Met-Leu-Phe (FMLP) [1,2], effects which in the neutrophil are mediated by a rise in intracellular calcium ($[Ca^{2+}]_i$) [3–5].

A recent study of these DMSO-differentiated HL-60 cells, in which they were proposed as a convenient model for the neutrophil, showed that one of the first actions of FMLP was the phospholipase C-mediated breakdown of phosphatidylino-

sitol 4,5-bisphosphate [PI(4,5)P$_2$] to form inositol trisphosphate (IP$_3$) [2].

(1,4,5)IP$_3$, the expected breakdown product of phosphodiesteratic attack of PI(4,5)P$_2$, has recently been shown to induce Ca release from ATP-dependent non-mitochondrial vesicular pool of certain permeable cell preparations [6–8] and from liver [9] and insulinoma microsomes [18]. In light of these findings it has been suggested that (1,4,5)IP$_3$ may be a messenger which signals intracellular Ca release after Ca-mobilising hormone stimulation of these tissues.

Here, we have shown that (1,4,5)IP$_3$ also causes Ca release from permeable DMSO-differentiated HL-60 cells. The Ca is released from an ATP-dependent non-mitochondrial pool likely to be a component of the ER. These findings support the suggestion that (1,4,5)IP$_3$ may also be the messenger which causes intracellular Ca-mobilisation in neutrophils [2] after activation of their plasma-membrane receptors with FMLP and other Ca-mobilising agonists.

2. METHODS

2.1. HL-60 cell culture

HL-60 myelomonocytic leukemia cells were cultured as described previously [2]. The cells were differentiated into granulocytes by culturing for 6–7 days in the presence of DMSO (1.3%, v/v) and dexamethasone (1 μM).

2.2. Preparation of permeable DMSO-differentiated HL-60 cells

The differentiated cells were centrifuged and resuspended in a cytosolic-type medium [10] which had the following composition (mM): NaCl, 20.0; KCl, 100; MgSO$_4$, 5.0; NaH$_2$PO$_4$, 0.96; NaHCO$_3$, 25; EGTA, 1.0 and 2% albumin at pH 7.2 and 37°C. The cells were then exposed to saponin (150 μg/ml) for a period of 5–10 min until more than 99% of the cells became permeable to trypan blue. The cells were then washed and resuspended in a medium of the same composition but without saponin at a cell density of ~2 × 10^7 cells/ml. At this stage the medium was usually also supplemented with antimycin (10 μM) to prevent substrate oxidation, and an ATP-regenerating system consisting of creatine phosphate (5 mM) and creatine phosphokinase (5 U/ml) to keep the level of any added ATP constant (for further details, see figure legends for each experiment). Ca–EGTA buffers were used to set free Ca concentrations as described previously [10].

This treatment causes the plasma membranes of cells to become permeable to small molecules and ions while leaving the membranes of the intracellular organelles intact [10]. The advantage of such a permeable cell preparation is that the composition of the medium surrounding the intracellular organelles can be controlled in what is a semi-intact system.

2.3. Measurement of ^{45}Ca contents of saponin-treated, DMSO-differentiated HL-60 cells

Cells were incubated with 1 μCi/ml ^{45}Ca at a cell density of ~1 mg/ml cellular protein for the times indicated in individual experiments. Contents of ^{45}Ca were determined by diluting 100-μl aliquots of cell suspension in 10 ml ice-cold isotonic sucrose (310 mM) containing EGTA (4 mM) and tracer amounts (0.5 μCi/ml) of [^{3}H] mannose for determination of trapped volumes. The samples were rapidly filtered through GF/C filters and washed with 10 ml of ice-cold isotonic sucrose. The radioactivity on the filters was then determined by liquid scintillation spectrophotometry.

2.4. Preparation of inositol phosphates

(1,4,5)IP$_3$ and inositol 4,5-bisphosphate [I(4,5)P$_2$] were prepared from brain inositol fractions as described originally [11,12] with modifications as in [13].

2.4. Materials

Radioactive materials were obtained from New England Nuclear. All other compounds were obtained from Sigma.

3. RESULTS AND DISCUSSION

To examine the hypothesis that (1,4,5)IP$_3$ might be involved in the mobilisation of intracellular Ca in neutrophils, purified (1,4,5)IP$_3$ was applied to saponin-treated DMSO-differentiated HL-60 cells. In the experiments in fig.1 these permeable cells were incubated in the cytosolic-type medium
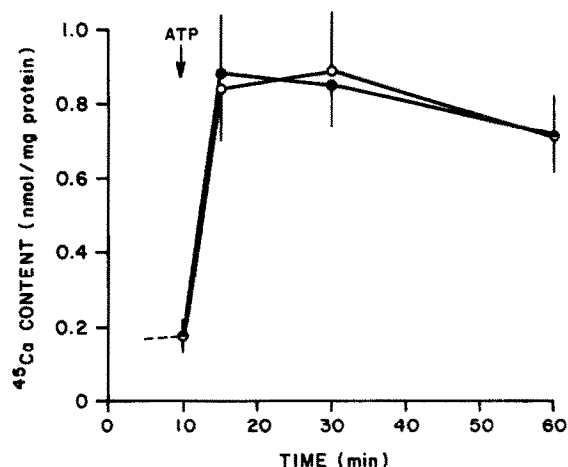

Fig.1. Uptake of ^{45}Ca by saponin-treated DMSO-differentiated HL-60 cells. At time zero ^{45}Ca was added to a suspension of cells incubated in the cytosolic-type medium described in section 2 containing antimycin, the ATP-regenerating system and with [Ca^{2+}] set at 180 nM. At 10 min ATP was added and there was a rapid uptake of ^{45}Ca by the cells. ●, control; ○, 0.5 mM DNP and 10 μM oligomycin present from time zero. The data were averaged and the bars indicate SE. $n = 3$.

described previously, with Ca^{2+} buffered to 180 nM, plus tracer amounts of [45]Ca. This $[Ca^{2+}]$ was chosen because it has been shown in neutrophils [5] and other tissues [10,14] that $[Ca^{2+}]_i$, in intact unstimulated cells, lies between 100 and 200 nM. On addition of ATP (1.5 mM) the permeable cells accumulated [45]Ca to a level of about 0.9 ± 0.16 nmol/mg protein ($n = 3$) from a basal pre-ATP value of about 0.2 nmol/mg protein. This uptake was not affected if the mitochondrial inhibitors 2,4-dinitrophenol (DNP) and oligomycin were included in the medium. This indicates that at this $[Ca^{2+}]$ the mitochondria of the permeable cells were unable to accumulate Ca and that the [45]Ca was probably being taken up into the ER. This is in agreement with results obtained in other permeable cell preparations where it has been found that only the ER can take up Ca when the $[Ca^{2+}]$ in the medium is set around 200 nM (i.e., resting $[Ca^{2+}]_i$), and where it has also been found that the mitochondria can accumulate Ca only when the medium $[Ca^{2+}]$ is greater than $1 \mu M$ [10,15,16].

The effect of $5 \mu M$ (1,4,5)IP$_3$, a concentration which is maximally effective in permeable hepatocytes [7,8,17] and other permeable cells [6,13], on the steady-state accumulation of [45]Ca in the permeable HL-60 cells, incubated as described in fig.1, is shown in fig.2. The (1,4,5)IP$_3$ induced a
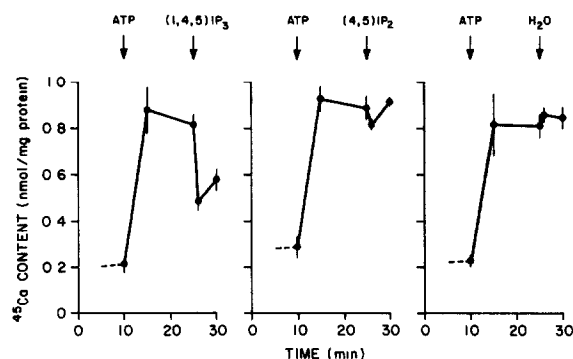
rapid efflux of about 0.3 nmol [45]Ca/mg protein, or about 50% of the [45]Ca taken up by the cells in response to ATP. The effect was maximal at 1 min, which was the first time point taken. Another inositol phosphate, (4,5)IP$_2$, was tested to determine whether there were any structural requirements for Ca-releasing ability. (4,5)IP$_2$, which was a much less potent Ca-releasing agonist in permeable hepatocytes [17] or Swiss 3T3 cells [13], did not cause any significant loss of [45]Ca from the permeable DMSO-differentiated HL-60 cells when applied at $5 \mu M$.

In the experiments shown in fig.3 the permeable cells were incubated as in fig.1,2, with 180 nM Ca^{2+} in the medium but in the absence of added ATP and the ATP regenerating system. The cells bound 0.36 ± 0.05 nmol [45]Ca/mg protein in an ATP-independent manner, and this was not affected by the addition of (1,4,5)IP$_3$ ($5 \mu M$). The experiment shown in fig.3 indicates that the (1,4,5)IP$_3$ did not cause the permeable cells to lose [45]Ca in a non-specific way by either binding or displacing the [45]Ca.

In conclusion, these results show that (1,4,5)IP$_3$ can release [45]Ca sequestered in an ATP-dependent

Fig.2. The effect of (1,4,5)IP$_3$ and (4,5)IP$_2$ on the [45]Ca content of saponin-treated DMSO-differentiated HL-60 cells. The cells were incubated as described in fig.1. When a steady-state had been achieved after addition of ATP, (1,4,5)IP$_3$ ($5 \mu M$), (4,5)IP$_2$ ($5 \mu M$) or an equivalent volume of H_2O were added. (1,4,5)IP$_3$ caused a rapid net efflux of [45]Ca from the cells whereas (4,5)IP$_2$ had no significant effect. Data were averaged and bars indicate SE. $n = 7$ for (1,4,5)IP$_3$ and H_2O and 3 for (4,5)IP$_2$.
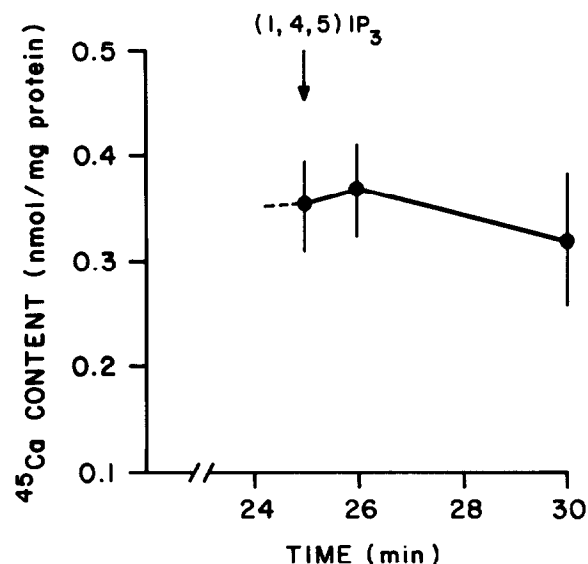
Fig.3. Effect of (1,4,5)IP$_3$ on the [45]Ca associated with the permeabilised cells in the absence of ATP. The cells were incubated as described in fig.1 but without the ATP regenerating system or any added ATP. (1,4,5)IP$_3$ ($5 \mu M$) was without effect. Data were averaged and bars indicate SE. $n = 3$.

manner by a non-mitochondrial pool likely to be a component of the ER of permeable DMSO-differentiated HL-60 cells. The mechanism by which it does so seems fairly specific as it will not release Ca bound to the cells in an ATP-independent manner and another inositol phosphate, $(4,5)IP_2$, does not release Ca when applied at the same concentration as $(1,4,5)IP_3$. This suggests that there is a receptor on which the $(1,4,5)IP_3$ acts, which may, for example, increase the permeability of the ER to Ca (see [17]). It has been demonstrated in intact DMSO-differentiated HL-60 cells that FMLP-induced break-down of $PI(4,5)P_2$, which is the source of the increased level of IP_3, is not mediated by the agonist-induced rise in $[Ca^{2+}]_i$ [2]. As it has also been shown that IP_3 is formed rapidly enough in these cells after stimulation with FMLP so that its formation might preceed FMLP-induced degranulation [2], and as intracellular Ca release is involved in the agonist-mediated rise in $[Ca^{2+}]_i$ in neutrophils [5], the findings in this study suggest that as in other tissues [6–9], $(1,4,5)IP_3$ is the messenger which signals agonist-induced Ca mobilisation in DMSO-differentiated HL-60 cells and by analogy in neutrophils.

ACKNOWLEDGEMENTS

REFERENCES

[1] Fontana, J.A., Wright, D.G., Schiffmann, E., Corcoran, B.A. and Deisseroth, A.B. (1980) Proc. Natl. Acad. Sci. USA 77, 3664–3668.

[2] Dougherty, R.W., Godfrey, P.P., Hoyle, P.C., Putney, J.W. jr and Freer, R.J. (1984) Biochem. J. 222, in press.

[3] Weissmann, G., Korchak, H.M., Perez, H.D., Smolen, E., Goldstein, I.M. and Hoffstein, S.T. (1979) in: Advances in Inflammation Research (Weissmann, G. et al. eds) vol. 1, pp. 95–112, Raven, New York.

[4] Petroski, R.J., Naccache, P.H., Becker, E.L. and Sha'afi, R.I. (1979) Am. J. Physiol. 237, C43–C49.

[5] 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.

[6] Streb, H., Irvine, R.F., Berridge, M.J. and Schulz, I. (1983) Nature (Lond.) 306, 67–69.

[7] Burgess, G.M., Godfrey, P.P., McKinney, J.S., Berridge, M.J., Irvine, R.F. and Putney, J.W. jr (1984) Nature 309, 63–66.

[8] Joseph, S.K., Thomas, A.P., Williams, R.J., Irvine, R.F. and Williamson, J.R. (1984) J. Biol. Chem. 259, 3077–3081.

[9] Dawson, A.P. and Irvine, R.F. (1984) Biochem. Biophys. Res. Commun. 120, 858–864.

[10] Burgess, G.M., McKinney, J.S., Fabiato, A., Leslie, B.A. and Putney, J.W. jr (1983) J. Biol. Chem. 258, 5716–5725.

[11] Grado, C. and Ballou, C.E. (1961) J. Biol. Chem. 236, 54–60.

[12] Tomlinson, R.V. and Ballou, C.E. (1961) J. Biol. Chem. 236, 1902–1906.

[13] Irvine, R.F., Brown, K.D. and Berridge, M.J. (1984) Biochem. J., in press.

[14] Tsien, R.Y., Pozzan, T. and Rink, T.J. (1982) J. Cell Biol. 94, 325–334.

[15] Blaustein, M.P., McGraw, C.F., Somlyo, A.V. and Schweitzer, E.S. (1980) J. Physiol. (Paris) 76, 459–470.

[16] Wakasugi, H., Kimura, T., Haase, W., Kribben, A., Kaufmann, B. and Schulz, I. (1982) J. Membrane Biol. 65, 205–220.

[17] Burgess, G.M., Irvine, R.F., Berridge, M.J., McKinney, J.S. and Putney, J.W. jr (1983) Biochem. J., in press.

[18] Prentki, M., Biden, T.J., Janjic, D., Irvine, R.F., Berridge, M.J. and Wollheim, C.B. (1984) Nature 309, 562–564.