Free Cytoplasmic Calcium Concentration and the Mitogenic Stimulation of Lymphocytes*

(RECEIVED FOR PUBLICATION, JUNE 23, 1982)

T. ROBIN HESKETH, GERRY A. SMITH, JOHN P. MOORE, MICHAEL V. TAYLOR, AND JAMES C. METCALFE

From the Department of Biochemistry, University of Cambridge, Cambridge, CB2 1QW, United Kingdom

The effects of the lectins concanavalin A, succinylated concanavalin A, and wheat germ agglutinin on the free cytoplasmic Ca$^{2+}$ concentration in mouse thymocytes were measured using the fluorescent Ca$^{2+}$ indicator "quin 2" (Tsien, R. Y. (1980) Biochemistry 19, 2396-2404) and compared with the metabolic and mitogenic effects of the lectins on the cells. Within 1 min of adding each ligand, there is a dose-dependent increase in the free cytoplasmic Ca$^{2+}$ concentration reported by quin 2. This response is selective for Ca$^{2+}$, but it does not coincide closely with the subsequent mitogenic stimulation at 48 h by concanavalin A or succinyl concanavalin A. The nonmitogenic lectin wheat germ agglutinin also causes an increase in free cytoplasmic Ca$^{2+}$ concentration and early metabolic stimulation of the cells, but stimulation is self-aborted before DNA synthesis occurs. At the intracellular concentrations of quin 2 required for measurement of the free Ca$^{2+}$ concentration, the chelator causes early metabolic stimulation of the cells very similar to that produced by concanavalin A and the mitogenic Ca$^{2+}$ ionophore A23187. Thus, phosphatidylinositol metabolism and lactate production are stimulated in mouse thymocytes and pig lymphocytes within 1 h of loading with quin 2 and significant increases in RNA synthesis occur after 8 h. Quin 2 causes mitogenic stimulation of pig lymphocytes measured as increased [3H]thyidine uptake at 48 h, that is variable but substantial in most experiments (up to 100% of the stimulation by A23187). The chelator itself has no significant activity as a Ca$^{2+}$ ionophore, but the apparent free Ca$^{2+}$ concentration in the cells increases both with the concentration of intracellular quin 2 and with the extracellular Ca$^{2+}$ concentration. These data leave open the possibility that quin 2 itself affects the concentration of free Ca$^{2+}$ or other cations in the cells.

Many studies have implicated Ca$^{2+}$ in the mitogenic stimulation of lymphocytes (1). It is well established that Ca$^{2+}$ must be present in the culture medium at above $10^{-4}$ M for maximal mitogenic stimulation of the cells (2) and the demonstration that the Ca$^{2+}$ ionophores A23187 (2, 3) and ionomycin (4) are potent mitogens provided suggestive evidence that Ca$^{2+}$ influxes into the cells might act as a primary mitogenic signal. Measurements of $^{45}$Ca$^{2+}$ fluxes have indicated that mitogenic lectins such as concanavalin A can cause substantial increases in $^{45}$Ca$^{2+}$ associated with the cells, but the magnitude and duration of the effect and its relevance to mitogenic stimulation are highly controversial (5-7). We have proposed as a working hypothesis that an increase in the free cytoplasmic Ca$^{2+}$ concentration ([Ca$^{2+}$]) within the range $10^{-7}$ to $10^{-5}$ M constitutes the primary mitogenic signal for the transition out of the resting state (Go) into the cell cycle and that this signal must persist for about 20 h to commit the maximum proportion of cells to DNA synthesis (1). If [Ca$^{2+}$] is lowered back into the Go range (for example by removing the mitogen from the cells) before commitment to DNA synthesis has occurred, then the cells will return to the resting (Go) state. If [Ca$^{2+}$] is raised above about $10^{-4}$ M, however, mitogenic stimulation is reversibly inhibited in the short term and toxic effects may occur if [Ca$^{2+}$] is maintained at high concentrations for long periods.

Recently, it has been demonstrated that new Ca$^{2+}$ chelators derived from EGTA1 can be introduced into a variety of cells, using the acetoxymethyl ester derivatives of the chelators (8). The nonpolar ester derivatives cross the plasma membrane and are hydrolyzed intracellular to the parent tetracarboxylic anions which are membrane-impermeant. One of the chelators developed by Tsien (9) was recently used as a fluorescent indicator to detect increases in [Ca$^{2+}$] in lymphocytes within 1 min of the addition of ConA or phytohemagglutinin (10). The increase in [Ca$^{2+}$], indicated by quin 2 from about $1.2 \times 10^{-7}$ M in control cells to about $2.4 \times 10^{-7}$ M after addition of mitogenic concentrations of the lectins is consistent with the "calcium hypothesis" for the primary mitogenic signal summarized above.

To assess further the relationship between the early [Ca$^{2+}$], changes and subsequent mitogenic stimulation, we have compared the two responses as a function of the concentration of mitogenic and nonmitogenic lectins. However, we found that intracellular quin 2 at the concentrations required to measure [Ca$^{2+}$], itself caused immediate metabolic and subsequent mitogenic stimulation of lymphocytes which were very similar to those produced by the mitogens ConA and A23187. These data imply that all of the measurements of [Ca$^{2+}$], using quin 2 are made in cells already stimulated by the presence of the chelator. We have therefore examined the effect of the concentration of intracellular quin 2 and of extracellular Ca$^{2+}$ on the indicated [Ca$^{2+}$], on the assumption that [Ca$^{2+}$] would remain constant if the cells were not perturbed by quin 2.

1 The abbreviations used are: EGTA, ethylene glycol bis(2-aminoethyl ether)-N,N,N',N'-tetracetic acid; [Ca$^{2+}$], free cytoplasmic calcium concentration; ConA, concanavalin A; AM, acetoxymethyl; PI, phosphatidylinositol; M$^{+}$, polyvalent cation; WGA, wheat germ agglutinin; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore hereby be marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2 Recipients of Medical Research Council Studentships.

§ Supported by grants from the Science Research Council.
**EXPERIMENTAL PROCEDURES**

**RESULTS**

($[Ca]$, in Thymocytes Loaded with quin 2)—The estimation of the per cent saturation of quin 2 by $Ca^{2+}$ is illustrated in Fig. 1. The cells were treated with 50 nM ionomycin which causes the intracellular quin 2 to be saturated with $Ca^{2+}$ from the medium (1.0 mM). The background signal from the cells (autofluorescence and scatter) was determined by adding 0.5 mM MnCl$_2$. The affinity of Mn$^{2+}$ for quin 2 is 10$^2$ greater than the affinity of Ca$^{2+}$ (Table IS in Miniprint) and therefore demonstrates that the apparent per cent saturation by Mn$^{2+}$ ions in the presence of ionomycin. The per cent saturation of quin 2 by Ca$^{2+}$ was calculated from the expression given in the legend to Fig. 1.

Within the range 0.6 to 4.8 mM internal quin 2, the per cent saturation is constant within experimental error and corresponds to a $[Ca]$, of 1.1 $\times 10^{-2}$ M (±0.5; n = 58) assuming a value of log $K_{ca}$ for quin 2 of 7.05 at 37 °C.$^3$ At lower quin 2 concentrations, however, the apparent per cent saturation by Ca$^{2+}$ decreases substantially (see Fig. 1S in Miniprint).

**Effect of ConA and Succinyl-ConA on $[Ca]$,—Tsien et al.** (10) reported that mitogenic concentrations of ConA and phytohemagglutinin cause a rise in $[Ca]$, in thymocytes of about 2-fold with a delay of approximately 30 s. This effect is shown for ConA in Fig. 2A. The increases in fluorescence induced by ConA were observed with internal quin 2 concentrations ranging from about 0.1 to 2.0 mM, with similar delays and time courses for the fluorescence increases at all quin 2 concentrations. Control experiments in which 0.1 mM Mn$^{2+}$ (or 0.01 mM Ce$^{3+}$) was added before ConA showed that the fluorescence increase is similar to that in the absence of quenching Mn$^{2+}$ ions (Fig. 2B). This indicates that the fluorescence increase is not due to a significant leakage of quin 2 out of the cells when ConA binds to the cell surface. Further control experiments confirmed that $[H]quin$ leakage caused by the addition of ConA accounts for only a minor part (<20%) of the fluorescence increase in response to the ligand. The observation that the quin 2 fluorescence increase in response to ConA is obtained in the presence of external Mn$^{2+}$ or Ce$^{3+}$ shows that the increase in intracellular cation concentration is highly selective for Ca$^{2+}$ compared with the fluorescence quenching ions (see Table IS in Miniprint).

The ion selectivity of the effect of ConA is consistent with an increase in $[Ca]$, as a specific response of thymocytes to the ligand, rather than a general increase in ion permeability of the plasma membrane as a result of the cross-linking of proteins by ConA. This prompts the question of whether the increase in $[Ca]$, reported by quin 2 is correlated with mitogenic stimulation by the lectin. In Fig. 3A, the increase in per cent saturation of quin 2 by Ca$^{2+}$ is shown as a function of the amount of ConA added. Serial additions of 0.05 μg/ml of ConA give progressive increases in fluorescence, with the maximal fluorescence increase remaining constant between 0.5 and 10 μg/ml of ConA. This profile can be compared with the mitogenic stimulation of the same cells by ConA (Fig. 3B), which shows (<10% of maximal stimulation below 0.3 μg/ml or above 2 μg/ml of ConA. The concentration of ConA required for maximal mitogenic stimulation therefore coincides quite closely with the lowest ConA concentration required to produce the maximum increase in quin 2 fluorescence. On the other hand, the, self-inhibition of mitogenic stimulation by supraoptimal concentrations of ConA cannot be attributed to an excessive early increase in $[Ca]$,.

**The results for ConA may be compared with the corresponding data for succinyl-ConA (Fig. 3, A and B). Succinyl-ConA causes maximal mitogenic stimulation over a wider range of concentrations than ConA and at 10 μg/ml caused a similar increase in $[Ca]$, to optimal ConA. It should be noted, however, that the dose-response curves for the increase in $[Ca]$, and mitogenic stimulation by succinyl-ConA do not coincide closely (compare A and B of Fig. 3). In marked contrast, the threshold concentrations of both ConA and succinyl-ConA for the stimulation of PI metabolism and mitogenic stimulation are closely correlated (compare B and C of Fig. 3).

**Effect of Wheat Germ Agglutinin—**The response to WGA is of interest in that it causes an increase in $[Ca]$, with a concentration dependence intermediate between ConA and succinyl-ConA, although it is not mitogenic (Fig. 3, A and B). WGA does not cause a further increase in quin 2 fluorescence after the maximum quin 2 response to ConA has been elicited and concentrations of WGA which cause maximal or submaximal increases in $[Ca]$, completely block the mitogenic action of optimal concentrations of ConA (Fig. 3B). Since WGA is

---

1 Portions of this paper (including "Experimental Procedures," part of "Results," Figs. 1S-3S, and Table IS) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-1674, cite the authors, and include a check or money order for $4.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

2 J. Rogers, unpublished data.
Fig. 2. The effect of Con A on [Ca], A, per cent Ca\(^2+\)–quin 2 in thymocytes (6 \times 10^6/ml) at 37 °C loaded with 1.0 mM [\(^3\)H]quin 2 as described under "Experimental Procedures." The calibration of \(\Delta F\) with 50 nM ionomycin is also shown. B, the per cent Ca\(^2+\)–quin 2 in a sample of the same thymocyte preparation as in A as a function of time after the addition of 1 mg/ml of Con A in the presence of 0.1 mM Mn\(^2+\). The immediate decrease in fluorescence after the addition of Mn\(^2+\) is due to extracellular quin 2; the subsequent slow decrease is due to a combination of Mn\(^2+\) permeation into the cells and leakage of Mn\(^2+\) from the cells (see Miniprint).

Fig. 3. Responses of thymocytes to lectins. A, per cent Ca\(^2+\)–quin 2 in thymocytes (6 \times 10^6/ml) at 37 °C loaded with 0.8 to 1.2 mM [\(^3\)H]quin 2 as a function of lectin concentration. O, ConA; □, succinyl-ConA; Δ, WGA. The fluorescence measurements were made 5 min not a mitogen, but causes an increase in [Ca], similar to the mitogenic lectins, its effects on PI metabolism were compared with those of the mitogens. It can be seen from Fig. 3C that WGA also stimulates PI metabolism with a dose-response curve which lies between those for ConA and succinyl-ConA.

Metabolic and Mitogenic Responses to Quin 2—In the course of the above experiments it was found that quin 2 itself caused early metabolic stimulation of mouse thymocytes and pig lymphocytes. In pig lymphocytes, these early responses ultimately lead to increased DNA synthesis at 48 h. Since the mechanism of mitogenic stimulation by quin 2 is of considerable interest, the progressive responses of the cells to quin 2 were characterized.

2-Phosphatidylinositol Metabolism—A well established early response in T cells to mitogens is an increase in the turnover of PI (see above), usually assayed as an increase in the incorporation of \(^32\)P into phospholipid and detectable within 10 min of the addition of mitogen (11). The effect on the incorporation of [\(^3\)H]inositol into PI of incubating mouse thymocytes and pig lymphocytes for 1 h with increasing concentrations of quin 2 AM ester is shown in Fig. 4. It can be seen that at concentrations above 0.1 \(\mu\)M quin 2 AM ester there is a substantial increase in [\(^3\)H]PI obtained from both cell types. It is clear that loading the cells with quin 2 using concentrations of the quin 2 AM ester in the range 0.1–1 \(\mu\)M causes increases in [\(^3\)H]PI similar to those observed with mitogenic concentrations of Con A and A23187 (compare with Fig. 4S in Miniprint).

Lactate Production—Lymphocytes respond to ConA and other polyvalent lectins at optimal mitogenic concentrations with an increase in lactate output which follows the time course of increased DNA synthesis (12). Fig. 5 shows the lactate output of mouse thymocytes and pig lymph node lymphocytes 1 h after the cells had been loaded with increasing concentrations of [\(^3\)H]quin 2. There is a large stimulation of lactate output from mouse thymocytes which is maximal at about 0.4 mM internal [\(^3\)H]quin 2. Thus substantial stimulation of lactate output occurs at internal quin 2 concentrations after each addition of lectin. B, mitogenic stimulation measured as [\(^3\)H]thymidine incorporation at 42 to 48 h after the addition of lectins (symbols as in A) as described under “Experimental Procedures.” In one experiment, WGA (O) (10 \(\mu\)g/ml) was added to optimal ConA (1.0 \(\mu\)g/ml). C, incorporation of [\(^3\)H]inositol into phosphatidylinositol in thymocytes measured 1 h after the addition of lectins (symbols as in A) as described under "Experimental Procedures." The incorporation is expressed relative to control thymocyte samples without lectins (100%). The increase in [\(^3\)H]inositol incorporation varied between 20 and 80% at 1 \(\mu\)g/ml of ConA in different cell preparations.
Cytoplasmic Calcium Concentration and Lymphocyte Stimulation

FIG. 4. Stimulation of PI metabolism by quin 2. Incorporation of [3H]inositol into phosphatidylinositol in mouse thymocytes (MT, ○) and pig lymphocytes (PL, △) measured 1 h after the addition of quin 2 AM ester, as described under “Experimental Procedures.” The incorporation is expressed relative to control cell samples without quin 2 AM ester.

FIG. 5. Stimulation of lactate production by quin 2. Lactate production in mouse thymocytes (MT, ○) and pig lymphocytes (PL, △) 1 h after loading with [3H]quin 2, as described under “Experimental Procedures.” Lactate production is expressed relative to control samples without [3H]quin 2 (100% = 9–12 fmol/cell/h for pig lymphocytes and 2–3 fmol/cell/h for mouse thymocytes).

which have no detectable effect on the ATP level (see Fig. 1S in Miniprint). However, it should be emphasized that although both quin 2 and other mitogens induce early changes in lactate production, many factors can influence glycolysis. For example, inhibition of mitochondrial ATP synthesis by quin 2 might stimulate glycolysis to cause the large increases in lactate production shown in Fig. 5 for thymocytes, and the decrease in lactate production above 0.4 mM quin 2 may result from the progressive loss in cell viability at high internal quin 2 concentrations, as evidenced by the decrease in cellular ATP levels (Fig. 1S in Miniprint). In contrast to the mouse thymocytes, there was only a small stimulation of lactate output from pig lymphocytes (up to 25%), but a significant inhibition of output to below the level of unloaded control cells occurred at above about 1 mM internal quin 2.

Stimulation of Uridine Uptake by quin 2—While the relationship between the early metabolic changes which occur within an hour of the addition of mitogens and the subsequent stimulation of DNA synthesis remains obscure (1), the prior stimulation of RNA synthesis is apparently essential for mitogenic stimulation. The specific inhibition of RNA synthesis by α-amanitin causes complete and reversible block of the subsequent stimulation of DNA synthesis by mitogens (13). Fig. 6 shows that quin 2 caused a large increase in uptake of [3H]uridine into pig lymphocytes measured at 18–19 h after loading the cells. Quin 2 caused small but significant increases in [3H]uridine uptake into both pig and mouse lymphocytes measured at 8 to 9 h. No effect was observed in mouse cells at the later time due to toxic effects of quin 2 on these cells.

Stimulation of Thymidine Uptake by quine 2—Mitogenic stimulation of pig lymphocytes measured as [3H]thymidine uptake 42 to 48 h after the addition of quin 2 AM ester is shown in Fig. 7. Significant stimulation was observed in most experiments ($n = 27$), varying from 15 to 100% of the mitogenic stimulation by A23187 of the same pig lymphocyte prepara-
tions in the absence of quin 2 (Fig. 7). Both A23187 and quin 2 AM ester show maximal mitogenic activity at about 0.1 μM.

**DISCUSSION**

**Correlation of [Ca], Changes with Mitogenic Stimulation—**Concentrations of ConA and succinyl-ConA which cause optimal mitogenic stimulation also cause the maximal increase in [Ca], as indicated by quin 2. The two responses do not, however, coincide closely for either ligand. Suboptimal concentrations of ConA caused detectable increases in [Ca], whereas no increase in [Ca], was observed at suboptimal succinyl-ConA concentrations which caused substantial mitogenic stimulation. In contrast, the dose-response curves for PI metabolism are closely correlated with mitogenic stimulation by ConA and succinyl-ConA in the suboptimal concentration range. Until it can be established whether quin 2 itself affects [Ca], and its response to this ligand, the correlation between changes in [Ca], and mitogenic stimulation remains uncertain. It is significant, however, that the increase in [Ca], in response to ConA is a specific effect and not attributable to a general increase in permeability to M" + ions.

At supramitogenic concentrations of ConA and succinyl-ConA, all of the early responses associated with mitogenic stimulation are observed (i.e. increases in [Ca], PI metabolism, and lactate production). The increase in [Ca], is the same at mitogenic and supramitogenic concentrations of ConA, suggesting that self-inhibition of mitogenic stimulation cannot be attributed to an excessive early increase in [Ca], although a subsequent slow increase in [Ca], at supramitogenic concentrations cannot be excluded.

We have suggested previously (14) that self-inhibition of mitogenic stimulation by supraoptimal ConA concentrations may result from premature removal of the ligand and its receptors from the cell surface by rapid cap formation. This terminates the primary mitogenic signal and the cells return to G0 before commitment to DNA synthesis can occur. We have shown here that WGA stimulates both early [Ca], increases and PI metabolism, but is not mitogenic. Since WGA has been reported previously to cap rapidly on lymphocytes (15), we compared the rates of cap formation by fluorescein-labeled WGA and ConA at the minimum concentration of each ligand which produced the maximum increase in [Ca], (10 μg/ml and 0.8 μg/ml, respectively). The WGA capped with a half-time of 30 min compared with 9 h for ConA. We have shown previously that supramitogenic concentrations of ConA (>10 μg/ml) cap with a half-time of less than 4 h and we therefore suggest that WGA is not mitogenic because it self-inhibits mitogenic stimulation by capping prematurely at concentrations which produce the required increase in [Ca], or other primary mitogenic signals.

**Metabolic and Mitogenic Stimulation by quin 2—Ambiguity in correlating [Ca], changes with mitogenic stimulation may arise because all of the changes in [Ca], in response to mitogens are measured in cells already stimulated metabolically by quin 2. The correlation of metabolic stimulation by intracellular chelators with their affinities for Ca2+ suggests that quin 2 may stimulate the cells by affecting the intracellular concentration of Ca2+ or other M+ ions (see Miniprint). The large Ca2+ fluxes across the plasma membrane of thymocytes, equivalent to 0.2 mm/cell/min (see Miniprint) imply that extracellular Ca2+ equilibrate rapidly with intracellular quin 2. Under these conditions, [Ca], is determined by the kinetic parameters of the Ca2+ fluxes across the plasma membrane and it is possible in principle to load the cells with increasing concentrations of quin 2 without affecting [Ca], at steady state. Thus, quin 2 would act purely as an indicator without affecting [Ca], However, the apparent fractional saturation of quin 2 by Ca2+ increased as the intracellular concentration of quin 2 was increased from 0.1 to 0.5 mM. This may indicate either that quin 2 causes an increase in [Ca], or that there are impermanent M+ ions in the cells which quench a significant proportion of the quin 2 fluorescence at low quin 2 concentrations. Either mechanism implies a perturbation of intracellular cation concentrations by quin 2. It is possible to distinguish these mechanisms from each other and from any systematic experimental errors in the use of quin 2 at low intracellular concentrations (see Miniprint) by using more sensitive analogues of quin 2.

Perturbation of [Ca], homeostasis by quin 2 may account for the increase in [Ca], indicated by quin 2 in response to increases in Ca2+ concentration in the medium (see Miniprint). The increase in [Ca], when the external Ca2+ concentration is raised from 0.1 to 1 mM is comparable to that produced by mitogenic concentrations of lectins. However, the same change in external Ca2+ concentration does not cause stimulation of PI metabolism, lactate production, or mitogenesis in cells without quin 2. The key question is whether significant [Ca], changes occur in cells without quin 2 in response to changes in external Ca2+ concentration or whether strict [Ca], homeostasis is maintained in unstimulated cells as would be expected if the primary mitogenic signal involves the small increase in [Ca], indicated by quin 2.

The mitogenic action of quin 2 on pig lymphocytes does not bear directly on the Ca2+ hypothesis, since any changes in free cytoplasmic Ca2+ concentration induced by quin 2 remain to be established using more sensitive indicators. The pattern of metabolic responses of the cells to quin 2 is very similar to that of well-characterized mitogens and the experiments described in the miniprint section show that it is the hydrolyzed form of quin 2 AM ester inside the cells which causes stimulation. The upper limit to the concentration of intracellular quin 2 required for optimal mitogenic stimulation of pig lymphocytes is about 0.1 mM, assuming that all of the quin 2 AM ester is taken up into the cells and hydrolyzed to quin 2. This concentration of intracellular quin 2 causes early metabolic concentrations of ConA and A23187.

We conclude that while the ionic specificity and pattern of responses of [Ca], indicated by quin 2 to response to mitogens are consistent with the increase in [Ca], at mitogenic and supramitogenic concentrations of ConA, as a primary mitogenic response, a critical assessment of this hypothesis requires a more sensitive indicator of [Ca], than quin 2 that does not perturb the resting cells.

**REFERENCES**

1. Metcalfe, J. C., Pozzan, T., Smith, G. A., and Henketh, T. R. (1980) Biochem. Soc. Symp. 46, 1-26
2. Maino, V. C., Green, N. M., and Crompton, M. J. (1974) Nature 251, 324-337
3. Löw, E., White, J. E., Smith, J. K., and Gomperts, R. (1975) Biochemistry 14, 247-255
4. Hesketh, T. R., Smith, G. A., Macdonald, M. D., and Warren, O. G., and Metcalfe, J. C. (1977) Nature 267, 490-494
5. Tsien, R. Y. (1981) Nature 290, 527-529
6. Hesketh, T. R., Smith, G. A., Macdonald, M. D., and Warren, O. G., and Metcalfe, J. C. (1980) Nature 285, 378-382
7. Maino, V. C., Hayman, M. J., and Metcalfe, J. C. (1981) Nature 295, 68-71
8. Tsien, R. Y., Pozzan, T., and Raina, T. J. (1982) Nature 305, 51-61
9. Maino, V. C., Hayman, M. J., and Crompton, M. J. (1979) Biochem. J. 148, 247-255
10. Hesketh, T. R., Smith, G. A., Macdonald, M. D., and Warren, O. G., and Metcalfe, J. C. (1980) Nature 285, 527-528
11. Hesketh, T. R., and Metcalfe, J. C. (1981) Exp. Cell Res. 134, 395-408
12. Lastik, S., and Planin, D. H. (1976) Nature 260, 149-150
13. Irving, H., and Frausto da Silva, D. D. (1962) J. Chem. Soc. 3338
14. Gunning, G. K., Wang, J. L., Yahara, I., Cunningham, B. A., and Edelmann, G. M. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 1012-1016
15. Beppu, T., Tono, T., and Osawa, T. (1979) J. Biochem. 85, 1275-1277
16. Pozzan, T., Corradi, A., Henketh, T. R., and Metcalfe, J. C. (1980) J. Biochem. 88, 592-594
17. Marshell, J. R. (1978) Nature 275, 660-661
18. Pozzan, T., Corradi, A. N., Henketh, T. R., and Metcalfe, J. C. (1981) Exp. Cell Res. 134, 395-408
19. Lastik, S., and Planin, D. H. (1976) Nature 260, 149-150
20. Beppu, T., Tono, T., and Osawa, T. (1979) J. Biochem. 85, 1275-1277
21. Reed, P. W., and Rosly, H. A. (1972) J. Biol. Chem. 247, 6970-6977
22. Liu, C.-M., and Harman, T. E. (1978) J. Biol. Chem. 253, 5892-5904

Downloaded from http://www.jbc.org/ by guest on March 21, 2020
Supplementary Material: FREE CYTOSOLIC CALCIUM CONCENTRATION AND THE MECHANISTIC STUDY OF LYMPHOCYTES

T. Rehn-Hernandez, Jerry A. Slemp, John P. Moore, Michael V. Taylor and James C. Nystrom

EXPERIMENTAL PROCEDURES

Preparation of Concanavalin A and PHA

The intracellular levels of quin 2-2 (3) and BAPTA (1,2,2,3-tetraazacyclodecane-1,2,2,3-tetraacetic acid) were prepared essentially as described by Jones et al.

The compounds were dissolved in water and the product dissolved in water (phosphate-buffered saline, PBS). The quin 2-2 solution was then used to treat the lymphocytes in a 1:1 dilution of the original stock solution. The BAPTA solution was also diluted in water and the product dissolved in water (phosphate-buffered saline, PBS). The quin 2-2 solution was then used to treat the lymphocytes in a 1:1 dilution of the original stock solution.

Preparation of Lymphocytes

The lymphocytes were isolated from the blood of normal healthy donors and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum. The lymphocytes were then used to perform the experiments.

Preparation of Con A and PHA

The Con A and PHA solutions were prepared according to the method of Jones et al. (1980). The Con A solution was prepared by dissolving the dry powder in PBS at a concentration of 100 μg/ml. The PHA solution was prepared by dissolving the dry powder in PBS at a concentration of 100 μg/ml.

RESULTS

Effect of Interleukin-1 on Intracellular Ca2+

The effect of interleukin-1 (IL-1) on intracellular calcium (Ca2+) levels was measured using a fluorescent calcium indicator, Fluo-3 AM, in Jurkat T cells. The IL-1 concentration was adjusted to 100 ng/ml. The results showed that IL-1 treatment caused a significant increase in intracellular Ca2+ levels, as determined by fluorescence microscopy.

Preparation of Lymphocytes

The lymphocytes were isolated from the blood of normal healthy donors and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum. The lymphocytes were then used to perform the experiments.

Preparation of Con A and PHA

The Con A and PHA solutions were prepared according to the method of Jones et al. (1980). The Con A solution was prepared by dissolving the dry powder in PBS at a concentration of 100 μg/ml. The PHA solution was prepared by dissolving the dry powder in PBS at a concentration of 100 μg/ml.

RESULTS

Effect of Interleukin-1 on Intracellular Ca2+

The effect of interleukin-1 (IL-1) on intracellular calcium (Ca2+) levels was measured using a fluorescent calcium indicator, Fluo-3 AM, in Jurkat T cells. The IL-1 concentration was adjusted to 100 ng/ml. The results showed that IL-1 treatment caused a significant increase in intracellular Ca2+ levels, as determined by fluorescence microscopy.
Cytoplasmic Calcium Concentration and Lymphocyte Stimulation

Effect of extracellular \(Ca^{2+}\) on (a).

The extracellular \(Ca^{2+}\) concentration causes rapid increases in \(Ca^{2+}\), indicated by point 1. The peak in the amount of \(Ca^{2+}\) bound to point 2 is due to a significant increase in the calcium content of the cells. The subsequent decrease in point 3 is due to a decrease in the calcium content of the cells. The peak in point 4 is due to a decrease in the calcium content of the cells. The peak in point 5 is due to a decrease in the calcium content of the cells. The peak in point 6 is due to a decrease in the calcium content of the cells. The peak in point 7 is due to a decrease in the calcium content of the cells. The peak in point 8 is due to a decrease in the calcium content of the cells. The peak in point 9 is due to a decrease in the calcium content of the cells. The peak in point 10 is due to a decrease in the calcium content of the cells. The peak in point 11 is due to a decrease in the calcium content of the cells. The peak in point 12 is due to a decrease in the calcium content of the cells. The peak in point 13 is due to a decrease in the calcium content of the cells. The peak in point 14 is due to a decrease in the calcium content of the cells. The peak in point 15 is due to a decrease in the calcium content of the cells. The peak in point 16 is due to a decrease in the calcium content of the cells. The peak in point 17 is due to a decrease in the calcium content of the cells. The peak in point 18 is due to a decrease in the calcium content of the cells. The peak in point 19 is due to a decrease in the calcium content of the cells. The peak in point 20 is due to a decrease in the calcium content of the cells. The peak in point 21 is due to a decrease in the calcium content of the cells. The peak in point 22 is due to a decrease in the calcium content of the cells. The peak in point 23 is due to a decrease in the calcium content of the cells. The peak in point 24 is due to a decrease in the calcium content of the cells. The peak in point 25 is due to a decrease in the calcium content of the cells. The peak in point 26 is due to a decrease in the calcium content of the cells. The peak in point 27 is due to a decrease in the calcium content of the cells. The peak in point 28 is due to a decrease in the calcium content of the cells. The peak in point 29 is due to a decrease in the calcium content of the cells. The peak in point 30 is due to a decrease in the calcium content of the cells. The peak in point 31 is due to a decrease in the calcium content of the cells. The peak in point 32 is due to a decrease in the calcium content of the cells. The peak in point 33 is due to a decrease in the calcium content of the cells. The peak in point 34 is due to a decrease in the calcium content of the cells. The peak in point 35 is due to a decrease in the calcium content of the cells. The peak in point 36 is due to a decrease in the calcium content of the cells. The peak in point 37 is due to a decrease in the calcium content of the cells. The peak in point 38 is due to a decrease in the calcium content of the cells. The peak in point 39 is due to a decrease in the calcium content of the cells. The peak in point 40 is due to a decrease in the calcium content of the cells. The peak in point 41 is due to a decrease in the calcium content of the cells. The peak in point 42 is due to a decrease in the calcium content of the cells. The peak in point 43 is due to a decrease in the calcium content of the cells. The peak in point 44 is due to a decrease in the calcium content of the cells. The peak in point 45 is due to a decrease in the calcium content of the cells. The peak in point 46 is due to a decrease in the calcium content of the cells. The peak in point 47 is due to a decrease in the calcium content of the cells. The peak in point 48 is due to a decrease in the calcium content of the cells. The peak in point 49 is due to a decrease in the calcium content of the cells. The peak in point 50 is due to a decrease in the calcium content of the cells. The peak in point 51 is due to a decrease in the calcium content of the cells. The peak in point 52 is due to a decrease in the calcium content of the cells. The peak in point 53 is due to a decrease in the calcium content of the cells. The peak in point 54 is due to a decrease in the calcium content of the cells. The peak in point 55 is due to a decrease in the calcium content of the cells. The peak in point 56 is due to a decrease in the calcium content of the cells. The peak in point 57 is due to a decrease in the calcium content of the cells. The peak in point 58 is due to a decrease in the calcium content of the cells. The peak in point 59 is due to a decrease in the calcium content of the cells. The peak in point 60 is due to a decrease in the calcium content of the cells. The peak in point 61 is due to a decrease in the calcium content of the cells. The peak in point 62 is due to a decrease in the calcium content of the cells. The peak in point 63 is due to a decrease in the calcium content of the cells. The peak in point 64 is due to a decrease in the calcium content of the cells. The peak in point 65 is due to a decrease in the calcium content of the cells. The peak in point 66 is due to a decrease in the calcium content of the cells. The peak in point 67 is due to a decrease in the calcium content of the cells. The peak in point 68 is due to a decrease in the calcium content of the cells. The peak in point 69 is due to a decrease in the calcium content of the cells. The peak in point 70 is due to a decrease in the calcium content of the cells. The peak in point 71 is due to a decrease in the calcium content of the cells. The peak in point 72 is due to a decrease in the calcium content of the cells. The peak in point 73 is due to a decrease in the calcium content of the cells. The peak in point 74 is due to a decrease in the calcium content of the cells. The peak in point 75 is due to a decrease in the calcium content of the cells. The peak in point 76 is due to a decrease in the calcium content of the cells. The peak in point 77 is due to a decrease in the calcium content of the cells. The peak in point 78 is due to a decrease in the calcium content of the cells. The peak in point 79 is due to a decrease in the calcium content of the cells. The peak in point 80 is due to a decrease in the calcium content of the cells. The peak in point 81 is due to a decrease in the calcium content of the cells. The peak in point 82 is due to a decrease in the calcium content of the cells. The peak in point 83 is due to a decrease in the calcium content of the cells. The peak in point 84 is due to a decrease in the calcium content of the cells. The peak in point 85 is due to a decrease in the calcium content of the cells. The peak in point 86 is due to a decrease in the calcium content of the cells. The peak in point 87 is due to a decrease in the calcium content of the cells. The peak in point 88 is due to a decrease in the calcium content of the cells. The peak in point 89 is due to a decrease in the calcium content of the cells. The peak in point 90 is due to a decrease in the calcium content of the cells. The peak in point 91 is due to a decrease in the calcium content of the cells. The peak in point 92 is due to a decrease in the calcium content of the cells. The peak in point 93 is due to a decrease in the calcium content of the cells. The peak in point 94 is due to a decrease in the calcium content of the cells. The peak in point 95 is due to a decrease in the calcium content of the cells. The peak in point 96 is due to a decrease in the calcium content of the cells. The peak in point 97 is due to a decrease in the calcium content of the cells. The peak in point 98 is due to a decrease in the calcium content of the cells. The peak in point 99 is due to a decrease in the calcium content of the cells. The peak in point 100 is due to a decrease in the calcium content of the cells.
Free cytoplasmic calcium concentration and the mitogenic stimulation of lymphocytes.
T R Hesketh, G A Smith, J P Moore, M V Taylor and J C Metcalfe
J. Biol. Chem. 1983, 258:4876-4882.

Access the most updated version of this article at http://www.jbc.org/content/258/8/4876

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/258/8/4876.full.html#ref-list-1