Clin Biochem, Vol. 23, pp. 159-166, 1990
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0009-9120/90 $3.00 + .00
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The Calcium Signal and Neutrophil Activation

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The cytosolic free calcium concentration, $[Ca^{2+}]_i$ in phagocytic cells (*e.g.* neutrophils, human leukemic cell line HL-60) is an important determinant of cellular activity. In resting phagocytes $[Ca^{2+}]_i$ is low (approximately 100 nM), but in response to occupation of cell surface receptors, it rises to micromolar levels, thereby activating a variety of cellular functions. The increases in $[Ca^{2+}]_i$ consist of two components: an immediate that is independent of extracellular Ca^{2+}, and a more delayed that is abolished by the removal of extracellular Ca^{2+}. These two components reflect the involvement of two subcellular structures in intracellular Ca^{2+} homeostasis: an intracellular Ca^{2+} store, referred to as the calciosome; and the plasma membrane. The function of the intracellular Ca^{2+}-store depends on a Ca^{2+}-pump, functionally and immunologically related to the cardiac sarcopiasmic reticulum Ca^{2+}-ATPase, a Ca^{2+}-storage protein, similar to muscle calsequestrin, and a Ca^{2+}-release channel, which is sensitive to inostol 1,4,5-trisphosphate. The Ca^{2+}-regulatory function of the plasma membrane depends on a Ca^{2+} pump, similar to the erythrocyte-type Ca^{2+}-ATPase, and a Ca^{2+} channel; the activity of the Ca^{2+} channel is closely coupled to phosphatidylinositol turnover.

KEY WORDS: neutrophil granulocytes; calcium homeostasis; calciosomes; calcium channels; calsequestrin; Ca^{2+}-ATPase; phosphatidylinositol.

Introduction

Neutrophils are mobile, phagocytic white blood cells that play an important role in the host defense against bacterial and fungal infections (1, 2). Differentiation of neutrophils from the stem cell in the bone marrow takes approximately 5-7 days, while the average circulation time of a mature neutrophil in the blood stream is only around 8 h. The mature neutrophil is a terminally differentiated cell, unable to divide, and virtually depleted of the protein synthesizing apparatus (endoplasmic reticulum, ribosomes). Neutrophils have various cell surface receptors with specialized functions, such as, chemotaxis, adherence and phagocytosis. Due to these receptors, neutrophils sense distant microorganisms, crawl towards them, adhere, engulf the microorganisms and kill them by the combined action of various microbicidal systems.

At least three different intracellular messenger systems are involved in these events: (a) An increase in $[Ca^{2+}]_i$ activates various neutrophil functions (3-5); (b) Protein kinase C probably has a dual regulatory role, stimulating and inhibiting neutrophil functions (6-8); (c) cyclic adenosine monophosphate, cAMP, is an intracellular inhibitor of neutrophil function (9-11). The involvement of other intracellular messenger systems is likely, but unproven.

In the present review, we will focus on the Ca^{2+}-messenger system in neutrophils. We will not address the question of how $[Ca^{2+}]_i$ regulates neutrophil functions, but rather how $[Ca^{2+}]_i$ is regulated during neutrophil activation. The studies discussed here are done in mature neutrophils or in the promyelocytic human leukemic cell line HL-60 that can be induced to differentiate to neutrophil-like cells. We will also refer to some relevant observations in other cell types.

Time course of Ca^{2+}-transients and dependence on extracellular Ca^{2+}: studies with fluorescent Ca^{2+}-sensitive dyes

The introduction of fluorescent indicators of $[Ca^{2+}]_i$, such as quin2 and fura2, led to the first analysis of Ca^{2+}-transients during neutrophil activation. Studies in populations of neutrophils (3) showed an average resting $[Ca^{2+}]_i$ of approximately 100 nM. In response to stimulation with f-met-leu-phe (a synthetic formyl-peptide that resembles products of bacterial metabolism), $[Ca^{2+}]_i$ increases to micromolar concentrations and returns to baseline within approximately 10-15 min. This $[Ca^{2+}]_i$ increase is partially dependent on extracellular Ca^{2+}, (*i.e.* the initial rate of the $[Ca^{2+}]_i$ increase is unchanged in a Ca^{2+}-free solution, while the prolonged period of the $[Ca^{2+}]_i$ increase is abolished under these conditions) (Figure 1) (3,12). These results lead to the conclusion that the $[Ca^{2+}]_i$ increase in response to chemoattractants consists of two components, an initial release of Ca^{2+} from an intracellular storage site and a more delayed influx

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Manuscript received March 31, 1989; revised August 8, 1989; accepted September 19, 1989.

Abbreviations: $[Ca^{2+}]_i$ = cytosolic free Ca^{2+} concentration; cAMP = cyclic adenosine monophosphate; Ins P = inositol phosphate.

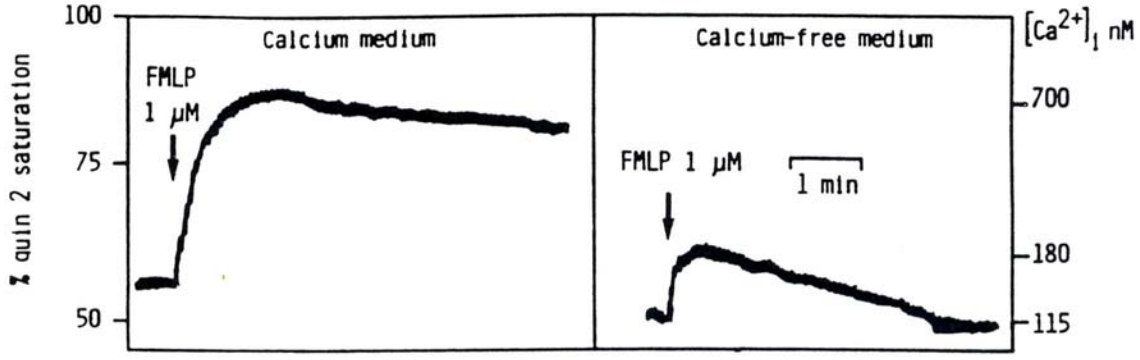

Figure 1 – Evidence for chemotactic peptide induced Ca^{2+}-release and C^{2+}-influx in neutrophils. Human neutrophils were loaded with quin 2 as described (3). Changes of the cytosolic free Ca^{2+} concentration, $[Ca^{2+}]_i$, during stimulation with the chemotactic peptide f-met-leu-phe (FMLP), were monitored in the presence Geft panel) or absence (right panel) of Ca^{2+} in the extracellular medium. The results indicate the existence of both, Ca^{2+}-influx and Ca^{2+}-release.

of Ca^{2+} across the plasma membrane. The Ca^{2+}-ionophore ionomycin can induce $[Ca^{2+}]_i$ increases in the absence of extracellular Ca^{2+}, as is seen for f-met-leu-phe stimulation (Figure 1), suggesting that the intracellular Ca^{2+}-storage site is a vesicular organelle (3).

Studies in single cells show that Ca^{2+}-homeostasis is more complicated than assumed in large populations of neutrophils. Spatial and temporal aspects of Ca^{2+} signaling become evident. During locomotion and phagocytosis, Ca^{2+} increases only in a restricted area of the cell (13). When neutrophils are stimulated with chemoattractants, low concentrations lead to oscillating $[Ca^{2+}]_i$ increases, while high concentrations lead to continuous $[Ca^{2+}]_i$ increases (14).

The mediation of Ca^{2+}-transients: studies on the production and action of inositol phosphates

Phosphatidylinositol turnover is closely coupled to the action of Ca^{2+}-mobilizing agonists in a variety of cell types (15). In neutrophils the following evidence argues for inositol 1,4,5-trisphosphate (Ins 1,4,5-P_3) as an intracellular mediator of Ca^{2+}-release: (a) Ins 1,4,5-P_3 is produced in response to Ca^{2+}-mobilizing messengers, such as f-met-leu-phe or Leukotriene B_4 (16,17); (b) Ins 1,4,5-P_3 generation does not depend on a previous increase in $[Ca^{2+}]_i$ (18); (c) Inhibition of receptor mediated Ins 1,4,5-Pa production by pertussis toxin abolishes Ca^{2+} increases (19); (d) Purified Ins 1,4,5-Pa induces Ca^{2+} release from permeabilized neutrophils (20). Ca^{2+} influx also seems to be closely coupled to phosphatidylinositol turnover, but the mechanism remains elusive (see below).

The intracellular pool of rapidly releasable Ca^{2+}: subcellular fractionation studies and unmuno electron microscopy

Clearly at least two subcellular structures play a crucial role in Ca^{2+} homeostasis in neutrophils: the plasma membrane and a vesicular, intracellular Ins 1,4,5-P_3-sensitive Ca^{2+} store. In other non-muscle cells, this intracellular store was thought to be the endoplasmic reticulum (15,21). However, this seems unlikely in neutrophils because they are virtually devoid of endoplasmic reticulum (22). Subcellular fractionation studies in neutrophils and HL-60 cells revealed that the Ca^{2+}-pumping, Ins 1,4,5-P_3-responsive organelle could be separated from endoplasmic reticulum, mitochondria, golgi apparatus and other known organelles (23,24). The Ca^{2+}-pumping activity and Ins 1,4,5-P_3-induced Ca^{2+}-release activity copurified with a 60 kDa Ca^{2+}-binding protein that showed biochemical similarities and immunological cross-reactivity with calsequestrin from muscle sarcoplasmic reticulum (24). Thus, neutrophils and HL-60 cells may possess a sarcoplasmic reticulum-like organelle, referred to as the calciosome. Morphological studies (24) showed the calsequestrin-like protein to be contained in 100–250 nm vesicles, distinct from previously described organelles. Similar results were obtained in other non-muscle cells, such as, liver and pancreatic cells. This observation raises the question of whether previous results, that identified the endoplasmic reticulum as the intracellular store of rapidly releasable Ca^{2+}, were due to contamination of endoplasmic reticulum-fractions by calciosomes. However, the evidence for the calsequestrin-like protein containing vesicles being the Ins 1,4,5-P_3-responsive organelle is based on the copurification of Ins 1,4,5-P_3 response and the calsequestrin-like protein in the subcellular fractions of HL-60 cells and, is thus, circumstantial.

Proposed model of Ca^{2+}-homeostasis in neutrophils

To regulate Ca^{2+} as described above, the neutrophil needs, in the simplest model, five proteins (Figure 2). Although a variety of direct and indirect experimental evidence supporting this scheme has been obtained (25,26) it is not yet established. In

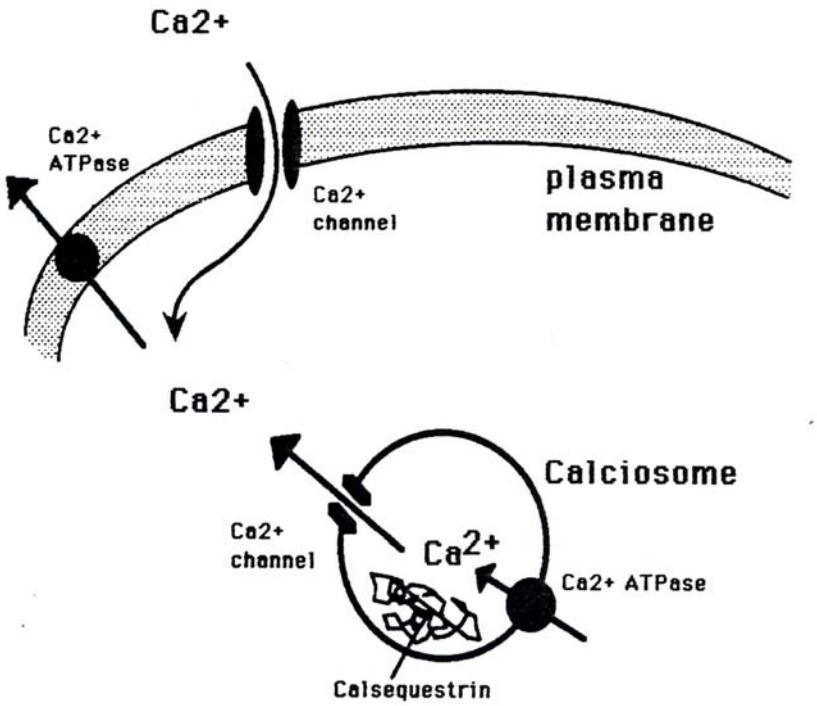

Figure 2 – Proposed model of Ca^{2+}-homeostasis in neutrophils. During cellular activation, an internal Ins 1,4,5-P3-sensitive Ca^{2+} channel and a plasma membrane Ca^{2+} channel are opened, leading to Ca^{2+} release -from the internal storage site and Ca^{2+} influx across the plasma membrane. To terminate the Ca^{2+} signal, the two Ca^{2+} channels close, a plasma membrane Ca^{2+}-ATPase pumps Ca^{2+} to the extracellular space and an internal Ca^{2+}-ATPase pumps Ca^{2+} into the internal Ca^{2+} store (the calciosome), where it is stored by a calsequestrin-like Ca^{2+}-binding protein.

particular, this model does not account for observations that suggest the existence of Ins 1,4,5-P3-insensitive Ca^{2+} pools (27-29). There may be intracellular organelles endowed with Ca^{2+}-ATPase and calsequestrin that do not posses an Ins 1,4,5-P3-sensitive Ca^{2+}-channel (26).

Ca^{2+}-regulatory proteins of the internal Ca^{2+} store

CA^{2+}-ATPASE

Functional studies in permeabilized neutrophils and in neutrophil homogenates (20,23) demonstrate that the Ca^{2+} pump of the intracellular Ca^{2+} store is a Ca^{2+}-ATPase, functionally similar to the Ca^{2+}-ATPase of the sarcoplasmic reticulum of muscle cells; (a) Ca^{2+}-uptake is ATP-dependent; (b) Ca^{2+}-uptake can be inhibited by millimolar, but not micromolar concentrations of vanadate; (c) Ca^{2+}-uptake is not inhibited by calmodulin-inhibitors. Immunological studies in HL-60 cells show a 105 kDa protein that crossreacts with monoclonal antibodies against slow-twitch/cardiac Ca^{2+}-ATPase, but not fast-twitch Ca^{2+}-ATPase (25). Tne subcellular distribution of this protein parallels the distribution of Ca^{2+} pumping and Ins 1,4,5-P3 response in subcellular fractions of HL-60 cells (25). It is likely that this protein is the Ca^{2+}-pump of the intracellular Ca^{2+}-store in HL-60 cells. This assumption is supported by recent studies demonstrating cDNA clones from kidney, brain and stomach that code for two alternatively spliced products of the cardiac Ca^{2+}-ATPase gene (30,31) and by studies in liver and pancreas showing a phosphorylated intermediate of a Ca^{2+}-ATPase with a molecular weight similar to the HL-60 protein (32,33). However, purification of the enzyme and functional studies will be necessary to more precisely define the role of this protein in HL-60 cells.

In liver cells, a 100 kDa protein that crossreacts with polyclonal antibodies against fast type skeletal muscle Ca^{2+}-ATPase has been described (34). Immunogold electron microscopy studies in liver and pancreas suggest that these antibodies recognize the same intracellular structure as anti-calsequestrin antibodies (35). These tissues might express a fast-twitch Ca^{2+}-ATPase, although cross-reaction of a slow-twitch Ca^{2+}-ATPase with polyclonal fast-twitch Ca^{2+}-ATPase antibodies might also account for these results.

CALCIUM-STORAGE PROTEIN

Calsequestrin from both skeletal and cardiac muscle sarcoplasmic reticulum has been shown to bind Ca^{2+} with high capacity (up to 45 moles Ca^{2+}/mole calsequestrin) and relatively low affinity with Kd

100 μM in the absence, and 1 mM in the presence, of 100 mM KCl, respectively (36). These Ca^{2+}-binding properties of calsequestrin are thought to be essential for the function of the sarcoplasmic reticulum. During muscle relaxation, the Ca^{2+}-release channel of the sarcoplasmic reticulum is closed and the Ca^{2+}-ATPase pumps Ca^{2+} from the cytosol into the lumen of the organelle. Under these conditions, calsequestrin will bind large amounts ofCa2"1" due to its high capacity. During muscle contraction, on the other hand, the Ca^{2+}-release channel opens and Ca^{2+} is released to the cytosol, facilitated by the low affinity of the Ca^{2+}-calsequestrin interaction. The primary structure of both skeletal muscle and cardiac calsequestrin has been deduced by cDNA cloning (37,38). Neither protein has the typical Ca^{2+}-binding structures which are seen with many cytosolic Ca^{2+}-binding proteins, such as, calmodulin. Both have a large proportion of acidic, negatively charged amino acids, which are thought to be responsible for the Ca^{2+}-binding. Surprisingly, the comparison of the primary structure of cardiac and skeletal muscle calsequestrin showed only 60% homology between the two proteins. The latter result has an important implication for our concept of calsequestrin. Rather than a single protein with homogeneous structure in all cell types, a family of proteins with similar function, but varied primary structure, seems likely.

The biochemical properties that justify a classification of a protein as a member of the family of calsequestrins (36) are: (a) High capacity, low affinity Ca^{2+}-binding; (b) Metachromatically blue staining with the carbocyanine dye Stains-all; (c) Change of apparent molecular mass with the pH; (d) High proportion of acidic negatively charged amino acids. Less commonly applied criteria are: good solubility in ammonium sulfate, pH- and Ca^{2+}-dependent precipitation, and decreased hydrophobicity at high Ca^{2+}-concentrations (39). Cross reactivity with antibodies against other calsequestrins are also suggestive of a protein being a calsequestrin, but not definitive.

The following properties have been demonstrated for the calsequestrin-like protein of HL-60 cells (24-26): Ca^{2+}-binding (however, capacity and affinity are unknown); metachromatical staining with Stains-all (Figure 3); Change of apparent molecular weight with pH; good solubility in ammonium sulfate; pH-dependent precipitation; crossreaction with antibodies against skeletal muscle calsequestrin. It is thus likely that the CS-like protein from HL-60 cells is related to calsequestrin; however, Ca^{2+}-binding properties, Ca^{2+}-dependent changes of hydrophobicity and primary structure remain to be studied.

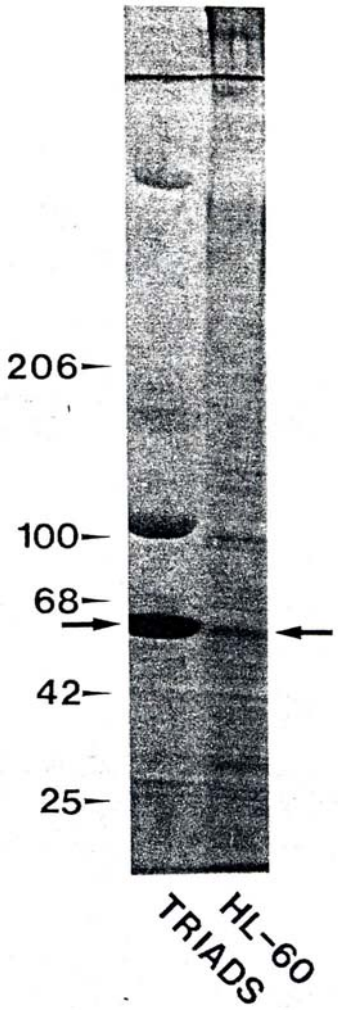

Figure 3 – Stains-all stained gel of skeletal muscle sarcoplasmic reticulum and HL-60 cells. A sarcoplasmic reticulum preparation of rabbit skeletal muscle (left lane) or whole HL-60 cells (right lane) were analyzed by SDS-polyacrylamide electrophoresis (3-12% gradient gels) and stained with the carbocyanine dye Stains-all. The arrow indicates the metachromatically blue staining muscle calsequestrin (left) or the metachromatically blue staining calsequestrin-like protein from HL-60 cells (right).

Ca^{2+} RELEASE CHANNEL

Ca^{2+} release from the internal Ca^{2+} store is mediated by Ins 1,4,5-P_3, which suggests that the Ca^{2+} release channel has binding site(s) for Ins 1,4,5-P_3. Alternatively, Ins 1,4,5-P_3 could bind to a different structure, which controls the activity state of the channel.

Studies in HL-60 cells show that the Ins 1,4,5-P_3-induced Ca^{2+} release, the calsequestrin-like protein and the Ca^{2+}-ATPase all copurify in subcellular fractions (25). This observation suggests, but does not prove, that all three proteins are located in the same subcellular structure. Recent studies indicate, that binding of tritiated Ins 1,4,5-P_3 parallels the Ins l,4,5-P_3-induced Ca^{2+} release in subcellular fractions (D. Pittet and D.P. Lew, unpublished).

An Ins l,4,5-P_3-binding protein has been purified from rat brain, raising the possibility that this protein is an Ins l,4,5-P_3-sensitive Ca^{2+} channel

(40). However no Ca^{2+} channel activity of this protein has been reported.

In contrast to the cross-reactivity of the calsequestrin-like protein and the Ca^{2+}-ATPase with corresponding muscle proteins, no HL-60 protein that crossreacts with antibodies against the Ca^{2+} release channel of muscle sarcoplasmic reticulum was found (K-H. Krause and K. P. Campbell, manuscript in preparation).

Ca^{2+}-regulatory proteins of the plasma membrane

CA^{2+}-ATPASE

Our present knowledge of the plasma membrane pump of neutrophils is based upon studies in inside-out plasma membrane vesicles (41,42). The enzyme seems to be, similar to the intracellular Ca^{2+}-pump, a Ca^{2+}-ATPase, requiring ATP-hydrolysis for activity. However, unlike the intracellular Ca^{2+}-ATPase, the plasma membrane Ca^{2+} pump shares several properties with the erythrocytes type Ca^{2+}-ATPase; (a) Ca^{2+}-uptake is inhibited by low concentrations of vanadate (30 mM); (b) Ca^{2+}-uptake is inhibited by calmodulin inhibitors; (c) addition of exogenous calmodulin increases Ca^{2+}-pumping. Recently a plasma membrane Ca^{2+}-ATPase from human teratoma cells has been cloned (43). Supporting the functional data in neutrophils and other cell types, a calmodulin binding site was demonstrated. The plasma membrane Ca^{2+} pump of neutrophils can be activated by phorbol esters (42), suggesting that this protein is, directly or indirectly, regulated by protein kinase C in addition to its Ca^{2+} /calmodulin regulation.

There is no evidence for the involvement of a Na^{+}/Ca^{2+}-exchanger in Ca^{2+} transport to the extracellular space in neutrophils (44).

PLASMA MEMBRANE CA^{2+} CHANNEL

There are no convincing direct electrophysiological measurements of Ca^{2+} influx (neither single channel nor whole cell recordings) in non-excitable cells, such as neutrophils. However, Ca^{2+} influx in response to receptor activation can clearly be demonstrated in non-excitable cells, both with fluorescent cytosolic Ca^{2+} indicators and by Ca^{2+} effects on Ca^{2+}-activated plasma membrane ion-channels (3, 12, 45–48). The molecular mechanism of the receptor-operated Ca^{2+}-influx in non-excitable tissues is not yet understood. However, it can be clearly distinguished from Ca^{2+} influx through voltage gated Ca^{2+} channels in excitable tissues: (a) It cannot be elicited by plasma membrane depolarization (12); (b) It is increased by hyperpolarization during receptor activation (49); (c) It is not inhibited by blockers of voltage gated Ca^{2+}-channels, such as 1,4-dihydropyridines (12).

A variety of mechanisms have been proposed for receptor-operated Ca^{2+}-influx (Figure 4). None of these hypotheses has been proven or excluded. Evidence in a variety of systems is suggestive of a second messenger-operated mechanism (50); however, in some systems, direct receptor coupled channel activity has been shown (51). The following three putative second messengers for Ca^{2+} influx have received most attention: Ca^{2+}; Ins 1,4,5-P_3; the combination of Ins 1,4,5-P_3 with Ins 1,3,4,5-P_4 (the product of phosphorylation of Ins 1,4,5-P_3 by a Ca^{2+}/calmodulin-dependent kinase).

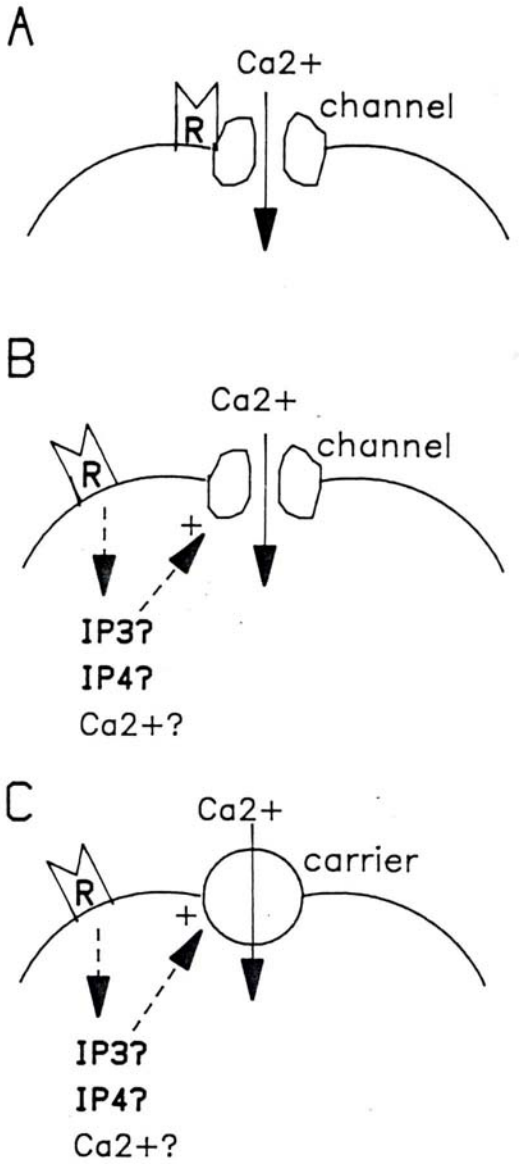

Figure 4–Possible mechanisms of receptor operated Ca^{2+}-influx. Ca^{2+} has been proposed to enter the cell from the extracellular space by A) a directly receptor coupled Ca^{2+}-channel; B) a second messenger-operated Ca^{2+}-channel; or, C) a second messenger operated Ca^{2+}-carrier. Ins l,4,5P_3, Ins 1,3,4,5-P_4 and Ca^{2+} have been proposed to be second messengers mediating Ca^{2+} influx.

We are working on three independent approaches to study this question:

1) Single channel recordings in neutrophils;

2) Correlation of time course of receptor-mediated Ca^{2+}-influx with time course of receptor-mediated second messenger generation;

3) Use of xenopus leavis oocytes as a model system, in which the putative second messengers can be injected into intact cells.

Concerning 1), in single channel experiments we were unable to identify an ionic channel that is a good candidate for the mediation of the above described Ca^{2+} influx. A Ca^{2+}-activated non-specific cation channel has been observed in human neutrophils (52) and has been proposed to be the receptor-operated Ca^{2+} channel. However, two points argue against the physiological importance of this channel: (a) Addition of extracellular Ca^{2+} to cells stimulated in Ca^{2+}-free medium leads to Ca^{2+}-influx, even if Ca^{2+}-release is already terminated and $[Ca^{2+}]_i$ has returned to baseline (12,53), arguing against Ca^{2+} -release as a cause of Ca^{2+}-influx, and (b) such a non-specific cation channel would allow sodium influx. Ca^{2+}-influx and depolarization would then be two inseparable events in neutrophil activation. This is clearly not the case as shown in neutrophils from patients with chronic granulomatous disease (54).

An Ins 1,4,5-P_3 activated non-specific channel has been described in T-lymphocytes and was proposed to be the receptor-operated Ca^{2+}-channel (55). Although the idea of an Ins 1,4,5-P_3 activated plasma membrane Ca^{2+}-channel would fit well with a variety of experimental results, the following points should be considered: (a) As discussed above, Ca^{2+} influx is — at least in neutrophils — more likely to occur through a specific Ca^{2+}-channel, than through a non-specific channel; (b) A channel with similar conductivity, selectivity and kinetic properties has been described in mast cells, but no Ins 1,4,5-P_3 activation of this channel could be found, and the activity state of this channel did not correlate with receptor mediated Ca^{2+} influx, as assessed with the Ca^{2+}-sensitive fluorescent dye fura 2 (45).

Given that the actual amount of Ca^{2+} influx during cellular activation is very low, the receptor-operated Ca^{2+} channel might, provided it is very selective for Ca^{2+}, have a very low conductivity and thus, might not be detected by classical patch clamp methods (45). Alternatively, the possibility of Ca^{2+} entering the cell by a carrier protein, rather than a channel should be considered.

Concerning 2), comparison of the time course of production of various inositol phosphates and of Ca^{2+} influx after stimulation of cells with f-met-leu-phe showed a good correlation of Ca^{2+}-influx with Ins 1,3,4,5-P_4, but not with any of the Ins P_3 isomers (47).

Concerning 3), in Xenopus laevis oocytes (48) microinjection of various Ins P_3 isomers, but not of Ins 1,3,4,5-P_4 or Ca^{2+}, induced Ca^{2+}-influx. Intriguingly, injection of the Ins 2,4,5-P_3 isomer, that cannot be phosphorylated to Ins P_4, produced an even longer lasting Ca^{2+}-influx than Ins 1,4,5-P_3.

The microinjection results in oocytes and the inositol phosphate time course in neutrophils seem contradictory. On the one hand, Xenopus laevis oocytes might have different types of second messenger-regulated Ca^{2+} channels; there are indications for different types of receptor-operated Ca^{2+} channels (56). On the other hand, the good temporal correlation between Ca^{2+} influx and Ins P_4 generation in neutrophils does not prove the causal relationship. Clearly, further studies will be necessary to elucidate the mechanism of Ca^{2+} influx and to identify the protein in the plasma membrane of neutrophils that mediates Ca^{2+} influx.

Acknowledgements

Karl-Heinz Krause is a recipient of a fellowship of the "Deutsche Forschungsgemeinschaft." The studies were supported by the following grants: NIH HL29851; NIH (PPG) HL14388; and Swiss National Foundation 3.839.0.87.

References

1. Klebanoff SJ, Clark RA. *The neutrophil: function and clinical disorders.* Amsterdam: North Holland, 1978.
2. Krause K-H, Lew PD. Bacterial toxins and phagocyte dysfunction. *Semin Hematol* 1988; **25:** 112-22.
3. Pozzan T, Lew PD, Wollheim CB, Tsien RY; Is cytosolic ionized calcium regulating neutrophil activation? *Science* 1983; **221:** 1413-5.
4. Lew PD, Wollheim CB, Waldvogel FA, Pozzan T. Modulation of cytosolic free calcium transients by changes in intracellular calcium buffering capacity: correlation with exocytosis and O_2-production in human neutrophils. *J Cell Biol* 1984; **99:** 1212-20.
5. Lew PD, Monod A, Waldvogel FA, Dewald B, Baggiolini M, Pozzan T. Quantitative analysis of the cytosolic free calcium dependency of exocytosis from three subcellular compartments in intact human neutrophils. *J Cell Biol* 1986; **102:** 2197-204.
6. Di Virgilio F, Lew PD, Pozzan T. Protein kinase C activation of physiological processes in human neutrophils at vanishingly small cytosolic Ca^{2+}-levels. *Nature* 1984; **310:**691-3.
7. Andersson T, Dahlgren C, Lew PD, Stendahl O. Cell surface expression of f-met-leu-phe receptors in human neutrophils. Correlation to changes in the cytosolic free Ca^{2+}-level and action of phorbol myristate acetate. *J Clin Invest* 1987; **79:** 1226-33.
8. Smith CD, Uhing RJ, Snyderman R. Nucleotide regulatory protein-mediated activation of phospholipase C in human polymorphonuclear leukocytes is disrupted by phorbol esters. *J Biol Chem* 1987; **262:** 6121-7.
9. Ignarro LJ, Paddock RJ, George WJ. Hormonal control of neutrophil lysosomal enzyme release: Effect of epinephrine on adenosine 3,5-monophosphate. *Science* 1974; **183:** 855-7.
10. Rivkin I, Rosenblatt J, Becker EL. The role of cyclic

AMP in the chemotactic responsiveness and spontaneous mobility of rabbit peritoneal neutrophils. *J Immunol* 1975; **115:** 1126-32.
11. De Togni P, Cabrini G, Di Virgilio F. Cyclic AMP inhibition of f-met-leu-phe-dependent metabolic responses in human neutrophils is not due to its effects on cytosolic Ca^{2+}. *Biochem J* 1984; **224:** 629-35.
12. Andersson T, Dahlgren C, Pozzan T, Stendahl 0, Lew PD. Characterization of f-met-leu-phe receptor mediated Ca^{2+} influx across the plasma membrane of human neutrophils. *Mol Pharmacol* 1986; **30:** 437-43.
13. Sawyer DY, Sullivan JA, Mandell GL. Intracellular free calcium localization in neutrophils during phagocytosis. *Science* 1985; **230:** 663-6.
14. Jaconi MEE, Rivest RW, Schlegel W, Wollheim CB, Pittet D, Lew PD. Spontaneous and chemoattractant-induced oscillations of cytosolic free calcium in single adherent human neutrophils. *J Biol Chem* 1988; **263:** 10557-60.
15. Berridge MJ. Inositol trisphosphate and diacylglycerol: two interacting second messengers. *Annu Rev Biochem* 1987; **56:** 159-93.
16. Bradford PG, Rubin RP. Characterization of f-met-leu-phe stimulation of inositol trisphosphate accumulation in rabbit neutrophils. *Mol Pharmacol* 1985; **27:** 74—8.
17. Andersson T, Schlegel W, Monod A, Krause K-H, Stendahl O, Lew PD. Leukotriene B_4 stimulation of phagocytes results in the formation of inositol 1,4,5-trisphosphate: a second messenger for Ca^{2+} mobilization. *Biochem J* 1986; **240:** 333-40.
18. Lew PD, Monod A, Krause K-H, Waldvogel FA, Biden TJ, Schlegel W. The role of cytosolic free calcium in the generation of inositol. 1,4,5-trisphosphate and inositol 1,3,4-trisphosphate in HL-60 cells: differential effects of chemotactic peptide receptor stimulation at distinct Ca^{2+} levels. *J Biol Chem* 1986; **261:** 13121-8.
19. Krause K-H, Schlegel W, Wollheim CB, Andersson T, Waldvogel FA, Lew PD. Chemotactic peptide activation of human neutrophils and HL-60 cells: pertussis toxin reveals the correlation between inositol trisphosphate generation, calcium ion transients and cellular activation. *J Clin Invest* 1985; **76:** 1348-54.
20. Prentki M, Wollheim CB, Lew PD. Ca^{2+} homeostasis in permeabilized human neutrophils: characterization of Ca^{2+} sequestering pools and the action of inositol 1,4,5-trisphosphate. *J Biol Chem* 1984; **259:** 13777-82.
21. Carafoli E. Intracellular calcium homeostasis. *Annu Rev Biochem* 1987; **56:** 395-135.
22. Binton DF, Ullyot JL, Farquhar MG. The development of neutrophilic polymorphonuclear leukocytes in human bone marrow. *J Exp Med* 1971; **134:** 907-34.
23. Krause K-H, Lew PD. Subcellular distribution of calcium pumping sites in human neutrophils. *J Clin Invest* 1987; **80:** 107-16.
24. Voipe P, Krause K-H, Hashimoto S *et al.* Calciosome, a cytoplasmic organelle: the inositol 1,4,5-trisphosphate-sensitive Ca^{2+} store of non-muscle cells? *Proc Natl Acad Sci USA* 1988; **85:** 1091-5.
25. Krause K-H, Pittet D, Voipe P, Pozzan T, Meldolesi J, Lew PD. Calciosome, a sarcoplasmic-reticulum-like organelle involved in intracellular Ca^{2+}-handling by non-muscle cells: studies in human neutrophils and HL-60 cells. *Cell Calcium* 1989; **10:** 351-61.
26. Pozzan T, Voipe P, Zorzato F, *et al.* The Ins(1,4,5)P_3-sensitive Ca^{2+} store of non-muscle cells: endoplasmic reticulum or calciosomes? *J Exp Biol* 1988; **139:** 181-93.
27. Berridge MJ, Irvine RF. Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* 1984; **312:** 315-21.
28. Mullaney JM, Yu M, Ghosh TK, Gill DL. A GTP-regulatory mechanism mediates Ca^{2+}-entry into the inositol 1,4,5-trisphosphate-releasable calcium pool. *Proc Natl Acad Sci USA* 1988; **85:** 2499-503.
29. Lipscombe D, Madison DV, Poenie M, Reuter H, Tsien RY, Tsien RW. Spatial distribution of calcium channels and cytosolic calcium transients in growth cones and cell bodies of sympathetic neurons. *Proc Natl Acad Sci USA* 1988; **85:** 2398-402.
30. Lytton J, MacLennan DH. Molecular cloning of cDNAs from human kidney coding for two alternatively spliced products of the cardiac Ca^{2+}-ATPase gene. *J Biol Chem* 1988; **263:** 15024-31.
31. Gunteski-Hamblin AM, Greeb J, Shull GE. A novel Ca^{2+}-pump expressed in brain, kidney, and stomach is encoded by an alternative transcript of the slow-twitch muscle sarcoplasmic reticulum Ca^{2+}-ATPase gene. *J Biol Chem* 1988; **263:** 15032-40.
32. Spamer C, Heilmann C, Gerok W. Ca^{2+}-activated ATPase in microsomes from human liver. *J Biol Chem* 1987; **262:**7782-9.
33. Kemmer TP, Bayerdorfer E, Will H, Schuiz I. Anion dependence of Ca^{2+}-transport and (Ca^{2+}/K^{+}) stimulated Mg^{2+}-dependent transport ATPase in rat pancreatic endoplasmic reticulum. *J Biol Chem* 1987; **262:** 13758-64.
34. Damiani E, Spamer C, Heilman C, Salvatori S, Margeth A. Endoplasmic reticulum of rat liver contains two proteins closely related to skeletal sarcoplasmic reticulum Ca^{2+}-ATPase and calsequestrin. *J Biol Chem* 1988; **263:** 340-3.
35. Hashimoto S, Bruno B,"Lew DP, Pozzan T, Voipe P, Meldolesi J. Immunocytochemistry of calciosomes-in liver and pancreas. *J Cell Biol* 1988; **107:** 2523-31.
36. MacLennan DH, Campbell KP, Reithmeier RAF. Calsequestrin. In: Cheung WY, ed. *Calcium cell function Vol. 4.* Pp. 151-73. New York, Academic Press, 1983.
37. Fliegel L, Ohnishi M, Carpenter MR, Khanna VK, Reithmeier RAF, MacLennan DH. Amino acid sequence of rabbit fast-twitch skeletal muscle calsequestrin deduced from cDNA and peptide sequencing. *Proc Natl Acad Sci USA* 1987; **84:** 1167-71.
38. Scott BT, Simmerman HKB, Collins JH, Nadal-Ginard B, Jones LR. Complete amino acid sequence of canine cardiac calsequestrin deduced by cDNA cloning. *J Biol Chem* 1988; **263:** 8958-64.
39. Cala SE, Jones LR. Rapid purification of calsequestrin from cardiac and skeletal muscle sarcoplasmic reticulum vesicles by Ca^{2+}-dependent elution from phenyl sepharose. *J Biol Chem* 1983; **258:** 11932-6.
40. Supattapone S, Worley PF, Baraban JM, Snyder SH. Solubilization, purification and characterization of an inositol trisphosphate receptor. *J Biol Chem* 1988; **263:** 1530-4.
41. Lagast H, Lew PD, Waldvogel FA. ATP-dependent Ca^{2+} pump in the plasma membrane of guinea pig and human neutrophils. *J Clin Invest* 1984; **73:** 107-15.

42. Lagast H, Pozzan T, Waldvogel FA, Lew PD. Phorbol myristate acetate stimulates the ATP-dependent calcium transport by the plasma membrane of neutrophils. *J Clin Invest* 1984; **73:** 878-83.
43. Verma AK, Filoteo AG, Stanford CR *et al.* Complete primary structure of a human plasma membrane Ca^{2+}-pump. *J Biol Chem* 1988; **263:** 14152-9.
44. Nasmith PE, Grinstein S. Phorbol ester induced changes in cytoplasmic Ca^{2+} in human neutrophils. Involvement of a pertussis toxin sensitive G-protein. *J Biol Chem* 1987; **262:** 13558-66.
45. Penner R, Matthews G, Neher E. Regulation of calcium influx by second messengers in rat mast cells. *Nature* 1988; **334:** 499-504.
46. Morris AP, Gallacher DV, Irvine RF, Peterson OH. Synergism ofinositol trisphosphate and tetrakisphosphate in activating Ca^{2+}-dependent K^channels. *Nature* 1987; **330:** 653-5.
47. Pittet D, Lew DP, Mayr GW, Monod A, Schlegel W. Chemoattractant receptor promotion of Ca^{2+} influx across the plasma membrane of HL-60 cells. *J Biol Chem* 1989; **264:** 7251-61.
48. Snyder PM, Krause K-H, Welsh MJ. Inositol trisphosphate isomers, but not inositol 1,3,4,5-tetrakisphosphate, induce calcium influx in xenopus laevis oocytes. *J Biol Chem* 1988; **263:** 11048-51.
49. Di Virgilio F, Lew PD, Andersson T, Pozzan T. Plasma membrane potential modulates chemotactic peptide-stimulated cytosolic free Ca^{2+}-changes in human neutrophils. *J Biol Chem* 1987; **262:** 4574-9.
50. Pozzan T, Meldolesi J. Receptor operated Ca^{2+}-channels. *J Exp Cell Res* 1983; **171:** 271-83.
51. Young JDE, Unkeless JC, Young TM, Mauro A, Cohn ZA. Role for mouse macrophage IgG Fc receptor as ligand-dependent ion channel. *Nature* 1983; **306:**186-9.
52. Von Tscharmer V, Prod'hom B, Baggiolini M, Reuter H. Ion channel in human neutrophils activated by rise in free cytosolic calcium concentration. *Nature* 1986; **324:** 369-72.
53. Nasmith PE, Grinstein S. Are Ca^{2+}-channels in neutrophils activated by a rise in cytosolic free Ca^{2+}? *FEBS Lett* 1987; **221:** 95-100.
54. Lew PD, Wollheim CB, Seger RA, Pozzan T. Cytosolic free calcium changes induced by chemotactic peptide in neutrophils from patients with chronic granulomatous disease. *Blood* 1983; **63:** 231-3.
55. Kuno M, Gardener P. Ion-channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T-lymphocytes. *Nature* 1987; **326:** 301-4.
56. Merrit JE, Hallam TJ. Platelets and parotid acinar cells have different mechanisms for agonist-stimulated divalent cation entry. *J Biol Chem* 1988; **263:** 6161-4.