Mechanisms of IL-8-induced Ca^{2+} signaling in human neutrophil granulocytes

Walter Schorr, Dieter Swandulla and Hanns Ulrich Zeilhofer

Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Universität Erlangen-Nürnberg, Erlangen, Germany

Interleukin-8 (IL-8) plays an important role in the activation of neutrophil granulocytes. Although intracellular Ca^{2+} signals are essential in this process, they have not been studied in great detail so far. Here, we have measured IL-8-induced Ca^{2+} signals in single human neutrophil granulocytes using the Ca^{2+} indicator dye FURA-2 AM and we have investigated the signal transduction that leads to these Ca^{2+} signals with various pharmacological tools. Our results indicate that IL-8-induced Ca^{2+} signals consist of at least two components. An initial fast component was followed by a smaller and more persistent one. The initial Ca^{2+} signal was independent of extracellular Ca^{2+}. It required the activation of phospholipase C via a pertussis toxin sensitive G-protein and was due to activation of IP_3 receptor-coupled Ca^{2+} release channels. The late phase of the Ca^{2+} signal was suppressed when extracellular Ca^{2+} was removed suggesting that it was generated by Ca^{2+} influx through Ca^{2+} release-activated Ca^{2+} (CRAC) channels. This Ca^{2+} influx may prolong IL-8-induced Ca^{2+} signals during granulocyte activation.

Key words: Chemokine / Neutrophil / Ca^{2+} current / Second messenger / Ca^{2+} signaling

Received	12/11/98
Revised	15/12/98
Accepted	15/12/98

1 Introduction

The recruitment of leukocytes at a site of inflammation is a multistep process including tethering and rolling, activation, firm adhesion and (trans-)migration of granulocytes [1, 2]. The initial tethering and the “rolling” along the surface of activated endothelial cells of the blood vessel wall requires a transient binding of leukocytes to the endothelium, which is mediated by the selectin family of adhesion molecules [3–5]. Subsequent events including firm adhesion, shape change, migration through the tissue to a site of inflammation and respiratory burst are at least in part mediated by chemokines like IL-8 [6, 7] released from cells of the immune system (*e.g.* macrophages) and auxilliary cells (*e.g.* endothelial cells) [7, 8, 9].

IL-8 binds to membrane receptors of the CXC chemokine receptor family which possess seven transmembrane domains [10]. These receptors couple to pertussis toxin-sensitive G_i proteins. One of the early intracellular events following activation of IL-8 receptors is the release of Ca^{2+} from intracellular stores. The relevance of these Ca^{2+} signals for the different activation steps is still controversial. Although many activation processes like the initial spreading of granulocytes on a substrate appear to be correlated with the occurrence of intracellular Ca^{2+} signals [11], buffering of intracellular Ca^{2+} to very low levels with BAPTA-AM had little effect on these events [12]. One process that is meanwhile generally believed to require increases in $[Ca^{2+}]_i$ is the detachment of pseudopods during directed migration, which involves the activation of the calcium-dependent phosphatase calcineurin by Ca^{2+} transients [13, 14].

Although the consequences of these Ca^{2+} signals have already attracted much attention, the signal transduction that leads to IL-8-induced Ca^{2+} signals has not been studied in great detail so far. Here, we have investigated IL-8-induced Ca^{2+} signaling in FURA-2 AM-loaded single human neutrophil granulocytes using digital ratiometric Ca^{2+} measurements and various pharmacological tools.

[I 18964]

0014-2980/99/0303-897$17.50+.50/0

2 Results and discussion

2.1 IL-8-induced Ca^{2+} signals in individual human granulocytes

Most of the experiments on Ca^{2+} signals in blood cells have been performed with cells in suspension, a situation which resembles the physiological situation through most of a blood cell's life span. However, in the case of granulocytes and also of many other blood cells, *e.g.* lymphocytes, one of the initial events initiating the migratory process to an inflammatory site is the binding to adhesion molecules presented on the membrane of activated endothelial cells [1, 2]. To mimic this situation we have used cells adherent to the plastic surface of standard culture dishes or to culture dishes coated with E-selectin × Ig chimera. This method together with the use of digital Ca^{2+} imaging allowed us to investigate the time course of the Ca^{2+} signals in individual acutely isolated granulocytes.

When human recombinant IL-8 (15-μl microdrops of 0.625 μM IL-8 added to a total culture dish volume of 1.5 ml) was applied increases in the intracellular concentration of free Ca^{2+} ($[Ca^{2+}]_i$) were reliably elicited. Fig. 1 shows an experiment where Ca^{2+} signals were recorded in up to 100 cells simultaneously. Most neutrophils were attached to the culture dish and were round and phase contrast-positive before application of IL-8 (Fig. 1A). A fluorescence image excited at the Ca^{2+}-insensitive wavelength of 360 nm in the same field shows that most granulocytes were rather homogeneously loaded with FURA-2 (Fig. 1B). Under these conditions IL-8 increased the fluorescence ratio (F350/F380), which was used as a measure of $[Ca^{2+}]_i$ in more than 90 % of the cells tested from a value of 0.51 ± 0.013 to 1.30 ± 0.058, under resting conditions and at the peak of the response, respectively (Fig. 1C–E). These values roughly correspond to $[Ca^{2+}]_i$ of 160–200 nM and 1.0 μM, respectively. This initial steep rise in $[Ca^{2+}]_i$ was followed by a smaller persistent elevation of $[Ca^{2+}]_i$.

2.2 Effect of thapsigargine and carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP) on IL-8-induced Ca^{2+} signals

Initial Ca^{2+} signals of similar amplitudes were observed in nominally Ca^{2+}-free medium (0 mM Ca^{2+}, 4 mM EGTA), indicating that IL-8-induced Ca^{2+} rises were primarily due to release of Ca^{2+} from intracellular stores and that extracellular Ca^{2+} was not required for receptor activation (Fig. 2A). Since Ca^{2+} can be released from different Ca^{2+} storing organelles, *e.g.* the endoplasmic reticulum and the mitochondria [15, 16], which are both present in neutrophils, we have tested the effect of thapsigargine, an inhibitor of sarcoplasmic reticulum Ca^{2+} (SERCA) pumps [17], and of the protonophore FCCP, which causes depletion of mitochondrial Ca^{2+} [18, 19]. When applied extracellularly in Ca^{2+}-free extracellular solution containing 4 mM EGTA, thapsigargine (2 μM) evoked a slow increase in $[Ca^{2+}]_i$ to not more than 200 nM lasting for several minutes. After a 15-min exposure to thapsigargine neutrophils were unable to respond to IL-8 with a Ca^{2+} signal (Fig. 2B). In contrast, preincubation with FCCP (5 μM, for 20 min) had no effect on IL-8-evoked increases in $[Ca^{2+}]_i$ (Fig. 2C). These observations suggest that IL-8-induced Ca^{2+} signals were mainly due to Ca^{2+} release from the endoplasmic reticulum and that mitochondria did not significantly contribute to this release. Fig. 2D summarizes these results.

2.3 Identification of the Ca^{2+} release channel involved in IL-8-induced Ca^{2+} signals

Ca^{2+} ions can be released from the endoplasmic or sarcoplasmic reticulum via different Ca^{2+} release channels [15, 16, 20]. Ca^{2+} release from the endoplasmic reticulum of electrically non-excitable cells is thought to be predominantly mediated by inositol trisphosphate (IP_3) receptor-coupled Ca^{2+} release channels.

IP_3 receptors can be blocked by intracellular heparin [21, 22]. However, heparin does not penetrate the cell membrane and could therefore not be used in our experiments. We have instead used two other blockers of IP_3 receptors. Caffeine has long been known as an inhibitor of IP_3-dependent Ca^{2+} release [23, 24] and very recently arachidonic acid has been identified as an inhibitor of IP_3 receptor-coupled Ca^{2+} release channels in lipid bilayers [25]. Application of caffeine (20 mM) for 30 min had no effect on resting $[Ca^{2+}]_i$, but completely blocked IL-8-induced rises in $[Ca^{2+}]_i$ in a reversible manner (Fig. 3A). IL-8-induced Ca^{2+} signals could also be blocked reversibly and in a concentration-dependent manner with arachidonic acid (25–100 μM) (Fig. 3B). Both findings indicate that IP_3 receptor activation is necessary for the generation of IL-8-induced Ca^{2+} signals. The concentrations of arachidonic acid needed to block IL-8-induced Ca^{2+} signals in our experiments were considerably higher than those sufficient to block IP_3 receptor channels in artificial lipid bilayer experiments [25]. We do not know at present what intracellular concentrations of arachidonic acid were achieved under our experimental conditions, nor do we know whether physiological levels of arachidonic acid are sufficient to regulate the activity of IP_3 receptors.

When applied in combination with acetylsalicylic acid (ASS, 150 μM) to prevent the degradation of arachidonic

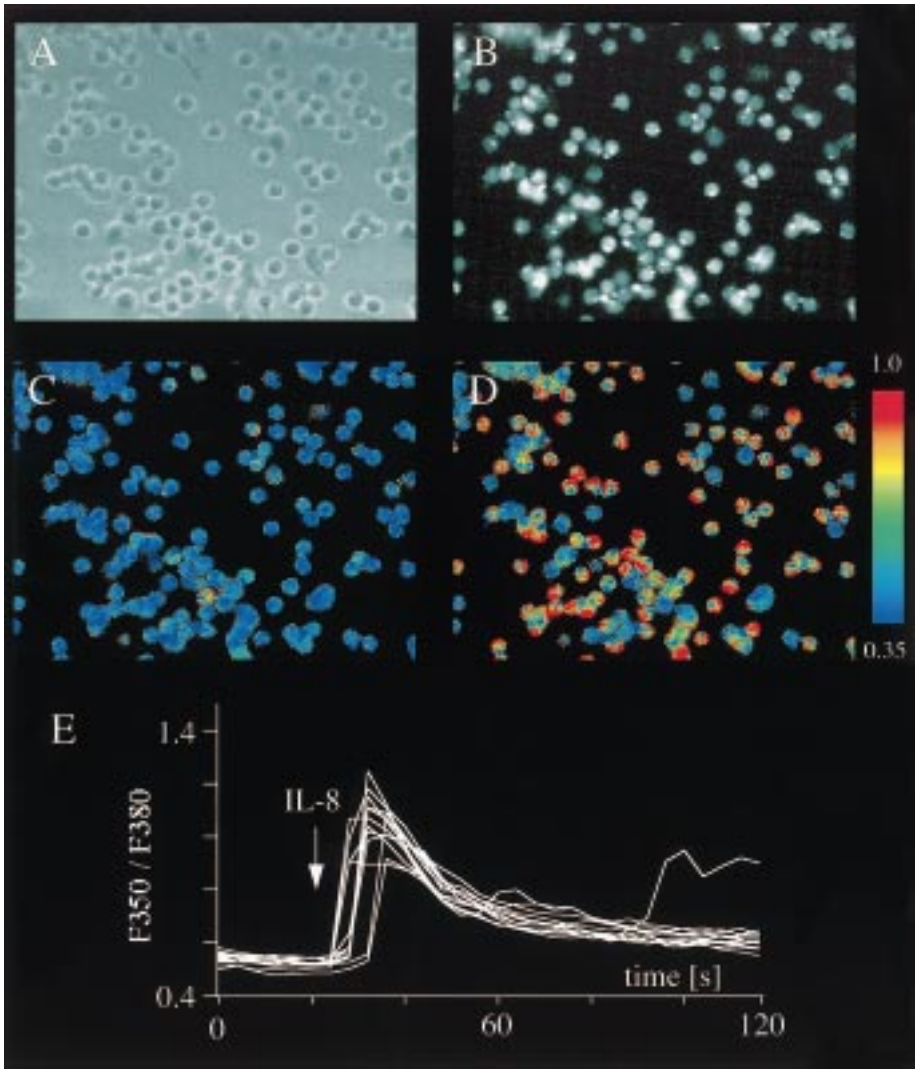

Figure 1. Digital Ca^{2+} imaging in single human granulocytes. (A) Phase contrast image of acutely isolated human granulocytes 15 min after plating on a culture dish. (B) Fluorescence image of FURA-2 AM-loaded human granulocytes, excited at 360 nm. Same field as A. (C and D) Pseudocolor-coded image of the fluorescence ratio F350/F380. Cold and warm colors correspond to low and high ratio and $[Ca^{2+}]_i$, respectively. C before and D during stimulation of the cells with IL-8. (E) Time course of the complete experiment. Ratiometric Ca^{2+} measurements were taken every 4 s.

acid to prostaglandins by cyclooxygenases [26], arachidonic acid was still effective. It is therefore likely that the effects of arachidonic acid seen in our experiments are not mediated by cyclooxygenase products of arachidonic acid. A contribution of arachidonic acid metabolites generated by the lipoxygenase pathway, *i.e.* leukotrienes, cannot be ruled out, but leukotriene B4 has been reported to be without effect on IP_3 receptors [25].

Apart from IP_3 receptors other Ca^{2+} release mechanisms might contribute to IL-8-induced Ca^{2+} signals [15, 16]. Several reports have suggested the existence of ryanodine receptor-like Ca^{2+} release channels in electrically non-excitable cells, including neutrophil granulocytes [27–29]. In these cells chemotaxis evoked by IL-8 and N-formyl-methionine-leucine-phenylalanine (FMLP) has been shown to be reduced by ryanodine [29].

In electrically non-excitable cells ryanodine receptors might be activated by increases in $[Ca^{2+}]_i$ primarily elicited via Ca^{2+} release through IP_3 receptor-coupled channels. Alternatively, ryanodine receptors might be activated by intracellular messengers different from Ca^{2+}. Cyclic-ADP-ribose has been suggested to be such an endogenous

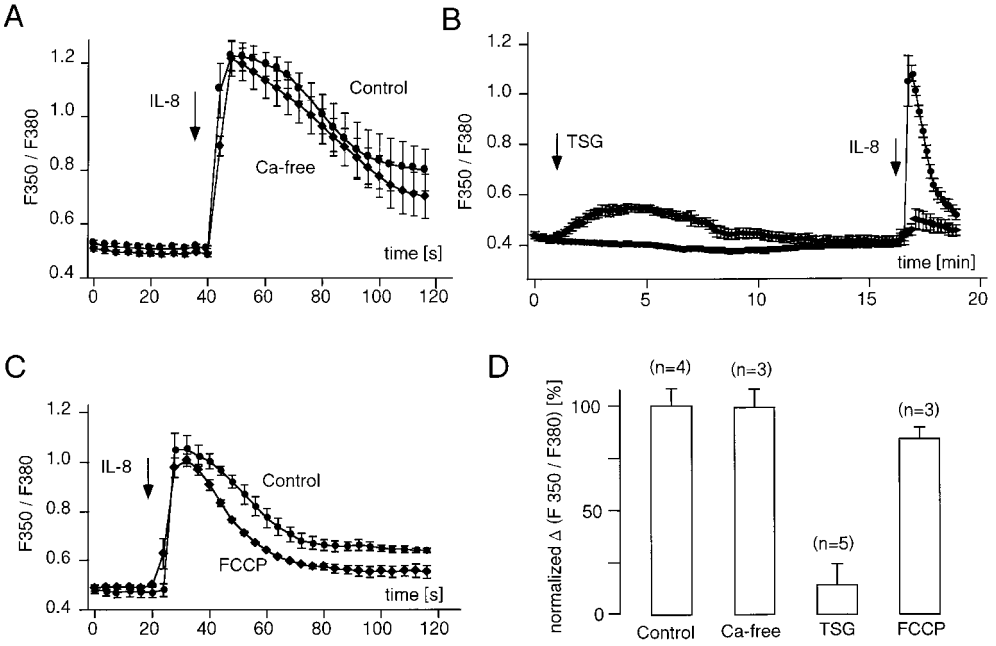

Figure 2. IL-8-induced Ca^{2+} signals in human neutrophil granulocytes (averages of four experiments with 20 cells each ± SEM). (A) IL-8-induced Ca^{2+} signals (F350/F380 ratios) in the presence (2 mM Ca^{2+}; ●) and absence (0 mM Ca^{2+}, 4 mM EGTA; ◆) of extracellular Ca^{2+}. (B) Incubation with thapsigargine (TSG; 2 μM in Ca^{2+}-free extracellular solution containing 4 mM EGTA) induced a small Ca^{2+} signal in human granulocytes and after 15 min almost completely inhibited Ca^{2+} release by IL-8 (◆). Control cells, which had been incubated with vehicle for the same time period, exhibited normal Ca^{2+} responses upon stimulation with IL-8 (●). (C) Preincubation with FCCP (5 μM for 20 min; ◆) had no effect on the initial Ca^{2+} signal induced by IL-8. The recovery of the Ca^{2+} signal from IL-8 stimulation was significantly facilitated in the presence of FCCP compared with control cells treated with vehicle (ethanol 0.1 %; ●). (D) Summary of the experiments shown in A to C. n: number of independent experiments with 20 cells each.

activator of ryanodine-sensitive Ca^{2+} release channels [30, 31], but Ca^{2+} still appears to be the most important activator in the majority of cells. Ca^{2+} signals evoked by IP_3 receptor-dependent Ca^{2+} release might therefore be boosted by ryanodine-sensitive Ca^{2+} release.

We have tested the involvement of Ca^{2+} release channels of the ryanodine type (ryanodine receptors) in IL-8-induced Ca^{2+} signaling with ryanodine (15 μM), which at this concentration blocks the channels [20, 21] and with ruthenium red (3 μM), another but rather nonspecific blocker of ryanodine receptors [20, 21] (Fig. 3C). Both substances failed to inhibit Ca^{2+} signaling, indicating that ryanodine-sensitive Ca^{2+} release channels are not involved in IL-8 signal transduction. In this context it is interesting to note that caffeine, which activates ryanodine receptors in many cell types [21, 32, 33], did not induce Ca^{2+} signals itself. As shown in Fig. 3A caffeine had no effect on the resting $[Ca^{2+}]_i$, which argues against the existence of typical ryanodine receptors in human neutrophil granulocytes. This is in line with our observation that ryanodine, which at low concentrations can open ryanodine receptor channels [32], had no effect on the resting $[Ca^{2+}]_i$ of neutrophils.

2.4 *Bordetella pertussis* toxin (PTX) and inhibitors of phospholipase C (PLC) block IL-8-induced Ca^{2+} signals

Further support for the involvement of IP_3 as a relevant intracellular messenger in IL-8-induced Ca^{2+} signals comes from experiments with the PLC inhibitor U-73122 (1-[6-[[17 beta-3-methoxyestra-1, 3, 5(10)-trien-17-yl] amino]hexyl]-1H-pyrrole-2, 5-dione) [34]. Preincubation of human neutrophils for 30 min with U-73122 (7 μM) led to a complete block of IL-8-induced Ca^{2+} signals, whereas its inactive analog U-73343 (1-[6-[17 beta-3-methoxyestra-1, 3, 5(10)-trien-17-yl]amino]hexyl]-1H-pyrrolidine-2, 5-dione) (7 μM) failed to affect Ca^{2+} signals (Fig. 4).

Activation of PLC can be triggered by PTX-sensitive and -insensitive G-proteins (PLC-β) as well as by tyrosine

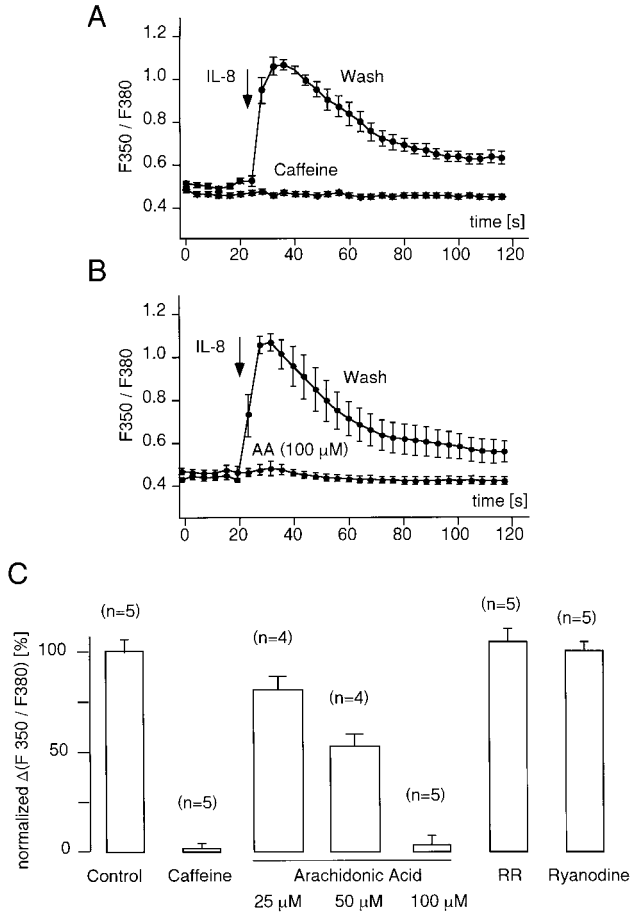

Figure 3. IL-8-induced Ca^{2+} signals in human neutrophil granulocytes are blocked by inhibitors of IP_3 receptor channels. (A) Preincubation of granulocytes with caffeine (20 mM for 30 min; ◆) completely and reversibly blocked IL-8-induced Ca^{2+} signals. After extensive washing the same cells responded to application of IL-8 (wash; ●) similar to untreated controls (compare *e.g.* Fig. 2A). (B) Arachidonic acid (AA; ◆) reversibly blocked IL-8-induced Ca^{2+} signaling in a concentration-dependent manner. The same cells responded to IL-8 after complete removal of arachidonic acid (wash; ●). (C) Summary of the experiments shown in A and B. While caffeine (20 mM) and arachidonic acid (100 μM) completely inhibited IL-8-induced Ca^{2+} signals, ruthenium red (RR; 3 μM) and ryanodine (15 μM) had no effect. n: number of independent experiments with 20 cells each.

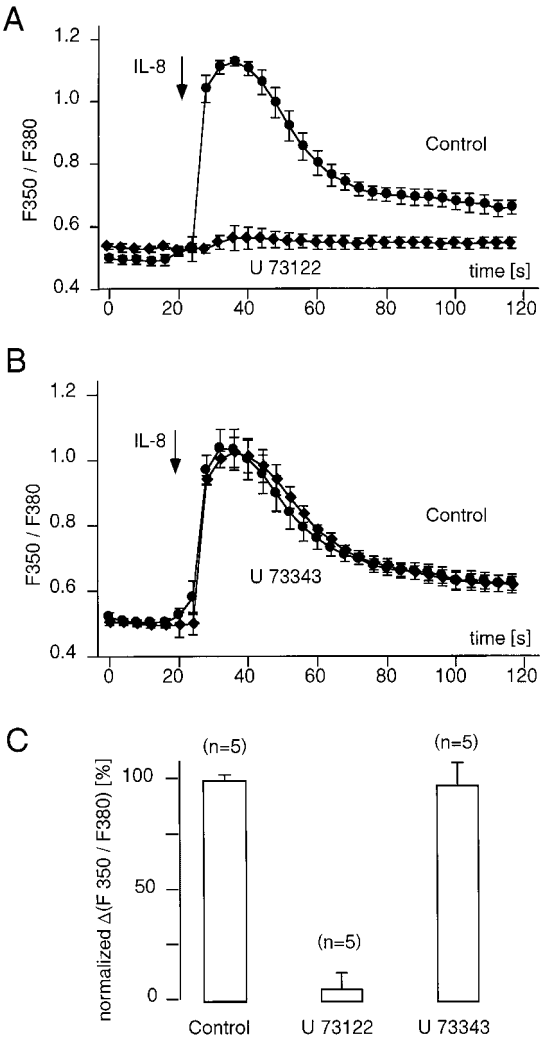

Figure 4. Inhibition of IL-8-induced Ca^{2+} signals by blockers of PLC. (A) Preincubation with U-73122 (30 min, 7 μM; ◆) completely blocked IL-8-induced Ca^{2+} signals. ●: Vehicle (0.1 % DMSO)-treated control. (B) Its inactive analog U-73343 (30 min, 7 μM; ◆) had no significant effect on IL-8-induced Ca^{2+} signals. ●: Vehicle (0.1 % DMSO)-treated control. (C) Summary of the experiments (mean ± SEM) shown in A and B. n: number of independent experiments with 20 cells each.

kinases (PLC-γ) [35]. IL-8 can activate several types of G proteins including the G_i type [36]. In our experiments pretreatment with PTX (4 μg/ml for 4–5 h at 37 °C) caused a complete block of IL-8-induced Ca^{2+} responses in the vast majority of the cells (Fig. 5), similarly to what has been described for Ca^{2+} measurements performed in cuvettes, where the average Ca^{2+} increase of a whole cell population has been measured [37].

2.5 Contribution of a Ca^{2+} release-activated Ca^{2+} current

Release of Ca^{2+} from intracellular stores can lead to the activation of Ca^{2+} release-activated Ca^{2+} influx (I_{CRAC}) [30, 38]. To test for a possible contribution of such Ca^{2+} influx mechanisms we have monitored the time course of IL-8-induced Ca^{2+} signals in the presence and absence of extracellular Ca^{2+} (0 mM Ca^{2+}, 1 mM EGTA). Recovery of IL-8-induced Ca^{2+} signals was significantly faster in Ca^{2+}-free medium than under control conditions.

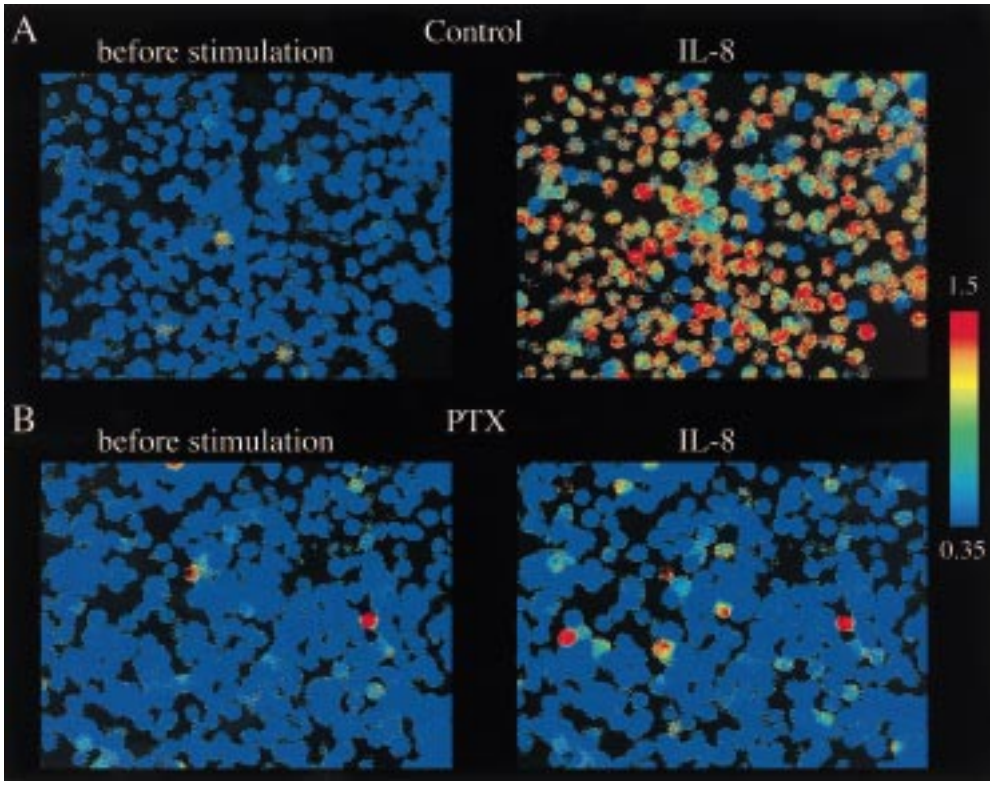

Figure 5. Inhibition of IL-8-induced Ca^{2+} signaling by PTX. Pseudocolor-coded image of the fluorescence ratio F350/F380. Cold and warm colors correspond to low and high ratio and $[Ca^{2+}]_i$, respectively. IL-8-induced Ca^{2+} responses under control conditions (A) and after preincubation with PTX (4 μg/ml for 4–5 h at 37 °C; B).

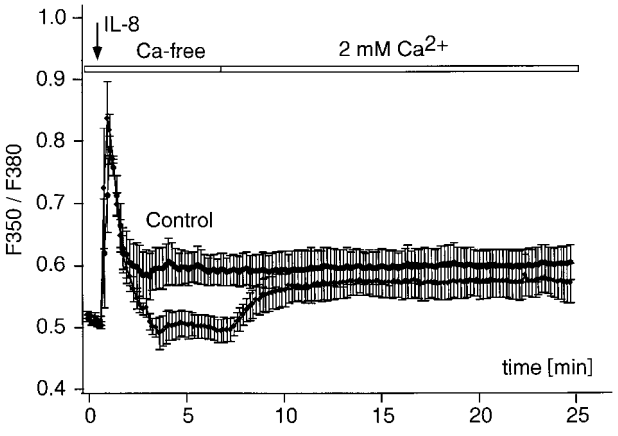

Figure 6. Ca^{2+} influx contributes to the late phase of IL-8-induced Ca^{2+} signals. IL-8 was applied either in the presence of 2 mM Ca^{2+} (control; ●) or in the absence of Ca^{2+} (0 mM Ca^{2+}, 1 mM EGTA; ◆). No significant difference was seen in the initial part of the IL-8-induced Ca^{2+} signal, while the late phase was dependent on the presence of external Ca^{2+}. When Ca^{2+} was re-added after 7 min the difference of both traces became insignificant.

Although the initial Ca^{2+} signal remained unaffected when extracellular Ca^{2+} was removed, the late phase (> 2 min) was reversibly abolished when extracellular Ca^{2+} was removed. In the presence of extracellular Ca^{2+}, $[Ca^{2+}]_i$ remained elevated over the complete time course of the experiment (20 min). These findings indeed suggest the presence of a Ca^{2+} influx mechanism most likely activated by depletion of intracellular Ca^{2+} stores. This influx is probably mediated by Ca^{2+} release-activated Ca^{2+} (CRAC) channels [38], which have meanwhile been described in a variety of different cell types including mast cells and T lymphocytes [39].

In summary, we have found that IL-8 triggers an initial Ca^{2+} release via a PTX-sensitive G protein-dependent activation of PLC, which in turn leads to the activation of IP_3 receptor-coupled Ca^{2+} release channels at thapsigargine-sensitive Ca^{2+} stores. This initial Ca^{2+} release was followed by a sustained Ca^{2+} influx probably mediated via CRAC channels. Besides facilitating the refilling of intracellular Ca^{2+} stores this Ca^{2+} influx may contribute to ongoing activation of granulocytes, *e.g.* on their way to a site of inflammation.

3 Materials and methods

3.1 Cell preparation and measurement of Ca^{2+} signals

Human neutrophil granulocytes were isolated from blood samples of healthy young male volunteers aged between 25 and 34 years as described previously [27]. Cells in suspension were loaded with FURA-2 AM [3 μM] at 37 °C for 30 min to measure $[Ca^{2+}]_i$ [40]. Cells were washed twice and stored on ice for up to 5 h. Immediately before the Ca^{2+} measurement experiments 100 μl of a cell suspension, which contained 1×10^6 cells/ml were dropped onto the center of a 35-mm uncoated culture dish (Nunc, Denmark) and cells were then allowed to attach for 5 min. One milliliter of standard external recording solution (136 mM NaCl, 2.8 mM KCl, 2 mM $CaCl_2$, 20 mM Hepes, 5 mM glucose, pH 7.40) was added, and the cells were then transferred to the microscope for the measurement of Ca^{2+} signals.

$[Ca^{2+}]_i$ was measured using a commercially available Ca^{2+} imaging system consisting of a slow-scan CCD camera coupled to a monochromator (TILL Photonics, Planegg, München, Germany) both connected to an upright Zeiss microscope Axioscope FS equipped with a 40× water immersion objective. This experimental set-up allowed for the 2-dimensional representation of Ca^{2+} signals in up to 100 cells simultaneously.

$[Ca^{2+}]_i$ is presented as fluorescence ratio determined from background-corrected fluorescence images excited alternately at 350 and 380 nm (F350/F380) [41]. IL-8 was applied by microdrop application (15 μl; 0.625 μM) in the close proximity of the water immersion objective.

3.2 Chemicals

All inorganic compounds were from Merck, Darmstadt, Germany. FURA-2 AM was from Molecular Probes Europe BV, Leiden, The Netherlands. Acetylsalicylic acid, arachidonic acid, caffeine, DMSO, EGTA, FCCP, Hepes, IL-8, and thapsigargine were from Sigma, Deisenhofen, Germany. Ruthenium red, ryanodine, pertussis toxin, U-73122 and U-73343 were from Alexis Deutschland GmbH, Germany. Thapsigargine, FCCP, U-73122 and U-73343 were dissolved in DMSO (0.1 %). Arachidonic acid was dissolved in ethanol (0.1 %). All other substances were dissolved in standard external solution.

Acknowledgments: We thank Mrs. Susanne Gabriel and Mrs. Alexandra Schmidt for excellent technical assistance and Dr. D. Vestweber and Dr. R. Hallmann for the mouse E-selectin × human Ig chimera. Supported in part by the Deutsche Forschungsgemeinschaft Ze 377/4-1 to HUZ and SFB 353 to DS. W.S. was supported by a stipend of the Graduiertenkolleg of the Deutsche Forschungsgemeinschaft.

4 References

1 **Butcher, E. C.,** Leukocyte-endothelial cell recognition: Three (or more) steps to specificity and diversity. *Cell* 1991. **67:** 1033–1036.

2 **Springer, T. A.,** Traffic signals on endothelium for leukocyte recirculation and leukocyte emigration. *Annu. Rev. Physiol.* 1995. **57:** 827–872.

3 **Lasky, L. A.,** Selectin-carbohydrate interactions and the initiation of the inflammatory response. *Annu. Rev. Biochem.* 1995. **64:** 113–139.

4 **McEver, R. P.,** Leukocyte adhesion through selectins under flow. *Immunologist* 1998. **6:** 61–66.

5 **Tözeren, A. and Ley, K.,** How do selectins mediate rolling in venules? *Biophys. J.* 1992. **63:** 700–709.

6 **Baggiolini, M., Loetscher, P. and Moser, B.,** Interleukin-8 and the chemokine family. *Int. J. Immunopharmacol.* 1995. **17:** 103–108.

7 **Walz, A., Peveri, P., Aschauer, H. and Baggiolini, M.,** Purification and amino acid sequencing of NAF, a novel neutrophil-activating factor produced by monocytes. *Biochem. Biophys. Res. Commun.* 1987. **149:** 755–761.

8 **Baggiolini, M., Walz, A. and Kunkel, S. L.,** Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. *J. Clin. Invest.* 1989. **84:** 1045–1049.

9 **Cassatella, M. A., Bazzoni, F., Ceska, M., Ferro, I., Baggiolini, M. and Berton, G.,** IL-8 production by human polymorphonuclear leukocytes. *J. Immunol.* 1992. **148:** 3216–3230.

10 **Baggiolini, M., Dewald, B. and Moser, B.,** Human chemokines: An update. *Annu. Rev. Immunol.* 1997. **15:** 675–705.

11 **Marks, P. W. and Maxfield, F. R.,** Local and global changes in cytosolic free calcium in neutrophils during chemotaxis and phagocytosis. *Cell Calcium* 1990. **11:** 181–190.

12 **Hendey, B. and Maxfield, F. R.,** Regulation of neutrophil motility and adhesion by intracellular calcium transients. *Blood Cells* 1993. **19:** 143–164.

13 **Hendey, B., Klee, C. B. and Maxfield, F. R.,** Inhibition of neutrophil chemokinesis on vitronectin by inhibitors of calcineurin. *Science* 1992. **258:** 296–299.

14 **Lawson, M. A. and Maxfield, F. R.,** Ca^{2+}- and calcineurin-dependent recycling of an integrin to the front of migrating neutrophils. *Nature* 1995. **377:** 75–79.

15 **Pozzan, T., Rizzuto, R., Volpe, P. and Meldolesi, J.,** Molecular and cellular physiology of intracellular calcium stores. *Physiol. Rev.* 1994. **74:** 595–636.

16 **Tsien, R. W. and Tsien, R. Y.,** Calcium channels, stores, and oscillations. *Annu. Rev. Cell Biol.* 1990. **6:** 715–760.

17 **Lytton, J., Westlin, M. and Hanley, M. R.,** Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum Ca-ATPase family of calcium pumps. *Biol. Chem.* 1991. **266:** 17067–17071.

18 **Heytler, P. G.,** Uncouplers of oxidative phosphorylation. *Methods Enzymol.* 1979. **55:** 462–542.

19 **Joseph, S. K., Coll, K. E., Cooper, R. H., Marks, J. S. and Williamson, J. R.,** Mechanisms underlying calcium homeostasis in isolated hepatocytes. *Biol. Chem.* 1983. **258:** 731–741.

20 **Ehrlich, B. E., Kaftan, E., Bezprozvannaya, S. and Bezprozvanny, I.,** The pharmacology of intracellular Ca^{2+}-release channels. *Trends Pharmacol. Sci.* 1994. **15:** 145–149.

21 **Ehrlich, B. E. and Watras, J.,** Inositol 1, 4, 5-trisphosphate activates a channel from smooth muscle sarcoplasmic reticulum. *Nature* 1988. **336:** 583–586.

22 **Supattapone, S., Worley, P. F., Baraban, J. M. and Snyder, S. H.,** Solubilization, purification, and characterization of an inositol trisphosphate receptor. *J. Biol. Chem.* 1988. **263:** 1530–1534.

23 **Hirose, K., Iino, M. and Endo, M.,** Caffeine inhibits Ca^{2+}-mediated potentiation of inositol 1, 4, 5-trisphosphate-induced Ca^{2+} release in permeabilized vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.* 1993. **194:** 726–732.

24 **Parker, I. and Ivorra, I.,** Caffeine inhibits inositol trisphosphate-mediated liberation of intracellular calcium in Xenopus oocytes. *J. Physiol. (Lond.)* 1991. **433:** 229–240.

25 **Striggow, F. and Ehrlich, B. E.,** Regulation of intracellular calcium release channel function by arachidonic acid and leukotriene B4. *Biochem. Biophys. Res. Commun.* 1997. **237:** 413–418.

26 **Ferreira, S. H., Moncada, S. and Vane, J., R.,** Indomethacin and aspirin abolish prostaglandin release from the spleen. *Nat. New Biol.* 1971. **231:** 237–239.

27 **Bang, H., Muller, W., Hans, M., Brune, K. and Swandulla, D.,** Activation of Ca^{2+} signaling in neutrophils by the mast cell-released immunophilin FKBP12. *Proc. Natl. Acad. Sci. USA* 1995. **92:** 3435–3438.

28 **Larini, F., Menegazzi, P., Baricordi, O., Zorzato, F. and Treves, S.,** A ryanodine receptor-like Ca^{2+} channel is expressed in nonexcitable cells. *Mol. Pharmacol.* 1995. **47:** 21–28.

29 **Elferink, J. G. and De Koster, B. M.,** Ryanodine as inhibitor of chemotactic peptide-induced chemotaxis in human neutrophils. *Biochem. Pharmacol.* 1995. **50:** 975–979.

30 **Meszaros, L. G., Bak, J. and Chu, A.,** Cyclic ADP-ribose as an endogenous regulator of the non-skeletal type ryanodine receptor Ca^{2+} channel. *Nature* 1993. **364:** 76–79.

31 **Lee, H. C., Walseth, T. F., Bratt, G. T., Hayes, R. N. and Clapper, D. L.,** Structural determination of a cyclic metabolite of NAD^+ with intracellular Ca^{2+}-mobilizing activity. *J. Biol. Chem.* 1989. **264:** 1608–1615.

32 **Smith, J. S., Imagawa, T., Ma, J., Fill, M., Campbell, K. P. and Coronado, R.,** Purified ryanodine receptor from rabbit skeletal muscle is the calcium-release channel of sarcoplasmic reticulum. *J. Gen. Physiol.* 1988. **92:** 1–26.

33 **Sitsapesan, R. and Williams, A. J.,** Mechanisms of caffeine activation of single calcium-release channels of sheep cardiac sarcoplasmic reticulum. *J. Physiol.* 1990. **423:** 425–439.

34 **Bleasdale, J. E., Thakur, N. R., Gremban, R. S., Bundy, G. L., Fitzpatrick, F. A., Smith, R. J. and Bunting, S.,** Selective inhibition of receptor-coupled phospholipase C-dependent processes in human platelets and polymorphonuclear neutrophils. *J. Pharmacol. Exp. Ther.* 1990. **255:** 756–768.

35 **Clapham, D. E.,** Calcium Signaling. *Cell* 1995. **80:** 259–268.

36 **Wu, D., LaRosa, G. J. and Simon, M. I.,** G-protein-coupled signal transduction pathways for interleukin-8. *Science* 1993. **261:** 101–103.

37 **Thelen, M., Peveri, P., Kernen, P., von Tscharner, V., Walz, A. and Baggiolini, M.,** Mechanism of neutrophil activation by NAF, a novel monocyte-derived peptide agonist. *FASEB J.* 1988. **2:** 2702–2706.

38 **Hoth, M. and Penner, R.,** Depletion of intracellular calcium stores activates a calcium current in mast cell. *Nature* 1992. **355:** 561–563.

39 **Parekh, A. B. and Penner, R.,** Store depletion and calcium influx. *Physiol. Rev.* 1997. **77:** 801–930.

40 **Grynkiewicz, G., Poenie, M. and Tsien, R. Y.,** A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *Biol. Chem.* 1985. **260:** 3440–3450.

41 **Zeilhofer, H. U., Kress, M. and Swandulla, D.,** Fractional Ca^{2+} currents through capsaicin- and proton-activated ion channels in rat dorsal root ganglion neurones. *J. Physiol.* 1997. **503:** 67–78.

Correspondence: Hanns Ulrich Zeilhofer, Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Universitätsstrasse 22, D-91054 Erlangen, Germany
Fax: +49-9131-206119
E-mail: uz@macpost.pharmakologie.uni-erlangen.de