Regulation of Hormone-Induced Ca\textsuperscript{2+} Mobilization in the Human Platelets

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\textbf{Introduction}

In many mammalian cell types, stimulation of specific receptors by agonists is accompanied by a nonvoltage-regulated mobilization of intracellular Ca\textsuperscript{2+} stores. This results in the elevation of the cytosolic Ca\textsuperscript{2+} concentration, which can evoke cellular responses such as in the platelet, secretion, shape change, and aggregation. The Ca\textsuperscript{2+} appears to originate from specific, nonmitochondrial sites in the cell, probably the endoplasmic reticulum or the platelet-dense tubular system.

The mechanism by which agonists cause a receptor-mediated Ca\textsuperscript{2+}-mobilization has been extensively studied over recent years. It appears that the transduction mechanism of occupied receptors is the phospholipase C-catalyzed hydrolysis of inositol phospholipid, specifically, phosphatidylinositol-4,5-bisphosphate (PIP\textsubscript{2}). The immediate products of this reaction are diacylglycerol (DAG), which can activate protein kinase C \cite{1} and inositol trisphosphate (IP\textsubscript{3}), which will mobilize the hormone-sensitive Ca\textsuperscript{2+} stores when applied to permeabilized cells \cite{2}.

Therefore, the connection between receptor and Ca\textsuperscript{2+} store mobilization has been quite convincingly established. The questions that we wanted to address were a) what are the kinetics of Ca\textsuperscript{2+} mobilization in the platelet; b) did these correlate with changes in the IP\textsubscript{3} levels; and c) where in the chain of events following the agonist’s receptor occupation is the release of Ca\textsuperscript{2+} modulated or controlled? We have discussed these points elsewhere \cite{3}.

\textbf{Platelet Ca\textsuperscript{2+} Responses to Agonists}

We chose three different agonists: \(\alpha\)-thrombin, \(\gamma\)-thrombin, and the platelet-activating factor (PAF) to study Ca\textsuperscript{2+} mobilization in the platelet. Each of these agents was able to mobilize Ca\textsuperscript{2+} from intracellular Ca\textsuperscript{2+} stores, but to ranging degrees and rates (Fig. 1). \(\alpha\)-thrombin was most effective, whereas \(\gamma\)-thrombin produced a smaller and slower response. PAF produced a rapid Ca\textsuperscript{2+} signal, but had a smaller magnitude than that of \(\gamma\)-thrombin.

Each of these responses was quite transient, despite the continued presence of the agonist; after the return to baseline levels, the cytosolic Ca\textsuperscript{2+} level could not be reelevated by a second addition of the same agonist at the same concentration (Fig. 1). Thus, the loss of response was not due to degradation of the agonist and could not be explained by the agonists fully depleting the Ca\textsuperscript{2+} stores, since PAF was a much less effective agonist than \(\alpha\)- or \(\gamma\)-thrombin. The Ca\textsuperscript{2+} response to PAF returned most quickly to basal levels and could not be restimulated with PAF.

These responses appeared to be a desensitization of the receptors or of a post-receptor mechanism. To determine which of these two possibilities was more...
likely, the ability of a second agonist to elicit the release of Ca\textsuperscript{2+} stores was examined. After the α- or γ-thrombin induced Ca\textsuperscript{2+} release was complete, the cytosolic Ca\textsuperscript{2+} concentration had returned to basal levels, and the addition of the same agonist at the same concentration was shown to have no effect. PAF was still able to induce a small but significant release of Ca\textsuperscript{2+} stores (Fig. 1). The smaller response to PAF after the thrombin rather than to PAF alone is probably because the thrombin has already depleted much of the Ca\textsuperscript{2+} store, and the PAF can act to release only a much-reduced pool of Ca\textsuperscript{2+}.

**Thrombin Receptor Desensitization**

Because thrombin and PAF are both thought to couple through the same second messenger system (i.e., phospholipase C), this suggests that the desensitization observed is homologous and occurs at the receptor-effector site rather than a post-receptor site. It has been shown previously (4) that activation of protein kinase C, which occurs during stimulation of platelets with thrombin or PAF, is able to increase the degredation of cytosolic IP\textsubscript{3} by elevating IP\textsubscript{3} phosphatase activity. This could decrease the receptor-activated Ca\textsuperscript{2+} release. However, the activation of protein kinase C does not appear to be the mechanism of the desensitization observed here since this would cause a heterologous desensitization. The desensitization is better explained by being related to the generation of the Ca\textsuperscript{2+}-mobilizing signal (i.e., IP\textsubscript{3}) rather than to the fate of IP\textsubscript{3} once it formed.

**Thrombin Effects Are Restored by Epinephrine**

To examine this effect further, we have used epinephrine, which is able to potentiate the action of many platelet agonists without having a direct effect on platelet responses. Epinephrine acts via α\textsubscript{2}-adrenergic receptors, since yohimbine was found to totally inhibit its actions. We found that epinephrine alone had no effect on platelet Ca\textsuperscript{2+} levels. However, when added to platelets previously desensitized to thrombin, epinephrine could elicit a relatively large mobilization of Ca\textsuperscript{2+} stores (Fig. 2). Thus, epinephrine was able to re-sensitize the thrombin receptor to the generation of a Ca\textsuperscript{2+} signal.

**Phospholipase C Activation by Thrombin**

In supporting the role of IP\textsubscript{3} in mobilizing intracellular Ca\textsuperscript{2+} stores, the desensitization of the thrombin-induced Ca\textsuperscript{2+} release and resensitization by epinephrine were paralleled by desensitization and resensitization of the α-thrombin-induced IP\textsubscript{3} formation (Fig. 3). This showed us that the thrombin receptor did desensitize at the level of receptor activation of phospholipase C and hydrolysis of PIP\textsubscript{2}, but that the α\textsubscript{2}-adrenergic receptor could re-sensitize thrombin receptor coupling. Those effects were able to fully explain our Ca\textsuperscript{2+} data.
Down-Regulation by Protein Kinase C

The next part of our study was to try to assess what caused the homologous desensitization. Since each desensitization was coupled to specific receptors but showed similar characteristics, it was likely that the agonists induced a local activation of a second messenger, which was itself responsible for the receptor inactivation. It is known from studies of other cell types that protein kinase C activation can be inhibitory to hormone-induced phospholipase C. In the platelet, diacylglycerol, the endogenous protein kinase C activator, is rapidly converted to phosphatidic acid. Thus, diacylglycerol may well be produced as a local event and also locally activate protein kinase C near the occupied receptors.

When we treated platelets with the protein kinase C-activating phorbol ester, phorbol-12,13-dibutyrate (PdBu), we found a reduction in the thrombin-induced release of Ca\textsuperscript{2+} stores and an abolition of resensitization of thrombin action by epinephrine (Fig. 4). This effect of PdBu was half maximal at 1 nM and occurred within 30 sec, with both parameters being consistent with a specific activation of protein kinase C. From these results, we suggest that a local elevation in the DAG near the occupied receptor causes a local activation and translocation of protein kinase C so that only the occupied receptor is desensitized.
Conclusion

We believe that receptor desensitization in the human platelet represents an important negative feedback system in controlling platelet responses, including the mobilization of intracellular Ca\(^{2+}\) stores and activation of phospholipase C. This desensitization appears to be homologous and mediated by hormone-induced activation of protein kinase C. Our current work (3) implicates the inhibitory GTP-binding protein, G\(_i\), as the substrate of protein kinase C involved in receptor-phospholipase C coupling.

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