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THE ROLE OF CALCIUM IN STIMULUS–SECRETION COUPLING IN EXCITABLE AND NON-EXCITABLE CELLS

BY REINHOLD PENNER AND ERWIN NEHER

Max-Planck-Institut für Biophysikalische Chemie, Am Faßberg, D-3400 Göttingen, FRG

Summary

Secretion of vesicular contents by exocytosis is a common feature of excitable (neurones, chromaffin cells, beta cells) and non-excitable cells (platelets, neutrophils, mast cells). The simplistic view that the universal mechanism controlling secretion is elevation of $[Ca^{2+}]_i$ – whatever the source of this second messenger may be – is no longer tenable in view of recent reports demonstrating secretion at basal or even reduced $[Ca^{2+}]_i$. It is nevertheless clear that in excitable cells an increase in $[Ca^{2+}]_i$ is the triggering event that induces secretion. In non-excitable cells, secretion is presumably triggered by other second messengers, although $[Ca^{2+}]_i$ appears to act as an important modulator of the rate of secretion. Conversely, these second messenger systems may serve a regulatory function in excitable cells.

Given the relative importance of $[Ca^{2+}]_i$ in the regulation of cellular functions in excitable and non-excitable cells, it is not surprising that several mechanisms are expressed in these cells to regulate intracellular calcium concentration. The major pathway for Ca^{2+} in excitable cells is by voltage-activated Ca^{2+} channels, but release of Ca^{2+} from intracellular stores, *via* second messengers, predominates in non-excitable cells, and may also be important in excitable cells. In addition, receptor-operated channels and second messenger-gated conductances may prove to be important. All of these pathways are subject to regulation by a variety of interactive second messenger systems, which provide necessary tuning for an appropriate control of intracellular calcium level.

Introduction

There is hardly any cellular function that is not influenced directly or indirectly by the universal second messenger calcium. Particularly, the release of vesicular contents by exocytosis has become the most extensively studied process for establishing the crucial role of calcium as the triggering and controlling event. Historically, this view emerged from studies on quantal transmitter release at the neuromuscular junction (Katz, 1969). It was extended to other excitable cells, such

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as adrenal chromaffin cells and pancreatic beta cells, and soon became the universal mechanism for control of secretion (Douglas, 1968). The following main observations corroborated the 'calcium hypothesis' of exocytosis: (1) extracellular calcium is required to evoke transmitter release, (2) voltage-activated Ca^{2+} channels allow influx of Ca^{2+}, the magnitude of which determines the amount of transmitter release, (3) procedures that elevate intracellular calcium concentration ($[Ca^{2+}]_i$) induce exocytosis, and blocking Ca^{2+} influx abolishes secretion.

The concept of Ca^{2+} control of exocytosis also appeared to apply to non-excitable cells which seem to be devoid of voltage-activated Ca^{2+} channels. This was primarily based on observations that closely resemble those mentioned above: (1) antigenic stimulation of mast cells or rat basophil leukaemic cells depends on extracellular $[Ca^{2+}]$ and is paralleled by Ca^{2+} uptake (Foreman, Hallett & Mongar, 1977), (2) injection of Ca^{2+} into mast cells has been reported to induce secretion (Kanno, Cochrane & Douglas, 1973), (3) Ca^{2+} ionophores, which translocate external Ca^{2+} into the cytosol, produce secretory responses in mast cells (Cochrane & Douglas, 1974; Penner & Neher, 1988), RBL cells (Beaven *et al.* 1987), neutrophils (Rubin, Sink & Freer, 1981) and platelets (Feinman & Detwiler, 1974). Furthermore, studies with fluorescent Ca^{2+} indicator dyes have revealed increases in $[Ca^{2+}]_i$ following stimulation with secretagogues in virtually every type of non-excitable cell investigated (Tsien, Pozzan & Rink, 1984). It soon became clear that in many of these cells the source of calcium was not extracellular Ca^{2+}, but was storage organelles that sequester Ca^{2+} and release it upon stimulation by the recently discovered phosphatidylinositol pathway (Berridge & Irvine, 1984). This did not temper, but instead corroborated, the overwhelming evidence in support of the dominant role of $[Ca^{2+}]_i$ in the secretory process, although now Ca^{2+} released from internal stores by a second messenger had to be considered as a 'third messenger'.

Some findings, however, disturb the simplistic view that an increase in $[Ca^{2+}]_i$ induces secretion in a straightforward manner. (1) In neutrophils, platelets, RINm5F cells and mast cells secretion can be induced at the cells' resting $[Ca^{2+}]_i$ level or even in the almost complete absence of $[Ca^{2+}]_i$ (Sha'afi *et al.* 1983; Rink, Sanchez & Hallam, 1983; Di Virgilio, Lew & Pozzan, 1984; Barrowman, Cockroft & Gomperts, 1986; Wollheim, Ullrich, Meda & Vallar, 1987; Neher & Almers, 1986; Neher, 1988) and (2) in Ca^{2+}-ionophore-treated RBL cells (Beaven *et al.* 1987) or patch-clamped mast cells (Penner & Neher, 1988) a rise of $[Ca^{2+}]_i$ into the range produced by physiological stimulation does not elicit secretion. This would suggest that in some non-excitable cells $[Ca^{2+}]_i$ is neither a necessary nor sufficient stimulus for secretion.

This article aims to review the role of $[Ca^{2+}]_i$ as a second messenger in the secretory responses of excitable and non-excitable cells. Special attention will be paid to the mechanisms by which these cell types bring about changes in $[Ca^{2+}]_i$ and the modulations exerted by other second messenger systems. It is not intended, and in fact not possible, to give a full account of the overwhelming literature being published on this topic. Instead, this is an attempt to sketch

roughly what we think are the most important and most intriguing features in the regulation of $[Ca^{2+}]_i$.

The role of $[Ca^{2+}]_i$ in stimulus–secretion coupling

Although the formulation of the 'calcium hypothesis' dates back almost 20 years, only recently developed methodological approaches provide a proper quantitative evaluation of cell calcium function and regulation, namely: (1) permeabilization techniques which have enabled control of the composition of the cell's intracellular milieu (Gomperts & Fernandez, 1985; Knight & Scrutton, 1986) and the introduction of defined Ca^{2+} concentrations, (2) fluorescent Ca^{2+} indicator dyes that have been extensively used to monitor changes in $[Ca^{2+}]_i$ following stimulation with secretagogues (Tsien *et al.* 1984) and (3) the patch-clamp technique which enables the effective control of the composition of the cytosol and at the same time measures cell membrane parameters such as membrane current or capacitance (Neher, 1988). Although the current gives an indication of transmembrane ion fluxes, the capacitance reflects cell membrane area. The fusion of vesicles with the plasma membrane brings about an increase in membrane area, thus reflecting the exocytotic activity of a single cell (Neher & Marty, 1982). The combined application of the patch-clamp technique and fluorescence measurement provides the most effective methodological assay to monitor simultaneously $[Ca^{2+}]_i$, membrane currents and secretion.

The relationship between $[Ca^{2+}]_i$ and secretory responses in three different cell types is illustrated in Fig. 1. To compare these responses, nearly identical compositions of extracellular and intracellular solutions were used. The cells were challenged by dialysing the cytosol through the orifice of a patch pipette containing free Ca^{2+} (buffered to about $1\,\mu mol\,l^{-1}$ with the aid of EGTA). Secretory responses were monitored by measuring the cell membrane capacitance. With excitable cells, such as bovine adrenal chromaffin cells (Fig. 1A) and mouse pancreatic beta cells (Fig. 1B), the elevated Ca^{2+} level is sufficient stimulus to produce secretion, as witnessed by an increase in cell membrane capacitance. The different magnitudes of these capacitance changes presumably reflect differences in the number of vesicles contained in chromaffin and beta cells or different ratios of exocytosis to endocytosis, both of which determine the measured capacitance changes. With non-excitable mast cells (Fig. 1C), a similar increase in Ca^{2+} concentration is not sufficient to induce sizeable secretory responses in patch-clamp experiments.

Ca^{2+}-induced secretion in mast cells can be achieved by clamping $[Ca^{2+}]_i$, in the high micromolar range, or by adding Ca^{2+} ionophores to produce large increases in $[Ca^{2+}]_i$ (Penner & Neher, 1988). However, the apparent requirement for $[Ca^{2+}]_i$ in mast cells (Penner & Neher, 1988) and RBL cells (Beaven *et al.* 1987) is about an order of magnitude higher than that for Ca^{2+}-induced exocytosis in permeabilized chromaffin cells (Knight & Baker, 1982). It is also much higher than $[Ca^{2+}]_i$ typically observed during degranulation in physiologically stimulated cells.

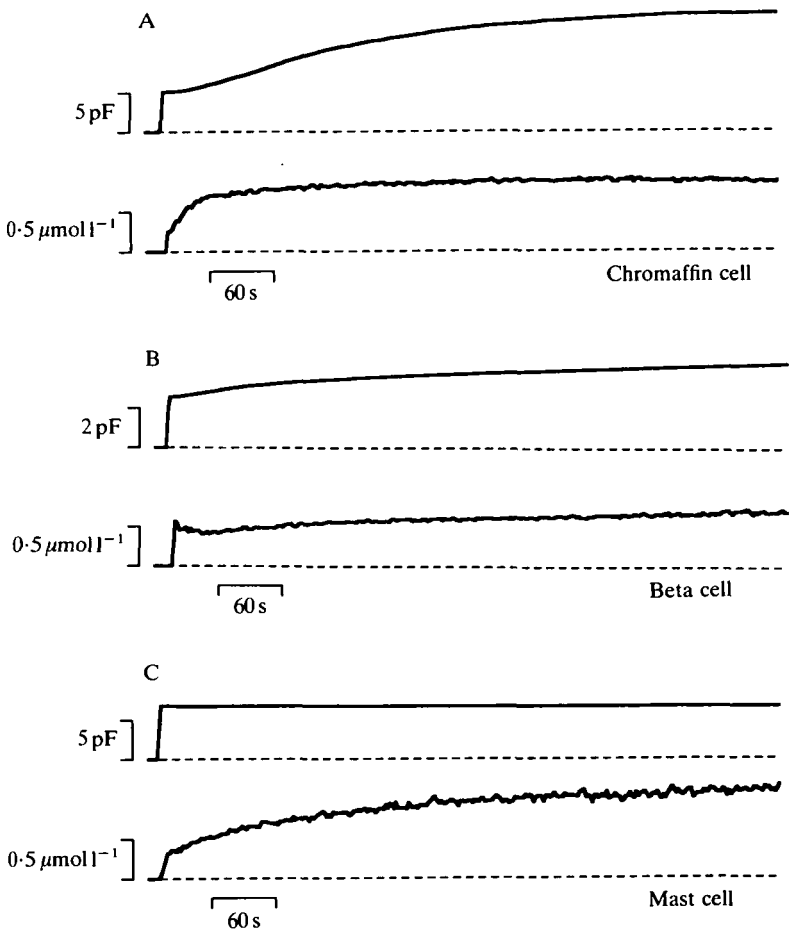

Fig. 1. Secretory responses of different cell types stimulated by injection of micromolar concentrations of intracellular calcium. Bovine adrenal chromaffin cells, mouse pancreatic beta cells and rat peritoneal mast cells were obtained and kept as described (Fenwick, Marty & Neher, 1982; Rorsman & Trube, 1985; Penner, Pusch & Neher, 1987). Experiments were performed at room temperature using the whole-cell configuration of the patch-clamp technique (see Neher, 1988 for details). Fura-2 was used to monitor $[Ca^{2+}]_i$ (Grynkiewicz, Poenie & Tsien, 1985) and changes in cell membrane capacitance reflect increases in membrane area as a result of exocytosis (Neher & Marty, 1982). (A) The cell was dialysed with pipettes containing standard intracellular solution (in mmol l^{-1}): potassium glutamate, 145; NaCl, 8; $MgCl_2$, 1; KOH-Hepes, 10; Na_2-ATP, 0·5; fura-2 pentapotassium salt, 0·1; pH 7·2, which additionally contained GTP (300 μmol l^{-1}) and a combination of Ca^{2+}-EGTA/EGTA at a ratio of 7:1. The extracellular solution (solution *A*) contained (in mmol l^{-1}): NaCl, 140; KCl, 2·8; $CaCl_2$, 2; $MgCl_2$, 1; glucose, 11; Hepes-NaOH, 10; pH 7·2. (B) Same solution as in A except that the EGTA buffer ratio in the internal solution was 6:1. (C) Same solution as in B except that the extracellular solution (solution *B*) contained (in mmol l^{-1}): NaCl, 140; KCl, 2·5; $CaCl_2$, 2; $MgCl_2$, 5; glucose, 11; Hepes-NaOH, 10; pH 7·2.

Thus in chromaffin cells, secretion may be induced by elevating $[Ca^{2+}]_i$ into the physiological range, 0·4–1·5 μmol l^{-1}, whereas unphysiologically high $[Ca^{2+}]_i$ levels of several micromolar are required to 'force' secretion in mast cells. These findings raise the question whether non-excitable cells have developed alternative means either to bring about secretion by Ca^{2+}-independent mechanisms or to vary the Ca^{2+} dependence of secretion in terms of lowering the Ca^{2+} requirement of the secretory process in response to the adequate stimulus. In fact, in permeabilized mast cells, a variety of $[Ca^{2+}]_i$ concentration–response relationships can be obtained which cover the range of physiologically observed $[Ca^{2+}]_i$ levels, depending on the additional provision of different nucleotides (Howell, Cockcroft & Gomperts, 1987). Similarly, there is a shift of the concentration–response curve for Ca^{2+}-induced secretion in the presence of phorbol esters which mediate activation of protein kinase C (Heiman & Crews, 1985). These findings indicate that the dual pathway may act synergistically to promote secretion in non-excitable cells.

The role of G-proteins in stimulus–secretion coupling

In non-excitable cells, the transduction cascade is initiated by agonist-induced receptor stimulation, followed normally by activation of G-proteins which mediate activation of membrane-associated enzymes and eventual execution of cellular functions (Berridge, 1987). It is, therefore, not surprising that one may bypass and mimic receptor stimulation by directly activating G-proteins with non-hydrolysable GTP-analogues such as GTP-γ-S. Dramatic secretory responses leading to complete degranulation of mast cells (Fig. 2A) can be elicited by internally perfusing the cells with GTP-γ-S (Fernandez, Neher & Gomperts, 1984). The action of GTP-γ-S, which irreversibly activates GTP-binding proteins, results in activation of phospholipase C leading to the generation of inositol trisphosphate ($InsP_3$) and diacylglycerol (DAG). The transient rise of $[Ca^{2+}]_i$ seen in Fig. 2A presumably reflects the $InsP_3$-mediated release of Ca^{2+} from internal stores. Similar Ca^{2+} transients can also be induced by intracellular perfusion of mast cells with $InsP_3$ alone (Neher, 1986) or by externally applied secretagogues which are known to cause polyphosphoinositide breakdown (Neher & Penner, 1988). They can readily be elicited in the absence of extracellular calcium (Neher & Almers, 1986). Ironically, similar Ca^{2+} transients are very often observed when removing divalent ions from the external medium in the absence of any additional stimulus (R. Penner & E. Neher, unpublished observations).

Whether the secretory response is a consequence of DAG-mediated activation of protein kinase C is not clear. However, secretion does not seem to be mediated by the rise in $[Ca^{2+}]_i$ since little capacitance increase is associated with the Ca^{2+} transient, and degranulation proceeds at near-resting levels of $[Ca^{2+}]_i$. In fact, degranulation of mast cells does not require Ca^{2+} transients at all when stimulating with GTP-γ-S or compound 48/80, as demonstrated by experiments in which cells were dialysed with Ca^{2+} buffers that kept $[Ca^{2+}]_i$ at near-resting levels (Neher &

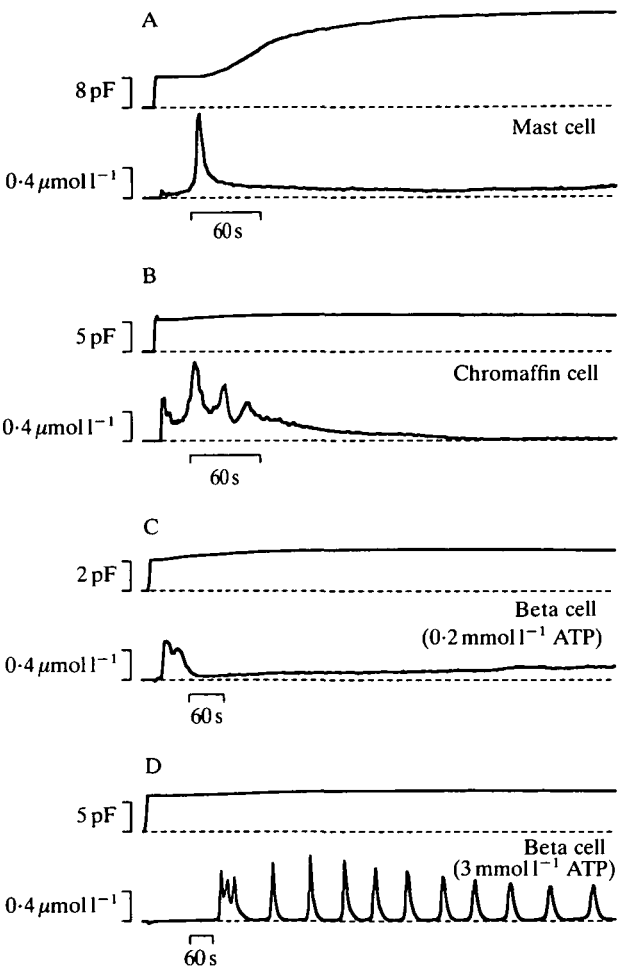

Fig. 2. Secretory responses and typical changes in $[Ca^{2+}]_i$ of different cell types stimulated by injection of GTP-γ-S. (A) The mast cell was stimulated with 40 μmol l^{-1} GTP-γ-S added to the standard internal solution (bath solution *B*, see Fig. 1, was used). (B) The same as A except that in this chromaffin cell GTP-γ-S was used at 100 μmol l^{-1} and bath solution *A* was employed. (C,D) The same as B, except that 0·2 mmol l^{-1} MgATP (in C) and 3 mmol l^{-1} MgATP (in D) were present in the pipette filling-solution.

Penner, 1988; Neher, 1988). Apart from mast cells, the ability of non-excitable cells to secrete at basal $[Ca^{2+}]_i$ levels following stimulation has also been shown to exist in RBL cells (Sagi-Eisenberg, Lieman & Pecht, 1985), platelets (Rink *et al.* 1983; Haslam & Davidson, 1984) and parotid cells (Takemura, 1985). In the case of GTP-γ-S-stimulation, secretion can be induced even in the presence of 10 mmol l^{-1} internal EGTA, albeit delayed and at a slow rate (Neher, 1988). Apparently, there is a signal being provided by the stimulus to enable the cell to secrete at resting or even reduced levels of $[Ca^{2+}]_i$. In neutrophils a novel GTP-

binding protein G_E has been postulated to mediate such Ca^{2+}-independent secretion (Barrowman *et al.* 1986).

It is clear, however, that elevated levels of $[Ca^{2+}]_i$ raise the rate of secretion following stimulation. Mast cells become gradually more responsive to Ca^{2+} after stimulation (Neher, 1988), suggesting synergistic actions of $[Ca^{2+}]_i$ and another signal delivered by GTP-γ-S. Another hint of such a hidden signal is the observation that 48/80-induced secretion is rapidly lost in a whole-cell patch-clamp recording, whereas Ca^{2+} transients are immune to such washout (Penner, Pusch & Neher, 1987). It should be noted that permeabilized mast cells have been reported to show an essential synergy between Ca^{2+} and guanine nucleotides (Howell *et al.* 1987), whereas in patch-clamped mast cells this synergy is not essential. GTP-γ-S-induced secretion in the almost complete absence of $[Ca^{2+}]_i$ has also been shown to occur in insulin-secreting RINm5F cells (Wollheim *et al.* 1987) and neutrophils (Barrowman *et al.* 1986).

Neuronal tissue is known to possess the phospholipid composition, the G-proteins and the enzymatic machinery to employ the polyphosphoinositide–protein kinase C pathway in the regulation of cellular functions (Fisher & Agranoff, 1986). Furthermore, a growing number of receptors that are known to activate phospholipid turnover is found in excitable cells. However, little information is available as to what purpose the dual-signal pathway serves in neuronal cells. Activation of protein kinase C through phorbol esters has been reported to augment transmitter release at the neuromuscular junction (Shapira, Silberberg, Ginsburg & Rahamimoff, 1987), to induce secretion in permeabilized adrenal chromaffin cells (Knight & Baker, 1983; Pocotte *et al.* 1985) and to mimic cellular responses normally associated with long-term potentiation in brain slices (Hu *et al.* 1987).

If this signal transduction pathway included a G-protein it should be possible to mimic receptor-mediated responses by introducing GTP-γ-S into the cell. Consequently, it would be anticipated that secretion could be induced by GTP-γ-S through activation of protein kinase C, since this is the case following direct activation of this enzyme by phorbol esters. However, GTP-γ-S does not induce secretion in permeabilized chromaffin cells, but instead causes a rightward inhibitory shift of the concentration–response relationship of Ca^{2+}-dependent secretion (Knight, 1987). Other reports, demonstrating that phorbol esters caused a moderate leftward shift of the curve (Knight & Baker, 1983), have made it difficult to interpret secretion in terms of a G-protein-mediated activation of protein kinase C in these cells.

In Fig. 2 the responses of two excitable cell types are compared with a typical mast cell response following stimulation by internally administered GTP-γ-S. In adrenal chromaffin cells, repetitive Ca^{2+} transients can be elicited by GTP-γ-S (Fig. 2B). These Ca^{2+} transients are likely to reflect the generation of $InsP_3$ due to the G-protein-mediated activation of phospholipase C. This response closely resembles the actions of GTP-γ-S in mast cells (Fig. 2A). However, there is no secretory response induced by the nucleotide apart from a small capacitance

increase during the transient increases in $[Ca^{2+}]_i$. This clearly contrasts with the case of non-excitable mast cells where during the initial Ca^{2+} transients no appreciable secretion occurs, but instead degranulation proceeds as $[Ca^{2+}]_i$ returns to resting levels.

Pancreatic beta cells also show Ca^{2+} transients when stimulated by GTP-γ-S. The pattern of $[Ca^{2+}]_i$ changes depends on the ATP concentration provided by the pipette solution with which the cells are dialysed. At low ATP concentrations ($0{\cdot}2\,mmol\,l^{-1}$) in the intracellular pipette solution, GTP-γ-S usually evokes a single long-lasting Ca^{2+} transient (Fig. 2C). Nevertheless, capacitance increases can be seen after $[Ca^{2+}]_i$ has returned to pre-stimulus levels. In the presence of high levels of ATP ($3\,mmol\,l^{-1}$), a series of Ca^{2+} transients can be elicited which would suggest a major role for ATP in the cycling of uptake and release of intracellular Ca^{2+}. Interestingly enough, the pattern of intracellular Ca^{2+} transients induced by GTP-γ-S under hyperpolarizing voltage-clamp conditions is quite similar to the well-known repetitive bursting activity recorded under current-clamp (Matthews & Sakamoto, 1975), in spite of the fact that voltage-activated Ca^{2+} channels remain closed. It will be interesting to learn how excitable cells, and particularly beta cells, coordinate release from internal stores with Ca^{2+} influx driven by voltage-dependent Ca^{2+} channels. In the case of beta cells, it seems that secretion may be under dual control: elevated physiological levels of $[Ca^{2+}]_i$ are as effective as G-protein-mediated stimulation in inducing secretion. Parallel and synergistic actions of these two pathways provide an effective means for controlling insulin release through neurotransmitters as well as hormones or glucose.

It is evident, from what has been discussed above, that both excitable and non-excitable cells utilize Ca^{2+} in control of secretion. Whereas in excitable cells Ca^{2+} alone is sufficient to trigger secretion, it appears likely that non-excitable cells use Ca^{2+} as a modulator of secretion in addition to a synergistic signal provided by another second messenger system (Fig. 3). So far, the discussion has centred around the role of $[Ca^{2+}]_i$ in secretion. But what are the mechanisms by which excitable and non-excitable cells bring about changes in intracellular calcium concentration?

Voltage-gated calcium channels

In excitable cells, the predominant mechanism by which an increase in $[Ca^{2+}]_i$ is accomplished is the pathway provided by voltage-activated Ca^{2+} channels that allow extracellular Ca^{2+} to permeate the plasma membrane down its electrochemical gradient (Hagiwara, 1983). At least three different types of voltage-activated Ca^{2+} channels may be distinguished based upon their electrophysiological and pharmacological properties (Nowycky, Fox & Tsien, 1985). The trigger for the opening of these channels is provided by the depolarization occurring during the action potential.

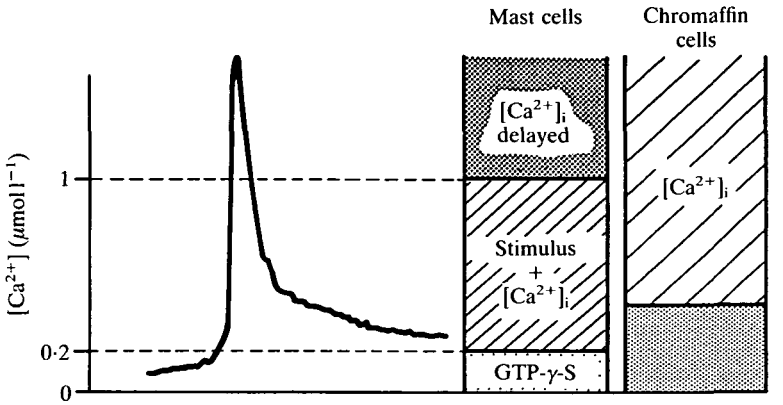

Fig. 3. Comparison of the Ca^{2+} requirements for exocytosis between non-excitable cells (mast cells) and excitable cells (chromaffin cells). The bar graphs indicate $[Ca^{2+}]_i$ ranges and conditions under which secretion occurs. For chromaffin cells no stimuli have yet been found that would induce secretion at $[Ca^{2+}]_i$ below basal values. At higher concentrations, calcium alone is a sufficient stimulus. In mast cells GTP-γ-S can induce secretion at sub-basal $[Ca^{2+}]_i$. In an intermediate range calcium enhances secretion induced by secretagogues. Only at unphysiologically high $[Ca^{2+}]_i$ is calcium a secretagogue by itself, acting only after a delay of some tens of seconds. The left part shows a typical calcium signal for comparison (see also Figs 2, 4). In mast cells the spiking portion of the transient is too short-lived to induce secretion by itself, whereas the shoulder is ideally timed to enhance secretion (see also Fig. 4C).

In Fig. 4A the effect of membrane depolarization on $[Ca^{2+}]_i$ is exemplified for adrenal chromaffin cells. In this experiment the cell was clamped at a holding potential of −70 mV and subjected to depolarizing voltage pulses for periods of 4 s. Three different potential levels were chosen to illustrate the basic properties of voltage-activated Ca^{2+} currents. The first sequence of voltage pulses clamped the membrane potential from −70 mV to −30 mV which is just above the threshold for Ca^{2+} channel activation. In the $[Ca^{2+}]_i$ trace this is accompanied by small increases in $[Ca^{2+}]_i$ during the depolarizing episodes, and decreases in $[Ca^{2+}]_i$ as the cell is repolarized to −70 mV. A sequence of stronger depolarizations to 0 mV, which fully activate Ca^{2+} channels, elicits larger Ca^{2+} transients during the depolarization. Stronger depolarizations into the range of +50 mV, which also fully activate Ca^{2+} channels, are not as effective in increasing $[Ca^{2+}]_i$, because approaching the reversal potential for Ca^{2+} reduces the driving force for Ca^{2+}. Thus, the observed changes in $[Ca^{2+}]_i$ reflect the current–voltage relationship of voltage-activated calcium currents. A similar parallelism has been observed for currents and capacitance changes (Clapham & Neher, 1984).

Fluxes through Ca^{2+} channels are regulated by several mechanisms. Hyperpolarization, through voltage changes and/or by Ca^{2+}-activated K^+ currents, deactivates Ca^{2+} channels, whereas increased $[Ca^{2+}]_i$ provides negative feedback control by inactivating Ca^{2+} channels (Eckert & Chad, 1984). These mechanisms constitute an effective and fast safety device to prevent Ca^{2+} overload. In

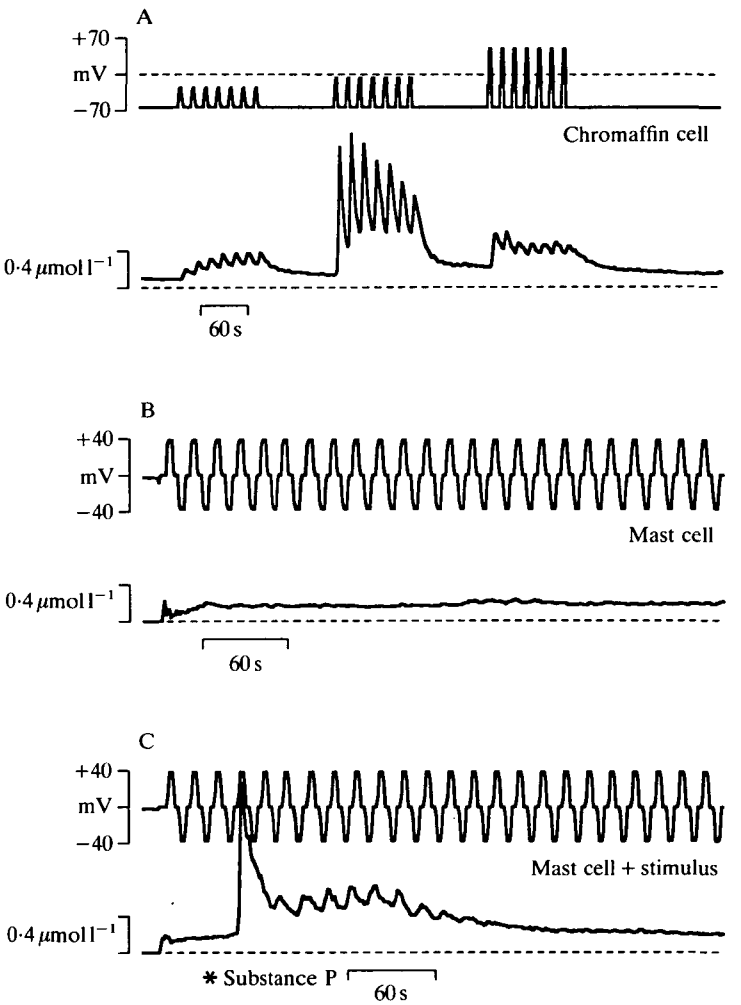

Fig. 4. Voltage-dependence of $[Ca^{2+}]_i$ in excitable cells and non-excitable cells. (A) The chromaffin cell was subjected to various depolarizing voltage pulses of 4 s duration as indicated by the voltage trace (see text for details). Bath solution *A* and standard internal solution (see Fig. 1) were used. (B) The mast cell (exposed to bath solution *B* and standard internal solution, see Fig. 1) was stepped to various depolarized and hyperpolarized voltages as indicated in the voltage trace. No voltage-dependent changes in $[Ca^{2+}]_i$ are associated with either depolarization or hyperpolarization. (C) Same conditions as in B, except that the mast cell was stimulated with substance P (50 $\mu g\,ml^{-1}$) at the indicated time (*). Following the initial fast Ca^{2+} transient due to phospholipid turnover there is a phase in which $[Ca^{2+}]_i$ becomes voltage-dependent. Presumably, second messenger-activated channels are opened and $[Ca^{2+}]_i$ increases as the driving force for Ca^{2+} is high (during the hyperpolarized episodes) (see Matthews, Neher & Penner, 1988).

addition, long-term modulatory mechanisms regulate the amount of Ca^{2+} influx by affecting the open probability of Ca^{2+} channels. Most prominent is the capability of cyclic nucleotides (cyclic AMP and cyclic GMP) or catalytic subunits of cyclic-AMP-dependent and cyclic-GMP-dependent protein kinase to increase Ca^{2+} currents *via* phosphorylation (Hescheler, Kameyama & Trautwein, 1986; Paupardin-Tritsch *et al.* 1986). Similarly, injection of protein kinase C into mollusc neurones causes an increase of Ca^{2+} current (DeRiemer *et al.* 1985), whereas injection into chick dorsal root ganglion neurones causes a decrease in Ca^{2+} current (Rane & Dunlap, 1986). Interestingly, purified exogenous G-proteins added to a recording patch pipette in the whole-cell configuration can substitute for endogenous G-proteins in the modulation of Ca^{2+} channels by opioids (Hescheler *et al.* 1986). This suggests that G-proteins may interact directly with Ca^{2+} channels, in analogy with the case of K^+ channels in heart cells, as discussed by Dunlap, Holz & Rane (1987).

Release of calcium from intracellular stores

Since, by definition, non-excitable cells lack voltage-activated Ca^{2+} channels, other mechanisms for $[Ca^{2+}]_i$ control must be present. In recent years a new second messenger system has gained much attention as it provides a mechanism to increase $[Ca^{2+}]_i$ by releasing Ca^{2+} from internal stores. No electrical activity and no external source for Ca^{2+} is required for this process. Following receptor occupancy, a G-protein mediates the activation of phospholipase C which hydrolyses membrane-integral polyphosphoinositides yielding two second messengers: inositol-(1,4,5)-trisphosphate (Ins(1,4,5)P_3) and diacylglycerol (DAG) (see Berridge & Irvine, 1984 for a review). InsP_3 is well established as a cause of release of Ca^{2+} from the endoplasmic reticulum while DAG activates protein kinase C such that its affinity towards Ca^{2+} is increased. This dual signal is predestined to promote cellular responses synergistically.

Due to the limited accessibility of intracellular organelles, little information and much speculation is available about how release of Ca^{2+} from internal stores is regulated. It is believed that InsP_3 mediates the opening of some channel-like structure through which Ca^{2+} flows down its concentration gradient. The InsP_3-induced Ca^{2+} release may be caused by putative Ca^{2+} channels in the endoplasmic reticulum membrane, either directly gated by InsP_3 or possibly indirectly activated through a GTP-binding protein (Dawson, 1985). In mast cells, however, agonist-induced release of Ca^{2+} from internal stores occurs even at GTP levels that fail to support secretion (Penner *et al.* 1987).

InsP_3-induced release of Ca^{2+} from internal stores is typically transient in nature (see Fig. 2). Several mechanisms could curtail Ca^{2+} release induced by InsP_3. These include Ca^{2+}-dependent or time-dependent inactivation of putative endoplasmic reticulum Ca^{2+} channels in analogy to plasma membrane Ca^{2+} channels, and desensitization of the InsP_3 receptor. Modulation of polyphosphoinositide breakdown may also occur at the level of phospholipase C. So far,

evidence for inhibition of phosphoinositide (PI) breakdown by cyclic AMP and cyclic GMP has been presented (Knight & Scrutton, 1984). Furthermore, the phospholipase A_2 pathway through arachidonic acid and its metabolites may interact with the phospholipase C pathway by enhancing or reducing PI breakdown (see Rubin, 1986, and references cited therein). The removal of the agonist InsP_3 may serve as an additional mechanism for terminating Ca^{2+} release. Fast degradation of InsP_3 due to phosphatases as well as the generation of a number of different inositol phosphates, including other forms of InsP_3, InsP_4 and cyclic derivatives, are known (see Irvine, 1986 for a review). Finally, by analogy with desensitization of the β-adrenergic receptor (Sibley & Lefkowitz, 1985), receptor desensitization has been reported to occur following activation of protein kinase C by phorbol esters (Kelleher, Pessin, Ruoho & Johnson, 1984). As a result, receptor stimulation is ineffective in releasing inositol phosphates (Orellana, Solski & Brown, 1985; Watson & Lapetina, 1985). Neither degradation nor negative feedback can be responsible for the decline in $[Ca^{2+}]_i$ seen in experiments where InsP_3 was directly injected into mast cells (Neher, 1986), since InsP_3 is constantly supplied from the recording pipette in the absence of receptor stimulation.

Another modulatory component of the Ca^{2+} release process has emerged from the finding that replenishment of intracellular stores may involve complicated regulation. Apparently, the stores cannot be refilled in the sustained presence of receptor agonists (Berridge & Fain, 1979). Furthermore, the emptying and refilling of the internal stores, as proposed by the capacitative model of Ca^{2+} release and uptake (Putney, 1986), has been suggested to be under the control of InsP_3 or InsP_4 or a combination of both (Putney, 1987). InsP_3 and InsP_4 are also candidates as agonists for the gating of plasma membrane Ca^{2+} channels (see Houslay, 1987).

Second messenger and receptor-operated calcium channels

As emphasized above, excitable cells mainly utilize voltage-activated Ca^{2+} channels to bring about changes in $[Ca^{2+}]_i$. In addition, they are able to release Ca^{2+} from internal stores by using the ubiquitous second messenger system of PI turnover. Yet another conceptually distinct way of allowing changes in $[Ca^{2+}]_i$ is provided by so-called receptor-operated channels in excitable cells and may possibly be realized also in non-excitable cells. Some classical receptor-operated channels like the acetylcholine or glutamate receptor are known to be permeant also to Ca^{2+} (Lewis, 1979; MacDermott *et al.* 1986). Fluxes through the acetylcholine receptor channel are modulated by $[Ca^{2+}]_i$ (Nastuk, 1977; Miledi, 1980), cyclic AMP (presumably *via* phosphorylation through cyclic-AMP-dependent kinase) (Huganir, Delcour, Greengard & Hess, 1986) and GTP-γ-S (presumably *via* phosphorylation through DAG-activated protein kinase C) (Eusebi, Grassi, Molinaro & Zani, 1987). In addition to their Ca^{2+} permeability these

channels determine the general excitability of cells, such that their regulation also affects the Ca^{2+} influx through voltage-operated Ca^{2+} channels. More recently, a more Ca^{2+}-specific receptor-operated channel has been described in smooth muscle cells (Benham & Tsien, 1987). This channel is gated by the putative sympathetic neurotransmitter ATP, apparently without the involvement of second messengers. Presumably, more receptor-operated Ca^{2+} channels in excitable and non-excitable cells (where they have been postulated based on tracer flux studies) await discovery. In analogy to these receptor-operated channels which are gated by external ligands, there are channels that are gated by internal ligands and may be termed second messenger-activated channels. Well-known examples of such channels are the cyclic GMP-dependent conductance in photoreceptors (Fesenko, Kolesnikov & Lyubarski, 1985) and the cyclic AMP-dependent currents in olfactory receptor cells (Nakamura & Gold, 1987).

In non-excitable cells, Ca^{2+} release from internal stores through second messengers appears to be the primary mechanism by which $[Ca^{2+}]_i$ is regulated. However, as originally proposed (Michell, 1975), and only recently overshadowed by the excitement caused by the discovery of internal Ca^{2+} release, increasing evidence suggests that Ca^{2+} influx from the extracellular space may be just as important. In many cell types $[Ca^{2+}]_i$ shows a characteristic diphasic behaviour (Rasmussen & Barrett, 1984). An initial transient increase in $[Ca^{2+}]_i$ that is independent of extracellular Ca^{2+} is followed by a more sustained phase which depends on external $[Ca^{2+}]$ and is believed to result from changes in plasma membrane permeability for Ca^{2+} induced by second messengers. There is only little but promising information available about the gating of these so-called second messenger-activated channels in the plasma membrane. One mechanism of Ca^{2+} influx present in neutrophils may depend on $[Ca^{2+}]_i$ itself (presumably brought about by initial receptor-stimulated release of Ca^{2+} from intracellular stores) in such a way that $[Ca^{2+}]_i$-gated cation channels permeant to Ca^{2+} are activated (von Tscharner, Prod'hom, Baggiolini & Reuter, 1986). In other cells, InsP_3 may open not only Ca^{2+} channels in the endoplasmic reticulum but also Ca^{2+}-permeant channels in the plasma membrane, as reported in lymphocytes (Kuno & Gardner, 1987). A membrane current with similar properties has been observed in mast cells (Matthews, Neher & Penner, 1988). Modulation of a class of Ca^{2+} channels by InsP_3 has also been suggested to occur in *Xenopus* oocytes (Parker & Miledi, 1987*a*). In the same cells, injection of InsP_4 (physiologically derived from InsP_3 and subsequent phosphorylation by the InsP_3-kinase) is believed to activate voltage-sensitive Ca^{2+} channels *via* a process that may require 'priming' by InsP_3 (Parker & Miledi, 1987*b*). This is reminiscent of the findings in sea urchin eggs, where InsP_4 was suggested to control Ca^{2+} entry across the plasma membrane (Irvine & Moor, 1986). Similarly, synergistic actions of InsP_3 and InsP_4 have been reported in lacrimal gland cells (Morris, Gallacher, Irvine & Petersen, 1987). In these, InsP_4 greatly augments and prolongs InsP_3-induced $[Ca^{2+}]_i$ increases (as measured by increased activity of Ca^{2+}-activated K^+ channels). In another study, however, injection of GTP-γ-S or InsP_3 alone

produced notable $[Ca^{2+}]_i$ responses that were dependent on extracellular Ca^{2+} and membrane voltage (Llano, Marty & Tanguy, 1987).

In conclusion, it may appear surprising how many diverse and elaborate mechanisms have evolved in controlling and modulating the intracellular calcium concentration. However, given the central role of $[Ca^{2+}]_i$ in so many cellular processes, we should be prepared to learn in the not too distant future that our current understanding of the mechanisms involved in Ca^{2+} regulation is far too simplistic.

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References

BARROWMAN, M. M., COCKCROFT, S. & GOMPERTS, B. D. (1986). Two roles for guanine nucleotides in the stimulus–secretion sequence of neutrophils. *Nature, Lond.* **319**, 504–507.

BEAVAN, M. A., GUTHRIE, D. F., MOORE, J. P., SMITH, G. A., HESKETH, T. R. & METCALFE, J. C. (1987). Synergistic signals in the mechanism of antigen-induced exocytosis in 2H3 cells: Evidence for an unidentified signal required for histamine release. *J. Cell Biol.* **105**, 1129–1136.

BENHAM, C. D. & TSIEN, R. W. (1987). A novel receptor-operated Ca^{2+}-permeable channel activated by ATP in smooth muscle. *Nature, Lond.* **328**, 275–278.

BERRIDGE, M. J. (1987). Inositol trisphosphate and diacylglycerol: Two interacting second messengers. *A. Rev. Biochem.* **56**, 159–193.

BERRIDGE, M. J. & FAIN, J. N. (1979). Inhibition of phosphatidylinositol synthesis and the inactivation of calcium entry after prolonged exposure of the blowfly salivary gland to 5-hydroxytryptamine. *Biochem. J.* **178**, 59–69.

BERRIDGE, M. J. & IRVINE, R. F. (1984). Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature, Lond.* **312**, 315–321.

CLAPHAM, D. E. & NEHER, E. (1984). Trifluoperazine reduces inward ionic currents and secretion by separate mechanisms in bovine chromaffin cells. *J. Physiol., Lond.* **353**, 541–564.

COCHRANE, D. E. & DOUGLAS, W. W. (1974). Calcium-induced extrusion of secretory granules (exocytosis) in mast cells exposed to 48/80 or the ionophores A-23187 and X-537A. *Proc. natn. Acad. Sci. U.S.A.* **71**, 408–412.

DAWSON, A. P. (1985). GTP enhances inositol trisphosphate-stimulated Ca^{2+} release from rat liver microsomes. *FEBS Letts* **185**, 147–150.

DERIEMER, S. A., STRONG, J. A., ALBERT, K. A., GREENGARD, P. & KACZMAREK, L. K. (1985). Enhancement of calcium current in *Aplysia* neurones by phorbol ester and protein kinase C. *Nature, Lond.* **313**, 313–316.

DI VIRGILIO, F., LEW, D. P. & POZZAN, T. (1984). Protein kinase C activation of physiological processes in human neutrophils at vanishingly small cytosolic Ca^{2+} levels. *Nature, Lond.* **310**, 691–693.

DOUGLAS, W. W. (1968). Stimulus–secretion coupling: The concept and clues from chromaffin and other cells. *Br. J. Pharmac.* **34**, 451–474.

DUNLAP, K., HOLZ, G. G. & RANE, S. G. (1987). G proteins as regulators of ion channel function. *Trends Neurosci.* **10**, 241–244.

ECKERT, R. & CHAD, J. E. (1984). Inactivation of Ca channels. *Prog. Biophys. molec. Biol.* **44**, 215–267.

EUSEBI, F., GRASSI, F., MOLINARO, M. & ZANI, B. M. (1987). Acetylcholine regulation of nicotinic receptor channels through a putative G protein in chick myotubes. *J. Physiol., Lond.* **393**, 635–645.

FEINMAN, R. D. & DETWILER, T. C. (1974). Platelet secretion induced by divalent cation ionophores. *Nature, Lond.* **249**, 172–173.

FENWICK, E. M., MARTY, A. & NEHER, E. (1982). A patch-clamp study of bovine chromaffin cells and of their sensitivity to acetylcholine. *J. Physiol., Lond.* **331**, 577–597.

FERNANDEZ, J. M., NEHER, E. & GOMPERTS, B. D. (1984). Capacitance measurements reveal stepwise fusion events in degranulating mast cells. *Nature, Lond.* **312**, 453–455.

FESENKO, E. E., KOLESNIKOV, S. S. & LYUBARSKI, A. L. (1985). Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segment. *Nature, Lond.* **313**, 310–313.

FISHER, S. K. & AGRANOFF, B. W. (1986). Phosphoinositide turnover in the CNS and in neural-related tissues. In *Receptor Biochemistry and Methodology* (ed. J. C. Venter & L. C. Harrison), vol. 7, *Phosphoinositides and Receptor Mechanisms* (ed. J. W. Putney), pp. 219–243. New York: A. R. Liss.

FOREMAN, J. C., HALLETT, M. B. & MONGAR, J. L. (1977). The relationship between histamine secretion and ^{45}calcium uptake by mast cells. *J. Physiol., Lond.* **271**, 193–214.

GOMPERTS, B. D. & FERNANDEZ, J. M. (1985). Techniques for membrane permeabilization. *Trends biochem. Sci.* **10**, 414–417.

GRYNKIEWICZ, G., POENIE, M. & TSIEN, R. Y. (1985). A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. biol. Chem.* **260**, 3440–3450.

HAGIWARA, S. (1983). *Membrane Potential-dependent Ion Channels in Cell Membrane.* New York: Raven Press.

HASLAM, R. J. & DAVIDSON, M. L. (1984). Guanine nucleotides decrease the free $[Ca^{2+}]$ required for secretion of serotonin from permeabilized blood platelets. Evidence of a role for a GTP-binding protein in platelet activation. *FEBS Letts* **174**, 90–95.

HEIMAN, A. S. & CREWS, F. T. (1985). Characterization of the effects of phorbol esters on rat mast cell secretion. *J. Immunol.* **134**, 548–554.

HESCHELER, J., KAMEYAMA, M. & TRAUTWEIN, W. (1986). On the mechanism of muscarinic inhibition of the cardiac Ca current. *Pflügers Arch. ges. Physiol.* **407**, 182–189.

HOUSLAY, M. D. (1987). Egg activation unscrambles a potential role for IP_4. *Trends biochem. Sci.* **12**, 1–2.

HOWELL, T. W., COCKCROFT, S. & GOMPERTS, B. D. (1987). Essential synergy between Ca^{2+} and guanine nucleotides in exocytotic secretion from permeabilized rat mast cells. *J. Cell Biol.* **105**, 191–197.

HU, G.-Y., HVALBY, Ø., WALAAS, S. I., ALBERT, K. A., SKJEFLO, P., ANDERSEN, P. & GREENGARD, P. (1987). Protein kinase C injection into hippocampal pyramidal cells elicits features of long term potentiation. *Nature, Lond.* **328**, 426–429.

HUGANIR, L. R., DELCOUR, A. H., GREENGARD, P. & HESS, G. P. (1986). Phosphorylation of the nicotinic acetylcholine receptor regulates its rate of desensitization. *Nature, Lond.* **321**, 774–776.

IRVINE, R. F. (1986). The structure, metabolism, and analysis of inositol lipids and inositol phosphates. In *Receptor Biochemistry and Methodology* (ed. J. C. Venter & L. C. Harrison), vol. 7, *Phosphoinositides and Receptor Mechanisms* (ed. J. W. Putney), pp. 89–107. New York: A. R. Liss.

IRVINE, R. F. & MOOR, R. M. (1986). Micro-injection of inositol 1,3,4,5-tetrakisphosphate activates sea urchin eggs by a mechanism dependent on external calcium. *Biochem. J.* **240**, 917–920.

KANNO, T., COCHRANE, D. E. & DOUGLAS, W. W. (1973). Exocytosis (secretory granule extrusion) induced by injection of calcium into mast cells. *Can. J. Physiol. Pharmac.* **51**, 1001–1004.

KATZ, B. (1969). *The Release of Neural Transmitter Substances.* Liverpool: Liverpool University Press.

KELLEHER, D. J., PESSIN, J. E., RUOHO, A. E. & JOHNSON, G. L. (1984). Phorbol ester induces desensitization of adenylate cyclase and phosphorylation of the β-adrenergic receptor in turkey erythrocytes. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4316–4320.

KNIGHT, D. E. (1987). Calcium and diacylglycerol control of secretion. *Biosci. Rep.* **7**, 355–367.

KNIGHT, D. E. & BAKER, P. F. (1982). Calcium-dependence of catecholamine release from bovine adrenal medullary cells after exposure to intense electric fields. *J. Membr. Biol.* **68**, 107–140.

KNIGHT, D. E. & BAKER, P. F. (1983). The phorbol ester TPA increases the affinity of exocytosis for calcium in "leaky" adrenal medullary cells. *FEBS Letts* **160**, 98–100.

KNIGHT, D. E. & SCRUTTON, M. C. (1984). Cyclic nucleotides control a system which regulates Ca^{2+} sensitivity of platelet secretion. *Nature, Lond.* **309**, 66–68.

KNIGHT, D. E. & SCRUTTON, M. C. (1986). Gaining access to the cytosol: the technique and some applications of electropermeabilization. *Biochem. J.* **234**, 497–506.

KUNO, M. & GARDNER, PH. (1987). Ion channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T-lymphocytes. *Nature, Lond.* **326**, 301–304.

LEWIS, C. A. (1979). Ion-concentration dependence of the reversal potential and the single channel conductance of ion channels at the frog neuromuscular junction. *J. Physiol., Lond.* **286**, 417–445.

LLANO, I., MARTY, A. & TANGUY, J. (1987). Dependence of intracellular effects of GTP-γ-S and inositoltrisphosphate on cell membrane potential and on external Ca ions. *Pflügers Arch. ges. Physiol.* **409**, 499–506.

MACDERMOTT, A. B., MAYER, M. L., WESTBROOK, G. L., SMITH, S. J. & BARKER, J. L. (1986). NMDA-receptor activation elevates cytoplasmic calcium in cultured spinal cord neurones. *Nature, Lond.* **321**, 519–522.

MATTHEWS, E. K. & SAKAMOTO, Y. (1975). Electrical characteristics of pancreatic islet cells. *J. Physiol., Lond.* **246**, 421–437.

MATTHEWS, G. G., NEHER, E. & PENNER, R. (1988). Second messengers activate ionic currents in rat peritoneal mast cells. *Pflügers Arch. ges. Physiol.* (in press).

MICHELL, R. H. (1975). Inositol phospholipids and cell surface receptor function. *Biochim. biophys. Acta* **415**, 81–147.

MILEDI, R. (1980). Intracellular calcium and desensitization of acetylcholine receptors. *Proc. R. Soc. Ser.* B **209**, 447–452.

MORRIS, A. P., GALLACHER, D. V., IRVINE, R. F. & PETERSEN, O. H. (1987). Synergism of inositol trisphosphate and tetrakisphosphate in activating Ca^{2+}-dependent K^+ channels. *Nature, Lond.* **330**, 653–655.

NAKAMURA, T. & GOLD, G. H. (1987). A cyclic nucleotide-gated conductance in olfactory receptor cilia. *Nature, Lond.* **325**, 442–444.

NASTUK, W. L. (1977). Cholinergic receptor desensitization. In *Synapses* (ed. G. A. Cottrel & P. N. R. Usherwood), pp. 177–201. London: Blackie.

NEHER, E. (1986). Inositol 1,4,5-trisphosphate and GTP-γ-S induce calcium transients in isolated rat peritoneal mast cells. *J. Physiol., Lond.* **381**, 71P.

NEHER, E. (1988). The influence of intracellular calcium concentration on degranulation of dialyzed mast cells from rat peritoneum. *J. Physiol., Lond.* **395**, 193–214.

NEHER, E. & ALMERS, W. (1986). Fast calcium transients in rat peritoneal mast cells are not sufficient to trigger exocytosis. *EMBO J.* **5**, 51–53.

NEHER, E. & MARTY, A. (1982). Discrete changes of cell membrane capacitance observed under conditions of enhanced secretion in bovine adrenal chromaffin cells. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6712–6716.

NEHER, E. & PENNER, R. (1988). The influence of intracellular calcium concentration on the secretory response of mast cells. *Proc. A. Benzon Symp.* (in press).

NOWYCKY, M. C., FOX, A. P. & TSIEN, R. W. (1985). Three types of neuronal calcium channel with different calcium agonist sensitivity. *Nature, Lond.* **316**, 440–443.

ORELLANA, S. A., SOLSKI, P. A. & BROWN, J. H. (1985). Phorbol ester inhibits phosphoinositide hydrolysis and calcium mobilization in cultured astrocytoma cells. *J. biol. Chem.* **260**, 5236–5239.

PARKER, I. & MILEDI, R. (1987*a*). Inositol trisphosphate activates a voltage-dependent calcium influx in *Xenopus* oocytes. *Proc. R. Soc. Ser.* B **231**, 27–36.

PARKER, I. & MILEDI, R. (1987*b*). Injection of inositol 1,3,4,5-tetrakisphosphate into *Xenopus* oocytes generates a chloride current dependent upon intracellular calcium. *Proc. R. Soc. Ser.* B **232**, 59–70.

PAUPARDIN-TRITSCH, D., HAMMOND, C., GERSCHENFELD, H. M., NAIRN, A. & GREENGARD, P. (1986). cGMP-dependent protein kinase enhances Ca^{2+} current and potentiates the serotonin-induced Ca^{2+} current increase in snail neurones. *Nature, Lond.* **323**, 812–814.

PENNER, R. & NEHER, E. (1988). Secretory responses of rat peritoneal mast cells to high intracellular calcium. *FEBS Letts* **226**, 307–313.

PENNER, R., PUSCH, M. & NEHER, E. (1987). Washout phenomena in dialyzed mast cells allow discrimination of different steps in stimulus–secretion coupling. *Biosci. Rep.* **7**, 313–321.

POCOTTE, S. L., FRYE, R. A., SENTER, R. A., TERBUSH, D. R., LEE, S. A. & HOLZ, R. W. (1985). Effects of phorbol ester on catecholamine secretion and protein phosphorylation in adrenal medullary cell cultures. *Proc. natn. Acad. Sci. U.S.A.* **82**, 930–934.

PUTNEY, J. W., JR (1986). A model for receptor-regulated calcium entry. *Cell Calcium* **7**, 1–12.

PUTNEY, J. W., JR (1987). Calcium-mobilizing receptors. *Trends pharmac. Sci.* **8**, 481–486.

RANE, S. G. & DUNLAP, K. (1986). Kinase C activator 1,2-oleoylacetylglycerol attenuates voltage-dependent calcium current in sensory neurons. *Proc. natn. Acad. Sci. U.S.A.* **83**, 184–188.

RASMUSSEN, H. & BARRETT, P. Q. (1984). Calcium messenger system: An integrated view. *Physiol. Rev.* **64**, 938–984.

RINK, T. J., SANCHEZ, A. & HALLAM, T. J. (1983). Diacylglycerol and phorbol ester stimulate secretion without raising cytoplasmic free calcium in human platelets. *Nature, Lond.* **305**, 317–319.

RORSMAN, P. & TRUBE, G. (1985). Glucose dependent K^+-channels in pancreatic β-cells are regulated by intracellular ATP. *Pflügers Arch. ges. Physiol.* **405**, 305–309.

RUBIN, R. P. (1986). Inositol lipids and cell secretion. In *Receptor Biochemistry and Methodology* (ed. J. C. Venter & L. C. Harrison), vol. 7, *Phosphoinositides and Receptor Mechanisms* (ed. J. W. Putney), pp. 149–162. New York: A. R. Liss.

RUBIN, R. P., SINK, L. E. & FREER, R. J. (1981). Activation of (arachidonyl)phosphatidylinositol turnover in rabbit neutrophils by the calcium ionophore A23187. *Biochem. J.* **194**, 497–505.

SAGI-EISENBERG, R., LIEMAN, H. & PECHT, I. (1985). Protein kinase C regulation of the receptor-coupled calcium signal in histamine-secreting rat basophilic leukaemia cells. *Nature, Lond.* **313**, 59–60.

SHA'AFI, R. I., WHITE, J. R., MOLSKI, T. F. P., SHEFCYK, J., VOLPI, M., NACCACHE, P. H. & FEINSTEIN, M. B. (1983). Phorbol 12-myristate 13-acetate activates rabbit neutrophils without an apparent rise in the level of intracellular free calcium. *Biochem. biophys. Res. Commun.* **114**, 638–645.

SHAPIRA, R., SILBERBERG, S. D., GINSBURG, S. & RAHAMIMOFF, R. (1987). Activation of protein kinase C augments evoked transmitter release. *Nature, Lond.* **325**, 58–60.

SIBLEY, D. R. & LEFKOWITZ, R. J. (1985). Molecular mechanisms of receptor desensitization using the β-adrenergic receptor-coupled adenylate cyclase system as a model. *Nature, Lond.* **317**, 124–129.

TAKEMURA, H. (1985). Changes in free cytosolic calcium concentration in isolated rat parotid cells by cholinergic and β-adrenergic agonists. *Biochem. biophys. Res. Commun.* **131**, 1048–1055.

TSIEN, R. Y., POZZAN, T. & RINK, T. J. (1984). Measuring and manipulating cytosolic Ca^{2+} with trapped indicators. *Trends biochem. Sci.* **9**, 263–266.

VON TSCHARNER, V., PROD'HOM, B., BAGGIOLINI, M. & REUTER, H. (1986). Ion channels in human neutrophils activated by a rise in free cytosolic calcium concentration. *Nature, Lond.* **324**, 369–372.

WATSON, S. P. & LAPETINA, E. L. (1985). 1,2-Diacylglycerol and phorbol ester inhibit agonist-induced formation of inositol phosphates in human platelets: Possible implications for negative feedback regulation of inositol phospholipid hydrolysis. *Proc. natn. Acad. Sci. U.S.A.* **82**, 2623–2626.

WOLLHEIM, C. B., ULLRICH, S., MEDA, P. & VALLAR, L. (1987). Regulation of exocytosis in electrically permeabilized insulin-secreting cells. Evidence for Ca^{2+} dependent and independent secretion. *Biosci. Rep.* **7**, 443–454.