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SHORT REVIEW

CALCIUM SIGNALLING IN SECRETORY CELLS

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ABSTRACT

Stimulation of secretory cells with muscarinic agonists leads to an increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_{i)}$, which activates protein secretion through exocytosis and causes closure of gap junctions between adjacent cells. In addition, the increase in $[Ca^{2+}]_i$ activates three different kinds of ion channels: large K^+ channels, Cl^- channels and non-specific cation channels. The opening of those channels leads to an increase of $[Na^+]$ and a decrease of $[Cl^-]$ and $[K^+]$ in the cell.

The two components that contribute to the increase in $[Ca^{2+}]_i$ are calcium release from intracellular stores, localised in the endoplasmic reticulum and calcium influx through the plasma membrane. Several models for the regulation of $[Ca^{2+}]_i$ have been proposed, including a recently suggested model whereby a distinct pathway involving arachidonic acid is added to the well-established capacitative model. Different hypotheses concerning coupling between the intracellular calcium stores and membrane channels co-exist.

In addition to a historical overview, recent developments and future challenges are discussed in this review.

KEYWORDS: Calcium signalling, muscarinic agonists, secretion, Inositol 1, 4, 5-triphosphate, capacitive model.

INTRODUCTION

The overall homeostasis of the body is maintained in different ways. Ion and fluid secretion mechanisms both are necessary for homeostasis and therefore these mechanisms are of great importance. The process of secretion may seem rather simple at first sight, but the wide variety in secretion mechanisms for different body functions and the intricate underlying intracellular signalling systems betray its actual complexity. This review will deal with ion and fluid secretion control in exocrine glands. An important underlying intracellular signalling system for the regulation of secretion involves changes in the cytosolic Ca^{2+} concentration $[Ca^{2+}]_i$. Different models for control of those changes in $[Ca^{2+}]_i$ exist.

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Besides an historical overview of the research in this field, the most recent developments and future challenges are given in this review.

IONIC SECRETORY MECHANISM

Many researchers investigated the secretory mechanism during the 1980s and 1990s. During the 1980s, it became clear that muscarinic agonists cause conductance changes via Ca^{2+} signalling. A large increase in available information about the ionic mechanisms has occurred with the introduction of fluorescent probes in the early 1980s. This revealed a lot about the spatial and temporal fluctuations in $[Ca^{2+}]_i$ levels.

The rise in cytosolic Ca^{2+} concentration, both from internal release of Ca^{2+} from stores localised in the endoplasmatic reticulum and from Ca^{2+} influx through the plasma membrane activates three types of channels: large K^+, Cl^- and monovalent cation channels (Marty et

al., 1986). The opening of those channels initiates secretory activity. As a result of ion channel opening, the intracellular concentration of K^+ and Cl^- decreases, which leads to cell shrinkage because water leaves the cell osmotically. Whether water loss is also directly regulated by $[Ca^{2+}]_i$ is still unclear (Ishikawa et al., 1998).

In addition, the intracellular conversion of CO_2 into H^+ and HCO_3^- causes a Na^+ gain via a Na^+/H^+ exchange mechanism that works parallel with a Cl^-/HCO_3^- exchange mechanism. This Na^+ increase activates the Na^+/K^+-pump, which results in loss of Na^+ from the cell. Besides, the $Na^+/H^+/2\ Cl^-$ cotransporter is responsible for maintaining a high intracellular concentration of Cl^-. The secreted fluid is plasmalike and contains high concentrations of Na^+ and Cl^-. This model of fluid secretion has proven to be a sufficient answer to research questions concerning fluid composition and ionic mechanisms that have been formulated in the mid-1980s.

CONTROL OF CA^{2+} CONCENTRATION

Although extensive study in the 1990s into the mechanisms in secretory cells that underlie secretion activity has clarified a lot about the role of $[Ca^{2+}]_i$, new assumptions and model adaptations are still being made. The different signalling pathways that are involved in regulating the intracellular Ca^{2+} concentration are very complicated and closely interrelated. During the last five years, a lot of research was performed into the coupling between stimulation with muscarinic agonists and both the release and the influx of Ca^{2+}. In recent years, increasingly detailed knowledge concerning the chain of intracellular events following muscarinic stimulation became available.

$[Ca^{2+}]_i$ in resting cells is maintained at around 100 nM, while extracellular Ca^{2+} concentrations are about four orders of magnitude higher. Thus, in order to maintain this low intracellular concentration, the cell has to resist a strong driving force for Ca^{2+} influx. This is achieved by Ca^{2+} pumps in the plasma membrane and in the endoplasmatic reticulum, that serve to pump Ca^{2+} from the cytosol either out of the cell or into the endoplasmatic reticulum (Ambudkar, 2000). Ca^{2+} influx is mediated through channels in the cell membrane and in the endoplasmatic reticular membrane. Those mechanisms interact closely to regulate signalling changes and to prevent increases in $[Ca^{2+}]_i$ to toxic levels.

Two types of Ca^{2+} signalling responses can follow stimulation. Which one occurs depends on the level of agonist concentration. If this concentration is relatively high, the induced $[Ca^{2+}]_i$ signal consists of an initial rapid rise, which is independent of the extracellular Ca^{2+} concentration, but which is caused by release from intracellular stores (Takemura & Putney, 1989). This increase is followed by a decrease to a constant level: the so-called ‘plateau’. Because of the limited amount of intracellular stored Ca^{2+}, this plateau can only be sustained by entry from extracellular Ca^{2+} (Putney, 1986).

As a result of extensive study of the mechanism that controls Ca^{2+} release an important messenger, inositol 1, 4, 5 triphosphate ($InsP_3$), has been discovered. The first link between muscarinic stimulation and an increase in inositol was found in 1964 in an experiment on cells from the avian salt-secreting nasal gland (Hokin & Hokin, 1964). In this experiment, stimulation led to an increase in inositol-containing membrane phospholipids. It took 15 years before a direct causal relation between a Ca^{2+} increase and inositol could be shown (Shuttleworth, 1997). A landmark in the field of calcium signalling was the discovery that $InsP_3$ releases Ca^{2+} from the intracellular stores via $InsP_3$ receptors on the endoplasmatic reticulum (Streb et al., 1983). This study related the increase in inositol-containing phospholipids to intracellular Ca^{2+} release. This combination underlies the capacitative model, as will be explained below.

In the mid-1990s, the anatomy of the $InsP_3$ receptors was investigated. This receptor consists of a protein with a long N-terminal domain which contains the $InsP_3$ binding site. The $InsP_3$ binding site is directed towards the cytosol. The C-terminal part of the protein contains the membrane-spanning domains. The localisation of $InsP_3$ receptors is consistent with the non-homogeneous distribution of the Ca^{2+} signal; the receptors appear to be concentrated in the luminal part of the cell (Ambudkar, 2000).

The role of $InsP_3$ integrated with G-proteins like GTP-gamma-S became subject of research in 1986. Muscarinic stimulation activates GTP-binding proteins that trigger production of $InsP_3$. An increase in $InsP_3$ concentration causes Ca^{2+} release from the endoplasmatic reticulum (Marty, 1987). In addition, Nauntofte concludes that receptors on the plasma membrane are able to activate the $InsP_3$ metabolism, followed by Ca^{2+} release (Nauntofte, 1992). Other results that confirm the causality between $InsP_3$ and Ca^{2+} release include research from Jones (Jones et al., 1990) but even so the coupling between $InsP_3$ and $InsP_3$-dependent Ca^{2+} stores and the coupling between receptors and membrane phosphoinositides were still under investigation in 1994 (Martinez, 1994).

CAPACITATIVE MODEL

The role of the $InsP_3$-receptors on the endoplasmatic reticulum in regulating the Ca^{2+} concentration can be explained as follows. The initial rapid increase in Ca^{2+} is caused by release from the intracellular stores, resulting in depletion of these stores. A sustained level of $[Ca^{2+}]_i$ can only be reached through influx from extracellular Ca^{2+}, as mentioned above. The capacitative model of Ca^{2+} influx states that depletion of the stores is the triggering event for membrane channels to open, causing extracellular Ca^{2+} entry to refill the stores. According to the capacitative model, the $InsP_3$-receptor functions as detector of the status of the intracellular stores. The capacitative model for Ca^{2+} influx is well established, although Shuttleworth describes a number of experimental observations that contradict the capacitative model.

Coupling between the intracellular Ca^{2+} stores and membrane channels

In the capacitative model, it is assumed that the status of Ca^{2+} stores directly regulates Ca^{2+} influx. Although many researchers have studied possible models for this store-operated calcium influx mechanism and although new methods for measuring Ca^{2+} selective currents became available in the early 1990s, data that conclusively support one of those models are presently lacking (Ambudkar, 2000). Two main models (the conformational coupling hypothesis and the diffusible activator hypothesis) have been taken into consideration in recent years (Ambudkar, 2000). They are described in the following two sections.

Conformational coupling hypothesis

The first formulation of the conformational coupling hypothesis was based on the excitation-contraction mechanism in muscle cells, whereby the depolarization signal is transmitted to the ryanodine receptor in the sarcoplasmatic reticulum where Ca^{2+} is released (Berridge, 1995). The foot structures that have been observed on the ryanodine receptor span the region between the endoplasmatic reticulum and the T-tubule membrane, which makes protein-protein interaction likely. The conformation hypothesis is based on the homology between the ryanodine receptor and the $InsP_3$ receptor. They share many structural properties and may have evolved from the same ancestor.

Coupling takes place via interaction of Ca^{2+} influx channels with the N-terminal of the $InsP_3$ receptor. Ca^{2+} depletion in the endoplasmatic reticulum lumen is detected by the $InsP_3$ receptor, inducing its conformational change. This change activates $InsP_4$-receptors in the plasma membrane (Irvine, 1990). However, in 1996 experimental results showed that a specific kind of $InsP_3$ receptor is situated in the plasma membrane and thus might function as a direct channel, gated by intracellular $InsP_3$ and not as a store-operated channel (Khan et al., 1996).

Diffusable activator hypothesis

The diffusible activator hypothesis involves the release of a diffusible metabolite from the endoplasmatic reticulum concomitant with the release of Ca^{2+} (Berridge 1993). The metabolite in question remains to be identified, but different reports suggest the existence of a Calcium Influx Factor (CIF) which transmits information about the status of the endoplasmatic reticulum lumen to the Ca^{2+} influx channels hypothesis (Ambudkar, 2000; Jaconi et al., 1997; Ribiero et al., 1997). Whether this CIF also diffuses to adjacent cells is still unclear (Ambudkar, 2000). A study by Jaconi et al. (1997) which confirms this hypothesis suggests that the physical approximity of the endoplasmatic reticulum and plasma membrane is critical to the activation of store-operated Ca^{2+} influx. Moreover, reagents that drastically affect cytoskeletal arrangement do not influence Ca^{2+} influx (Ribiero et al., 1997). If coupling mechanisms use protein-protein interaction, a disturbance of the cytoskeleton would have affected Ca^{2+} influx. The influence of physical distance between the endoplasmatic reticulum and the membrane thus is compliant with the diffusible activator hypothesis.

NON-CAPACITATIVE MODEL

One type of Ca^{2+} concentration response has already been discussed above. The other type occurs if the agonist concentration is relatively low. In that case, $[Ca^{2+}]$ oscillations with a frequency proportional to the agonist concentration appear. Physiologically, agonist concentrations are probably relatively low, which implies that oscillating Ca^{2+} levels are responsible for the control of cellular activity (Shuttleworth, 1997).

Modelling Ca^{2+} oscillation signalling has appeared to be rather complex. The probability curve for channels in the stores to open when $InsP_3$ is present as a function of $[Ca^{2+}]_i$ is bell-shaped (Bezprozvanny et al., 1991), which is essential for oscillation. First, $InsP_3$ triggers Ca^{2+} release from the stores, thereby inducing further Ca^{2+} release. When $[Ca^{2+}]_i$, especially near the

$InsP_3$ receptor, becomes too high, the probability of the channels to be open decreases, inducing a decrease in $[Ca^{2+}]_i$. This explains the oscillatory Ca^{2+} signal. The molecular mechanism involved in the regulation of channel opening by Ca^{2+} is still not well understood (Ambudkar, 2000). The above mentioned capacitative model suggests that Ca^{2+} influx is the driving force for the oscillation.

Experimental results from the late 1990s cannot be matched with the capacitative model as far as the oscillation-type response is concerned. The first indication that the capacitative model could be invalid came from experiments where the quenching of intracellular Indo-1 by Mn^{2+} was used as a measure for the rate of Ca^{2+} entry. Mn^{2+} can be used as a surrogate for Ca^{2+} and has the advantage that it does not affect the Ca^{2+} pumps. Thus, it can be used as a measure for pure entry. The quenching increases and decreases with $[Ca^{2+}]_i$ during oscillations, but quenching remains constant during emptying and refilling of the stores, which implies that the rate of Ca^{2+} entry remains constant. It ia concluded that at least one of the components of agonist-activated Ca^{2+} entry is independent of the status of the stores (Martin and Shuttleworth, 1994).

More evidence resulted from experiments where Ca^{2+} entry was blocked during oscillations. After restoration of the Ca^{2+} entry, oscillation immediately continues, suggesting that the Ca^{2+} entry pathway remains active under conditions where the stores are not depleted (Shuttleworth & Thompson, 1996).

The capacitative model was further contradicted by experiments that inhibited Ca^{2+} entry, resulting in an increased delay of the initiation of the Ca^{2+} response. Because the capacitative model assumes that depletion of stores must precede Ca^{2+} entry, the beginning of the response should not be affected by inhibition of Ca^{2+} entry (Shuttleworth, 1997).

An experiment has been performed by Shuttleworth to investigate the Ca^{2+} influx pathway in a direct way. After stimulation with a low agonist concentration to arouse the oscillatory response, exposing the cell to a high K^+ medium has blocked Ca^{2+} influx. Subsequently, atropine was added to displace the receptors from the agonist and to allow the $InsP_3$ concentration to return to resting level. Next, the extracellular concentration of Ca^{2+} was raised to increase capacitative Ca^{2+} influx. Because the receptors are not occupied and the $InsP_3$ concentration is at resting level, this influx would be purely capacitative. The expected influx did not occur, indicating that the capacitative model is indeed invalid. In cells that were stimulated with high agonist concentrations, such that the response was of the plateau-type, this influx did occur (Shuttleworth, 1997).

Shuttleworth suggests two alternative mechanisms that could explain these results. The first mechanism consists of a different way of Ca^{2+} entry control by an element of the inositol triphosphate pathway that affects the plasma membrane. The second mechanism consists of a distinct signalling pathway, activated by the agonist and independently responsible for control of Ca^{2+} entry. In this pathway, activation of a phospholipase A_2 that leads to generation of arachidonic acid (AA), which partially controls Ca^{2+} entry, is involved. Besides the controlling function of AA, inhibition of oscillations by tetrandrine can be reversed by AA (Clark et al., 1991).

With this new pathway, a new model for the patterns in $[Ca^{2+}]_i$ has been proposed by Shuttleworth. He suggests that the capacitative model accounts for the refilling of the stores, while the stores are responsible for oscillations. Consequently, the $InsP_3$-receptor on the intracellular Ca^{2+} stores controls oscillations. $InsP_3$ and AA act together as co-agonists to make fine-tuning of the oscillatory pattern possible. Stimulation with agonists induces an increase in the $InsP_3$-level via G-proteins. This increase makes $InsP_3$-receptors on the stores more sensitive to Ca^{2+}. Parallel, agonists cause production of AA via the phospholipase A_2 pathway. AA production stimulates Ca^{2+} entry which results in an increase in $[Ca^{2+}]_i$ that triggers the sensitive receptors on the stores in order to release more Ca^{2+}. In this way, the integration of AA and $InsP_3$ controls the oscillatory pattern (Shuttleworth, 1997).

Clearly, no consensus has been reached yet with respect to oscillation modelling. Ambudkar mentions four possible models in a recent review article (Ambudkar, 2000). The first model attributes the oscillations solely to the biphasic effect of Ca^{2+} on the $InsP_3$-receptor, which is the only release site in the cell. The second model involves a second Ca^{2+} store that consists of either another $InsP_3$-receptor with a different sensitivity to Ca^{2+} or a ryanodine receptor. In the third and fourth model, the Ca^{2+} influx is repeatedly activated and inactivated, either direct by the intracellular $[Ca^{2+}]$ or by refilling of intracellular stores. The latter is consistent with the capacitative model.

Obviously, much research is required to investigate which models can be applied to the different types of cells.

In addition to the above described factors that influence $[Ca^{2+}]_i$, the pH is also of importance. An extracellular pH > 7.4 increases Ca^{2+} influx while a pH $<$

7.4 causes a decrease. The pH level does not directly control the influx; it only modulates the rate of influx. It has been suggested that pH modulates influx via effects on carboxyl groups of proteins, associated with the influx system (Lockwich et al., 1993). In addition, a high pH value might contribute to the sustained opening of Cl^- channels during secretion (Park & Brown, 1995).

ANATOMICAL ASPECTS

Apart from research into the biochemical chains of events that underlie secretion mechanisms, extensive study has been done concerning the anatomy of secretion cells. Marty (1987) mentions study of the anatomical distribution of the Cl^-, K^+ and cation channels over the luminal and basolateral membrane and the composition of secreted fluid as challenges for future research (Marty, 1987). It took about five years before the distribution of the different types of channels involved in fluid secretion became clear. In 1992, the distribution of Cl^- and K^+ channels over luminal and basolateral membranes in lacrimal acinar cells has been studied. In these experiments, each of these two ion currents was measured separately by choosing a membrane potential close to the Nernst potential of the other. From the current densities, channel densities can be deduced if luminal and basolateral channel properties are assumed to be the same. K^+ and Cl^- channel densities on the luminal membrane are approximately ten times higher than densities on the basolateral membrane. Besides that, luminal Ca^{2+} release appears to be synchronous with the 'hump' of current response, whereas basolateral response is activated by the delayed current rise (Tan et al., 1992). This non-homogeneous calcium response has been confirmed by many other experiments. However, in 1992, Nauntofte finds a simultaneous luminal and basolateral Ca^{2+} rise in salivary acinar cells and in 1997, Gromada et al. find a synchronous calcium rise throughout pancreal gland cells (Nauntofte, 1992; Gromada et al., 1997).

Recently, five different classes of Cl^- channels with different gating mechanisms have been found (Melvin, 1999). One of them is dependent on Ca^{2+}; another uses cAMP as messenger. According to Melvin, an increase in $[Ca^{2+}]_i$ is the dominant triggering event while cAMP may be required for efficient reabsorption of NaCl. The other classes of Cl^- channels are regulated by agonist-induced changes in the plasma membrane or by cell volume (Melvin, 1999). Cell swelling stimulates Cl^- channels to open. In addition, cAMP acts as a second messenger in stimulating Cl^- channels to open while it inhibits K^+ secretion (Schultheiss & Diener, 1998).

FUTURE CHALLENGES

As this review demonstrates a lot of research in the field of secretory mechanisms has been done since the 1960s. Although much is clarified, the underlying regulation of Ca^{2+} concentration changes that induce ionic secretion mechanisms is still subject of much research.

A critical and yet unanswered question concerning store-operated Ca^{2+} influx deserving further attention in the near future is: What is the molecular nature of the mechanism which conveys the status of the endoplasmatic reticulum to the plasma membrane to regulate Ca^{2+} influx? Another important issue is the molecular identification of the Ca^{2+} influx mediating channels themselves. More data is necessary to be able to support or rule out one of the proposed models for store operated calcium gating. The distribution and architecture of the involved proteins can learn us a lot. In addition, identification of those proteins is an important challenge.

Recently a different pattern, consisting of spikes in Ca^{2+} concentration has been observed. The spikes appear to initiate regenerative waves that can spread to neighbouring cells (Berridge, 1997). These spikes could play a role in signalling between adjacent cells. The physiological basis of these transient and localised patterns should be investigated.

Another important question is the development of the involved receptors and their gene expression.

A multidisciplinary approach that integrates molecular biology, chemistry and cell physiology can clarify a lot in this field within the next few years.

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