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REVIEW

Calcium Dependencies of Regulated Exocytosis in Different Endocrine Cells

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Summary

Exocytotic machinery in neuronal and endocrine tissues is sensitive to changes in intracellular Ca^{2+} concentration. Endocrine cell models, that are most frequently used to study the mechanisms of regulated exocytosis, are pancreatic beta cells, adrenal chromaffin cells and pituitary cells. To reliably study the Ca^{2+} sensitivity in endocrine cells, accurate and fast determination of Ca^{2+} dependence in each tested cell is required. With slow photo-release it is possible to induce ramp-like increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) that leads to a robust exocytotic activity. Slow increases in the $[Ca^{2+}]_i$ revealed exocytotic phases with different Ca^{2+} sensitivities that have been largely masked in step-like flash photo-release experiments. Strikingly, in the cells of the three described model endocrine tissues (beta, chromaffin and melanotroph cells), distinct Ca^{2+} sensitivity 'classes' of secretory vesicles have been observed: a highly Ca^{2+}-sensitive, a medium Ca^{2+}-sensitive and a low Ca^{2+}-sensitive kinetic phase of secretory vesicle exocytosis. We discuss that a physiological modulation of a cellular activity, e.g. by activating cAMP/PKA transduction pathway, can switch the secretory vesicles between Ca^{2+} sensitivity classes. This significantly alters late steps in the secretory release of hormones even without utilization of an additional Ca^{2+} sensor protein.

Key words

Calcium sensitivity • Exocytosis • Insulin-secreting cells • Chromaffin cells • Melanotrophs

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Introduction

Increase in intracellular calcium concentration ($[Ca^{2+}]_i$) is a key signal to trigger exocytosis in most neuronal and endocrine tissues. Experimentally the best described endocrine cell models to study the role of Ca^{2+} in the regulated exocytosis are pancreatic beta cells (Barg *et al.* 2001, Takahashi *et al.* 1997, Wan *et al.* 2004), adrenal chromaffin cells (Augustine and Neher 1992, Neher and Zucker 1993, Voets 2000, Voets *et al.* 1999) and pituitary melanotroph cells (Sedej *et al.* 2005). A whole range of different physiological stimuli (like glucose, acetylcholine, etc.) can trigger increase in $[Ca^{2+}]_i$ and induce exocytosis. The exocytotic activity itself can also be further modulated by a range of physiological signals (like GLP-1, dopamine, etc).

The role of cytosolic Ca^{2+} has been shown to be multiple, on one hand Ca^{2+} is directly involved in the fusion of the secretory vesicles with plasma membrane (Katz and Miledi 1965), and on the other hand, these ions also function in the several distinct maturation steps of these secretory vesicles prior to fusion (Voets 2000). Some of these Ca^{2+}-dependent steps in the late stages of the secretory activity have not yet been described in detail. The aforementioned biochemical maturation steps have been attempted to be separated into distinct functional states or pools using different experimental approaches to increase cytosolic Ca^{2+}, like whole-cell dialysis, trains of depolarizing pulses or caged Ca^{2+} photolysis. Based on the depolarization studies it has

been found that insulin granules (Rorsman and Renstrom 2003), similar to secretory vesicles in numerous other cell types, exist within the cell in various pools. These, so called, releasable pools of vesicles have been termed readily releasable (RRP), immediately releasable (IRP) and slowly releasable pool (SRP) (described in detail in Mears 2004, Rorsman and Renström 2003 for beta cells, in Voets 2000, Voets *et al.* 1999 for chromaffin cells and in Sedej *et al.* 2005 for melanotroph cells). Most of previous experiments did not focus on establishing Ca^{2+} sensitivity per se, rather focusing on distinguishing pools of vesicles upon their putative biochemical state or localisation within the cell. The biochemical maturation state and localisation were supposed to define the limiting conditions for the release of the vesicles from corresponding releasable pools. In addition, these studies largely assumed relatively low sensitivity to Ca^{2+}, utilizing Ca^{2+} microdomain concept, with Ca^{2+} sensitive proteins on secretory vesicles facing the inner mouth of the voltage-activated Ca^{2+} channels (Neher and Sakaba 2008). No matter from which releasable pool they originate, vesicle exocytosis in endocrine cells, as in synapse, is managed by SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) fusion machinery (Easom 2000, Lang 1999).

Biochemical studies suggested numerous Ca^{2+} sensor proteins with vast variability in their sensitivity to Ca^{2+}. Synaptotagmins appear to be a primary Ca^{2+} sensor proteins, however some forms of Ca^{2+} dependent exocytosis may use yet unidentified sensors (Pang and Südhof 2010). Alternatively to having different synaptotagmin isoforms to cover a whole range of Ca^{2+} sensitivities of the secretory vesicles, we can have ways to significantly influence the Ca^{2+} sensitivity of a given synaptotagmin by changing the physiological context of the cell.

In previous studies few simple questions have been largely overlooked: what is the precise $[Ca^{2+}]_i$ threshold to trigger fusion of the secretory vesicles? How much do cells in different endocrine tissues differ in their Ca^{2+} sensitivities? And finally, can we physiologically influence the Ca^{2+} sensitivity within these different endocrine cells?

Previous studies on Ca^{2+} sensitivity of secretory vesicle exocytosis

Differences in Ca^{2+} sensitivities among different endocrine cells types were also readily observed in depolarization experiments where trains of depolarizing pulses were used to mimic the electrical activity and therefore physiological stimulation in different endocrine cells (Figure 1). The depolarization pulses opened voltage-activated Ca^{2+} channels. Subsequent influx of Ca^{2+} through these channels increased $[Ca^{2+}]_i$. Due to localisation of voltage-gated calcium channels at the plasma membrane, spatial Ca^{2+} concentration gradients should build up (Neher and Augustine 1992) to trigger Ca^{2+}-dependent exocytosis. Such exocytotic events can be adequately measured as membrane capacitance (C_m) changes (Neher and Marty 1982) since this parameter is linearly proportional to the membrane surface area.

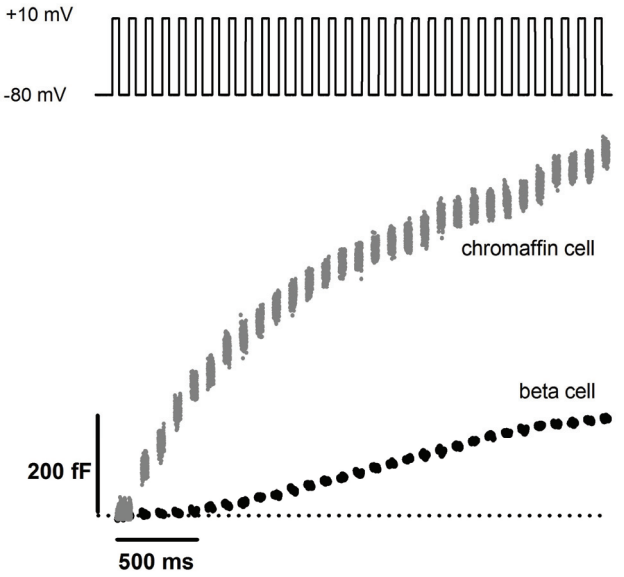

Fig. 1. A train of depolarizing pulses triggers higher C_m response in chromaffin cells compared to beta cells. Train of depolarizations (upper panel) elicited a cumulative C_m change (lower panel) in pancreatic beta cell (black) and chromaffin cell (gray). The train consisted of thirty 40 ms depolarization pulses from –80 mV to +10 mV at a frequency of 10 Hz. Already the first depolarization pulse triggered a several times larger C_m increase in chromaffin compared to beta cells suggesting higher sensitivity of the secretory machinery to Ca^{2+}.

Already after the first depolarization pulse a several times larger increase in C_m has been observed in chromaffin cells compared to pancreatic beta cells (Figure 1). Chromaffin cells express a higher density of voltage-activated Ca^{2+} channels as beta cells; therefore a comparable influx of Ca^{2+} ions has not been expected. Nevertheless, this experiment may also indicate that the secretory vesicles in chromaffin cells may be more sensitive to Ca^{2+} than in beta cells.

A variety of depolarization protocols have been

utilized to trigger Ca^{2+} regulated exocytosis (Barg *et al.* 2001, Barg *et al.* 2001, Chow *et al.* 1996, Ge *et al.* 2006, Marengo 2005, Voets *et al.* 1999). Lengths of the depolarization pulses varied largely in these experiments. Since majority of experiments were done on isolated cells in which significant portion of Ca^{2+} and Na^{+} channels were lost due to cell isolation procedures, unphysiologically long depolarization pulses were mostly required (500 ms) to stimulate Ca^{2+}-dependent exocytosis (Barg *et al.* 2002, Barg *et al.* 2001).

For depolarization pulses experiments it is difficult to determine actual values of $[Ca^{2+}]_i$ sensed by the secretory vesicles, therefore Ca^{2+} sensitivity studies are not straightforward using this method. Ca^{2+} influx and Ca^{2+} concentration gradients spanning near the plasma membrane during depolarizations were reported in several model cells (Chow *et al.* 1994, Klingauf and Neher 1997, Neher and Augustine 1992, Rose *et al.* 2007). In some experiments on chromaffin cells $[Ca^{2+}]_i$ was back-calculated from C_m and amperometry data assuming the same kinetic dependence of secretion upon Ca^{2+} concentration as obtained from experiments where Ca^{2+} has been manipulated by instantaneous release from buffer cages (Chow *et al.* 1994). The calculated $[Ca^{2+}]_i$ values during brief depolarizations have been as high as 10 μM close to the plasma membrane. This relatively high $[Ca^{2+}]_i$ indicated that the Ca^{2+} sensitivity may be low affinity but no more precise information could be elucidated from these experiments. More recently a confocal imaging approach has been used to quantify Ca^{2+} gradients during depolarization induced C_m changes in chromaffin cells (Marengo 2005). In this study a lower peak $[Ca^{2+}]_i$ has been determined (~1 μM), with an even lower threshold $[Ca^{2+}]_i$ (<1 μM) for C_m response indicating high sensitivity of the secretory machinery.

These latter results are in the agreement with earlier experiments studying Ca^{2+} sensitivities of the vesicle exocytosis, utilizing whole-cell patch-clamp dialysis of intracellular solution with different concentrations of Ca^{2+} to stimulate regulated exocytosis (Augustine and Neher 1992, Rupnik *et al.* 1995). In this manner the $[Ca^{2+}]_i$ was raised slowly (tens of seconds) as defined by the limitation in the diffusion between the patch pipette and the cell interior. The obtained Ca^{2+} dependency curves indicated that endocrine cells were very sensitive to Ca^{2+}. Triggering Ca^{2+} concentration has been reported to be 0.2 μM in chromaffin cells. However, the maximal rate of fusion has been reached no sooner than two orders of magnitude higher $[Ca^{2+}]_i$ (10 μM) (Augustine and Neher 1992). Similar values in high $[Cl^{-}]_i$ conditions have been reported for both triggering and saturating $[Ca^{2+}]_i$ in rat melanotrophs using the same approach (Rupnik *et al.* 1995). In addition, a low threshold for secretion (<1 μM) was observed in digitonin permealized chromaffin cells (Teraoka *et al.* 1993). On the other hand, triggering $[Ca^{2+}]_i$ has been reported to be significantly higher at 2 μM in beta cells (Barg *et al.* 2001).

Photo-release of caged Ca^{2+} compounds

The application of photo-release of caged Ca^{2+} compounds (e.g. NP-EGTA) has immensely contributed to our understanding of Ca^{2+}-dependent exocytosis (Neher and Zucker 1993). When cell is dialyzed with the intracellular solution containing Ca^{2+}-NP-EGTA, the subsequent UV illumination releases Ca^{2+} ions from the buffer cage. There are major advantages in using photo-release approach. Photo-release of caged Ca^{2+} increases $[Ca^{2+}]_i$ uniformly throughout the cell. This avoids the problem of loss of Ca^{2+} and Na^{+} channels during cell isolation procedures or up-regulation of protein expression during cell culturing. Furthermore, the photo-release approach bypasses any modulatory effects that act on electrical excitability and Ca^{2+} influx (Neher 2006). In pancreatic beta cells at least one third of cells did not show voltage-dependent inward current during the voltage protocol (Speier and Rupnik 2003).

So far most widely used form of photo-release was the instant uncaging of Ca^{2+} ions by strong flash of UV light (flash photolysis). This approach has been applied to several secretory cells including pancreatic beta cells (Ge *et al.* 2006, Takahashi *et al.* 1997, Wan *et al.* 2004), chromaffin cells (Heinemann *et al.* 1994, Neher and Zucker 1993, Voets 2000) and melanotrophs (Rupnik *et al.* 2000). Most of these experiments in endocrine tissues were done using flash photolysis of Ca^{2+} to several tens of μM (Chow *et al.* 1994, Ge *et al.* 2006, Neher and Zucker 1993, Takahashi *et al.* 1997, Voets 2000, Wan *et al.* 2004). These relatively high Ca^{2+} concentrations were used in an attempt to completely deplete releasable pools of vesicles and study the kinetics of their release.

In some of the photo-release studies attempts to determine threshold Ca^{2+} concentration have been made, however the data reported have not been consistent. In adrenal slices the triggering concentration >3 μM was

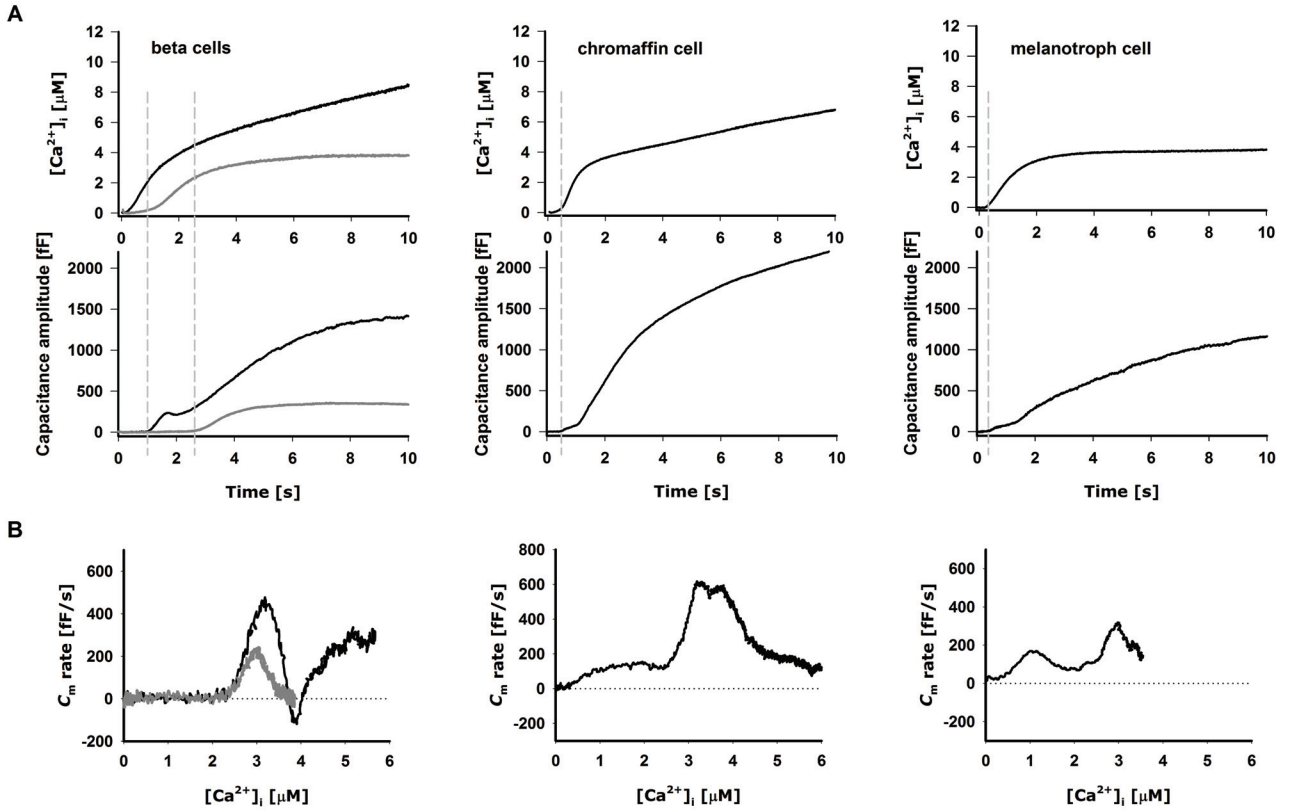

Fig. 2. Slow photo-release of Ca-NP-EGTA induced a several kinetic phases of C_m increase in a beta cell, a chromaffin cell and a melanotroph cell. (**A**) Slow photo-release of caged Ca^{2+} produced a ramp-like increase in intracellular calcium concentration ($[Ca^{2+}]_i$) (upper panels) in beta cell (first column), chromaffin cell (middle column) and melanotroph cell (right column). After reaching the triggering $[Ca^{2+}]_i$ (Ca_{tr} – dashed lines) mostly a biphasic membrane capacitance (C_m) was triggered (lower panels) (**B**) Rate of C_m change depended on $[Ca^{2+}]_i$. In beta cells the two phases of C_m response were triggered at 2.6 μM and 4.2 μM calcium concentrations, respectively. In chromaffin and melanotroph cell a low Ca^{2+} triggered kinetic phase is observed with triggering Ca^{2+}concentration ~0.5 μM. Representative responses of cells for each endocrine tissue are shown.

reported (Voets 2000) which is one order of magnitude higher than reported from dialysis experiments (Augustine and Neher 1992). In pancreatic beta cells even results obtained with the same experimental design are confusing. In some studies exocytosis was triggered at $[Ca^{2+}]_i$ as low as 2-3 μM (Barg *et al.* 2001, Wan *et al.* 2004) while in others more that 10 μM of Ca^{2+} was needed to evoke C_m change (Barg *et al.* 2001). The aforementioned inconsistencies strongly suggest that the flash photolysis approach is not a method of choice to study the Ca^{2+} sensitivity of secretory machinery.

Based on the results of flash photolysis experiments on beta cells, two distinct secretory vesicle populations with different Ca^{2+} sensitivities were suggested (Barg and Rorsman 2004, Wan *et al.* 2004, Yang and Gillis 2004). The authors suggested a secretory mechanism where granules with low-affinity Ca^{2+} sensor would need to be associated with voltage-gated Ca^{2+} channels while granules with high-affinity Ca^{2+} sensor would be able to undergo exocytosis after global elevation of $[Ca^{2+}]_i$. However, whether microdomains are really necessary for the release of secretory vesicles at low Ca^{2+} concentrations in all endocrine models still needs to be confirmed.

Slow photo-release – a Ca^{2+} ramp based measurement of Ca^{2+} sensitivity of secretory vesicle exocytosis

To reliably study Ca^{2+} sensitivity of the regulated exocytosis, accurate and fast determination of the triggering $[Ca^{2+}]_i$ and Ca^{2+} dependence in each tested cell is required. With slow photo-release of caged Ca^{2+} it is possible to induce ramp-like increase in $[Ca^{2+}]_i$ that leads to a robust secretory activity (Liu *et al.* 2008, Paulmann *et al*. 2009). Gradually increasing $[Ca^{2+}]_i$ inside the cell provides a test with a continuous $[Ca^{2+}]_i$ levels within a single experiment. In contrast, in the flash photolysis experiments only a single value of Ca^{2+} concentration (usually maximal value) is sampled per

cell. Slowly changing $[Ca^{2+}]_i$ can reveal the triggering of several exocytotic kinetic phases with different Ca^{2+} sensitivities that may be masked in flash photolysis experiments, where a rapid and large jump in $[Ca^{2+}]_i$ would trigger all the kinetic phases simultaneously, preventing their discrimination.

The slow photo-release of caged Ca^{2+} has been applied in three different mouse endocrine cell types: beta cells from pancreatic islets of Langerhans, chromaffin cells from adrenal medulla and melanotroph cells from the intermediate lobe of the pituitary gland (Figure 2). During the Ca^{2+} ramp the $[Ca^{2+}]_i$ gradually increased from the resting levels to 3-10 μM in approximately ten seconds. In addition to $[Ca^{2+}]_i$ we simultaneously measured net change in C_m (method described in detail in Skelin and Rupnik, 2011). When $[Ca^{2+}]_i$ reached a threshold level, typically a distinguishable C_m change has been observed. At lower $[Ca^{2+}]_i$, only one kinetic phase has been observed (Figure 2A). However, during the Ca^{2+} ramps to higher $[Ca^{2+}]_i$, two separate kinetic exocytotic components have been typically recorded. We named them first and second kinetic phase. For the first kinetic phase of exocytosis the time derivatives for the C_m traces (C_m rate) reached a maximum value after which the change in C_m rate either decreased or, sometimes and typically only in isolated cells, even endocytosis occurred. This indicates that the ramp change in $[Ca^{2+}]_i$ was slow enough to mainly deplete the most Ca^{2+} sensitive phase that was activated at triggering $[Ca^{2+}]_i$. Further increasing $[Ca^{2+}]_i$ during the Ca^{2+} ramp activated a distinct C_m change.

Separation of exocytotic kinetic phases

Separation of exocytotic kinetic phases with different Ca^{2+} sensitivities can be readily observed when C_m rate is plotted against the $[Ca^{2+}]_i$ values obtained with the Ca^{2+} ramp. Rate of secretion is sensitive to $[Ca^{2+}]_i$, accelerating in several orders of magnitude as concentration is elevated – as also previously reported (Augustine and Neher 1992). The rate of secretion should provide the better indicator of the actual affinity of the exocytotic apparatus for $[Ca^{2+}]_i$ since the total amount of C_m may be limited by the total number of vesicles available for exocytosis.

Detailed analysis of the obtained Ca^{2+} sensitivities revealed that the initial level of $[Ca^{2+}]_i$ (Ca_{tr}) to trigger the first kinetic phase of C_m increase was on average 2.6 μM, 0.4 μM and 0.4 μM in beta cells, chromaffin cells and melanotroph cells, respectively. The value of Ca_{tr} has been typical and highly reproducible for each cell type. For beta cells (Wan *et al.* 2004) and chromaffin cells (Liu *et al.* 2008) this result is in accordance with previously published data. The maximal rate during the first kinetic phase was 325±40 fF/s, 243±31 fF/s and 271±110 fF/s, respectively. For beta cells the maximal rate during the first phase was comparable with the results obtained from rat islets in pancreatic slices where depolarization was used to trigger exocytosis (Rose *et al.* 2007). The rate of secretion was few times larger in mouse adrenal slice preparation compared to respective $[Ca^{2+}]_i$ in isolated bovine chromaffin cells (Augustine and Neher 1992, Heinemann *et al.* 1994). However, in melanotroph cell the maximal rate of the first kinetic phase was slower compared to the one determined using depolarization pulses (Sedej *et al.* 2005).

The rate of vesicle fusion during the first kinetic phase may also be expressed as number of large dense-core vesicles (LDCV) per second based on average LCDV size (Rorsman and Renstrom 2003, Thomas *et al.* 1993). The observed rates of C_m change (90 LCDVs per second) were in accordance with previously published data in beta cells (Neher and Sakaba 2008). However, pancreatic beta cells were reported to possess two distinct types of secretory vesicles, synaptic-like microvesicles (SLMV) and large dense core vesicles (LDCV), both of which undergo calcium-dependent exocytosis (Takahashi *et al.* 1997, Thomas-Reetz and De Camilli 1994). There is an indication that uncaging of Ca^{2+} released both types of vesicles (Hatakeyama *et al.* 2007). Therefore we preferred to express the rate of the release in fF per second to avoid unresolved dilemma regarding the involvement of SLMV in the regulated exocytotic.

Interestingly, in chromaffin cells maximal rates of fusion were well correlated when comparing flash photolysis experiments (Heinemann *et al.* 1994) and Ca^{2+} dialysis experiments (Augustine and Neher 1992) despite the fact that responses were obtained on different time scales (tens of miliseconds and seconds, respectively). The reason may be that both were performed on single isolated chromaffin cells. On the other hand, the photolysis experiments on chromaffin cells in adrenal slices provided several times larger C_m rate. Cell dissociation has been found to disturb some cell functions as demonstrated in adrenal chromaffin cells (Moser and Neher 1997), pituitary melanotrophs (Sedej *et al.* 2005) and rodent beta cells (Speier and Rupnik 2003). In slice preparation local cell-cell contacts as well as the cell

architecture are preserved therefore providing a more physiological preparation. However, not all the functions seem to be perturbed since we did not observe any difference in Ca^{2+} sensitivity of the secretory machinery in pancreatic beta cells comparing cells in tissue slices to those isolated into cell culture (Skelin and Rupnik 2011). In beta cells the amplitude of the first phase, representing a number of fused vesicles, was 310±37 fF. Similar number of secretory vesicles has been found also in isolated beta cells (Barg *et al.* 2001, Takahashi *et al.* 1997, Wan *et al.* 2004).

Second kinetic phase of the exocytotic response

Determining the triggering Ca^{2+} concentration for the second phase of the biphasic response is a problem inherent to the whole-cell patch-clamp based approach to measure C_m. During the Ca^{2+} ramp the more sensitive kinetic phases would likely mask the onset of the less Ca^{2+}-sensitive phases. In this way the triggering concentration for the second kinetic phase would most likely be overestimated. In an attempt to overcome the shortcomings of the C_m measurement we carefully monitored other cellular parameters that may be influenced by the fusion of the secretory vesicles. As shown in Figure 3, slow photo-release of caged Ca^{2+} triggered, besides C_m change, also an inward current with two distinguishable phases. The first issue regarding these currents in all three tested cell types is that the inward current does not closely follow the time course of the change in $[Ca^{2+}]_i$. The time course of any current that would merely depend on $[Ca^{2+}]_i$ should mirror the time course of a slow Ca^{2+} ramp. Such Ca^{2+}-dependent currents have been previously described in beta cells and pituitary cells, however they were, at least in beta cells, outward at the holding potential of –80 mV (Gopel *et al.* 1999). An alternative explanation for our observation is that the secretory vesicles carry ion channels that, after fusion, add to the resting membrane current. This, in fact, may hold true for the second kinetic phase of the secretory activity in beta cells as well as for both kinetic phases in chromaffin cell and melanotrophs (Figure 3). Therefore, this change in the whole cell membrane current could be used to distinguish the onset of fusion of the secretory vesicles belonging to the second kinetic phase and to assess its triggering Ca^{2+} concentration. Triggering $[Ca^{2+}]$ for the second kinetic phase of secretion was therefore 4.2±0.2 μM, 2.6±0.1 μM, 1.8±0.1 μM in beta cells, chromaffin cells and melanotroph cells, respectively.

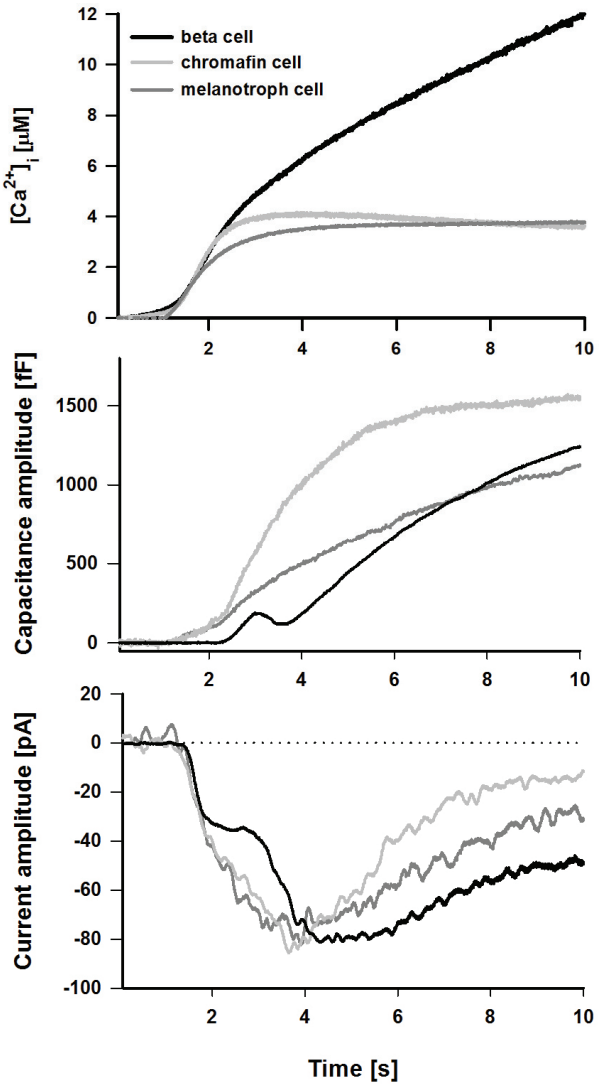

Fig. 3. Slow Ca^{2+} ramp triggered a biphasic increase in C_m and biphasic inward current change. Increase in $[Ca^{2+}]_i$ (upper panel) triggered a biphasic C_m change (middle panel) and a biphasic inward current change (I_m) in beta cell, chromaffin cell and melanotroph cell. In the latter two the first phase of I_m change was triggered simultaneously with the first kinetic phase of C_m change. In contrast, in beta cell the first phase of I_m change was triggered at $[Ca^{2+}]_i$ which is 2 μM lower than $[Ca^{2+}]_i$ at which the first kinetic phase of C_m was triggered. The second kinetic phase of I_m change was triggered simultaneously with the second kinetic phase of C_m change in all three different endocrine cells. Representative responses of cells for each endocine tissues are shown.

However, a puzzle remained regarding the inward current triggered by Ca^{2+} that has not been associated with the C_m change (Figure 3). In beta cells only, the whole-cell current observed during stimulation with Ca^{2+} ramp occurred before the onset of C_m change. The first phase of I_m change was triggered at 0.5 μM

$[Ca^{2+}]_i$ (almost 2 μM lower than $[Ca^{2+}]_i$ triggering C_m change). One possible explanation is that the I_m current represents a current through the fusion pore. Such microscopic fusion pore conductances can coincide with the corresponding changes in C_m (Vardjan *et al.* 2007). It is possible to detect also fusion pore related conductance events that do not have a simultaneous C_m change, due to the fact that the fusion pore is still too tight to support the measurement of the C_m change related to this fused vesicle. This fact would also be supported by an observation that C_m measurements miss some of the exocytotic events that could be picked up by simultaneous amperometry (Oberhauser *et al.* 1996)

At a triggering $[Ca^{2+}]_i$, a stable fusion pore complex could be formed but the $[Ca^{2+}]_i$ would not be sufficient to support the more complete fusion of the secretory vesicle. Interestingly, the fusion pore stability can be physiologically modulated. Stimulation of cAMP/PKA transduction pathway in beta cells resulted in the C_m change that appeared simultaneously with the first phase of I_m change (see below; Skelin and Rupnik 2011). It is plausible that the simultaneous onset of both C_m and I_m changes in chromaffin and melanotroph cells shows a higher level of the cAMP/PKA transduction pathway activation in these cell types. We suggest that so far unknown cAMP/PKA dependent process has the ability to destabilize the tight form of the fusion pore thereby fully fusing secretory vesicles with the plasma membrane at significantly lower $[Ca^{2+}]_i$.

Ca^{2+} sensitivity 'classes' in different endocrine cells

In the three model endocrine cells tested by the slow Ca^{2+} ramps, three distinct Ca^{2+} sensitivity 'classes' were observed (Figure 4): a highly Ca^{2+}-sensitive (triggering $[Ca^{2+}]_i$ around ~0.5 μM), a medium Ca^{2+}-sensitive (triggering $[Ca^{2+}]_i$ around 2-3 μM) and a low Ca^{2+}-sensitive (triggering $[Ca^{2+}]_i$ around 4-5 μM) kinetic phases of exocytosis. Mouse chromaffin cells and melanotroph cells show expression of both the highly and medium Ca^{2+}-sensitive phases, on the other hand beta cells at low cAMP availability have only the two less sensitive kinetic phases. Previous studies in beta cells did report a high Ca^{2+} sensitivity (Barg *et al.* 2001, Ge *et al.* 2006, Okazaki *et al.* 1994, Wan *et al.* 2004, Yang and Gillis 2004, Yaseen *et al.* 1982). The secretory vesicles with such a low Ca^{2+} sensitivity usually produces a very small change in C_m amplitude (<20 fF) in beta cells.

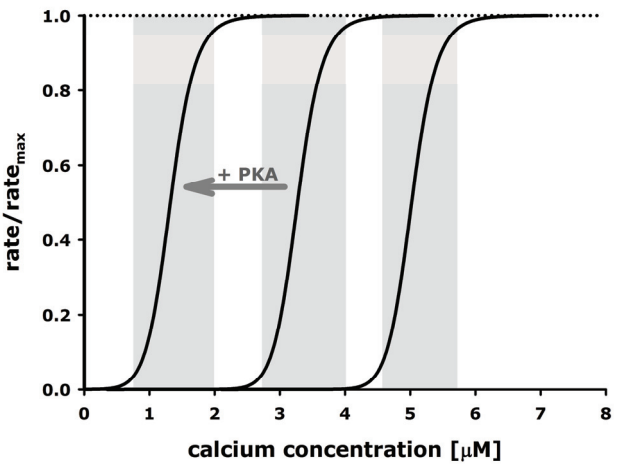

Fig. 4. A model describing Ca^{2+} sensitivity 'classes' in the endocrine cells. Based on triggering $[Ca^{2+}]_i$, in beta cells, chromaffin cells and melanotrophs, we propose a model consisting of three distinct 'classes' of Ca^{2+} sensitivities. A highly Ca^{2+}-sensitive (triggering $[Ca^{2+}]$ around ~0.5 μM, left trace), a medium Ca^{2+}-sensitive (triggering $[Ca^{2+}]$ around 2-3 μM, middle trace) and a low Ca^{2+}-sensitive (triggering $[Ca^{2+}]$ 4-5 μM, right trace) kinetic phases of exocytosis. A physiological stimulation, like activation of cAMP/PKA transduction pathway transits medium- to highly Ca^{2+} sensitive kinetic phase in beta cells. Rates of C_m changes are depicted relative to the maximal rate.

Furthermore only a fraction of tested beta cells possessed such sensitivity (Ge *et al.* 2006, Wan *et al.* 2004). In most flash photolysis experiments this phase has been overlooked when $[Ca^{2+}]_i$ has been instantly elevated to high (>10 μM) $[Ca^{2+}]_i$ values, the less sensitive but larger in amplitude kinetic phases masked the more Ca^{2+} sensitive phases.

In beta cells specific activation of cAMP/PKA transduction pathway (by addition of cAMP or by direct activation of PKA) facilitated the expression of the high Ca^{2+}-sensitivity kinetic phase (Skelin and Rupnik 2011). The fusion of the vesicles that has been triggered at 2.6 μM $[Ca^{2+}]_i$ was in high cAMP condition triggered at ~0.5 μM, shifting their sensitivity 'class' to the highly Ca^{2+}-sensitive kinetic phase (Figure 4). Modulation of the high Ca^{2+}-sensitive phase by cAMP was observed also in the flash photolysis experiments (Wan *et al.* 2004). Since the cAMP is one of the major physiological modulators in intracellular signalling pathways, such a shift in the Ca^{2+} sensitivity represents a way to modulate the level of exocytosis. This would significantly influence the release of hormones, like insulin. Modulation of secretory activity by different physiological stimuli (e.g. GLP-1) increasing cAMP levels in the beta cells could act through the sensitization of the secretory vesicles to Ca^{2+}. Such modulation of the Ca^{2+} sensitivity by GLP-1 could

also help us understand the cellular mechanism underlying the treatment of a diabetic organism, enhancing the availability of insulin. Further experiments are underway to assess the physiological modulation of the fusion pore stability and shifts between the Ca^{2+} sensitivity classes. In future, we might not discover new Ca^{2+} sensitive proteins, but understand that known players, like synaptotagmins, can operate differently depending on physiological context.

Conflict of Interest

There is no conflict of interest.

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