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Scinderin-derived actin-binding peptides inhibit Ca^{2+}- and GTPγS-dependent exocytosis in mouse pancreatic β-cells

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Abstract

Using capacitance measurements, we have explored the effects of three different scinderin actin-binding peptides (Sc^{77-89}; $Sc^{138-146}$; $Sc^{511-523}$) on Ca^{2+}- and GTPγS-induced exocytosis in single mouse pancreatic β-cells. Sc^{77-89} (10 μM) reduced exocytosis by 43% in whole-cell experiments in which secretion was triggered by intracellular dialysis with a Ca^{2+}-EGTA buffer with a free Ca^{2+} concentration of 2 μM. A more pronounced reduction of the rate of exocytosis was observed with $Sc^{138-146}$ (72%) but not with $Sc^{511-523}$ (39%). $Sc^{138-146}$ also reduced depolarisation-induced exocytosis by 61% without affecting the whole-cell Ca^{2+} current. When exocytosis was triggered by infusion of GTPγS, all scinderin-binding peptides reduced exocytosis by 59–75%. These data suggest that scinderin might be important for controlling cortical actin network dynamics in mouse pancreatic β-cells and that scinderin-induced cortical filamentous actin disassembly is required for insulin secretion.

Keywords: Cytoskeleton; Exocytosis; Insulin; Scinderin

1. Introduction

The pancreatic β-cell contains about 13,000 secretory granules (Dean, 1973) of which only a fraction is accessible for release during stimulation (Eliasson et al., 1997). Studies on both pituitary and chromaffin cells have indicated that the granules pass a series of functional states before undergoing exocytosis and a similar situation exists in the β-cell. The bulk of granules (> 95%) thus belong to a reserve pool and are not immediately available for release. In the β-cell, the number of granules that belong to the readily releasable pool is small and has been estimated as 100 granules per cell (Eliasson et al., 1997). The regulation of the transfer of the granules between the reserve pool and the readily releasable pool remains obscure but there is evidence suggesting the involvement of cytoskeletal components (Li et al., 1994). Immunofluorescence and cytochemical studies have described the presence of a mesh of filamentous actin (F-actin) underneath the plasma membrane (Li et al., 1994). In chromaffin cells, it has been proposed that the actin network acts as a barrier to the secretory granules and that stimulation is associated with a focal and transient disassembly of the actin network (Rodriguez et al., 1990; Zhang et al., 1996). This allows the movement of granules from the reserve pool to release sites on the plasma membrane. Thus, the cortical actin network controls the size of the readily releasable pool and consequently the rate of exocytosis. Cortical actin network dynamics is controlled by scinderin, a F-actin-severing protein (Zhang et al., 1996; Trifaró et al., 2000). The scinderin gene has been cloned and nucleotide and amino acid sequence analysis indicates that scinderin has three actin-binding sites (Zhang et al., 1996; Marcu et al., 1998). We demonstrate here using capacitance measurements of exocytosis that scinderin might play a role for insulin secretion since inclusion of peptide fragments to each of the three actin-binding domains on scinderin reduced the rate of exocytosis.

2. Materials and methods

Cultures of mouse pancreatic β-cells were prepared as described elsewhere (Eliasson et al., 1997). Exocytosis was monitored as increases in cell membrane capacitance using the standard whole-cell configuration of the patch-clamp

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technique. The recordings of cell capacitance depicted in Fig. 1 were carried out using an EPC-9 patch-clamp amplifier and the Pulse software (v. 8.01; HEKA Elektronik, Lamprecht/Pfalz, Germany). The interval between two successive points was 0.2 s and the measurements of cell capacitance were initiated < 5 s following establishment of the whole-cell configuration. The extracellular medium consisted of (in mM) 138 NaCl, 5.6 KCl, 2.6 $CaCl_2$, 1.2 $MgCl_2$, 5 HEPES (pH 7.4 with NaOH) and 5 D-glucose, whereas the electrode solution consisted of (in mM) 125 potassium glutamate, 10 KCl, 10 NaCl, 1 $MgCl_2$, 5 HEPES, 3 Mg–ATP, 10 EGTA, 9 $CaCl_2$ (pH 7.15 with KOH). The free Ca^{2+} concentration of the resulting buffer was 1.96 μM using the binding constants of Martell and Smith (1971). For GTPγS-evoked exocytosis (Fig. 3), the pipette solution contained no Ca^{2+} and 50 μM GTPγS. In Fig. 2, changes in cell capacitance were elicited by voltage-clamp depolarisations to zero from a holding potential of −70 mV using an EPC-7 patch-clamp amplifier (List Elektronik, Darmstadt, Germany) and in-house software written in AxoBasic (Axon Instrumens, Foster City, CA, USA) as detailed elsewhere (Ämmälä et al., 1993). In these experiments, the extracellular solution contained (in mM) 118 NaCl, 20 tetraethylammonium–Cl, 5.6 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 5 HEPES (pH 7.40 with NaOH) and 5 D-glucose. Tetraethylammonium–Cl was included in the medium to block the outward delayed rectifying K^+-current, which otherwise obscures the smaller Ca^{2+}-current (Rorsman and Trube, 1986). The pipette solution contained (in mM) 125 Cs-glutamate, 10 CsCl, 10 NaCl, 1 $MgCl_2$, 5 HEPES, 0.05 EGTA, 3 Mg–ATP, 0.1 cAMP and 0.01 GTP (pH 7.15 with CsOH). The scinderin actin-binding peptides were synthesised by Schafer-N (Copenhagen, Denmark) with sequences identical to the active actin-binding domains of scinderin (Zhang et al., 1996; Marcu et al., 1998): Sc^{77-89} (AAAIFTVQMDDYL); $Sc^{138-146}$ (RLLHVKGPR) and $Sc^{511-523}$ (RLFQVRRNLASIT). Scrambled peptides were R-Sc^{77-89} (YAALMFDIDATQV), R-$Sc^{138-146}$ (LVRGKRPLH) and R-$Sc^{511-523}$ (AVNIRLRFSTLQR). The molecular weight of the scinderin-binding peptides are < 2000 Da. Experiments using dextran-conjugated fura-2 with a molecular weight of 3000 Da (Molecular Probes, Eugene, OR, USA) suggest that solution exchange between the pipette and the cell interior is $> 75\%$ complete in < 30 s. All other chemicals were purchased from Sigma. Results are presented as mean values ± S.E.M. for indicated number of experiments. Statistical significance was evaluated using Student's *t*-test.

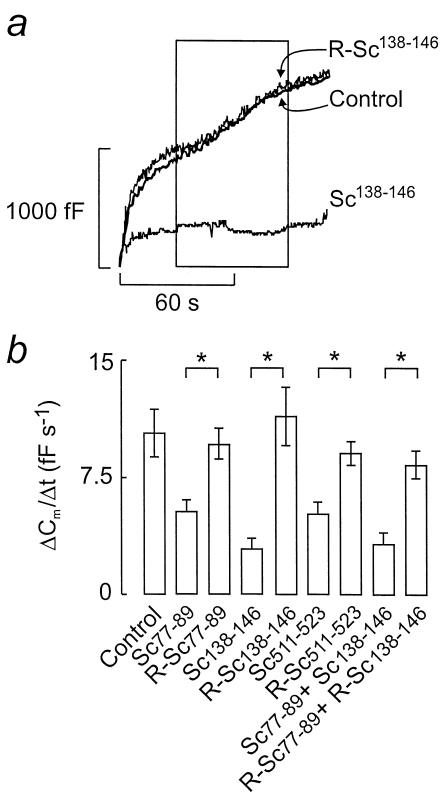

Fig. 1. Effects of scinderin-derived actin-binding peptides on exocytosis in single mouse pancreatic β-cells evoked by infusion of Ca^{2+} through the recording pipette. (a) Increases in cell capacitance elicited by intracellular infusion with a Ca^{2+}-EGTA buffer with a free Ca^{2+} concentration of 2 μM in the absence (control) or presence of 10 μM of either Sc^{77-89} or scrambled Sc^{77-89} (R-Sc^{77-89}). Throughout the recording, the cell was clamped at −70 mV in order to avoid activation of the voltage-dependent Ca^{2+} channels that would otherwise interfere with the measurement. (b) Histogram showing the average rates of increase in cell capacitance ($\Delta C_m/\Delta t$) measured from 30 to 90 s after establishment of the whole-cell configuration under control conditions and in the presence of 10 μM of either Sc^{77-89}; $Sc^{138-146}$; $Sc^{511-523}$ or the corresponding scrambled peptides. In the simultaneous presence of both Sc^{77-89} and $Sc^{138-146}$, 5 μM of each peptide was added to the pipette solution. Data are mean values ± S.E.M. of 6–8 cells. * $P < 0.01$.

3. Results

The effects of peptides with sequences corresponding to the actin-binding sites of scinderin (Sc^{77-89}; $Sc^{138-146}$; $Sc^{511-523}$) were investigated on Ca^{2+}-evoked exocytosis using standard whole-cell recordings. Intracellular dialysis with a Ca^{2+}-EGTA buffer with a free Ca^{2+} concentration of 2 μM stimulated exocytosis (reflected as a gradual capacitance increase; Fig. 1a, control). In general, the increase in cell capacitance reached at a new steady-state level within 5–7 min. Inclusion of 10 μM Sc^{77-89} in the pipette solution reduced the increase in cell capacitance (Fig. 1a, Sc^{77-89}). The rates of capacitance increase were measured 30–90 s after the establishment of the whole-cell configuration (to ensure replacement of the cytoplasm with the pipette solution; see Section 2) and the results are depicted in Fig. 1b. Inclusion of Sc^{77-89} in the pipette solution reduced the rate of exocytosis by 43% ($P < 0.01$; $n = 6$). On the contrary, exocytosis was not affected in the presence of 10 μM of the scrambled peptide (R-Sc^{77-89}). A similar inhibition of Ca^{2+}-induced exocytosis was ob-

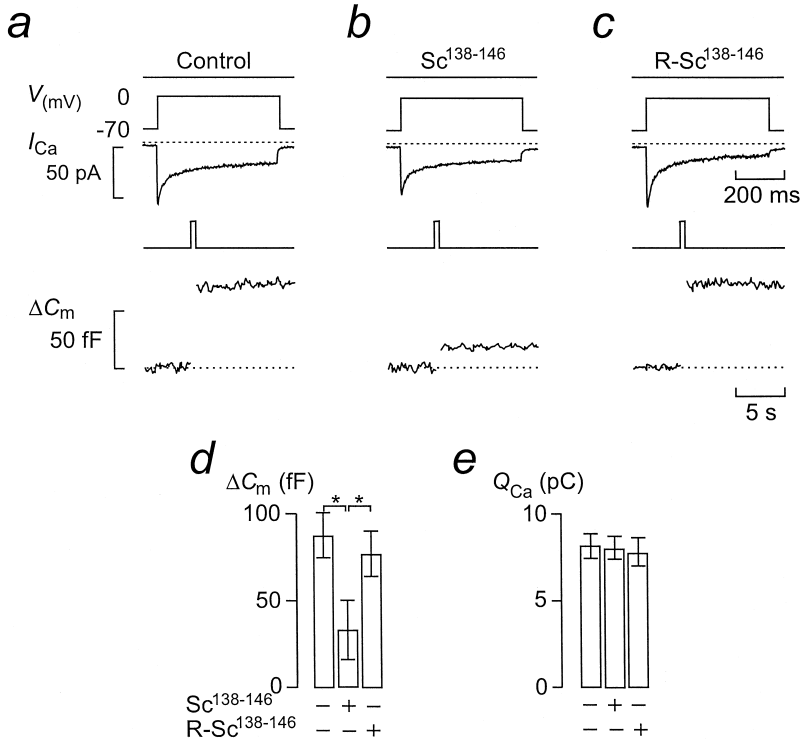

Fig. 2. $Sc^{138-146}$ inhibits depolarisation-induced exocytosis in mouse pancreatic β-cells. Ca^{2+} currents (middle) and capacitance increases (bottom) evoked by 500-ms depolarisations from −70 to 0 mV (top) in the absence (a) and presence of either 10 μM $Sc^{138-146}$ (b) or R-$Sc^{138-146}$ (c). Histograms summarising increases in cell capacitance (ΔC_m; d) and the integrated Ca^{2+} current (Q_{Ca}; e) in the absence (–) or presence of $Sc^{138-146}$ or R-$Sc^{138-146}$ (+). The voltage-clamp depolarisations were applied 2 min after the establishment of the standard whole-cell configuration in order to allow the pipette solution and the peptides to equilibrate with the cytoplasm. The data are mean ± S.E.M. of five cells. $^{*}P < 0.01$.

tained with 10 μM $Sc^{511-523}$ (39% reduction; $P < 0.01$; $n = 6$), whereas $Sc^{138-146}$ (10 μM) reduced exocytosis by 72% ($P < 0.01$; $n = 8$) (Fig. 1b). Moreover, when 5 μM Sc^{77-89} and 5 μM $Sc^{138-146}$ (total peptide concentration: 10 μM) were present together in the pipette solution, inhibition of exocytosis was similar to that observed in the presence of either peptide alone (51%; $P < 0.01$; $n = 7$; Fig. 1b).

In infusion experiments, such as those illustrated in Fig. 1, it is not possible to precisely control the peptide concentration since the wash-in of the pipette solution with the peptides requires some (variable) time. In Fig. 2, we have avoided this problem by eliciting exocytosis in response to 500 ms voltage-clamp depolarisation from −70 to 0 mV. The pipette solution was allowed to equilibrate with the cytoplasm for 2 min before the voltage-clamp depolarisation was applied, which under control conditions produced a capacitance increase of 87 fF (Fig. 2a). Inclusion of 10 μM $Sc^{138-146}$ (the most potent scinderin-binding peptide) in the pipette solution reduced the increase in cell capacitance in response to the membrane depolarisation to 24 fF (Fig. 2b). Exocytosis was not affected in the presence of 10 μM of R-$Sc^{138-146}$ (Fig. 2c). On average, $Sc^{138-146}$ reduced exocytosis by 61 ± 8% ($P < 0.01$; $n = 5$; Fig. 2d) without affecting the integrated Ca^{2+} current (Fig. 2e).

In mouse β-cells, increases in cell capacitance can be elicited both by a rise in $[Ca^{2+}]_i$ and by GTP or its non-hydrolysable analogue GTPγS (Proks et al., 1996). In the following, we have explored whether scinderin is also important for GTPγS-stimulated exocytosis. Fig. 3a shows that inclusion of 10 μM $Sc^{138-146}$ in the pipette solution reduced the GTPγS-induced exocytosis, whereas the scrambled peptide (10 μM) did not affect the secretion rate. On average (Fig. 3b), we found that Sc^{77-89} reduced exocytosis by 75% ($P < 0.01$; $n = 4$), not different from that observed with $Sc^{138-146}$ (69% inhibition; $P < 0.01$; $n = 7$) and $Sc^{511-523}$ (59% reduction; $P < 0.01$; $n = 7$).

4. Discussion

The dynamic changes in the F-actin network observed during exocytosis in (neuro)-endocrine cells is the result of activation of F-actin severing proteins. One such protein is scinderin, which is expressed only in tissues with high secretory activity (Rodriguez et al., 1990). However, the presence of scinderin in the pancreatic β-cell is not known. The effects of stimulation on F-actin disassembly and scinderin redistribution are Ca^{2+}-dependent and precede

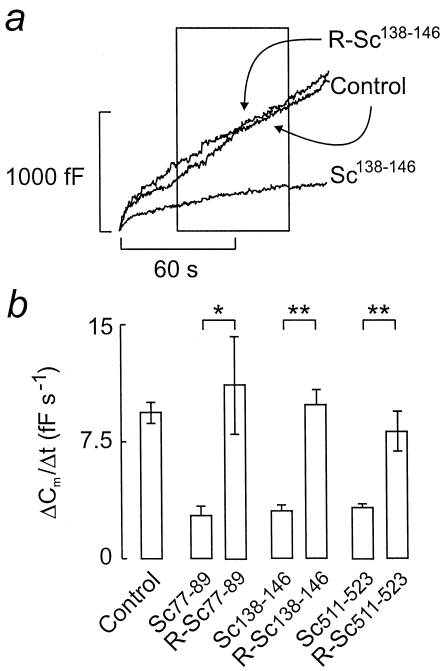

Fig. 3. Effect of Sc^{77-89}, $Sc^{138-146}$ and $Sc^{511-523}$ on GTPγS-evoked exocytosis in mouse pancreatic β-cells. Changes in cell capacitance were elicited by intracellular dialysis with a Ca^{2+}-free pipette solution supplemented with 50 μM GTPγS. (a) Increases in cell capacitance in the absence and presence of $Sc^{138-146}$ or R-$Sc^{138-146}$ (10 μM). (b) Histogram shows average rates of increase in cell capacitance ($\Delta C_m/\Delta t$) measured from 30–90 s after establishment of the whole-cell configuration. Data are mean ± S.E.M. of 4–7 cells. $^{*}P < 0.01$.

exocytosis. Our results demonstrate that scinderin might play an important role not only for Ca^{2+}- but also GTPγS-dependent exocytosis in mouse pancreatic β-cells since preventing the actin severing activity of scinderin with peptides with sequences identical to the active actin-binding sites strongly reduced the rate of exocytosis. This is consistent with previous observations that a F-actin network is localised in the cortical surface of pancreatic β-cells and might act as a barrier to the secretory vesicles, impeding their contact with the plasma membrane (Orci et al., 1972). In chromaffin cells, the actin-severing activity of scinderin is required for stimulation-induced disassembly of the actin network (Zhang et al., 1996) and it has been demonstrated that the concentrations of Ca^{2+} required to activate scinderin are in the range of Ca^{2+} concentrations expected to be found in the cytoplasm as a result of cell stimulation (Rodriguez et al., 1990). Our data suggest that inhibition of one actin-binding site is sufficient to inhibit exocytosis, suggesting that one actin-binding domain is sufficient for mediating F-actin disassembly and exocytosis.

The present experiments describe for the first time an important role for scinderin in GTPγS-induced exocytosis since the rate of secretion was reduced by $> 50\%$ in the presence of the three scinderin-derived actin-binding peptides. This is consistent with the previous observation that this GTP analogue induced a reduction of cortical actin filaments in rat peritoneal mast cells (Norman et al., 1994). This raises the interesting possibility that a heterotrimeric G-protein or a small GTPase participates in the scinderin-evoked changes in the actin cytoskeleton during cell stimulation.

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