cAMP Induces Stromal Interaction Molecule 1 (STIM1) Puncta but neither Orai1 Protein Clustering nor Store-operated Ca\(^{2+}\) Entry (SOCE) in Islet Cells*

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**Background:** Regulation of the ER Ca\(^{2+}\) sensor STIM1 and its association with the plasma membrane channel Orai1 to activate store-operated Ca\(^{2+}\) entry (SOCE) remains incompletely understood.

**Results:** cAMP induced plasma membrane translocation of STIM1 in islet cells without concomitant SOCE activation.

**Conclusion:** STIM1 translocation alone is insufficient to activate SOCE.

**Significance:** This study describes novel interaction between the components of cAMP and Ca\(^{2+}\) signaling cascades.

The events leading to the activation of store-operated Ca\(^{2+}\) entry (SOCE) involve Ca\(^{2+}\) depletion of the endoplasmic reticulum (ER) resulting in translocation of the transmembrane Ca\(^{2+}\) sensor protein, stromal interaction molecule 1 (STIM1), to the junctions between ER and the plasma membrane where it binds to the Ca\(^{2+}\) channel protein Orai1 to activate Ca\(^{2+}\) influx. Using confocal and total internal reflection fluorescence microscopy, we studied redistribution kinetics of fluorescence-tagged STIM1 and Orai1 as well as SOCE in insulin-releasing \(\beta\)-cells and glucagon-secreting \(\alpha\)-cells within intact mouse and human pancreatic islets. ER Ca\(^{2+}\) depletion triggered accumulation of STIM1 puncta in the subplasmalemmal ER where they co-clustered with Orai1 in the plasma membrane and activated SOCE. Glucose, which promotes Ca\(^{2+}\) store filling and inhibits SOCE, stimulated retranslocation of STIM1 to the bulk ER. This effect was evident at much lower glucose concentrations in \(\alpha\)-than in \(\beta\)-cells consistent with involvement of SOCE in the regulation of glucagon secretion. Epinephrine stimulated subplasmalemmal translocation of STIM1 in \(\alpha\)-cells and retranslocation in \(\beta\)-cells involving raising and lowering of cAMP, respectively. The cAMP effect was mediated both by protein kinase A and exchange protein directly activated by cAMP (Epac) in their N termini facing the ER lumen and are believed to sense the Ca\(^{2+}\) depletion that activates SOCE (3, 5, 6). Another molecule in the PM named Orai was identified as an important player in the store-operated mechanism (7–9). There are three isoforms of Orai (Orai1–3), and evidence has accumulated that Orai is the pore-forming unit of the store-operated channel (10–14). Emptying of Ca\(^{2+}\) from the ER results in dissociation of the ER from STIM1, which rapidly moves and aggregates in the subplasmalemmal ER where it forms distinct puncta (3, 12, 15, 16), and this is the site where STIM interacts with Orai to activate SOCE (11, 17, 18).

The store-operated pathway is also present in excitable cells like the insulin-releasing \(\beta\)- and glucagon-secreting \(\alpha\)-cells. Although these cells have more potent voltage-operated routes for Ca\(^{2+}\) entry, SOCE has been found to be important for different cellular processes. However, even maximally activated SOCE has modest effects on the cytoplasmic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]) in \(\beta\) (19–21) and \(\alpha\)-cells (22) and has mostly been attributed a functional role based on the depolarizing effect (22–24). Whereas Ca\(^{2+}\) depletion of the ER has little effect on the membrane potential (20) and insulin release (24)

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Based on studies in nonexcitable cells, Putney (1) proposed the existence of a store-operated or capacitative pathway for Ca\(^{2+}\) entry into cells. The store-operated Ca\(^{2+}\) entry (SOCE)

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\(^2\) The abbreviations used are: SOCE, store-operated Ca\(^{2+}\) entry; PM, plasma membrane; ER, endoplasmic reticulum; IP\(_3\), inositol 1,4,5-trisphosphate; SERCA, sarcoendoplasmic reticulum Ca\(^{2+}\)-ATPase; ROI, region of interest; TIRF, total internal reflection fluorescence.
from β-cells exposed to a sub-stimulatory glucose concentration (3 mM), such depletion further depolarizes glucose-stimulated β-cells (23) and amplifies insulin secretion (23, 24). Activation of SOCE has much more important effects on the α-cell by triggering Ca\(^{2+}\) entry through voltage-operated Ca\(^{2+}\) channels (VOCCs) (22) and glucagon release (24), and SOCE has been attributed to be a central function in epinephrine stimulation and glucagon inhibition of glucagon secretion (22, 24).

Studies of the store-operated mechanism in primary β-cells (21, 25) and α-cells (22) have been based on measurements of [Ca\(^{2+}\)]\(_i\), in Ca\(^{2+}\) omission-readdition (21, 22, 25) and Mn\(^{2+}\) quench (21, 25) experiments. Direct studies of molecules involved in the store-operated mechanism have so far been restricted to clonal MIN6 β-cells transfected with STIM1 tagged with enhanced yellow fluorescent protein (STIM1-YFP) showing that Ca\(^{2+}\) depletion of the ER triggers the expected PM association of the molecule (26). We have now utilized adenoviruses encoding STIM1-YFP and Orai1-mCherry (16) to infect pancreatic islets and study STIM1 translocation and association with Orai1 in primary pancreatic islet cells during conditions known to modulate hormone secretion and SOCE. Consistent with a role of SOCE in glucagon secretion, the Ca\(^{2+}\) entry was controlled by similar low glucose concentrations in α-cells as those regulating release of the glucose-elevating hormone. We also discovered that CAMP triggers STIM1 translocation to the subplasmalemmal regions but neither induces co-clustering of STIM1-Orai1 nor the activation of SOCE that occurs after calcium depletion of the ER. The data indicate that STIM1 translocation can occur independent of Orai1 clustering and SOCE.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—Epinephrine, cyclopiazonic acid (CPA), 2‘,5’-dideoxyadenosine (DDA), 3-isobutyl-1-methylxanthine (IBMX), carbachol, forskolin, poly-L-lysine, EGTA, HEPES, and methoxyverapamil were purchased from Sigma, and RPMI 1640 medium and fetal bovine serum were from Invitrogen. Biolog Life Science Institute (Bremen, Germany) supplied N\(^\circ\)-phenyladenosine-3’-5’-cyclic monophosphate, 8-(4-chlorophenyl-thio)adenosine-3’-5’-cyclic monophosphorothioate, S\(_p\) isomer (S\(_p\)-8-CPT-cAMPS), and 8-(4-chlorophenylthio)-2’-O-methyladenosine-3’,5’-cyclic monophosphate, acetoxymethyl ester. The acetoxymethyl ester of the Ca\(^{2+}\) indicator Fura-PE3 was obtained from TEF Labs (Austin, TX). Tris was from Merck; diazoxide was from Schering-Plough (Rathdum, Ireland); and serum-free protein block, rabbit anti-glucagon and guinea pig anti-insulin were bought from Dako (Glostrup, Denmark). The MACH 3\(^{TM}\) rabbit probe alkaline phosphatase polymer kit was from Biocare (Concord, CA). Mayer’s hematoxylin was bought from HistoLab (Gothenburg, Sweden). Adenoviruses expressing STIM1-YFP and Orai1-mCherry were produced as described previously (16).

**Islet Isolation, Cell Culture, and Virus Infection**—All procedures for animal handling, preparation, and use of pancreatic islets were approved by local animal and human ethical committees. Islets of Langerhans were isolated from C57BL/6J female mice. The animals were placed in a sealed container into which a stream of CO\(_2\) was delivered. When the animals became unconscious, they were exsanguinated by decapitation. After opening the peritoneal cavity, the splenic part of the pancreas was excised and cut into small pieces, which were digested with 1 mg/ml collagenase to obtain free islets of Langerhans. Human pancreatic islets from five normoglycemic cadaveric donors (two female, three male; aged 39–57 years) were generously provided by the Nordic Network for Clinical Islet Transplantation. The islets were isolated with semiautomated digestion filtration (27) and purified on a continuous density gradient in a refrigerated cell processor (COBE 2191; COBE Blood Component Technology, Lakewood, CO). After purification, the islets were kept for 2–5 days at 37 ºC in an atmosphere of 5% CO\(_2\) in CMRL 1066 culture medium (Mediatech, Herndon, VA) containing 5.5 mM glucose and supplemented with 10 mM nicotinamide, 10 mM HEPES, 0.25 µg/ml Fungizone, 50 µg/ml gentamicin, 2 mM glutamine, 10 µg/ml ciprofloxacin, and 10% fetal calf serum. The islets were subsequently cultured for 1–4 days in RPMI 1640 medium containing 5.5 mM glucose and supplemented with 10% fetal calf serum, 100 µg/ml penicillin, and 100 µg/ml streptomycin. The islets were then infected with adenovirus encoding STIM1-YFP and/or Orai1-mCherry using a multiplicity of infection of 10\(^5\) fluorescent focus-forming units/islet in culture medium. After 1 h of incubation at 37 ºC, the inoculum was removed, and the islets were washed twice, followed by further culture for 24 h. To check that fluorescence changes did not merely reflect alterations of membrane properties and/or cell adhesion, some measurements were performed after co-infection with adenovirus encoding cyan fluorescent protein (CFP) anchored to the PM as reference (28). Before the experiments, the islets were transferred to a buffer containing 125 mM NaCl, 4.8 mM KCl, 1.3 mM CaCl\(_2\), 1.2 mM MgCl\(_2\), and 25 mM HEPES (with pH adjusted to 7.40 with NaOH) and incubated for 30 min at 37 ºC. The islets were then allowed to attach to the center of polylysine-coated round 25-mm coverslips for 5 min. Some experiments were performed on single cells prepared by shaking the freshly isolated islets in a Ca\(^{2+}\)-deficient medium (29). After resuspension in the RPMI 1640 culture medium, the cells were allowed to attach to the center of round coverslips during 2–5 days of culture at 37 ºC in an atmosphere of 5% CO\(_2\) in humidified air.

**Measurements of STIM1-YFP Translocation and Redistribution of Orai1-mCherry**—The subcellular distribution of the STIM1-YFP fluorescence was analyzed with a Yokogawa CSU-10 spinning disk confocal system (Andor Technology, Belfast, Northern Ireland) attached to a TE2000 microscope (Nikon) with a ×60 1.40 NA objective (Nikon). The fluorescence was excited by the 514-nm line of an argon ion laser (ALC ×60, Creative Laser Corp, Munich, Germany). The laser beam was homogenized and expanded by a rotating light-shaping diffruser (Physical Optics Corp., Torrance, CA) before being refocused into the confocal scanhead. Fluorescence emission was selected by a 560–40-nm half-bandwidth filter (Semrock, Rochester, NY) and detected with a back-illuminated EMCCD camera (DU-888, Andor Technology) under MetaFluor (Molecular Devices Corp., Downingtown, PA) software control. An Eclipse Ti microscope (Nikon) with a total internal reflection fluorescence (TIRF) illuminator and a ×60 1.45 NA objective was used for measurements of the PM concentrations of...
STIM1-YFP, CFP-tagged PM marker, and Orai1-mCherry. The 514- and 458-nm lines of an argon laser (Creative Laser Production) were used for excitation of YFP and CFP, respectively, and the 561-nm line of a diode-pumped solid-state laser (Jive, Cobolt AB, Stockholm, Sweden) was used to excite mCherry. However, because of the properties of the dichroic mirror, the 488-nm line of the argon laser was used to excite YFP when measured in parallel with mCherry. Wavelengths were selected by interference filters (Semrock) mounted in a filter wheel (Sutter Instruments, Novato, CA), and the beam was coupled to the TIRF illuminator by an optical fiber (Oz Optics, Ottawa, Canada). Fluorescence was detected with a back-illuminated EMCCD camera (DU-887, Andor Technology) controlled by the MetaFluor software. Emission wavelengths were selected with interference filters (560-/40-nm half-bandwidth for YFP, 485-/25-nm for CFP; Semrock) or a glass filter (645-nm long pass for mCherry; Melles Griot) mounted in a filter wheel (Sutter Instruments). YFP or mCherry images and YFP/CFP or YFP/mCherry image pairs were acquired every 5 s. The beam was blocked by a shutter (Sutter Instruments) between image captures to minimize exposure of the cells to the potentially harmful laser light. The coverslips with the attached islets were used as exchangeable bottoms of an open custom-built 50-µl laminar flow chamber. The chamber holder on the microscope stage and the objective were thermostated at 37 °C. Islets in the chamber were superfused with medium at a rate of 0.3 ml/min.

Measurements of [Ca<sup>2+</sup>]:—For [Ca<sup>2+</sup>], measurements, the cells were preincubated in the presence of 1 µM of the acetoxyethyl ester of Fura-PE3. [Ca<sup>2+</sup>], imaging was performed with an inverted microscope (Nikon Diaphot) placed in a climate box maintained at 37 °C. The microscope was equipped for epifluorescence fluorometry with a 400-nm dichroic mirror and a ×40 1.3 NA Fluor oil immersion objective. Excitation light was delivered through a 5-mm diameter liquid light guide from an Optoscan monochromator (Cairn Research Ltd., Faversham, UK) with a 150-watt xenon arc lamp. The monochromator provided excitation light at 340 nm (2.5-nm half-bandwidth) and 380 nm (1.9-nm half-bandwidth), and emission was measured at 510 nm (40-nm half-bandwidth) using a EMCCD camera (DU-887, Andor Technology). The Metafluor software controlled the monochromator and the camera, acquiring image pairs every 2 s with 80–100-ms integration at each wavelength and <1 ms for changing wavelength and slits. To reduce photodamage, the specimens were illuminated only during image capture. Ratio frames were calculated after background subtraction, and [Ca<sup>2+</sup>] was estimated as described previously (30).
Cell Identification—Immediately after experiments, α- and β-cells remaining in position within the experimental chamber in the microscope were identified by immunostaining for glucagon and insulin. The cells were fixed by sequential 5-min exposures to 25, 50, 75, and 95% ethanol. After sequential rinsing with 3% H2O2 and Tris buffer (0.05 M, pH 7.4), protein block was added to reduce background staining. After 10 min, polyclonal rabbit anti-glucagon or guinea pig anti-swine insulin (1:100; DAKO) was added for 30 min followed by rinsing with Tris buffer. The MACH 3TM rabbit probe alkaline phosphatase (1:100; DAKO) was added for 30 min followed by rinsing with Tris buffer and distilled water, cell nuclei were stained with hematoxylin for 0.5–2 min. The α-cells are smaller than the β-cells, and the two cell types show opposite responses to epinephrine with regard to STIM1-YFP translocation between the bulk ER and the subplasmalemmal junctions (see “Results”). Therefore, the size of the cell footprint together with the translocation response to epinephrine was used for cell identification in most experiments. These criteria should eliminate the small somatostatin-releasing δ-cells with β-cell-like domination of α2-α3-adrenoreceptors (31) as small cells and cells with small footprints were never taken as β-cells. Because epinephrine mobilizes intracellular Ca2+ in α- but not β-cells (22), this response together with cell size was used for cell identification in most measurements of [Ca2+]i.

Data and Statistical Analysis—Image analysis was made using the MetaFluor or ImageJ (W. S. Rasband, National Institutes of Health, rsb.info.nih.gov) software. The STIM1-YFP concentration in the subplasmalemmal region was evaluated as the fluorescence intensity F in relation to the initial fluorescence intensity F0 after subtraction of background (F/F0). To quantify the redistribution of Orai1 in the PM, we analyzed the intensity variability in the Orai1 images by calculating the variation coefficient of pixel intensities. The pixel size was 266 nm, which is close to the theoretical 222-nm optical resolution in 645 nm of light. Data are presented as means ± S.E. Statistical comparisons were assessed with Student’s t test.

RESULTS

Depletion of ER Ca2+ Induces Subplasmalemmal STIM1 Accumulation—Peripheral cells in isolated mouse islets expressing STIM1-YFP and exposed to 3 mM glucose showed diffuse fluorescence over the cytoplasm in confocal microscopy (Fig. 1A), but also some subplasmalemmal fluorescence puncta were observed with TIRF microscopy (Fig. 1B). Depletion of the ER Ca2+ stores by inhibition of the sarcoendoplasmic reticulum Ca2+-ATPase (SERCA) with CPA induces gradual formation of much more conspicuous subplasmalemmal puncta (52 ± 4 s rise time; Fig. 1, A and B). This CPA effect was delayed 118 ± 21 s (n = 13). Omission of extracellular Ca2+ with addition of EGTA induced a less marked subplasmalemmal accumulation of STIM1-YFP that was further enhanced by CPA (Fig. 1C).

The cholinergic agonist carbachol (50 μM), which raises IP3 and mobilizes ER Ca2+ both in α-cells (22) and β-cells (19), induced sustained subplasmalemmal accumulation of STIM1-YFP preceded (3 of 8) or not by an initial peak in most islet cells exposed to 3 mM glucose (Fig. 1D). Both the delay before the onset of STIM1 translocation (28 ± 4 s; n = 8) and the rise time (22 ± 4 s) were much shorter than after CPA exposure (4- and 2.4-fold difference, respectively, p < 0.001).

Epinephrine Induces Opposite Translocation of STIM1 in α- and β-Cells—In the presence of 3 mM glucose, the addition of 5 μM epinephrine induced opposite responses in different cells. In the majority of the cells (27 of 41), TIRF microscopy revealed epinephrine-induced loss of PM-associated STIM1-YFP fluorescence indicating retranslocation of the protein from the subplasmalemmal region into the bulk ER (Fig. 2A). In 5 of the 41 cells that had smaller footprints, epinephrine instead induced pronounced subplasmalemmal accumulation of STIM1-YFP with formation of distinct puncta. Fig. 2B shows that one of the latter cells also responded to CPA with similar STIM1-YFP translocation to the PM. There was no epinephrine response in the remaining nine cells (data not shown). Immunostaining for

\[ \text{Ca}^{2+} \]

FIGURE 2. Epinephrine triggers opposite translocation of STIM1-YFP in pancreatic α- and β-cells. A, TIRF images and intensity recordings showing accumulation of the subplasmalemmal STIM1-YFP in response to 5 μM epinephrine (Epi) in a superficial islet cell with a small footprint (α-cell) and the loss of subplasmalemmal STIM1-YFP in an adjacent cell with larger footprint (β-cell) within an islet. The numbered arrowheads indicate when respective images were taken. B, TIRF intensity recording of a superficial islet cell (α-cell) responding to 100 μM CPA and 5 μM epinephrine with subplasmalemmal accumulation of STIM1-YFP. C, immunostaining showing that a small islet cell responding to epinephrine with subplasmalemmal accumulation of STIM1-YFP is a glucagon-positive α-cell (left). Staining for insulin showed that the larger cells with opposite responses to epinephrine are β-cells (data not shown). The glucose concentration was 3 mM in all panels and the image scale bars indicate 10 μm.
insulin and glucagon revealed that the epinephrine-induced decrease and increase of subplasmalemmal STIM1-YFP occurred in β-cells (data not shown) and α-cells (Fig. 2C), respectively.

Glucose Stimulates Retranslocation of STIM1 to Bulk ER—Increase of the glucose concentration from 3 to 20 mM resulted in loss of subplasmalemmal STIM1-YFP fluorescence in β-cells identified by size and epinephrine response (21 of 32; Fig. 3). In most cases, this glucose effect was partially reversed after reintroduction of 3 mM glucose (A) and complete reversal required glucose omission in β-cells (B and C). D, TIRF intensity recording of a β-cell with STIM1-YFP oscillations in 3 mM glucose (Gluc) that are reversibly inhibited by 20 mM glucose. E, TIRF intensity recording of the effect of increasing glucose concentrations within the 0–20 mM range on reduction of subplasmalemmal STIM1-YFP in a single β-cell. F, dose-response relationships for glucose-induced reduction of subplasmalemmal STIM1-YFP in β-cells. Mean values ± S.E., n = 9.
starting to respond to reduction of PM-associated STIM1-YFP fluorescence at 1 and 1.5 mM glucose, respectively, and Fig. 4D summarizes the concentration dependence with half-maximal and maximal effects at 1.3 and 3 mM. Some α-cells showed STIM1-YFP oscillations in the absence of glucose (C). D, dose-response relationships for glucose-induced reduction of subplasmalemmal STIM1-YFP in α-cells. Mean values ± S.E., n = 5.

FIGURE 4. Glucose reduction of subplasmalemmal STIM1-YFP fluorescence in islet α-cells is maximal already at 3 mM. A–C, TIRF intensity recordings on epinephrine-identified (5 μM) α-cells showing that 3 mM glucose is sufficient for maximal reduction of subplasmalemmal STIM1-YFP fluorescence. Some α-cells showed STIM1-YFP oscillations in the absence of glucose (C). D, dose-response relationships for glucose-induced reduction of subplasmalemmal STIM1-YFP in α-cells. Mean values ± S.E., n = 5.

FIGURE 5. Glucose-induced reduction of subplasmalemmal STIM1-YFP fluorescence in human β- and α-cells. A, TIRF intensity recording of an epinephrine-identified (5 μM) β-cell showing that the glucose-induced reduction of subplasmalemmal STIM1-YFP fluorescence is interrupted by peaks of STIM1-YFP increase. B, monotonous reduction of subplasmalemmal STIM1-YFP fluorescence by increasing glucose concentrations after hyperpolarization of an epinephrine-identified (Epi, 5 μM) β-cell with diazoxide. C, reduction of subplasmalemmal STIM1-YFP fluorescence in an epinephrine-identified (5 μM) α-cell is maximal already at 3 mM glucose.

cAMP Regulates STIM1 but Neither Orai1 nor SOCE

PM fluorescence in all five β-cells (Fig. 5A). This phenomenon may reflect activation of the store-operated pathway by Ca^{2+} release from the ER triggered by Ca^{2+} entry through VOCCs. We therefore repeated experiments under conditions preventing such entry. As shown in Fig. 5B, increasing glucose concentrations monotonously reduced subplasmalemmal STIM1-YFP fluorescence in the presence of the hyperpolarizing ATP-sensitive K⁺ (K_{ATP}) channel activator diazoxide with maximal effect at 7–11 mM of the sugar in two studied β-cells. Also, four human α-cells behaved similarly to mouse α-cells with maximal loss of subplasmalemmal fluorescence already with 3 mM glucose (Fig. 5C).

cAMP Induces Subplasmalemmal STIM1 Accumulation Involving PKA and Epac—Because epinephrine acts on α₁ (32) and β-adrenoceptors (32, 33) in α-cells to increase IP₃ and cAMP (34) resulting in Ca^{2+} release from the ER, it was not surprising that epinephrine induced subplasmalemmal accumulation of STIM1-YFP. However, the opposite effect in β-cells was unexpected considering that epinephrine acts on α₂-adrenoceptors (33, 35) to lower cAMP (34). We therefore tested whether changes in cAMP might be involved in the STIM1 translocation responses of the two cell types. These experiments were done at a basal glucose concentration to prevent cAMP from promoting IP₃ receptor-mediated Ca^{2+} release, which only occurs in β-cells exposed to higher concen-
**cAMP Regulates STIM1 but Neither Orai1 nor SOCE**

FIGURE 6. *cAMP induces subplasmalemmal accumulation of STIM1-YFP.* TIRF intensity recordings of subplasmalemmal fluorescence of STIM1-YFP in cells within pancreatic islets. *A* and *B*, adenylyl cyclase activator forskolin (5 μM) increases subplasmalemmal fluorescence of STIM1-YFP in epinephrine-identified (Epi, 5 μM) α- and β-cells. *C*, phosphodiesterase inhibitor IBMX (50 μM) increases subplasmalemmal STIM1-YFP fluorescence, and the adenylyl cyclase inhibitor DDA (100 μM) reverses this effect in an epinephrine-identified (5 μM) β-cells. *D*, IBMX increases subplasmalemmal STIM1-YFP fluorescence, and DDA reverses this effect in an epinephrine-identified (5 μM) α-cells. *E*, specific PKA agonist N6-phenyladenosine-3',5'-cyclic monophosphate (6-Phe-cAMP) (100 μM) increases subplasmalemmal STIM1-YFP fluorescence and forskolin (10 μM) has additional effect in an epinephrine-identified (5 μM) β-cell. *F*, specific Epac agonist 8-(4-chlorophenylthio)-2'-O-methyladenosine-3',5'-cyclic monophosphate, acetoxymethyl ester (8-CPT-AM) (1 μM) increases subplasmalemmal STIM1-YFP fluorescence in an epinephrine-identified (5 μM) β-cell. The glucose concentration was 3 mM in all panels.

...trations of the sugar (36, 37). Because [Ca\(^{2+}\)]\(_i\) in β-cells is low and stable under these conditions, the reported effects of [Ca\(^{2+}\)]\(_i\), elevation on STIM1 translocation (38) should not influence the results. Fig. 6, *A* and *B*, shows that the rise of cAMP by activation of adenylyl cyclases with 5 μM forskolin increased STIM1-YFP translocation to the PM in 22 of 27 β-cells with opposite response to epinephrine. Also, most epinephrine-identified α-cells (6 of 8) reacted to forskolin in a similar manner (Fig. 6*B*), and the remaining α-cells did not respond (Fig. 6*A*). Fig. 6, *C* and *D*, illustrates that STIM1-YFP translocation to the PM after rise of cAMP by phosphodiesterase inhibition with 50 μM IBMX is reversed by 100 μM of the adenylyl cyclase inhibitor DDA in all of 10 β-cells (Fig. 6*C*) and all of 9 α-cells (Fig. 6*D*) identified with epinephrine. Also, the specific PKA agonists N6-phenyladenosine-3',5'-cyclic monophosphate (100 μM) induced STIM1-YFP translocation to the PM in 11 of 17 β-cells (Fig. 6*E*) and 6 of 10 α-cells (data not shown) identified with epinephrine, and similar effects were seen with another PKA activator (100 μM Sp-8-CPT-cAMPS; data not shown). However, elevation of cAMP with forskolin induced additional PM translocation of STIM1-YFP (Fig. 6*E*, 6 of 8 β-cells). We therefore also tested the effect of 1–2 μM of the Epac activator 8-(4-chlorophenylthio)-2'-O-methyladenosine-3',5'-cyclic monophosphate, acetoxymethyl ester (8-CPT-AM) (1 μM) increases subplasmalemmal STIM1-YFP fluorescence in an epinephrine-identified (5 μM) β-cell. The glucose concentration was 3 mM in all panels.

**cAMP-stimulated STIM1 Translocation Does Not Activate SOCE**—The effects of cAMP modulation on SOCE were studied using a Ca\(^{2+}\) omission-readition approach in cells that were hyperpolarized with dazoxoxide and exposed to the Ca\(^{2+}\) channel blocker methoxyverapamil to prevent voltage-operated Ca\(^{2+}\) entry. The β-cell in Fig. 7*A* was initially exposed to 1.3 mM Ca\(^{2+}\) and 3 mM glucose, which causes less than half-maximal Ca\(^{2+}\) filling of the ER (19) associated with partial inactivation of the store-operated pathway (21, 25). When subsequent omission of extracellular Ca\(^{2+}\) had lowered [Ca\(^{2+}\)]\(_i\) to a stable level, the introduction of 10 mM Ca\(^{2+}\) induced 18 ± 4 nm...
cAMP Regulates STIM1 but Neither Orai1 nor SOCE

The effect of cAMP elevation on SOCE was also tested in the presence of 20 mM glucose that maximally fills the ER with Ca\(^{2+}\) and the cells hyperpolarized with diazoxide to prevent triggering of Ca\(^{2+}\)-induced Ca\(^{2+}\) release, there were few STIM1-YFP puncta at the PM (Fig. 8A, top panels). The number of puncta increased markedly in response to forskolin, and the effect was even more striking after depletion of ER Ca\(^{2+}\) with CPA. The more pronounced effect of CPA than of forskolin was preferentially observed when STIM1 was co-expressed with Orai-1. In cells expressing STIM1-YFP alone, the total PM-associated STIM1-YFP fluorescence increased 1.64 ± 0.19-fold in the presence of forskolin and 1.82 ± 0.21-fold (not significant, \(n = 6\)) of control during subsequent exposure to CPA. However, after co-expression with Orai1-mCherry, the total PM-associated STIM1-YFP fluorescence in response to forskolin was 1.31 ± 0.16-fold and that induced by CPA was 2.25 ± 0.24-fold (\(p < 0.001; n = 8\)) of control.

Under control conditions, Orai1-mCherry showed a diffuse distribution with even PM fluorescence and no apparent effect of exposure to forskolin. The variation coefficients in pixel intensities under these conditions were identical (10.2 ± 0.3% for control and 10.3 ± 0.2% for forskolin). However, subsequent SERCA inhibition with CPA induced Orai1-mCherry redistribution with formation of a punctate pattern (Fig. 8A, middle panels) resulting in a highly significant (\(p = 0.001\)) increase of the variation coefficient to 13.5 ± 0.4%. The CPA-induced Orai1-mCherry puncta co-localized with those of STIM1-YFP, whereas STIM1-YFP puncta formed in response to forskolin did not associate with increased Orai1-mCherry (Fig. 8A, bottom panels). To quantify the changes in STIM1 and Orai1, regions of interest (ROIs) were selected corresponding to STIM1 puncta formed either in response to CPA or forskolin, and the STIM1-YFP and Orai1-mCherry fluorescence was then measured in these ROIs. Fig. 8B shows that the STIM1-YFP fluorescence within ROIs defined by CPA-induced STIM1-YFP puncta showed a less pronounced increase during exposure to forskolin. However, the Orai1-mCherry fluorescence within these ROIs only increased in response to CPA (Fig. 8C). When the ROIs were instead defined by STIM1-YFP puncta formed in response to forskolin, the STIM1-YFP fluorescence within these ROIs also increased after exposure to CPA (Fig. 8D). The Orai1-mCherry fluorescence within the same ROIs was not different under basal conditions and in the presence of forskolin but tended to decrease slightly after exposure to CPA (Fig. 8E). Orai1 consequently only associates with STIM1 after Ca\(^{2+}\) depletion of the ER and preferentially at sites other than those where STIM1 forms puncta in response to forskolin. This conclusion was supported by comparing the location of STIM1 puncta formed in response to forskolin and CPA. As seen in Fig. 8F, there was relatively little overlap (yellow) between STIM1-YFP puncta formed in response to forskolin (displayed in red) and those formed after exposure to CPA (displayed in green).

DISCUSSION

This study shows that Ca\(^{2+}\) release from the ER and sequestration of the ion in this organelle in the insulin- and glucagon-releasing pancreatic islet cells cause the characteristic translocations of STIM1 to and from the subplasmalemmal junctions,
respectively. It also demonstrates that epinephrine mimics the effect of Ca
store depletion in inducing subplasmalemmal accumulation of STIM1 in α-cells but that the opposite effect is observed in β-cells. Searching for the underlying mechanism, we found that cAMP, which increases and decreases in response to epinephrine in α- and β-cells, respectively (34), is involved in the subplasmalemmal accumulation of STIM1. Because the dynamin-related mitochondrial protein mitofusin 2, which was recently implicated in the trafficking of STIM1 to the ER-PM junctions (39), is regulated by PKA (40), we speculate that this mechanism partakes in the cAMP-induced STIM1 translocation. Additional processes are likely involved, as translocation was induced both by specific PKA and Epac agonists. However, although the STIM1 translocation determined by the filling state of the ER affected STIM1-Orai1 co-clustering and modulated SOCE as expected, translocation determined by cAMP occurred without effect on Orai1 and Ca
entry. Activation of SOCE by Ca
store depletion has been found to involve a conformational transition that releases the Orai-activating region of STIM1 from an intramolecular clamp (41, 42). Our data indicate that cAMP induces STIM1 translocation independent of such a conformational change.

Glucose is the major physiological stimulator of insulin secretion. The sugar is taken up and metabolized by the β-cells resulting in increased ATP production. An early effect of glucose stimulation is therefore to energize the SERCA pump causing calcium sequestration in the ER, lowering of [Ca
], (20, 43) and initial inhibition of insulin secretion (44, 45). The increased ATP/ADP ratio also closes KATP channels in the PM with ensuing depolarization. Subsequent opening of L-type VOCCs results in entry of Ca
and a rise of [Ca
], that trig-

FIGURE 8. Ca
store depletion but not cAMP induces STIM1-Orai1 co-clustering in islet cells. A, TIRF images of an islet cell co-expressing STIM1-YFP (green) and Orai1-mCherry (red). The cell was initially exposed to 20 mM glucose while hyperpolarized with 250 μM of the KATP channel activator diazoxide (basal). Subsequently 5 μM forskolin and 50 μM CPA were added. The top and middle rows show STIM1 and Orai1 images, respectively, and the bottom row shows a STIM1/Orai1 overlay. The images represent averages of 25 consecutive frames under each condition. B and C, changes in mean STIM1-YFP and Orai1-mCherry fluorescence intensity ± S.E. in 25 ROIs based on selection of STIM1 puncta that were formed after exposure to CPA. D and E, changes in mean STIM1-YFP and Orai1-mCherry fluorescence intensity ± S.E. in 18 ROIs based on selection of STIM1 puncta that were formed after exposure to forskolin. F, overlay image of STIM1 after exposure to forskolin (red) and CPA (green). Image scale bars indicate 1 μm.
CAMP Regulates STIM1 but Neither Orai1 nor SOCE

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MARCH 23, 2012 • VOLUME 287 • NUMBER 13

JOURNAL OF BIOLOGICAL CHEMISTRY 9871
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