Increased Intracellular Calcium Is Required for Spreading of Rat Islet β-Cells on Extracellular Matrix

Domenico Bosco, Carmen Gonelle-Gispert, Claes B. Wollheim, Philippe A. Halban, and Dominique G. Rouiller

Rat islet β-cells spread in response to glucose when attached on the matrix produced by a rat bladder carcinoma cell line (804G). Furthermore, in a mixed population of cells, it has been observed previously that spread cells secrete more insulin acutely in response to glucose, compared with cells that remain rounded. These results suggest bi-directional signaling between the islet β-cell and the extracellular matrix. In the present study, the role of increased intracellular free Ca²⁺ concentration [Ca²⁺]i as an intracellular step linking glucose stimulation and β-cell spreading (inside-out signaling) was investigated. Purified rat β-cells were attached to this matrix and incubated under various conditions known to affect [Ca²⁺]i. The effect of glucose on β-cell spreading was mimicked by 25 mmol/l KCl (which induces calcium influx) and inhibited by diazoxide (which impairs depolarization and calcium entry) and by the L-type Ca²⁺ channel blocker SR-7037. When a 24-h incubation at 16.7 glucose was followed by 24 h at 2.8 mmol/l, β-cells that had first spread regained a round phenotype. In the presence of thapsigargin, spreading progressed throughout the experiment, suggesting that capture of calcium by the endoplasmic reticulum is involved in the reversibility of spreading previously induced by glucose. Spreading was still observed in degranulated β-cells and in botulinum neurotoxin E-expressing β-cells when exocytosis was prevented. In summary, the results indicate that increased [Ca²⁺]i is required for the glucose-induced spreading of β-cells on 804G matrix and that it is not a consequence of exocytotic processes that follow elevation of [Ca²⁺]i.

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xtracellular matrix (ECM) surrounds pancreatic islets and forms the basal lamina, which separates endocrine cells from capillaries. Although the molecular components of these basal laminae remain to be fully investigated in islets, there is evidence indicating that some of the molecules generally found in epithelial basal lamina play a role in the growth and differentiation of β-cells. Fetal pancreatic epithelia cultured in Matrigel or collagen I gel differentiated into endocrine cells and form islet structures (1); neonatal rat islet cells embedded in collagen I formed well-organized islet-like organoids (2); and adult human β-cells or associated islet cells proliferated when attached on 804G matrix produced by a rat bladder carcinoma cell line (3,4). More recently, it has been shown that laminin I specifically promotes differentiation of mouse β-cells from precursor cells in vitro. This finding is of physiological relevance because laminin I is expressed in basement membranes of the mouse pancreas from ~15 days of intrauterine life (5).

The importance of cell-matrix interactions for an optimal regulated insulin secretion is supported by a number of studies on β-cells cultured on various crude matrixes or their purified extracts (6–14). In a recent study, we observed that β-cells attached to a matrix produced by a rat bladder carcinoma cell line 804G (hereafter referred to as 804G matrix) secreted more insulin than control cells. We also noticed that on 804G matrix, cells that had spread expressed higher amounts of α6β1 integrins and secreted more insulin than cells that had remained round (15). The importance of cell-matrix interactions for regulated insulin secretion was further demonstrated by the fact that secretagogues, including glucose, increased the number of spread cells (15).

The mechanism leading stimulated β-cell to spread on 804G matrix is totally unknown. In the present study, we raise the hypothesis that some of the intracellular pathways governing stimulation-secretion coupling of β-cells might be shared for coupling stimulation and spreading. Intracellular free Ca²⁺ concentration [Ca²⁺]i plays a central role in the consensus model for stimulus-secretion coupling, which may be summarized as follows: in response to glucose, β-cell metabolism is stimulated, resulting in increased levels of ATP produced by the mitochondria. ATP-sensitive K⁺ (KATP) channels then close, thereby triggering membrane depolarization. The subsequent opening of voltage-dependent Ca²⁺ channels leads to an increase in [Ca²⁺]i, which is the main trigger for insulin secretion (16). In the present study, we show that increased [Ca²⁺]i is also a key factor in glucose-induced spreading of rat β-cells on 804G matrix.

RESEARCH DESIGN AND METHODS

**Iset isolation and β-cell purification.** Islets of Langerhans were isolated by collagenase digestion of pancreata from male Sprague-Dawley rats (180–200 g wt), followed by Ficoll purification using a modification of previously

DIABETES, VOL. 50, MAY 2001 1039
of this preparation were injected in Cunningham’s chambers. After 3 h of incubation at 37°C, the chambers were first rinsed with KRBB containing either 2.8 or 16.7 mmol/l glucose and then filled with the same buffer, supplemented with a heat-inactivated (45 min at 50°C) anti-insulin guinea pig serum (1:50 dilution) (22). After 1 h at 37°C, chambers were rinsed with KRBB containing 2.8 mmol/l glucose, filled with the same buffer containing guinea pig complement (1:40) (Behring Institute), and incubated at 37°C for 1 h. Chambers were then filled with a 0.04% (w/v) solution of trypsin blue in KRBB, rinsed with KRBB, and filled with 4% paraformaldehyde.

Analysis was restricted to single cells that excluded trypsin blue at the end of the plaque assay. Fluorescent (GFP-positive) cells and nonfluorescent (GFP-negative) cells were analyzed separately. Results are expressed as percentage of cells surrounded by an hemolytic plaque (20).

Degranulation of β-cells. To maximally reduce their insulin content, β-cells were attached to 804G-coated Petri dishes and incubated for 6 to 12 h in DMEM supplemented with a cocktail of secretagogues (16.7 mmol/l glucose, 100 mmol/l IBMX, 1 mmol/l IBMX, and 10 mmol/l forskolin). After trypsinization, cells were rinsed with DMEM, resuspended in DMEM containing either 2.8 or 16.7 mmol/l glucose, and spreading was analyzed.

Immunofluorescence. β-cells were fixed using Bouin’s solution. After 24 h, cells were rinsed three times with PBS, dehydrated and permeabilized with graded concentrations of ethanol, and incubated for 2 h at room temperature with an anti-insulin antibody (Linco) diluted 1:1,000 in PBS. After rinsing, slides were incubated for 1 h at room temperature with a fluorescein-labeled goat anti–guinea pig second antibody (1:400) (Jackson Immuno Research Laboratories). After rinsing in PBS, slides were covered with 0.02% p-phenylene diamine in PBS-glycerol (1:2, v/v) and screened by fluorescence microscopy.

RESULTS

Basic conditions of β-cell spreading and secretion in response to glucose. High glucose concentrations (16.7 or 22.2 mmol/l) induce spreading of β-cells attached to 804G matrix, as previously reported (15). Cells rapidly develop lamellipodia, and spreading becomes readily apparent for a number of cells by 3 h and for most of them at 24 h (Fig. 1B and Table 1). However, spreading did not occur when β-cells were cultured on plastic (not shown) or in the absence of a stimulatory concentration of glucose (2.8 mmol/l) (Fig. 1A, Table 1), even after 24 h, suggesting

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described procedures (17,18). For cell preparation, the isolated islets were rinsed 3 times with Mg²⁺- and Ca²⁺-free phosphate-buffered saline (PBS) and resuspended in 1.5 ml of the same buffer containing 0.01% of trypsin (activity against casein 1:250) and 0.006% EDTA (Gibco, Life Technologies, Paisley, Scotland). Digestion (with occasional pipetting) occurred for 6 min at 37°C and was stopped by the addition of 10 ml ice-cold Krebs-Ringer-bicarbonate buffer (KRBB), pH 7.4, containing 0.5% bovine serum albumin (BSA), 2.8 mmol/l glucose, and 10 mmol/l HEPES. β-cells were then separated from non-β-cells by autofluorescence-activated sorting using a FACStar-Plus cell sorter (Becton Dickinson), as previously described (18,19).

Cell culture. Sorted β-cells were washed twice in 10–15 ml sterile Dulbecco’s minimum essential medium (DMEM) (Gibco, Life Technologies) containing 11.1 mmol/l glucose and 10% heat-inactivated fetal calf serum (FCS) and supplemented with 110 U/ml penicillin, 110 μg/ml streptomycin, and 50 μg/ml gentamicin. After centrifugation for 10 min at 130 g, aliquots of 10⁶ cells were seeded in nonadherent 60-mm diameter Petri dishes containing 3 ml medium. Cells were then incubated for 20 h at 37°C to allow full recovery of any cell surface molecules that may have been lost or damaged during islet isolation or cell purification.

804G matrix preparation and coating of Petri dishes. 804G matrix was prepared as previously described (15). Briefly, 804G cells (Desmos, San Diego, CA) were grown in DMEM containing 10% FCS and 5.6 mmol/l glucose. At confluence, cells were rinsed and maintained for an additional 3 days in the same medium. Conditioned medium (hereafter referred to as 804G matrix) was collected and used to coat 35-mm culture Petri dishes. To this end, 60-μl aliquots of 804G matrix were layered at the center of 35-mm culture Petri dishes, which were kept in a damp box at 37°C for 18–20 h before being rinsed three times with sterile water and air-dried. Uncoated Petri dishes were used as controls.

Spreading test. β-cells cultured for 20 h in nonadherent Petri dishes were centrifuged for 5 min at 130 g and resuspended at a density of 10⁶ cells per ml in DMEM containing 10% FCS. The medium was supplemented with either glucose (concentration as indicated), KCl (25 mmol/l), glucagon (1 μmol/l), isobutylmethylxanthine (IBMX) (0.5 mmol/l), diazoxide (200 μmol/l), SB-7037 (1 μmol/l), or phorbol 12-myristate 13-acetate (PMA) (100 μmol/l). We plated 60-μl aliquots of this suspension as droplets at the center of Petri dishes coated with 804G matrix, then incubated them at 37°C. Cell morphology was generally analyzed after 1-h, 3-h, and 24-h incubations. Phase-contrast views of different fields were photographed after 3 and/or 24 h of incubation. The percentage of spreading cells was scored either under microscope or on pictures (≥100 cells per condition were analyzed). Data were expressed as means ± SE.

In experiments with thapsigargin, cells were first allowed to attach to coated dishes. The medium was then replaced with 1 ml medium supplemented with or without 0.5 μmol/l thapsigargin, allowing us to study the same fields before and after incubation with the drug. Spreading was quantified by measuring an area profile of cells on numerous images using the National Institutes of Health image software (version 1.62).

Cultured β-cells transfected with a green fluorescent protein (GFP)-containing vector were recovered with trypsin as described above, rinsed with DMEM, and resuspended in DMEM containing either 2.8 or 16.7 mmol/l glucose. Aliquots of this suspension were plated as droplets at the center of Petri dishes, coated with 804G matrix, and incubated at 37°C. Cell morphology was analyzed after 24 h of incubation. The percentage of spreading cells was analyzed separately for fluorescent (GFP-expressing) and nonfluorescent (GFP-negative nontransfected) cells.

Insulin secretion by radioimmunoassay. After 1.5 or 24 h of incubation, medium (∼50 μl) was collected and centrifuged to remove any detached cells and debris. Aliquots were stored at −20°C for subsequent insulin measurement performed by classical radioimmunoassay (RIA). Data were expressed as means ± SE.

β-cell transfection. β-cells cultured for 20 h in nonadherent Petri dishes were centrifuged for 10 min at 130 g and resuspended at a density of 10⁶ cells per ml in DMEM containing 10% FCS and 11.1 mmol/l glucose. We plated 60-μl aliquots of this suspension as droplets at the center of Petri dishes coated with 804G matrix and incubated them at 37°C for 3 h. Cells were cotransfected with 0.8 μg of the DNA constructs pCMV5-BoNT/E (provided by H. Niemann) and a pEGFP vector (Clontech) using the TransFast reagent (Promega, Madison, WI), according to the manufacturer’s instructions. After 48 h, cells were detached with trypsin, rinsed with KRBB, and used either for a spreading test or measurement of insulin secretion by reverse hemolytic plaque assay (RHPA).

Insulin secretion by RHPA. Insulin secretion of transfected β-cells was assessed by an RHPA, as previously described (20,21,15). Briefly, β-cells were diluted in KRBB (pH 7.4) supplemented with 0.1% BSA and 2.8 mmol/l glucose. Packed sheep erythrocytes (5% v/v) (Behring Institute, Marburg, Germany) previously coated with protein A were then mixed with β-cells, and 50–60 μl

FIG. 1. Effect of secretagogues on β-cell spreading. β-cells were attached on 804G matrix and incubated for 24 h with 2.8 mmol/l glucose, 22.2 mmol/l glucose, 0.5 mmol/l IBMX, or 25 mmol/l KCl. Under basal conditions (A), cells remained rounded. Upon stimulation with either glucose (B), IBMX (C), or KCl (D), spreading was induced. Pictures are representative fields of at least three independent experiments. Bar, 30 μmol/l.

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synergism between secretagogues and signals from the extracellular matrix. Insulin measured in the incubation medium (Fig. 2A) confirmed good responsiveness of these cells to glucose.

**Contribution played by protein kinases A and C to β-cell spreading.** Results from the current study (Fig. 1C, Table 1) and those of a previous study (15) show that IBMX, which stimulates insulin secretion consequent to elevation of cAMP, stimulates β-cell spreading, suggesting the contribution of protein kinase A (PKA) in β-cell spreading. This hypothesis is supported by the effect of glucagon (1 μmol/l), which induced spreading prevented by the PKA inhibitor Rp-cAMP (0.5 mmol/l) (Fig. 3C and D, Table 1). However, Rp-cAMP had no effect on glucose-induced β-cell spreading (Fig. 3B, Table 1), which could have been expected because the studies by Persaud et al. (23) showed that in β-cells, PKA is neither activated by glucose nor involved in glucose-stimulated insulin secretion.

The possibility that IBMX increases [Ca$^{2+}$], by releasing calcium from endoplasmic reticulum (ER) has also been tested. To this end, β-cells attached to 804G matrix were incubated for 1 h with 1 μmol/l thapsigargin to deplete Ca$^{2+}$ from intracellular stores (24), then cells were incubated in the presence 0.5 mmol/l IBMX. The experiment showed that after 1–3 h, IBMX-induced spreading was identical in both control and thapsigargin-treated β-cells (data not shown).

The role of protein kinase C (PKC) in β-cell spreading was studied by incubating cells in the presence of 100

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**TABLE 1**

Effect of secretagogues and inhibitors on cell spreading

<table>
<thead>
<tr>
<th>Percentage of spreading cells</th>
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<tr>
<td>Control</td>
</tr>
<tr>
<td>2.8 mmol/l glucose</td>
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<tr>
<td>22.2 mmol/l glucose</td>
</tr>
<tr>
<td>0.5 mmol/l IBMX</td>
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<tr>
<td>25 mmol/l KCl</td>
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<tr>
<td>1 μmol/l glucagon</td>
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Values are means ± SEM. Values in parentheses are the number of experiments. In each experiment, ≥100 cell were analyzed.

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**FIG. 2.** Insulin secretion in the presence of various secretagogues and channel blockers. Total β-cell insulin secretion measured by radioimmunoassay at the end of a 24-h (A–E) or 1.5-h (F) incubation with various secretagogues in the presence or absence of different inhibitors: A: Insulin secretion was increased with a high glucose concentration (22.2 vs. 2.8 mmol/l). B: Insulin secretion of cells incubated with 2.8 mmol/l glucose (2.8 mmol/l) was increased by the addition of 25 mmol/l KCl, although not to the same extent as with 22.2 mmol/l glucose. C: In the presence of diazoxide, insulin secretion of cells incubated with 22.2 mmol/l glucose was inhibited, whereas secretion of cells incubated with 2.8 mmol/l glucose was not affected, and secretion of cells incubated with IBMX was only slightly decreased. D: In the presence of SR-7037, insulin secretion of cells incubated with 22.2 mmol/l glucose was inhibited, whereas the drug had no effect on the secretion of cells incubated with 2.8 mmol/l glucose or with IBMX. E and F: Insulin secretion of cells incubated for 3 h with 2.8 mmol/l glucose was increased by the addition of 100 mmol/l PMA to the same extent as with 16.7 mmol/l glucose (F); the effect of PMA became almost undetectable by 24 h (E).
nmol/l PMA. Within 1–3 h of incubation at low glucose (2.8 mmol/l) with 100 nmol/l PMA (Fig. 4B), spreading was increased to the same extent as in response to 16.7 mmol/l glucose (Fig. 4C). However, after 24 h of incubation in the presence of PMA, spreading had not augmented (Fig. 4E), in contrast to the progressive spreading induced by 16.7 mmol/l glucose (Fig. 4F). This is attributed to the known depletion of PKC after long-term exposure to PMA. Likewise, the insulin response to PMA was similar to 16.7 mmol/l glucose after 90 min of stimulation (Fig. 2F), but it was significantly lower at 24 h (Fig. 2E). To analyze the role of PKC in glucose-induced spreading, β-cells were treated for 24 h with 500 nmol/l PMA to downregulate PKC, then shifted to 16.7 mmol/l glucose (in the absence or presence of PMA). Under these conditions, β-cells spread out within 3 or 24 h, as did the cells incubated in the absence of PMA (not shown). These results suggest that PKA or PKC pathways are not necessary for glucose-induced β-cell spreading.

**Ca^{2+} influx caused β-cell spreading.** The results presented above suggest that at least three different pathways (glucose, PKA, and PKC) converge to invoke β-cell spreading. To determine whether β-cell spreading was dependent on Ca^{2+} influx, a high concentration of KCl was used to depolarize cells, thus opening L-type Ca^{2+} channels and raising [Ca^{2+}]_i (25,16). The results show that in the presence of 2.8 mmol/l glucose, 25 mmol/l KCl was able to promote cell spreading as efficiently as did other secretagogues (Fig. 1D, Table 1). This effect was observed at both 3 and 18 h of incubation. Insulin secretion, as expected, was also stimulated by KCl (Fig. 2B).

**Effect of diazoxide and SR-7037 on glucose- and IBMX-induced β-cell spreading.** To further assess the putative role of Ca^{2+} influx in β-cell spreading, we used the sulphonamide diazoxide (which activates K_{ATP} channels), thus impairing both glucose-induced depolarization of the membrane and the entry of calcium ions. As shown in Fig. 5 and Table 1, 200 μmol/l diazoxide added to cells incubated in the presence of 22.2 mmol/l glucose inhibited spreading. However, the agent only slightly affected spreading induced by 0.5 mmol/l IBMX (Fig. 5E), which could have been expected because cAMP and PKA activate calcium entry downstream of the K_{ATP} channels. As predicted, glucose-induced insulin secretion was inhibited in the presence of diazoxide, whereas IBMX-induced insulin secretion decreased only slightly (Fig. 2C). These results support the hypothesis that increased [Ca^{2+}]_i represents a necessary step in secretagogue-induced β-cell spreading.

To further address the role of calcium influx through the L-type voltage-gated Ca^{2+} channels in β-cell spreading, L-type Ca^{2+} channels were blocked with the specific agent SR-7037 (belfosdil). As shown in Fig. 5C and Table 1, 1
Spreading. Spread abolished in the presence of thapsigargin (B). Represent the quantitative measurements of the results; ~100 cells were measured for each interval and expressed as percentage of maximum spreading. Spread β-cells regained a round morphology when shifted from a high to low glucose concentration (A). This reversibility was abolished in the presence of thapsigargin (B).

μmol/l SR-7037 abolished β-cell spreading induced by 22.2 mmol/l glucose and decreased the spreading induced by 0.5 mmol/l IBMX (Fig. 5F). As expected, KCl-induced spreading was also inhibited by SR-7037 (Table 1). In all conditions, the effect was already visible by 3 h of incubation. In the presence of the Ca²⁺ channel blocker, insulin response to glucose was greatly decreased, whereas the response to IBMX was only slightly diminished (Fig. 2D).

**Reversibility of β-cell spreading was inhibited by thapsigargin.** β-Cells that had spread regained a round morphology when shifted from high (22.7 mmol/l) to low (2.8 mmol/l) glucose concentrations. The effect was detected by 3 h, and it extended to all cells within 24 h. To determine whether the reticular Ca²⁺ pump that dissipates elevation of [Ca²⁺], was involved in the reversibility of spreading induced by glucose, the specific pump inhibitor thapsigargin was added to culture medium concomitantly with the switch to low glucose. The results showed that the reversibility of spreading was prevented at both 3 h and 24 h by 0.5 μmol/l thapsigargin (Fig. 6). When thapsigargin was added to round β-cells incubated at 2.8 mmol/l glucose, spreading was not observed at 3 h and increased only slightly after 24 h (data not shown).

**β-cell spreading is not related to exocytotic events.** All conditions tested suggested that increased [Ca²⁺], is a prerequisite to β-cell spreading. However, increased [Ca²⁺], is also associated with increased insulin secretion, raising the possibility that spreading is in fact secondary to exocytotic processes. To try to resolve this conundrum, three approaches were used.

First, exogenous insulin was added at different concentrations (from 1 to 20 μg/ml) to the incubation medium, producing no effect on β-cell spreading at either 2.8 or 22.2 mmol/l glucose when compared with cells without added insulin. Furthermore, conditioned medium collected after a 24-h incubation of 2 × 10⁶ β-cells per ml at 2.8 mmol/l glucose failed to induce spreading of cells seeded at 2 × 10⁶ cells per ml, suggesting that spreading was not affected by insulin or other factors constitutively released by cells.

Second, β-cells were degranulated by a 12- to 24-h incubation in the presence of 16.7 mmol/l glucose supplemented with 100 nmol/l PMA, 10 μmol/l forskolin, and 0.5 mmol/l IBMX. Cells were then trypsinized and attached on petri dishes coated with 804G matrix. Under these conditions, they were still able to maximally spread out in response to 16.7 mmol/l glucose (Fig. 7).

Finally, sorted β-cells were transfected with botulinum neurotoxin E (BoNT/E), which is known to hydrolyze a 25-kDa synaptosome-associated protein, leading to inhibition of exocytosis (26,27). Transfected β-cells were tagged by cotransfection with a GFP vector. Insulin secretion of transfected cells was then analyzed using an RHPA (Fig. 8A and B). Following a 1-h stimulation with 16.7 mmol/l glucose, 9% (7 of 78, total of three different experiments) of GFP-positive cells were surrounded by a hemolytic plaque, compared with 75% of GFP-negative cells analyzed in the same preparation, indicating that secretion was inhibited in most transfected cells. Nevertheless, after 18 h of incubation on 804G matrix in the presence of 16.7 mmol/l glucose (Fig. 8C–H), 50% of 100 GFP-labeled cells were spread (n = 2) in the exact same proportion (50%) as observed for nontransfected cells.

These results strongly suggest that spreading of β-cells requires increased [Ca²⁺], but is not dependent on the consequence of increased insulin secretion.

**DISCUSSION**

In previous studies, we showed that rat islet β-cells spread in response to glucose when attached on the matrix
produced by a rat bladder carcinoma cell line (804G) (15). Furthermore, in a mixed population of cells, it was observed that spread cells secrete more insulin acutely in response to glucose compared with cells that remain rounded. These results suggest bi-directional signaling between the islet β-cell and the extracellular matrix.

The primary purpose of the present study was to get insight into possible intracellular pathways linking glucose stimulation, β-cell spreading, and insulin secretion. The experiments demonstrate that a sustained rise in \([\text{Ca}^{2+}]\), is required for glucose-induced rat β-cell spreading on 804G matrix. The effect is mimicked by any maneuver which raises \([\text{Ca}^{2+}]\), (IBMX, KCl, PMA) and is prevented by blocking L-type calcium channels (Belfosdil). Diazoxide, which activates KATP channels, also prevented glucose-mediated spreading and secretion.

The effect of PKA and PKC activation on \([\text{Ca}^{2+}]\) is still a matter of controversy, particularly in the absence of glucose. However, both PKA (28) and PKC (29,30) have been shown to activate \([\text{Ca}^{2+}]\) currents and/or L-type calcium channels in control conditions. Even though PKA and PKC increase both \([\text{Ca}^{2+}]\), and insulin secretion, it is thought that the \([\text{Ca}^{2+}]\), increase induced by these kinases is not necessary for insulin release (28,29). This does not exclude the possibility that a \([\text{Ca}^{2+}]\), increase after PMA and IBMX treatment can be involved in cell spreading. In our study, the effect of IBMX was only partially blocked by diazoxide, probably because PKA activates calcium entry downstream of the KATP channels. However, because the effect is also partially blocked by SR7037, we cannot exclude that PKA-induced spreading could be partially independent of \([\text{Ca}^{2+}]\). The possibility that IBMX increases \([\text{Ca}^{2+}]\), by releasing calcium from ER has been tested. The experiment showed that IBMX induced the same extent of spreading in both control and thapsigargin-treated β-cells, suggesting that IBMX does not induce spreading by releasing \([\text{Ca}^{2+}]\), from the ER.

Although both PKA or PKC were shown to increase spreading, an obligatory role for one or the other in glucose-induced spreading is unlikely because the effect of glucose persists in the absence of activity of the two enzymes. In fact, our results show that after long-term PMA treatment (PKC downregulation) and in the presence of an inhibitor of PKA (RpAMP), glucose is still able to induce spreading of β-cells. In this work, the mechanisms by which PKA and PKC activate spreading have not been investigated beyond the possible role in glucose-induced spreading.

Spreading of β-cells in response to glucose is probably related to glucose metabolism and not to an autocrine effect secondary to granule exocytosis because it was not abolished in degranulated cells. That the effect of glucose is not related to exocytosis is supported by the observa-
tion that it persists in β-cells transfected with botulinus toxin, which prevents secretion by >90%, as previously shown (31) and confirmed in the current study. These experiments also indicate that spreading is not the result of presentation of adhesion molecules at the cell surface during exocytosis.

The present results suggest a signaling pathway by which glucose-increased [Ca\textsuperscript{2+}] modulates cell-matrix interactions (inside-out signaling). A role of [Ca\textsuperscript{2+}] in inside-out signaling has been described previously. A correlation between intracellular calcium and integrin-mediated cell adhesion has been reported in other cell types (32–35). Cytosolic calcium modulates integrin affinity (36–38), and prevention of a rise in [Ca\textsuperscript{2+}] rises alters integrin-mediated adhesion of neutrophils (39,40). Whether calcium acts through changes in integrin conformation and/or post-receptor events, such as integrin-cytoskeleton association, is not completely understood. The cytoplasmic domain of integrins is the target of cytoplasmic modulators of integrin affinity (41). Ca\textsuperscript{2+}-binding proteins that associate with integrins include calreticulin, calcineurin, calpain, and calmodulin, which are all present within β-cells. Studies are now under way to assess whether they are involved in glucose-mediated β-cell spreading. A rise in [Ca\textsuperscript{2+}] may not be a consequence of spreading in response to glucose because a rise in [Ca\textsuperscript{2+}], in response to glucose has been observed in β-cells in suspension (42), and it has been routinely observed in β-cells attached to plastic or glass, on which they do not spread.

The cell surface molecules responsible for transducing cell matrix signaling in β-cells is unknown. We and others have reported the presence of β1 integrins on rat and human β-cells. Interestingly, it has been found that αβ1, but not α3β1, expression is increased at the surface of β-cells in response to glucose, and anti-α6 antibodies greatly reduce β-cell spreading (15); thus the hypothesis was that integrin αβ1 might be the β-cell transmembrane protein responsible for cell-matrix signaling by glucose. Our previous studies showed a correlation between spreading and insulin secretion of individual cells (outside-in signaling). Interestingly, calcium influx is also known to increase in response to endothelial cell spreading (36,38,43). This suggests a positive feedback mechanism involving the extracellular matrix and resulting in improved insulin response to glucose. An increased [Ca\textsuperscript{2+}] in response to αβ1 ligation would support this hypothesis.

In conclusion, the present study demonstrated a crucial role for calcium influx through the L-type voltage-gated Ca\textsuperscript{2+} channels in rat islet β-cell spreading in response to secretagogues, including glucose (inside-out signaling). Further studies are needed to determine to what extent the induced spreading plays a role in the regulation of secretion, and whether increased [Ca\textsuperscript{2+}] is also involved in outside-in signaling.

ACKNOWLEDGMENTS
This work was supported by Grant no. 1–1998-213 from the Juvenile Diabetes Foundation International (D.G.R.). Additional support was provided by the Swiss Diabetes Association (D.G.R.); the Raymond Berger Foundation, Lausanne, Switzerland (D.G.R.); and Swiss National Sci-

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