Absence of *Shb* impairs insulin secretion by elevated FAK activity in pancreatic islets

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Abstract

The Src homology-2 domain containing protein B (SHB) has previously been shown to function as a pleiotropic adapter protein, conveying signals from receptor tyrosine kinases to intracellular signaling intermediates. The overexpression of *Shb* in β -cells promotes β -cell proliferation by increased insulin receptor substrate (IRS) and focal adhesion kinase (FAK) activity, whereas *Shb* deficiency causes moderate glucose intolerance and impaired first-peak insulin secretion. Using an array of techniques, including live-cell imaging, patch-clamping, immunoblotting, and semi-quantitative PCR, we presently investigated the causes of the abnormal insulin secretory characteristics in *Shb*-knockout mice. *Shb*-knockout islets displayed an abnormal signaling signature with increased activities of FAK, IRS, and AKT. β -catenin protein expression was elevated and it showed increased nuclear localization. However, there were no major alterations in the gene expression of various proteins involved in the β -cell secretory machinery. Nor was *Shb* deficiency associated with changes in glucose-induced ATP generation or cytoplasmic Ca^{2+} handling. In contrast, the glucose-induced rise in cAMP, known to be important for the insulin secretory response, was delayed in the *Shb*-knockout compared with WT control. Inhibition of FAK increased the submembrane cAMP concentration, implicating FAK activity in the regulation of insulin exocytosis. In conclusion, *Shb* deficiency causes a chronic increase in β -cell FAK activity that perturbs the normal insulin secretory characteristics of β -cells, suggesting multi-faceted effects of FAK on insulin secretion depending on the mechanism of FAK activation.

Key Words

- Src homology 2
- FAK
- insulin secretion
- cAMP

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Introduction

Src homology-2 domain containing protein B (SHB) is a pleiotropic adapter protein, generating signaling complexes in response to tyrosine kinase activation via multi-domain interactions (Annerén *et al.* 2003). These complexes are context dependent and exert different roles in different settings. SHB has been found to regulate

apoptosis, proliferation, differentiation, and the cytoskeleton (Annerén *et al.* 2003). In insulin-secreting pancreatic β -cells, SHB overexpression not only increases focal adhesion kinase (FAK) and insulin receptor substrate 1/2 (IRS1/2) signaling (Welsh *et al.* 2002) and proliferation (Annerén 2002), but also increases apoptosis in response

to stress (Welsh *et al.* 1999). FAK is a tyrosine kinase, operating in submembranous focal adhesions connecting the extracellular matrix with intracellular signal transduction and the cytoskeleton (Parsons 2003). Cues from both extracellular matrix proteins and intracellular signaling events stimulate FAK activity, which in turn acts as a scaffold in focal adhesions and activates various downstream signaling pathways, such as AKT and ERK (Parsons 2003). In β -cells, FAK has been shown to convey signals from the extracellular matrix (Hammar *et al.* 2004), promoting β -cell survival, and from glucose (Rondas *et al.* 2011, 2012, Arous *et al.* 2013), stimulating insulin secretion both *in vitro* and *in vivo* (Cai *et al.* 2012).

Absence of *Shb* has no effect on β -cell proliferation, but reduces cell death in response to cytotoxic agents (Åkerblom *et al.* 2009, Mokhtari *et al.* 2009). In addition, *Shb*-deficient mice show mild glucose intolerance due to a reduction in insulin secretion (Åkerblom *et al.* 2009). The mechanisms underlying this secretory defect are unknown. Glucose stimulation of insulin secretion involves uptake and metabolism of the sugar, and the resulting production of ATP causes membrane depolarization by an inhibitory action on ATP-sensitive K^+ channels (Rorsman & Braun 2013). The depolarization activates voltage-dependent Ca^{2+} channels and the concomitant increase in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) triggers exocytosis of insulin secretory granules (Rorsman & Braun 2013). Glucose metabolism also stimulates the generation of cAMP and other metabolic coupling factors, which amplify the Ca^{2+} -triggered exocytosis response (Dyachok *et al.* 2008, Henquin 2009). The glucose response is typically biphasic with a pronounced first phase lasting a few minutes followed by a lower rate of secretion that is sustained or slowly increasing (second phase). *Shb* knockout seems to affect primarily the first phase of insulin secretion (Åkerblom *et al.* 2009).

In this study, we investigated whether the absence of *Shb* was associated with changes in ATP generation, $[Ca^{2+}]_i$, cAMP, and tyrosine kinase signaling and gene expression of certain exocytotic proteins in isolated islets. There were little abnormalities in *Shb*-knockout islets. However, we observed elevated constitutive FAK activity that provides an explanation for the similarly increased activities of AKT and β -catenin. Moreover, glucose-induced cAMP elevation was delayed, a finding which probably underlies the reduced first-peak insulin secretion, and which may occur as a consequence of increased FAK activity in *Shb*-deficient islets.

Materials and methods

Mice

Two- to five-month-old WT and *Shb*-knockout mice (Kriz *et al.* 2007) on a mixed genetic background (FVB/C57Bl6/129Sv) were used for islet isolation. Approval for breeding and killing the mice had been given by the local animal ethics committee.

Islet isolation

The animals were killed and pancreata were removed and suspended in Hanks' solution (SVA, Uppsala, Sweden). Each pancreas was cut into pieces with scissors and subjected to collagenase digestion (1 mg/ml Hanks per pancreas, Collagenase A, 10-154 121, Roche) for 20 min at 37 °C, on a shaking water bath, then washed twice with wash buffer (Ringer-acetate Fresenius-Kabi, Uppsala, Sweden, supplemented with 4.5 mM $NaHCO_3$, 2.5 mM glucose and penicillin–streptomycin), and suspended in Hanks' solution. The hand-picked islets were cultured at 5.6 or 11.1 mM glucose in RPMI 1640 + 10% fetal bovine serum + antibiotics for at least 24 h before experimentation. For ATP and cAMP imaging experiments, the islets were infected for 1–2 h with adenovirus expressing the respective protein-based signaling biosensors at a concentration of 20 plaque-forming units/cell in a medium containing 2% (v/v) serum, followed by washing with a regular complete medium and further culture for 16–20 h before use.

Immunoblotting

After 15 min incubation in Hanks' solution at 37 °C, 30–50 islets were washed with ice-cold PBS and directly lysed in SDS-sample buffer, boiled for 5 min, and separated on SDS-PAGE. The proteins were electrophoretically transferred to Hybond-P filters (GE Healthcare, Uppsala, Sweden). The filters were blocked in 5% BSA for 1 h, after which they were probed with phospho-202/204 ERK; phospho-473 AKT; phospho-612 IRS1/2; phospho-Y397 FAK, AKT, IRS2, FAK; β -catenin; and ERK (Cell Signaling, Beverly, MA, USA for most antibodies; pYFAK and pYIRS, Invitrogen; ERK, Santa Cruz; β -catenin, Abcam, Cambridge, UK; and IRS2, Upstate Biotechnology, Lake Placid, NY, USA) antibodies. The bound antibodies were removed from filters by incubating for 40 min at 55 °C in 2% w/v SDS and 0.1 mM β -mercaptoethanol. HRP-linked goat anti-rabbit or anti-mouse was used as a secondary antibody.

Immunodetection was performed as described for the ECL prime immunoblotting detection system (GE Healthcare) using the Kodak ImageStation 4000MM. The intensities of the bands were quantified by densitometric scanning using Kodak Digital Science ID software (Eastman Kodak).

Gene expression analysis

Total RNA was prepared by using the RNeasy Mini Kit (Qiagen) with on-column DNase digestion with RNase-Free DNase set (Qiagen), according to the manufacturer's descriptions. For RNA isolation, 30 islets/sample were collected, briefly washed in PBS, and then lysed and homogenized. RNA yield was determined using a spectrophotometer at 260 nm. One-step quantitative real-time RT-PCR was carried out using the QuantiTect SYBR Green RT-PCR Kit (Qiagen) on a LightCycler (Roche Diagnostics).

The PCR conditions were according to kit supplier's instructions: RT 20 min at 50 °C, denaturation 15 min at 95 °C, cycling: denaturation 15 s at 94 °C, annealing 30 s at 55–60 °C, and extension 30 s at 72 °C. Crossing point (Ct) values were determined with the LightCycler Software v3.5 (Roche Diagnostics). Gene expression was normalized by subtracting the corresponding β -actin Ct value. Statistical comparisons were made on normalized Ct values.

Measurements of cytoplasmic ATP dynamics

The islets were preincubated for 30–60 min in an experimental buffer containing NaCl 138 mM, KCl 4.8 mM, MgCl₂ 1.2 mM, CaCl₂ 1.3 mM, glucose 3 mM, HEPES 25 mM (pH 7.40), and 0.5 mg/ml BSA. The islets were then placed on a poly-L-lysine-coated coverslip in the superfusion chamber on the thermostated stage of a total internal reflection fluorescence microscope (Eclipse Ti with a 60 $\times$, 1.45-NA objective, Nikon, Japan). A diode-pumped solid-state laser (Cobolt AB, Solna, Sweden) provided 491 nm excitation light and the emission was selected by a 527/27-nm (center wavelength/half-bandwidth) interference filter (Semrock, Rochester, NY, USA) and recorded by an EMCCD camera (DU-897; Andor, Belfast, UK) controlled by MetaFluor software (Molecular Devices, Sunnyvale, CA, USA). All imaging experiments were carried out at 37 °C, with a medium superfusion rate of 0.12–0.20 ml/min and with images acquired every 2–5 s.

Recordings of [Ca²⁺]_i

For the measurements of [Ca²⁺]_i, islets were pre-incubated for 30–40 min in the presence of 1 μ mol/l acetoxymethyl

ester of the Ca²⁺ indicator Fura-PE3. Imaging of the Fura-PE3-loaded cells was carried out with an inverted microscope equipped with a 40 $\times$ 1.3-NA objective (Nikon) and an epifluorescence illuminator (Cairn Research Ltd, Faversham, UK) connected through a 5 mm diameter liquid light guide to an Optoscan monochromator (Cairn Research Ltd) with a 150-W xenon arc lamp. The monochromator provided excitation light at 340 and 380 nm, which was reflected by a 400-nm dichroic beam splitter, and emission was measured at 510 nm/40 nm half-bandwidth using a CCD camera (Orca, Hamamatsu, Hamamatsu City, Japan) with an image intensifier (C8600, Hamamatsu). The Metafluor software (Molecular Devices) controlled the monochromator and the camera, acquiring image pairs every 2 s with 100–400 ms integration at each wavelength and <1 ms for changing wavelength and slits. To minimize bleaching and photodamage, the monochromator slits were closed until the start of the next acquisition cycle. The ratio images (340/380 nm) were obtained after subtraction of background and [Ca²⁺]_i values calculated as previously described (Gryniewicz *et al.* 1985).

Real-time measurements of cAMP

The measurements of the sub-plasma membrane concentration of cAMP were carried out with a CFP/YFP-based translocation reporter and TIRF microscopy as previously described (Tian *et al.* 2011). The microscope setup was the same as that described for ATP measurements except that CFP and YFP excitation was provided by the 457- and 515-nm lines of diode-pumped solid-state lasers (Cobolt), and fluorescence was detected at 485/25 and 560/40 nm (Semrock).

Patch-clamp analysis of Ca²⁺ currents

The electrophysiological recordings were done using whole-cell patch-clamp recordings of cells in intact islets. The extracellular solution contained 140 mM NaCl, 3.6 mM KCl, 2 mM NaHCO₃, 0.5 mM NaH₂PO₄, 5 mM HEPES (pH 7.40 with NaOH), and 2.6 mM CaCl₂. Glucose was present at 5 mM in all experiments. The pipette solution consists of 76 mM K₂SO₄, 10 mM NaCl, 10 mM KCl, 1 mM MgCl₂, and 5 mM HEPES (pH 7.35 with KOH). In order to record only inward Ca²⁺ currents, outward currents were suppressed by the inclusion of tetraethylammonium chloride at a concentration of 20 mM in the extracellular medium (NaCl was correspondingly reduced to maintain isosmolarity) and by replacing K₂SO₄

in the pipette-filling solution with an equimolar amount of CsSO₄. The recording pipettes were made from borosilicate glass capillaries (Harvard Apparatus, Holliston, MA, USA) and had a resistance of 3–5 MΩ when filled with the pipette solution. The holding potential was set at –60 mV and then stepped to the experimental potential for 200 ms. The current recordings were done using an Axopatch 200B amplifier, filtered at 2 kHz, sampled at 10 Hz by an analog-to-digital converter, and stored in a computer. The recordings were then analyzed with the pClamp 9 software (Molecular Devices).

Statistical analyses

The means $\pm$ S.E.M. for the indicated number of observations are given. For testing differences, Student's *t*-test was used (unpaired or paired depending on the experimental conditions) as indicated.

Results

Characteristics of tyrosine kinase signal transduction in *Shb*-knockout islets

As *Shb* is a signal transduction protein downstream of tyrosine kinase receptors and as altered signaling characteristics were previously observed in *Shb*-deficient cells, we decided to investigate FAK, IRS1/2, AKT, and ERK signaling in *Shb*-knockout islets by using phospho-specific antibodies recognizing active forms of these signaling intermediates (Fig. 1). Basal FAK activity at 5.6 mM glucose was elevated as a consequence of absence of *Shb*. The increase was modest (42%) but typical for the level of increase previously observed as a consequence of *Shb* deficiency (Funa *et al.* 2009, Gustafsson *et al.* 2013). Downstream of FAK in β -cells are IRS-proteins (Baron *et al.* 1998). We assessed IRS1/2 activity using an antibody that recognizes the phosphotyrosine characteristic of the active form of both proteins (pY612). On the basis of the total IRS2 protein reactivity on the blots, the predominant phospho-reactive component in the current setting appeared to be IRS2 (Fig. 1). IRS2 activity was increased by 19%. AKT and ERK are downstream of both FAK and IRS2. AKT activity was increased by 51%, whereas ERK activity showed a large variability with an average increase of 36% that failed to reach statistical significance.

One target of AKT is glycogen synthase kinase 3-beta (GSK-3b), which becomes inhibited through AKT phosphorylation (Woodgett 2005). GSK-3b is a negative regulator of β -catenin and consequently β -catenin levels

Figure 1

Activities of FAK, IRS2, AKT, and ERK in WT and *Shb*-knockout islets as determined by western blotting analysis using phospho-specific antibodies recognizing active forms. The isolated islets were cultured overnight in RPMI 1640 + 10% fetal bovine serum, incubated at 37 °C for 15 min in Hanks' salt solution containing 5.6 mM glucose before lysis, electrophoresis, blotting, and incubation with appropriate antibodies. Relative values after densitometry are given as means $\pm$ S.E.M. for five to six separate experiments (separate islet isolation and blot). In the pERK and total ERK blots, bands from separate parts of the same blot with an identical exposure were juxtaposed. **P* < 0.05 with a paired Student's *t*-test.

increase and β -catenin translocates to the nucleus where it alters gene transcription upon AKT activation (Woodgett 2005). As AKT activity was increased in *Shb*-knockout islets, we found it relevant to investigate β -catenin levels and nuclear translocation (Fig. 2). These were both increased in *Shb*-deficient islets, suggesting that the increase in FAK activity has implications for β -catenin-dependent gene transcription via enhanced AKT activity.

Gene expression of proteins involved in exocytosis

We next determined gene expression levels of various proteins relevant to the exocytotic process using real-time RT-PCR. The genes analyzed were *Rab3a*, synaptosomal-associated protein 25 (*Snapt25*), vesicle-associated membrane protein 2 (*Vamp2*), *Munc18-1* (syntaxin-binding protein 1 (*Stxbp1*)), and Syntaxin1a (*Stx1a*) (Table 1). None of these were differentially expressed in *Shb*-knockout islets compared with control islets, suggesting that altered expression of these exocytosis proteins are not likely explanations for the reduced first-phase insulin secretory response.

Sub-membrane ATP levels and intracellular Ca²⁺ handling in *Shb*-knockout islets

To investigate whether abnormalities in glucose stimulus-secretion coupling can explain the secretory defect, we measured the submembrane ATP concentration using

Figure 2

Contents and nuclear translocation of β -catenin in WT and *Shb*-knockout islets. For expression, islets were analyzed as shown in Fig. 1. Values are relative expression after normalization for total ERK. Nuclear translocation was determined as shown in images, in which DAPI-positive nuclei (blue) were also positive for β -catenin staining (green). Fractions of nuclei positive for β -catenin were quantified. Means $\pm$ S.E.M. for six separate experiments are shown. * $P < 0.05$ and ** $P < 0.01$ using a paired Student's *t*-test.

TIRF microscopy and the fluorescent ATP sensor Perceval in islets exposed to a step increase of the glucose concentration from 3 to 20 mM. This stimulation resulted in a pronounced rise in ATP (Fig. 3), which was of similar magnitude in both WT ($48 \pm 3\%$ increase in Perceval fluorescence) and *Shb*-knockout ($50 \pm 2\%$) islets ($P > 0.6$), suggesting that the absence of *Shb* does not impair the ability of β -cells to generate ATP in response to glucose stimulation.

The rise in sub-membrane ATP triggers depolarization and voltage-dependent Ca^{2+} influx in β -cells. The resulting increase in $[\text{Ca}^{2+}]_i$ was investigated as a potential explanation for the aberrant insulin secretory characteristics. Slight tendencies to lower $[\text{Ca}^{2+}]_i$ in

Shb-knockout islets in the presence of both 3 and 20 mM glucose did not reach statistical significance (Fig. 4). Moreover, closure of ATP-sensitive K^+ channels with 1 mM tolbutamide resulted in similar increases in $[\text{Ca}^{2+}]_i$. Patch-clamp analysis of whole-cell Ca^{2+} currents in the β -cells of intact islets also failed to demonstrate any difference between WT and *Shb*-knockout cells (Fig. 5), suggesting that the defective insulin secretion is not due to alterations in intracellular Ca^{2+} handling.

Glucose-induced cAMP generation is delayed in *Shb*-knockout islets

First-peak insulin secretion has been found to be related with cAMP generation (Idevall-Hagren *et al.* 2010) and we therefore investigated the dynamics of glucose-induced cAMP generation. Interestingly, the rise in cAMP, triggered by an elevation of the glucose concentration from 3 to 20 mM, was delayed by ~ 1 min in *Shb*-knockout islets compared with control (Fig. 6). This provides a potential explanation for the impaired first-peak insulin secretory response observed in these islets.

Inhibition of FAK triggers rise in sub-membrane cAMP

Increased FAK activity has previously been reported to stimulate insulin secretion in response to glucose stimulation (Rondas *et al.* 2011). We decided to address whether FAK activity can be related with insulin secretion also in the current setting by monitoring the submembrane cAMP concentration because active FAK has been shown to recruit a cAMP-degrading phosphodiesterase (Serrels *et al.* 2010, 2011). Addition of 10 μM of the FAK inhibitor 14 (Golubovskaya *et al.* 2008, Gustafsson *et al.* 2013) to WT islets, and *Shb*-knockout islets exposed to 3 mM glucose caused a progressive increase in the basal cAMP concentration (Fig. 6), suggesting that FAK indeed may exert negative control of cAMP signaling.

Table 1 Expression of genes coding for exocytotic proteins in *Shb*-knockout islets. Values are Ct values after subtracting the corresponding β -actin Ct values. Means $\pm$ S.E.M. are shown for 6–11 separate experiments

Gene	WT	<i>Shb</i> -knockout
<i>Rab3a</i>	5.04 ± 0.42	5.15 ± 0.39
<i>Snap25</i>	3.79 ± 0.47	4.09 ± 0.31
<i>Munc18-1 (Stxbp1)</i>	3.99 ± 0.25	4.34 ± 0.32
<i>Vamp2</i>	2.86 ± 0.61	2.99 ± 0.56
<i>Syntaxin1a (Stx1a)</i>	10.42 ± 0.96	10.25 ± 0.97

Figure 3

Glucose-induced ATP generation in WT and *Shb*-knockout islets. Representative TIRF microscopy recordings of the sub-membrane ATP concentration in superficially located cells in Perceval-expressing WT and *Shb*-knockout islets during an increase in the glucose concentration from 3 to 20 mM.

Discussion

Shb was originally described as a serum-inducible gene in β -cells (Welsh *et al.* 1994), but subsequently found to encode a ubiquitously expressed adapter protein with pleiotropic functions (Annerén *et al.* 2003). The multi-domain structure of SHB allows the generation of signaling complexes and these can propagate signals of great diversity. A recent study has described the binding of four key β -cell transcription factors (*PDX1*, *NKX2.2*, *FOXA2*, and *NKX6.1*) to a cluster of binding sites in the *SHB* gene (Pasquali *et al.* 2014), suggesting the importance of *Shb* in relationship with β -cell function and possibly also for the development of type 2 diabetes.

SHB has been found to interact with FAK (Holmqvist *et al.* 2003). It is thought that *SHB* bridges an active tyrosine kinase receptor and FAK while simultaneously binding c-SRC, thus promoting FAK activation within this multi-component complex (Annerén *et al.* 2003, Holmqvist *et al.* 2003). In insulin-producing cells, *SHB* overexpression increases the association between FAK and IRS1, thus augmenting IRS signaling (Welsh *et al.* 2002). Surprisingly, *Shb*-deficient cells commonly display elevated basal FAK

activity (Funa *et al.* 2009, Gustafsson *et al.* 2013, Zang *et al.* 2013) with reduced ligand responsiveness, but the reason behind this response has not been clarified. One possible interpretation of those findings is that the elevated basal FAK activity is an adaptive response to loss of the ability of ligands to induce FAK activation, although it is also possible that SHB associates with or regulates the activity of other signaling components that downregulate FAK activity. The presently described moderate increase in basal FAK activity is in line with studies on endothelial and hematopoietic cells demonstrating a similar effect of *Shb* deficiency (Funa *et al.* 2009, Gustafsson *et al.* 2013).

The increases in IRS2 and AKT activities are likely the consequences of elevated FAK phosphorylation (Cai *et al.* 2012). Absence of *Shb* renders β -cells less prone to apoptosis upon cytokine exposure and this is likely due to the

Figure 4

[Ca²⁺]_i recordings from WT and *Shb*-knockout islets. The islets were sequentially exposed to 0.25 mM diazoxide, 1 mM tolbutamide, and 20 mM glucose as indicated. The bar diagram shows means $\pm$ S.E.M. for the amplitudes of the initial glucose- and tolbutamide-triggered [Ca²⁺]_i increases from four to eight experiments. Basal [Ca²⁺]_i was 114 $\pm$ 8 nM for WT and 92 $\pm$ 6 nM for *Shb*-knockout.

Figure 5

Ca²⁺-channel activity in WT and *Shb*-knockout islets. The data points show the average $\pm$ s.e.m. whole-cell current response from WT ($n=7$, black circle) and KO ($n=10$, gray square) cells. The holding potential was -60 mV and the test pulse was applied for 200 ms.

activation of AKT (Bernal-Mizrachi *et al.* 2001, Elghazi *et al.* 2007). One consequence of AKT activation is phosphorylation of the β -catenin regulator GSK-3 β , leading to increased expression of β -catenin and its nuclear translocation (Elghazi *et al.* 2007). This will participate in the anti-apoptotic effect of AKT via changes in gene transcription (Elghazi *et al.* 2007).

Even if the expression of many genes can be expected to be altered as a consequence of *Shb*-gene knockout and the associated increase in FAK and AKT activities, we did not find changes in the expression of genes directly involved in the exocytosis machinery, suggesting that alterations in insulin secretion had other causes. Investigation of stimulus–secretion coupling by live-cell imaging of various signaling events showed that glucose-induced ATP formation and voltage-dependent Ca²⁺ influx were normal in *Shb*-knockout β -cells. Instead, the most striking finding in these cells was delayed glucose-induced cAMP production. Since cAMP is crucial not only in amplification of insulin secretion by incretin hormones, but also for the normal glucose-induced insulin secretory response (Dyachok *et al.* 2008), we suggest that this defect may explain the reduced first-phase insulin secretion in the absence of *Shb*. The mechanism underlying the altered cAMP signaling is unknown. However, it is interesting to note that a consequence of elevated FAK activity is the recruitment of phosphodiesterase 4D5 to focal adhesions,

invoking a decreased cAMP concentration in the submembrane compartment adjacent to focal adhesions (Serrels *et al.* 2010, 2011). A similar mechanism whereby FAK regulates the submembrane cAMP concentration may be present also in β -cells and FAK-inhibition was accordingly found to cause an increase in the basal cAMP concentration, possibly by release of phosphodiesterases from the dissolving focal adhesions.

FAK has been shown to exert positive cues in glucose-stimulated insulin secretion (Rondas *et al.* 2011, 2012, Cai *et al.* 2012, Arous *et al.* 2013), but the present data indicate that FAK may serve an inhibitory role in this process as well. FAK inhibition reduced both first- and second-phase glucose-stimulated insulin secretion and reduced the number of docked granules (Rondas *et al.* 2011, 2012), indicating that the prevailing effect of FAK is to promote the exocytotic process. *Shb*-knockout islets exhibiting elevated basal FAK activity, on the other hand, display elevated basal insulin secretion, reduced first-phase secretion, and reduced numbers of docked granules (Åkerblom *et al.* 2009), suggesting that the dual effect of FAK becomes most apparent when related with the significant reduction in first-phase secretion noted in the *Shb*-knockout system. This would imply that chronic FAK

Figure 6

Delayed increase in cAMP in response to 20 mM glucose in *Shb*-knockout islets. (A) TIRF microscopy recordings of the sub-membrane cAMP concentration in single cells within intact WT (black trace) and *Shb* knockout (dashed gray trace) islets during increase in the glucose concentration from 3 to 20 mM. (B) Quantification of the delay between glucose stimulation and rise in cAMP in WT and *Shb*-knockout islet cells. Means $\pm$ s.e.m. for six separate experiments are shown. * $P<0.05$ with an unpaired Student's *t*-test. (C) Effect of 10 μ M of FAK inhibitor 14 on cAMP in a WT islet exposed to 3 mM glucose. (D) Corresponding results from a *Shb*-knockout islet.

stimulation exerts compensatory changes that are detrimental to the first-phase secretory process, whereas stimulatory effects of FAK prevail under the other situations. The most apparent consequence of the altered FAK response in the *Shb*-knockout islets is the delayed cAMP response to glucose-stimulation, providing a plausible explanation for the reduced first-phase response.

In summary, the absence of *Shb* causes a chronic activation of FAK by an unknown mechanism. This will change certain signaling characteristics, such as activation of IRS2, AKT, and β -catenin. The β -cell undergoes adaptive responses with reduced cell toxicity and first-phase glucose-induced insulin secretion. The latter seems to be a consequence of FAK-mediated suppression of submembrane cAMP signaling.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

A T and M W designed the experiments, I A, O D, G T, J L, S M, Y J, and M W performed the experiments, I A, O D, G T, Y J, B B, A T, and M W analyzed the data and A T and M W wrote the paper.

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