

Research Article

A role for PAK1 mediated phosphorylation of β -catenin Ser552 in the regulation of insulin secretion

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The presence of adherens junctions and the associated protein β -catenin are requirements for the development of glucose-stimulated insulin secretion (GSIS) in β -cells. Evidence indicates that modulation of β -catenin function in response to changes in glucose levels can modulate the levels of insulin secretion from β -cells but the role of β -catenin phosphorylation in this process has not been established. We find that a Ser552Ala version of β -catenin attenuates glucose-stimulated insulin secretion indicating a functional role for Ser552 phosphorylation of β -catenin in insulin secretion. This is associated with alterations F/G actin ratio but not the transcriptional activity of β -catenin. Both glucose and GLP-1 stimulated phosphorylation of the serine 552 residue on β -catenin. We investigated the possibility that an EPAC-PAK1 pathway might be involved in this phosphorylation event. We find that reduction in PAK1 levels using siRNA attenuates both glucose and GLP-1 stimulated phosphorylation of β -catenin Ser552 and the effects of these on insulin secretion in β -cell models. Furthermore, both the EPAC inhibitor ESI-09 and the PAK1 inhibitor IPA3 do the same in both β -cell models and mouse islets. Together this identifies phosphorylation of β -catenin at Ser552 as part of a cell signalling mechanism linking nutrient and hormonal regulation of β -catenin to modulation of insulin secretory capacity of β -cells and indicates this phosphorylation event is regulated downstream of EPAC and PAK1 in β -cells.

Introduction

Glucose is the key trigger for insulin secretion from β -cells and its effects are modulated by incretins including GLP-1 [1,2]. It is known that the formation of adherens junction proteins are important to allow β -cells to properly control the secretion of insulin but the full mechanisms by which this is achieved are not completely understood [3,4]. Evidence indicates that the adherens junction protein β -catenin plays an important role in a range of regulated vesicle trafficking processes including that of synaptic vesicles [5], glucose and GLP-1 stimulated insulin secretion [6–8] and insulin-stimulated translocation of GLUT4 [9,10]. One question that arises is whether signalling pathways are able to acutely regulate the function of β -catenin in these vesicle trafficking processes, for example by phosphorylation. Other than the well characterised N-terminal sites in β -catenin associated with triggering ubiquitin-mediated degradation [11], two common C-terminal serine phosphorylation sites have been identified at Serine 552 and Serine 675 [12–15]. The functional consequences of β -catenin phosphorylation for β -cell function are not known; although in some cell types the phosphorylation of Ser552 or Ser675 increases β -catenin transcriptional activity [12–15]. Recently we have demonstrated that phosphorylation of Ser552 in β -catenin is involved in the mechanisms by which insulin regulates the translocation of GLUT4 to the plasma membrane in muscle, indicating a role for this in mechanisms regulating vesicle trafficking in cells [10].

Received: 3 November 2020
 Revised: 17 February 2021
 Accepted: 18 February 2021

Accepted Manuscript online:
 19 February 2021
 Version of Record published:
 28 April 2021

We have shown that glucose induces phosphorylation of β -catenin on Ser552 but not the Ser675 site in β -cells via a mechanism that at least in part involves Protein kinase A (PKA) [6], but those studies suggest that other signalling mechanisms are also involved. We note that the Ser552 site fits a consensus site for p21-activated protein kinase-1 (PAK1) [16]. A pathway linking glucose and GLP-1 to PAK1 has been defined in β -cells which involves EPAC; a known mediator of glucose and GLP-1 effects on insulin secretion [17]. Glucose, GLP-1 and activation of EPAC are known to cause activation of small GTP-ases including Rac and Cdc42 [18]. These in turn are known to regulate insulin secretion via the activation of PAK1 [18–20]. The functional targets of PAK1 in β -cells are not fully understood.

Here we provide evidence that Ser552 phosphorylation of β -catenin contributes to the regulation of rearrangement of the actin cytoskeleton and of insulin secretion. We go on to investigate the mechanisms by which this Ser552 phosphorylation occurs in β -cells and find that GLP-1 enhances glucose-induced phosphorylation at Ser552 and that both require the activity of EPAC and PAK1. Together this identifies a new component of the complex mechanisms by which glucose and GLP-1 regulate insulin secretion.

Materials and methods

Inhibitors and drugs used

The following reagents and inhibitors were used in these studies; Exendin-4 (Byetta[®]), GLP-1(7–36) amide (GenScript), Exendin-3(9–39) amide (Tocris), H-89 (LC Laboratories), ESI-09 (BioLog), IPA3 (Sigma–Aldrich).

Culture of immortalised beta-cell lines

INS-832/3 (kindly provided by Professor C.B. Newgard, Duke University) and INS-1E (kindly provided by Professor C.B. Wollheim) cells were maintained in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM HEPES, 50 μ M 2-mercaptoethanol, 100 U/ml penicillin and 100 μ g/ml streptomycin. Insulin secretion experiments and treatment of cells for western blot analysis was performed on confluent cells in six-well or 12-well culture plates following a 1 h glucose- and serum-starvation.

Plasmid and siRNA transfection

For siRNA knockdown experiments cells were transfected at 70% confluence with validated specific siRNA or with control siRNA (Stealth[™] RNAi siRNA Negative Control, Med GC). Transfections were performed in OptiMEM Reduced Serum Medium using Lipofectamine[®] 2000 and contained siRNA to a final concentration of 30 nM. For overexpression of β -catenin, cells were transfected with pcDNA3- β -catenin-FLAG cDNA expression plasmid (Addgene 16828). At 70% confluence cells were transfected with plasmid DNA using Lipofectamine[®] 3000 at a ratio of 1 μ g DNA to 2 μ l reagent. Prior to transfection mutagenic primers were used to introduce the S552A amino acid change into the pcDNA3- β -catenin-FLAG plasmid. Primer sequences were: 5'-AAGACATCACTGAGCCTGCC-3' (forward) and 5'-GGCACGAGAGACTTGAGCTT-3' (reverse). Mutagenesis PCR was performed using the GENEART[®] Site-Directed Mutagenesis Kit according to manufacturer's instructions and the integrity of all vectors was confirmed by sequencing of the β -catenin and FLAG cDNA regions. All transfections and subsequent experiments were performed using medium lacking antibiotics. All reagents for transfection experiments were from Life Technologies and used according to the manufacturer's instructions.

Insulin secretion assay

Prior to insulin secretion experiments cells were starved in modified Krebs-Ringer bicarbonate HEPES (KRBH) buffer (119 mM NaCl, 4.74 mM KCl, 1.19 mM MgSO₄, 25 mM NaHCO₃, 1.19 mM KH₂PO₄, 2.54 mM CaCl₂ and 20 mM HEPES) pH 7.4, containing 0.2% (w/v) BSA for 1 h. When required, cells were pre-treated with inhibitor during the final 30 min of starvation. Following starvation the medium was replaced with KRBH containing the indicated glucose concentration and inhibitor. Following incubation for 2 h an aliquot of medium was collected, diluted appropriately and the insulin content was determined using the AlphaLISA Insulin Assay Kit (PerkinElmer), according to the manufacturer's instructions.

Islet isolation and inhibitor treatment

All experiments involving mice were approved by the University of Auckland Animal Ethics Committee (AEC 001732) and performed at the Faculty of Medical and Health Sciences, University of Auckland. Mice were euthanised through CO₂ inhalation following AVMA 2013 guidelines with a gradual chamber fill rate of 30%/min, following unconsciousness blood withdrawal was performed via cardiac puncture. Islets were isolated by collagenase digestion from male CD1 (8–10 weeks) mice and then purified using Histopaque as described previously [21]. Following isolation, islets were transferred into a non-tissue culture-treated Petri dish and cultured overnight at 11 mM glucose in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 1% penicillin/streptomycin (v/v) and 50 μM 2-mercaptoethanol at 27°C for the first 24 h. Then media was changed and islets were then incubated at 37°C. Islets were used within 2–3 days of isolation. For GSIS, 10–15 size-matched islets (per tube) were aliquoted into low-retention microtubes and incubated for 30 min in KRBH buffer supplemented with 0.2% BSA and 2.8 mM glucose. The islets were sequentially incubated with 150 μl KRBH buffer supplemented with 0.2% BSA and 2.8 mM glucose then 16.7 mM glucose and finally 16.7 mM Glucose + Exendin for 30 min. Incubations were performed in the presence of either vehicle control (DMSO), ESI-09 (30 μM) or IPA3 (90 μM). Supernatants were collected at the end of each incubation and insulin concentrations were measured by performing AlphaLISA Insulin Assay Kit (PerkinElmer), according to the manufacturer's instructions. For western blot analysis, 35–50 size-matched islets (per tube) were incubated in KRBH buffer supplemented with 0.2% BSA and 2.8 mM glucose for 1 h, a buffer was then replaced with KRBH buffer supplemented with 0.2% BSA and 16.7 mM glucose with either vehicle control (DMSO), ESI-09 (30 μM) or IPA3 (90 μM) and incubated for a further hour. Buffer was removed and islets were prepared for western blot.

Cell lysates and western blot analysis

Cells were rinsed twice with ice-cold PBS and cellular lysates were harvested in buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerol phosphate, 1 mM vanadate, 100 mM NaF, 1 mM AESBF, 4 μg/ml aprotinin, 0.4 μg/ml pepstatin, 4 μg/ml leupeptin and 30 μM ALLN. Lysates were collected, centrifuged at 16 100×g for 10 min and cleared supernatants were subject to polyacrylamide gel electrophoresis and analysed by western blotting. Following overnight incubation in primary antibody, western blot membranes were washed and incubated with anti-mouse (1 : 25 000; Sigma-Aldrich) or anti-sheep (1 : 15 000; Dako) IgG-horseradish peroxidase conjugated antibody for 1 h at room temperature and developed using Clarity™ Western ECL substrate (Bio-Rad Laboratories). Western blotting was carried out with antibodies specific against: Phospho-473 Akt (1 : 1000; Cell Signaling Technologies), Total Akt (1 : 1000; Cell Signaling Technologies), Phospho-552 β-catenin (1 : 1000; Cell Signaling Technologies), Total β-catenin (1 : 2000; Symansis), Phospho-133 CREB (1 : 1000; Cell Signaling Technologies), Total CREB (1 : 1000; Cell Signaling Technologies), FLAG (1 : 2000; Sigma-Aldrich), Phospho-44/42 ERK1/2 (1 : 1000; Cell Signaling Technologies), Total ERK1/2 (1 : 1000; Cell Signaling Technologies), PKA-Cα (1 : 1000; Cell Signaling Technologies), PKA-Cβ (1 : 1000; Abcam), Total PAK1 (1 : 1000; Cell Signaling Technologies), β-actin (1 : 10 000; Sigma-Aldrich), α-tubulin (1 : 20 000; Sigma-Aldrich), GAPDH (1 : 20 000; Abcam).

Assessing F/G actin ratio

INS-1E cells were co-transfected with either WT FLAG tagged β-catenin (Human) or S552A FLAG β-catenin (Human) and rat β-catenin siRNA using reverse transfection method. 48 h after siRNA transfection cells were lysed with actin solubilization buffer (50 mM NaCl, 5 mM MgCl₂, 50 mM PIPES, 5 mM EGTA, 5% (v/v) glycerol, 0.1% Nonidet P-40, pH 6.9, 0.1% Triton X-100, 0.1% Tween 20, 0.1% 2-mercaptoethanol, 0.001% Antifoam A, 1 mM ATP and protease inhibitors). Cell lysates were homogenised by passing through a 24-gauge needle. Cell lysates were incubated at 37°C for 10 min and cells debris were separated by centrifuging at 2000×g for 5 min. The G-actin was separated by centrifuging the supernatants at 100 000×g for 1 h at 37°C. F-Actin pellet was resuspended in actin destabilisation buffer (Milli-Q water containing 10 μM of cytochalasin D) and incubated in ice for 1 h. F-actin and G-actin were analysed by performing western blot using β-actin antibody.

Statistical analysis

Results are presented as means ± S.E.M. Statistical analyses were performed using statistical software package GraphPad Prism 6.0 (GraphPad Software Inc.). Statistical analysis for each experiment is described in figure legends.

Results

We investigated whether phosphorylation of β -catenin on Ser552 is important in the processes regulating insulin secretion. We were able to overexpress both wild type and Ser552Ala variant in INS-1E cells and show that overexpression of the alanine variant resulted in lower levels of Ser552 phosphorylation (Figure 1A). We find that glucose stimulation of insulin secretion is attenuated in INS-1E cells overexpressing a Ser552Ala mutant version of β -catenin compared with cells overexpressing wild-type β -catenin, supporting a role for Ser552 phosphorylation in regulating insulin secretion (Figure 1B). This was associated with an increase in F-actin (Figure 1C,D) consistent with phosphorylation of Ser552 being involved in the regulation of rearrangements of the actin cytoskeleton. We did not observe any impact of expression of the Ser552Ala variant on the

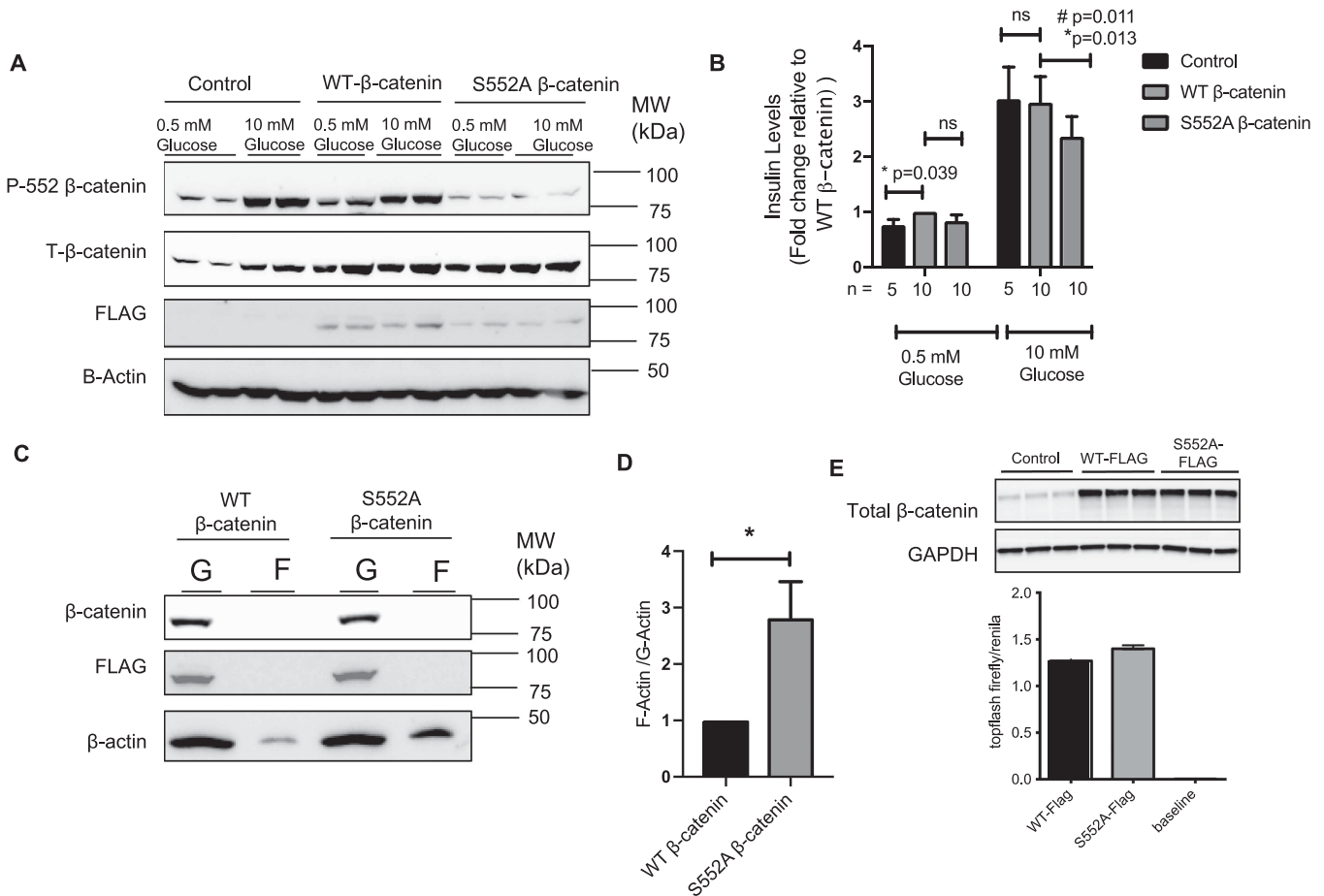


Figure 1. Ser552Ala β -catenin has lower insulin secretion compared with wild-type β -catenin in β -cells and does not inhibit the transcriptional role of β -catenin.

(A and B) INS-1E β -cells were transfected with wild-type- β -catenin-FLAG (WT-FLAG), mutant S552A- β -catenin-FLAG (S552A-FLAG) plasmid or were mock transfected (control). Forty-eight hours after transfection cells were serum- and glucose- starved for 1 h in KRBH buffer and treated with 0.5 mM or 10 mM glucose for 2 h. (A) Cell lysates were used for western blot analysis and (B) supernatants were collected for determination of insulin concentrations using alphaLISA. Results are mean \pm S.E.M of the indicated number of independent experiments, each performed with at least duplicate technical replicates. * $P < 0.05$ as compared with the WT-FLAG transfected cells of the same glucose condition, as assessed by paired t -test and Wilcoxon test (# $P < 0.05$). (C) F-actin and G-actin were separated in INS-1E cells transfected with either WT or S552A β -catenin and analysed by western blot using β -actin antibody (D) and F/G actin ratios were analysed. Results are mean \pm S.E.M of three independent experiments. * $P < 0.05$ as compared with the WT-FLAG transfected cells as assessed by unpaired t -test. (E) HEK293 cells were co-transfected with TOPFlash firefly and renilla luciferase plasmids alongside WT-FLAG or mutant S552A-FLAG plasmid. Control refers to mock transfected cells. Cells were lysed 48 h after transfection and luciferase activity measured using the Dual-Luciferase Reporter Assay system. Similar results were obtained in at least three independent experiments.

expression of a β -catenin gene reporter (Figure 1E). We were not able to develop conditions for successfully transfecting the GLP-1 responsive INS-832/3 cells with β -catenin despite trying a range of conditions.

We find that the previously demonstrated increase in phosphorylation of Ser552 in response to glucose exposure is strongly enhanced by addition of GLP-1 or the GLP-1 receptor agonist Exendin-4 in INS-832/3 cells (Figure 2A,B). The GLP-1R antagonist Exendin-3(9–39) had no effect on Ser552 phosphorylation (Figure 2C,D). The increase in phosphorylation of CREB was indicative of the production of cAMP by glucose and subsequent activation of PKA by GLP-1 and Exendin-4. The glucose effect on Ser552 phosphorylation is partially attenuated by the PKA inhibitor H-89 (Figure 3A,B) and by PKA-C siRNA (Figure 3C,D) consistent with previous data [6]. In contrast, the effect of GLP-1 on Ser552 phosphorylation is not attenuated by inhibition of PKA (Figure 3B–D). GLP-1 signals through G α s and causes significant increases in cAMP in β -cells and this concentrations of cAMP generated could allow additional signalling through EPAC1 or EPAC2 to activate small GTP binding proteins [17]. In support of this we find that the EPAC inhibitor ESI-09 attenuated both glucose and GLP-1 mediated increase in Ser552 phosphorylation on β -catenin (Figure 3E,F) and attenuation of insulin secretion in parallel (Figure 3G).

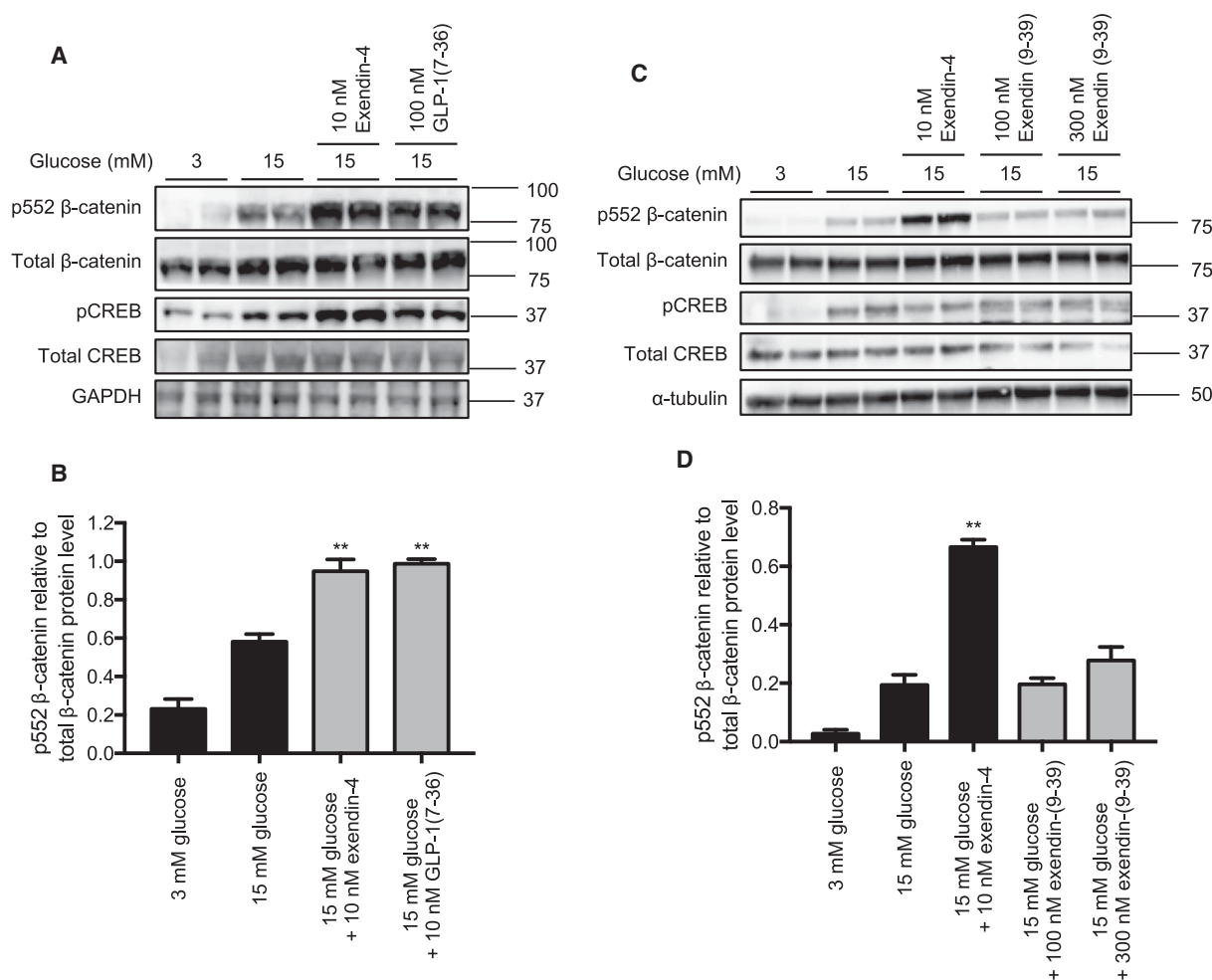


Figure 2. GLP-1 potentiates the glucose induced phosphorylation of Ser552 β -catenin in β -cells.

INS-832/3 cells were glucose- and serum-starvation for 1 h in KRBH buffer then treated with 3 mM glucose, 15 mM glucose and 15 mM glucose with (A and C) 10 nM GLP-1 receptor agonist Exendin-4, (A) 100 nM GLP-1 (7–36) amide, or (C) 100 nM GLP-1 receptor antagonist Exendin-(9–39) for 2 h and lysates subject to western blot analysis. (B and D) Densitometry analysis of Serine 552 β -catenin phosphorylation level relative to total β -catenin protein in western blot images in A, C. Results are mean \pm S.E.M. ** $P < 0.01$ compared with 15 mM glucose condition, as assessed by one-way ANOVA with Tukey’s post hoc test. Similar results were obtained in at least three independent experiments.

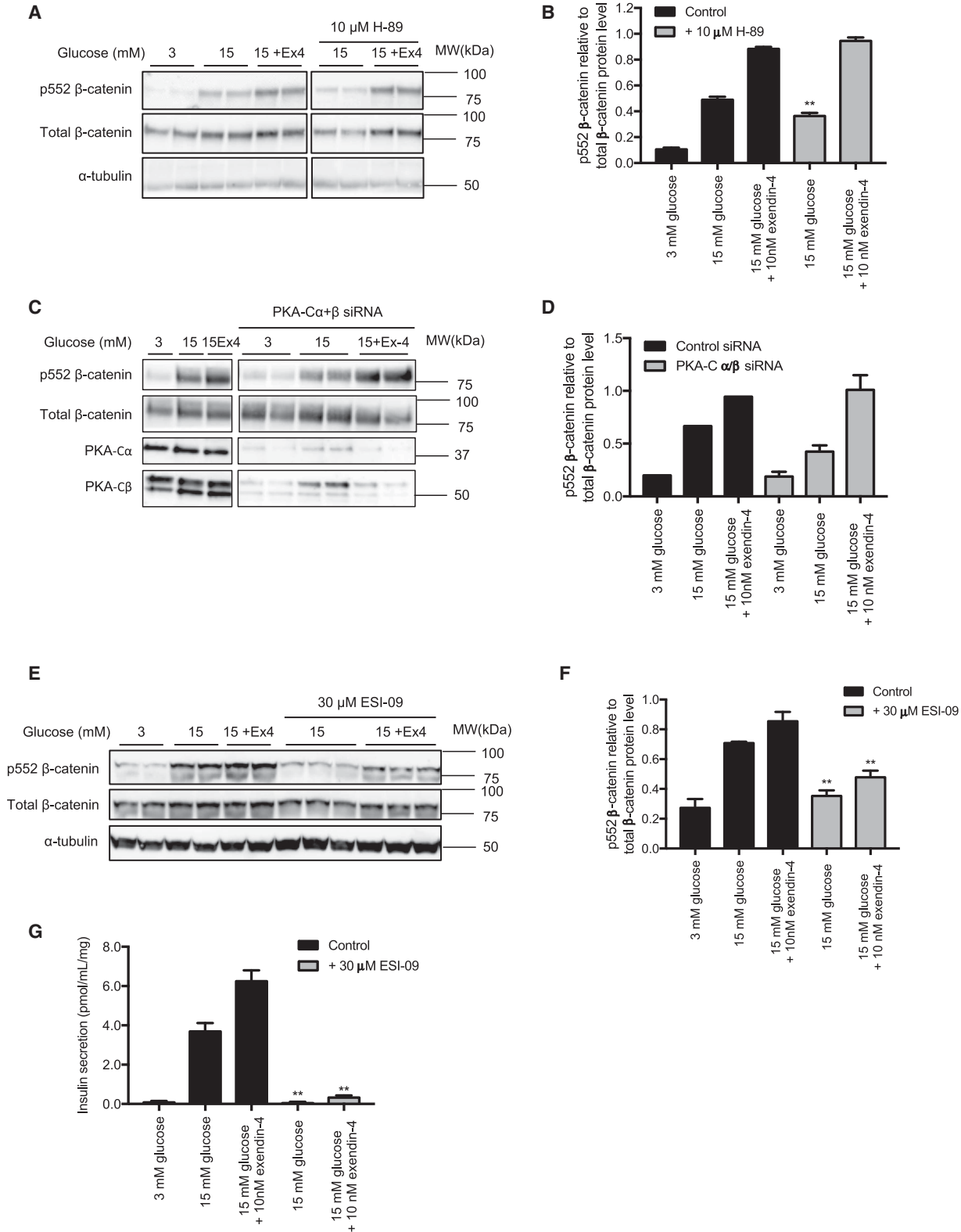


Figure 3. Glucose induced Ser552 phosphorylation of β -catenin involves PKA and GLP-1 induced Ser552 phosphorylation involves EPAC.

Part 1 of 2

INS-832/3 cells were glucose- and serum-starved for 1 h and then incubated in KRBH buffer containing 3 mM glucose, 15 mM glucose, or 15 mM glucose +10 nM Exendin-4 for 2 h. Treatment buffer contained (A and B) 10 μ M PKA inhibitor H-89 or (C and D) Cells were transfected with siRNA specific to

Figure 3. Glucose induced Ser552 phosphorylation of β -catenin involves PKA and GLP-1 induced Ser552 phosphorylation involves EPAC.

Part 2 of 2

PKA-C α + β 48 h prior to use. (E–G) 30 μ M EPAC inhibitor ESI-09 as indicated. (A,C,E) Cell lysates were collected and used for western blotting analysis. (B,D,F) Densitometry quantification of western blot images in A, C, E. (G) Buffer was collected for determination of insulin concentration using AlphaLISA. Results are mean \pm S.E.M. ** $P < 0.01$ as compared with the control treated cells of the same glucose/Exendin-4 condition, as assessed by one-way ANOVA with Tukey's post hoc test. Similar results were obtained in at least three independent experiments.

We next investigated whether phosphorylation of Ser552 required PAK1 activity. We find that both the PAK1 inhibitor IPA3 (Figure 4A,B) and siRNA targeting PAK1 (Figure 4D,E) block glucose and GLP-1 induced phosphorylation of β -catenin on Ser552 in INS-832/3 cells. The effects on Ser552 phosphorylation closely parallel the effects of the IPA3 and the PAK1 siRNA on insulin secretion (Figure 4C,F).

Similarly, when we treated mouse islets with EPAC inhibitor ESI-09 or the PAK inhibitor IPA3, glucose induced increase in Ser552 β -catenin is attenuated (Figure 5A,B). Consistent with this, in ESI-09

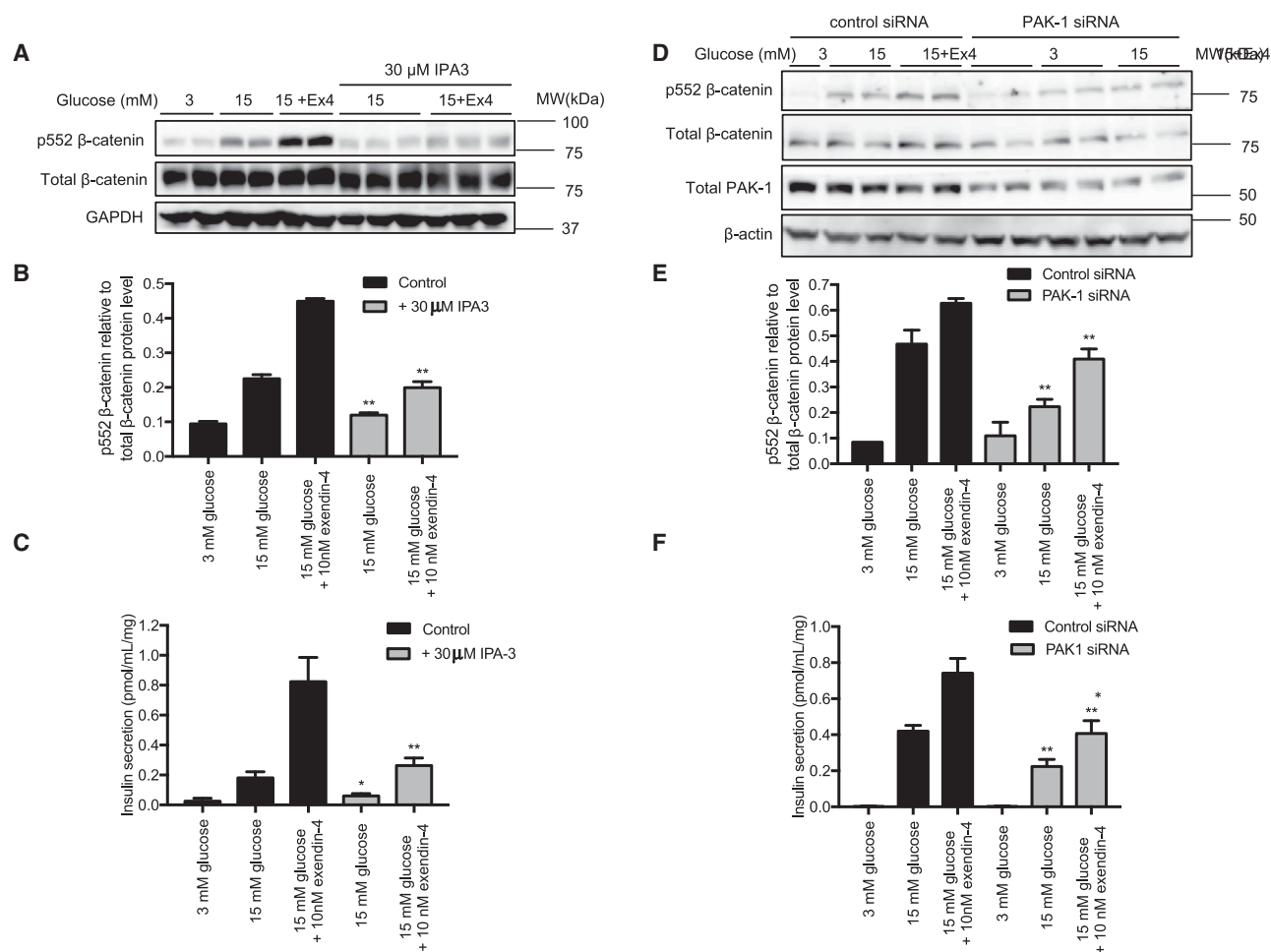


Figure 4. Inhibition of PAK-1 attenuates Ser552 phosphorylation of β -catenin.

INS-832/3 cells were glucose- and serum-starved for 1 h and then incubated in KRBH buffer containing 3 mM glucose, 15 mM glucose, or 15 mM glucose + 10 nM Exendin-4 for 2 h. (A–C) Treatment buffer contained 30 μ M PAK-1 inhibitor IPA3 where indicated. (D–F) Forty-eight hours prior to use cells were transfected with siRNA specific to PAK-1 or control siRNA. (A and D) Cell lysates were collected and used for western blotting analysis. (B and E) Densitometry analysis of blot images in A, D. (C and F) Buffer was collected for determination of insulin concentration by alphaLISA. Results are mean \pm S.E.M. * $P < 0.05$ and ** $P < 0.01$ as compared with the control treated cells of the same glucose/Exendin-4 condition, as assessed by one-way ANOVA with Tukey's post hoc test. Similar results were obtained in at least three independent experiments.

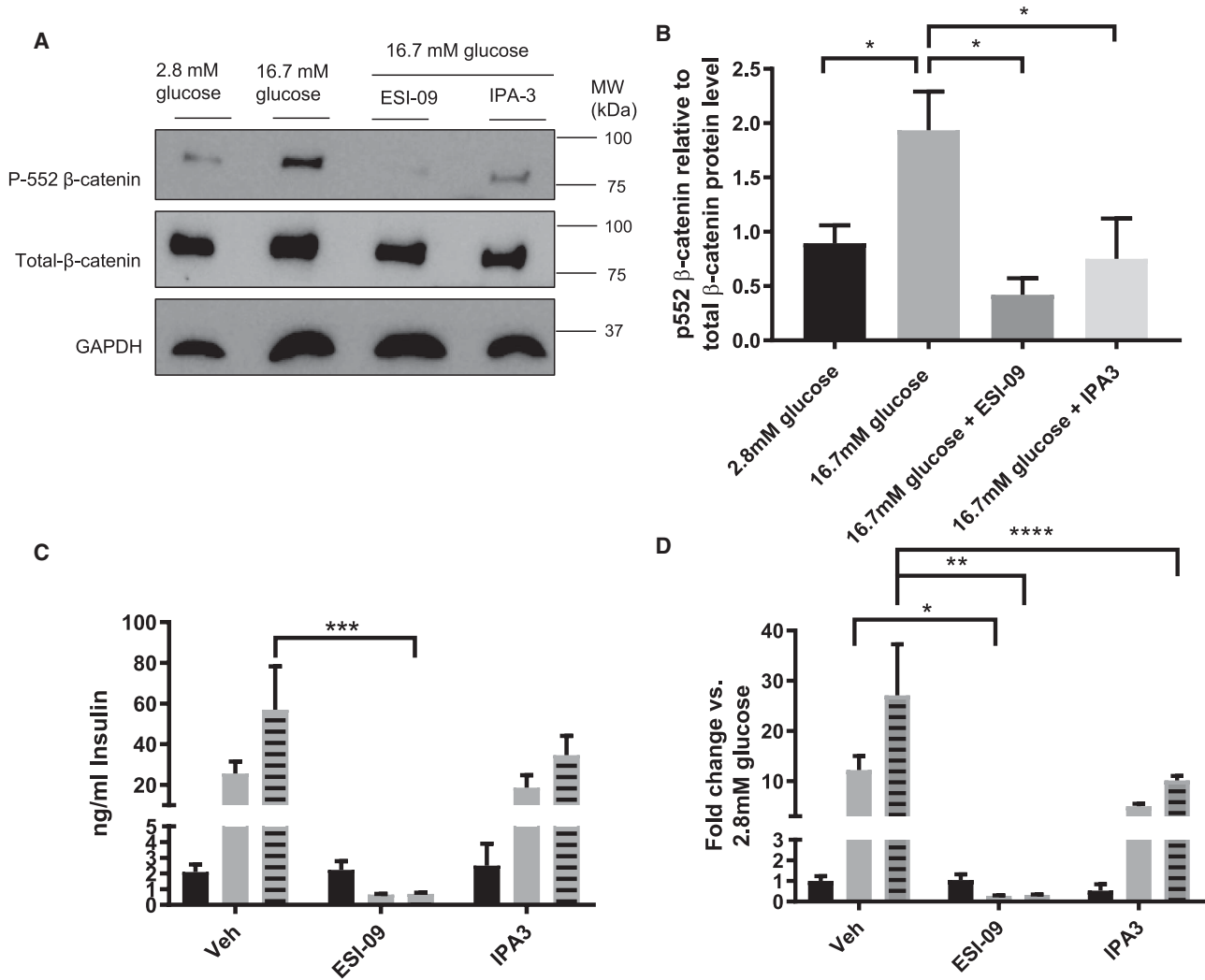


Figure 5. Inhibition of EPAC and PAK1 attenuates glucose induced increase in Ser552 phosphorylation in islets.

(A) Islets from male CD1 mice (8–10 weeks) were glucose and serum starved for 1 h and then incubated with buffer containing 2.8 mM glucose, 16.7 mM Glucose, 16.7 mM Glucose with ESI-09 or 16.7 mM Glucose with IPA3 for 1 h. Cell lysates were collected and used for western blot analysis. Representative western blot image is shown. (B) Densitometry analysis of Ser-552 β-catenin protein level relative to total β-catenin level. Results are mean ± S.E.M of three independent experiments. Statistics are one-way ANOVA with Holm–Sidak’s multiple comparisons test vs. 16.7 mM with no inhibitor. (C and D) AlphaLISA assays for insulin were performed on supernatants collected from mouse islets treated with vehicle control, ESI-09 or IPA3 with 2.8 mM Glucose (black bars), 16.7 mM Glucose (grey bars) and 16.7 mM Glucose + 10 nM Exendin-4 (striped bars). Absolute insulin concentrations (C) and fold change vs. 2.8 mM (D) are shown. Results are mean ± S.E.M of four independent experiments, statistics are two-way ANOVA with Dunnett’s multiple comparisons testing inhibitors vs. vehicle control within each secretagogue treatment (2.8 mM, 16.7 mM, and 16.7 + Exendin-4).

and IPA3 treated islet samples both glucose and GLP-1 stimulated insulin secretion were reduced (Figure 5C,D). This indicates the PAK1 dependent phosphorylation of Ser552 β-catenin wasn’t restricted to cultured cell models.

Discussion

Here we present evidence that phosphorylation of β-catenin Ser552 can play a role in modulating levels of insulin secretion and we go on to investigate the signaling pathways involved. The expression of the mutant form of β-catenin partially attenuates the GSIS in the face of continued expression of wild-type β-catenin suggesting a

mechanism whereby the unphosphorylated form actively blocks vesicle trafficking to the cell surface; essentially behaving in a dominant-negative fashion. It is not clear in β -cells what the details of this phosphorylation-dependent mechanism are. In other cell types, β -catenin Ser552 phosphorylation is associated with increased TCF-dependent transcriptional activation [12,13,22] which raises the question of whether a similar mechanism could be playing a role in regulating vesicle trafficking in β -cells. While we haven't directly tested this we believe it is unlikely to explain the mechanism for the effects on insulin secretion in β -cells. This is because we have previously shown that β -catenin's effects on insulin secretion [7] and GLUT4 translocation [10] are not dependent on TCF-mediated gene expression. Furthermore, we have previously shown that overexpression of TCF7L2 in β -cell lines actually reduces GSIS rather than stimulating it [7]. This is consistent with *in vivo* studies showing the tissue specific deletion of TCF7L2 in β -cells in adult animals does not impact on β -cell functions including insulin secretion. Together this suggests the effects of β -catenin on gene expression may not be as important to the function of fully differentiated β -cells to the extent they are in the developmental stages of these cells [23].

A more likely explanation for the effects of Ser552 phosphorylation in β -cells is that it is involved as part of mechanisms regulating the actin cytoskeleton required for the insulin secretory vesicles to translocate to and fuse with the plasma membrane [24]. This is consistent with it being part of the PAK1 signalling pathway in β -cells as this is known to be involved in regulating actin remodelling and insulin vesicle trafficking [18]. In support of this, we have shown that β -catenin plays a role in rearrangement of actin cytoskeleton in parallel with effects on vesicle trafficking in muscle and β -cells [7,10]. Here we extend these findings to show that overexpression of the Ser552Ala version perturbs the F/G actin ratios. We have also reported very similar effects in muscle cells [10]. Together this supports a role for Ser552 phosphorylation regulating actin cytoskeleton. The detail of this mechanism remains to be determined, however, in muscle we also found the phosphorylation of Ser552 to favour binding to M-cadherin [10]. Given that adherens junctions are required for the development of proper insulin secretion [3,4], this suggests that binding of β -catenin to the N- and E-cadherins found in β -cells could be regulated by a similar mechanism.

Our findings lead us to study the mechanisms by which β -catenin Ser552 is becoming phosphorylated in β -cells. cAMP production downstream of glucose or incretins such as GLP-1 plays a crucial role in dictating the overall amount of insulin secreted [1]. Evidence has been presented for roles of both PKA [25–27] and EPAC [27–30] downstream of cAMP in these processes. Here we have investigated the potential role of phosphorylation of Ser552 on β -catenin in this process. The Ser552 site fits as a PKA consensus site and PKA has been implicated in phosphorylating this site in a range of cell types [12,13,15], including in cells stimulated with GLP-1 and glucagon [31–33]. However, we find here PKA doesn't fully explain the effects of glucose and GLP-1 on phosphorylation of β -catenin on Ser552. Another candidate kinase would be PAK1 as this is activated downstream of glucose in β -cells and is known to be important in mediating the effects of glucose on insulin secretion [18]. Here we find that both the glucose and GLP-1 induced increases in the phosphorylation of β -catenin on Ser552 are attenuated by a PAK1 selective small-molecule inhibitor IPA3 and also by siRNA to PAK1 indicating the involvement of PAK1 in this phosphorylation event. This is also consistent with the presence of a pathway linking EPAC to PAK1 activation in β -cells [30].

In summary, our studies provide direct evidence that phosphorylation of Ser552 on β -catenin is important for modulating insulin secretion in β -cell models. By demonstrating that this phosphorylation event is regulated by an EPAC-PAK1 dependent mechanism in β -cells our findings also identify a previously undescribed mechanism that provides an explanation as to how PAK1 could possibly mediate effects on insulin secretion [18]. The findings also identify a potential convergence point between glucose and GLP-1 in the regulation of insulin secretion.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

F.H. was supported by the China Scholarship Council. B.S. was supported by a Rutherford Foundation Post Doctoral Fellowship. The Health Research Council of New Zealand provided support for experimental reagents.

Open Access Statement

Open access for this article was enabled by the participation of University of Auckland in an all-inclusive *Read & Publish* pilot with Portland Press and the Biochemical Society under a transformative agreement with CAUL.

CRedit Contribution

Peter R. Shepherd: Conceptualization, Formal analysis, Supervision, Funding acquisition, Writing — review and editing. **Brie Sorrenson:** Conceptualization, Formal analysis, Investigation. **Waruni C. Dissanayake:** Formal analysis, Investigation, Methodology. **Fengyun Hu:** Conceptualization, Investigation, Methodology. **Kate L. Lee:** Formal analysis, Investigation, Methodology.

Data Availability

There are no large data sets associated with this manuscript. Reagents described in this study available on reasonable request.

Acknowledgements

We thank Betty Shih for assistance with Western blotting.

Abbreviations

GSIS, glucose stimulated insulin secretion; KRBH, Krebs-Ringer bicarbonate HEPES; PAK1, p21-activated protein kinase-1; PKA, Protein kinase A.

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