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PACAP stimulates insulin secretion by PAC_1 receptor and ion channels in β-cells

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ABSTRACT

Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) plays a crucial role in the endocrine system. The present study aimed to investigate the effect of PACAP38 on insulin secretion and the underlying mechanism in rat pancreatic β-cells. The insulin secretion results showed that PACAP38 stimulated insulin secretion in a glucose- and dose-dependent manner. The insulinotropic effect was mediated by PAC_1 receptor, but not by $VPAC_1$ and $VPAC_2$ receptors. Inhibition of adenylyl cyclase and protein kinase A suppressed PACAP38-augmented insulin secretion. Glucose-regulated insulin secretion is dependent on a series of electrophysiological activities. Current-clamp technology suggested that PACAP38 prolonged action potential duration. Voltage-clamp recordings revealed that PACAP38 blocked voltage-dependent potassium currents, and this effect was reversed by inhibition of PAC_1 receptor, adenylyl cyclase, or protein kinase A. Activation of Ca^{2+} channels by PACAP38 was also observed, which could be antagonized by the PAC_1 receptor antagonist. In addition, calcium-imaging analysis indicated that PACAP38 increased intracellular Ca^{2+} concentration, which was decreased by PAC_1 receptor antagonist. These findings demonstrate that PACAP38 stimulates glucose-induced insulin secretion mainly by acting on PAC_1 receptor, inhibiting voltage-dependent potassium channels, activating Ca^{2+} channels and increasing intracellular Ca^{2+} concentration. Further, PACAP blocks voltage-dependent potassium currents via the adenylyl cyclase/protein kinase A signaling pathway.

1. Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP), a hypothalamic peptide, activates adenylate cyclase (AC) in rat pituitary cells [1]. PACAP is a member of the secretin-glucagon superfamily [2], including a 38-amino-acid form (PACAP38) and a shorter 27-amino-acid form (PACAP27). Chromatographic analyses have shown that the concentration of PACAP38 in extracted human pancreas is much higher than that of PACAP27 [3,4]. The peptide elicits its biological actions via regulating three types of class II G protein-coupled receptors (GPCRs): PAC_1 receptor (PAC1-R), $VPAC_1$ receptor (VPAC1-R), and $VPAC_2$ receptor (VPAC2-R) [5]. PAC1-R has a distinct high affinity for PACAP. Study has shown that PACAP and its receptors are widely distributed in the central nervous system (CNS) and peripheral organs [1].

Previous study suggested that PACAP is a potential therapeutic target for diabetes and its complications [5]. It has been proposed that PACAP regulates postprandial glucose homeostasis and potentiates glucose-dependent insulin secretion from isolated rat islets [6,7]. However, the underlying mechanism of this action is still unknown. Pancreatic β-cells possess electrical excitability. Glucose-induced insulin secretion from β-cells is mediated by a series of electrophysiological activities, which result in exocytosis of insulin-containing granules [8]. Hence, we proposed that electrogenic events were involved in PACAP38-modulated glucose-stimulated insulin secretion through acting on G protein-coupled receptor and AC/PKA signaling pathways.

In this study, we used primary rat pancreatic islets and dispersed islet cells to explore the effects of PACAP38 on β-cell function, including insulin secretion, electrical activity, and intracellular Ca^{2+} concentration. Based on insulin secretion experiments and patch-clamp technology, we identified the cellular and molecular mechanisms whereby PACAP38 amplified glucose-induced insulin secretion.

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2. Materials and methods

2.1. Animals

Adult male Wistar rats, weighing 240–260 g, were purchased from Beijing Weitong Lihua experimental animal center. Rats were housed with pellet-type food and tap water under temperature (25 ± 2 °C) and humidity (55–60%) condition with a 12 h-light/darkness cycle. All experimental procedures involving animals described below were in accordance with the ethical guidelines for animal research at Shanxi Medical University and were approved by the Animal Care and Use Committee of Shanxi Medical University (Taiyuan, PR China).

2.2. Isolation and culture of islets and cells

Rat pancreas was digested with 1 mg/mL collagenase P (Roche, Indianapolis, IN, USA) for 11 min at 37 °C. Islets were obtained by histopaque-1077 (Sigma-Aldrich, USA) density gradient centrifugation. Islet cells were separated from pancreatic islets by DispaseII (Roche, Indianapolis, IN, USA) digestion. Isolated islets and cells were cultured in Hyclone RPMI 1640 (Hyclone, Thermo Scientific, USA) medium with 10% fetal bovine serum, 100 mg/mL streptomycin, and 100 U/mL penicillin in a humidified atmosphere of 5% CO_2, 95% air at 37 °C.

2.3. Measurements of insulin secretion

Separated fresh pancreatic islets were cultured 24 h before the experiments. Islets (5/tube) were pre-incubated in Krebs Ringer bicarbonate-HEPES (KRBH) buffer with 2.8 mM glucose for 30 min at 37 °C, 5% CO_2. After discarding the supernatant, the islets were incubated in KRBH buffer for 30 min with different drugs and glucose concentrations. Incubated supernatant was collected and stocked at 4 °C. Insulin secretion was determined by Iodine [^{125}I] Insulin Radioimmunoassay Kit (North Biological Technology Research Institute of Beijing). KRBH buffer contained (mM): 128.8 NaCl; 4.8 KCl; 1.2 KH_2PO_4; 2.5 $CaCl_2$; 1.2 $MgSO_4$; 10 HEPES; 5 $NaHCO_3$ and 2% BSA at pH 7.4.

2.4. Patch-clamp experiment

Rat pancreatic islet cells were cultured on coverslips coated with cell adherent reagent (Applygen Technologies Inc., Beijing, China) in RPMI 1640 medium before the experiments. For patch-clamp recordings, coverslips containing islet cells were shifted to a recording chamber, installed on an inverted microscope (Nikon Diaphot-TMD), and perfused with extracellular recording solution. The resistances of patch pipettes loaded with pipette solution ranged between 4 and 7 MΩ. Cells were recorded by whole-cell patch-clamp technology performed using the EPC-10 amplifier and PULSE software from HEKA Electronik (Lambrecht, GER) at room temperature. Islets β-cells were recognized by cell capacitance > 7 pF [9]. To record voltage-dependent potassium (Kv) currents, β-cells were clamped to a holding potential of −70 mV using voltage-clamp and then elicited to test potentials ranging from −70 mV to 80 mV in 10 mV steps by 400 ms. Patch pipettes were filled with pipette solution as follows (mM): 0.3 MgATP; 140 KCl; 10 NaCl; 1 $MgCl_2$; 0.05 EGTA, and 10 HEPES (pH 7.25 with KOH). The extracellular solution contained (mM): 11.1 glucose; 141.9 NaCl; 5.6 KCl; 1.2 $MgCl_2$ and 5 HEPES (pH 7.4 with NaOH).

For depolarization-evoked Ca^{2+} currents recordings, β-cells were clamped to a holding potential of −70 mV using the voltage-clamp and then elicited to test potentials of −50 mV to 30 mV in 10 mV steps by 50 ms. The Ca^{2+} currents intracellular solution contained (mM): 120 CsCl; 20 TEA (Tetraethylammonium chloride, Sigma-Aldrich, USA); 5 MgATP; 1 $MgCl_2$; 0.05 EGTA; and 10 HEPES (pH 7.25 with CsOH). The extracellular solution consisted of (mM): 100 NaCl, 20 TEA, 20 $BaCl_2$, 4 CsCl, 1 $MgCl_2$, 5 HEPES, and 3 glucose (pH 7.4 with NaOH). Ca^{2+} was replaced with Ba^{2+} as the charge carrier in the extracellular solution to eliminate Ca^{2+}-dependent inactivation of the voltage-gated calcium channels and amplify current responses through Ca^{2+} channels during the depolarization stimulus.

In current-clamp mode, action potentials were recorded by stimulating β-cells at 150 pA with pulses of 4 ms duration. The calculation of action potential duration was the difference between the time from the start of the action potential until the time that the membrane potential returned to within 10 mV of the resting membrane potential.

2.5. Calcium imaging technology

Islet cells were cultured on coverslips coated with adhesion reagent in RPMI 1640 medium for 3 h before the experiments. Cells were incubated for 30 min in KRBH buffer with 2.8 mM glucose and 2 μM Fura 2-AM at 37 °C, with 5% CO_2. Subsequently, cells were washed twice with KRBH solution containing 2.8 mM glucose in a 35 mm dish. Intracellular calcium ions were measured at excitation wavelengths 380 nm and 340 nm, emission wavelength 510 nm, using OLYMPUS IX71 inverted microscope and Meta Fluor software 7.8 (Molecular Devices, USA) at 30 °C. The change in intracellular Ca^{2+} concentrations was recorded by the ratio of fluorescence intensity (F340/F380). $F-F_0$ value was used to compare the difference of Ca^{2+} concentrations under different drug conditions. (F: the average value during 30 s (15 s before and after the peak of F340/F380) for different treatment; F_0: the average value during 30 s (15 s before and after the nadir of F340/F380) for 2.8 mM glucose concentration). [10,11].

2.6. Statistical analysis

All experimental data are presented as mean ± SEM. Statistical analyses were performed by Student's *t*-test or ANOVA test. Significant difference was assumed at $P < .05$ using Sigma Plot (version 12.5).

3. Results

3.1. PACAP38 amplifies glucose-induced insulin secretion in rat pancreatic islets

To examine the effect of PACAP38 on insulin secretion, islets were stimulated with different doses of PACAP38 under 2.8 or 8.3 mM glucose. As shown in Fig. 1A, PACAP38 at various concentrations (0–100 nM) had no effect on insulin secretion under 2.8 mM glucose conditions. However, under conditions of 8.3 mM glucose, PACAP38 (10 and 100 nM) potentiated insulin secretion from isolated rat islets.

To confirm whether PACAP38-induced insulin secretion was glucose-dependent, islets were stimulated with 10 nM PACAP38 at different glucose concentrations. As depicted in Fig. 1B, PACAP38 augmented insulin secretion under high (8.3 or 16.7 mM), but not at low (2.8 mM) glucose concentrations. Based on the above results, we selected 10 nM PACAP38 and 8.3 mM glucose for the subsequent experiments.

3.2. PACAP38 stimulates insulin secretion through the PAC_1 receptor and AC/PKA signaling pathway

In numerous studies, PACAP has been shown to bind to three receptors, including PAC_1 receptor (PAC1-R), $VPAC_1$ receptor (VPAC1-R), and $VPAC_2$ receptor (VPAC2-R) in the pancreas [12,13]. PAC1-R played a significant role in glucose homeostasis during food intake [14]. Therefore, we examined whether PACAP38-induced glucose-dependent insulin secretion was dependent on PAC1-R. As expected, PACAP (6–38), the PAC1-R antagonist, significantly blocked PACAP38-potentiated insulin secretion at various concentrations (1 μM, 5 μM) (Fig. 2A). However, PG97–269 (the VPAC1-R antagonist) and PG99–465 (the VPAC2-R antagonist) had no influence on PACAP38-

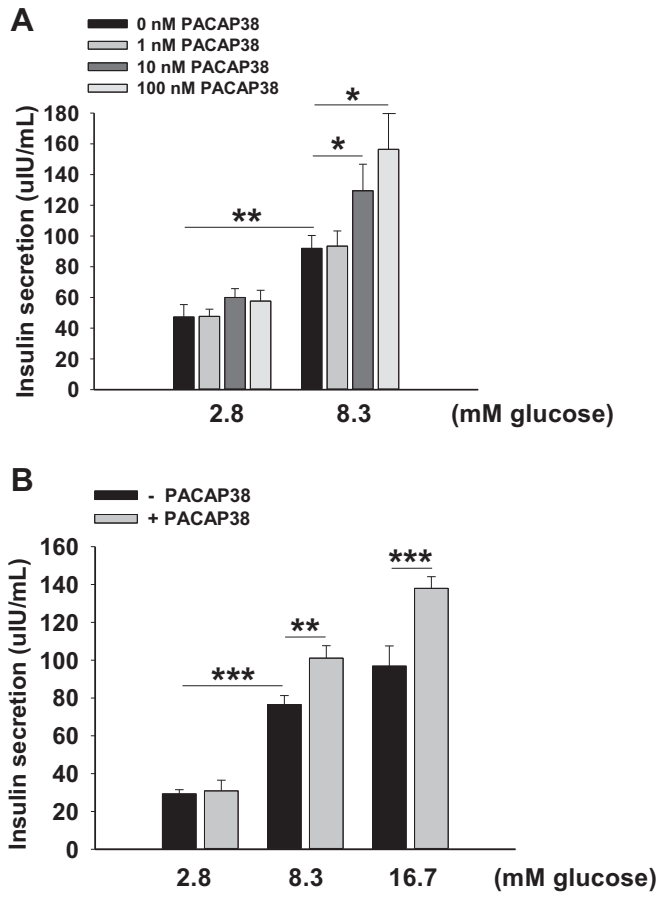

Fig. 1. Effect of pituitary adenylate cyclase-activating polypeptide 38 (PACAP38) on glucose-stimulated insulin secretion. (A) rat pancreatic islets were treated with various concentrations of PACAP38 under 2.8 mM glucose and 8.3 mM glucose. ($n = 6$). (B) islets were treated with 10 nM PACAP38 under different glucose concentrations. ($n = 9$). $^{*}P < .05$, $^{**}P < .01$ and $^{***}P < .001$.

stimulated insulin secretion. Moreover, the insulinotropic function of PACAP38 was not visibly inhibited by VIP (6–28), an nonselective inhibitor of VPAC1-R and VPAC2-R ($n = 7$, $P > .05$, Fig. 2B). These results suggest that the effect of PACAP38 on insulin secretion is dependent on PAC1-R activation in rat pancreatic islets.

It has been reported that PAC1-R activation increases cAMP levels in rodent pancreatic islets [15]. Hence, we investigated the relationship between the AC/PKA signaling pathway and PACAP38-regulated insulin secretion. Adenylyl cyclase (AC)is a crucial enzyme that can catalyze cAMP formation from ATP. As depicted in Fig. 2C, SQ 22536, an AC inhibitor, attenuated the effect of PACAP38 on insulin secretion. We further evaluated the role of cAMP downstream effector on PACAP38-modulated insulin secretion. The data showed that H89, a PKA inhibitor, dramatically weakened the effect of PACAP38 on insulin secretion (Fig. 2C), while SQ 22536 or H89 alone did not influence insulin secretion. These results indicate that the insulinotropic action of PACAP38 is related to the AC/PKA signaling pathway.

3.3. PACAP38 prolongs action potential duration via PAC1-R

Rat pancreatic β-cells are electrically excitatory. Action potential plays a significant role in the stimulus-secretion coupling of β-cells, and the prolongation of action potential duration (APD) promotes insulin secretion [16]. Hence, we next examined whether PACAP38 influenced action potentials to enhance insulin secretion. We applied whole-cell current-clamp technique in these experiments. Action potential was

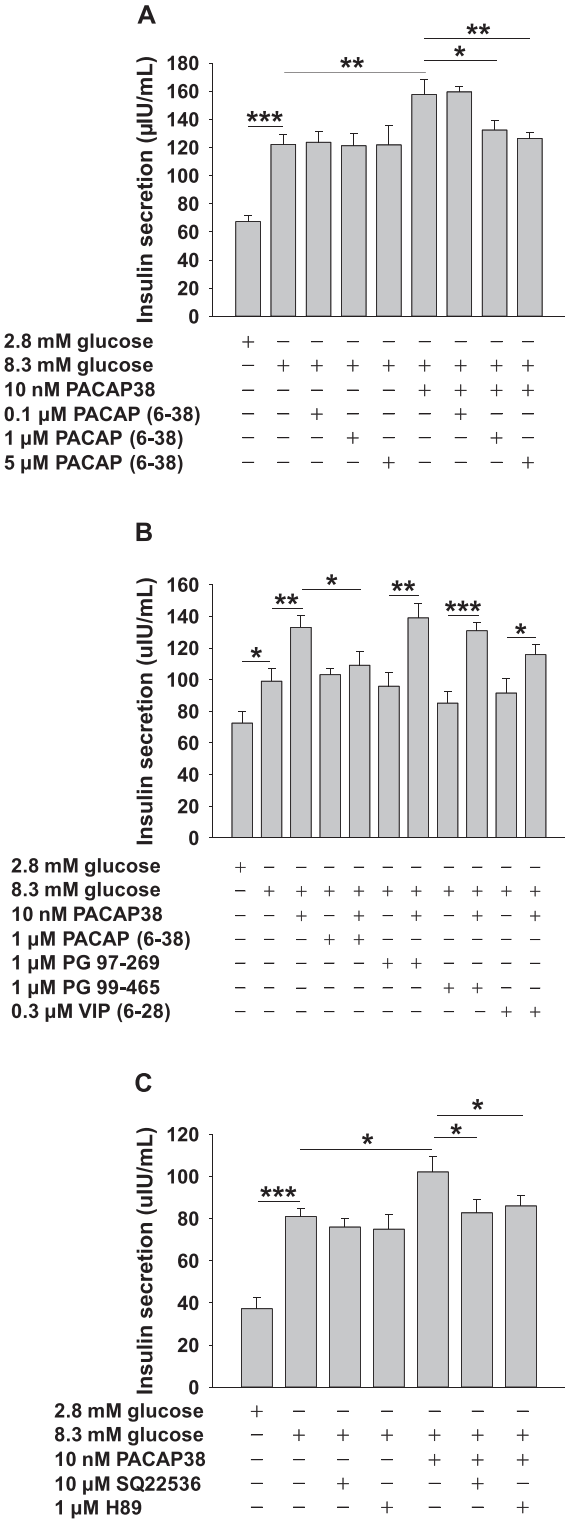

Fig. 2. PACAP38 stimulates glucoese-dependent insulin secretion through PAC1-R and, AC/PKA pathway. (A) islets were stimulated with different concentrations of PACAP (6–38). ($n = 6$). (B) PACAP38 (10 nM) enhanced glucose-induced insulin secretion in the presence orabsence of PACAP (6–38) (1 μM), PG 97–269 (1 μM), PG 99–465 (1 μM), VIP (6–28) (0.3 μM). ($n = 7$). (C) Effect of SQ 22536 (10 μM) and H89 (1 μM) on PACAP-agumented insulin secretion. (n = 7). $^{*}P < .05$, $^{**}P < .01$ and $^{***}P < .001$. (PACAP (6–38), as PAC_1 receptor inhibitor; PG 97–269, as $VPAC_1$ receptor inhibitor; PG 99–465, as $VPAC_2$ receptor inhibitor; VIP (6–28), as $VPAC_{1,2}$ receptor inhibitor; SQ 22536, as AC inhibitor; H89, as PKA inhibitor.)

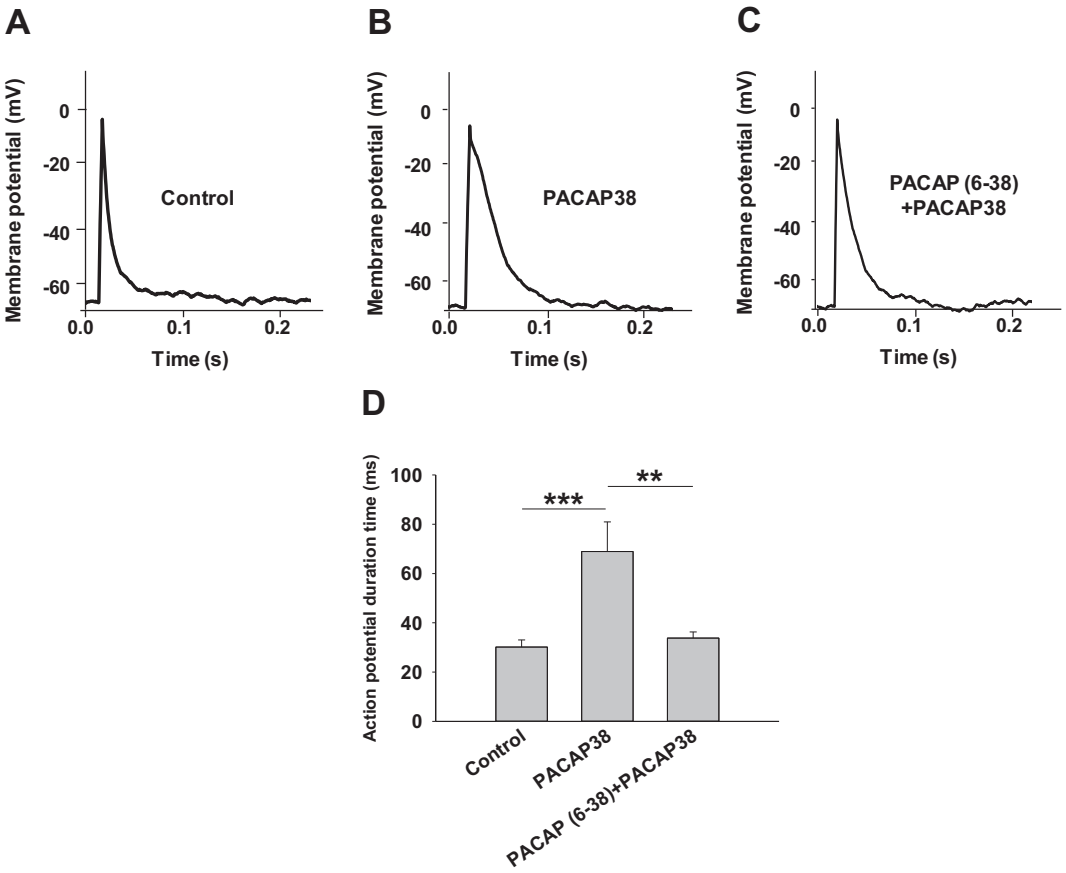

Fig. 3. PACAP38 prolongs action potential duration (APD) via PAC1-R. In whole-cell current-clamp mode, action potential were elicited by applying 4 ms, 150 pA current pulse injections. Representative action potential waveforms were shown for β-cells when treated without (A) or with PACAP38 (B). (C) Representative action potential waveforms were shown when treated with PACAP38 and PACAP (6–38). (D) Summary of the mean APDs. ($n = 6$). $^{**}P < .01$ and $^{***}P < .001$.

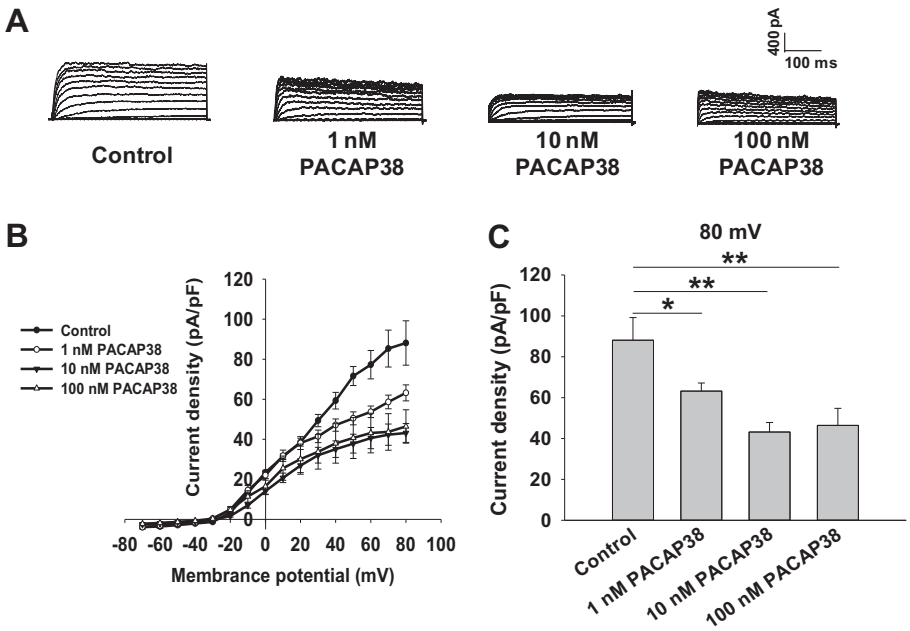

Fig. 4. Kv channels are inhibited by PACAP38 in pancreatic β-cells. Kv currents were recorded in conventional whole-cell configuration from a holding potential of −70 mV to various depolarizing voltages (−70 to 80 mV) in 10 mV increments. (A) Representative current trances recorded under various concentrations of PACAP38. (B) Current-voltage relationship curves of Kv channels from rat β-cells. (C) Summary of the mean current density of Kv channels recorded at 80 mV depolarization. ($n = 6$). $^{*}P < .05$ and $^{**}P < .01$.

recorded in the absence and presence of PACAP38. As predicted, PACAP38 significantly prolonged APD compared to controls (Fig. 3 A, B and D, $P < .001$).

The insulin secretion results showed that PAC1-R is involved in PACAP38-stimulated insulin release. Therefore, we next explored the relationship between PAC1-R and PACAP-prolonged APD. As shown in Fig. 3B, C and D, the prolongation of APD induced by PACAP38 was noticeably blocked by PACAP (6–38), indicating that the effect of PACAP38 on APD is dependent on PAC1-R activation.

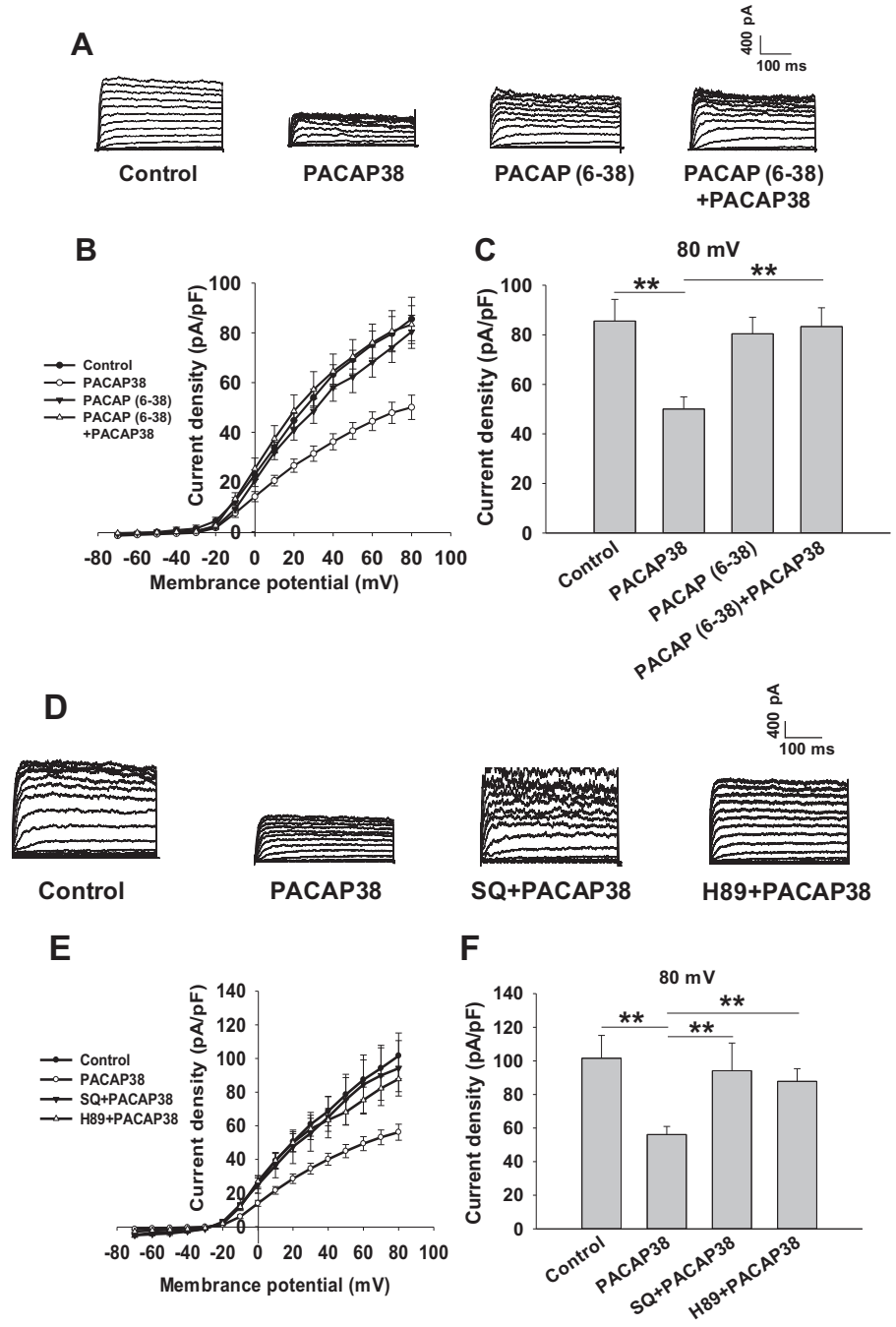

Fig. 5. PACAP38 inhibits Kv channels via PAC1-R and AC/PKA pathway. (A) Representative current trances recorded with PACAP38 in the prescence or absence of PACAP (6–38) (1 μM). (B, E) Current-voltage relationship curves of Kv channels from rat β-cells. (C, F) Summary of the mean current density of Kv channels recorded at 80 mV depolarization. (D) Representative current trances recorded with PACAP38 in the prescence or absence of SQ22536 (SQ, 10 μM) or H89 (1 μM). (n = 6). $^{**}P < .01$.

3.4. PACAP38 inhibits Kv channel currents in pancreatic β-cells

Kv channels participate in the repolarization of action potentials, and inhibition of Kv channels extends action potential duration [17,18]. Thus, we hypothesized that Kv channels were involved in PACAP38 modulated-action potential duration. Outward K^+ currents were evoked by depolarizing pulses in whole cell voltage-clamp experiments (Fig. 4A). As shown in Fig. 4B, PACAP38 decreased Kv current densities compared to controls. The current densities of controls were 88.2 ± 11.0 pA/pF at 80 mV. However, 10 nM PACAP38 significantly reduced current densities (43.1 ± 4.7 pA/pF) ($P < .01$) (Fig. 4C), which demonstrated that PACAP38 prolonged APD by inhibiting Kv channels.

3.5. PACAP38 inhibits Kv channels by regulating PAC1-R and AC/PKA signaling pathway

We then applied voltage-clamp technology to gain insights into the relationship between PACAP38-inhibited Kv channels and PAC1-R activation. As shown in Fig. 5A-C, PACAP (6–38), a PAC1-R antagonist, alone did not influence Kv currents notably. However, PACAP (6–38) significantly reversed PACAP38-inhibited Kv channels.

Further, we investigated whether the AC/PKA signaling pathway had an effect on PACAP-inhibited Kv channels. By applying SQ 22536,

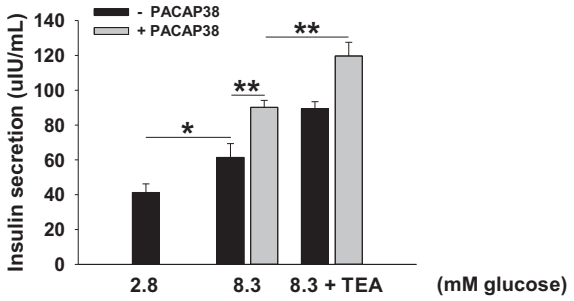

Fig. 6. Effect of PACAP38 on insulin secretion under different conditions. Rat pancreatic islets were stimulated with 10 nM PACAP38 in the presence or absence of TEA (20 mM). (n = 6). $^{*}P < .05$, $^{**}P < .01$. (TEA, Tetraethylammonium chloride, a inhibitor of Kv channels).

an AC inhibitor, and H89, a PKA blocker, we found that inhibition of Kv channels by PACAP38 was blocked in the presence of SQ 22536 or H89 (Fig. 5D-F). Additionally, SQ 22536, and H89 did not influence Kv channels (data not shown) [19]. Thus, our results indicate that the effect of PACAP38 on Kv channels is mediated by PAC1-R and the AC/PKA signaling pathway.

3.6. Kv channels partially mediated PACAP38-stimulated insulin secretion

To investigate whether PACAP-modulated insulin secretion was mediated only by Kv channels, we used Tetraethylammonium chloride (TEA), a potent inhibitor of Kv channels. As shown in Fig. 6, TEA enhanced insulin secretion at 8.3 mM glucose. PACAP38 still remarkably potentiated insulin secretion in the presence of TEA, which implies that PACAP-stimulated insulin secretion is mediated not only by Kv channels, but also other factors.

3.7. PACAP38 activates voltage-gated Ca^{2+} channels via PAC1-R

Voltage-gated Ca^{2+} channels are involved in the pathway that triggers insulin secretion in pancreatic β-cells [20]. We next explored if Ca^{2+} channels participated in PACAP38-regulated insulin secretion. Inward Ca^{2+} currents were elicited by depolarizing pulses. The current-voltage relationship revealed that PACAP38 increased Ca^{2+} channels activity when compared to the controls (Fig. 7B). The Ca^{2+} current density at 0 mV was −2.5638 ± 0.4210 pA/pF for PACAP38 (Fig. 7C), which was higher than that of the controls (−1.3502 ± 0.3452 pA/pF). Hence, PACAP38-modulated insulin secretion was also mediated by the activation of voltage-gated Ca^{2+} channels.

Because PAC1-R activation is required for PACAP38-stimulated insulin secretion, we further examined if PACAP38-activated Ca^{2+} current potentiation was mediated through PAC1-R. As illustrated in Fig. 7 A-C, in comparison with controls, PACAP38-activated Ca^{2+} channels were blocked when treated with PACAP (6–38). These findings demonstrated that PACAP38 potentiated Ca^{2+} channels by acting on PAC1-R.

3.8. PACAP38 increases intracellular Ca^{2+} ($[Ca^{2+}]_i$) concentration through PAC1-R

Intracellular calcium ion is a key factor in triggering insulin secretion in pancreatic β-cells. To assess the influence of PACAP38 on $[Ca^{2+}]_i$, we applied Fura 2-AM to detect changes in fluorescence intensity. As shown in Fig. 8, A and B, the fluorescence intensity increased under 8.3 mM glucose conditions, which showed a significant difference when compared to 2.8 mM glucose. Compared to 8.3 mM glucose, PACAP38 dramatically enhanced fluorescence intensity, which suggests that PACAP38 increased $[Ca^{2+}]_i$ concentration to stimulate insulin secretion.

In light of the above results, we further investigated whether PAC1-R was related to the PACAP38-elevated $[Ca^{2+}]_i$ level. PACAP (6–38) alone showed no change in fluorescence intensity (Fig. 8, C and D). However, the effect of PACAP38-augmented $[Ca^{2+}]_i$ concentration was counteracted by PACAP (6–38). These findings demonstrate that the PACAP38-increased $[Ca^{2+}]_i$ level is dependent on PAC1-R.

4. Discussion

PACAP is a widely known potential insulinotropic neuropeptide [21,22]. It has been reported that administration of PACAP in vivo significantly enhanced plasma insulin levels in mice [23], pigs [24], and humans [25]. And studies have reported that PACAP amplified insulin secretion in a glucose-dependent manner [21,26]. Of note, we found that PACAP38 had no effect on insulin secretion at a low glucose concentration. PACAP38 dose-dependently augmented insulin secretion under high glucose conditions. Therefore, PACAP could be potentially used as an insulinotropic agent without risk of hypoglycemia, which is a common adverse effect of diabetes mellitus therapies [27]. In this study, we investigated the effect of PACAP on insulin secretion and the

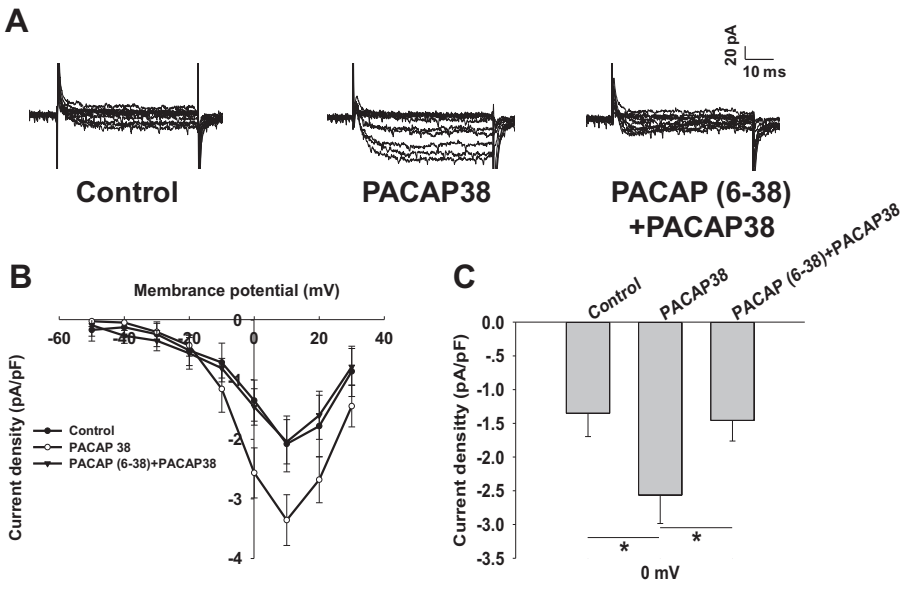

Fig. 7. Effect of PACAP38 on voltage-gated Ca^{2+} channels in the presence or absence of PACAP (6–38). (A) Representative current traces recorded under different conditions. (B) Current-voltage relationship curves of voltage-gated Ca^{2+} channels obtained from rat β-cells. (C) Summary of the mean current density recorded at 0 mV depolarization. ($n = 6$). $^{*}P < .05$.

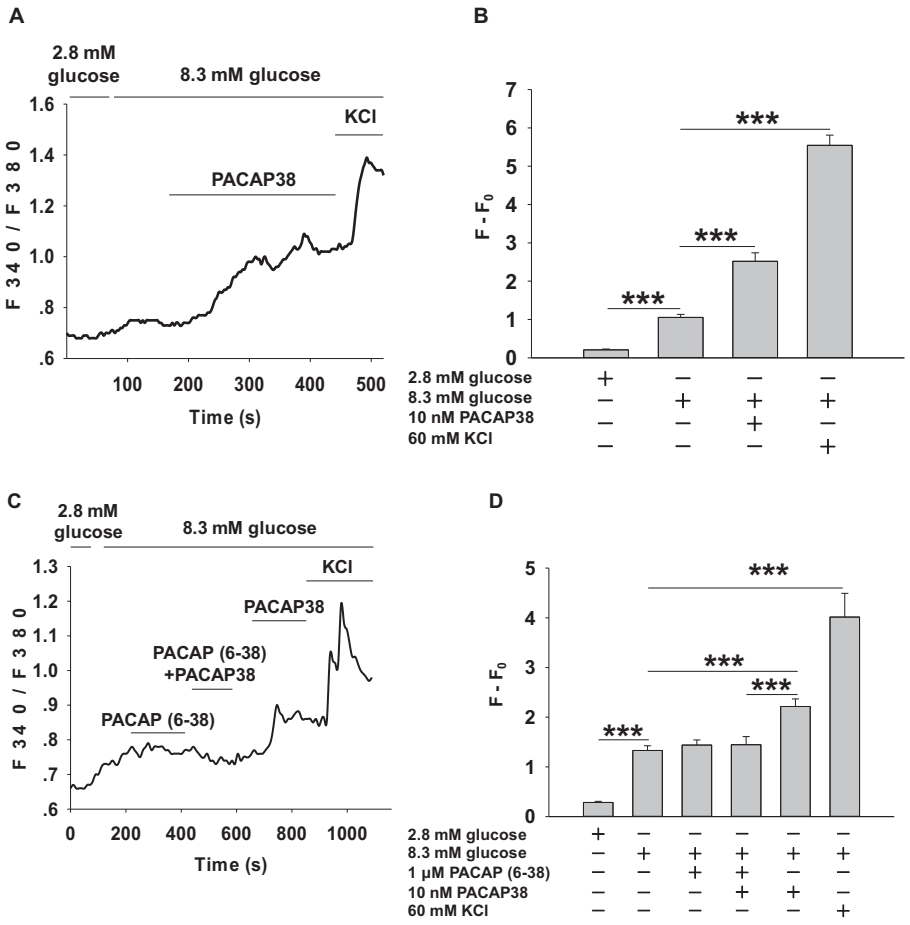

Fig. 8. PACAP38 increases the $[Ca^{2+}]_i$ in rat pancreastic β–cells through PAC1-R. (A) Cells were perfused with PACAP38. The level of $[Ca^{2+}]_i$ were plotted by the ratio of F340/F380. (B) The mean value of $F-F_0$ in response to PACAP38 as indicated. (F: the average value during 30 s (15 s before and after the peak of F340/F380) for different treatment; F_0: the average value during 30 s (15 s before and after the nadir of F340/F380) for 2.8 mM glucose concentration). (C) The level of $[Ca^{2+}]_i$ were plotted by the ratio of F340/F380 under different conditions. (D) The mean value of $F-F_0$ in response to PACAP38 in the presence or absence of PACAP (6–38) (1 μM) as indicated. KCl (60 mM) was used as positive control. ($n = 11$). $^{***}P < .001$.

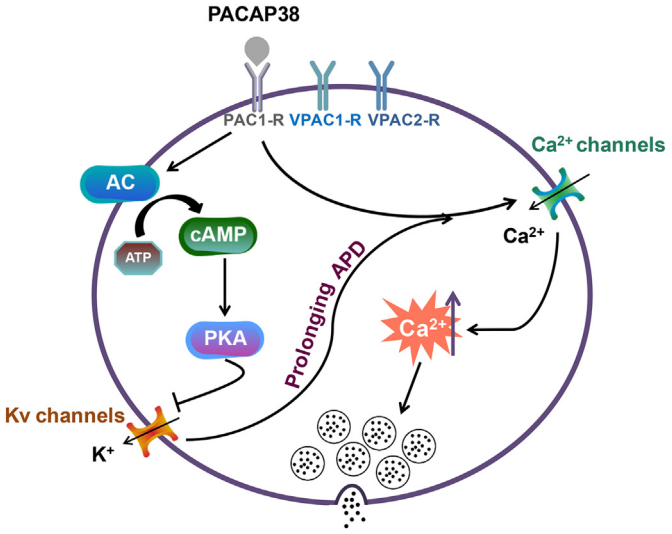

Fig. 9. The cellular and molecular mechanisms whereby PACAP38 amplified glucose-induced insulin secretion in rat pancreatic β-cells. At high glucose concentrations, PACAP38 binded to PAC_1 receptor on β-cell membrane, stimulating adenylate cyclase (AC) and its downstream effector protein kinase A (PKA), inhibiting Kv channels, prolonging action potential duration (APD), enhancing voltage-gated Ca^{2+} currents, increasing intracellular Ca^{2+} concentration and finally leading to insulin secretion. Meanwhile, PACAP38 binds to PAC_1 receptor, activating voltage-gated Ca^{2+} channels, elevating Ca^{2+} concentration, which also stimulates insulin release.

underlying mechanism in rat pancreatic β-cells.

The wide physiological actions of PACAP are produced through the activation of PAC1-R, VPAC1-R, and VPAC2-R [28]. However, it was not clear which receptor contributes to PACAP-modulated insulin secretion in primary rat pancreatic β-cells. In the human pancreas, only the RNA transcripts of VPAC2-R were expressed [29]. While previous studies have shown that PAC1-R, VPAC1-R and VPAC2-R were expressed in rat pancreatic islets and insulin-secreting cell lines including MIN6, HIT-T15 and RINm5F [6,30,31]. Additionally, PAC1-R had a higher affinity for PACAP than VPAC1-R and VPAC2-R in rat pancreatic islets [30]. Here, we show that PACAP38 magnified glucose-potentiated insulin secretion through PAC1-R primarily, but not by VPAC1-R or VPAC2-R, suggesting that PAC1-R plays a vital role in PACAP-stimulated insulin secretion. PAC1-R as a G protein-coupled receptor preferentially stimulates AC and augments intracellular cAMP levels [28]. The increase in cAMP, in turn, activates PKA [32,33]. Our results clearly suggested that PACAP38-stimulated insulin secretion was mediated by AC and its downstream effector PKA.

Glucose-induced insulin secretion is mediated by a series of electrogenic events in β-cells. The underlying mechanism is that ATP-sensitive K^+ (K_{ATP}) channels are closed by the rise in circulating glucose concentration. This phenomenon stimulates membrane depolarization, resulting in voltage-gated Ca^{2+} channels opening, which increases intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and finally stimulates insulin secretion. Under membrane depolarization, voltage-dependent K^+ (K_V) channels open to promote action potential repolarization, limiting free Ca^{2+} entry into β-cells through Ca^{2+} channels and decreasing insulin secretion [8]. Blockade of Kv channels prolongs action potential duration, resulting in glucose-dependent insulin secretion [17,34].

However, it is still mostly unknown the underlying electrophysiological mechanism of PACAP-regulated insulin secretion.

Furthermore, the relationship between PACAP receptors and ion channels in primary rat pancreatic β-cells also remains unclear. The present study strongly indicated that outward K^+ currents were suppressed by PACAP38, and this action was attenuated in pancreatic β-cells by PAC1-R, AC, and PKA inhibitors. Moreover, our study showed that PACAP prolonged action potential duration via PAC1-R, and that the underlying mechanism could be attributable to inhibition of Kv channels. Taken together, our results suggest that inhibition of Kv channels is associated with PACAP38-regulated glucose-augmented insulin secretion by acting on the PAC1-R and AC/PKA signaling pathways. In addition, Kv2.1 protein is highly expressed in islets and insulin-secreting cell lines [35,36]. As a major subtype of Kv channels, Kv2.1 has been reported to play an important role in the regulation of insulin secretion [35]. Therefore, Kv2.1 channels might be involved in PACAP-stimulated insulin secretion.

It is well known that inhibition of K_{ATP} channels stimulates insulin secretion in a glucose-independent manner [37]. While the blockage of Kv channels potentiates insulin secretion in a glucose-dependent manner [38]. Our results suggested that PACAP38 had no effect on insulin secretion at low glucose concentration. Therefore, we speculated that K_{ATP} channels are likely not involved in PACAP-stimulated insulin secretion.

In the present study, we found that TEA, a potent inhibitor of Kv channels, potentiated insulin secretion at the 8.3 mM glucose concentration. Interestingly, PACAP38 still elicited an additive enhancement of insulin secretion in the presence of TEA. These findings imply that PACAP38-augmented insulin secretion is not exclusively mediated by Kv channels in rat pancreatic β-cells. We found that PACAP38 increased currents of voltage-gated Ca^{2+} channels, which suggest that voltage-gated Ca^{2+} channels also participate in PACAP-stimulated insulin secretion. PACAP exists in two biologically active forms: PACAP38 and PACAP27. Similarly, it has been reported that PACAP27 activates voltage-gated Ca^{2+} channels from INS-1 cells [39], which is consistent with our data. Furthermore, the present results indicated that PACAP38 activated Ca^{2+} channels via PAC1-R. It is well known that voltage-gated calcium channels include L-type Ca^{2+} channels and T-type Ca^{2+} channels in rat pancreatic β-cells [40]. T-type Ca^{2+} channels are low-voltage-activated and L-type Ca^{2+} channels are high-voltage-activated. Moreover, L-type Ca^{2+} channels played a dominant role in stimulating insulin secretion by regulating Ca^{2+} influx [41,42]. Thus, PACAP-stimulated insulin secretion might be related to L-type Ca^{2+} channels.

In HIT-T15 cells, the insulinotropic action of PACAP was linked to additional ion channels, including Na^+ channels [43]. However, in rat pancreatic β-cells, up to 89% of Na^+ channels were silent at resting potential (−70 mV). Further, inactivation of Na^+ channels did not influence depolarization-induced insulin secretion at −70 mV [44]. Therefore, we speculate that Na^+ channels were not involved in the action of PACAP-induced insulin secretion in rat β-cells.

In summary, we have identified that the effect of PACAP-amplified glucose-induced insulin secretion was modulated by acting upon PAC1-R, activating AC and its downstream effector PKA, inhibiting Kv channels, prolonging action potential duration, and increasing Ca^{2+} currents and $[Ca^{2+}]_i$ concentration in rat pancreatic β-cells (Fig. 9). Our data suggest that PAC1-R plays a more significant role in PACAP-stimulated insulin secretion than VPAC1-R and VPAC2-R, which provides a useful clue to further investigate PAC1-R agonists for type 2 Diabetes treatment.

Declaration of interest

None.

Author contribution statement

Y Z, Y L and M L conceived and designed the study; M L, X Y, T B, Z L, T L and L C carried out the experiments; M L, X Y and T B contributed to analyze the data and interpret the results of the experiments; Y Z, Y L and M L wrote the manuscript; Y Z, Y L, M L and Y W revised the manuscript. All authors read and approved the final version.

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