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Action potentials and insulin secretion: new insights into the role of Kv channels

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Abstract

Coordinated electrical activity allows pancreatic β-cells to respond to secretagogues with calcium entry followed by insulin secretion. Metabolism of glucose affects multiple membrane proteins including ion channels, transporters and pumps that collaborate in a cascade of electrical activity resulting in insulin release. Glucose induces β-cell depolarization resulting in the firing of action potentials (APs), which are the primary electrical signal of the β-cell. They are shaped by orchestrated activation of ion channels. Here we give an overview of the voltage-gated potassium (Kv) channels of the β-cell, which are responsible in part for the falling phase of the AP, and how their regulation affects insulin secretion. β cells contain several Kv channels allowing dynamic integration of multiple signals on repolarization of glucose-stimulated APs. Recent studies on Kv channel regulation by cAMP and arachidonic acid and on the Kv2.1 null mouse have greatly increased our understanding of β-cell excitation–secretion coupling.

Keywords

delayed rectifier; electrical activity; hannatoxin; islet; Kv2.1; potassium channel; stromatoxin

Introduction

Glucose-induced electrical activity of the pancreatic β-cell is organized into slow depolarizing waves with a plateau from which action potentials (APs) rapidly fire. They are separated by quiescent periods at potentials below the AP threshold (figure 1). The β-cell AP is generated primarily through cation flux through ion channels modulated by glucose metabolism [1–4]. The produced ATP binds to the inward rectifier potassium channel complex (K_{ATP}) reducing its conductance of potassium as a reflection of the ATP/ADP ratio, thus causing an increase in the β-cell membrane potential [5,6]. The resulting depolarization activates voltage-gated channels that lead to the rising phase of the AP, including the L-type calcium channel and sodium channel(s). Continued depolarization also activates voltage-gated potassium channels (Kv), in particular Kv2.1, regulating membrane repolarization, with effects on AP width and frequency. Calcium entry additionally regulates calcium-activated potassium channels (Kca) that can also affect AP repolarization and the duration of

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the slow wave. The timing of AP firing is also regulated by the influence and extent of repolarizing vs. depolarizing ion flux around the activation threshold for voltage-gated channels. Although simple in shape, the AP is generated by a complex set of coordinated ion movements through several ion channels.

Initiation of the AP: K_{ATP}, Ca^{2+} and Na^{+} Channels

The resting membrane potential of the pancreatic β-cell is set by the activity of K_{ATP} [5,6]. K_{ATP} is an inward rectifier K^+ channel (discussed in detail elsewhere in this volume) made of a hetero-octomer of the sulphonylurea receptor 1 (SUR1, *ABCC8*) and Kir6.2 (*KCNJ11*) membrane proteins [5,6]. This channel sets the membrane potential of the β-cell near −70 mV, determined by the equilibrium potential of K^+ (E_K). This is because of the activity of K_{ATP} allowing inward K^+ flow when the cell is hyperpolarized and outward flow when the cell is slightly positive of E_K. K_{ATP} is also regulated by polyamines and Mg^{2+} binding to the channel [7]. The K_{ATP} channel is sensitive to ATP and when the ratio of ATP/ADP increases, as following augmented glucose metabolism, flux through the channel is inhibited [5,6]. ATP inhibition of K_{ATP} allows the β-cell to reach the AP threshold by reducing the outward K^+ flow and resulting in an intracellular accumulation of cations. Mutations in the genes encoding SUR1 (*ABCC8*) and Kir6.2 (*KCNJ11*) are rare but important causes of both persistent hyperinsulinaemia of infancy and neonatal diabetes, and polymorphisms in *KCNJ11* have been associated with type 2 diabetes and gestational diabetes. While K_{ATP} channels are expressed in the brain, muscle and elsewhere, it seems that only in the β-cell do these channels function in just this way, with the possible exception of glucose-sensing neurons. More severe mutations do indeed have muscle and neurological features and this has been termed the developmental delay, epilepsy and neonatal diabetes (DEND) syndrome [8].

As K_{ATP} activity is reduced following an increase in the ATP/ADP ratio, the β-cell is slowly depolarized and reaches an activation threshold for voltage-dependent calcium channels (VDCC) [9]. Once activated, the VDCC of the β-cell, primarily the L-type calcium channels Cav1.2 and/or Cav1.3 (depending on species), allows inward Ca^{2+} flux down its electrochemical activity gradient [9–17]. Pharmacological inhibition of VDCC eliminates insulin secretion, so clearly they play an essential role in islet electrical activity and insulin secretion [9]. When Ca^{2+} entry is blocked with nifedipine or cobalt, the AP fails to fire even while the threshold for firing is met by glucose-induced K_{ATP} inhibition [18,19]. Following activation with glucose, Ca^{2+} influx shows an oscillatory pattern that is significantly slower than the duration of an individual AP with a time course that has been linked to the oscillatory pattern of islet insulin secretion [13–16]. Intracellular calcium $[Ca^{2+}]_i$ changes within the β-cell do not alone account for the APs, which in some species is also clearly connected to Na^+ influx. VDCC activity regulates Ca^{2+}-activated K^+ channels, which can influence AP repolarization and firing pattern [17].

The amount of Ca^{2+} entry also affects the duration of glucose-induced slow waves. When external Ca^{2+} is reduced to 1 mM, the islet slow waves are extended with more APs and reduced repolarization during quiescent periods [18]. However, when the extracellular Ca^{2+} is elevated to supra-physiological levels (5–10 mM), islets have shorter slow waves with

fewer APs per wave and longer quiescent periods with greater repolarization between each slow wave [18,20]. These changes directly correlate with the amount of Ca^{2+} entry into the β-cell during glucose-induced depolarization, and thus the Ca^{2+} levels of the β-cell can determine many of the responses to glucose downstream of electrical activity [21–24].

Extracellular Na^+ is also required for normal insulin secretion from both human and rodent islets [25–30]. Rodent β-cell APs fire in the absence of extracellular Ca^{2+} during glucose-induced depolarization; however, when Na^+ is also removed with Ca^{2+} the APs fail to fire, implicating an important role for Na^+ influx during the AP [30,31]. Human and canine β-cells on the other hand require external Na^+ regardless of extracellular Ca^{2+} for a normal pattern of glucose-induced APs [29]. Two voltage-gated Na^+ currents have been identified in rodent β-cells based on their differences in biophysical activation and inactivation [10,32]. Both are inhibited with a toxin specific for voltage-gated Na^+ channels, tetrodotoxin (TTX, from *Fugu rubripes*). Interestingly, islet electrical activity is most affected by TTX under low glucose conditions (5–6 mM glucose), whereas TTX has minimal effect on islet electrical activity above 10 mM [32]. As normal blood glucose rarely climbs above 10 mM, activation of voltage-gated Na^+ channels during glucose-induced depolarization will influence the upstroke of the resulting AP. Indeed, TTX treatment of β-cells undergoing glucose-induced (at 5 mM) APs causes a reduction in AP amplitude and broadens its width [32]. Although TTX-sensitive voltage-gated Na^+ currents have been identified in the β-cell, other Na^+ channels such as NaV1.7 and NaV1.3 may also combine to modulate glucose-induced sodium influx and resulting cellular excitability [10,33]. It remains uncertain which β-cell Na^+ channels are functionally important, but it does seem that their contribution is species-specific and has greater importance in human than rodent β-cells.

Potassium Channels that Regulate Repolarization of the AP

Calcium-activated Potassium Channels

The three main groups of calcium-activated K^+ (Kca) channels of the β-cell are large conductance (BK) channels, small and intermediate conductance (SK and IK) channels and a slowly activated K^+ conductance termed K_{slow} [34–36]. The kinetics of the β-cell Kca currents allow for roles in both AP repolarization and slow wave duration. Although the molecular identity of K_{slow} is undefined, both SK and BK channels are expressed in β-cells. BK channel transcripts with multiple splice variants are highly expressed in the islet [37]. SK channels are also expressed in the rodent β-cell [36]. SK1, -2, -3 and -4/IK1 cDNAs have been identified in rodent islets and insulinoma cells by RT-PCR [36]. SK2 and SK3 protein expression is also found in mouse islets [36]. The activity of both SK and BK channels in various tissues modulate AP firing and shape and thus these channels may also affect glucose-induced APs of the β-cell.

The contribution of Kca channels to individual glucose-induced AP remains incompletely understood. Inhibitors of SK4/IK1 and BK channels including charybdotoxin, iberiotoxin and tetraethylammonium (TEA) have been used to decipher the roles of these two channels [38,39]. Glucose-induced APs are not affected by charybdotoxin or low concentrations of TEA [38–40]. However, when iberiotoxin is used to block BK channels when Kv2.1 is also blocked, there is a significant increase in AP amplitude [41]. The AP duration and the

quiescent period between APs are also increased during combined blockade of Kv2.1 and BK channels during glucose stimulation [41]. This indicates that there may be multiple repolarizing K^+ channels activated following the AP upstroke and depolarization including BK.

Small conductance Kca channels of the pancreatic β-cell may also influence islet insulin secretion. Apamin, a specific inhibitor of cloned SK1–3 channels, has no significant effect on glucose-induced electrical activity of rat islets [42]. However, glucose-induced Ca^{2+} fluctuations in mouse islets are increased in amplitude and frequency with apamin [36]. This effect was modulated in part through the SK3 channel. This was shown by knockdown of this subunit in a transgenic mouse model in which SK3 regulation with doxycycline was possible by introduction of the tet response element. Lowering SK3 expression reduced the Ca^{2+} changes induced by apamin [36]. Thus, SK3 channels play a detectable but modest role on islet Ca^{2+} fluctuations as long as other Kv channels are functional.

Calcium also regulates the duration of the slow wave of depolarization, from which APs fire, in part through its regulation of the Kca channel K_{slow}. Although the molecular identity of this channel remains elusive, K^+ efflux from the cell has been extensively studied during the termination of the slow wave. A transient increase in K^+ permeability has been shown to precede the termination of the slow wave [43,44]. The current responsible for the termination of the slow wave has been studied in intact islets [35]. The current induced, termed K_{slow}, is K^+ permeable and inactivates over a time course similar to the quiescent period between glucose-activated slow waves [35]. The amount of K_{slow} current closely follows the Ca^{2+} levels and its inactivation follows a time course similar to the termination of the Ca^{2+} wave at the end of the slow wave [35]. Inhibition of Ca^{2+} influx eliminates the K_{slow} current and removal of extracellular Ca^{2+} induces a continued slow wave of depolarization [17,30,31,35,45].

The molecular identity of the K_{slow} current remains obscure. The current is inhibited up to 70% by high concentrations of the non-specific K^+ channel inhibitor TEA [35]. K_{slow} from primary β-cells is insensitive to charybdotoxin and apamin; thus, the current is not composed of BK or SK subunits except that it is inhibited by UCL 1684, a non-peptide SK channel blocker. This indicates that the current could still be in the SK channel family [35,46]. UCL 1684 significantly inhibits SK channels as well as K_{slow} currents in β-cells, suggesting that the molecular identity of the K_{slow} current may be a heteromultimer.

Voltage-gated Potassium Channels

One of the primary functions of voltage-gated K^+ channels is repolarization of the AP. They are encoded by the largest family of ion channel genes. Of the eleven families of Kv-related α-subunit genes so far reported, at least three families encode channel isoforms with currents superficially similar to those found in β-cells (table 1) [47,48]. These were originally named according to homology to three *Drosophila* genes: Shaker (Kv1.*x*), Shab (Kv2.*x*) and Shaw (Kv3.*x*). Members of Kv1, -2 and -3 give rise to delayed rectifier-type currents (figure 2). A fourth family, Shal (Kv4.*x*) comprises only rapidly activating and inactivating channels (similar to Kv1.4) and therefore Kv4.1–3 are thought unlikely to be expressed in β-cells (figure 2) [49]. K^+ channel families five to eleven include some (Kv6–9)

that do not express functional channels in mammalian cells on their own. Some subunits also form heteromultimers, generating additional current types with combinatorial properties [49,50]. The ability of isoforms to heteromultimerize is usually restricted and family-specific. For example, Kv1.*x* can combine with other Kv1.*x* but not with Kv2.*x* [49]. Some examples of 'promiscuous coupling' of Kv isoforms have been reported, such as between Kv5.*x*–Kv9.*x* (inactive as homomultimers) and Kv2.1 [51,52]. Kv5.*x*, Kv6.*x*, Kv8.*x* and Kv9.*x* may actually be subunits that are primarily involved in modulating Kv2.*x* expression or function. The heteromultimers produce channels that attenuate the amplitude of Kv2.*x* currents. Each modulatory subunit has its own specific properties of regulation of the functional Kv2 subunits, and they can lead to extensive inhibitions, to large changes in kinetics and/or to large shifts in the voltage dependencies of the inactivation process.

The β-cell-delayed rectifier current activates slowly and inactivates slowly (figure 2). Rapidly inactivating Kv currents are not usually seen in primary β-cells (figure 2, upper panel). The Kv current is sensitive to TEA in the 5–7 mM range. Several investigators have made an effort to define the K^+ channel gene or genes encoding this current. A summary of all the K^+ channel genes known and their expression in β-cells is shown in table 1. Seven different isoforms of the Kv1.*x* gene family were initially identified in insulinoma cell lines and whole islets using the RT-PCR method [53]. These channels were identified by their sequence similarity to the rat brain K^+ channels [54,55]. Kv1.5 (also termed hPCN1, *KCNA5*) was found to be highly expressed in human insulinomas [56]. Insulinoma cells overexpressing this channel had an ablated glucose-induced Ca^{2+} response, and transgenic mice overexpressing Kv1.5 specifically in β-cells exhibited reduced glucose tolerance [56]. Inhibiting Kv1 channels with a dominant-negative construct has little to no effect on β-cell repolarization [56]. Kv3.2 channel RNA and protein has also been identified in β-cells; however, their TEA-sensitive currents do not reflect the low sensitivity of the β-cell Kv currents to TEA. Kv2.1 channels are the primary β-cell delayed rectifier current [47,57,58], indicated by the voltage-gated kinetics of the current, as well as by RT-PCR, Western blot and immunohistochemical confirmation of its expression specifically in the endocrine β-cell [58–60]. Accordingly, most of the voltage-gated K^+ current is eliminated in rat β-cells expressing a Kv2 dominant-negative construct [59].

Spider toxins that inhibit Kv2 channels also inhibit a majority of the Kv current of rodent and human β-cells [61–63]; these include the spider peptides stromatoxin, hannatoxin, guangxitoxin and SGTx1. These toxins are termed cystine knot toxins because of their disulphide bond-constrained structure; they bind to the S3–S4 extracellular loop of Kv2 channels, reducing channel activity by restricting the movement of the transmembrane domain S4 [61–63]. The toxins also inhibit Kv2.2 and Kv4 channels. As these channels are expressed in non-β-cells of the islet, their inhibition could also affect islet electrical activity and/or hormone secretion together with the inhibition of Kv2.1.

Inhibition of Kv channels with TEA or stromatoxin during trains of glucose-induced APs results in a significant increase in amplitude and duration of the AP with a slowing of the firing frequency (figures 3 and 4) [63]. Islets from a mouse model with an ablated Kv2.1 gene also show increased glucose-induced AP duration and reduced firing frequency compared with controls (unpublished observation). This change in electrical activity is

reflected in the Ca^{2+} fluctuations of the islet, which usually show increased rates in response to inhibition of Kv2.1 during glucose stimulation [62,63]. Similarly, glucose-stimulated insulin secretion is also increased when Kv2.1 channels are inhibited [41,63]. Kv2.1 would thus be a therapeutic target if it could be specifically blocked in the islet [41,47,63], allowing increased glucose-induced insulin secretion in patients with type 2 diabetes. However, the Kv2.1 channel is expressed in many other tissues besides the pancreatic β-cell, including the brain, and thus blocking Kv2.1 specifically in the β-cell is a difficult task.

Interestingly, the incretin glucagon-like peptide 1 and related agonists have been shown to affect Kv2.1 activity. This peptide activates a G-protein-coupled receptor expressed on the β-cell, and the ensuing kinase cascade has been shown to reduce Kv2.1 currents [64]. Neuronal Kv2.1 channel activity in the brain is also dynamically modulated by its phosphorylation status in a Ca^{2+}-dependent manner [65]. Thus, modulation of β-cell function through second messengers may also provide β-cell-specific inhibition of Kv2.1, allowing increased glucose-induced insulin secretion.

Kv2.1 activity is also modulated by the polyunsaturated fatty acids arachidonate and linoleic acid [66–68]. Non-esterified arachidonate and linoleic acid reduce β-cell Kv2.1-like currents and accelerate their inactivation [67,68]. Arachidonate also causes increased Ca^{2+} fluctuations from glucose-treated islets [68]. Hydrolysis of esterified linoleic acid and arachidonic acid amplifies glucose-induced insulin secretion [69]. Therefore, dynamic modulation of Kv2.1 channel function by fatty acids may play an important role in regulating the electrical responses of β-cells to glucose.

Conclusions

We have reviewed the important Ca^{2+}, Na^{+} and K^{+} channels of the β-cell with a focus on new insights on the role of Kv channels. The growing intense focus on the role of the β-cell in all types of diabetes has increased the importance of understanding how electrical activity induced by glucose and other agonists is established and how it is altered in various pathological states. Pharmacologic intervention in hyperinsulinaemic, diabetic and hypoglycaemic states would also be aided by a better set of tools to specifically augment or inhibit β-cell electrical activity.

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Fig. 1.

Electrical activity of a mouse islet treated with 14 mM glucose, exhibiting typical depolarizing waves topped with rapidly firing action potentials.

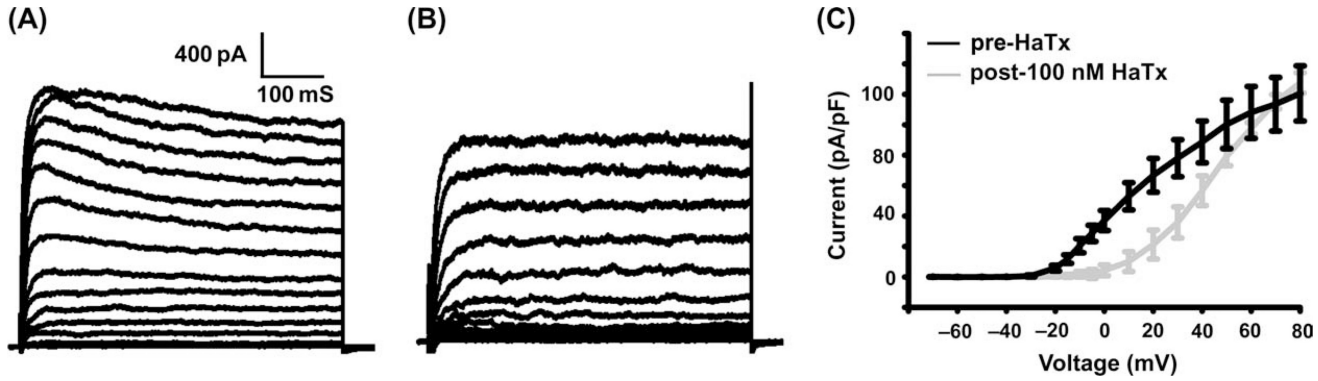

Fig. 2.

Hannatoxin significantly reduces β-cell voltage-gated potassium (Kv)-like currents. (A) Control β-cell Kv-like currents recorded in voltage clamp with voltage steps from −80 to +80 in 10 mV increments. (B) Control β-cell Kv currents 10 min following addition of 100 nM hannatoxin (HaTx). (C) Current vs. voltage plots for β-cells recorded as in A (black bar) and B (grey bar) ± s.e.m.'s (n > 7 for each condition).

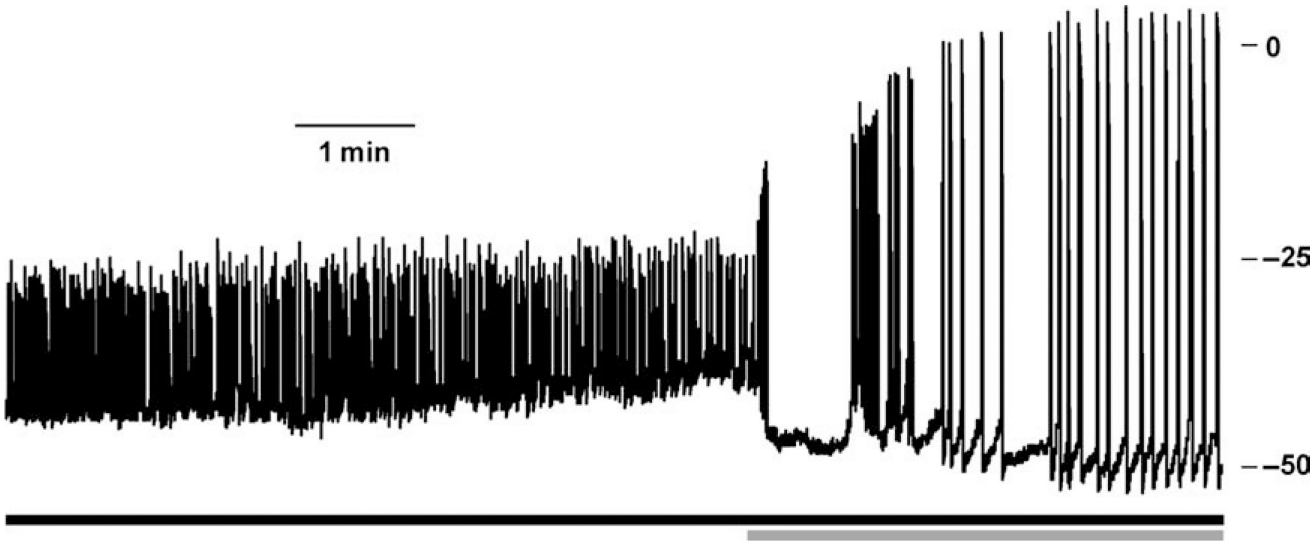

Fig. 3.

Islets respond to tetraethylammonium (TEA) with increased glucose-induced action potential (AP) amplitude and duration. C57 mouse islet glucose-induced (14 mM, black bar) APs treated with 15 mM TEA (grey bar).

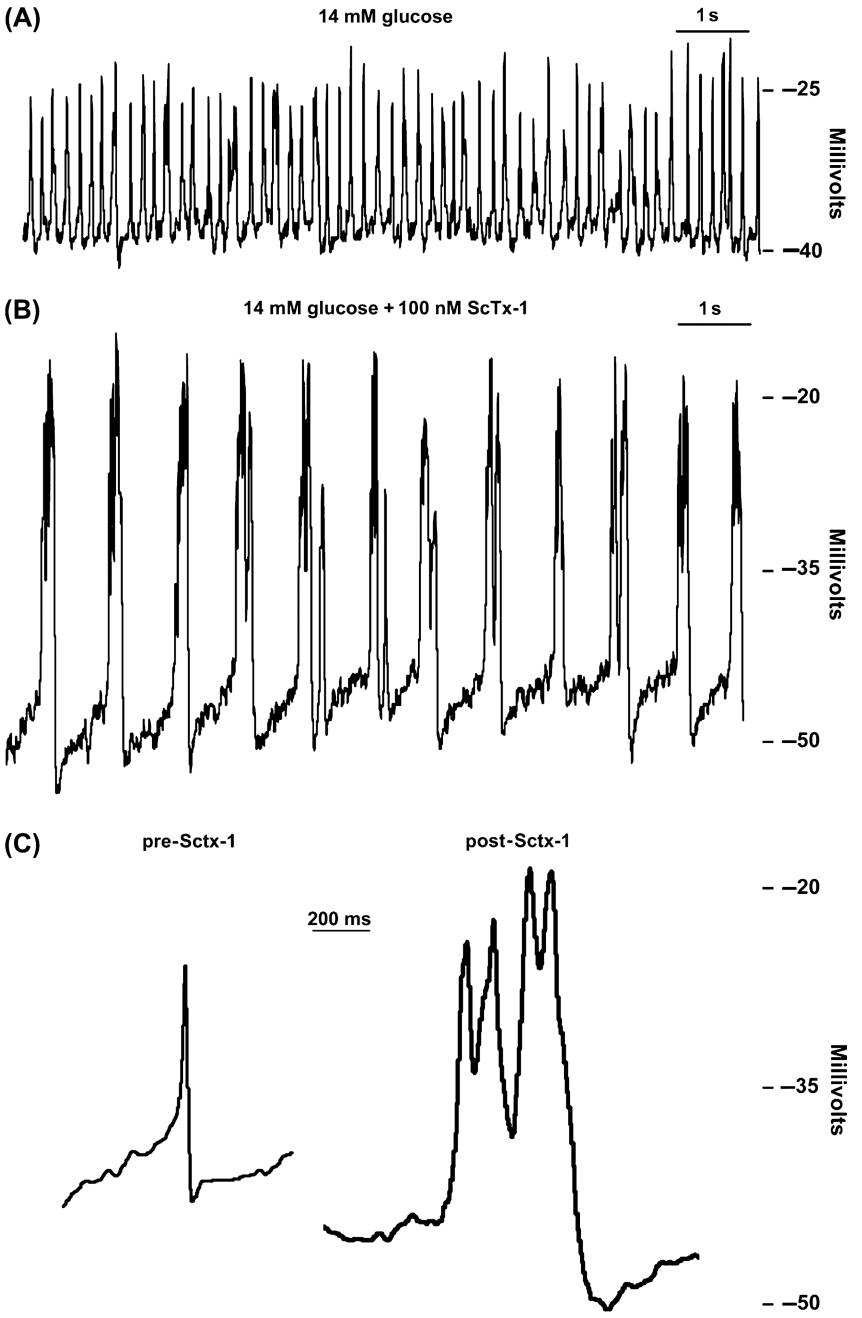

Fig. 4.

Stromatoxin (ScTx-1) significantly affects β-cell action potentials (APs). (A) Islet APs recorded in whole cell current clamp mode from an intact β-cell treated with 14 mM glucose. (B) APs from the same islet in panel (A) 2 min post-treatment with 100 nM ScTx-1. (C) Single APs recorded in 14 mM glucose alone (pre-ScTx-1) or with 14 mM glucose and 100 nM ScTx-1 (post-ScTx-1).

Table 1

Expression of voltage-gated potassium (Kv) channels in pancreatic islets and expression of modulatory/silent Kv subunits in islets

Gene	Alias	Expression	Detection	Reference
KCNA1	Kv1.1	None	PCR, IB	[59]
KCNA2	Kv1.2	None	PCR, IB	[59]
KCNA3	Kv1.3	None	PCR, IB	[59]
KCNA4	Kv1.4	β cell	PCR, IB	[59]
KCNA5	Kv1.5	β cell	PCR	[56,70]
KCNA6	Kv1.6	β cell	PCR, IB	[59]
KCNA7	Kv1.7	Islet	PCR	[48]
KCNA10	Kv1.10	ND		
KCNB1	Kv2.1	β cell	PCR, IB, IHC	[48,59,62]
KCNB2	Kv2.2	Delta cell	PCR, IHC	[48]
KCNC1	Kv3.1	Alpha cell	PCR, ISH	[48]
KCNC2	Kv3.2	β cell	PCR, ISH, IHC	[48,58]
KCNC3	Kv3.3	None	PCR	[48]
KCNC4	Kv3.4	Delta cell	PCR, IHC	[48,71]
KCND1	Kv4.1	Pancreas	PCR	[48]
KCND2	Kv4.2	Islet	PCR, IB	[64]
KCND3	Kv4.3	Alpha cell	PCR, IHC	[71]
KCNH1	Kv10.1	Islet	PCR	[48]
KCNH2	Kv11.1	Islet	PCR	[48]
KCNF1	Kv5.1	None	PCR	[48]
KCNG1	Kv6.1	Alpha cell	PCR, ISH	[48]
KCNG2	Kv6.2	β cell	PCR, ISH	[48]
KCNG3	Kv6.3	None	PCR	[48]
KCNG4	Kv6.4	ND		
KCNS1	Kv9.1	ND		
KCNS2	Kv9.2	Islet	PCR, ISH	[48]
KCNS3	Kv9.3	β cell	PCR, ISH	[48]

IB, immunoblot; IHC, immunohistochemistry; ISH, *in situ* hybridization; ND, not determined.