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Functional expression of TREK-2 in insulin-secreting MIN6 cells

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Abstract

Insulin secretion from pancreatic β cells is partly regulated by cell membrane potential. Background K^+ channels that stabilize the resting membrane potential would suppress excitability and insulin secretion. Recent studies show that members of the two-pore domain K^+ (K_{2P}) channel family behave as background K^+ channels in many excitable cells. Therefore, the expression of K_{2P} channels was studied in insulin-secreting MIN6 cells. Reverse transcriptase PCR showed that, among nine K_{2P} channels tested, *TASK-1*, *TASK-2*, *TASK-3*, *TREK-2*, and *TRESK-2* were expressed in MIN6 cells. Cell-attached recordings on MIN6 cells revealed five types of K^+ channels that were open at rest. Two were ATP-sensitive and Ca^{2+}-activated K^+ channels, as judged by their sensitivity to ATP and Ca^{2+}, respectively, and single-channel conductance. Among five K_{2P} channels, only TREK-2 could be clearly identified in MIN6 cells. The molecular identity of two other K^+ channels is not yet known. TREK-2 in MIN6 cells was activated by arachidonic acid, membrane stretch, and low pH solution (pH 5.8). Arachidonic acid increased Ba^{2+}-sensitive whole-cell current in MIN6 cell. These results suggest that TREK-2 contributes to the background K^+ conductance in MIN6 cells, and may regulate depolarization-induced secretion of insulin.

Keywords: Pancreatic β cell; Two-pore K^+ channel; Insulin; Arachidonic acid; pH; MIN6

Pancreatic β cells express various ion channels that help to regulate excitability and insulin secretion. In these cells, the normally active ATP-sensitive K^+ (K_{ATP}) channel is thought to keep the resting membrane potential below the firing threshold when plasma glucose concentration is low. Inhibition of the ATP-sensitive K^+ channels by glucose-mediated increase in ATP/ADP ratio depolarizes the cell, producing bursts of action potential spikes and influx of Ca^{2+} via voltage-dependent Ca^{2+} channels [1–5]. Elevation of intracellular [Ca^{2+}] sets in motion a cascade of events that ultimately lead to secretion of insulin, and help to maintain the proper balance between plasma glucose concentration and glucose uptake into tissues. Pancreatic β cells also express voltage-gated (K_v) and several types of Ca^{2+}-activated (K_{Ca}) K^+ channels that regulate excitability [6–9]. K_v channels mediate action potential repolarization. K_{Ca} channels are believed to modulate glucose-induced Ca^{2+} response and membrane potential, regulate pacemaker activity, and Ca^{2+} oscillation [2,9].

Recently, molecular cloning has identified a family of K^+ channels known as two-pore domain K^+ (K_{2P}) channels [10–13]. K_{2P} channels are structurally distinct from those of K_{ATP} and K_v channels [14]. K_{ATP} channels belong to the inward rectifier K^+ channel family (K_{ir}) whose individual subunit has one pore (P)-forming domain and two transmembrane (TM) segments. A K_v channel subunit has one P domain and six TM segments. In contrast, each K_{2P} channel subunit has two P domains and four TM segments. Because four P domains form a functional K^+ channel, K_{2P} channels are most likely homodimers and heterodimers [15,16]. Most

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K_{2P} channels exhibit characteristics of background K^+ channels, as they are open at a physiological range of membrane potentials with little or no voltage and time dependence. Functional K_{2P} channels expressed in any cell would therefore help to suppress depolarization and reduce excitability.

Studies so far have shown that mRNA transcripts of several K_{2P} channels (*TWIK-1*, *TASK-1*, *TASK-5*, *TALK-1*, *TALK-2*, *THIK-2*, *TASK-2*, and *TREK-2*) are expressed in human pancreas, as judged by Northern blot analysis and RT-PCR [17–21]. In rat pancreas, mRNA expression of TRESK-2, TRAAK, and TREK-2 has been reported [22–24]. These results suggest that the resting membrane potential of certain cells in the pancreas would be regulated by some of these K_{2P} channels. Whether any of the K_{2P} channels are expressed in insulin-secreting β cells is not known. If background K_{2P} channels are expressed in pancreatic β cells, they will tend to oppose glucose-mediated inhibition of K_{2P} channels and reduce insulin secretion. Therefore, the goal of this study was to determine whether and which K_{2P} channels are functionally expressed in pancreatic β cells. MIN6, a pancreatic β cell line, was used, as it has been shown to possess physiological characteristics of normal β cells including insulin synthesis, secretion and β cell development, and morphological characteristics [25,26].

Materials and methods

Cell culture. MIN6 cell line, a pancreatic β cell line derived from mouse, was obtained from Dr. Robert Tsushima (University of Toronto). The cells (passage 39) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 25 mM glucose, 20% fetal bovine serum, penicillin/streptomycin (100 U/ml), and 50 mM β-mercaptoethanol at 37 °C in a humidified incubator gassed with a 95% air–5% CO_2 mixture. The medium was changed every 3 days. The monolayers were trypsinized (0.1% trypsin, 0.02% EDTA) when 80–90% confluent. The trypsinized cells were plated on glass coverslips at a density of 1×10^5 cells^{-2} per 35 mm-dish ~16 h prior to experiment.

Reverse transcriptase-polymerase chain reaction analysis. First-strand cDNA was synthesized from total RNA isolated from MIN6 cells using oligo(dT) (Superscript preamplification system, Invitrogen, Rockville, MD) and used as template for polymerase chain reaction (PCR) amplification. We used nine K_{2P} channel-specific primers (see Table 1 for primer sequences). PCR amplification was performed with *Taq* polymerase (Takara, Japan). PCR conditions were initial denaturation at 94 °C for 4 min, then 30 cycles at 94 °C for 45 s, 55 °C for 1 min, 72 °C for 2 min, and a final extension step at 72 °C for 10 min. In some PCRs, the annealing temperature was 49 and 60 °C. The DNA fragments obtained by reverse transcriptase (RT)-PCR using MIN6 cells were subcloned into pCR2.1 TOPO vector by TA cloning. Subcloned DNA fragments were sequenced on both strands with the dideoxynucleotide chain termination method.

Electrophysiological studies. Electrophysiological recording was performed using a patch-clamp amplifier (Axopatch 200, Axon Instruments, Union City, CA). All recordings were performed at room temperature (24 °C). Single channel currents were digitized with a digital data recorder (VR10, Instrutech, Great Neck, NY) and stored on videotape. The recorded signal was filtered at 2 kHz using an 8-pole Bessel filter (−3 dB; Frequency Devices, Haverhill, MA) and transferred to a computer (Dell) using the Digidata 1200 interface (Axon Instruments) at a sampling rate of 20 kHz. Threshold detection of channel openings was set at 50%. Whole-cell currents were recorded after cancelling the capacitative transients. Whole-cell and single-channel currents were analyzed with the pCLAMP program (Version 8). For single-channel analysis, the filter dead time was 100 μs (0.3/cutoff frequency) such that events shorter than 50 μs in duration would be missed. Data were analyzed to obtain a duration histogram, amplitude histogram, and channel activity (NP_o, where N is the number of channels in the patch and P_o is the probability of a channel being open). NP_o was determined from several minutes of current recording. The single-channel current tracings shown in the figures were filtered at 2 kHz. In experiments using cell-attached and excised patches, pipette and bath solutions contained (mM): 150 KCl, 1 $MgCl_2$, 5 EGTA, and 10 Hepes (pH 7.3). In whole-cell recordings, bath solution contained (mM): 135 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 5 glucose, and 10 Hepes (pH 7.3). All other chemicals were purchased

Table 1
Primer sequences used for RT-PCR

Gene	GenBank No.	Primer sequences (5′–3′)	Expected size (bp)
TASK-1	NM010608	Sense: TGTTCTGCATGTTCTACGCG Antisense: TGGAGTACTGCAGCTTCTCG	676
TASK-3	AF192366	Sense: TGACTACTATAGGGTTCGGCG Antisense: AAGTAGGTGTTCCTCAGCACG	517
TREK-1	U73488	Sense: TGGAAACATCTCCCCACGAACTGAAGG Antisense: TCACAGACAGGTCCTCATACACGGAGT	670
TREK-2	AF196965	Sense: GGCTAATGTCACTGCTGAGTTCC Antisense: ACAAGACAACACATAGTCCAATTCC	625
TRAAK	AF302842	Sense: CACCACTGTAGGCTTTGGCGATTATG Antisense: ACTCTGCGTGTCTGAGGACTCGTCG	445
TALK-1	AF358909	Sense: GGGACGCTGGTCATTCTCATCTTCC Antisense: TTGGACTCCTCTTGCTGCTGTAGAGCC	321
TALK-2	AF358910	Sense: TCCACCATCACCACCATTGGC Antisense: CTAGCTGTCCTTGCCACAGCCTGCAGCGTG AGCA	667
TASK-2	AF319542	Sense: CCTCATCAAACAGATTGGGAAGAAGG Antisense: TCAGGTTGTCCTCCACAGAAGACTTAG	428
TRESK-2	AY325301	Sense: ATGGAGGCTGAGGAGCCACCTGAG Antisense: AAAGGAGAGCCTGGAACCGACTGT	544

from Sigma Chemical (St. Louis, MO). For statistics, Student's t test was used with $p < 0.05$ as the criterion for significance. Data are represented as means ± standard deviation.

Results

Expression of K_{2P} channels in MIN6 cells

Expression of K_{2P} channel mRNA in MIN6 cells was studied by RT-PCR. To compare the results with the expression in native tissue, RT-PCR was also done using first-strand cDNA prepared from rat pancreas. Expression of nine members of the K_{2P} channel family was studied using specific primers designed to amplify short DNA fragments of each K_{2P} channel. Among them, PCR products for *TASK-1*, *TASK-2*, *TASK-3*, *TREK-2*, and *TRESK-2* were obtained and confirmed by sequencing in both MIN6 and the pancreatic tissue (Fig. 1). PCR products of *TRAAK* and *TREK-1* were not detected in MIN6 and pancreas, whereas they were detected using cDNAs prepared from rat brain and heart

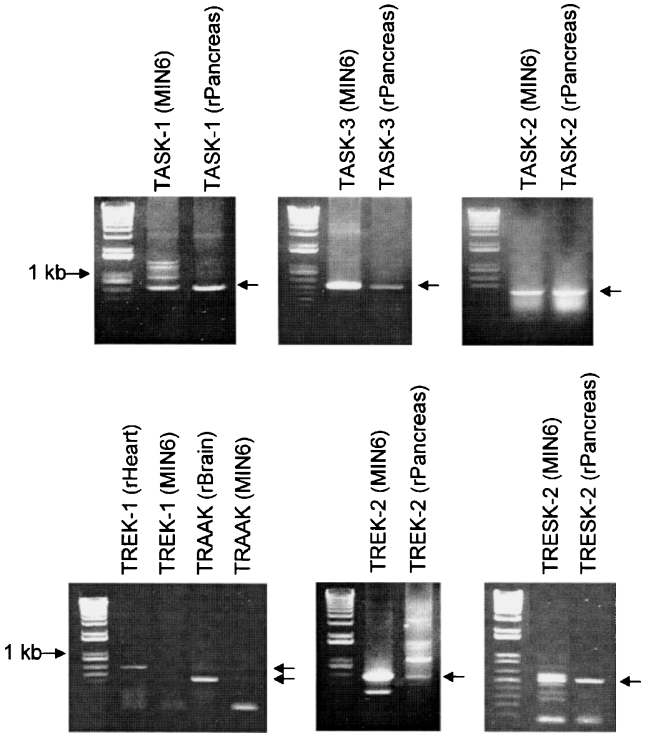

Fig. 1. Expression of K_{2P} channels in MIN6 cells. Total RNA extracted from MIN6 cells and other tissues was used to prepare first-strand cDNA. Primers specific for each K_{2P} channel were used for PCR. DNA fragments of expected sizes were extracted, inserted into pCR2.1 by TA cloning, and sequenced for confirmation. Expected band sizes indicated by arrow were 676 bp (TASK-1), 517 bp (TASK-3), 428 bp (TASK-2), 670 bp (TREK-1), 445 bp (TRAAK), 625 bp (TREK-2), and 544 bp (TRESK-2). The first lane shows the 1-kb DNA ladder.

that were known to express *TRAAK* and *TREK-1*, respectively [23,27,28]. *TALK-1* and *TALK-2* were not detected either in MIN6 or the whole rat pancreas (data not shown). Because *TASK-1*, *TASK-2*, *TASK-3*, *TREK-2*, and *TRESK-2* all form functional channels in expression systems, we tested whether these K_{2P} channels are indeed functionally expressed in MIN6 cells.

Background K^+ channels in MIN6 cells

A photomicrograph of MIN6 cells grown on plastic culture dish is shown in Fig. 2A. The cell shapes were irregular and polymorphic, as described previously [25,26]. To identify functional K_{2P} channels that contribute to the background K^+ conductance in MIN6 cells, cell-attached patches were formed and all channels open at −60 and +60 mV were recorded in bath and pipette solutions containing 150 mM KCl. From 182 patches studied, five different background K^+ channels were identified, as judged by their differences in single-channel conductance and opening kinetics. Ion selectivity, pharmacology, and channel kinetics were studied for each channel type and described below.

K_{ATP} channel was the first easily identifiable channel because of its sensitivity to glybenclamide and ATP (Fig. 2B). Opening of this K^+ channel was observed frequently in cell-attached patches (27% of patches). The single-channel conductance was 80 ± 4 pS at −60 mV and 44 ± 2 pS at +60 mV ($n = 5$), producing a weakly inward rectifying current–voltage relationship that is typical for the K_{ATP} channel [5,29–32]. K_{ATP} channel activity increased markedly upon patch excision, presumably due to washout of cytosolic ATP. In such inside-out patches, bath application of 100 μM glybenclamide or 2 mM ATP inhibited opening of these channels, confirming that they were K_{ATP} channels. In inside-out patches, reducing the $[K^+]$ in the bath solution from 150 to 30 mM KCl shifted the reversal potential from 0 to +37 ± 6 mV, as expected of a K^+-selective ion channel.

Ca^{2+}-activated K^+ (K_{Ca}) channel was recorded in only eight patches. Open probability of K_{Ca} channels was very low in cell-attached patches, presumably due to low cytosolic $[Ca^{2+}]$ at rest. The current–voltage relationship was nearly linear with a single-channel conductance of 217 ± 9 pS at −60 mV ($n = 8$), similar to the large conductance Ca^{2+}-activated K^+ channel recorded in other cell types [33,34]. As predicted, this K^+ channel was activated by elevation of cytosolic $[Ca^{2+}]$ to 10 μM. Applying negative pressure to the patch membrane or arachidonic acid also activated the K^+ channel, further identifying it as the large conductance K_{Ca} channel [35]. In inside-out patches, reducing the $[K^+]$ in the bath solution from 150 to 30 mM KCl shifted the reversal potential from zero to +37 ± 4 mV.

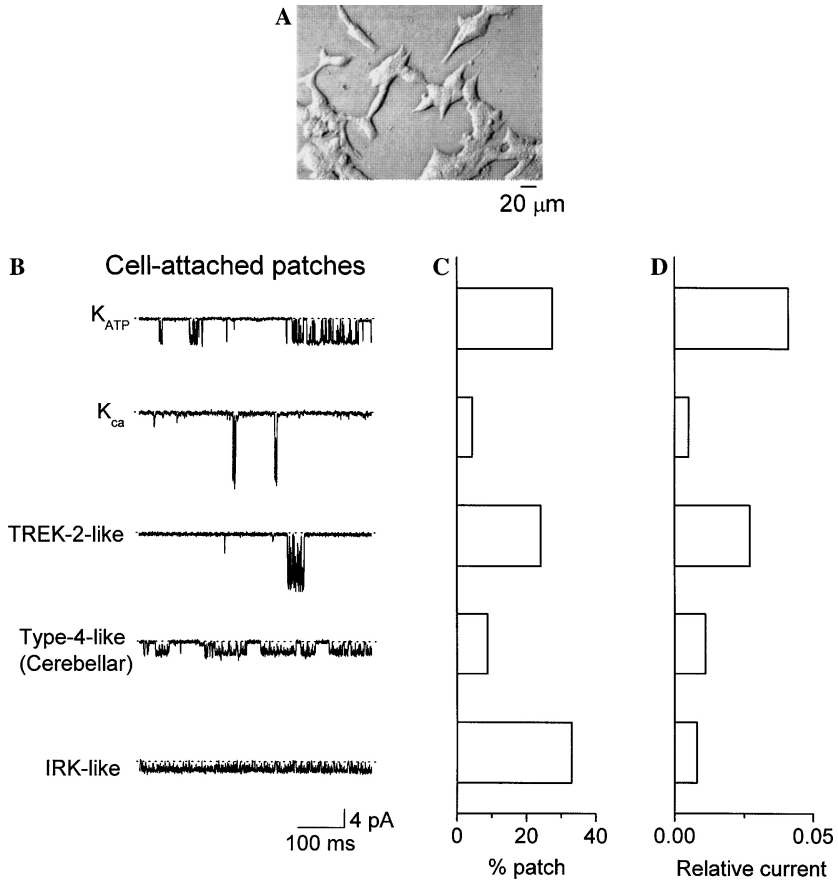

Fig. 2. Single-channel currents in cell-attached patches of MIN6 cells. (A) A photomicrograph shows MIN6 cells grown on a tissue culture dish. (B) Five types of channels with distinct opening kinetics are shown. The cell membrane potential was held at −60 mV. Pipette and bath solutions contained 150 mM KCl. Each channel type was determined to be K^+ selective by measurement of reversal potential shifts after changing bath [KCl] and by lack of outward current in Na^+-containing solution. The abbreviations used are: K_{ATP}, ATP-sensitive K^+ channel; K_{Ca}, Ca^{2+} activated K^+ channel; and IRK, inwardly rectifying K^+ channel. (C) The bar graph shows the percent of patches showing each type of K^+ channel. (D) Estimated relative outward current averaged from 182 patches is shown.

The third K^+ channel recorded from cell-attached patches had a single-channel conductance of 146 ± 6 pS at −60 mV and 56 ± 5 pS at +60 mV. The current–voltage relationship showed an inward rectification that was stronger than that of the K_{ATP} channel. Again, this ion channel was K^+-selective, because the reversal potential shifted by 37 ± 3 mV to the right when bath [KCl] was lowered from 150 to 30 mM in inside-out patches. This K^+ channel opened in bursts and showed large current fluctuations within each burst, typical of those observed with TREK-type K^+ channels. The identity of this K^+ channel is further examined in the next section.

The fourth ion channel recorded exhibited channel behavior very similar to that of a previously described K^+ channel (referred to as "Type 4 channel") in cerebellar granule neurons [36]. The K^+-selectivity of this channel was confirmed by the rightward shift of 34 ± 7 mV in reversal potential upon changing the bath [KCl] from 150 to 30 mM KCl in inside-out patches. No outward current was recorded when K^+ was replaced with Na^+, indicating that the channel was not a non-selective cation channel. Like the Type 4 K^+ channel in cerebellar granule neurons, this K^+ channel showed linear current–voltage relationship with a single-channel conductance of 37 ± 4 pS at −60 mV. This K^+ channel was observed infrequently.

The fifth ion channel recorded from cell-attached patches had the lowest single-channel conductance (28 ± 2 pS at −60 mV and 11 ± 3 pS at +60 mV) among the five channels but was most frequently observed (33% of patches). The K^+-selectivity of this channel was also confirmed by the rightward shift of 31 ± 7 mV in reversal potential upon changing the bath [KCl] from 150 to 30 mM KCl in inside-out patches. No outward current was observed when the bath KCl was replaced with NaCl in inside-out patches, indicating that the channel was not a non-selective cation channel. In patches that contained this K^+ channel, the opening was nearly always long-lasting (>100 ms) and also showed many closings within each long burst of opening. The molecular identity of this K^+ channel is not known, but is similar in behavior to that of the classical inward rectifier K^+

channel (IRK). Fig. 2C shows the percent of patches in which the five channels were observed, and Fig. 2D shows estimated relative current calculated by multiplying channel activity and current amplitude at +60 mV (outward current), and then dividing by the total number of patches studied. These estimates indicate that several K^+ channels contribute to the background K^+ conductance at rest.

Molecular identity of the TREK-like K^+ channel

Of the five K^+ channels recorded from MIN6 cells, one showed ion channel characteristics similar to those of TREK-2 and this was observed in 24% of patches. To further confirm that the TREK-like K^+ channel observed in MIN6 cells is indeed encoded by *TREK-2*, we compared the basic electrophysiological properties of the single-channel current expressed in MIN6 cells with those in COS-7 cells transfected with cloned rat TREK-2, under identical experimental conditions. Single-channel openings at various membrane potentials in symmetrical 150 mM K^+ solution are shown in Fig. 3A. The mean open times were 1.2 ± 0.2 and 1.0 ± 0.1 ms in MIN6 cells and COS-7 cells, respectively ($n = 5$). Amplitude and open time duration histograms are shown in Fig. 3B. The open time duration histograms obtained at −40 mV could be fitted with a single exponential function for both MIN6 K^+ channels and TREK-2 in COS-7 cells.

The current–voltage relationships of the MIN6 K^+ channel and TREK-2 in COS-7 cells were indistinguishable and showed mild inward rectification. The single-channel conductance of MIN6 K^+ channel was 122 ± 10 pS and 64 ± 10 pS at −40 and +40 mV, respectively. The single-channel conductance of TREK-2 in COS-7 cells was 116 ± 8 and 62 ± 5 pS at −40 and +40 mV, respectively. Previous studies have shown that TREK-2 is more active at depolarized than at hyperpolarized potentials [22]. MIN6 K^+ channels also showed such voltage-dependence at high KCl solution.

The hallmark of members of the TREK K_{2P} family is their activation by unsaturated free fatty acids, negative pressure applied to the membrane, and acidification of the intracellular medium [13,37]. Therefore, these properties of MIN6 K^+ channel were examined. In a large fraction of cell-attached patches from MIN6, the TREK-like K^+ channel activity was generally low or absent, despite the presence of several channels in the patch membrane ($P_o < 0.01$). However, the channel activity increased several-fold when inside-out patches were formed (Fig. 4A). This is also a well-observed phenomenon for TREK-1 and TREK-2 expressed in COS-7 cells. This may occur due to disruption of certain cytoskeletal elements during patch excision and subsequent

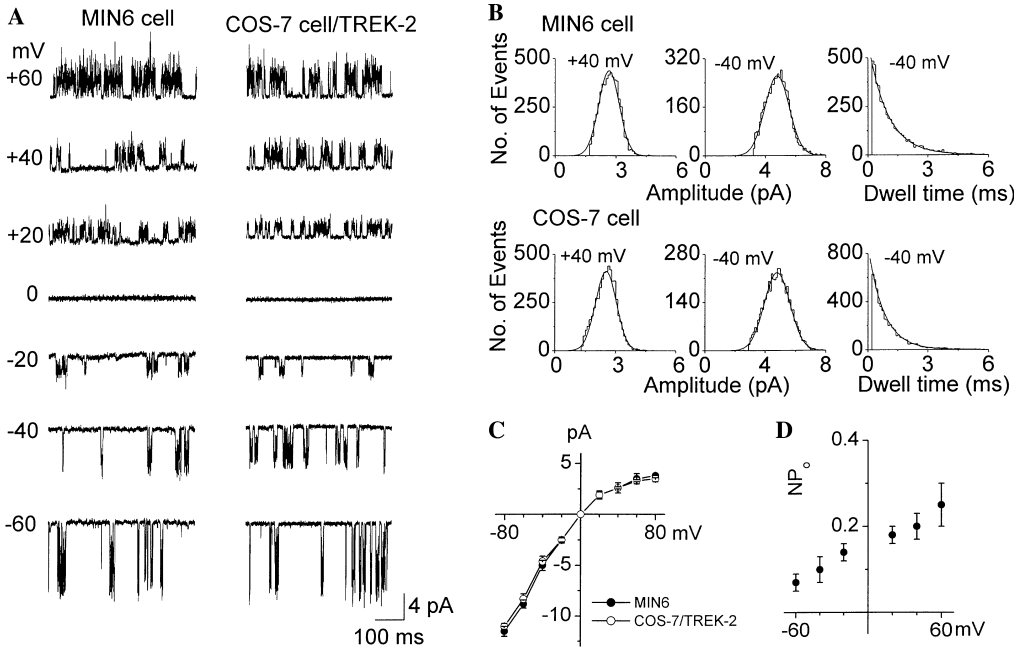

Fig. 3. Single-channel properties of TREK-like K^+ channel in MIN6 cells. (A) Current recording shows single-channel openings at different potentials in an inside-out patch in a MIN6 cell and in a COS-7 cell transfected with TREK-2. (B) Graphs show amplitude histograms of channel openings recorded at two membrane potentials (−40 and +40 mV), and open-time histograms determined from single-channel openings at −40 mV in a MIN6 cells and a COS-7 cell transfected with TREK-2. (C) Current amplitudes from the first open level were determined (mean ± SD, $n = 5$) and plotted to obtain the current–voltage relationships for MIN6 cell (filled circle) and COS-7 cells (open circle) transfected with TREK-2. (D) Channel activity at different membrane potentials in MIN6 cells is shown. Each point is the mean ± SD of five determinations.

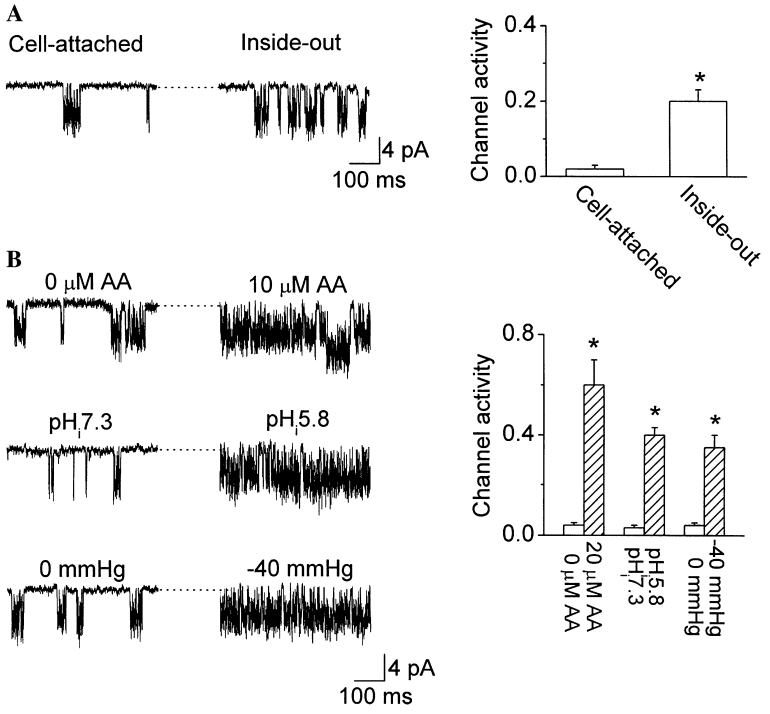

Fig. 4. Activation of TREK-2 by AA (acid), and negative pressure in MIN6 cells. (A) Channel openings of TREK-2 in cell-attached and inside-out patches are shown. The cell membrane potential was held at −40 mV. Pipette and bath solutions contained 150 mM KCl. The bar graph shows channel activity determined at −40 mV. Asterisk indicates a significant difference from the value at cell-attached patches ($p < 0.05$). Each bar is the mean ± SD of 10 determinations. (B) Inside-out patches were formed and arachidonic acid (10 μM), low pH solution (pH_i 5.8), and negative pressure (−40 mmHg) were applied to the patch membrane. Activation by the three stimuli was reversible in every patch. The holding potential was −40 mV. The bar graph shows channel activity before and after application of each activator. Asterisks indicate a significant difference from the corresponding control value ($p < 0.05$).

changes in membrane tension. In such patches containing active MIN6 K^+ channels, application of 10 μM AA, negative pressure (−40 mmHg), and acid solution (pH_i 5.8) to the bath produced marked and reversible increases in channel activity (Fig. 4B). These results, combined with the single-channel kinetics, provide strong evidence to indicate that this MIN6 K^+ channel is a functional correlate of TREK-2.

Whole-cell current studies

As fatty acid-sensitive K^+ channel (TREK-2) is expressed in MIN6 cells, whether arachidonic acid increases the whole-cell current was tested. MIN6 cells under whole-cell configuration were held at −80 mV and a ramp potential (−120 to +60 mV; 865 ms duration) was applied in physiological solution containing 5 mM KCl. The reversal potential was ∼−75 mV in control cells, presumably due to several active K^+ channels near resting potential. Ramp voltage steps elicited large outward currents that probably include activation of voltage-gated K^+ channels such as Kv2.1 that is the predominant K_v channel in MIN6 cells [38]. Addition of 10 μM arachidonic acid to the bath solution increased the whole-cell current by 74% (from 429 ± 26 to 747 ± 149 pA at +30 mV; $n = 5$; Fig. 5A). Ba^{2+} (3 mM)

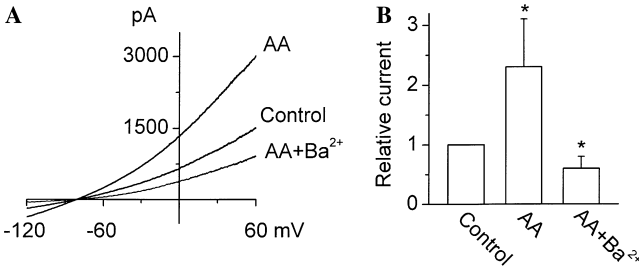

Fig. 5. Whole-cell current activated by arachidonic acid. (A) Whole-cell currents were recorded following application of ramp pulses from −120 to +60 mV. Arachidonic acid was applied to the bath or pipette solution. Holding potential was −80 mV. Ba^{2+} (3 mM) was then added together with arachidonic acid to the bath solution to block K^+ current. (B) Currents at +30 mV were determined and plotted. Each bar represents the mean ± SD of five determinations. Asterisk indicates a significant difference from the basal value ($p < 0.05$).

inhibited the increase in current, indicating that K^+ channels were activated. Because arachidonic acid is more effective in activating TREK-2 from the cytoplasmic side of the membrane, we also dialyzed the perfusing pipette with 10 μM arachidonic acid. In 19 of 28 cells, this maneuver produced a stronger activation (2.3-fold) of the whole-cell current (Fig. 5B). Arachidonic acid also caused a small inhibition of the current in seven cells, and no significant change in two cells. The reason

for such different effects of arachidonic acid on the whole-cell current is not clear, and may be due to widely varying levels of cell-to-cell expression of TREK-2 and other K^+ channels in our cultured MIN6 cells. If TREK-2 expression is low in some cells, arachidonic acid would cause inhibition of the whole-cell current, as the free fatty acids inhibit K_{ATP} channels from the cytoplasmic side [39]. The increase in Ba^{2+}-sensitive outward current by AA in a majority of cells is consistent with the expression and strong activation of TREK-2 in MIN6 cells.

Discussion

The expression of K_{2P} channels at the levels of mRNA and functional protein was studied in MIN6 cell, a pancreatic β cell line. MIN6 cells secrete insulin in response to glucose and other secretagogues [25,40], and have been used extensively to study the mechanisms of insulin secretion. Our results show that MIN6 cells express TASK-1, TASK-2, TASK-3, TREK-2, and TRESK-2 mRNAs, as judged by RT-PCR. Among these K_{2P} channels, however, TREK-2 was the only one that could be clearly identified in MIN6 cells by patch-clamp analysis. Because TREK-2 is activated by free fatty acids, low intracellular pH, and cell swelling [13,37], our findings suggest that TREK-2 may regulate insulin release during certain metabolically stressed conditions.

Background K^+ channels in MIN6 cells

Five types of K^+ channels were recorded in cell-attached patches of MIN6 cells and are referred to here as 80-pS (K_{ATP}), 217-pS (K_{Ca}), 146-pS (TREK-2), 37-pS, and 28-pS channels. The K_{ATP} channel and TREK-2 together provided ~75% of the K^+ current at rest, based on the number of patches they were observed and their channel activity in each patch at a positive membrane potential of +60 mV. Whether these K^+ channels also contribute to the background K^+ conductance in native pancreatic β cells remains to be determined. The degree of expression of these K^+ channels may be different in native pancreatic β cells. Their contribution to the background K^+ conductance may also depend on the metabolic state of the cell. Plasma free fatty acid concentration is generally high (~1 mM). However, the concentration of unbound free fatty acids in the plasma is low between 0.01 and 10 μM, due to their tight binding to serum albumin [41,42]. When free fatty acid levels are in the high range (0.5–10 μM), TREK-2 may be activated and its contribution to the background K^+ conductance may increase greatly.

Glucose-induced depolarization is the result of inhibition of K_{ATP} channel by intracellular ATP. TREK-2 and the 28-pS channel may help to prevent the membrane potential from over-depolarizing. In fact, TREK-2 becomes more active as the membrane potential becomes more positive (depolarized), providing a progressively stronger effect against glucose-mediated depolarization. Such a "break" mechanism (or desensitization) is normally observed in many physiological processes to help prevent over-stimulation by an agonist. Thus, these background K^+ channels may be important in helping to limit the increase in $[Ca^{2+}]_i$ and insulin secretion.

The molecular identity of the 37- and 28-pS channels is not known. A K^+ channel with single channel kinetics and conductance similar to those of MIN6 37-pS channel has been recorded earlier in cerebellar granule cells, in magnocellular neurosecretory cells from the supraoptic nucleus, and in untransfected COS-7 cells [36,43]. In magnocellular neurosecretory cells, cytoplasmic application of arachidonic acid increases the activity of the 37-pS channel [43]. We also found that arachidonic acid increased the 37-pS channel in MIN6 cells. The 28-pS channel behaves like a classical inward rectifying K^+ channel (IRK) that shows long-lasting openings with brief closures within each opening. Whether the 28-pS K^+ channel belongs to the IRK family remains to be determined.

In human pancreas, TALK-1 and TALK-2 K_{2P} channel mRNAs were highly expressed, as judged by Northern blot analysis [20]. However, our RT-PCR studies failed to detect expression of these two K_{2P} channels in MIN6 cells. Single-channels kinetic properties of TALK-1 and TALK-2 have recently been determined in our laboratory [44]; under identical experimental conditions, ion channels with similar kinetics were not detected in MIN6 cells in cell-attached or inside-out patches. It seems likely that the level of mRNA expression of TALK-1 and TALK-2 is extremely low in MIN6 cells, or that they are expressed in cell types other than β cells in the pancreas.

RT-PCR analysis clearly shows that mRNA for TASK-1, TASK-2, TASK-3, and TRESK-2 are expressed in MIN6 cells. Single-channel openings with conductance and open times similar to TASK-1 (~16 pS, ~1 ms) and TASK-3 (~36 pS, ~1 ms) were present in a few patches, but their open probabilities were too low for any reasonable analysis to be performed. For example, one such opening would be present once every 5 s or more. Channel openings with properties similar to TASK-2, TREK-1, TRAAK, and TRESK-2 were not observed. Thus, TREK-2 was the only measurable K_{2P} channel in MIN6 cells.

Potential role of TREK-2 in MIN6 cells

TREK-2 is now well known to be a target of various physical and chemical factors that include free fatty acids, pressure, protons, heat, G proteins, and volatile

anesthetics [18,22]. Therefore, in addition to serving as a background K^+ channel, TREK-2 is likely to transduce various signals into changes in membrane potential and excitability. Because of its sensitivity to free fatty acids in particular, TREK-2 could mediate some of the effects of elevated free fatty acid levels on cell excitability and insulin secretion.

An acute increase in free fatty acids (1–30 μM) such as oleic, linoleic, linolenic, and arachidonic acids has been shown to elevate insulin secretion from pancreatic β cells [45,46]. Recent studies show that free fatty acids bind to an orphan G protein-coupled receptor (GPR40) that is coupled to Gq/11 [46]. Free fatty acids elicited an increase in $[Ca^{2+}]_i$ as expected of an agonist that acts on Gq/11 coupled receptor that stimulates phospholipase C and increases IP3 production. Thus, this pathway does not involve inhibition of K_{ATP} channels, membrane depolarization, and Ca^{2+} influx via voltage-dependent Ca^{2+} channels, but probably involves Ca^{2+} release from intracellular stores. Free fatty acid-induced insulin secretion was greatly increased in the presence of glucose (5.5–11 mM), presumably due to a large elevation of $[Ca^{2+}]_i$ [46].

Our studies on transfected COS-7 cells and in MIN6 cells show clearly that free fatty acids such as arachidonic acid increase TREK-2 activity. This would help to stabilize the resting membrane potential and reduce glucose-induced depolarization. Other factors that augment TREK-2 activity would produce similar changes. Inhibition of TREK-2 may therefore help to amplify glucose-stimulated insulin secretion. Because free fatty acids increase insulin secretion via a mechanism independent of changes in membrane potential (via GPR40), inhibition of TREK-2 would not be expected to affect fatty acid-induced insulin secretion. Unfortunately, these possibilities could not be tested, as no specific inhibitor of TREK-2 or an antagonist of the GPR40 receptor is currently available. Nevertheless, a drug that specifically inhibits TREK-2 could be of potential therapeutic value for the treatment of certain types of diabetes.

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