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Y-26763: ATP-sensitive K^+ channel activation and the inhibition of insulin release from human pancreatic β-cells

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Abstract

The effect of Y-26763 [(−)-(3*S*,4*R*)-4-(*N*-acetyl-*N*-hydroxyamino)-6-cyano-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ol], a novel ATP-sensitive K^+ (K_{ATP}) channel activator, was tested on insulin secretion from human pancreatic islets in vitro. Y-26763 was able to inhibit both glucose- and tolbutamide-induced insulin secretion from islets as assessed by radioimmunoassay. The mechanism for inhibition of insulin secretion was characterised using patch clamp electrophysiology on dispersed human pancreatic β-cells which express K_{ATP} channels comprised of Kir6.2 and SUR1, and the NES2Y human β-cell line, transfected with Kir6.2ΔC26. Y-26763 activated K_{ATP} channels in a reversible manner with a similar activity to diazoxide. This required the presence of hydrolysable nucleotides and appeared to be mediated by interaction of Y-26763 with SUR1 since: (a) tolbutamide was able to reverse the actions of Y-26763 and (b) Y-26763 failed to activate Kir6.2ΔC26 in the absence of SUR1. We conclude that Y-26763-induced inhibition of insulin release is dependent upon the activation of K_{ATP} channels in human β-cells.

Keywords: Y-26763; K_{ATP} channel; Insulin secretion; Human β-cell; NES2Y

1. Introduction

ATP-sensitive K^+ (K_{ATP}) channels are hetero-octomeric structures composed of two different subunits, a pore forming inward rectifier K^+ conducting protein Kir6.2 or Kir6.1, and the regulatory sulphonylurea receptor protein, SUR1, SUR2A, SUR2B or SUR2C. These proteins are differentially expressed in a wide range of tissues including pancreatic β-cells (Kir6.2 and SUR1), cardiac myocytes (Kir6.2 and SUR2A), and smooth muscle (Kir6.1 and SUR2B) (Lawson and Dunne, 2001). The synthetic K_{ATP} channel activator Y-26763 [(−)-(3*S*,4*R*)-4-(*N*-acetyl-*N*-hydroxyamino)-6-cyano-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ol] is the active metabolite of the pro-drug Y-27152 which has been previously examined for actions on blood pressure in healthy volunteers (Uematsu et al., 1996). Y-26763 has been tested in a number of different tissues and reported to open K_{ATP} channels in canine and rat myocardium (Fukunari et al., 1997; Rahman et al., 1996; Tanabe et al., 2000), rat cerebral arteries (Kitayama et al., 2002a,b), guinea pig coronary arteries (Yajima et al., 1999) and in guinea pig bladder smooth muscle (Hashitani et al., 1996).

K_{ATP} channels play a pivotal role in controlling β-cell responses to glucose by maintaining the resting membrane potential of the cell close to −70 mV. Following glucose uptake and metabolism, increases in the intracellular ratio of ATP:ADP cause closure of K_{ATP} channels. The reduced efflux of K^+ ions leads to membrane depolarisation and the subsequent activation of voltage-gated Ca^{2+} channels and voltage-gated K^+ channels. Calcium entry through voltage-gated Ca^{2+} channels triggers exocytosis and insulin release whilst activation of voltage-gated K^+ channels promotes repolarisation of the membrane back towards resting levels (Dunne et al., 1999). Pharmacological activation of K_{ATP}

Abbreviations: K_{ATP}, ATP-sensitive K^+; Kir, K^+ inward rectifier; SUR, sulphonylurea receptor; Y-26763, [(−)-(3*S*,4*R*)-4-(*N*-acetyl-*N*-hydroxyamino)-6-cyano-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ol].

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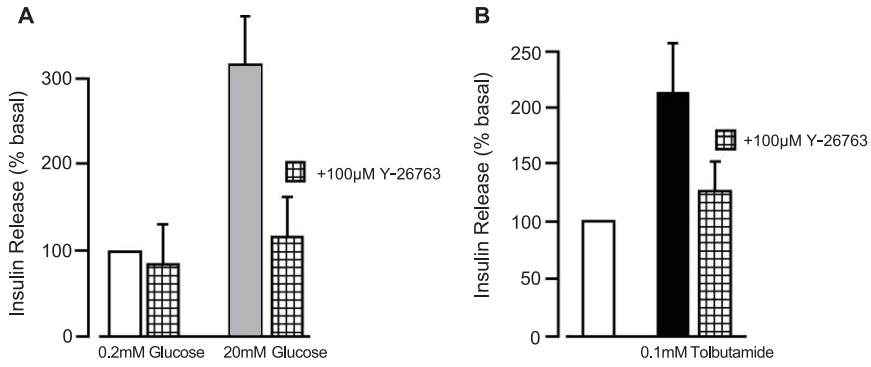

Fig. 1. Y-26763 inhibits insulin secretion in human islets. Panel A: Y-26763 (100 μM) inhibited glucose-induced (20 mM) insulin secretion but had no significant effect on basal insulin secretion ($n=6$; $*p<0.05$). Panel B: In the presence of 2 mM glucose, Y-26763 was also able to attenuate tolbutamide-induced (100 μM) insulin secretion ($n=6$; $*p<0.05$).

channels therefore inhibits depolarisation–response coupling in β-cells, by promoting membrane hyperpolarisation, and results in reduced insulin output at a given glucose concentration. Clinically, the K_{ATP} channel opener, diazoxide is used in the treatment of hyperinsulinism as a consequence of hyperinsulinism in infancy or pancreatic insulinoma (Cosgrove et al., 2002; Hussain et al., 2002). However, the clinical effectiveness of diazoxide in the treatment of hyperinsulinism is highly variable due to diverse defects of K_{ATP} channels in these patients (Touati et al., 1998; Shepherd et al., 2000) and as a consequence of adverse side effects the compound can be poorly tolerated in vivo (Aynsley-Green et al., 2000). A role for K_{ATP} channel activators in the treatment of type 2 diabetes mellitus has also been proposed since diazoxide was found to reduce weight gain, improve glucose uptake and modify central neuropeptide Y levels in Zucker (fa/fa) rats (Alemzadeh et al., 1993, 1996). Furthermore, diazoxide administration combined with intense insulin treatment to induce β-cell rest has now been shown to prevent or delay the progression of type 1 diabetes in newly diagnosed patients (Bjork et al., 1996, 1998). More recently developed K_{ATP} channel openers including BPDZ 154 (Cosgrove et al., 2002) and NNC 55-0118 (Dabrowski et al., 2002; Nielsen et al., 2002) have previously been shown to be more potent and selective for β-cell K_{ATP} channels than diazoxide in vitro, but clinical trial data is not yet available for these compounds. In this study we have examined the actions of Y-26763, a novel K_{ATP} channel opener, on human β-cells and show that the compound inhibits insulin release via the activation of K_{ATP} channels.

2. Materials and methods

2.1. Tissue preparation

Human islets were obtained with permission from human cadaver organ donors ($n=6$). Islets of Langerhans were isolated using a controlled collagenase digestion procedure and were dispersed into single cells as previously described (Straub et al., 2001; Cosgrove et al., 2002). Dispersed cells

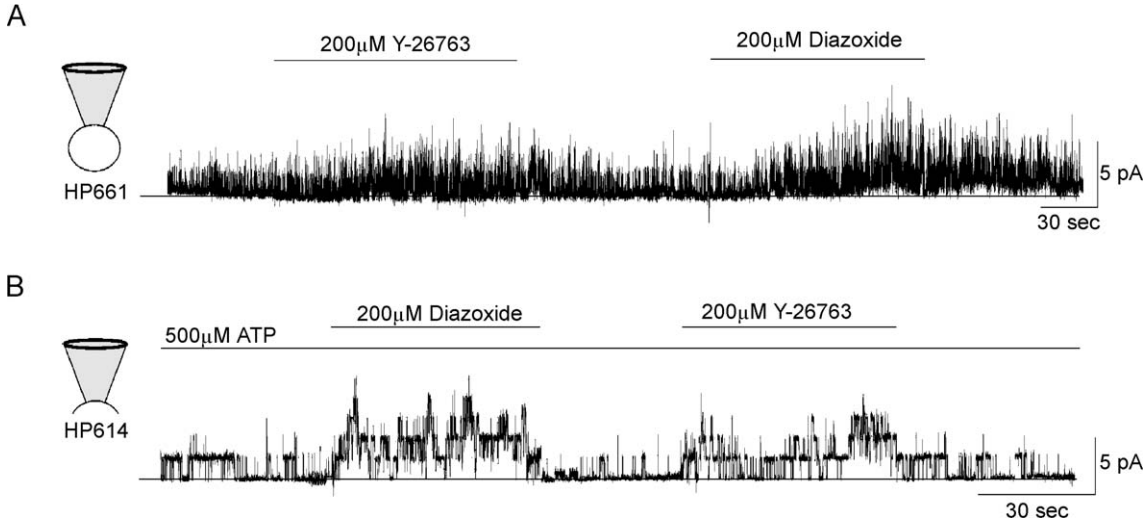

Fig. 2. Effects of diazoxide and Y-26763 on K_{ATP} channels in human β-cells. Panel A: Both Y-26763 (200 μM) and diazoxide (200 μM) were able to activate K_{ATP} channels in cell-attached patches. Illustrated data are typical of $n=4$ separate recordings. Panel B: Both drugs were able to activate K_{ATP} channels in inside-out patches in the presence of 500 μM ATP. Data are typical of $n=14$ separate recordings.

were incubated at 37 °C in a humidified atmosphere of 5% CO_2/air mixture for up to 4 days. NES2Y cells, transfected with cDNA encoding Kir6.2ΔC26, were maintained as described previously (MacFarlane et al., 1999, 2000). Islets and cells were maintained under standard tissue culture conditions in RPMI 1640 medium (Sigma, Poole, UK) supplemented with 10% v/v foetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Transfected NES2Y cells were maintained in the presence of G418 as described previously (MacFarlane et al., 1999).

2.2. Insulin secretion

Insulin secretion was measured from static incubations using batches of five human islets and triplicate determinations (as previously described, Straub et al., 2001). Islets were pre-incubated at 37 °C in Krebs–Ringer HEPES buffer composed of (in mM): 137 NaCl, 5.36 KCl, 0.81 $MgSO_4$, 0.34 Na_2HPO_4, 0.44 KH_2PO_4, 4.17 $NaHCO_3$, 10 HEPES, and 1.26 $CaCl_2$, with 2 or 20 mM glucose as described previously (Straub et al., 2001). Subsequently the islets were exposed for 30 min to the test substances. Following incubation aliquots of the buffer were removed and stored at −20 °C until radioimmunoassay was performed using a charcoal separation method (Herbert et al., 1965). An anti-porcine insulin antibody was used (Linco, MO, USA), ^{125}I Insulin was purchased from New England Nuclear (Boston, MA), and the human insulin standard with the reference number 66/304 was purchased from NIBSC (Hertfordshire, UK).

2.3. Electrophysiology

All data were obtained using cell-attached or inside-out recording configurations of the patch-clamp technique as described (Hamill et al., 1981). The pipette contained a standard NaCl-rich bathing solution containing (in mM): 140 NaCl; 4.7 KCl; 2.5 $CaCl_2$, 1.13 $MgCl_2$, 10 HEPES, 2.5 glucose (pH 7.4 with NaOH) and the bath solution contained (in mM): 140 KCl, 10 NaCl, 1.13 $MgCl_2$, 1 EGTA, 2.5 glucose, 10 HEPES (pH 7.2 with KOH) for all recordings. All illustrated records are displayed according to the convention with upward deflections representing outward currents. In patches with a suitable number of K_{ATP} channel events, open-state probability was assessed as described previously (Monks et al., 1999).

2.4. Drugs

Y-26763 was a gift from Yoshitomi Pharmaceutical Industries (Osaka, Japan). ATP, α-β-Me-ATP, ADP, tolbutamide and diazoxide were obtained from Sigma. ADP was

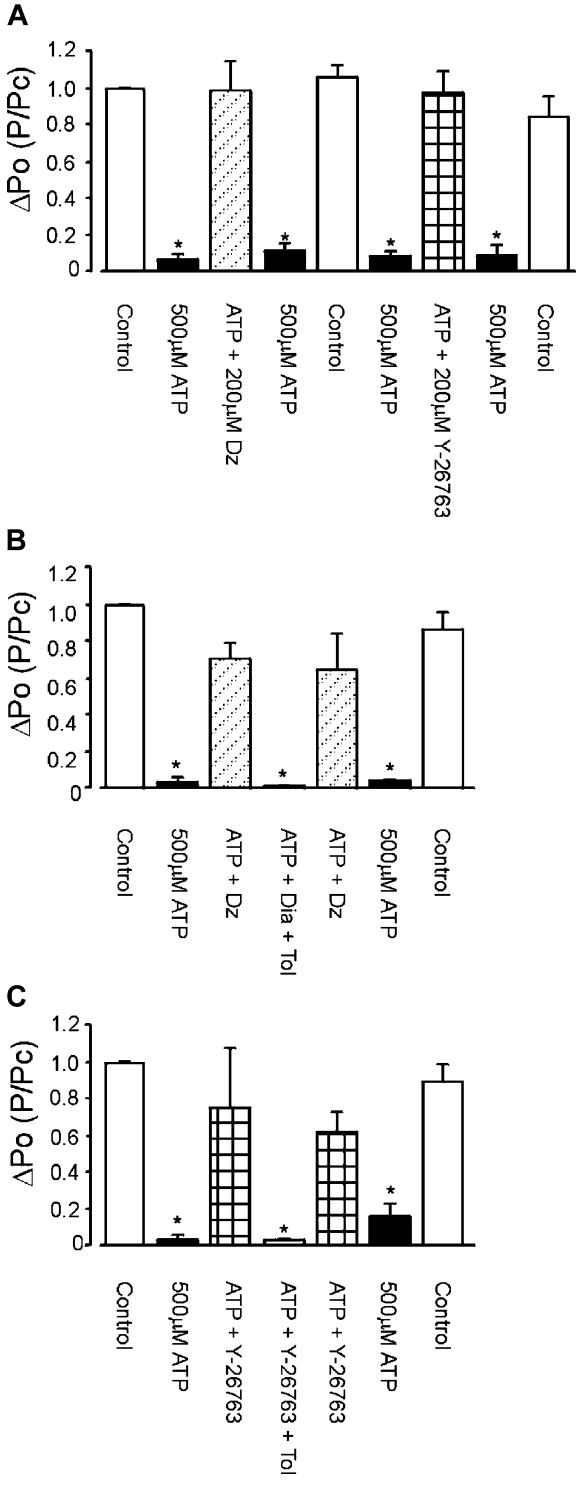

Fig. 3. Reversal of diazoxide and Y-26763 effects by tolbutamide in human β-cells. Panel A: Summary of open-state probability (osp) values (expressed as a percentage of the control values, osp = 0.35 ± 0.07) for the effects of diazoxide (values calculated for $n = 5$ separate recordings) and Y-26763 ($n = 4$ separate recordings) on K_{ATP} channels in inside-out patches. All drug applications were made sequentially to each of the patches as described for a minimum of 1 min with a washout period of 2–3 min per experimental procedure. Panel B: Summary of open-state probability values illustrating reversal of diazoxide (100 μM) activation by tolbutamide (100 μM). Data obtained for $n = 5$ separate recordings, control osp = 0.23 ± 0.09. Panel C: Summary of open-state probability values illustrating reversal of Y-26763 (100 μM) activation by tolbutamide (100 μM). Data obtained for $n = 4$ separate recordings, control osp = 0.58 ± 0.14; *$p < 0.05$.

obtained from Fluka, UK. Agents were added at concentrations as indicated in the text. Diazoxide and Y-26763 were added as a stock solution dissolved in dimethylsulphoxide (Sigma) giving a final concentration of less than 0.1% v/v.

2.5. Statistics

Statistical comparisons were made using analysis of variance or paired Student's *t*-tests with values of $p < 0.05$ taken to be significant. All data are expressed as mean values ± standard error of the mean (S.E.M.).

3. Results

3.1. Y-26763 inhibits stimulated insulin secretion from human islets of Langerhans

Insulin secretion was assessed from intact human islets of Langerhans incubated under low and high glucose conditions (2 vs. 20 mM) in the absence and presence of Y-26763 (100 μM; Fig. 1A; $n = 6$). Although Y-26763 had no significant effect upon basal insulin secretion (1.8–2.5 ng/5 islets/60 min), glucose-induced insulin secretion was inhibited to near basal levels. Similarly, Y-26763 (100 μM) inhibited tolbutamide-induced insulin secretion to basal levels (Fig. 1B; $n = 6$).

3.2. Activation of K_{ATP} channels by Y-26763 in human β-cells

The mechanism of inhibition of insulin secretion was investigated using patch-clamp electrophysiology to study the effects of Y-26763 on K_{ATP} channels in isolated single human β-cells. K_{ATP} channel currents were activated in intact cells following application of Y-26763 in the cell-attached recording configuration (200 μM; $n = 4$; Fig. 2A). Following excision to form inside-out patches, application of Y-26763 (100 μM or 200 μM), in the presence of ATP (500 μM) also led to an increase in the frequency of openings of K_{ATP} channels (Fig. 2B; $n = 14$; Fig. 3). These effects were similar to those produced by identical concentrations of diazoxide in membrane patches (Fig. 2A, B; $n = 5$). Open-state probability data from a number of similar experiments are summarised in Fig. 3. On average, ATP (500 μM) reduced the control current by approximately 90% ($p < 0.05$) and this inhibition was almost fully reversed by the addition of Y-26763 or diazoxide, Fig. 3A. The sulphonylurea tolbutamide (100 μM) was able to consistently antagonise the effects of both diazoxide (Fig. 3B, $n = 5$) and Y-26763 (Fig. 3C, $n = 4$).

3.3. SUR1 is required for the actions of Y-26763 in human β-cells

The human β-cell-derived cell line NES2Y, which has no functional K_{ATP} channels and reflects the properties of acutely isolated β-cells from patients with Hyperinsulinism in Infancy, was transfected with Kir6.2ΔC26 and used to study the effects of Y-26763 in the absence of functional SUR1. Currents recorded from Kir6.2ΔC26 channels were inhibited by ATP (Fig. 4A,B); however, neither diazoxide (Tucker et al., 1997, data not shown) nor Y-26763 (100 μM; Fig. 4, $n = 9$) were able to reverse the inhibition by ATP. These data indicate that SUR1 is required for pharmacological activation of the channel.

3.4. Hydrolysable nucleotides are required for the actions of Y-26763 in human β-cells

To further investigate the role of SUR1 in the action of Y-26763 on β-cell K_{ATP} channels, we studied the action of the

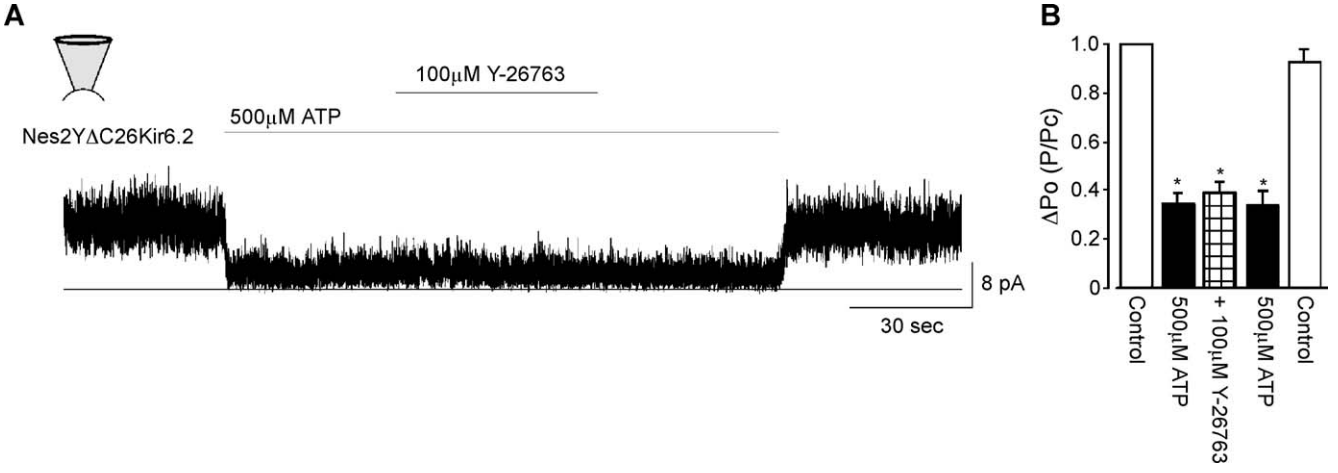

Fig. 4. SUR1 is required for the actions of Y-26763 on β-cell K_{ATP} channels. Panel A: A typical inside-out recording from a NES2Y cell expressing Kir6.2ΔC26 channels which were blocked by 500 μM ATP. Y-26763 (100 μM) had no effect. This recording is typical of $n = 9$ recordings. In addition, and not shown here, Y-26763 (200 μM) did not activate Kir6.2ΔC26 channels ($n = 10$). Panel B: Summary of open-state probability values for the lack of effect of Y-26763 (100 μM) on Kir6.2ΔC26 channels (control osp = 0.4 ± 0.04, $n = 4$); *$p < 0.05$.

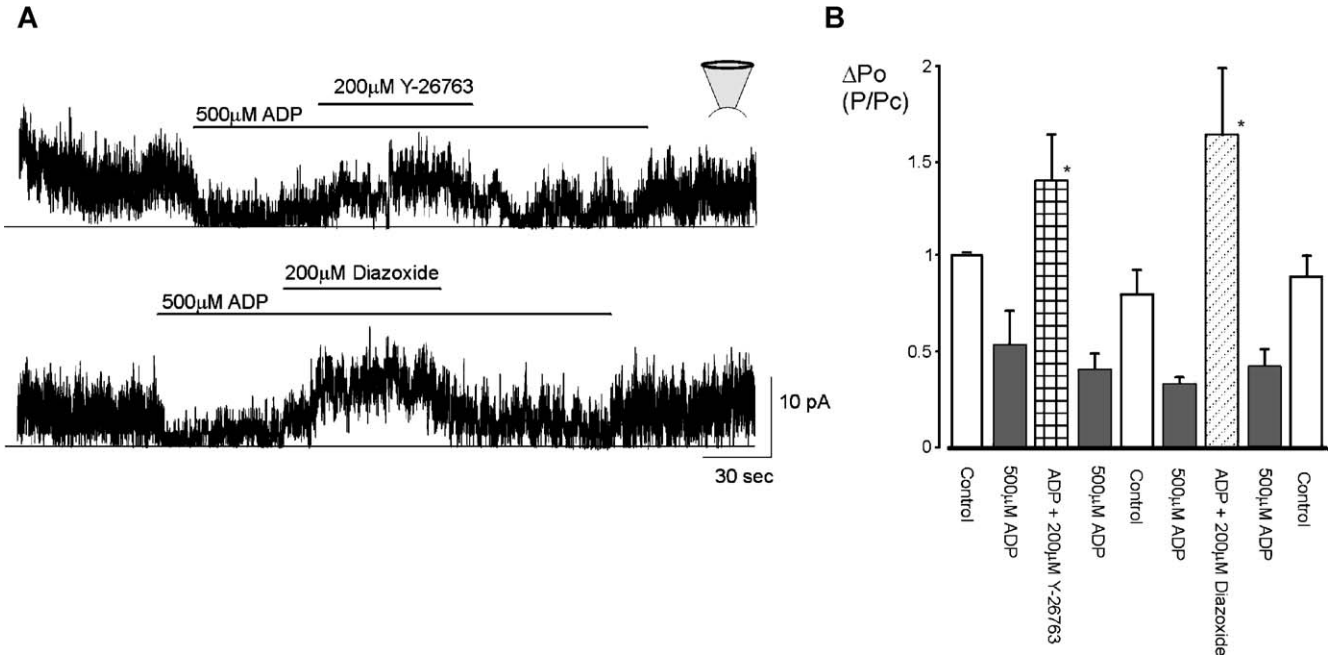

Fig. 5. Activation of K_{ATP} channels by Y-26763, in the presence of ADP, in human β-cells. Panel A: Following inhibition of K_{ATP} channel activity by 500 μM ADP, Y-26763 (200 μM) was able to activate channel activity. Data illustrated are typical of $n = 7$ separate recordings. Similarly, diazoxide (200 μM) was also able to reverse K_{ATP} channel block by 500 μM ADP. Data illustrated are typical of $n = 7$ separate recordings. Panel B: Summary of open-state probability values illustrating channel block by 500 μM ADP and reversal by both Y-26763 and diazoxide (control osp = 0.37 ± 0.08, $n = 4$); $^*p < 0.05$.

drug in the presence of either ADP (500 μM) or a non-hydrolysable analogue of ATP, α-β-Me-ATP (500 μM) in human β-cells. Following inhibition by ADP (500 μM; $n = 7/11$), both Y-26763 (200 μM) and diazoxide (200 μM; Fig. 5A; $n = 7$ for each) were able to stimulate K_{ATP} channels to a similar degree of activity. ADP is known to have a dual action on K_{ATP} channels with activatory or inhibitory effects depending on the concentration of nucleotide used and level of K_{ATP} channel run-down (Dunne and Petersen, 1986; Kakei et al., 1986). During the experiments described here, we found that when ADP (500 μM) activated K_{ATP} channels ($n = 9/11$ patches), both Y-26763 (200 μM) and diazoxide (200 μM) further enhanced channel activity (data not shown).

In contrast to the effects of the K^+ channel openers in the presence of hydrolysable nucleotides such as ATP and ADP, inhibition of K_{ATP} channel activity by α-β-Me-ATP (500 μM), a non-hydrolysable analogue of ATP, could not be reversed by application of either Y-26763 (200 μM) or diazoxide (200 μM; Fig. 6; $n = 10$).

4. Discussion

Pancreatic β-cell K_{ATP} channels are composed of Kir6.2 and SUR1 subunits, unlike K_{ATP} channels found in cardiac and smooth muscle which are formed by Kir6.2 and SUR2A or SUR2B, respectively (see Aguilar-Bryan et al., 1998; Ashcroft and Gribble, 1998; Seino and Miki, 2003 for reviews). The novel K^+ channel opener, Y-26763, has previously been found to activate K_{ATP} channels in both cardiac myocytes and in smooth muscle cells from various tissues, suggesting that the compound has a non-specific effect on SUR2 channel subunits (Hashitani et al., 1996; Rahman et al., 1996; Fukunari et al., 1997; Yajima et al., 1999; Tanabe et al., 2000; Kitayama et al., 2002a, 2002b).

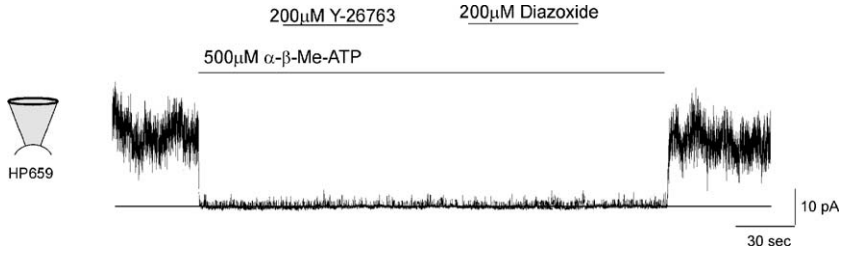

Fig. 6. Nucleotide hydrolysis is required for activation of K_{ATP} channels by Y-26763. Both Y-26763 (200 μM) and diazoxide (200 μM) were unable to reverse K_{ATP} channel inhibition by 500 μM α-β-Me-ATP, a non-hydrolysable analogue of ATP. The illustrated record is typical of $n = 13$ separate recordings.

We studied the effects of Y-26763 in human islets and β-cells, to investigate whether this compound also activated the SUR1-containing K_{ATP} channels, by examination of insulin release and K_{ATP} channel function.

During static incubations of human islets of Langerhans we found that although Y-26763 did not affect basal insulin secretion, it was able to significantly inhibit both glucose- and tolbutamide-induced insulin secretion, both of which exert their effects through closure of K_{ATP} channels (Fig. 1).

Further studies were performed to confirm that Y-26763 inhibited insulin secretion via activation of K_{ATP} channels in isolated β-cells. Our results clearly show that Y-26763 modulated β-cell K_{ATP} channels to a similar degree of activity to that seen for diazoxide when applied at the same concentrations in both cell-attached and inside-out patch recordings (Figs. 2, 3). Furthermore, the sulphonylurea receptor was shown to be important in the mechanism of Y-26763 activation of K_{ATP} channels. First, inside-out patch recordings of K_{ATP} channels revealed that the effects of Y-26763 could be reversed by application of tolbutamide, a sulphonylurea drug which binds to specific amino acid sequences in SUR1 (Fig. 3). Secondly, expression of Kir6.2ΔC26 in the NES2Y cell line, which has an absence of functional SUR1, resulted in channels which could not be activated by Y-26763 (Fig. 4). Given the previously reported activity of Y-26763 on K_{ATP} channels in other tissues (Hashitani et al., 1996; Rahman et al., 1996; Fukunari et al., 1997; Yajima et al., 1999; Tanabe et al., 2000; Kitayama et al., 2002a, 2002b), the presence of a sulphonylurea receptor (SUR1 or SUR2), therefore seems critical for the activity of Y-26763.

Finally, the mechanism of activation of K_{ATP} channels by Y-26763 required the presence of hydrolysable nucleotides since inhibition of channels by a non-hydrolysable analogue of ATP, α-β-Me-ATP, could not be reversed by Y-26763 (Fig. 6). As nucleotide hydrolysis is thought to be mediated by the intrinsic activity of SUR (Schwanstecher et al., 1998), this represents further evidence of the importance of SUR in the mechanism of Y-26763 activation. Hence, there are clear parallels with the action of diazoxide on K_{ATP} channels which similarly activates channels by mechanisms that rely upon nucleotide hydrolysis and ATP/ADP availability (Dunne and Petersen, 1991).

In summary, this study describes the actions of Y-26763, a novel K^+ channel opener on insulin secretion in human islets of Langerhans. Inhibition of glucose- and tolbutamide-stimulated insulin secretion by Y-26763 was brought about through activation of K_{ATP} channels in human β-cells. The compound is selective for K_{ATP} channels but its actions are not β-cell-specific since Y-26763 also activates SUR2 containing K_{ATP} channels. Sulphonylurea receptor subunits are critically required for K_{ATP} channel activation by Y-26763. The activatory site for Y-26763 was not identified in this study but is likely to be distinct from that for ADP since these compounds have an additive effect; moreover, the binding site is likely to be conserved in the structures of the different SUR proteins (SUR1, SUR2A, SUR2B). The findings described here suggest that in vivo, treatment with the pro-drug Y-27152 may cause hyperglycaemia and symptoms of diabetes mellitus, and this should be considered in the development of this drug and related compounds. Nevertheless, Y-26763 represents a useful pharmacological tool as a broad-acting K_{ATP} channel activator in the study of K_{ATP} channel function in vitro.

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