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Selective Inhibition of Glucose-Stimulated β-Cell Activity by an Anion Channel Inhibitor

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Abstract. 4,4′-dithiocyanatostilbene-2,2′-disulfonic acid (DIDS), an inhibitor of the volume-sensitive anion channel, was used to investigate the role of this channel in the stimulation of rat pancreatic β-cells by glucose and by tolbutamide. Glucose-stimulated electrical activity in β-cells was markedly and reversibly inhibited by DIDS. The increase in cytosolic [Ca^{2+}] and stimulated insulin release evoked by glucose were also inhibited by DIDS. In contrast to its inhibitory effect on glucose-induced β-cell activity, DIDS had no effect on electrical activity, the rise in $[Ca^{2+}]_i$ or insulin release induced by tolbutamide.

DIDS failed to increase β-cell input conductance, an index of whole-cell K_{ATP} channel activity, or the rate of efflux of $^{86}Rb^+$ from perifused islets, a measure of net K^+ permeability. Furthermore, DIDS had no effect on intracellular pH or on regulatory volume increase following exposure of cells to hypertonic solutions, indicating that the effects of DIDS were not the result of inhibition of Cl^- transport systems. It is suggested that the DIDS-induced repolarization is caused by inactivation of the volume-sensitive anion channel. The stimulation of β-cell electrical and secretory activity by glucose, but not tolbutamide, may therefore involve activation of the anion channel.

Key words: Islet — Pancreatic β-cell — Electrical activity — Cytosolic [Ca^{2+}] — Insulin release — Chloride channel

Introduction

The stimulation of insulin secretion by glucose and several other types of stimulus is intimately associated with electrical activity which consists of bursts of Ca^{2+}-dependent action potentials (*see* Ashcroft & Rorsman, 1989; Cook, Satin & Hopkins, 1991 for reviews). The induction of electrical activity by glucose requires metabolism of the sugar and the generation of one or more signals that couple glucose metabolism to depolarization of the plasma membrane. This leads to opening of voltage-sensitive Ca^{2+} channels, Ca^{2+} entry and exocytosis. The exact nature of this coupling mechanism is not entirely certain, but the consensus model proposes that a rise in [ATP] or ATP/ADP ratio inhibits the activity of K_{ATP} channels thus producing a net depolarization of the β-cell membrane (Rorsman & Trube, 1985; Henquin, 1988).

Hypoglycemic sulfonylureas such as tolbutamide and glibenclamide directly stimulate insulin release from the β-cell and, like glucose, induce electrical activity (Gylfe, Hellman & Sehlin, 1984; Cook & Ikeuchi, 1989). In this case, it is thought that the sulfonylurea inhibits directly the K_{ATP} channel, leading to β-cell depolarization (Sturgess et al., 1985; Cook & Ikeuchi, 1989). Thus, glucose and sulfonylureas are thought to share a common pathway in stimulating electrical and secretory activity in the β-cell (Cook & Ikeuchi, 1989). A more recent study, however, indicates that the ability of tolbutamide to mimic the effects of glucose on β-cell electrical activity requires low concentrations of the hexose, suggesting that inhibition of K_{ATP} channels is not the sole mechanism whereby glucose controls electrical activity (Henquin, 1998).

We and others have described a volume-sensitive anion channel in insulin-secreting cells that could also regulate electrical and secretory activity in the pancreatic β-cell (Kinard & Satin, 1995; Best, Sheader & Brown, 1996*b*). Activation of this channel by cell swelling causes depolarization, presumably due to anion efflux, leading to electrical activity and insulin release (Best, Miley & Yates, 1996*a*). We have also provided evidence that glucose and other nutrient stimuli can activate

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this channel (Best, 1997, 1999), possibly by causing β-cell swelling (Miley et al., 1997). The results of the present study demonstrate that DIDS, at a concentration previously shown to inhibit the volume-sensitive anion channel (Kinard & Satin, 1995; Best, Sheader & Brown, 1996*b;* Best, Miley & Yates, 1996*a*), inhibits glucose-induced, but not sulfonylurea-induced activation of rat pancreatic β-cells. This finding suggests that a mechanism other than closure of K_{ATP} channels contributes towards the induction of β-cell electrical activity by glucose and that this mechanism could involve activation of the volume-sensitive anion channel.

Materials and Methods

Pancreatic islets were prepared from Sprague-Dawley rats by collagenase digestion (Lacy & Kostianovsky, 1967). For patch-clamp and fluorescence studies, islets were dispersed into single cells by a brief (~5 min) exposure to Ca^{2+}-free medium containing (in mM): NaCl (135), KCl (5), $MgSO_4$ (1), NaH_2PO_4 (1), glucose (4), EGTA (1) and HEPES-NaOH (10; pH 7.4). Cells were suspended in HEPES-buffered RPMI medium (Gibco, Paisley, Scotland) and cultured in polystyrene dishes for up to 7 days. A standard incubation medium containing (in mM): NaCl (135), KCl (5), $MgSO_4$ (1), NaH_2PO_4 (1), $CaCl_2$ (1.2), glucose (4) and HEPES-NaOH (10; pH 7.4) was used for all experiments. A 33% hypertonic solution was prepared by addition of 100 mM mannitol. For insulin secretion studies, 1 mg/ml bovine serum albumin was added to the medium.

Changes in cell membrane potential were measured in single isolated β-cells by means of the 'perforated patch' technique (Rae et al., 1991) using a List EPC-7 amplifier (List, Darmstadt, Germany) under current-clamp conditions. The pipette solution contained (in mM): K_2SO_4 (60); KCl (10), NaCl (10), HEPES-NaOH, (10; pH 7.2) and amphotericin B (240 μg/ml). Only cells that showed a depolarization and electrical activity in response to stimulation were used for this study.

Whole-cell K_{ATP} channel activity in single β-cells was measured also under perforated patch conditions using a method described previously (Smith, Ashcroft & Rorsman, 1990). Briefly, cells were voltage-clamped at −70 mV and subjected to 50 msec pulses of ±10 mV at 2 sec intervals. K_{ATP} channel activity was estimated from the input conductance, the major component of which represents whole-cell K_{ATP} channel activity (Ashcroft, 1988; Smith et al., 1990). In all cases, access resistance was <25 MΩ and whole-cell capacitance within the range 9–14 pF. Efflux of $^{86}Rb^+$ from preloaded perifused rat islets was used to assess net K^+ channel activity as described previously (Best et al., 1988).

Changes in cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_i$) in single adherent cells or small cell clusters were assessed from the 340:380 fluorescence ratio (510 emission) in fura-2 loaded cells essentially as described previously (Elliott et al., 1993). Similarly, cytosolic pH was assessed from the 500/450 fluorescence ratio (530 emission) in cells loaded with 2′7′-bis(carboxyethyl) 5′(6′)-carboxyfluorescein (Best & Elliott, 1995). Changes in β-cell volume were measured using a video-imaging technique as described previously (Miley et al., 1997, 1998). Insulin release was measured in groups of 10 islets incubated for 90 min in 1 ml medium. The insulin content of the medium was measured by radioimmunoassay. The patch-clamp experiments were performed at 30–32°C and all other experiments at 37°C.

^{125}I-insulin was obtained from the Radiochemical Centre, Amersham, UK, collagenase (type 4) from Worthington Biochemicals (Freehold, NJ) and all chemicals from the Sigma Chemical, Poole, UK. Statistical significance was assessed using Student's *t*-test.

Results

A rise in the concentration of glucose from 4 to 16 mM resulted in depolarization leading to a characteristic pattern of 'spiking' electrical activity in isolated rat pancreatic β-cells (Fig. 1). The subsequent addition of the anion channel inhibitor DIDS (100 μM) in the continued presence of the high glucose concentration rapidly repolarized the cell and terminated electrical activity. This inhibitory effect of DIDS on glucose-induced electrical activity was observed in 5/5 cells tested and was fully reversible; the withdrawal of DIDS from the medium resulted in the gradual reappearance of electrical activity. In 4/4 cells, application of DIDS prior to increasing the glucose concentration markedly impaired the glucose-induced depolarization and prevented electrical activity (Fig. 1, *inset*). Exposure of β-cells to the sulfonylurea tolbutamide (100 μM) also caused β-cell depolarization and evoked electrical activity (Fig. 1). In this case, however, the subsequent addition of DIDS had no apparent effect in 5/5 cells. DIDS also had no effect on glibenclamide-induced electrical activity, nor on the depolarization evoked by exposure to 25 mM KCl (*not shown*).

Electrical activity in the β-cell is associated with Ca^{2+} entry via voltage-sensitive Ca^{2+} channels and thus a rise in $[Ca^{2+}]_i$. The next series of experiments therefore examined the effects of DIDS on glucose- and tolbutamide-induced changes in $[Ca^{2+}]_i$. As shown in Fig. 2 (*upper panel*), a rise in glucose concentration from 4 to 16 mM caused a sustained increase in $[Ca^{2+}]_i$, consistent with the pattern of electrical activity. In 10 cells, the mean 340/380 ratio in the presence of 4 mM glucose was 0.76 ± 0.03. Upon exposure to 16 mM glucose, a significant ($P < 0.005$) increase occurred in the above ratio to 1.07 ± 0.07 ($n = 5$). Application of DIDS caused a rapid and significant ($P < 0.05$) reduction in ratio to 0.81 ± 0.08 ($n = 5$; Fig. 2, *center panel*). Consistent with previous studies (Martin, Reig, & Soria, 1995), the effect of tolbutamide on β-cell $[Ca^{2+}]_i$ was found to be transient, as has also been shown to be the case with tolbutamide-induced insulin release from rat islets (Henquin, 1980; Martin et al., 1995). We therefore studied the effect of tolbutamide on $[Ca^{2+}]_i$ in the presence and absence of DIDS. As shown in Fig. 2 (*lower panel*), the rise in $[Ca^{2+}]_i$ in response to tolbutamide was virtually unaffected by the anion channel inhibitor. Peak values for the 340/380 ratio were 1.30 ± 0.10 and 1.31 ± 0.09 in the absence and presence of DIDS respectively (both $n = 5$).

The inhibition by DIDS of glucose-induced electrical activity and increase in $[Ca^{2+}]_i$ was associated with a marked impairment of glucose-induced insulin secretion

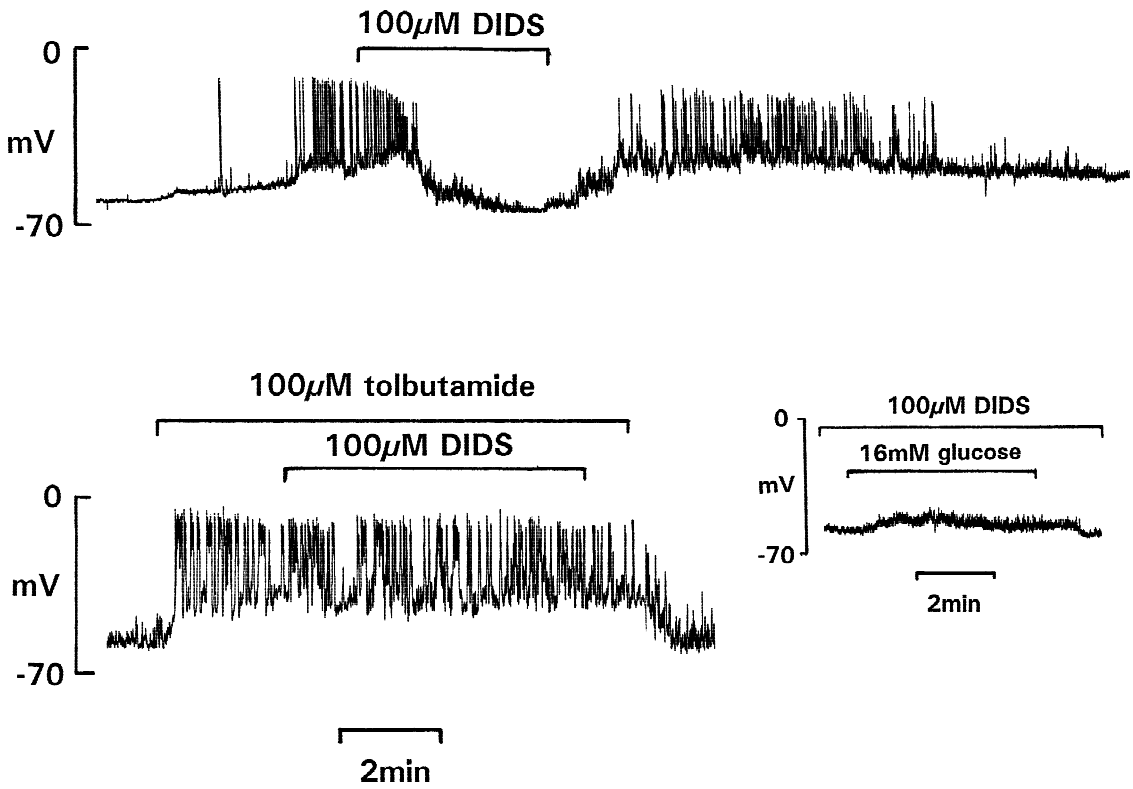

Fig. 1. Effects of DIDS on β-cell electrical activity. Membrane potential was recorded in rat pancreatic β-cells using the perforated patch recording technique. Effects of DIDS on electrical activity evoked by glucose (*upper panel* and *inset*) and tolbutamide (*lower panel*). Qualitatively similar results were obtained in five similar experiments.

(Fig. 3). In contrast, neither basal insulin release nor the stimulation of secretion by 100 μM tolbutamide were significantly affected by the presence of DIDS.

To ascertain whether the inhibitory actions of DIDS on glucose-induced β-cell activity involved an increase in K^+ permeability, two types of experiments were carried out. First, the effects of DIDS were examined on β-cell input conductance, an index of whole-cell K_{ATP} channel activity. Figure 4 shows examples of recordings from single β-cells voltage-clamped at −70 mV and subjected to 50 msec pulses of ±10 mV at 2 sec intervals. After approximately 10 min exposure to a glucose-free medium, input conductance in the cells was 3.36 ± 0.45 nS ($n = 5$). Addition of 10 mM glucose or 100 μM tolbutamide significantly ($P < 0.01$) reduced input conductance to 0.91 ± 0.18 and 1.20 ± 0.08 nS respectively, (both $n = 5$), consistent with an inhibition of K_{ATP} channel activity (Fig. 4, *upper* and *lower traces*). Addition of 100 μM DIDS had no significant effect on input conductance either in the presence (Fig. 4, *upper trace*) or absence (Fig. 4, *middle trace*) of glucose, the corresponding values being 0.88 ± 0.15 and 3.15 ± 0.35 nS (both $n = 5$).

The lack of a stimulatory effect of DIDS on K_{ATP} channel activity was confirmed by measuring $^{86}Rb^+$ efflux from preloaded, perifused islets, an index of net K^+ permeability (Fig. 5). Application of 100 μM DIDS failed to increase the fractional outflow rate of $^{86}Rb^+$ in the absence of glucose (Fig. 5, *upper panel*), although tolbutamide caused a marked inhibition of efflux under such conditions (Fig. 5, *center panel*), reflecting inhibition of K_{ATP} channel activity. After approximately 5 min exposure to DIDS in the absence of glucose, a small, gradual inhibition of $^{86}Rb^+$ efflux became apparent (Fig. 5, *upper panel*), possibly reflecting inhibition of K_{ATP} channel activity by DIDS (Furukawa et al., 1993). The rate of $^{86}Rb^+$ efflux was also reduced by 10 mM glucose (Fig. 5, *lower panel*), again presumably due to inhibition of K_{ATP} channel activity. DIDS also failed to affect significantly $^{86}Rb^+$ efflux in the presence of 10 mM glucose (Fig. 5, lower panel).

DIDS is known to inhibit some cellular Cl^--coupled transport systems, notably Cl^-/HCO_3^- exchange (Aicken & Brading, 1984), while an effect on $Na^+/K^+/2Cl^-$ cotransport is also possible. We therefore examined the effects of DIDS on intracellular pH (pH_i) in BCECF-loaded cells in order to assess changes in Cl^-/HCO_3^- exchange (Fig. 6*A*). In the absence of DIDS, the mean 500/450 ratio was 1.71 ± 0.17, a value not significantly different from that recorded in the presence of 100 μM

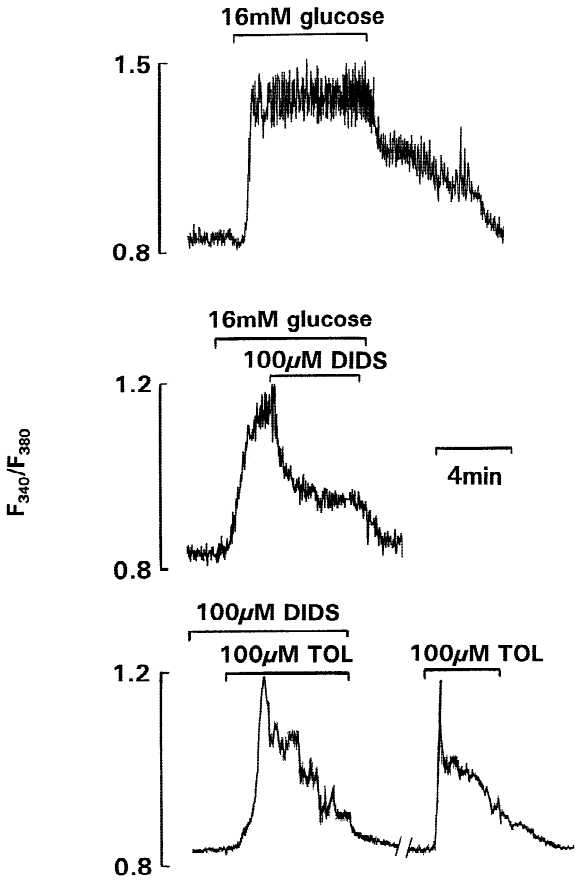

Fig. 2. Effects of DIDS on changes in $[Ca^{2+}]_i$ (assessed by F_{340}/F_{380} fluorescence ratio in β-cells loaded with fura-2) evoked by glucose (*upper and center panels*) and tolbutamide (*lower panel*). The two traces in the *lower panel* were from the same cell: similar results were obtained when the experiment was carried in reverse order. In each case, qualitatively similar results were obtained in five similar experiments.

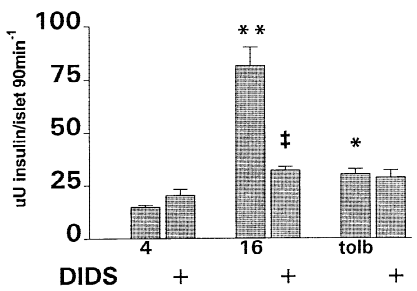

Fig. 3. Effects of 100 μM DIDS on insulin release from rat islets in the presence of 4 mM glucose (4), 16 mM glucose (16) and 4 mM glucose plus 100 μM tolbutamide (tolb). Data shown are mean ± SEM of 6–9 separate determinations. *$P < 0.05$ compared to column 1; **$P < 0.001$ compared to column 1; ‡$P < 0.001$ compared to column 3.

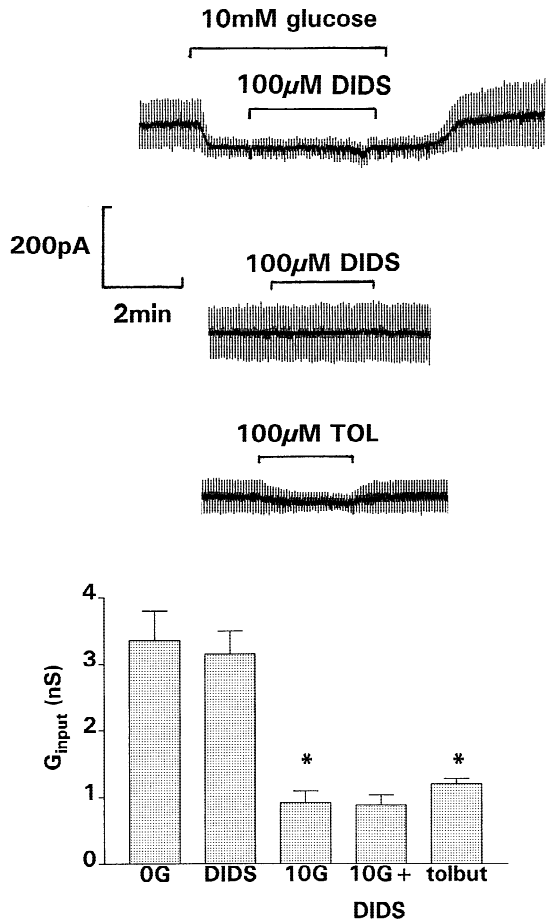

Fig. 4. Effects of DIDS on input conductance (G_{input}) in isolated rat pancreatic β-cells in the presence (*upper trace*) and absence (*middle trace*) of glucose. *Lower trace:* effect of 100 μM tolbutamide on G_{input}. Cells were voltage-clamped under perforated patch conditions at −70 mV and subjected to 50 msec pulses of ±10 mV. Data from 5 separate experiments (mean ± SEM) are summarized in the lower panel. *$P <$ 0.01 compared to column 1.

DIDS (1.64 ± 0.26; $n = 5$ in both cases). In contrast, alkalinization of the cell interior by application of 10 mM NH_4Cl resulted in a significant ($P < 0.01$) increase in the ratio to 2.99 ± 0.38. $Na^+/K^+/2Cl^-$ cotransport activity plays an essential role in regulatory volume increase (RVI) in HCO_3^--free conditions as used in the present study (Miley et al., 1998). Possible effects of DIDS on activity of the cotransporter were therefore assessed by measuring changes in cell volume following hypertonically-induced cell shrinkage. As shown in Fig. 6*B*, exposure of cells to a 33% hypertonic solution resulted in a rapid cell shrinkage followed by RVI. The latter response was unaffected in the presence of 100 μM DIDS, indicating that the drug did not affect activity of the $Na^+/K^+/2Cl^-$ cotransporter.

Discussion

In this study, we used a concentration of DIDS (100 μM) previously shown to be effective in inhibiting the vol-

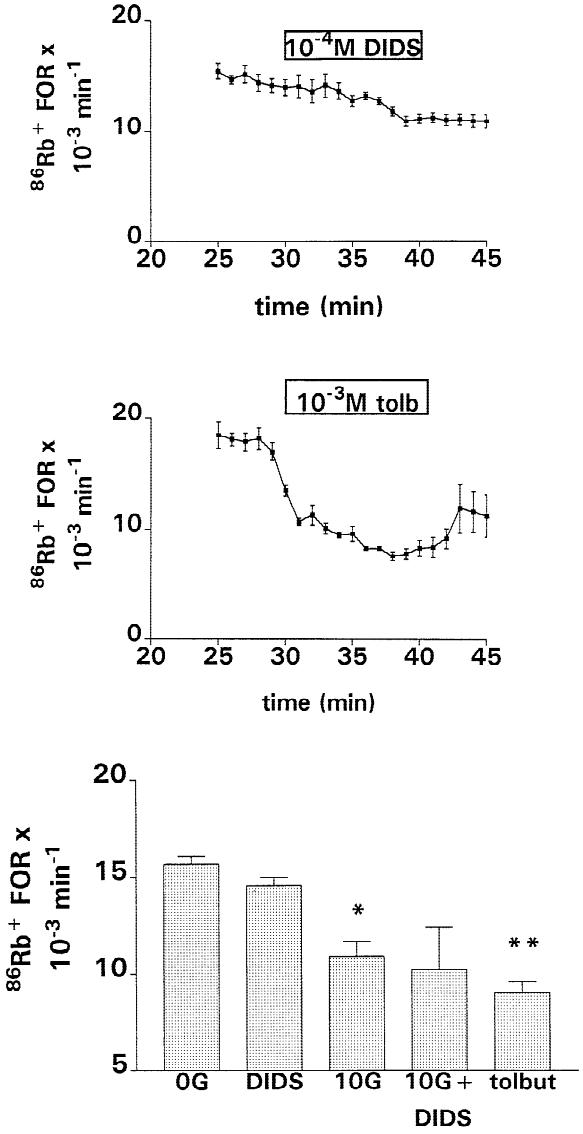

Fig. 5. Effect of DIDS (*upper panel*) and tolbutamide (*middle panel*) on the fractional outflow rate (FOR) of $^{86}Rb^+$ in preloaded, perifused rat islets in the absence of 10 mM glucose. Data from 6 separate experiments (mean ± SEM) are summarized in the *lower panel*. *$P < 0.01$; **$P < 0.001$ compared to column 1.

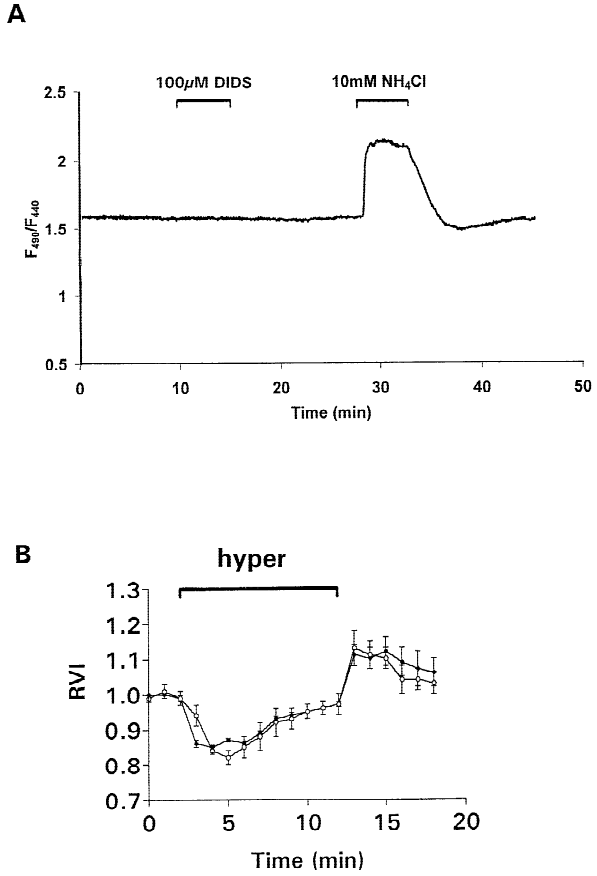

Fig. 6. (*A*) Effect of DIDS on intracellular pH (pH_i) assessed from the 500/450 nm fluorescence ratio in β-cells loaded with BCECF. The trace shown is typical of five similar experiments. (*B*) Regulatory volume increase (RVI) in β-cells following exposure to a 33% hypertonic solution (hyper) in the absence (○; $n = 5$) and presence (●; $n = 4$) of 100 μM DIDS.

ume-sensitive anion channel (Best et al., 1996*a* and *b*). Lower concentrations have been found to be poorly effective in inhibiting the inward current component (*unpublished observations*), while higher concentrations were avoided to minimize possible effects of the drug on other membrane channels (Furukawa et al., 1993) or transport systems (Aicken & Brading, 1984). The results demonstrate that DIDS selectively inhibits the effects of glucose on electrical activity, $[Ca^{2+}]_i$ and insulin release in rat pancreatic β-cells. These inhibitory effects were probably the result of a repolarization of the membrane potential by DIDS. Since DIDS failed to increase input conductance or the rate of $^{86}Rb^+$ efflux, it is unlikely that the repolarization was due to increased β-cell K^+ permeability. Indeed, the small reduction in $^{86}Rb^+$ efflux rate which was apparent after 5–10 min exposure to DIDS would be expected to exert a modest depolarizing influence. Since the inhibition by glucose of K_{ATP} channel activity requires metabolism of the sugar, it is also unlikely that DIDS exerted its inhibitory actions by interfering with glucose metabolism. The finding that DIDS specifically and reversibly inhibited glucose-stimulated β-cell activity would also argue against any nonspecific or toxic effects of the drug on β-cell function. The lack of effect of DIDS on electrical activity and changes in $[Ca^{2+}]_i$ in response to tolbutamide also indicates that the drug does not affect voltage-sensitive Ca^{2+} channels or any other aspect of cellular Ca^{2+} handling. DIDS has been reported to inhibit the Cl^-/HCO_3^- exchanger (Aicken & Brading, 1984) and can therefore influence intracellular pH. Indeed, in an earlier report, an enhancement by DIDS of glucose-induced β-cell electrical ac-

tivity in intact mouse islets was attributed to changes in intracellular pH resulting from an inhibition of Cl^-/HCO_3^- exchange (Pace & Tarvin, 1983). Similar findings and conclusions were reached by Eddlestone and Beigelman (1983) using another stilbene disulfonate, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS). In contrast to the above studies, HCO_3^- was deliberately omitted from the incubation medium in the present study in order to minimize Cl^-/HCO_3^- exchange, although modest changes in pH_i might persist under such conditions due to the generation of endogenous HCO_3^- from glucose metabolism. It was therefore confirmed that DIDS had no influence on intracellular pH, assessed by BCECF fluorescence, under the conditions used in the present study. Thus, it is extremely unlikely that the repolarization evoked by DIDS was due to inhibition of anion exchange and associated changes in intracellular pH. The presence or absence of HCO_3^- could, at least in part, explain the apparent discrepancy between the above reports and the results of the present study. The lack of effect of DIDS on RVI in hypertonically-shrunken cells also indicates that the drug did not inhibit glucose-induced β-cell activity by inhibiting $Na^+/K^+/2Cl^-$ cotransport.

We therefore suggest that the inhibition by DIDS of glucose-stimulated β-cell activity is the result of inhibition by the drug of the volume-sensitive anion channel (Kinard & Satin, 1995; Best et al., 1996*a*,*b*). It has been previously demonstrated that activation of this channel by hypotonically-induced cell swelling leads to depolarization (Best et al., 1996*a*), presumably due to increased Cl^- efflux. Consistent with this finding, intracellular $[Cl^-]$ is reported to be maintained at concentrations higher than its electrochemical equilibrium, possibly as a result of activity of a $Na^+/K^+/2Cl^-$ cotransporter (Sehlin, 1978). Thus, inhibition of the channel would be predicted to exert a repolarizing action as demonstrated in the present study.

The finding that DIDS inhibits glucose-stimulated β-cell function leads us to suggest that glucose induces β-cell depolarization and hence electrical and secretory activity, at least in part, by activating the volume-sensitive anion channel. Consistent with this suggestion, it has recently been shown that glucose causes β-cell swelling to an extent which could activate the volume-sensitive anion channel (Miley et al., 1997), while glucose generates an inward current which resembles that evoked during hypotonically-induced cell swelling (Best, 1997, 1999). Such a mechanism could also account for the K_{ATP} channel-independent modulation of β-cell electrical activity by glucose (Henquin, 1998).

In contrast to its inhibitory actions on glucose-stimulated β-cell activity, DIDS failed to affect the stimulatory actions of tolbutamide. We have previously demonstrated a similar lack of effect of DIDS on glibenclamide-stimulated β-cell function (Best & Benington, 1998). These findings are inconsistent with the suggestion that the volume-sensitive anion channel can be stimulated via the sulfonylurea receptor, based on the observation that glibenclamide could cause channel activation (Kinard & Satin, 1995). We have recently provided evidence that this effect of glibenclamide is a non-specific effect of this particular drug (Best & Benington, 1998).

In conclusion, the anion channel inhibitor DIDS impairs pancreatic β-cell function in response to glucose, but not to tolbutamide. It is suggested that this inhibitory effect of DIDS results from inhibition of the volume-sensitive anion channel. Thus, activation of this channel by glucose could be an important step in the induction of β-cell electrical and secretory activity.

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