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Methylglyoxal Causes Swelling and Activation of a Volume-Sensitive Anion Conductance in Rat Pancreatic β-Cells

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Abstract. Membrane potential and whole-cell current were studied in rat pancreatic β-cells using the 'perforated patch' technique and cell volume measured by a video-imaging method. Exposure of β-cells to the α-ketoaldehyde methylglyoxal (1 mM) resulted in depolarization and electrical activity. In cells voltage-clamped at −70 mV, this effect was accompanied by the development of inward current noise. In voltage-pulse experiments, methylglyoxal activated an outwardly rectifying conductance which was virtually identical to the volume-sensitive anion conductance previously described in these cells. Two inhibitors of this conductance, 4,4′-dithiocyanatostilbene-2,2′-disulfonic acid (DIDS) and 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), also inhibited the depolarization and inward current evoked by methylglyoxal. Methylglyoxal increased β-cell volume to a relative value of 1.33 after 10 min with a gradual return towards basal levels following withdrawal of the α-ketoaldehyde. None of the effects of methylglyoxal was observed in response to *t*-butylglyoxal which, unlike methylglyoxal, is a poor substrate for the glyoxalase pathway. Methylglyoxal had no apparent effect on β-cell K^+ channel activity. It is suggested that the metabolism of methylglyoxal to D-lactate causes β-cell swelling and activation of the volume-sensitive anion channel, leading to depolarization. These findings could be relevant to the stimulatory action of D-glucose, the metabolism of which generates significant quantities of L-lactate.

Key words: Islet — Pancreatic β-cells — Methylglyoxal — Cell volume — Anion channel

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Introduction

Methylglyoxal, a three-carbon α-ketoaldehyde, is formed spontaneously during glycolysis as a result of transformation of triose phosphates (Phillips & Thornalley, 1993). It is a cytotoxic substance due to its high reactivity with cellular proteins (Thornalley, 1990), and is effectively metabolized in cells via the ubiquitous glyoxalase pathway (*see* Thornalley, 1993 for review).

Previous studies in this laboratory have demonstrated that methylglyoxal has rapid and profound effects on pancreatic β-cells (Cook et al., 1998), namely depolarization of the membrane potential leading to electrical activity and a rise in cytosolic $[Ca^{2+}]$, probably as a result of Ca^{2+} entry via voltage-sensitive Ca^{2+} channels. These effects were accompanied by a marked intracellular acidification. None of the above actions of methylglyoxal was observed upon exposure to *t*-butylglyoxal which, unlike methylglyoxal, is a poor substrate for the glyoxalase pathway (Vander Jagt et al., 1975). This led us to suggest that the metabolism of methylglyoxal to D-lactate could be an important step leading to β-cell depolarization. We have previously shown that rat islet cells produced large quantities of D-lactate when incubated with methylglyoxal (Cook et al., 1998).

The purpose of the present study was to investigate the mechanism of β-cell activation by methylglyoxal by studying changes in whole-cell current which underlie the depolarization. Evidence is presented that methylglyoxal activates a volume-sensitive anion channel. It is suggested that this effect results from intracellular generation of D-lactate and β-cell swelling.

Materials and Methods

Rat pancreatic islets from Sprague-Dawley rats (either sex) were prepared by collagenase digestion. Islets were dispersed into single cells

and small clusters by 10–15 min. exposure to Ca^{2+}-free medium supplemented with 1 mM EGTA (*see* below). Cells were suspended in HEPES-buffered RPMI medium (Gibco, Paisley, Scotland) and cultured in polystyrene dishes for up to 10 days. Single, isolated β-cells were used for all experiments to avoid contaminating currents from adjacent electrically coupled cells. The incubation medium contained (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).

Changes in cell membrane potential and whole-cell current were measured using the 'perforated patch' configuration of the patch-clamp technique (Rae et al., 1991). The pipette solution contained K_2SO_4 (60); KCl (10), NaCl (10), HEPES-NaOH, (10; pH 7.2) and amphotericin B (240 μg/ml). Membrane potential was recorded using a List EPC-7 amplifier (List, Darmstadt, Germany) under current-clamp conditions. At certain intervals, the amplifier was switched to voltage-clamp in order to record whole-cell current at a holding potential of –70 mV. At this potential, voltage-dependent Ca^{2+} and Na^+ channels should be inactive and K^+ conductances minimal, whereas anionic currents should be accentuated. A number of current recordings were carried out under similar conditions except that K^+ in the pipette solution and Ca^{2+} in the bath solution were replaced by Cs^+ and Co^{2+} respectively in order to eliminate K^+ and Ca^{2+} currents. These conditions were also used for voltage-pulse experiments. In the latter, cells were held at –30 mV and exposed to 500 msec voltage pulses from –100 to +100 mV in 20 mV increments. Reversal potentials in these experiments were measured by switching from voltage-clamp to current-clamp. K_{ATP} channel activity in β-cells under perforated patch conditions was measured using the method previously described by Smith, Ashcroft & Rorsman (1990). Briefly, cells were held 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. In all cases, access resistance was <250 MΩ and whole-cell capacitance within the range 10–16 pF. All experiments were performed at 30–32°C. Efflux of $^{86}Rb^+$ from preloaded perifused rat islets was used to assess net K^+ channel activity essentially as described previously (Best et al., 1988). β-cell volume was measured using a video-imaging technique as described previously (Best, Sheader & Brown, 1996*b*). For volume measurements, cells were used on days 1–2 after preparation to avoid excessive 'flattening' of the cells.

Methylglyoxal and *tert*-butylglyoxal were prepared and purified according to the methods of McLellan and Thornalley (1992) and Fuson et al. (1939), respectively. All other chemicals were obtained from Sigma Chemical, Poole, UK.

Results

The application of 1 mM methylglyoxal to rat pancreatic β-cells caused a rapid and reversible depolarization, leading to electrical activity (Fig. 1*A*). This experiment also illustrates the changes in whole-cell current in response to methylglyoxal. In the absence of stimulation, resting membrane potential was approximately –60 mV and whole-cell current was close to zero. In contrast, the electrical activity evoked by methylglyoxal was associated with the generation of inward current noise. Withdrawal of methylglyoxal was followed by a gradual repolarization of the cell and a return of whole-cell current towards the resting level. Figure 1*B* illustrates the reversible induction of inward current noise in a single β-cell voltage-clamped at –70 mV and using a Cs^+-rich pipette solution to further eliminate K^+ currents. It should be noted that this reversibility was seldom observed if the cells were exposed to methylglyoxal for periods in excess of 5 min. As mentioned earlier, depolarization of the β-cell membrane potential was not observed in response to *t*-butylglyoxal (Cook et al., 1998). It was therefore of interest to note that the latter compound also elicited little if any inward current (Fig. 1*C*). This suggests that induction of this current requires metabolism of methylglyoxal via the glyoxalase pathway, and is not a nonspecific response to α-ketoaldehydes. The pattern of inward current noise induced by methylglyoxal closely resembles that previously reported in β-cells exposed to hypotonic solutions (Best, Miley & Yates, 1996*a;* Best, 1997). This suggested that the current could represent activation of a volume-sensitive anion conductance recently described in β-cells (Kinard & Satin, 1995; Best et al., 1996*a,b*). To further investigate this possibility, voltage-pulse experiments were performed. As shown in Fig. 2, methylglyoxal resulted in activation of an outwardly rectifying conductance virtually identical to that previously demonstrated in response to swelling in β-cells (Kinard & Satin, 1995; Best et al., 1996*a,b*). This effect was accompanied by a shift in reversal potential from –17.8 ± 2.0 mV to –11.9 ± 1.8 mV ($n = 8$, $P < 0.05$). Figure 2 (*inset*) also shows that activation of the conductance by methylglyoxal occurred progressively over a period of 6 min. Cells which were exposed to the α-ketoaldehyde for periods of 8 min or more frequently became 'leaky'. No activation of the conductance was observed upon application of *t*-butylglyoxal (Fig. 2).

We have previously demonstrated that the volume-sensitive anion channel in β-cells is inhibited by the anion channel blockers DIDS and NPPB (Best et al., 1996*b*). We therefore investigated whether these compounds affected the actions of methylglyoxal. As shown in Fig. 3, both DIDS and NPPB (10^{-4} M) reversibly inhibited the electrical activity evoked by methylglyoxal and also reduced the inward current. These findings are consistent with the suggestion that methylglyoxal activates the volume-sensitive anion channel. This suggestion raised the further possibility that the α-ketoaldehyde causes β-cell swelling. As shown in Fig. 4*A,* this was in fact the case. Exposure of cells to methylglyoxal resulted in a progressive increase in cell volume to a relative value of 1.33 ± 0.03 ($n = 4$) at 10 min. Following withdrawal of methylglyoxal, cell volume gradually returned towards basal values, although more prolonged exposure often resulted in visible cell bursting. Exposure of cells to *t*-butylglyoxal, in contrast, had no effect on cell volume (Fig. 4*B*).

To examine the effects of methylglyoxal on β-cell K^+ channel activities, K_{ATP} channel activity in isolated rat β-cells and $^{86}Rb^+$ efflux from preloaded perifused

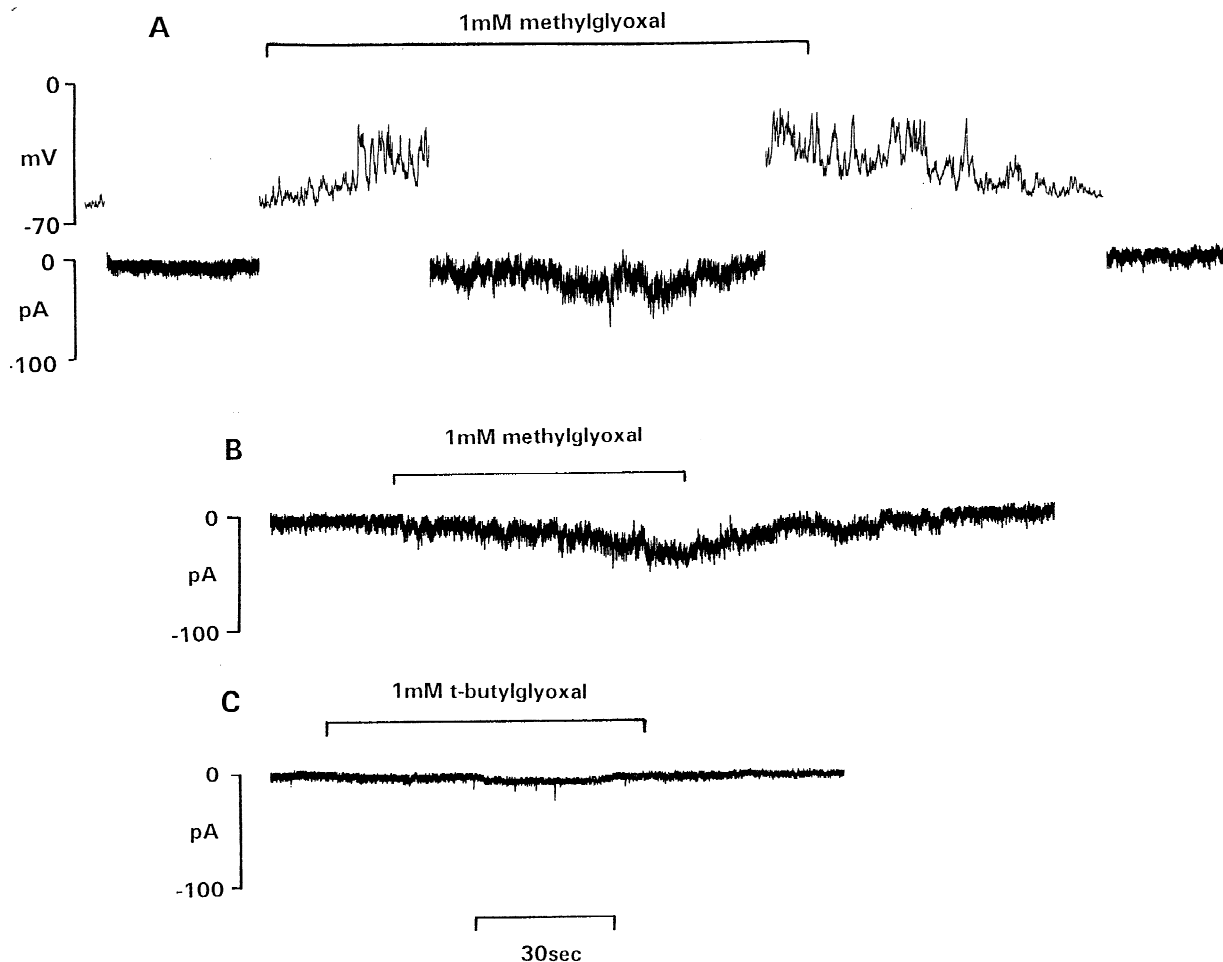

Fig. 1. (*A*) Effect of methylglyoxal on membrane potential (upper trace) and whole-cell current (lower trace) in a single isolated rat pancreatic β-cell under perforated patch conditions. A K^+-rich pipette solution was used. The amplifier was switched from current-clamp (zero current) to voltage-clamp (holding potential −70 mV) for the periods indicated. (*B*) Effect of methylglyoxal and (*C*) *t*-butylglyoxal on whole-cell current in single rat β-cells voltage clamped at −70 mV under perforated patch conditions and using a Cs^+-rich pipette solution. The traces are representative of a total of 4–7 cells showing essentially similar results.

islets were measured. In the former case, K_{ATP} channel activity in the presence of 4 mM glucose was estimated at 1.75 ± 0.15 and 1.85 ± 0.23 pS (n = 4) in the absence and presence of 1 mM methylglyoxal, respectively. Similarly, the fractional outflow rates for $^{86}Rb^+$ in the absence and presence of methylglyoxal were 0.024 ± 0.001 and 0.026 ± 0.004 min^{-1} (n = 6) respectively. Taken together, these findings suggest that alterations in K^+ channel activities are unlikely to play any significant role in β-cell depolarization by methylglyoxal.

Discussion

The results of the present study provide insight concerning the regulation and role of the volume-sensitive anion conductance in pancreatic β-cells. It has been previously demonstrated that this conductance can be activated in intact β-cells by exposure to hypotonic solutions (Best et al., 1996*a*). This in turn generates a net inward, depolarizing current, presumably because E_{Cl} is positive with respect to the resting membrane potential (*see* Best, Brown & Tomlinson, 1997 for discussion of this topic). The present study demonstrates that activation of this conductance can also be induced by the α-ketoaldehyde methylglyoxal. It is likely that this effect of methylglyoxal resulted, at least in part, from the marked increase in β-cell volume. The increase in cell volume, like activation of the anion conductance, occurred progressively and to a considerable degree (up to 33% after 10 min). It has been previously demonstrated that swelling the cells to a similar extent by exposure to a hypotonic solution causes a comparable activation of the anion conductance under perforated patch conditions (Best et al.,

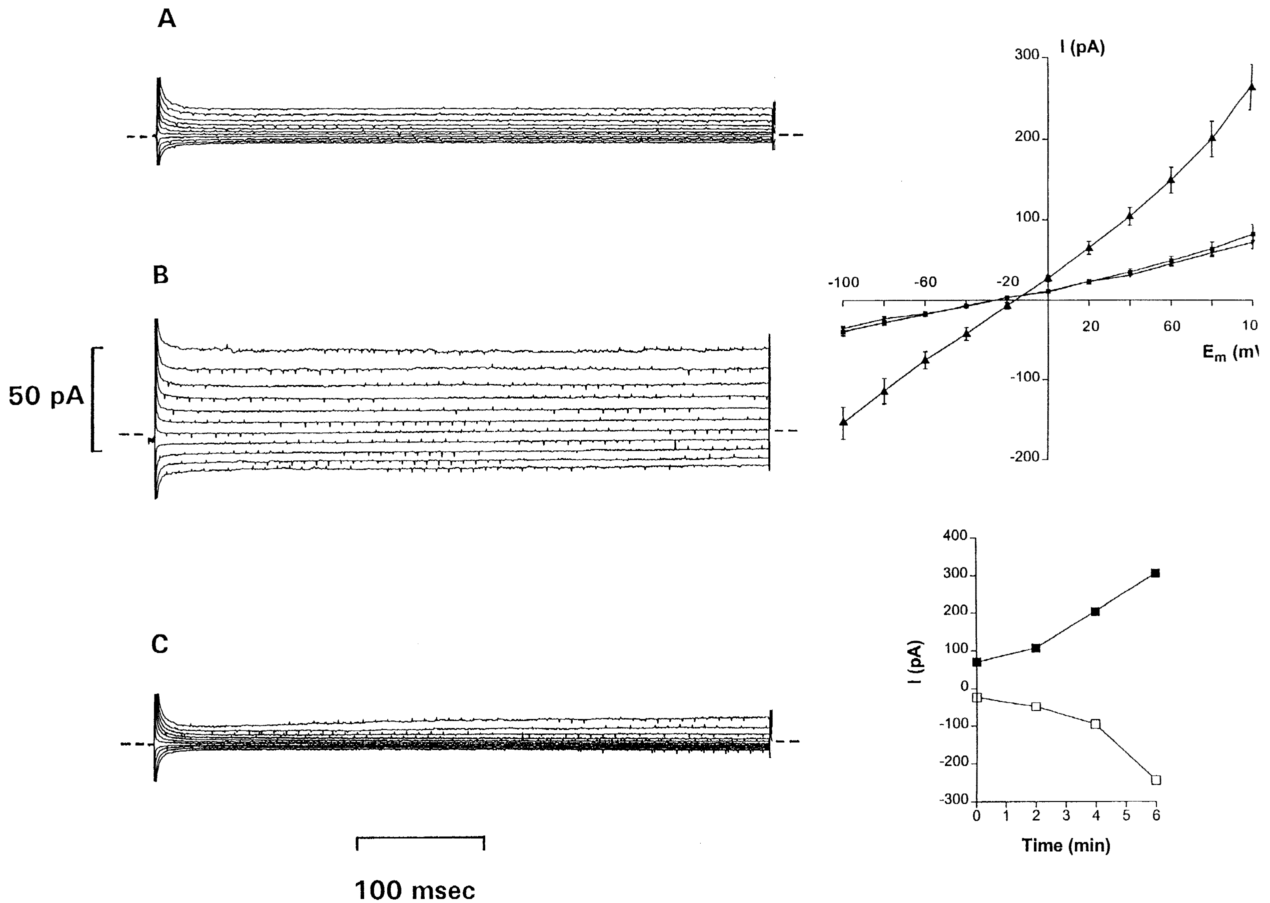

Fig. 2. Activation of outwardly rectifying conductance in single isolated rat β-cells by methylglyoxal under perforated patch conditions. K^+ in the pipette solution and Ca^{2+} in the bath solution were replaced with Cs^+ and Co^{2+}, respectively. Cells were held at −30 mV and exposed to 500 msec voltage pulses from −100 to +100 mV. *Left panel:* response of one typical cell; (*A*) basal, (*B*) 1 mmol/L methylglyoxal (4 min), (*C*) 1 mmol/L *t*-butylglyoxal (4 min). *Right panel:* Current voltage relationships; ■ basal, ▲ methylglyoxal, ▼ *t*-butyl-glyoxal. Each point represents the mean ± SEM of four different cells. The zero current levels are represented by dotted lines. *Inset:* time-course of activation of the conductance. The points represent amplitudes of inward (−ve) and outward (+ve) currents evoked by pulses of −100 and +100 mV, respectively.

1996*a*). The suggestion that increased cell volume leads to activation of the anion conductance and subsequent depolarization is supported by the observed temporal relationship between these events. It has been shown that a small change (~10%) in cell volume is sufficient to activate the anion conductance and cause depolarization (Best, 1997; Miley et al., 1997). Given the high electrical resistance of the β-cell, a relatively small inward current would be sufficient to cause depolarization (*see* Ashcroft & Rorsman, 1989).

The observation that methylglyoxal causes β-cell swelling raises the question of how this effect is brought about. The finding that *t*-butylglyoxal, a poor substrate for the glyoxalase pathway (Vander Jagt et al., 1975), failed to affect cell volume (or activate the cells) suggests that the metabolism of methylglyoxal via this pathway to D-lactic acid could be a necessary step. It has been shown that β-cells express very low lactic acid transport activity (Best, Trebilcock & Tomlinson, 1992), so that significant accumulation of D-lactate would be predicted in β-cells exposed to methylglyoxal. Together with an increase in intracellular $[Na^+]$ which might be expected to arise from Na^+/H^+ exchange, accumulation of D-lactate and osmotic entry of water would be predicted to lead to increased cell volume.

A notable feature of activation of the volume-sensitive anion conductance by methylglyoxal was the relatively larger increase in inward current compared to that recorded when cell swelling was evoked by exposure to hypotonic solutions (Best et al., 1996*a*). It is possible that formation of D-lactate from methylglyoxal could also explain this phenomenon. D-lactate shows a significant permeation through the anion channel ($P_{lactate}/P_{Cl}$ approximately 0.7; Cook et al., 1998) so that exit of this anion via the anion channel would generate an inward current. This suggestion would also be consistent with the positive shift in reversal potential of the conductance when activated by methylglyoxal. Since

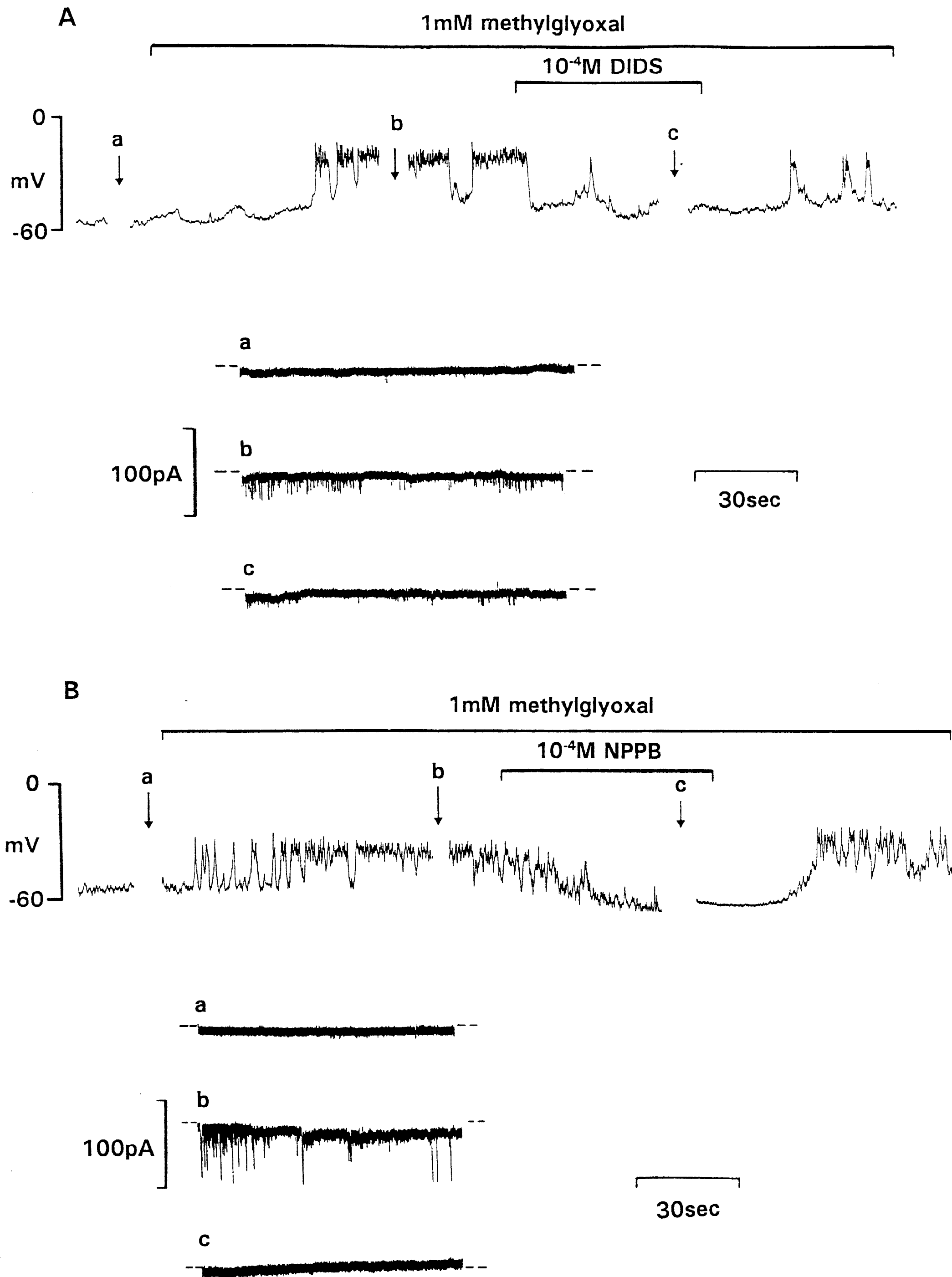

Fig. 3. Effects of DIDS (*A*) and NPPB (*B*) on methylglyoxal-induced electrical activity and whole-cell current in single β-cells under perforated patch conditions. A K^+-rich pipette solution was used. At the points marked a, b and c in the membrane potential recordings, the amplifier was switched from current-clamp (zero current) to voltage-clamp (holding potential –70 mV). Each trace is representative of a total of 4 similar experiments showing essentially similar results.

the intracellular lactate concentration is not known with any accuracy, it is not possible to calculate whether this shift in reversal potential is consistent with the calculated $P_{lactate}$ of the conductance.

Thus, we propose that metabolism of methylglyoxal via the glyoxalase pathway to D-lactate, and intracellular accumulation of the latter (possibly together with Na^+) leads to β-cell swelling. This results in activation of the

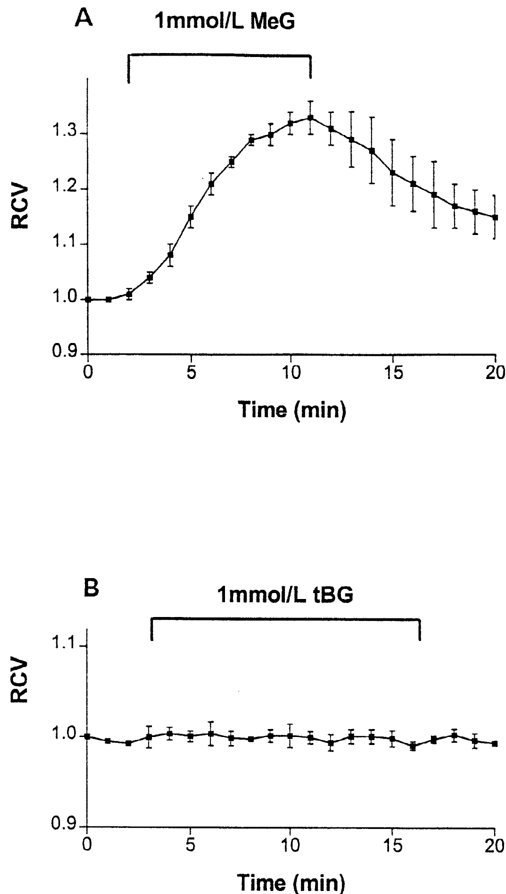

Fig. 4. Effect of methylglyoxal (MeG; A) and *t*-butylglyoxal (tBG; *B*) on relative cell volume (RCV) in single isolated rat β-cells, assessed by video-imaging. Each point represents the mean ± SEM from 4 different cells.

volume-sensitive anion conductance, generating an inward current. Efflux of D-lactate via the conductance could contribute towards this inward current, which causes depolarization of the cell membrane. As in our previous study (Cook et al., 1998), the concentrations of methylglyoxal used in these experiments are considerably greater than levels of free methylglyoxal detectable in the circulation. However, a recent report suggests that cellular levels of protein-bound methylglyoxal could approach the millimolar concentrations used in our studies (Chaplen, Fahl & Cameron, 1998), so that our findings could be of potential pathophysiological significance. In addition, the present study demonstrates that α-ketoaldehydes are potentially useful tools for probing the mechanisms controlling β-cell membrane potential and ion fluxes. It should be noted in particular that since methylglyoxal metabolism does not generate or consume nucleotide triphosphates, the depolarization evoked by the α-ketoaldehyde is unlikely to be accompanied by any significant acute changes in cellular levels of ATP or any other nucleotide. The above findings do imply, however, that the volume-sensitive anion conductance can be activated by the intracellular formation of D-lactate. This could be relevant to the mechanism of activation of the β-cell by glucose, the metabolism of which generates significant quantities of L-lactate (Malaisse et al., 1976; Best et al., 1989). In view of this, it is worth noting that both L- and D-lactate have been shown to cause depolarization in insulin-secreting cells (Best et al., 1989; Lynch et al., 1991; Best et al., 1994). Furthermore, the inward current noise induced by methylglyoxal resembles not only that evoked by hypotonically induced cell swelling but also shows similarity to an inward current induced by lactate (Best et al., 1994) and by raised concentrations of glucose (Best, 1997).

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