Biochemical Pharmacology, Vol. 55, pp. 1361–1367, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(97)00619-9

Effects of Methylglyoxal on Rat Pancreatic β-Cells

L. J. Cook, J. Davies,* A. P. Yates,§ A. C. Elliott,† J. Lovell,‡ J. A. Joule,‡ P. Pemberton,§ P. J. Thornalley‖ and L. Best*¶*

Departments of *Medicine, †Biological Sciences and ‡Chemistry, University of Manchester, Oxford Road, Manchester, M13 9WL; §Department of Clinical Biochemistry, Manchester Royal Infirmary; and ‖Department of Chemistry and Biological Chemistry, University of Essex, Wivenhoe Park, Colchester, CO4 3SQ, U.K.

ABSTRACT. The addition of the α-ketoaldehyde methylglyoxal (0.5 or 1 mmol/L) to single isolated rat pancreatic β-cells caused a rapid, marked depolarization resulting in electrical activity. This effect of methylglyoxal on β-cell was reversible upon removal of the α-ketoaldehyde, and could be inhibited by the anion channel blockers 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS) and 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB). Methylglyoxal also resulted in elevated cytosolic [Ca^{2+}] and an intracellular acidification in intact rat islets. In perifused islets, methylglyoxal provoked a modest, transient stimulation of secretion but inhibited glucose-induced insulin release. Incubation of islets with methylglyoxal resulted in the formation of large quantities of D-lactate, indicating metabolism of the α-ketoaldehyde via the glyoxalase pathway. The effects of methylglyoxal on β-cell membrane potential, cytosolic [Ca^{2+}] and intracellular pH were also observed in response to phenylglyoxal which is also effectively metabolized via the glyoxalase pathway. However, *t*-butylglyoxal which is poorly metabolized via the glyoxalase pathway, caused neither depolarization of the membrane potential nor intracellular acidification, but did inhibit glucose-induced insulin release. These findings suggests that the depolarization and acidification evoked by methyl- and phenylglyoxal are dependent upon their metabolism via the glyoxalase pathway. The possible mechanisms coupling α-ketoaldehyde metabolism via the glyoxalase pathway with membrane depolarization are discussed. BIOCHEM PHARMACOL **55**;9: 1361–1367, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. pancreatic islet; electrical activity; cytosolic [Ca^{2+}]; intracellular pH; stimulus-response coupling, methylglyoxal

Methylglyoxal is a three carbon α-ketoaldehyde formed from the spontaneous transformation of triose phosphates [1–3]. It is therefore an intrinsic component of glucose metabolism via the glycolytic pathway and has consequently been found in elevated concentrations in hyperglycaemia *in vitro* [4] and associated with diabetes mellitus [1, 5].

In virtually all cells and tissues studied, methylglyoxal is metabolized, via S-D-lactoylglutathione, to D-lactate by means of the enzymes glyoxalase I and II [1]. The maintenance of low levels of methylglyoxal by this glyoxalase pathway is likely to be of crucial importance since, like other α-ketoaldehydes, methylglyoxal is highly reactive, and can bind to and modify proteins by chemical interaction with arginyl, lysyl and sulphydryl groups [1, 6, 7]. As a result of such interactions with cellular proteins, α-ketoaldehydes can influence numerous aspects of cellular biochemistry and physiology including plasma membrane ATP-ases [7, 8], certain glycolytic enzymes [9] and inhibition of protein synthesis and cell growth [1].

In the present study, we describe rapid and pronounced effects on β-cell electrical activity and intracellular cation concentrations of the α-ketoaldehydes methylglyoxal and phenylglyoxal. A preliminary account of part of this work has been communicated to the Physiological Society [10].

MATERIALS AND METHODS

Rat islets were isolated from male or female Sprague–Dawley rats (300–400 g.) by collagenase digestion. For the patch-clamp experiments, islets were dispersed into single cells by a brief incubation in Ca^{2+}-free medium supplemented with 1 mM of EGTA. The incubation medium used for all experiments contained (in mmol/L) NaCl (135), KCl (5), $MgSO_4$ (1.2), $CaCl_2$ (1.2), NaH_2PO_4 (1.0), glucose (4) and HEPES (20) buffered to pH 7.4 with NaOH.

Changes in membrane potential were measured in single isolated β-cells superfused at room temperature using the "perforated patch" configuration of the patch-clamp technique, essentially as described previously [11]. Cytosolic calcium concentration ($[Ca^{2+}]_i$) was assessed from the

¶ Corresponding author: L. Best, Multipurpose Building, Manchester Royal Infirmary, Oxford Road, Manchester, M13 9WL, UK. Tel. +161 276 4338; FAX +161 275 5600; E-mail: lbest@fs1.scg.man.ac.uk.

Received 1 July 1997; accepted 16 October 1997.

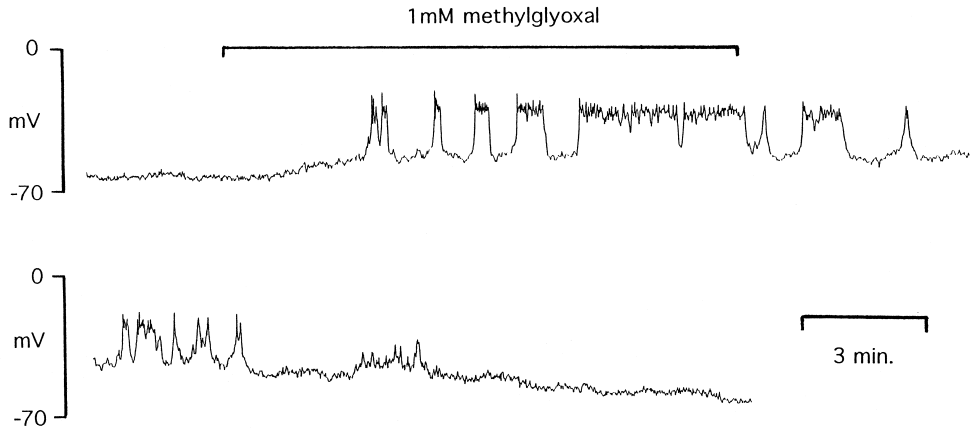

FIG. 1. Effects of 1 mM of methylglyoxal on membrane potential in a single rat β-cell under perforated patch conditions. The medium contained 4 mM of glucose. The patch pipette contained a K^+-rich solution and amphotericin (240 μg/mL). The trace is representative of six other similar recordings.

340:380 fluorescence ratio at 510 nm emission in intact islets loaded with fura-2, using a fluorescence microscope system [12]. No attempt was made to calibrate the fura-2 signals since this procedure is known to be extremely unreliable (for discussion of this topic, see ref. 12). Intracellular pH (pH_i) was measured in islets using the fluorescence microscope system. Islets were loaded with BCECF-AM (2 μmol/L) for 10 min at room temperature. Changes in pH_i were measured from the 500:450 fluorescence ratio at 530 nm emission. The signals were calibrated using a buffer containing 140 mmol/L of K^+ and nigericin (10 μmol/L) at different pH values [13]. Insulin release was measured by radioimmunoassay either from batch incubations of 25 islets in 1 mL of medium or from groups of 25 islets continuously perifused at a rate of 1 mL/min. The islets were perifused with control medium for a total of 30 min prior to the addition of methylglyoxal. A control experiment was carried out to ascertain that the insulin radioimmunoassay was not affected by the presence of the α-ketoaldehyde. Fluorescence and secretion experiments were carried out at 37° and electrophysiological recordings at 30°. The metabolism of methylglyoxal via the glyoxalase pathway was assessed by the spectrophotometric assay of D-lactate [14] produced by batches of 50 islets incubated for 45 min.

Fura-2 acetomethoxy (AM) ester was obtained from Cambridge Bioscience and BCECF-AM from Calbiochem. D-lactate, D-lactate dehydrogenase (staphylococcus epidermidis), phenylglyoxal and DIDS were supplied by the Sigma Chemical Co. NPPB was a generous gift from Professor R. Greger, University of Frieburg, Germany. ^{125}I-Insulin was supplied by Amersham International. Pure methylglyoxal was prepared and purified using the method of McLellan and Thornalley [15] and calibrated using an end-point spectrophotometric assay with glyoxalase I and II (Sigma) [14]. *Tert*-butylglyoxal was prepared according to the method of Fuson *et al.* [16].

RESULTS

The effect of methylglyoxal on membrane potential in single isolated β-cells is shown in Fig. 1. The cells' resting membrane potential under these conditions was −65 to −70 mV. The addition of methylglyoxal (1 mmol/L) caused a rapid, pronounced depolarization leading to the generation of electrical activity, consisting of bursts of action potentials (Fig. 1). The withdrawal of methylglyoxal resulted in a reduction in electrical activity and a gradual repolarization of the cells to the resting membrane potential. In a number of cells, a similar effect was also produced by 0.5 mmol/L of methylglyoxal although a lower concentration (0.1 mmol/L) had either no effect or otherwise caused a small depolarization with no electrical activity (not shown). The effect of methylglyoxal on β-cell electrical activity was markedly inhibited by application of the anion channel blockers DIDS or NPPB (Fig. 2).

A marked depolarization of the membrane potential was also observed in β-cells exposed to phenylglyoxal (Fig. 3A). In this case, the effect was invariably more pronounced than that elicited by methylglyoxal, resulting in a 'silent' depolarization with no accompanying bursts of action potentials. Since both methyglyoxal and phenylglyoxal are effective substrates for the glyoxalase system [17], we next investigated whether β-cell membrane potential was affected by *t*-butylglyoxal which is a relatively poor substrate for yeast glyoxalase 1 [17]. As shown in Fig. 3B, the addition of 1 mol/L of *t*-butylglyoxal to β-cells resulted in a reversible hyperpolarization rather than a depolarization of the membrane potential. A depolarization could, however, be elicited by 25 mM of K^+ in these cells.

Methylglyoxal and phenylglyoxal were also found to increase $[Ca^{2+}]_i$, assessed by fura-2 fluorescence, in intact rat islets (Fig. 4). At a concentration of 1 mmol/L, methylglyoxal caused a marked, rapid increase in $[Ca^{2+}]_i$

Abbreviations: BCECF, 2′,7′-bis(carboxyethyl)-5′(6′) carboxyfluorescein; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid; GSH, reduced glutathione; NPPB, 5-nitro-2-(3-phenylpropylamino) benzoic acid.

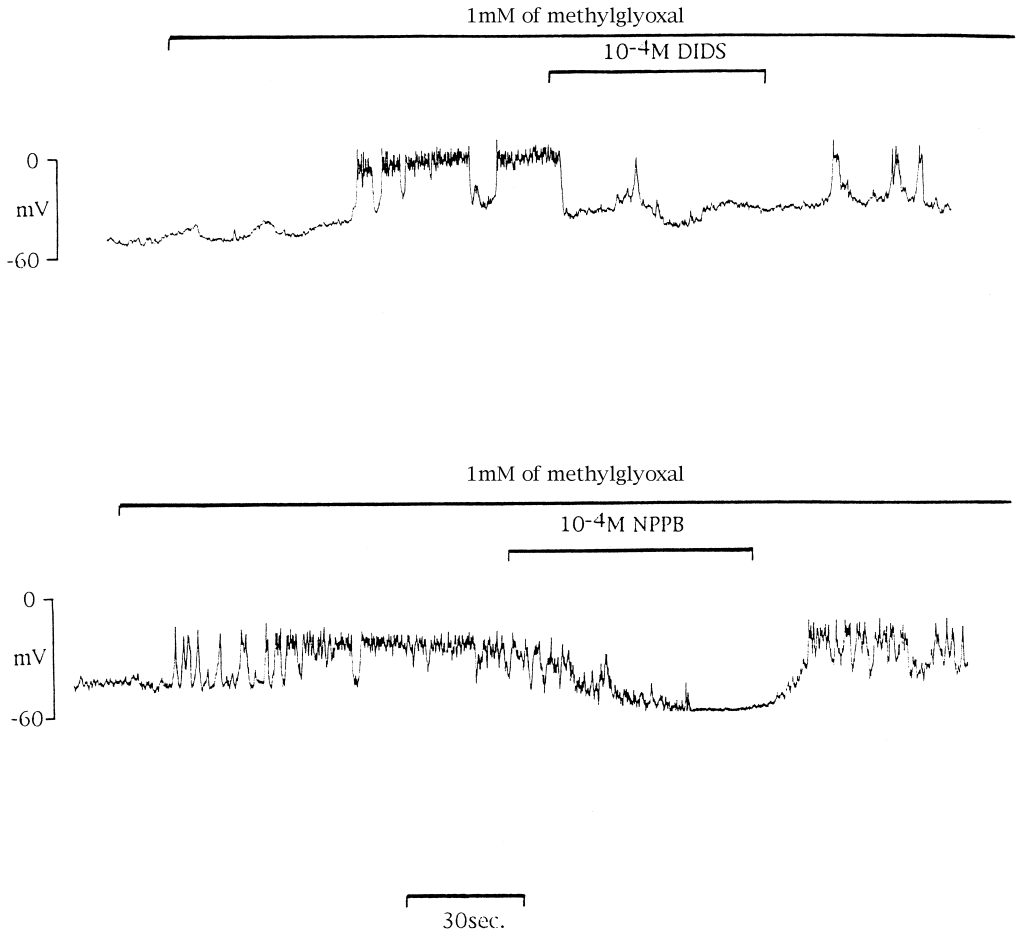

FIG. 2. The effects of 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS; 10^{-4} M, upper trace), and 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB; 10^{-4} M, lower trace) on electrical activity induced by 1 mM of methylglyoxal in a single rat β-cell. Each trace is representative of 2–3 similar recordings.

(Fig. 4A). The effect of a depolarizing concentration of K^+ (25 mmol/L) is shown for comparison. Phenylglyoxal was also found to raise $[Ca^{2+}]_i$ in islet cells (Fig. 4B). Consistent with its depolarizing action, phenylglyoxal had an extremely pronounced effect on $[Ca^{2+}]_i$. In contrast to methylglyoxal, the effect of phenylglyoxal on $[Ca^{2+}]_i$ was found to be irreversible. This could be due to inhibition of Ca^{2+}-ATPase by this α-ketoaldehyde [7, 8]. Addition of *t*-butylglyoxal to rat islets failed to increase $[Ca^{2+}]_i$ (Fig. 4C), a finding consistent with the observed lack of depolarization in response to this compound.

Figure 5 shows the effects of α-ketoaldehydes on pHi in intact rat islets, as assessed by BCECF fluorescence. Methylglyoxal caused a rapid, pronounced and reversible intracellular acidification (Fig. 5A). In the case of phenylglyoxal, the decrease in pHi appeared to be biphasic, the rapid early phase followed by a more gradual prolonged acidification (Fig. 5B). However, in contrast, *t*-butylglyoxal failed to induce an acidification in rat β-cells, although the subsequent application of the weak acid acetate resulted in a prompt fall in pHi followed by a slow recovery (Fig. 5C).

The secretion of insulin from islets in 'static' incubations was not significantly affected by 1 mmol/L of methylglyoxal (Table 1). However, under these conditions, methylglyoxal significantly reduced the stimulatory effect of 20 mmol/L of glucose. A marked inhibition of glucose-induced insulin release was also observed in response to *t*-butylglyoxal (Table 1). In order to study dynamic effects of methylglyoxal on insulin release, a continuous perifusion system was employed. As shown in Fig. 6, 1 mmol/L of methylglyoxal caused a modest and transient rise in the rate of insulin release. Thus, the relative amount of insulin released during the four minutes following the addition of methylglyoxal was 148 ± 7% of the control value of 100 ± 3% ($N = 8$, $P < 0.001$). In addition, the relative amount of insulin released at the peak of the response to the α-ketoaldehyde (5 min; 197 ± 43%) was significantly greater than the above mean control value ($P < 0.05$).

The formation of D-lactate from 1 mmol/L of methylglyoxal in batches of 50 rat islets amounted to 74.2 ± 16.6 pmol/min per islet ($N = 4$). This was significantly greater ($P < 0.001$) than the corresponding amount of D-lactate formed in the absence of added methylglyoxal (0.30 ± 0.01 pmol/min per islet; $N = 4$). Thus, rat islet cells appear to have an active glyoxalase system for the metabolism of

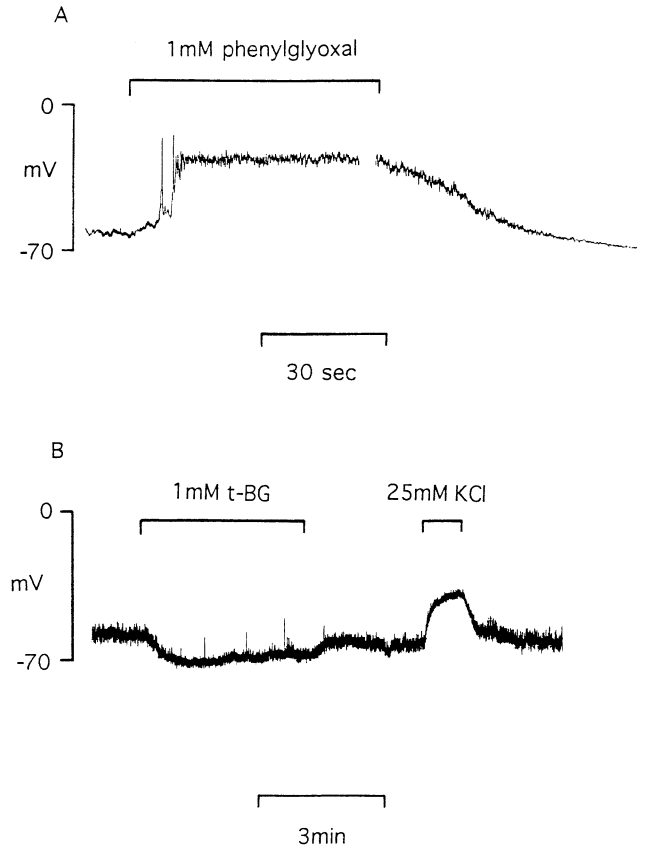

FIG. 3. The effects of phenylglyoxal (A) and *t*-butylglyoxal (B) on membrane potential in single rat β-cells under perforated patch conditions. Experimental conditions as in Fig. 1. The break in the recording in (A) represents a period of one minute. Note the difference in time scale in (B). Each trace is representative of those from two other experiments showing essentially similar results.

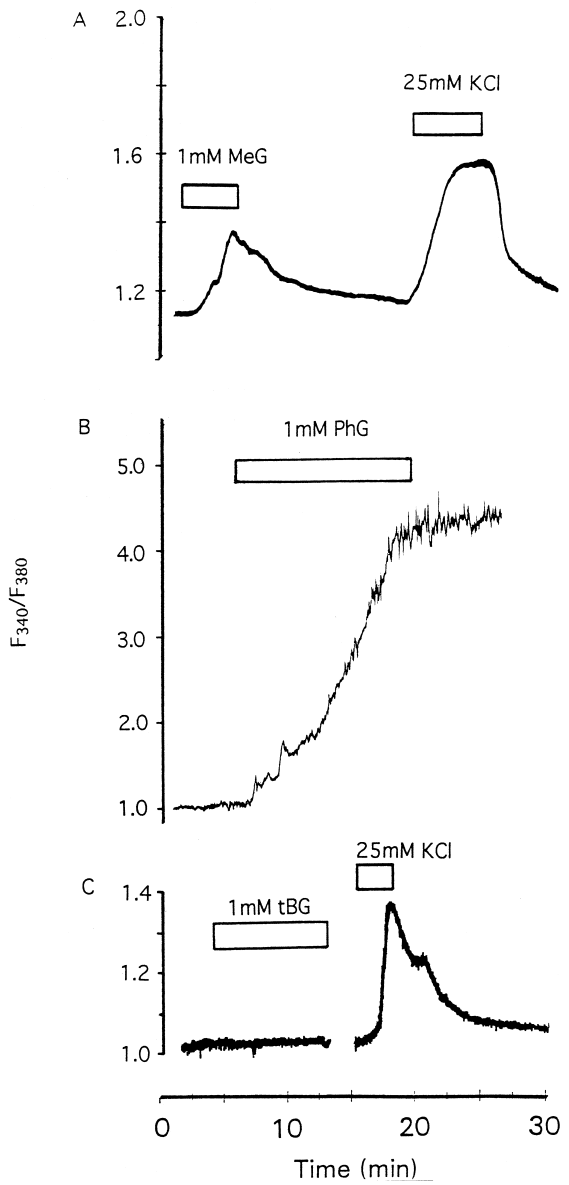

FIG. 4. The effects of methylglyoxal (MeG; A), phenylglyoxal (PhG; B) and *t*-butylglyoxal (tBG; C) on $[Ca^{2+}]_i$, assessed from the 340:380 fluorescence ratio, in rat islets loaded with fura-2. The medium contained 4 mM of glucose. The effect of a depolarizing concentration of K^+ (25 mmol/L) is shown in A and C for comparison. The break in trace C is approximately 4 min. The traces are representative of those from 2–5 other similar experiments.

methylglyoxal. It should be noted that this assay is specific for D-lactate and does not measure L-lactate formed from the glycolytic metabolism of D-glucose.

DISCUSSION

This study demonstrates that the α-ketoaldehydes methylglyoxal and phenylglyoxal exert rapid and marked effects on insulin-secreting cells, namely depolarization, a rise in $[Ca^{2+}]_i$ and intracellular acidification. It is likely that the rise in $[Ca^{2+}]_i$ is the direct result of depolarization and opening of voltage-sensitive Ca^{2+} channels. However, the mechanism whereby these α-ketoaldehydes initiate β-cell depolarization is uncertain. The finding that methylglyoxal is metabolized in islet cells via the glyoxalase pathway to D-lactate raised the possibility that the depolarization might be coupled in some way to metabolism of the α-ketoaldehyde. Phenylglyoxal has also been reported to be an effective substrate for the glyoxalase pathway [17] and was, if anything, more effective than methylglyoxal in causing depolarization and increased $[Ca^{2+}]_i$. It was consequently interesting to note that *t*-butylglyoxal, which is a poor substrate for the glyoxalase pathway [17], was ineffective in depolarizing β-cells, but rather caused a hyperpolarization. It should be borne in mind that the ineffectiveness of *t*-butylglyoxal as a substrate for glyoxalase I has so far only been documented in yeast. However, the fact that both methyl- and phenylglyoxal induced an intracellular acidification in islet cells, whereas *t*-butylglyoxal did not, suggests that metabolism of the latter compound via this pathway is relatively slow. Thus, it is suggested that depolarization of β-cells by methyl- and phenylglyoxal is dependent upon their metabolism via the glyoxalase pathway.

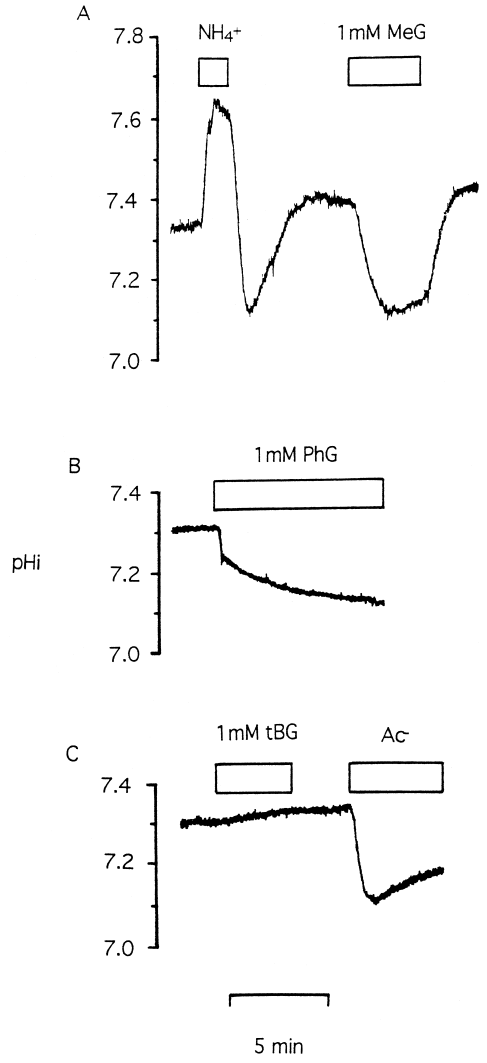

FIG. 5. The effects of methylglyoxal (MG; A), phenylglyoxal (PhG; C) and *t*-butylglyoxal (tBG; C) on pH_i, assessed from the 500/450 fluorescence ratio, in rat islets loaded with BCECF. The medium contained 4 mM of glucose. The application of either 10 mM of NH_4Cl (A) or 10 mM of NaAc (C) was carried out routinely to confirm that the cells' pH regulatory mechanisms were intact. The traces are representative of those from 2–3 other similar experiments.

A possible mechanism coupling α-ketoaldehyde metabolism to depolarization of the plasma membrane could involve intracellular accumulation of metabolites of these compounds (D-lactate and phenylglycolate formed from methyl- and phenylglyoxal respectively) and efflux of these metabolites from the cell via a conductance pathway. We have previously shown that rat β-cells do not express a lactic acid transport system [18], but are equipped with a non-selective anion conductance [19] which has a high permeability to both L- and D-lactate and a number of other organic metabolites (for example, $P_{D\text{-lact.}}/P_{Cl} \simeq 0.7$; our unpublished observations). Thus, efflux of an organic acid via this conductance would generate an inward current which could be responsible for depolarizing the cell. We have previously suggested that efflux of L-lactate (the major metabolite of glucose in β-cells) via this conductance could, in a similar way, couple glycolysis to β-cell depolarization [20]. The finding that the depolarizing action of methylglyoxal was reversed by DIDS and NPPB, two blockers of anion channels, adds further support to this mechanism, although the unproven specificity of these compounds should be borne in mind when interpreting these observations.

TABLE 1. Effects of methylglyoxal (MG; 1 mmol/L) and *t*-butylglyoxal (tBG; 1 mmol/L) on insulin release in batch incubations of 25 rat islets

Conditions	μU/islet in 90 min
4 mmol/L of glucose	27.3 ± 2.5 (4)
4 mmol/L of glucose + MG	38.9 ± 4.8 (4)
4 mmol/L of glucose + tBG	10.9 ± 2.9 (4)
	$P < 0.02$ vs line 1
20 mmol/L of glucose	71.7 ± 5.4 (4)
	$P < 0.001$ vs line 1
20 mmol/L of glucose + MG	49.1 ± 4.4 (4)
	$P < 0.02$ vs line 4
20 mmol/L of glucose + tBG	15.3 ± 3.5 (4)
	$P < 0.001$ vs line 4

The numbers are means ± SEM; the figures in parentheses represent the numbers of individual determinations. Statistical significance was ascribed using Student's *t*-test.

In order to further assess the role of metabolism of α-ketoaldehydes in β-cell depolarization, we investigated during the present study the effects of S-*p*-bromobenzylglutathione, an inhibitor of glyoxalase 1. At concentrations of 100–200 μM, this compound was found to inhibit production of D-lactate from methylglyoxal by up to 80%. However, at such concentrations, this drug was itself found to

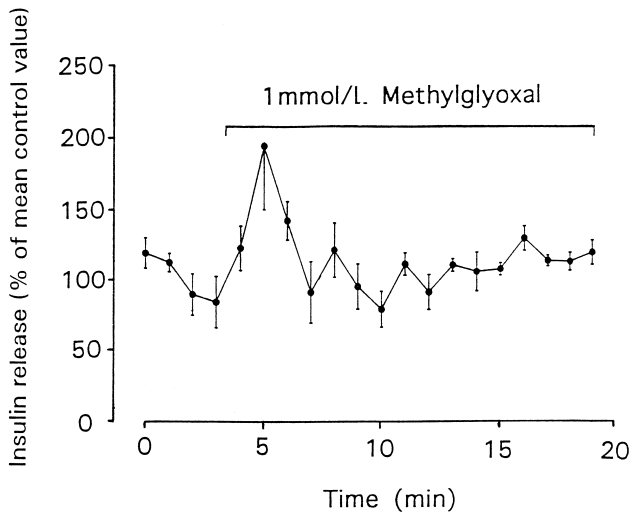

FIG. 6. The effect of methylglyoxal on insulin release from groups of 25 perifused rat islets. The medium contained 4 mM of glucose. Each point represents the mean ± SEM of 8 separate determinations. The islets were perifused for 30 min prior to the addition of methylglyoxal; only the last four 'control' points are shown.

rapidly depolarize the cells, so that its effects on α-ketoaldehyde-induced depolarization could not be investigated.

As mentioned above, the most probable explanation for the marked intracellular acidification in β-cells observed upon exposure to methyl- or phenylglyoxal is conversion via the glyoxalase pathway to D-lactic acid or phenylglycolic acid, respectively. In this context, it should be noted that rat β-cells do not express an active lactic acid transport system [18]. In view of the pronounced depolarization and increased $[Ca^{2+}]_i$ elicited by methylglyoxal, the finding that this compound evoked only a modest, transient stimulation of insulin release was initially surprising. However, it should be emphasised that dicarbonyl compounds are highly reactive substances which can interact with arginyl and lysyl residues and with SH groups [4, 7]. Thus, α-ketoaldehydes, especially at the high concentrations used in the present study, are likely to react non-enzymatically and rapidly with GSH [21], lowering its levels in the β-cell. Since insulin release has been reported to correlate closely with islet cell levels of GSH and thiol oxidants inhibit secretion [22], it is possible that the reaction of methylglyoxal with GSH in the β-cell could explain its failure to cause a prolonged stimulation of insulin release. Such an effect could also explain the inhibition of glucose-induced insulin release apparent in long-term incubations. Consistent with this suggestion, *t*-butylglyoxal, which would also be expected to react with cellular GSH, also inhibited glucose-induced insulin release. In fact the degree of inhibition by this compound was considerably greater than that observed with methylglyoxal, possibly due to significant metabolism of the latter via the glyoxalase pathway.

The effects of methylglyoxal on rat islet cells are highly reminiscent of the effects of D- and L-glyceraldehyde on HIT-T15 cells previously reported [23]. Indeed, we have recently obtained indirect evidence to suggest that commercial preparations of glyceraldehyde contain significant quantities of methylglyoxal [24]. Thus, the present study suggests that methylglyoxal contaminants in commercially available glyceraldehyde preparations could be responsible for some of the actions on β-cells previously ascribed to the triose.

The range of concentrations of methylglyoxal effective in inducing the acute effects reported here is considerably in excess of the levels of patho-physiological relevance [1, 25]. Thus, the β-cell is unlikely, under normal circumstances, to be exposed to the concentrations of methylglyoxal required to induce depolarization. However, in identifying a novel class of compound effective in causing these pronounced effects, study of the actions of methylglyoxal could be of value in dissecting the mechanisms which regulate β-cell membrane potential and ionic fluxes, and their interrelationship with exocytosis. The results of this study could also be relevant to the effects of long-term exposure to lower concentrations of α-ketoaldehydes, as occurs in diabetes mellitus.

In conclusion, methylglyoxal and phenylglyoxal, but not *t*-butylglyoxal, cause a rapid depolarization in rat pancreatic β-cells accompanied by a rise in $[Ca^{2+}]_i$ and an intracellular acidification, but only a modest, transient stimulation of insulin release.

This work was supported by the Wellcome Trust and the North Western Regional Health Authority.

References

1. Thornalley PJ, The glyoxalase system: New developments towards functional characterization of a metabolic pathway fundamental to biological life. *Biochem J* **269:** 1–11, 1990.
2. Richard JP, Mechanism for the formation of methylglyoxal from triose phosphates. *Biochem Soc Trans*. **21:** 549–553, 1993.
3. Phillips SA and Thornalley PJ, The formation of methylglyoxal from triose phosphates: Investigation using a specific assay for methylglyoxal. *Eur J Biochem* **212:** 101–105, 1993.
4. Thornalley PJ, Modification of the glyoxalase system in human red blood cells by glucose *in vitro*. *Biochem J* **254:** 751–755, 1988.
5. Atkins TW and Thornalley PJ, Erythrocyte glyoxalase activity in genetically obese (ob/ob) and streptozotocin diabetic mice. *Diab Res* **11:** 125–129, 1989.
6. Takahashi K, Further studies on the reaction of phenylglyoxal and related reagents with proteins. *J Biochem (Tokyo)* **81:** 403–414, 1977.
7. Mira ML, Martinho F, Azevedo MS and Manso CF, Oxidative inhibition of red blood cell ATPases by glyceraldehyde. *Biochim Biophys Acta* **1060:** 257–261, 1991.
8. Raess BU, Irreversible modification of red cell calcium transport by phenylglyoxal. *Mol Pharmacol* **44:** 399–404, 1993.
9. Leoncini G, Maresca M and Bonsignari A, The effect of methylglyoxal on the glycolytic enzymes. FEBS Lett **117:** 17–18, 1980.
10. Cook LJ and Best L, Efect of methylglyoxal on electrical activity in isolated rat pancreatic β-cells. *J Physiol* **489:** 49P, 1995.
11. Best L, Miley HE and Yates AP, Activation of an anion conductance and β-cell depolarization during hypotonically induced insulin release. *Exp Physiol* **81:** 927–933, 1996.
12. Best L and Elliott AC, Changes in BCECF, fura-2 and autofluorescence in intact rat pancreatic islets in response to nutrients and non-nutrients. *Mol Cell Endocrinol* **111:** 191–198, 1995.
13. Thomas JA, Buchsbaum RN, ZimniaK A and Racker E, Intracellular pH measurements in Ehrlich Ascites tumour cells utilizing spectroscopic probes generated *in situ*. *Biochemistry* **18:** 2211–2218, 1979.
14. McLellan AC, Phillips SA and Thornalley PJ, Fluorimetric assay of D-lactate. *Anal Biochem* **206:** 12–16, 1992.
15. McLellan AC and Thornalley PJ, Synthesis and chromatography of 1,2-diamino-4,5-dimethoxybenzene, 6,7-dimethoxy-2-methylquinoxaline and 6,7-dimethoxy-23-dimethylquinoxaline for use in liquid chromatographic fluorimetric assay of methylglyoxal. *Anal Chim Acta* **263:** 137–142, 1992.
16. Fuson RC, Gray H and Gouza JJ, Acyloins from *t*-butylglyoxal. *J Am Chem Soc* **61:** 1937–1940, 1939.
17. Van der Jagt DL, Daub E, Krohn JA and Han L-PB, Effects of pH and thiols on the kinetics of yeast glyoxalase 1. An evaluation of the random pathway mechanism. *Biochemistry* **14:** 3669–3675, 1975.
18. Best L, Trebilcock R and Tomlinson S, Lactate transport in insulin-secreting β-cells: contrast between rat islets and HIT-T15 insulinoma cells. *Mol Cell Endocr* **86:** 49–56, 1992.

19. Best L, Sheader EA and Brown PD, A volume-activated anion conductance in insulin-secreting cells. *Pfuger's Arch* **431:** 363–370, 1996.
20. Best L, Yates AP and Tomlinson S, Monocarboxylate production and transport: implications in pancreatic β-cell function. In: *Insulin Secretion and Pancreatic β-Cell Research* (Eds. PR Flatt and S Lenzen), pp. 191–198. Smith–Gordon, USA, 1994.
21. Marmstal E and Mannervik B, Evaluation of the two-substrate pathway of glyoxalase I from yeast by use of carbonic anhydrase and rapid kinetic studies. *FEBS Lett* **131:** 301–304, 1981.
22. Ammon HPT and Mark M, Thiols and pancreatic β-cell function. *Cell Biochem Function* **3:** 157–171, 1985.
23. Elliott AC, Trebilcock R, Yates AP and Best L, Stimulation of HIT-T15 insulinoma cells by glyceraldehyde does not require its metabolism. *Eur J Biochem* **213:** 359–365, 1993.
24. Davies J, Tomlinson S, Elliott AC and Best L, A possible role for glyceraldehyde transport in the stimulation of HIT-15 insulinoma cells. *Biochem J* **304:** 295–299, 1994.
25. McLellan AC, Thornalley PJ, Benn J and Sonksen PH, Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications. *Clin Sci* **87:** 21–29, 1994.