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COMMENTARY

Trioses and Related Substances: Tools for the Study of Pancreatic β-Cell Function

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ABSTRACT. Trioses, such as D-glyceraldehyde, have been used extensively for the study of stimulus-secretion coupling mechanisms in pancreatic β-cells. It is generally assumed that trioses enter the glycolytic pathway at the triose phosphate level, and stimulate insulin release in a manner analogous to glucose. This review focuses on a number of triose effects that are not entirely consistent with this model. These effects are likely to result, at least in part, from the actions of α-ketoaldehydes. One such compound, methylglyoxal, appears to be a major contaminant of triose preparations, and exerts effects on the β-cell identical to some of those evoked by glyceraldehyde. A related substance, hydroxypyruvaldehyde, is a product of triose autoxidation, which could exert similar effects. Study of the actions of trioses and α-ketoaldehydes could assist our understanding of cellular physiology, in general, and β-cell function, in particular. These substances are also likely to be of pathophysiological importance, especially in the context of sugar toxicity and autoxidative cell damage. BIOCHEM PHARMACOL **57**;6:583–588, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. pancreatic β-cell; glyceraldehyde; methylglyoxal; glyoxalase pathway; volume-sensitive anion channel

The most important stimulus of insulin release is probably glucose. A rise in the concentration of the hexose from non-stimulatory levels (~4 mM) to stimulatory levels (in the range of 6–20 mM) directly induces the secretion of insulin from the pancreatic β-cell. Briefly, this effect involves depolarization of the plasma membrane, leading to calcium-dependent electrical activity and exocytosis (see Ref. 1 for review). Glucose-stimulated insulin release depends entirely upon the metabolism of glucose in the β-cell, initially via the glycolytic pathway and subsequently via oxidative metabolism (see Refs. 2 and 3 for reviews). A long-standing question in β-cell physiology has been the nature of the coupling mechanism between increased nutrient metabolism and depolarization of the plasma membrane. The currently accepted model is that increased oxidative metabolism of glucose results in a rise in the β-cell ATP/ADP ratio [4], which inhibits K_{ATP} channels, thus promoting depolarization [1]. This model is supported by the observations that certain other substrates that are oxidized effectively by the β-cell are also potent insulinotropic agents. These include mannose and other glycolytic substrates (see Ref. 5 for review) and α-ketoisocaproate, a metabolite of leucine, which is oxidized in the mitochondria, and stimulates insulin release in a manner closely resembling that elicited by glucose [6]. Trioses, particularly D-glyceraldehyde, have also been used extensively in β-cell research as tools for the study of intracellular events. It is generally believed that these simple sugars enter the glycolytic pathway at the triose phosphate level, and thus activate the β-cell in a manner analogous to glucose. The purpose of this article is to focus attention on some of the anomalous actions of trioses and related substances on the pancreatic β-cell, and to suggest possible interpretations for some of these actions. This, in turn, may point to novel mechanisms of β-cell activation.

EFFECTS OF TRIOSES ON B-CELL FUNCTION

Although probably of little or no physiological importance, trioses have been used extensively to dissect the mechanism whereby nutrient stimuli act upon the pancreatic β-cell. Several reports have demonstrated stimulatory effects of D-glyceraldehyde and dihydroxyacetone on insulin release from mouse or rat islets of Langerhans [7–11]. It has generally been assumed that this effect of trioses involves their entry into the glycolytic pathway at the triose phosphate level and subsequent metabolism in a manner analogous to glucose [7]. Thus, trioses have been used extensively for the study of stimulus–secretion coupling in cultured β-cell lines, such as HIT-T15 and RINm5F, with poor or absent glucose responsiveness due to abnormal glucose transport and/or phosphorylation activities [12–14]. The stimulation of insulin-secreting cells by glyceraldehyde has been reported to involve inhibition of K_{ATP} channels [15], induction of electrical activity [16], and a rise in cytosolic $[Ca^{2+}]$ [13, 17].

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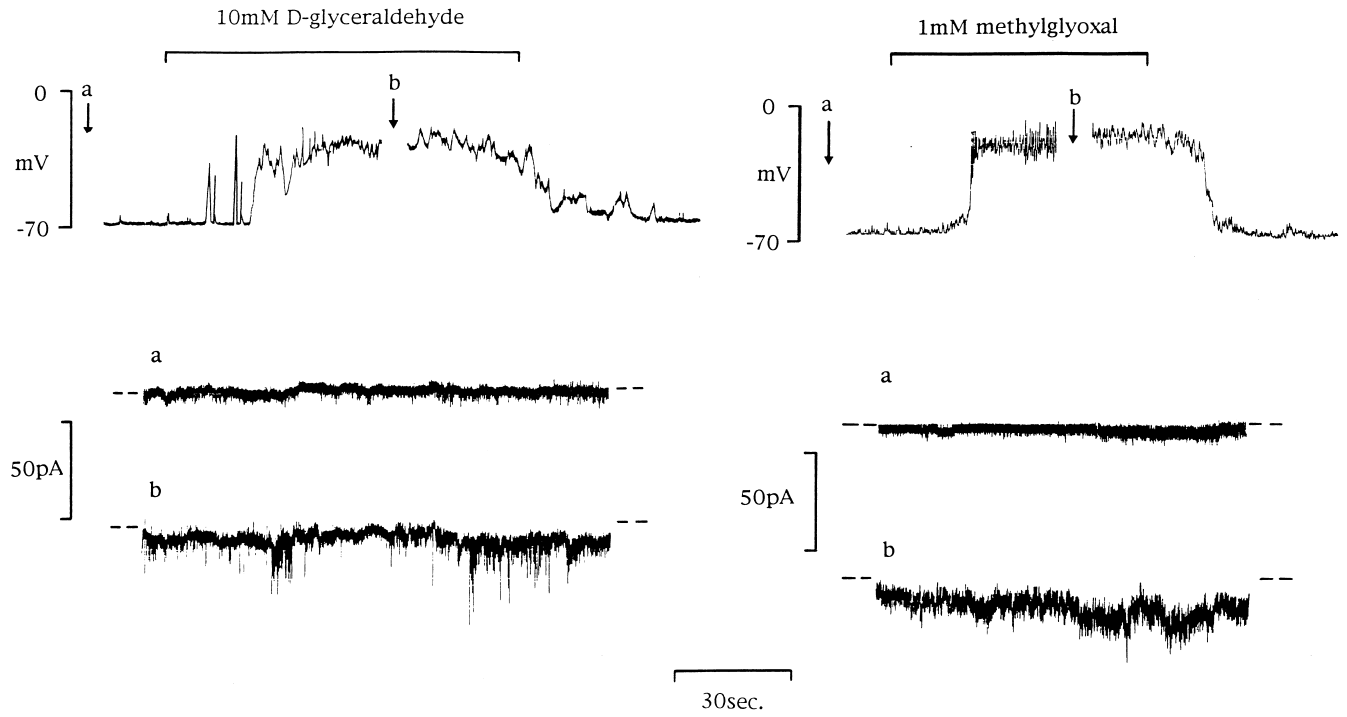

FIG. 1. Effects of D-glyceraldehyde and methylglyoxal on membrane potential (upper traces) and whole-cell current (bottom traces) in single, isolated rat pancreatic β-cells using the amphotericin "perforated patch" technique. At the points marked "a" and "b" in the membrane potential recordings, the amplifier was switched from current-clamp to voltage-clamp in order to record whole cell current at a holding potential of −70 mV.

Our interest in the actions of trioses on insulin-secreting cells arose from studies of the effects of glyceraldehyde on HIT-T15 cells [18, 19]. It was found that D-glyceraldehyde caused a marked and rapid depolarization of these cells (e.g. Fig. 1), a corresponding rise in cytosolic [Ca^{2+}] and [Na^+], and a pronounced intracellular acidification. An unexpected finding, however, was that these effects were all resistant to iodoacetate, an inhibitor of glyceraldehyde-3-phosphate dehydrogenase. Furthermore, all of the above effects of D-glyceraldehyde on HIT cells were produced by L-glyceraldehyde, which does not undergo significant metabolism via the glycolytic pathway [20]. The measurement of L-lactate and CO_2 formation from D-glyceraldehyde indicated that the rate of metabolism of the triose via glycolytic/oxidative metabolism was extremely modest compared with glucose metabolism. This could be due to the low activity of triose kinase, the enzyme responsible for the conversion of glyceraldehyde to glyceraldehyde-3-phosphate, reported in insulin-secreting cells [21]. Thus, despite the fact that significant rates of glyceraldehyde metabolism have been reported in intact pancreatic islets [7] and RINm5F insulinoma cells [22], there remains a question over the importance of glycolytic and oxidative metabolism of trioses in the stimulation of insulin-secreting cells.

An additional mechanism by which trioses might activate pancreatic β-cells is electrogenic transport into the cell, possibly via Na^+- or H^+-cotransport [18, 19]. Indeed, studies of D-glyceraldehyde transport have suggested that a component of triose transport may be electrogenic [19], and therefore could contribute towards β-cell depolarization. However, neither the plasma membrane system responsible for triose uptake in cells nor selective inhibitors of this process have been identified. Thus, the role of triose transport in the stimulation of insulin-secreting cells is, at present, unclear.

TRIOSE AUTOXIDATION

When considering the actions of trioses on cellular function, it should be borne in mind that these compounds are notoriously susceptible to autoxidation, a process that involves the generation of free radicals and results in the production of reactive α-ketoaldehydes [23]. Both of these products are potentially cytotoxic: free radicals by causing oxidative damage and α-ketoaldehydes due to their ability to glycosylate cellular proteins. The major product of glyceraldehyde autoxidation is hydroxypyruvaldehyde. This compound is expected to bind to proteins and initially to form a reversible hemithioacetal adduct with cysteine residues and glycosylamine adducts with lysine and arginine residues. Further irreversible reactions may lead to the formation of a bis(lysyl)imidazolium cross-link and 1-carboxy-2-hydroxyethyl derivatives of lysine and a hydroimidazolone derivative of arginine. This type of reaction is analogous to the reactions of methylglyoxal with proteins (see below and Fig. 2) and can exert profound effects on cellular function. For example, incubation of cultured cells with D-glyceraldehyde (1–10 mM) leads to the accumula-

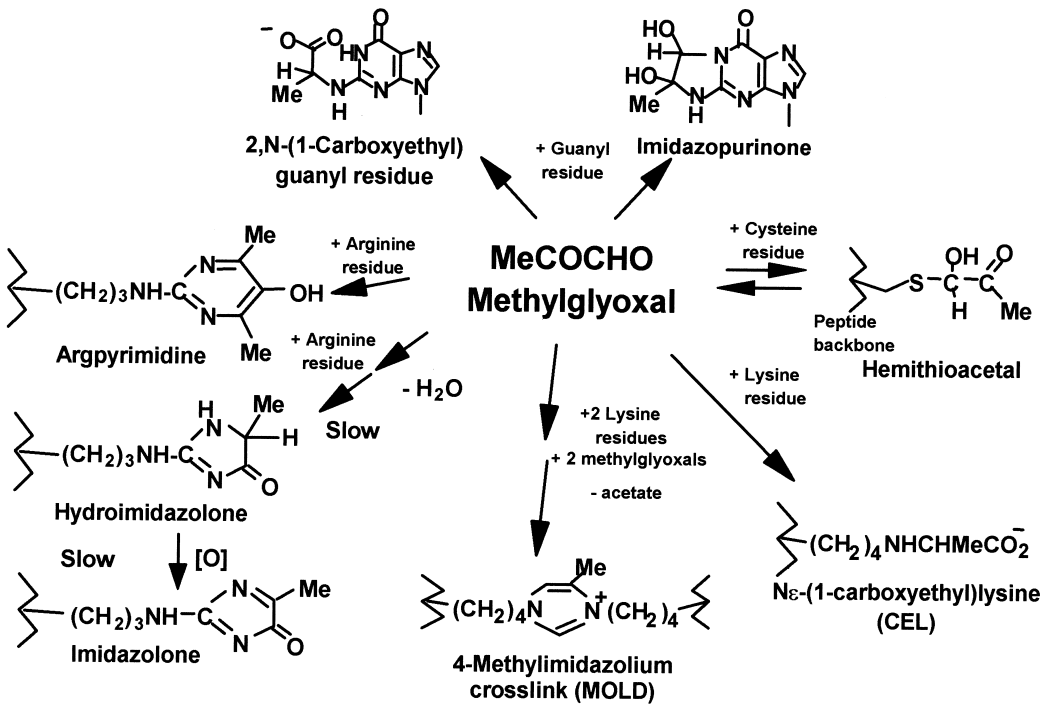

FIG. 2. Interactions of methylglyoxal with amino acid residues.

tion of α-ketoaldehydes (methylglyoxal and hydroxypyruvaldehyde), protein glycation, and mild oxidative stress [24]. In red cells, ATPase activities have been shown to be inhibited by glyceraldehyde, due to its autoxidation to hydroxypyruvaldehyde [25]. In the case of the pancreatic β-cell, a 60-min incubation with 6 mM glyceraldehyde was shown to inhibit specifically glucose-induced insulin release, probably as a result of glycosylation of the enzyme glucokinase [26].

A number of compounds closely related to the products of triose autoxidation can also be detected as contaminants in commercially available triose preparations. For example, there is evidence that the α-ketoaldehyde methylglyoxal is a major contaminant in two commonly available preparations of D- and L-glyceraldehyde [19]. This raises the possibility that methylglyoxal and related compounds might also exert profound effects on pancreatic β-cells.

METHYLGLYOXAL: FORMATION, CELLULAR ACTIONS AND METABOLISM

Methylglyoxal is a three-carbon α-ketoaldehyde that forms an intrinsic component of glucose metabolism. Its formation and metabolism have been reviewed extensively [27, 28]. Methylglyoxal is formed by the enzymatic and non-enzymatic fragmentation of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. It is also formed in ketone body metabolism from acetone catalyzed by cytochrome P450 2E1, and in the catabolism of threonine [29–33].

Methylglyoxal, in common with other α-ketoaldehydes, is a highly reactive substance. It can react with proteins under physiological conditions, initially by reversible reactions and subsequently by irreversible reactions, to form cross-links between amino acids ([34–36]; Fig. 2). At physiological concentrations of methylglyoxal (< 5 μM), the major irreversible modification of protein was of arginine residues. Methylglyoxal can also react with guanyl residues in DNA and RNA [37].

As might be predicted, such reactions with cellular components can result in a wide range of effects on cellular function. In common with glyceraldehyde [25], high concentrations of methylglyoxal (0.5 to 10 mM) have been shown to exert inhibitory effects on red blood cell ATPases [38], and also to inhibit glycolytic enzymes [39], mitochondrial respiration [40], microtubule assembly [41], and cell proliferation, particularly in tumours [42]. High concentrations of methylglyoxal have also been reported to cause apoptosis in HL60 cells *in vitro* [43]. In general, the interactions of methylglyoxal with cellular components, and the resulting cytotoxic actions, can be inhibited by substances such as aminoguanidine, which forms a cyclic adduct with α-ketoaldehyde [44]. It should be emphasized that the concentrations of methylglyoxal in human tissues and body fluids are usually low (0.1 to 1.0 μM; [28]) compared with the concentrations employed to elicit *in vitro* effects of the type described above.

Since methylglyoxal is a highly reactive and cytotoxic substance, it is important that cells are able to convert it to non-toxic metabolites. This process is the function of the glyoxalase pathway, which essentially involves two enzymatically catalyzed steps and occurs ubiquitously in mammalian cells. The first step, catalyzed by glyoxalase I,

converts methylglyoxal to *S*-D-lactoylglutathione. This intermediate is subsequently converted to D-lactic acid by the enzyme glyoxalase II. These two steps involve, respectively, the consumption and regeneration of reduced glutathione. There are additional metabolic transformations that methylglyoxal can undergo, resulting in the production of pyruvate, lactaldehyde, hydroxyacetone, acetyl-CoA, and formate, although these in total represent only a small proportion of the net metabolites of methylglyoxal.

EFFECTS OF METHYLGLYOXAL ON THE PANCREATIC β-CELL

In view of the finding that methylglyoxal can be a major contaminant in triose preparations, we were led to investigate the effects of this α-ketoaldehyde on insulin-secreting cells [45]. These studies used isolated rat β-cells, although essentially similar effects were observed with the HIT-T15 cells used in earlier studies (unpublished observations). To summarize, it was found that methylglyoxal exerts acute stimulatory actions that were virtually identical to the effects previously reported with D- and L-glyceraldehyde, namely depolarization (see Fig. 1), increased cytosolic [Ca^{2+}] and [Na^+], and intracellular acidification. A concentration of methylglyoxal of 1 mM induced effects comparable to those evoked by 10 mM glyceraldehyde (Fig. 1). Since the extent of methylglyoxal contamination in triose preparations was calculated to be in the order of 5–15% [19], this finding suggests that the activation of β-cells by trioses could be due largely to the α-ketoaldehyde content.

Insulin-secreting cells have an active glyoxalase system [19, 45], and thus produce large quantities of D-lactate when incubated with methylglyoxal. We therefore attempted to assess the role of the glyoxalase pathway in β-cell activation by methylglyoxal. It was found that all of the above effects of methylglyoxal could be reproduced by phenylglyoxal, another effective glyoxalase substrate. In contrast, none of these effects was evoked by *t*-butyl-glyoxal, which is a poor substrate for the glyoxalase pathway [46]. The simplest explanation for these findings is that the stimulatory action of methylglyoxal on the β-cell requires its metabolism via glyoxalase I and II to D-lactate. If this is the case, it raises the question of how generation of intracellular D-lactic acid produces the observed effects on β-cells. It is possible that the intracellular acidification is simply the result of the conversion of methylglyoxal to D-lactic acid. It is also conceivable that this process also leads to an elevation in intracellular [Na^+], through a process of Na^+/H^+ exchange. Investigations into the mechanism by which methylglyoxal induced depolarization and electrical activity in β-cells showed that this effect was sensitive to inhibition by the putative anion channel inhibitors 4,4′-dithiocyanatostilbene-2,2′-disulphonic acid (DIDS) and 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), raising the possibility that D-lactate generation could depolarize the cells by activating an anion channel. A volume-sensitive anion channel, which is activated by cell swelling, has been described recently in insulin-secreting cells [47–49]. Furthermore, activation of the channel generates an inward, depolarizing current due to Cl^- efflux [50]. We also found recently that methylglyoxal activates this channel in rat β-cells, inducing a similar inward current (Fig. 1, a and b), an effect accompanied by a significant increase in cell volume (unpublished data). Since β-cells express very low levels of the lactic acid transport system [51], it is possible that cell swelling results from intracellular accumulation of D-lactate, in addition to a rise in intracellular [Na^+] as a result of Na^+/H^+ exchange. Thus, it is suggested that the conversion of methylglyoxal to D-lactate, and accumulation of the latter, cause β-cell swelling and activation of the volume-sensitive anion channel, resulting in the generation of an inward current, leading to depolarization and electrical activity. The volume-sensitive anion channel appears to be permeable to several organic anions, including L- and D-lactate ([45]; our unpublished observations) in addition to Cl^-, and therefore could function as an efflux pathway for these anionic species. Such an electrogenic pathway would augment the inward current carried by Cl^- efflux. A diagram of this hypothetical model is shown in Fig. 3. Again, it should be noted that the concentrations of methylglyoxal used in the above studies are considerably greater than those found under patho-physiological conditions, so that β-cells probably produce negligible quantities

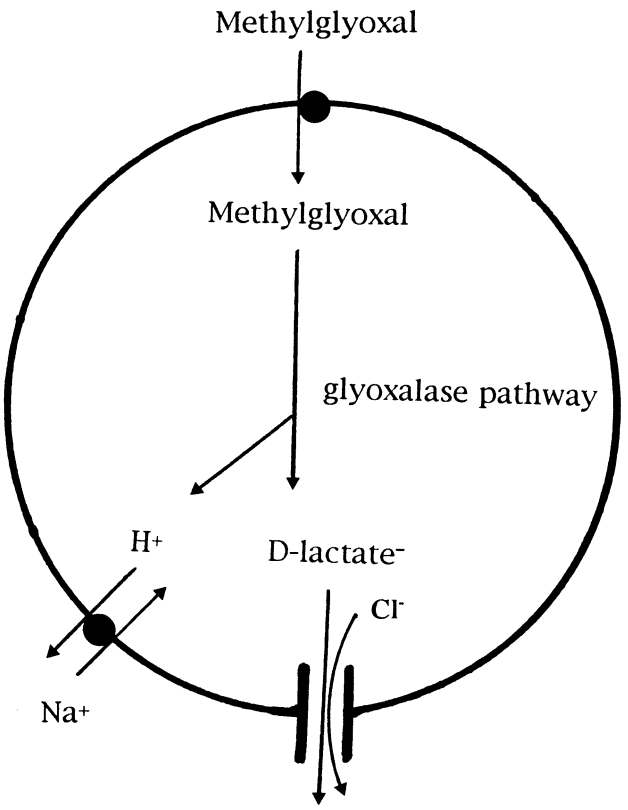

FIG. 3. Hypothetical model to explain the observed effects of methylglyoxal on the pancreatic β-cell. See text for explanatory details.

of D-lactate under normal physiological circumstances. However, the model depicted in Fig. 3 could be relevant to the stimulation of β-cell electrical activity by glucose, since metabolism of the hexose produces large amounts of L-lactate (see Ref. 52 for a further discussion of this topic). In fact, the characteristics of the inward current evoked by methylglyoxal (and glyceraldehyde; Fig. 1) closely resemble the inward current previously reported in cells stimulated by glucose or swollen by exposure to hypotonic solutions [53].

In view of the pronounced and rapid stimulatory effects of methylglyoxal on membrane potential and cytosolic [Ca^{2+}], it was surprising that α-ketoaldehyde evoked only a modest and transient stimulation of insulin release [45]. Indeed, we found that methylglyoxal significantly inhibited glucose-induced insulin release in longer-term experiments, as previously reported for glyceraldehyde [26]. It is likely that this inhibitory action is related to the cytotoxic actions of α-ketoaldehydes outlined earlier.

CONCLUSIONS

The interactions of trioses with the pancreatic β-cell are complex, and can result in acute stimulatory and more long-term inhibitory effects. The stimulatory actions could, at least in part, involve transmembrane transport of the triose, and its glycolytic and oxidative metabolism. However, it should be stressed that trioses are highly susceptible to autoxidation to form α-ketoaldehydes such as hydroxypyruvaldehyde. A closely related compound, methylglyoxal, is also present as a contaminant in triose preparations. These α-ketoaldehydes are highly reactive substances and can exert profound effects on cell function. For example, methylglyoxal exerts rapid and marked effects on the pancreatic β-cell that are strikingly similar to those evoked by glyceraldehyde. These substances can provide potentially interesting experimental tools for the dissection of certain aspects of β-cell physiology. Furthermore, a number of their actions are also likely to have a bearing on pathological processes, particularly those relating to glucose cytotoxicity and oxidative cellular damage.

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