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Glucose increases the synthesis of lipoxygenase-mediated metabolites of arachidonic acid in intact rat islets

(12-hydroperoxyicosatetraenoic acid/12-hydroxyicosatetraenoic acid/beta cell/insulin/HPLC)

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ABSTRACT **Previous studies suggested that products of a 12-lipoxygenase pathway in the pancreatic islet may promote insulin release. To determine whether glucose augments the production of such metabolites, intact rat islets prelabeled with [^{3}H]arachidonate were stimulated with glucose, and 12-hydroxy-5,8,10,14-icosatetraenoic acid (12-HETE) release was measured by using HPLC. D-Glucose (16.7 mM) augmented the enzymatic synthesis of 12-HETE by 271% above that seen with 0–1.7 mM glucose. The glucose effect was stereospecific and preferential for the α anomer; it was modestly potentiated by the cyclo-oxygenase inhibitor ibuprofen. Glucose-stimulated 12-HETE accumulation was abrogated by mannoheptulose and was reproduced by the trioses glyceraldehyde or dihydroxyacetone, suggesting that the metabolism of glucose to glucose 6-phosphate or triose phosphates (or both) is critical. Glucose also augmented [^{3}H]arachidonate labeling of islets, suggesting an action at the level of substrate release or re-uptake (or both). These features of islet 12-HETE synthesis accord well with other known effects of glucose on beta cell function and suggest that lipoxygenase-mediated metabolites of arachidonate may be suitable candidates to mediate or amplify glucose's effects on insulin release.**

Previously reported data suggest a critical role for the lipoxygenase pathway of arachidonic acid (AA) metabolism in glucose (Glc)-induced insulin release in rat islet cells (1–8). A 12-lipoxygenase pathway, assessed by the product 12-hydroxy-5,8,10,14-icosatetraenoic acid (12-HETE), was identified both in intact adult rat islets and in endocrine cell-enriched, dispersed, cultured neonatal rat pancreatic cells, through the use of HPLC with confirmation by gas chromatography/mass spectrometry (1, 7). Also, exogenously provided or endogenously released AA initiated insulin secretion at subthreshold Glc concentrations and potentiated it in the presence of a high Glc concentration (1, 4). The stimulatory effect of AA was abrogated by lipoxygenase inhibitors, which also totally blocked Glc-induced insulin release (in the absence of exogenously provided arachidonate) (1–3, 5). Kato and co-workers (6) and Turk *et al.* (8) have also observed that lipoxygenase or phospholipase inhibitors block Glc-induced insulin release from intact islets. However, a critical missing link in this formulation would be the demonstration that Glc can increase the production of specific lipoxygenase products. Recently, Turk and colleagues have described islet production of 12-HETE from endogenous AA (7, 8) and have reported its augmentation by Glc (8). Herein is presented additional evidence showing that a stimulatory Glc concentration augments the flux of AA into the lipoxygenase pathway.

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MATERIALS AND METHODS

Special Materials. [5,6,8,9,11,12,14,15-^{3}H]AA, 87.4 Ci/mmol (1 Ci = 37 GBq), was from New England Nuclear; collagenase, type IV, was from Worthington; and 11,12-epoxy-8- or 10-hydroxy[^{14}C]icosatrienoic acids and trihydroxyicosatrienoic acids were gifts of Robert Bryant (Washington, DC).

Isolation and Treatment of Rat Islets of Langerhans. Intact rat islets were isolated from 250- to 300-g fed male Sprague–Dawley rats by collagenase digestion of the pancreas (9), in conjunction with concentration on a Ficoll gradient (10). Islets were handpicked into Krebs/Ringer bicarbonate buffer (KRB buffer), containing 143 mM Na^+, 5.9 mM K^+, 1.2 mM Mg^{2+}, 1.2 mM phosphate, 128 mM chloride, 1.2 mM sulfate, and 25 mM bicarbonate (pH 7.4) with 1.7 mM of D-Glc, and gassed with 95% O_2/5% CO_2. The actual measured ionized Ca^{2+} concentration was 2.11–2.15 mM. Equal numbers of islets (70–130 islets per tube) of comparable sizes were aliquotted into 10×75 mm glass test tubes whose bases had been removed and replaced with polyester monofilament filters of 62-μm-pore size (Tetko, Elmsford, NY) held onto the tubes with a rim of Duco cement (DuPont). These filters retained the islets but allowed the medium to rapidly drain through. These inner tubes containing the islets were placed into standard glass outer tubes (16×100 mm) containing 1 ml of protein-free KRB buffer with 2 μCi of [^{3}H]AA per ml for labeling of the cell membranes and were incubated for 90 min at 34–36°C in a water bath. Islets were rinsed in fresh buffer containing 1.7 mM Glc and 0.2% delipidated bovine serum albumin in order to bind residual nonesterified AA. After a further 30-min preincubation period in 1.7 mM Glc, islets were incubated for 30 min in 1 ml of buffer in the presence or absence of various test compounds. Studies were terminated by addition of ice-cold ethanol or acetonitrile to the medium, and the tubes containing the islets were removed and discarded. Experimental compounds were generally added only during the final incubation step. For studies of the effect of mannoheptulose, 2-deoxyglucose (dGlc), 3-*O*-methylglucose (3-*O*-MeGlc), diethylmaleate, or bromphenacyl bromide on the response to 16.7 mM Glc, the experimental drug was also present in the preincubation period. In all studies, the control tubes (no drug) contained the same amount and nature of diluent as the experimental (drug) tubes. In the studies involving the anomers of D-Glc, the premeasured sugars were dissolved in medium immediately prior to initiation of the final incubation period to minimize mutarotation.

For all studies (except those involving the effect of Glc concentration on the incorporation of [^{3}H]AA into islet

Abbreviations: 12-HETE, 12-hydroxy-5,8,10,14-icosatetraenoic acid; 12-HPETE, 12-hydroperoxy-5,8,10,14-icosatetraenoic acid; AA, arachidonic acid; Glc, glucose; dGlc, 2-deoxyglucose; 3-*O*-MeGlc, 3-*O*-methylglucose; Glc-6-*P*, glucose 6-phosphate.

membranes), the labeling step was performed in the presence of 16.7 mM Glc. For the studies of the effect of Glc concentration on the incorporation of AA into membranes, the initial labeling step was performed as indicated above except that islets were labeled either in the presence of 1.7 or of 16.7 mM Glc; all islets were then incubated in the same Glc concentration (16.7 mM). Thus, in this series of studies, the only variable was the concentration of Glc in which the cells were exposed to [^{3}H]AA.

Extraction and HPLC. Following precipitation of cell proteins and removal of buffer and solvent *ex vacuo*, the sample was redissolved in 40% methanol in water, acidified to pH 3.0–3.5 with phosphoric acid, and transferred to an octadecasilyl minicolumn (Sep-Pak, Waters Associates) prewashed with 5 ml of 15% methanol in water. Compounds of interest were eluted from the column with 20 ml of pure methanol. The methanol was rotary-evaporated and the sample was injected onto the HPLC column (C_{18}, 5 μm, 4.6 mm × 25 cm, Altex Scientific, Berkeley, CA) in 500 μl of 70% methanol. Chromatography was carried out isocratically at 1 ml/min using 75–76% methanol in 24–25% water brought to pH 2.8 with aqueous phosphoric acid. Fractions were collected every minute for liquid scintillation spectrometry. Since the small amount of tissue used was not sufficient to generate UV-detectable peaks of 12-HETE, exogenous 12- and 15-HETEs were added to each sample prior to extraction to monitor retention times and act as internal standards for the calculation of recoveries. These internal standards were not added during the cell incubations since exogenous HETEs inhibit endogenous lipoxygenases in several cell systems, including that within the islet. HETE recoveries averaged 97% ± 13% (mean ± SD; $n = 75$). The recovery of exogenous 12-[^{14}C]HETE or unlabeled 12-HETE added during the final incubation step in the presence of viable islets was uninfluenced by Glc concentration; thus, the observed effect of Glc on 12-[^{3}H]HETE accumulation was largely independent of any effects on 12-HETE metabolism or re-uptake into islets. In other preliminary studies (data not shown) some 12-HETE (and some 5-HETE) was found, apparently esterified into islet lipids, as shown by cell sonification followed by alkaline hydrolysis in 0.5 M KOH, in 25% aqueous MeOH, under argon, with the anti-oxidant butylated hydroxytoluene at 250 μM (controls were treated identically except for the absence of KOH). This additional amount of 12-[^{3}H]HETE in islets incubated in 16.7 mM Glc was at least as great as, if not greater than that found in islets incubated at 1.7 mM Glc. Thus, the Glc effect to increase 12-HETE release (see *Results*) is not due to any reciprocal decrease of 12-HETE esterification and, indeed, may have been artefactually reduced by any such re-uptake.

Leukotrienes (11) or prostaglandin E_2 and trihydroxyicosatrienoic acids (1) were monitored with HPLC systems previously described.

Insulin Secretion. Ten islets per tube were preincubated at 1.7 mM Glc and then incubated for 30–60 min (with the compounds added as indicated) in KRB buffer containing 0.5% bovine serum albumin. Released insulin (i.e., values above those at 0–1.7 mM Glc) was measured by radioimmunoassay using rat insulin standards (Novo Research Institute, Bagsvaerd, Denmark).

Data Analysis. Experimental tubes were compared only to control tubes from the same day, using the same pool of islets, using paired *t* analysis (or Wilcoxon matched-pairs signed-ranks test where specifically indicated). The results are expressed in terms of the mean (±SEM) of cpm per 100 islets of total 12-HETE accumulating in the incubation medium, after correction for the recovery of HETE internal standards.

RESULTS

Identification of Islet Lipoxygenase Products. A single dominant peak was consistently found that co-eluted with authentic 12-HETE or with 12-HETE methyl ester after treatment with diazomethane (Fig. 1). In addition, smaller peaks were seen to comigrate with an isomeric mixture of 11,12-epoxy-8- or 10-hydroxy-5,8,14-icosatrienoic acids and with trihydroxyicosatrienoic acids, both further catabolic products of 12-hydroperoxy-5,8,10,14-icosatetraenoic acid (12-HPETE) (12). These latter two peaks had also been identified previously in endocrine cell-enriched cultures of neonatal rat pancreatic cells and, where quantifiable, appeared to be reduced by lipoxygenase blockers (1). However, additional definitive proof of their identity or derivation was not pursued further. No convincing evidence was found for the release of other enzymatically derived HETE isomers (such as 5-HETE) into the medium. Although it is conceivable that cells produced a small amount of 5-HETE that was all re-esterified (see *Materials and Methods*), release of 5-HETE into the medium was not induced by *p*-chloromercuribenzoate, an inhibitor of fatty acid esterification (13). Several nordihydroguaiaretic acid-inhibitable peaks were seen eluting very close to, but not precisely with, standards for leukotrienes or their *trans* isomers. Conceivably, these peaks could represent 12-lipoxygenase-derived leukotriene analogs or related compounds.

Quantitation of 12-HETE Release and the Effect of D-Glc and Other Sugars. Even using the special tubes, it was not possible to prevent a small percentage (2.8%) of 12-[^{3}H]HETE accumulating in the labeling medium from adsorbing to the tubes and being carried into the final incubation medium. The source of these preformed, nonsuppressible 12-[^{3}H]HETE cpm was found to be a combination of enzymatic synthesis by the islets during the labeling step, and auto-oxidatively derived cpm found in commercially obtained [^{3}H]AA or formed during incubation in aerobically gassed medium. This unavoidable background "noise" could be reproduced by incubating tubes with [^{3}H]AA in the *absence* of islets and was totally uninfluenced by Glc concentration (Glc: 0–1.7 mM, 62 ± 12 cpm; 16.7 mM, 56 ± 11 cpm; $n = 5$ each) or the presence of drugs in the incubation tubes. Thus, these background cpm are constant in all reported studies and do not influence any paired comparisons. This conclusion is supported by the additional observation (see Fig. 2 legend) that the nonenzymatically derived cpm seen in the *presence* of islets were exactly the same in the presence of high or low Glc concentrations.

Islets incubated in 1.7 mM Glc produced slightly but significantly more 12-HETE than those incubated at 0 mM of Glc (40 ± 8 vs. 63 ± 8 cpm; $n = 5$; $P < 0.05$). Islets incubated in 16.7 mM Glc accumulated considerably more 12-HETE than at 0–1.7 mM Glc ($P < 0.02$ by the *t* test or $P < 0.001$ by the Wilcoxon test; Fig. 2). The relative increase in enzymatically derived 12-[^{3}H]HETE induced by 16.7 mM Glc was 271% (Fig. 2). The incremental change in 12-HETE production induced by a stimulatory Glc concentration was enzymatically derived, since it was totally abrogated by boiling the islets or co-incubating them in the presence of lipoxygenase inhibitors (phenidone: −92% at 100 μg/ml; nordihydroguaiaretic acid: −81% at 100 μM and −100% at 250 μM) or a phospholipase inhibitor (bromphenacyl bromide: −100% at 100 μM). The Glc effect was stereospecific, since L-Glc was inactive. There was a clear anomeric preference for α-D-Glc over β-D-Glc with regard to 12-HETE formation at 6 mM ($\alpha = 370 \pm 55$ cpm; $\beta = 183 \pm 22$ cpm; $n = 5$; $P < 0.02$) (Fig. 3). A more modest preference for the α anomer was also seen at 8.3 mM ($P < 0.05$; Wilcoxon test). At 16.7 mM, β-D-Glc was insignificantly more effective than α-D-Glc (Fig. 3).

When added to 0–1.7 mM D-Glc, mannitol (control: 119 ±

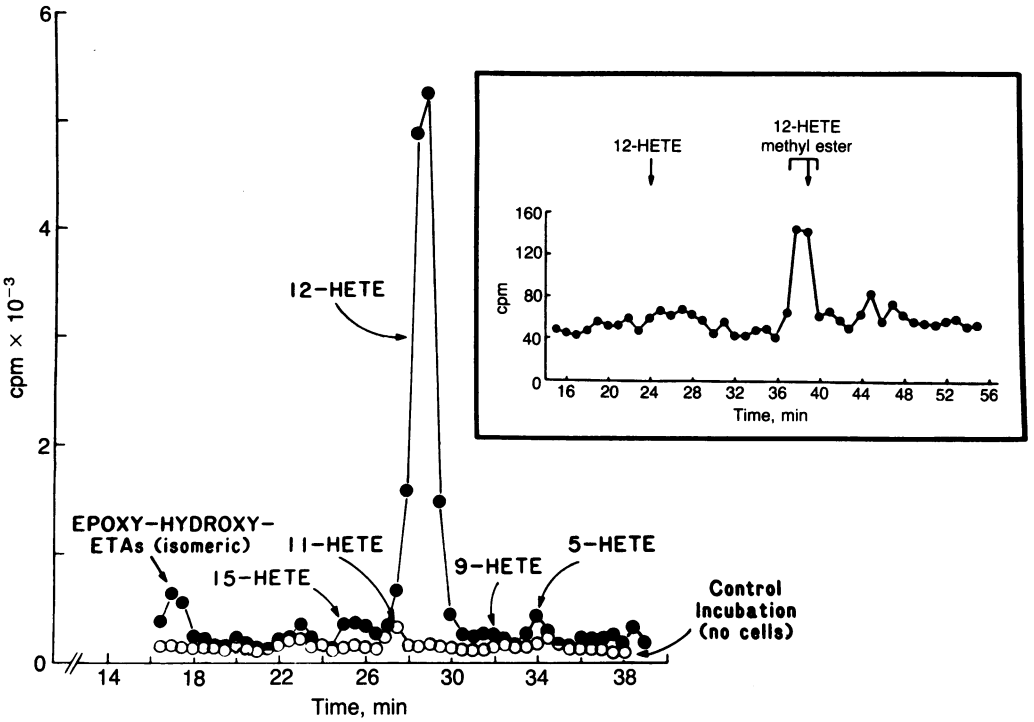

FIG. 1. Radiochromatogram depicting the major lipoxygenase product of intact rat islets. Islets were labeled with [^{3}H]AA in 16.7 mM Glc for 120 min and then maximally stimulated for an additional 30 min with the ionophore A23187, 10% fetal calf serum, and 16.7 mM Glc for the purposes of peak identification. "EPOXY-HYDROXY-ETA" refers to a smaller and more variable peak co-eluting with 11,12-epoxy-8- or 10-hydroxyicosatrienoic acids. (*Inset*) Results using a smaller sample (lacking A23187 and serum and excluding labeling medium) after collection of the fractions containing hydroxy acids, methylation of the sample using ethereal diazomethane, and rechromatography. Arrows indicate the UV peak of the indicated HETE standards or their methyl esters. The peak routinely found co-eluting with 12-HETE has disappeared and has reappeared as a peak migrating precisely with 12-HETE methyl ester.

15 cpm; mannitol: 130 ± 22 cpm; n = 8), dGlc (144 ± 43 vs. 134 ± 36 cpm; n = 5), and 3-*O*-MeGlc (129 ± 19 vs. 130 ± 22 cpm; n = 6) failed to support 12-HETE production or augment insulin release. The Glc epimer mannose (15 or 33.4 mM) had no effect on 12-HETE production (control: 117 ± 25 cpm; mannose: 118 ± 27 cpm; n = 11), even though 33.4 mM mannose supported insulin release (237 ± 29 microunits per 10 islets per hr; n = 29) nearly (79 ± 8%) as well as 16.7 mM Glc (312 ± 26; n = 39). DL-Glyceraldehyde (6.7 mM), however, could substitute for Glc (control: 115 ± 17 cpm; glyceraldehyde: 177 ± 33 cpm per 100 islets; n = 6; $P < 0.05$ by paired t or Wilcoxon test). Interestingly, at higher concentrations (13.3 mM), the effect on 12-HETE lost statistical significance, an observation which may relate to the loss of ability of glyceraldehyde to augment ATP accumulation (14) and stimulate insulin release (14) at higher concentrations. Dihydroxyacetone (16.7–22.3 mM) increased 12-HETE synthesis (control: 145 ± 15 cpm; dihydroxyacetone: 274 ± 52

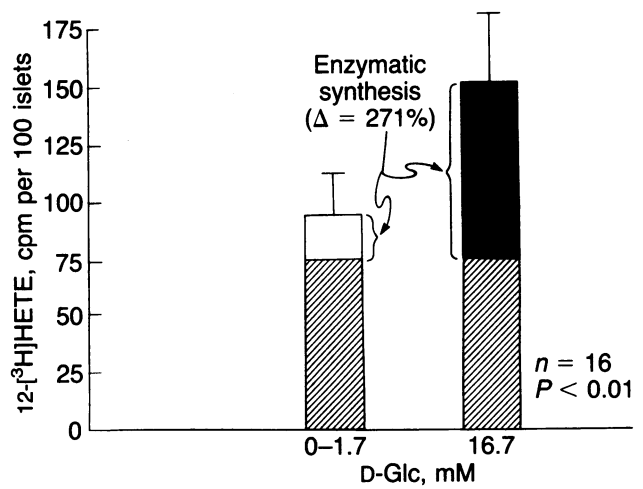

FIG. 2. Mean data (±SEM) from 16 studies in which net 12-HETE peaks at low and high D-Glc concentrations are analyzed in terms of enzymatic and nonenzymatic formation. Nonenzymatic formation at low Glc is approximated as the total peak (95 cpm) × the percentage resistant to inhibition by blockers of arachidonate metabolism (nordihydroguaiaretic acid, phenidone, SKF525a, metyrapone: −21% ± 5%; n = 13) = 75 cpm. Similarly, nonenzymatic formation at high Glc = 152 cpm (total peak) × 49% nonsuppressible fraction = 74 cpm. Thus, the nonenzymatic fraction is identical at high and low Glc concentrations, as verified directly by using incubations of [^{3}H]arachidonate in the absence of cells (see text). The inhibitable (i.e., enzymatically formed) fraction increases by a mean of 271% in the presence of 16.7 mM Glc.

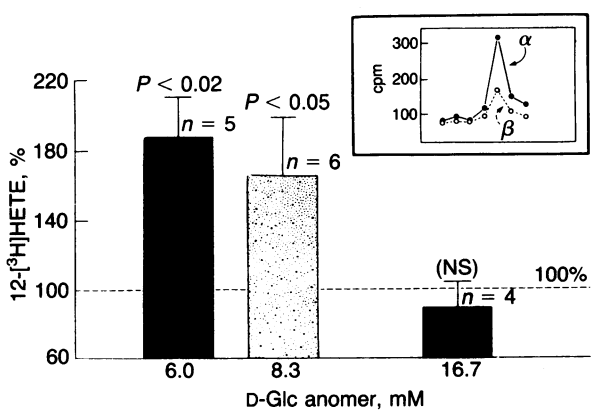

FIG. 3. Anomeric preference for 12-HETE accumulation at three concentrations of α-D-Glc (α) and β-D-Glc (β). Data are expressed as the ratio of cpm_α/cpm_β by using paired data from the same pool of islets studied on the same day. (*Inset*) A representative study at 6 mM α-D-Glc (α) and β-D-Glc (β). NS, not significant.

cpm; $n = 8$; $P < 0.02$; Wilcoxon test). DL-Glyceraldehyde stimulated insulin release (269 ± 69 microunits per 10 islets per hr; $n = 6$) as effectively as 16.7 mM Glc (208 ± 29); dihydroxyacetone was also effective, but, as expected (15), was only 47% as potent (130 ± 30 microunits per 10 islets per hr; $n = 18$) as Glc (254 ± 35; $n = 16$).

Effect of Inhibitors of Glc Metabolism, Prostaglandin Synthesis, and Glutathione Availability on Glc-Induced 12-HETE Production. At low Glc levels, 12-HETE was only minimally reduced (mean = −22% ± 5%; $n = 7$; $P < 0.01$) by mannoheptulose, an inhibitor of Glc phosphorylation. Inhibitors of AA oxygenation blocked nearly an identical percentage (−21% ± 5%; $n = 13$; Fig. 2 legend) at low Glc concentrations. Therefore, it appears likely that, although only a small amount of the 12-HETE peak seen at 0–1.7 mM Glc was enzymatically synthesized, this small percentage nevertheless was dependent on Glc metabolism. Mannoheptulose (16.7 mM) consistently reduced 12-HETE formation stimulated by Glc (control: 163 ± 49 cpm; mannoheptulose: 92 ± 23 cpm; $P < 0.05$) to values comparable to those seen in the presence of low Glc concentrations alone. The % inhibition by mannoheptulose for *total* (i.e., enzymatic plus nonenzymatic) 12-HETE accumulation at high Glc levels (−41% ± 4%; $P < 0.001$) was not significantly different from the reduction induced by enzymatic inhibitors of lipoxygenase (−51% ± 8%). Mannoheptulose blocked 82% ± 3% the Glc-induced *increment* in 12-HETE and completely abrogated Glc-induced insulin release. In contrast, dGlc (21.3 mM), which inhibits Glc metabolism primarily at steps distal to Glc phosphorylation (16), failed to modify "basal" or Glc-induced 12-HETE formation (control: 161 ± 29 cpm; dGlc: 181 ± 53 cpm; $n = 7$; P = not significant). dGlc usually inhibited Glc-induced insulin release, but, as expected for the molar ratio of dGlc to Glc used (17), this effect was quite variable (−53% ± 28%; $n = 4$ studies). Similarly, neither 18.0 mM 3-*O*-MeGlc ($n = 7$) nor 16.5 mM mannitol ($n = 6$) was able to reverse the Glc effect and, in fact, tended (nonsignificantly) to potentiate it. Thus, the mannoheptulose effect to reduce Glc-induced HETE formation was not due to nonspecific osmotic or hexose effects or simple interaction with the Glc transport mechanism.

Since the pancreatic islet is deficient in glutathione peroxidase (18), it is possible that the labile intermediate 12-HPETE could survive the cell incubations without complete reduction to the more stable by-product, 12-HETE; 12-HPETE, being unstable, might degrade in part to several polar products (12) not eluting with 12-HETE on HPLC. Therefore, I examined the effect of diethylmaleate, a selective binder of intracellular glutathione, which, in concentrations of 1–2 mM, depletes glutathione levels in many cell systems (12) and thereby diminishes conversion of HPETEs to HETEs (12). This agent failed to reduce the accumulation of 12-HETE at 16.7 mM Glc (control: 165 ± 46 cpm; diethylmaleate: 258 ± 95 cpm; $n = 4$) or at 1.7 mM Glc (control: 163 ± 22 cpm; diethylmaleate: 171 ± 38 cpm; $n = 4$).

Addition of ibuprofen (10 μg/ml) reduced production of prostaglandin E_2, the major cyclo-oxygenase product of the islet, by 79% ± 16% ($n = 5$; $P < 0.01$) from basal levels of 53 ± 20 cpm per 100 islets. This concentration of ibuprofen caused a small but significant increase of 12-HETE accumulation at 16.7 mM Glc (control: 199 ± 52 cpm; ibuprofen: 234 ± 49 cpm; $n = 6$; $P < 0.05$, Wilcoxon test), a potentiation of 30% ± 18%, but ibuprofen failed to increase 12-HETE accumulation when added to 1.7 mM Glc.

Effect of Glc on AA Incorporation. In each of 7 studies, cells labeled in the presence of a high Glc concentration released more 12-HETE when stimulated with 16.7 mM Glc (190 ± 62 cpm; $P < 0.02$) than did cells labeled in a substimulatory Glc concentration but then also incubated at 16.7 mM Glc (140 ± 54 cpm), a difference of 39% ± 13% ($P < 0.01$). This finding suggested that a high Glc concentration stimulated the esterification of AA into cell membranes.

DISCUSSION

These studies were designed to be as physiologic as possible in studying the effect of Glc on 12-HETE accumulation by intact islet cells with intact paracrine relationships. Despite the extremely small amount of tissue available (≈800 μg of wet weight per tube), these studies were able to demonstrate convincingly that D-Glc, in a concentration that maximally stimulates insulin release (15, 19), augments accumulation of 12-HETE and, by implication, its precursor 12-HPETE. During the course of these studies, Turk and colleagues, using independent methodology, reported a similar effect of Glc on 12-HETE formation (from endogenous arachidonate) by rat islets (8). Although the exact cell (or cells) synthesizing the 12-HETE is not identified by the current studies, islet endocrine cells appear to be the major source and retained platelets do not play a discernible role (1, 7). Previous studies (1, 4, 5) suggested that it is the labile precursor 12-HPETE (or a product of 12-HPETE) that might mediate (at least in part) the stimulatory action of AA (and of Glc itself) on insulin release. It has been observed (5, 8) that the stable reduction product of 12-HPETE (namely, 12-HETE) is an excellent marker for lipoxygenase activity in the islet. Inhibition of Glc-induced insulin release by dispersed neonatal rat islet cells using lipoxygenase blockers correlated closely ($r^2 = 0.96$) with inhibition of 12-HETE accumulation (5). The current studies do not address the question of whether the effect of Glc on AA flux through the lipoxygenase pathway is related to first, second, or both phases of insulin release.

D-Glc stereospecifically increased 12-HETE/HPETE accumulation. Further, at submaximal concentrations, the α anomer of D-Glc was considerably more potent than the β anomer in promoting 12-HETE formation, in parallel with the anomeric preference with regard to phosphorylation by glucokinase (20), glycolytic metabolism, and insulin secretion (21). The anomeric preference with regard to all four parameters occupies a narrow "window," with the difference being most obvious near the Glc threshold with the β anomer actually becoming slightly more potent at supramaximal hexose concentrations (22).

Almost all of the enzymatically synthesized 12-HETE appeared to be dependent on Glc metabolism, as shown by studies using mannoheptulose. Although these studies suggested that Glc must be phosphorylated to stimulate HETE formation, Glc 6-phosphate (Glc-6-*P*) is a common point of entry into more than one metabolic pathway. The fact that the triose products of glycolysis (dihydroxyacetone or glyceraldehyde) could replace Glc suggests a role for glycolysis and its products at or distal to the accumulation of the triose phosphates. However, dGlc (which primarily inhibits Glc metabolism and triose phosphate formation by inducing a block in glycolysis distal to the formation of Glc-6-*P*) (16) failed to mimic the inhibitory effect of mannoheptulose. Therefore, the possibility is raised that Glc-6-*P* itself, or another factor generated during its further metabolism (for example, NADPH generated in the hexose monophosphate shunt), can also mediate a part of the effect of Glc on 12-HETE production. Alternatively, since some glyceraldehyde 3-phosphate can be generated late in the pentose shunt, the possibility remains that generation of this one metabolite from Glc-6-*P* by either pathway can fully explain Glc's effect. The further glycolytic metabolism of the trioses is known to provide ATP; it also yields phospho*enol*pyruvate (23), which, like NADPH (24), has been suggested to be a controller of Ca^{2+} accumulation and consequent insulin release. Thus, the accumulation of calcium or of ATP, or β cell depolarization, may be the proximate signal(s) to arachidonate release (25, 26) and/or lipoxygenation (27) in the pancre-

atic islet. The failure of mannose to augment HETE accumulation at concentrations equipotent to Glc in causing release of insulin could be related to the fact that mannose 6-phosphate is converted primarily to fructose 6-phosphate, with little Glc-6-*P* accumulating (28).

Control studies showed that the Glc effect is not a nonspecific effect of osmolarity, simple phosphorylation of a hexose, or interaction with the hexose transport system, since mannose and dGlc are phosphorylated, and mannose, dGlc, and 3-*O*-MeGlc interact with the Glc transport system but do not mimic the Glc effect on HETE accumulation. The studies using diethylmaleate also suggest that reduced glutathione is not the Glc-dependent metabolite responsible for the increase of 12-HETE synthesis. Although other investigators have attributed a stimulatory effect of Glc on 12-HETE production in platelets to the effect of Glc as a biologic hydroxyl radical scavenger (29), the stronger hydroxyl scavenger mannitol failed to mimic the Glc effect in the current studies. Although a direct stimulatory effect of Glc on lipoxygenase cannot be excluded, other lipoxygenases appear to be inhibited by very high hexose or hexitol concentrations (30). Rather, current data, together with those of others (31, 32), suggest an action Glc, at least in part, at the level of AA release and/or reacylation steps.

The addition of ibuprofen to a high Glc concentration inhibited production of prostaglandin E_2 (the major islet cyclooxygenase product) and caused a small but significant potentiation of the maximal 12-HETE concentrations achieved. These observations may help to explain our findings (33) that several prostaglandin synthesis inhibitors (in particular, ibuprofen at the concentration used in the current study) augment insulin release, an effect prevented or reversed (3, 32) by lipoxygenase inhibitors. Furthermore, the ibuprofen data help to exclude the possibility that the measured 12-HETE production was due to cyclo-oxygenase-like enzymes (34) rather than a true lipoxygenase.

Although no attempt was made in these studies to correlate insulin release and 12-HETE formation over a range of Glc concentrations, the current data, and previous studies (5), suggest that there is not a simple, linear relationship between 12-HPETE production and insulin release. First, mannose, a potent insulin secretagogue, had no effect on 12-HETE production. Second, dGlc, which generally (15), although variably (17), inhibits insulin release, did not inhibit 12-HETE production at all. Furthermore, a small increment in 12-HETE production was discernible between 0 and 1.7 mM of Glc, although no augmentation of insulin secretion was observed. This increment was inhibitable by mannoheptulose and may be explained by observations that a (mannoheptulose-inhibitable) fraction of Glc metabolism does proceed (at about 1/4 of the maximal rate) at such low Glc concentrations (15) accompanied by other metabolic alterations, such as changes in adenylate charge and possibly Ca^{2+} efflux (19). Such dissociations between insulin and 12-HPETE release allow the conclusion that 12-HPETE synthesis is not merely secondary to the exocytosis of insulin-containing granules through cell membranes. Nonetheless, the same data also suggest that a (small) rise in 12-HPETE is not sufficient in and of itself to *initiate* insulin release. However, it is well known that substimulatory Glc concentrations are sufficient to markedly prime or potentiate the insulin secretory response to many non-Glc stimuli. Since AA can both initiate *and* potentiate insulin release (1, 4), it is possible that the small rise in 12-HPETE at 1.7 mM Glc could play a role in the potentiating ability of low Glc concentrations. Further studies will be needed to assess whether a threshold has to be exceeded for 12-HPETE (or other metabolites of AA) to directly stimulate insulin release or, alternatively, whether such metabolites play primarily only a permissive role in insulin secretion.

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