Inhibition of Arachidonate Release by Secretagogue-Stimulated Pancreatic Islets Suppresses both Insulin Secretion and the Rise in β-Cell Cytosolic Calcium Ion Concentration†

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ABSTRACT: Fuel secretagogues induce hydrolysis of esterified arachidonic acid from pancreatic islet cell phospholipids and accumulation of nonesterified arachidonate at concentrations up to 35 μM. Exogenous arachidonate (5–30 μM) amplifies depolarization-induced insulin secretion from islets. Fuel secretagogue-induced hydrolysis of arachidonate from islet phospholipids occurs in Ca^{2+}-free medium, suggesting the possible involvement of a Ca^{2+}-independent phospholipase. In the companion paper [Gross et al. (1993) *Biochemistry* (preceding paper in this issue)], we demonstrated that the major islet phospholipase A_2 is Ca^{2+}-independent, ATP-stimulated, and inhibited by the haloenol lactone suicide substrate (HELSS) (*E*)-6-(bromomethylene)-3-(1-naphthalenyl)-2*H*-tetrahydropyran-2-one. Here we demonstrate that HELSS suppressed both release of the arachidonate metabolite prostaglandin E_2 and insulin secretion from islets stimulated with D-glucose and the muscarinic agonist carbachol. Both prostaglandin E_2 release and insulin secretion were suppressed with similar concentration profiles and time courses. Islet oxidation of [^{14}C]-glucose to [^{14}C]CO_2, activities of islet lactate dehydrogenase and alanine and aspartate aminotransferases, and carbachol-induced inositol phosphate accumulation in islets were all unaffected by HELSS. Depolarization of isolated β-cells with 40 mM KCl induced a rise in cytosolic [Ca^{2+}] that was also unaffected by HELSS. In contrast, the 17 mM D-glucose-induced rise in β-cell [Ca^{2+}] was inhibited by HELSS in a concentration-dependent manner, but that induced by exogenous arachidonate (15 μM) was not. These results suggest that fuel secretagogues activate the islet Ca^{2+}-independent phospholipase A_2, resulting in release of nonesterified arachidonate, which facilitates Ca^{2+} entry into β-cells and promotes insulin secretion.

Pancreatic islet β-cells maintain the blood glucose concentration within a narrow range by modulating their insulin secretory rate in response to circulating blood glucose levels (Blackgard, 1986). This glucose sensor function appears to be intrinsic to islets, because islets isolated from human or rodent pancreata retain the ability to augment their insulin secretory rate in vitro in response to increases in perifusate glucose concentration (Malaisse et al., 1979; Ashcroft, 1980; Hedeskov, 1980; Wollheim & Scharp, 1981; Meglasson & Matschinsky, 1986; Prentki & Matschinsky, 1987; Turk et al., 1987; Bonner-Weir, 1988). Some insulin secretagogues, such as muscarinic agonists, induce insulin secretion via interaction with a β-cell plasma membrane receptor (Wollheim & Scharp, 1981; Prentki & Matschinsky, 1987), but glucose and certain other carbohydrates (e.g., mannose and glyceraldehyde) must be metabolized by islets in order to induce insulin secretion and are therefore designated "fuel secretagogues" (Malaisse et al., 1979; Ashcroft, 1980; Hedeskov, 1980; Wollheim & Scharp, 1981; Meglasson & Matschinsky, 1986). The biochemical mechanisms whereby islet glucose recognition is coupled to insulin secretion have been intensively studied because Type II diabetes mellitus is characterized by a relatively selective defect in glucose-induced insulin secretion despite nearly normal secretory responses to some other insulin secretagogues (Pfeiffer et al., 1981).

The nature of the metabolic signal which triggers insulin secretion is not known, but the possibility that ATP generated from metabolism of fuel secretagogues serves a second messenger function in β-cells has attracted recent interest. A K^+ channel (K_{ATP}) has been identified in β-cell plasma membranes which accounts for a major part of the permeability of the membrane to K^+ and which is inactivated by ATP (Ashcroft et al., 1984; Cook & Hales, 1984; Rorsman & Trube, 1985; Sturgress et al., 1985). Fuel secretagogue-induced inactivation of the K_{ATP} channel results in membrane depolarization (Ashcroft et al., 1984), which activates voltage-operated Ca^{2+} channels that mediate the influx of Ca^{2+} (Arkhammar et al., 1987; Gylfe, 1988a, 1988b; Keahey et al., 1989). The resultant rise in the β-cell cytosolic [Ca^{2+}] is thought to play a critical role in the induction of insulin secretion (Wollheim & Scharp, 1981; Ashcroft et al., 1984; Cook & Hales, 1984; Rorsman & Trube, 1985; Sturgess et al., 1985; Arkhammar et al., 1987; Prentki & Matschinsky, 1987; Gylfe, 1988a,b; Keahey et al., 1989).

Fuel secretagogues also induce hydrolysis of phospholipids in β-cell membranes, and this is reflected by accumulation of phospholipid-derived mediators including inositol 1,4,5-trisphosphate, free arachidonic acid, and arachidonate metabolites (Metz, 1983, 1984, 1985, 1988, 1991; Dunlop et al., 1984; Biden et al., 1987; Prentki & Matschinsky, 1987; Turk et al., 1987; Wolf et al., 1987, 1991; Robertson, 1988; Laychock, 1990). Fuel secretagogue-induced activation of β-cell phosphoinositide phospholipase C requires Ca^{2+} influx and is

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prevented by Ca^{2+} channel blockers or by removal of Ca^{2+} from the extracellular medium (Biden et al., 1987). In contrast, a major component of the accumulation of arachidonic acid and its metabolites in islets induced by fuel secretagogues is independent of Ca^{2+} influx and occurs in the presence of Ca^{2+} channel blockers and in the absence of extracellular Ca^{2+} (Wolf et al., 1991; Turk et al., 1992). At concentrations which accumulate in 17 mM D-glucose-stimulated pancreatic islets (up to 35–70 μM) (Wolf et al., 1986, 1991), arachidonate amplifies voltage-sensitive Ca^{2+} entry into prolactin-secreting cells (Vacher et al., 1989), induces a rise in β-cell cytosolic [Ca^{2+}] (Metz et al., 1987; Ramanadham et al., 1992), and amplifies depolarization-induced insulin secretion from islets (Wolf et al., 1991). The fuel secretagogue-induced accumulation of nonesterified arachidonate may therefore facilitate Ca^{2+} entry into β-cells and the insulin secretory response.

Although the biochemical mechanisms which induce the hydrolysis of β-cell membrane phospholipids upon stimulation with glucose are unknown, metabolism of fuel secretagogues to yield ATP is an obligatory event for induction of arachidonate release (Turk et al., 1992), just as it is for insulin secretion (Malaisse et al., 1979; Ashcroft, 1980; Hedeskov, 1980; Meglasson & Matschinksy, 1987). The fact that fuel secretagogue-induced hydrolysis of arachidonate from islet membrane phospholipids is in part independent of Ca^{2+} influx (Wolf et al., 1991; Turk et al., 1992) suggests that a Ca^{2+}-independent phospholipase may be involved in this process. A Ca^{2+}-independent phospholipase A_2 which selectively hydrolyzes arachidonic acid from cellular phospholipids and whose activity is regulated by ATP has recently been identified in canine myocardium (Wolf & Gross, 1985; Hazen et al., 1990, 1991a,b; Hazen & Gross, 1991). In the preceding paper (Gross et al., 1993), we have demonstrated that islets contain substantial amounts of a similar enzymatic activity and that this activity is inhibited by submicromolar concentrations of a haloenol lactone suicide substrate (HELSS)[1] in broken cell preparations. In this manuscript, we examine the effects of HELSS on secretagogue-induced insulin secretion and eicosanoid release from intact isolated pancreatic islets and on the secretagogue-induced rise in cytosolic [Ca^{2+}] in single β-cells isolated by fluorescence-activated cell sorting (FACS).

EXPERIMENTAL PROCEDURES

Materials. Male Sprague-Dawley rats (180–220 g body weight) were purchased from Sasco (O'Fallon, MO). Collagenase was obtained from Boehringer Mannheim (Indianapolis, IN). Tissue culture medium (CMRL-1066), penicillin, streptomycin, Hanks' balanced salt solution (HBSS), heat-inactivated fetal bovine serum, and L-glutamine were obtained from Gibco (Grand Island, NY). Pentex bovine serum albumin (BSA, fatty acid free, fraction V) was obtained from Miles Laboratories (Elkhart, IN). Fura-2AM (Molecular Probes, Eugene, OR) was dissolved in ethanol, and aliquots were air-dried and stored at −20 °C before dissolution in dimethyl sulfoxide. The compound 4-bromo-A23187 (Calbiochem, La Jolla, CA) was dissolved in dimethyl sulfoxide. Cell Tak was obtained from Collaborative Research Inc. (Bedford, MA). Rodent chow 5001 was purchased from Ralston Purina (St. Louis, MO). D-Glucose was purchased from the National Bureau of Standards (Washington, DC). Arachidonic acid was obtained from NuChek Prep (Elysian, MN), dissolved in 0.1 M Na_2CO_3 as a 0.2 mM solution just before use, and diluted in albumin-free buffer, as described (Wolf et al., 1991; Ramanadham et al., 1992). The haloenol lactone suicide substrate (HELSS) (*E*)-6-(bromomethylene)-3-(1-naphthalenyl)-2*H*-tetrahydropyran-2-one was prepared and purified as described previously (Hazen et al., 1991), transferred to its diluent vial as an ethanol solution, concentrated to dryness under nitrogen, and reconstituted in albumin-free buffer immediately before use. EGTA was obtained from Sigma (St. Louis, MO). ^{3}H-Labelled *myo*-[2-^{3}H]inositol (19 Ci/mmol, containing 10% ethanol) and potassium salts of D-*myo*-[2-^{3}H]inositol 1-phosphate (IP1), D-*myo*-[2-^{3}H]inositol 1,4-bisphosphate (IP2), D-*myo*-[2-^{3}H]inositol 1,4,5-trisphosphate (IP3), and D-*myo*-[2-^{3}H]inositol 1,3,4,5-tetrakisphosphate (IP4) standards (1 Ci/mmol each) were obtained from New England Nuclear.

Media. The various media used in these studies were constituted as follows: KRB (Krebs–Ringer bicarbonate buffer), 25 mM HEPES, pH 7.4, 115 mM NaCl, 24 mM $NaHCO_3$, 5 mM KCl, 2.5 mM $CaCl_2$, and 1 mM $MgCl_2$; nKRB ("normal" KRB), KRB containing 3 mM D-glucose; cCMRL-1066 ("complete" CMRL-1066), CMRL-1066 obtained from Gibco supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, and 1% (w/v) each of penicillin and streptomycin; and HBSS (Hanks' balanced salt solution), obtained from Gibco and supplemented with 0.5% penicillin–streptomycin).

Isolation and Perifusion of Islets. Islets were isolated by collagenase digestion of rat pancreata and density gradient centrifugation as described in detail in the companion paper (Gross et al., 1993). For perifusion studies, isolated, cultured islets were washed three times in nKRB medium saturated under an atmosphere of 95% air/5% CO_2 for 30 min and supplemented with 0.1% fatty acid free BSA. The islets were manually selected under stereomicroscopic visualization with a siliconized Pasteur pipette with an elongated tip and transferred onto a millipore filter of each chamber (150 islets per chamber) of a quadruple-chamber perifusion apparatus. The islets were then perifused with nKRB medium at 37 °C at a rate of 1 mL/min, as described elsewhere (Wolf et al., 1991). The composition of the medium was then changed to contain a higher D-glucose concentration (17 mM) or to contain another agent (such as 500 μM carbachol), and perifusion was then continued for 5–30 min. When perifusion experiments with arachidonate-containing solutions were performed, BSA was excluded from perifusion media but was added (final concentration 0.1% w/v) to perifusion effluent to facilitate recovery of secreted insulin. Perifusion effluent was collected continuously (1–5-mL aliquots) and was subsequently analyzed for insulin and PGE_2 content. Insulin was measured by radioimmunoassay as described in the companion paper (Gross et al., 1993).

Prostaglandin E_2 Measurement by Enzyme Immunoassay. Prostaglandin E_2 (PGE_2) was extracted from incubation medium essentially as described (Pradelles et al., 1985; Turk et al., 1992). In brief, 400 μL of the aqueous incubation medium from each condition was diluted with ethanol (100 μL) and pH was adjusted to 3 (1 N HCl). This solution was applied to an octadecylsilicic acid column (1 mL, Baker

[1] Abbreviations: HELSS, haloenol lactone suicide substrate [(*E*)-6-(bromomethylene)-3-(1-naphthalenyl)-2*H*-tetrahydropyran-2-one]; HBSS, Hanks' balanced salt solution; EGTA, [ethylenebis(oxyethylenenitrilo)]-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BSA, bovine serum albumin; PGE_2, prostaglandin E_2; FACS, fluorescence-activated cell sorting; KRB, Krebs–Ringer bicarbonate buffer; TEAB, triethylammonium hydrogen carbonate buffer; DAG, 1,2-diacyl-*sn*-glycerol; PKC, protein kinase C; $InsP_3$, inositol 1,4,5-trisphosphate; EIA, enzyme immunoassay.

Scientific, Phillipsburg, NJ) previously conditioned by elution with ethanol (3 mL) and then H_2O (3 mL). The columns were then washed with successive 3-mL aliquots of ethanol/H_2O (1/9), petroleum ether, and petroleum ether/$CHCl_3$ (65/35). PGE_2 was then eluted from the column with methyl formate (3 mL) and analyzed by enzyme immunoassay (EIA) as described (Pradelles et al., 1985). In brief, the methyl formate solution containing PGE_2 was concentrated to dryness under N_2 and reconstituted in buffer containing 100 mM potassium phosphate, 1.5 mM sodium azide, 400 mM NaCl, 1 mM tetrasodium EDTA, and 0.1% BSA, pH 7.4. Aliquots (50 μL) of each sample were then placed in wells of a microtiter plate (VWR Scientific, Batavia, IL) previously coated with mouse monoclonal anti-rabbit IgG (2 mg/mL, Biomol Laboratories, Philadelphia, PA). A tracer consisting of PGE_2 labeled with acetylcholinesterase (50 μL, Cayman Chemical, Ann Arbor, MI) and rabbit anti-PGE_2 antibody (50 μL, Biomol) were then added, and the plate was incubated at room temperature overnight. The plate was then washed (0.005% Tween in 100 mM potassium phosphate buffer, pH 7.4), and 200 μL of a developing reagent [700 mM acetylthiocholine iodide and 500 mM 5,5′-dithiobis(2-nitrobenzoic acid) in 100 mM potassium phosphate buffer] was added to each well. The plate was then covered with cellophane and agitated for 120 min. The absorbance at 414 nm was then determined on a Flow Systems microtiter plate reader, and the mass of PGE_2 in each well was calculated by interpolation from a standard curve prepared on the same plate. Fitting of the standard curve and calculation of the quantity of PGE_2 in the samples were performed with software provided by AIA reagents (Denver, CO) through the courtesy of Dr. Robert Murphy and Dr. Jacques Maclouf. The cross-reactivities of the anti-PGE_2 antibody specified by the manufacturer were as follows: PGA_1 <0.02%, PGB_1 <0.2%, PGB_2 <0.2%, PGD_2 <0.2%, PGE_1 5%, 15-keto-PGE_2 9.2%, PGF_{1a} <0.2%, 6-keto-PGF_{1a} <0.2%, PGF_{2a} <0.2%, 13,14-dihydro-15-keto-PGF_{2a} <0.2%, thromboxane B_2 <0.2%.

Preparation of Homogeneous Populations of β-Cells by Fluorescence-Activated Cell Sorting (FACS) and Measurement of β-Cell Cytosolic [Ca^{2+}] by Fura-2AM Loading and Microfluorometry. Isolated pancreatic islets were dispersed into individual cells with dispase and subjected to autofluorescence-activated cell sorting (FACS) to yield suspensions consisting predominantly (>90%) of single β-cells as described in detail in the companion paper (Gross et al., 1993). Cytosolic [Ca^{2+}] in the β-cells was measured by described techniques (Grynkiewicz, 1985; Tsien et al., 1985; Goligorsky et al., 1986; Wang & McDaniel, 1990). FACS-purified β-cells (10^5/mL) were plated onto 25-mm-diameter Cell Tak-coated glass cover slips and incubated in cCMRL-1066 medium overnight at 37 °C under an atmosphere of 5% CO_2 in air. The next day cCRML medium was replaced with nKRB medium, and β-cells were incubated (30 min, room temperature) with Fura-2 AM (10 μM) in the dark under aluminum foil (Grynkiewicz et al., 1985). The cells were washed twice with 1 mL of nKRB medium, and the cover slip with attached β-cells was transferred to a perifusion chamber apparatus. A thermostatic heating unit (37 °C) was placed around the chamber, and the cells were examined on a PTI DeltaScan instrument fitted with a Nikon microscope and a Fura lens attachment. A single cell was then selected for microfluorometric measurements. Dual-wavelength excitation was performed at 340 nm (Ca^{2+}-bound Fura-2) and 380 nm (unbound Fura-2), and emission was monitored at 505 nm while the cell was perifused. The ratio (R) of emission intensity at an excitation wavelength at 340 nm divided by that at 380 nm was determined as an index of the cytosolic [Ca^{2+}]. Calculation of the absolute [Ca^{2+}] from R was performed as previously described (Grynkiewicz, 1985; Wang & McDaniel, 1990), after the values of R_{min} and R_{max} were determined. R_{max} was defined operationally as the corrected value for R observed after addition of 4-bromo-A23187 (3 μM) to a Fura-2AM-loaded cell in Ca^{2+}-replete medium. R_{min} was defined as the corrected value for R observed after the subsequent addition of EGTA (1 mM). Corrections for Ca^{2+}-independent autofluorescence were performed after determining the value for R upon addition of $MnCl_2$, as described (Grynkiewicz, 1985; Wang & McDaniel, 1990).

Measurement of Islet Oxidation of [^{14}C]Glucose to [^{14}C]-CO_2. These measurements were performed essentially as previously described (McDaniel et al., 1974; Hughes et al., 1989). In brief, islets were cultured overnight at room temperature and washed three times in nKRB medium and manually selected under stereomicroscopic visualization into two populations. One population was incubated with the halenol lactone suicide substrate (HELSS, 25 μM) for 30 min at 37 °C and the other with vehicle in BSA-free medium. The islets were then washed with nKRB medium supplemented with 0.1% BSA, and islets from the HELSS-treated and control populations were manually selected under stereomicroscopic visualization and placed into a series of Beckman polyallomer tubes (30 islets/tube). At the appropriate time, the tubes were spun (5 s, 10000g) in a Beckman microfuge, and the supernatant was removed with an elongated Pasteur pipette and discarded. All solutions were maintained at 37 °C. A total of 0.2 mL of nKRB medium without BSA was then added to each tube, and the islets were gently resuspended and preincubated for 30 min at 37 °C in nKRB. The tubes were again spun for 5 s at 10000g on a Beckman microfuge. The supernatant was again removed and discarded, and 0.15 mL of either nKRB medium or KRB medium containing 17 mM uniformly labeled [^{14}C]glucose was added. The islets were again resuspended, and the polyallomer tubes were placed in scintillation vials containing a small piece of filter paper that just covered the bottom of the vial. The scintillation vial was then equilibrated with 95% O_2/5% CO_2, and the vials were sealed with lids containing gastight Teflon/silicone liners. The vials were then incubated for 2 h at 37 °C in a shaking water bath, during which time the islets converted a fraction of the [^{14}C]glucose to [^{14}C]CO_2. A total of 0.2 mL of hyamine base was then added to the scintillation vials with a syringe and needle by perforating the Teflon/silicone liners. The hyamine was applied to the filter paper outside the Beckman polyallomer tube. (The hyamine interacts with evolved [^{14}C]-CO_2 to entrap it in the filter paper.) Islet metabolism of [^{14}C]glucose was then terminated and dissolved [^{14}C]HCO_3^- was converted to [^{14}C]CO_2 by acidifying the medium inside the polyallomer tube with 0.2 N HCl (0.2 mL). The sealed scintillation vials were then incubated overnight at room temperature with shaking to allow the [^{14}C]CO_2 to escape from the incubation solution and to react with the hyamine impregnated in the filter paper. The next day the lids were removed from the scintillation vials, and the polyallomer tubes were removed with forceps. The exterior surface of the tubes was rinsed with 1 mL of liquid scintillation cocktail (ACS, Amersham), which was placed inside the scintillation vial. An additional 9 mL of liquid scintillation cocktail was added to each vial, and the [^{14}C] content of the vials was then measured by liquid scintillation spectrometry. Total [^{14}C] content of the stock [^{14}C]glucose solution was also determined,

as was "blank" conversion of [^{14}C]glucose to [^{14}C]CO_2 in the absence of islets. Blank values were found to account for less than 0.4% of total islet [^{14}C]CO_2 generation.

Measurement of Islet Enzyme Activities. Isolated islets were incubated either with HELSS (25 μM) for 30 min at 37 °C or with vehicle. The islets were then disrupted by sonication, and the sonicate was centrifuged to remove particulate debris. The supernatant was then either assayed directly or assayed after addition of HELSS (25 μM) for the activities of lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase by spectrophotometric measurements on a Technicon RAXT instrument. Lactate dehydrogenase was measured by described methods (Gay et al., 1968; Wacker, 1972) which involved the conversion of L-lactate plus NAD^+ to pyruvate plus NADH. Accumulation of NADH was followed spectrophotometrically at 340 nm. Aspartate aminotransferase was measured by a described method (Bergmeyer, 1978) involving coupled enzymatic reactions. The first reaction was catalyzed by aspartate aminotransferase and involved conversion of L-aspartate plus 2-ketoglutarate to L-glutamate plus oxaloacetate. The second reaction was catalyzed by malate dehydrogenase and involved conversion of oxaloacetate plus NADH to L-lactate plus NAD^+. Disappearance of NADH was followed spectrophotometrically by declining absorbance at 340 and 410 nm. Alanine aminotransferase was also measured by a described method (Bergmeyer, 1978) which involved coupled enzymatic reactions. The first reaction was catalyzed by alanine aminotransferase and involved conversion of L-alanine plus 2-ketoglutarate to L-glutamate plus pyruvate. The second reaction was catalyzed by lactate dehydrogenase and involved conversion of pyruvate plus NADH to L-lactate plus NAD^+. Disappearance of NADH was followed spectrophotometrically as above.

Islet Inositol Phosphate Generation. Isolated islets were incubated with 40 μCi (40 μL) of *myo*-[1,2-^{3}H]inositol (60 Ci/mmol) for 90 min at 37 °C with shaking in 0.31 mL of nKRB medium supplemented with 0.1% (w/v) BSA, as previously described (Turk et al., 1986b). The supernatant was then discarded, and the islets were rinsed three times with nKRB medium. Islet incorporation of radiolabel averaged 20%. For each experimental condition, 400 islets were then placed in separate polypropylene tubes with 1 mL of nKRB medium supplemented with LiCl (10 mM) and BSA (0.1%). After shaking at 37 °C for 30 min, the medium was removed, and 0.75 mL of nKRB medium ± 500 μM carbachol was added. Incubation was then continued with shaking at 37 °C for 5 min. Incubations were terminated by addition of freshly prepared ice-cold 15% (w/v) trichloroacetic acid (0.75 mL). Samples were vortex-mixed (15 s) and bath-sonicated on ice (1 min) three times, followed by bath sonication on ice (15 min) twice, at which point islet dissolution was complete as assessed under microscopic visualization. Samples were then transferred to 1.5-mL microfuge tubes, chilled on ice (20 min), and centrifuged (2000*g*, 20 min). The supernatant was placed in a polypropylene tube, extracted twice with diethyl ether (1 mL), adjusted to pH 7 with Tris (ca. 100 μL of 150 mg/mL), lyopholized, and reconstituted in 5 mL of 1 mM EDTA (pH 7.0). Samples were then analyzed by ion-exchange chromatography using Sep-Pak Accell Plus QMA disposable cartridges (Waters, Millipore, Milford, MA). Inositol phosphates were eluted, as illustrated in Figure 2A,B, by stepwise increases in the concentration of triethylammonium hydrogen carbonate buffer (TEAB, pH 8.0): from 0.1 M (IP1) to 0.3 M (IP2) to 0.4 M (IP3) to 0.5 M (IP4). Fractions (1 mL) eluting from the columns were collected and mixed with 5 volumes of liquid scintillation cocktail. The [^{3}H] content was quantitated by liquid scintillation spectrometry.

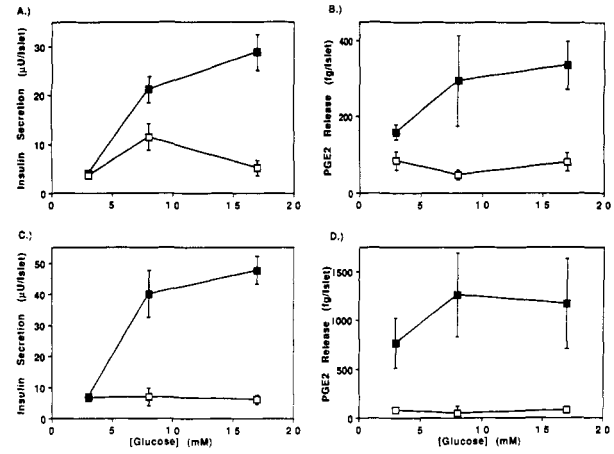

FIGURE 1: Glucose-induced and carbachol-amplified insulin secretion and prostaglandin E_2 release from isolated pancreatic islets preincubated with HELSS or vehicle. Isolated pancreatic islets were preincubated for 30 min at 37 °C with vehicle (closed symbols) or with 25 μM HELSS (open symbols) first in BSA-free KRB containing 17 mM glucose and then in KRB with 3 mM glucose (nKRB). The islets were then washed three times in nKRB supplemented with 0.1% (wv) BSA and hand-counted (150 per chamber) onto millipore filters of a quadruple-chambered perifusion apparatus and perifused with appropriate media. The perifusion medium contained D-glucose (3, 8, or 17 mM) without (panels A and B) or with carbachol (500 μM) (panels C and D). Perifusion was continued for 15 min, and the perifusion effluent was collected for measurement of insulin secretion (panels A and C) and PGE_2 release (panels B and D). Displayed values represent the mean (± SEM) of 18 measurements.

RESULTS

Basal release of insulin occurs from isolated pancreatic islets perifused with 3 mM D-glucose. As illustrated in Figure 1 (panel A), perifusion of islets with 8 and 17 mM D-glucose induced progressive increases in stimulation of insulin secretion. As previously reported, this was accompanied by hydrolysis of arachidonic acid from islet membrane phospholipid (Wolf et al., 1991), which was reflected by stimulation of release of the arachidonate metabolite PGE_2 (Figure 1, panel B). Both the insulin secretory response (panel C) and the release of PGE_2 (panel D) were further amplified by addition of the muscarinic agonist carbachol (500 μM) to the glucose stimulus, confirming previous observations (Turk et al., 1992). Preincubation of the islets for 30 min with the haloenol lactone suicide substrate (HELSS, 25 μM) resulted in substantial suppression of both insulin secretion (panel A) and eicosanoid release (panel B) induced by glucose and in suppression of the amplification of insulin secretion (panel C) and eicosanoid release (panel D) induced by carbachol.

Oxidation of [^{14}C]glucose to [^{14}C]CO_2 by islets requires both glycolytic metabolism and mitochondrial oxidation of the carbohydrate, and the rate of glucose utilization ordinarily closely parallels the rate of insulin secretion induced by D-glucose (Meglasson & Matschinsky, 1986). Similar relationships have been observed with a number of other carbohydrate secretagogues (Meglasson & Matschinsky, 1986). As illustrated in Table I, at a concentration of 3 mM [^{14}C]-D-glucose, islet oxidation of the substrate was 8.5 pmol/islet over a 2-h incubation, and the rate of oxidation rose to 32 pmol islet^{-1} (2 h)$^{-1}$ with 17 mM [^{14}C]-D-glucose, similar to previously reported values for these conditions (McDaniel et al., 1974; Hughes et al., 1989). Preincubation of the islets

Table I: Influence of HELSS on Rat Islet Oxidation of [^{14}C]-D-Glucose to [^{14}C]CO_2[a]

[^{14}C]glucose concn (mM)	HELSS concn (μM)	[^{14}C]CO_2 production (pmol/islet)
3	0	8.45 ± 2.71
3	25	9.70 ± 2.13
17	0	31.85 ± 6.70
17	25	31.85 ± 5.92

[a] Isolated pancreatic islets were preincubated for 30 min at 37 °C with 25 μM HELSS or with vehicle in KRB plus 17 mM glucose without BSA and then washed three times in KRB plus 3 mM glucose plus 0.1% (w/v) BSA. Islets were then hand-counted (30 per condition) into Beckman polyallomer tubes, and conversion of [^{14}C]glucose to [^{14}C]CO_2 was determined in a 2-h incubation at 37 °C, as described in Experimental Procedures. Displayed values represent the means ± SEM of three measurements.

Table II: Influence of HELSS on the Activity of Rat Islet Enzymes[a]

HELSS concn (μM)		enzyme activity (international units/L)		
preincubation	incubation	LDH	AST	ALT
0	0	20 ± 1	22 ± 1	2.0 ± 0.6
25	0	21 ± 1	21 ± 1	2.0 ± 0.0
0	25	17 ± 1	21 ± 1	1.7 ± 0.3

[a] Islets were hand-counted into siliconized glass vials (500 per condition) and homogenized by sonication. The activities of lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured in the homogenate as described in Experimental Procedures. Enzyme activities were measured in homogenates of islets that had been pretreated with HELSS (30 min, 37 °C) ("preincubation") or with vehicle. In addition, enzyme activities were measured in homogenates from islets that had not been pretreated with HELSS but in which HELSS was added to the homogenate during the period of measurement of enzymatic activity ("incubation"). Displayed values represent the mean ± SEM of three measurements.

for 30 min with the haloenol lactone suicide substrate (HELSS, 25 μM) under conditions which resulted in inhibition of insulin secretion and of eicosanoid release from glucose-stimulated islets (Figure 1) did not affect oxidation of either 3 or 17 mM [^{14}C]-D-glucose to [^{14}C]CO_2 (Table I). These observations indicate that HELSS does not impair islet glucose metabolism, in contrast to some other inhibitors of islet phospholipases and arachidonate oxygenases (Best, et al., 1984; Zawalich & Zawalich, 1985). Similarly, the activities of the islet enzymes lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase (Table II) were not influenced by 30-min preincubation of intact islets with 25 μM HELSS (Table II) or by adding HELSS (25 μM) to islet homogenates containing these enzymes (Table II).

Stimulation of islets with the muscarinic agonist carbachol induces activation of phosphoinositide phospholipase C and the accumulation of 1,2-diacyl-*sn*-glycerol (DAG) (Peter-Riesch et al., 1988; Wolf et al., 1989) and inositol polyphosphates including inositol 1,4,5-trisphosphate ($InsP_3$) (Dunlop & Larkins, 1986; Biden et al., 1987) in islets. Carbachol-induced DAG accumulation results in activation of islet protein kinase C (PKC) and amplifies D-glucose-induced insulin secretion (Easom et al., 1990) because D-glucose alone is a weak stimulus for islet DAG accumulation and PKC activation (Easom et al., 1990; Wolf et al., 1990). Carbachol-induced $InsP_3$ accumulation induces a transient rise in β-cell cytosolic [Ca^{2+}] reflecting Ca^{2+} release from intracellular sequestration sites. The inositol phosphates accumulating in carbachol-stimulated islets can be analyzed by ion-exchange chromatography (Turk et al., 1986b; Biden et al., 1987), as illustrated in Figure 2 (panel A). Carbachol-induced accumulation of inositol phosphates (Figure 2, panel B) including $InsP_3$ in

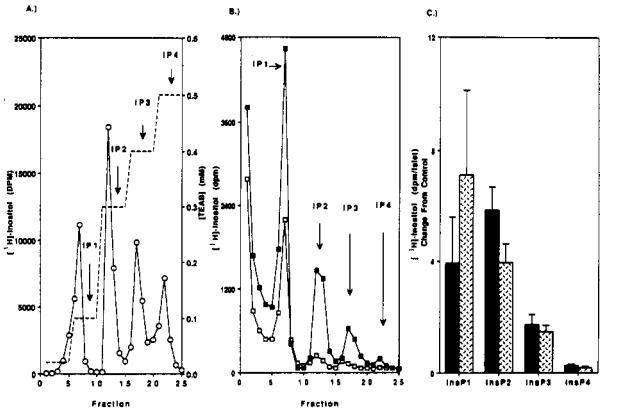

FIGURE 2: Carbachol-induced inositol phosphate accumulation in isolated pancreatic islets preincubated with HELSS or vehicle. Panel A illustrates the elution (solid line) of standard, [^{3}H]inositol-labeled inositol phosphates (IP1, [^{3}H]inositol 1-phosphate; IP2, [^{3}H]inositol 1,4-bisphosphate; IP3; [^{3}H]inositol 1,4,5-trisphosphate; and IP4, [^{3}H]-inositol 1,3,4,5-tetrakisphosphate) from ion-exchange columns by increasing concentrations of TEAB (triethylammonium hydrogen carbonate buffer, dashed line), as described under Experimental Procedures. Panel B illustrates the effect of carbachol (500 μM CCh, closed symbols) to stimulate accumulation of [^{3}H]inositol phosphates in *myo*-[^{3}H]inositol-prelabeled islets compared to islets incubated in control medium containing 3 mM D-glucose but without carbachol (open symbols). Panel C illustrates the difference in the [^{3}H]inositol content in the various inositol phosphate peaks obtained from islets incubated with carbachol (500 μM) plus D-glucose (3 mM) ± HELSS minus that in the peaks from islets incubated with 3 mM D-glucose alone ± HELSS and therefore reflects carbachol-induced [^{3}H]inositol phosphate accumulation in islets that had been pretreated with vehicle only (solid bars) or with 25 μM HELSS (stippled bars) under conditions described in Figure 1. Displayed values represent the means (±SEM) of five experiments.

islets was essentially unaffected by preincubation of islets with HELSS (25 μM) (Figure 2, panel B), although such pretreatment markedly attenuated the amplification of insulin secretion and eicosanoid release by carbachol (Figure 1). This observation indicates that HELSS did not inhibit the phosphoinositide phospholipase C activated by muscarinic receptor occupancy.

When islets were pretreated with varying HELSS concentrations (1, 5, or 25 μM), little influence was observed on basal insulin secretion or eicosanoid release at 3 mM D-glucose (Figure 3, panel A). Both insulin secretion and eicosanoid release induced by 17 mM D-glucose plus carbachol (500 μM) were progressively inhibited by the increasing HELSS concentrations, however, and the two processes were found to be inhibited with a similar HELSS concentration dependence (Figure 3, panel B). The same was true of both insulin secretion and eicosanoid release induced by 17 mM D-glucose alone, and 50% of the highest degree of inhibition of both processes was observed at a HELSS concentration between 1 and 5 μM (Figure 3, panel C). When islets were pretreated for various periods of time with a fixed concentration of HELSS (25 μM), the inhibition of both insulin secretion and eicosanoid release was found to occur rapidly and was essentially complete by 10 min (Figure 3, panel D), consistent with the relatively short half-life of the compound in aqueous solution (Hazen et al., 1991). Pretreatment (30 min) of islets with HELSS (25 μM) did not inhibit islet conversion of exogenous arachidonate (15 μM) to PGE_2 [3642 fg of PGE_2 islet^{-1} (5 min)$^{-1}$ in control islets vs 4470 fg islet^{-1} (5 min)$^{-1}$ in HELSS-pretreated islets].

The observations that D-glucose induces accumulation of nonesterified arachidonate in islets (Wolf et al., 1986, 1991), that nonesterified arachidonate induces a rise in the cytosolic

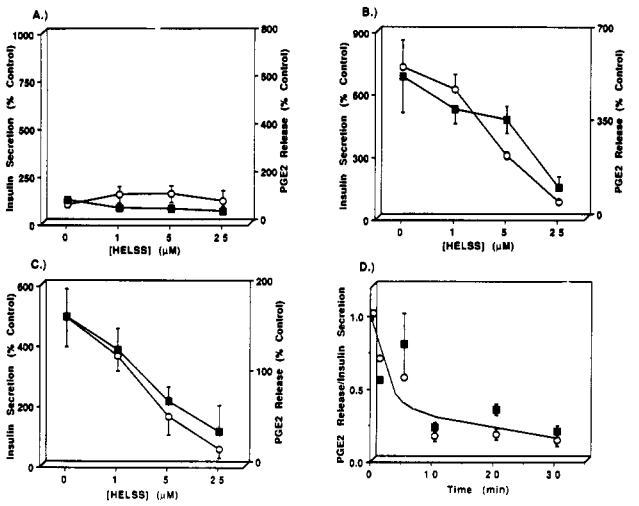

FIGURE 3: Concentration dependence and time course of inhibition of islet prostaglandin E_2 release and insulin secretion. Experiments were performed as described in Figure 1 except that the HELSS concentration in the pretreatment period was 0, 1, 5, or 25 μM in panels A, B, and C, and the duration of the pretreatment period with 25 μM HELSS was 0, 1, 5, 10, 20, or 30 min in panel D. In all panels insulin secretion is represented by open symbols and PGE_2 release by closed symbols. Islets were perifused with 3 mM D-glucose (panel A), 17 mM D-glucose plus 500 μM carbachol (panel B), or 17 mM D-glucose alone (panels C and D). Mean values (± SEM) of 5–8 measurements are displayed for each parameter normalized to the value obtained at 3 mM glucose without HELSS in each individual experiment.

$[Ca^{2+}]$ of isolated β-cells (Metz et al., 1987) by promoting Ca^{2+} influx (Ramanadham et al., 1992), and that the haloenol lactone suicide substrate HELSS inhibits arachidonate release from phospholipids catalyzed by an islet Ca^{2+}-independent phospholipase A_2 activity (Gross et al., 1993) all suggest that HELSS might attenuate the D-glucose-induced rise in β-cell cytosolic $[Ca^{2+}]$. The influence of HELSS on this phenomenon was therefore examined. As illustrated in Figure 4, depolarization of individual β-cells with 20 mM KCl induced a rise in the β-cell cytosolic $[Ca^{2+}]$ (panel A). This response was little affected by 3 μM HELSS (panel B). Similarly, the rise in β-cell cytosolic $[Ca^{2+}]$ induced by 500 μM carbachol alone (panel E) was not significantly affected by 3 μM HELSS (panel F). In contrast, the rise in β-cell cytosolic $[Ca^{2+}]$ induced by 17 mM D-glucose (panel C) was greatly attenuated by 3 μM HELSS (panel D). Combined stimulation of single β-cells with carbachol (500 μM) and 17 mM D-glucose induced a biphasic rise in cytosolic $[Ca^{2+}]$ (panel G), and the second phase of this response was diminished by 3 μM HELSS (panel H).

As illustrated in Figure 5, the mean rise in cytosolic $[Ca^{2+}]$ in β-cells stimulated with 17 mM D-glucose alone was inhibited by about 50% at a concentration of 3 μM HELSS and by 65% at concentrations of 5 or 10 μM HELSS (panel A). The inhibition of the 17 mM D-glucose-induced rise in β-cell cytosolic $[Ca^{2+}]$ by HELSS was significant at concentrations of 3 μM (Figure 5, panels A and B), 5 μM, and 10 μM (Figure 5, panel A). The mean rise in cytosolic $[Ca^{2+}]$ in β-cells stimulated by carbachol (500 μM) alone was slightly but not significantly reduced by 3 μM HELSS (Figure 5, panel B). HELSS (3 μM) also did not significantly affect the mean rise in cytosolic $[Ca^{2+}]$ in β-cells depolarized with 20 mM KCl (Figure 5, panel C) but did significantly suppress the mean magnitude of the second phase of the rise in cytosolic $[Ca^{2+}]$ in β-cells stimulated with the combination of carbachol (500 μM) plus 17 mM D-glucose (Figure 5, panel C).

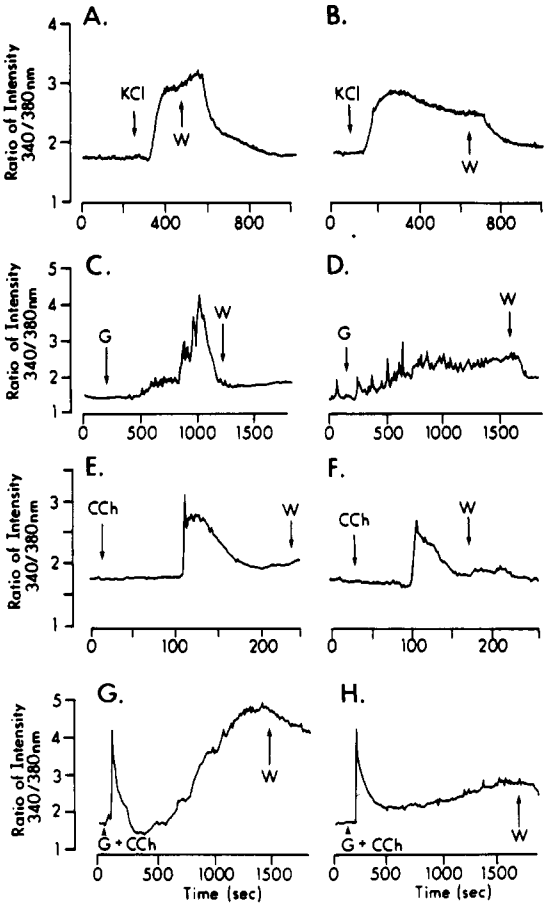

FIGURE 4: Changes in β-cell cytosolic $[Ca^{2+}]$ induced by depolarization, D-glucose, carbachol, and carbachol plus D-glucose without or with HELSS. Individual β-cells were prepared from dispersed islet cells by FACS, attached to glass cover slips, and loaded with Fura-2AM. The cytosolic $[Ca^{2+}]$ of the β-cells was determined as a function of time by dual-wavelength microfluorometry as described under Experimental Procedures. The cytosolic $[Ca^{2+}]$ is reflected by the ordinate value in the panels of the ratio of fluorescence intensity with excitation at 340 nm divided by that at 380 nm as described under Experimental Procedures. Cells in panels A, C, E, and G were pretreated with vehicle only, and cells in panels B, D, F, and H were pretreated with 3 μM HELSS for 10 min at 37 °C. The panels illustrate the rise in β-cell cytosolic $[Ca^{2+}]$ induced by four different stimuli: (1) depolarization induced by raising the extracellular KCl concentration from 5 to 20 mM (panels A and B), (2) 17 mM D-glucose (panels C and D), (3) 500 μM carbachol (panels E and F), and (4) carbachol (500 μM) plus 17 mM D-glucose (panels G and H). Arrows indicate the time of stimulus introduction and washout (W) with nKRB medium + 0.1% BSA.

We have demonstrated elsewhere that exogenous arachidonic acid induces a concentration-dependent (1–30 μM) rise in β-cell cytosolic $[Ca^{2+}]$ resulting from the influx of Ca^{2+}, that this effect is readily reversible upon washout of arachidonate, and that it is attributable to arachidonate itself rather than to a metabolite (Ramanadham et al., 1992). This effect is illustrated in Figure 6, panel A. Also illustrated in Figure 6 is the fact that, under conditions where pretreatment of isolated β-cells with 3 μM HELSS attenuated the rise in cytosolic $[Ca^{2+}]$ induced by 17 mM D-glucose (compare panels C and D), the rise in β-cell cytosolic $[Ca^{2+}]$ induced by exogenous arachidonic acid (15 μM) was unaffected (panel B). Coupled with the observation that HELSS pretreatment did not influence the depolarization-induced rise in the β-cytosolic $[Ca^{2+}]$ (Figure 4, panels A and B, and Figure 5, panel C), the observations in Figure 6 indicate that HELSS did not nonspecifically inhibit Ca^{2+} into β-cells from the extracellular space but rather induced a specific blockade in

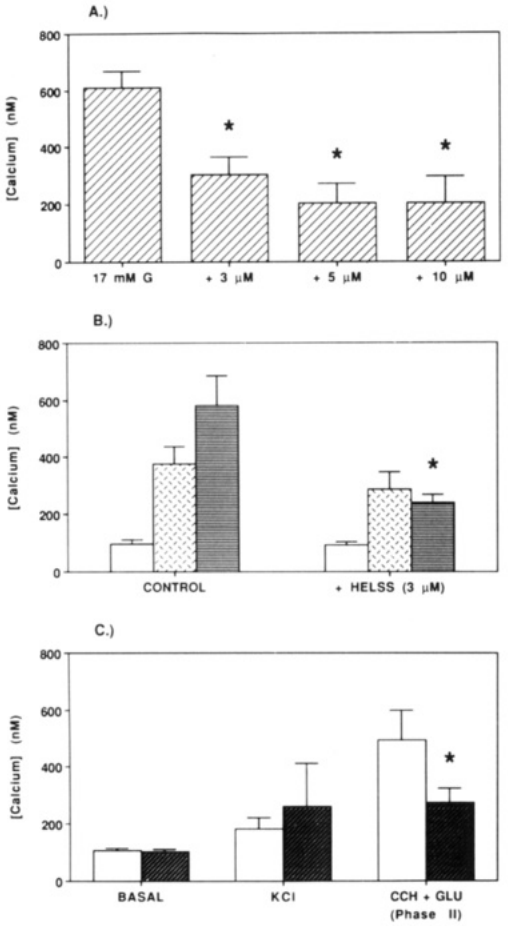

FIGURE 5: Mean data illustrating the rise in cytosolic [Ca^{2+}] induced by depolarization, D-glucose, carbachol, and carbachol plus D-glucose in isolated β-cells preincubated with HELSS or vehicle. Experiments were performed as in Figure 4 but were repeated 12 times, and mean values for the rise in cytosolic [Ca^{2+}] (±SEM) are indicated. In panel A, the HELSS concentration in the preincubation period was 0 μM (first bar), 3 μM (second bar), 5 μM (third bar), or 10 μM (fourth bar), and the cells were stimulated with 17 mM D-glucose. In panel B, experiments were performed as in panels C–F of Figure 4. In both the left set and the right set of three bars, the first bar (open) represents 3 mM D-glucose, and the second bar (stippled) represents 500 μM carbachol, and the third bar (horizontal) represents 17 mM D-glucose. The left set of three bars indicates the control responses, and the right set of three bars indicates the responses in β-cells pretreated with 3 μM HELSS. In panel C, experiments were performed as in panels A and B of Figure 4 (first set of two bars indicates the response to 5 mM KCl and 3 mM D-glucose; second set of two bars indicates the response to 20 mM KCl and 3 mM D-glucose) and as in panels G and H of Figure 4 (third set of two bars indicates the second phase of the response to combined stimulation with 17 mM D-glucose plus 500 μM carbachol). In each set of two bars, the first bar (open) indicates the control response to the stimulus, and the second bar (cross-hatched) indicates the response of β-cells pretreated with 3 μM HELSS.

the 17 mM D-glucose-induced rise in β-cell cytosolic [Ca^{2+}].

DISCUSSION

The haloenol lactone suicide substrate selectively inhibits the activity of a Ca^{2+}-independent phospholipase A_2 (Hazen et al., 1991a) first identified in canine myocardium (Wolf & Gross, 1985; Hazen et al., 1990, 1991b; Hazen & Gross, 1991), and this compound also inhibits a similar enzymatic activity in isolated pancreatic islets, as demonstrated in the preceding paper (Gross et al., 1993). The observations herein demonstrate that HELSS suppresses both insulin secretion and eicosanoid release from isolated islets stimulated with the fuel secretagogue D-glucose and with the muscarinic agonist

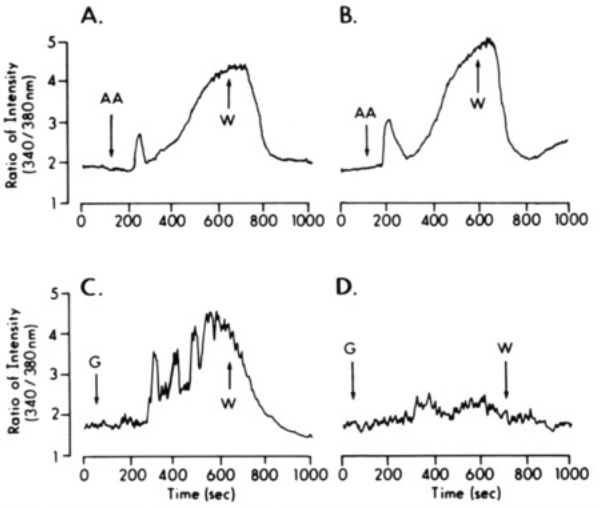

FIGURE 6: Contrasting effects of HELSS on the rise in β-cell cytosolic [Ca^{2+}] induced by arachidonic acid and by 17 mM D-glucose. Experiments were performed as in Figure 4 except that in panels A and B, cover-slip-attached, Fura-2-loaded β-cells were perifused with medium containing arachidonic acid (AA) (15 μM) without BSA and 3 mM D-glucose. In panels C and D, the Fura-2-loaded β-cells were perifused with medium containing 17 mM D-glucose (G) and no arachidonic acid. Washout solution (W) contained 0.1% (w/v) BSA. Tracings in panels A and C were obtained from β-cells pretreated with vehicle alone while those in panels B and D were obtained from β-cells that had been pretreated with 3 μM HELSS.

carbachol. Despite the clear suppression by HELSS of secretagogue-induced eicosanoid release and insulin secretion from isolated islets, HELSS exerted little effect on several other processes in islets, including oxidation of [^{14}C]-D-glucose to [^{14}C]CO_2, the activity of the islet enzymes lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase, carbachol-induced inositol trisphosphate accumulation; and the rise in β-cell cytosolic [Ca^{2+}] induced by depolarization with 20 mM KCl. These observations indicate that HELSS exerts a relatively specific effect on its target enzyme (the ATP-stimulated, Ca^{2+}-independent phospholipase A_2) in islets.

The inhibition of both eicosanoid release and insulin secretion from isolated islets exhibited a similar concentration and time dependence of exposure to HELSS. About 50% of the highest observed degree of inhibition of both 17 mM D-glucose-induced eicosanoid release and insulin secretion occurred at a HELSS concentration between 1 and 5 μM. In isolated β-cells, HELSS at a concentration of 3 μM also inhibited the D-glucose-induced rise in cytosolic [Ca^{2+}] by 50% but exerted no influence on the rise in cytosolic [Ca^{2+}] induced by exogenous arachidonate (15 μM) or by depolarization with KCl (20 mM). This suggests that Ca^{2+}-influx into β-cells induced by glucose is facilitated by arachidonate released by the action of the Ca^{2+}-independent phospholipase A_2. HELSS was observed in the preceding paper (Gross et al., 1993) to inhibit the activity of the islet Ca^{2+}-independent phospholipase A_2 at concentrations below 1 μM in broken cell preparations in which HELSS has direct access to the target enzyme. The slightly higher HELSS concentrations required in isolated β-cells and in intact islets probably reflect the barriers imposed by the β-cell plasma membrane and by the aggregated cell mass of isolated islets (which contain about 2000 cells within a limiting capsule) to access by HELSS to the target phospholipase A_2 enzyme.

These observations contribute to a growing body of data which suggest that modulation of release of nonesterified arachidonate from phospholipids in β-cells may participate in

the regulation of β-cell [Ca^{2+}] and of insulin secretion. Stimulation of islets with fuel secretagogues induces hydrolysis of islet membrane phospholipids (Turk et al., 1986a) and a rise in the mass of nonesterified arachidonate by an amount sufficient to achieve an increment in the cellular concentration by 35–70 μM, as measured by stable isotope dilution gas chromatography–mass spectrometry (Wolf et al., 1986, 1991; Turk et al., 1987). The accumulation of nonesterified arachidonate within islets is reflected by release of arachidonate metabolites such as PGE_2 into the perifusion medium (Wolf et al., 1991). PGE_2 itself does not stimulate and is thought to negatively modulate insulin secretion (Metz, 1988; Robertson, 1988). PGE_2 release nonetheless serves as a readily measured marker event for hydrolysis of arachidonate from islet membrane phospholipids (Wolf et al., 1991), which is the rate-limiting step for eicosanoid formation (Needleman et al., 1986).

A major component of the fuel secretagogue-induced hydrolysis of arachidonate from islet membrane phospholipids is independent of Ca^{2+} influx and occurs in Ca^{2+}-free medium and in the presence of Ca^{2+} channel blockers (Wolf et al., 1986; Turk et al., 1992). Induction of arachidonate hydrolysis from islet membrane phospholipids by fuel secretagogues requires islet metabolism of the secretagogue by processes which generate ATP (Turk et al., 1992). At concentrations which accumulate in D-glucose-stimulated islets, exogenous arachidonate (5–30 μM) induces a rise in the cytosolic [Ca^{2+}] of isolated β-cells (Metz et al., 1987; Ramanadham et al., 1992). A similar effect of exogenous arachidonate (5–30 μM) to raise the cytosolic [Ca^{2+}] has been observed in a clonal T-lymphocyte cell line (Chow & Jondal, 1990). At similar concentrations (5–30 μM), exogenous arachidonate has been observed to amplify voltage-dependent Ca^{2+} entry into a clonal prolactin-secreting pituitary cell line by shifting the voltage dependence of Ca^{2+} channel activation to lower transmembrane potentials (Vacher et al., 1989). In a similar manner, exogenous arachidonate (5–30 μM) has been found to amplify depolarization-induced insulin secretion from isolated islets by shifting the voltage dependence of this phenomenon to lower transmembrane potentials (Wolf et al., 1991) in a process that requires Ca^{2+} entry from the extracellular space (Turk et al., 1992).

Two properties of the hydrolysis of arachidonate from islet membrane phospholipids induced by fuel insulin secretagogues described above suggested that this process might involve the activity of a novel phospholipase A_2 enzyme similar to that recently described in canine myocardium (Wolf & Gross, 1985; Hazen et al., 1990, 1991a,b; Hazen & Gross, 1991). The first was that the fuel secretagogue-induced eicosanoid release in islets requires metabolism of the secretagogues to yield ATP (Turk et al., 1992). Second, a major component of this eicosanoid release occurs without Ca^{2+} influx (Wolf et al., 1991; Turk et al., 1992). The activity of the canine myocardial phospholipase A_2 enzyme is independent of Ca^{2+} (Wolf & Gross, 1985; Hazen et al., 1990, 1991a,b) and is activated by ATP (Hazen & Gross, 1991). This prompted a search for such an enzyme in islets, and in the preceding paper we have demonstrated that islets do contain substantial amounts of a very similar enzymatic activity (Gross et al., 1993). Both the myocardial (Hazen et al., 1991a) and the islet (Gross et al., 1993) phospholipase A_2 activities are inhibited by a haloenol lactone suicide substrate. The possible participation of this phospholipase A_2 in fuel secretagogue-induced eicosanoid release and insulin secretion is supported by the observations that HELSS inhibits both of these processes in islets and the secretagogue-induced rise in cytosolic [Ca^{2+}] in isolated β-cells. Previous reports have indicated that pharmacologic inhibitors of phospholipases suppress fuel secretagogue-induced insulin secretion from isolated islets (Kato et al., 1983; Laychock, 1983; Dunlop et al., 1984; Metz, 1984) but have not characterized the relative concentration dependence of suppression of eicosanoid release and insulin secretion, the specificity of the compounds with respect to glucose oxidation and other events in islets, or the effects of inhibition of arachidonate release on the β-cell cytosolic [Ca^{2+}].

The observation that fuel secretagogues must be metabolized by islets by ATP-generating processes in order to induce insulin secretion has long been interpreted to indicate that fuel sensors (Malaisse et al., 1979; Ashcroft, 1980; Hedeskov, 1980; Meglasson & Matschinsky, 1986) such as ATP response elements must be involved in fuel-induced insulin secretion. A novel ATP-inhibited K^+ channel (K_{ATP}) has recently been identified in β-cell plasma membranes and is thought to constitute one such ATP response element (Ashcroft et al., 1984; Cook & Hales, 1984; Rorsman & Trube, 1985; Sturgess et al., 1985). Stimulation of β-cells with fuel secretagogues induces the closure of the K_{ATP} channel, which accounts for the major part of the β-cell plasma membrane permeability to K^+. Since the β-cell transmembrane potential is largely a K^+ potential, the resulting decrement in the permeability to K^+ causes the membrane potential to rise toward zero. As the transmembrane potential rises, voltage-operated Ca^{2+} channels are activated, and Ca^{2+} enters the β-cell down its concentration gradient from the extracellular space. This results in a rise in the β-cell cytosolic [Ca^{2+}], which is thought to be critical in the induction of insulin secretion (Arkhammar et al., 1987; Gylfe, 1988a,b; Keahey et al., 1989).

All of the factors regulating these ionic events, however, are not clearly established. For example, although the islet K_{ATP} channel is clearly sensitive to ATP and the metabolism of glucose clearly generates ATP within the islet, the ATP concentration required to inhibit K_{ATP} channel activity by 50% is about 10 μM (Cook & Hales, 1984). Resting islet ATP levels are in the range of 1.5–3.5 mM (Meglasson et al., 1989; Ohta et al., 1990, 1991a,b, 1992; Ghosh et al., 1991), suggesting that the channel should be closed, and not open, in the resting state. A suggested explanation for this discrepancy is that many "spare" K_{ATP} channels exist, the vast majority of which are closed in the resting islet, and that closure of the small residual fraction of open channels requires ATP concentrations well above that required to close 50% of the entire channel population in patch-clamping studies (Cook et al., 1988). A second difficulty is that global ATP levels appear to change relatively little in the glucose-stimulated islet on the time scale when the initial insulin secretory response occurs (Ghosh et al., 1991), although a doubling of islet ATP levels has been observed after 30 min of glucose stimulation (Thankakitcharu et al., 1991; Zhou et al., 1991; Fadda et al., 1992). A suggested explanation for this discrepancy is that the K_{ATP} channel response to ATP is modulated by ADP (Kakei et al., 1986), the level of which may change more substantially in glucose-stimulated islets than that of ATP (Meglasson et al., 1989; Ohta et al., 1990, 1991a,b, 1992). Nonetheless, these considerations suggest that amplification mechanisms which magnify the effects of rather small changes in ATP levels or in membrane potential might be involved in producing the ionic and secretory responses to glucose stimulation and that ATP response elements in addition to the K_{ATP} channel might also participate in these processes.

It is possible that a Ca^{2+}-independent, ATP-activated phospholipase A_2 enzyme such as that first described in canine myocardium (Wolf & Gross, 1985; Hazen et al., 1990, 1991a,b; Hazen & Gross, 1991) and now in isolated islets (Gross et al., 1993) might constitute such an ATP response element and participate in the fuel-sensing apparatus of β-cells. Nonesterified arachidonate released from β-cell phospholipids by the action of such an enzyme might then accumulate within β-cell membranes and facilitate Ca^{2+} entry by reducing the activation requirement of voltage-operated Ca^{2+} channels to lower transmembrane potentials, as described in prolactin-secreting cells (Vacher et al., 1989). In this way, both the response to small, perhaps local, changes in ATP concentrations and the response to small changes in membrane potential could be amplified in the resultant change in β-cell cytosolic [Ca^{2+}]. With respect to the possibility that changes in ATP concentrations in localized compartments might occur, it is of interest that ATP derived from glycolysis has been found to interact with ATP-sensitive K^+ channels in cardiac myocytes in preference to ATP derived from mitochondrial oxidative phosphorylation (Weiss & Lamp, 1987), and a similar situation may obtain for the islet ATP-sensitive phospholipase A_2.

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