Glucose Activates the Multifunctional Ca\(^{2+}\)/Calmodulin-dependent Protein Kinase II in Isolated Rat Pancreatic Islets*

(Received for publication, August 9, 1993, and in revised form, November 1, 1993)

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The influence of the insulin secretagogues, glucose and K\(^+\), to activate the multifunctional, Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) in isolated rat pancreatic islets has been examined. Glucose (28 mM) and K\(^+\) (40 mM) were demonstrated to induce a 1.89 ± 0.19- and 1.75 ± 0.15-fold increase, respectively, in phosphorylation of a subunit of CaMKII immunoprecipitated by an anti-CaMKII antibody. In intact islets, glucose and K\(^+\) also induced the generation of an autonomous, Ca\(^{2+}\)/calmodulin-independent protein kinase II activity characteristic of autophosphorylated enzyme. Maximal activation, 2.9 ± 0.2- and 3.0 ± 0.5-fold for glucose and K\(^+\), respectively, relative to basal glucose control, was achieved at 2.5–5 min followed by a decline to near basal levels by 20 min. Glucose induced the production of autonomous CaMKII activity that, in terms of fold stimulation, correlated closely with the extent of insulin release over a glucose concentration range of 3–28 mM. This stimulated activity was completely prevented by an inhibitor of glucose metabolism, mannoheptulose. These data demonstrate that the exposure of islets to stimulatory glucose concentrations activates CaMKII. The close correlation of enzyme activation with insulin secretion is consistent with the hypothesis that CaMKII plays an important role in the regulation of insulin secretion or related β-cell processes.

D-Glucose is the primary physiological stimulator of insulin secretion from the pancreatic β-cell in man and rodents and induces biphasic secretion from isolated islets that mimics physiological secretion in vivo (Hedekov, 1980). It is clear that the metabolism of glucose is required for secretion, and it is thought that the first enzyme in this process, glucokinase, serves as the intracellular glucose recognition molecule (Megglasson and Matschinsky, 1986). Subsequent to metabolism, the principal response of the β-cell to glucose is an increased intracellular Ca\(^{2+}\), contributed to, in the most part, by the stimulation of Ca\(^{2+}\) influx (Frentk and Matschinsky, 1987; Wollheim and Sharp, 1981). The cellular mechanism linking these processes is thought to involve an increased intracellular ATP:ADP ratio as the result of glucose metabolism and the depolarization of the β-cell via the closing of ATP-sensitive K\(^+\) channels (K\(_{ATP}\) channels) (Rajan et al., 1990). Cell depolarization promotes the opening of L-type voltage-dependent Ca\(^{2+}\) channels on the plasma membrane allowing the influx of Ca\(^{2+}\) down its concentration gradient (Rajan et al., 1990).

In contrast to the recent progress in the understanding of these early events of the secretion process, the cellular events linking increased Ca\(^{2+}\) concentrations to the release of hormone are unknown. Several reports support a role of the cytoskeleton in insulin secretion (Howell and Tubyhurst, 1984; Stutchfield and Howell, 1984; Wang et al., 1990), which has led to the hypothesis that cytoskeletal-associated, Ca\(^{2+}\)-activated proteins, particularly protein kinases, may regulate insulin secretion (Ashcroft and Hughes, 1990). One enzyme implicated in this process is the Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII).

An increasing body of evidence has now established CaMKII as one of the major protein kinases orchestrating intracellular responses to extracellular signals (Hanson and Schulman, 1992). It is widespread in tissues and is characterized by the ability to phosphorylate a diverse array of substrates in vitro and in situ that contribute to a variety of cellular functions that include the regulation of cell metabolism, ion flux, Ca\(^{2+}\) homeostasis, and cytoskeletal function (Schulman and Hanson, 1987; Colbran et al., 1989). CaMKII activity is found in greatest concentration in neurons where it is involved in the regulation of neurotransmitter biosynthesis and release (Benfenati et al., 1992). In the forebrain, CaMKII exists as an oligomer of 8–12 similar subunits including an α-subunit of 50–54 kDa and a β-subunit of 58–60 kDa in a αβ ratio of 3:4:1 (Lin et al., 1987; Hanson and Schulman, 1992). This ratio, however, varies in other brain tissues (Miller and Kennedy, 1985; McGinness et al., 1985). Other isoforms, β' (generated by alternative splicing of the β gene) γ, and δ, have been identified by cloning and sequencing analysis, but no additional cellular function has been detected in these subunits (Tobimatsu and Fugisawa, 1989; Schulman and Hanson, 1993).

Activation of CaMKII by Ca\(^{2+}\) and calmodulin in vitro results in rapid enzyme autophosphorylation (Schulman and Hanson, 1993). The autophosphorylated enzyme is no longer dependent on Ca\(^{2+}\) and calmodulin for activity and performs further autophosphorylation or the phosphorylation of exogenous substrates in the absence of these cofactors (Saitob and Schwartz, 1986; Miller and Kennedy, 1986; Schreiber et al., 1989).
Glucose Activation of CaM Kinase II in Islets

Schröder et al., 1986). Enzyme autophosphorylation and generation of autonomous kinase II activity have since been reported in intact cells in response to agonists that increase intracellular Ca\(^{2+}\) concentration (Gorelick et al., 1988; Fukunaga et al., 1989; MacNicol et al., 1990; Jefferson et al., 1991; Ocorr and Schulman, 1991).

Isolated pancreatic islets have been shown to possess a cytoskeletal-associated Ca\(^{2+}\)- and calmodulin-dependent protein kinase activity (referred to as P53 kinase (Harrison and Ashcroft, 1982; Ashcroft and Hughes, 1990; Landt et al., 1982)), the major substrates of which are endogenous proteins of M\(_{r}\) 53,000 and 57,000 (Colca et al., 1983a). This P53 kinase has been purified to near homogeneity from RINm5F cells and the enzyme shown to possess properties reminiscent of the rat brain CaM kinase II including a low affinity for calmodulin (relative to other calmodulin-dependent enzymes) and the ability to phosphorylate known substrates of CaM kinase II. Furthermore, a P53 kinase in islets has recently been shown to possess kinetic properties similar to rat brain CaM kinase II (Hughes et al., 1993). Evidence from which a possible role for CaM kinase II in secretion has been evaluated is, however, purely circumstantial. In a preliminary study, the phosphorylation of the 53,000 molecular weight protein was increased, though only modestly, in glucose-stimulated islets (Colca et al., 1983a). The diabetogenic agent alloxan was further shown to inhibit P53 kinase activity and glucose-induced insulin secretion from islets in parallel (Colca et al., 1983b). In more recent efforts, the putative inhibitors of CaM kinase II, KN-62 (Wenham et al., 1990; Li et al., 1992), and KN-93 (Niki et al., 1993) have been shown to suppress insulin secretion. Experiments utilizing these pharmacological agents are, however, confused by additional effects of alloxan and KN-62 to influence glucokinase (Lenzen et al., 1988) and Ca\(^{2+}\) channel activity, respectively (Li et al., 1992).

The objective of the current study was to evaluate the ability of glucose to activate CaM kinase II in isolated islets by the induction of enzyme autophosphorylation and autonomous kinase activity. The correlation of CaM kinase II activation to glucose-induced insulin secretion over a similar concentration range supports the hypothesis that CaM kinase II plays an important role in glucose-regulated insulin secretion or related \(\beta\)-cell function.

**EXPERIMENTAL PROCEDURES**

**Materials**—Male Wistar rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN) and maintained on Tekland Rodent Diet (Indianapolis, IN) ad libitum for 7–10 days prior to use. CMRL-1066, glutamine, streptomycin, and fetal calf serum were purchased from Life Technologies, Inc., and Hanks' balanced salt solution was from GIBCO. Fetal calf serum, 5% (v/v) horse serum, 1% (v/v) heat-inactivated fetal calf serum, 50 units/ml streptomycin, and 100 \(\mu\)g/ml penicillin under an atmosphere of 95% air, 5% CO\(_2\) until use the same day.

**Preparation of Anti-CaM Kinase II Antibody-Protein A Conjugate**—Anti-CaM kinase II antibody was conjugated to prewashed, washed protein A-Sepharose by incubation at a ratio of 103.5 \(\mu\)l of antisera/4 mg of dry agarose for 16 h at 4°C with constant agitation. The conjugate was washed three times in Krebs-Ringer Hepes buffer (25 mM Hepes pH 7.4, 115 mM NaCl, 24 mM NaHCO\(_3\), 5 mM KCl, 2.5 mM CaCl\(_2\), 1 mM MgCl\(_2\) containing 3 mM glucose and 0.1% bovine serum albumin (KRB basal medium). Four hundred islets were individually selected under a stereomicroscope (Miji, San Jose, CA) and placed in polyclomer microcentrifuge tubes. The bathing medium was removed using a drawn out Pasteur pipette and replaced with KRB basal medium (300 \(\mu\)l) containing 400 \(\mu\)Ci of \([\beta\]P]orthophosphoric acid. The islets were preincubated for 90 min at 37°C under an atmosphere of 95% air, 5% CO\(_2\) with gentle agitation every 30 min to resuspend the islets. Incubations were then initiated by the addition of 131 \(\mu\)l of KRB basal medium (control) or KRB containing stimulatory concentrations of glucose or KCl to yield final concentrations of 28 and 40 mM, respectively. In the latter case, appropriate adjustments (i.e. the elimination of a compensatory concentration of NaCl) were made to maintain isosmotic balance. Incubations were continued for 2.5 min at 37°C. Reactions were terminated by brief centrifugation (5 s), aspiration of incubation medium, and immediate freezing in solid CO\(_2\). Islets were then lysed by sonication (10 pulses, setting 3, 30% duty cycle; Branson Ultrasonics, Danbury, CT) in 100 \(\mu\)l of ice-cold homogenization buffer (50 mM NaCl, 50 mM sodium pyrophosphate, 50 mM NaF, 1% SDS, 1% Triton X-100, 1% deoxycholate) was immediately added and the homogenate vortexed and then centrifuged for 2 min (4°C) at 8,000 \(\times\) g. Unincorporated nucleotides were removed by repeated (2 times) concentration of the supernatant using a Centricon-30 column (Amicon Inc., Beverly, MA) and subsequent washing in homogenate buffer (200 \(\mu\)l). Immunoprecipitation from the retentate was performed by the addition of 1 \(\mu\)l of anti-CaM kinase II antibody-protein A complex (see above) and constant rotation on a spin wheel for 2 h at 4°C. CaM kinase II-antibody conjugate was sedimented by centrifugation (Sorvall Microspin; 12,000 \(\times\) g, 5 min) and washed 4 times in homogenate buffer and 1 time in 20 mM NaPiO\(_4\), pH 7.2, 2.0 mM EDTA, 0.01% SDS, 0.05% Tween 20 buffer by resuspension and centrifugation. After the final wash, 50 \(\mu\)l of SDS sample buffer (186 mM Tris-HC\(_1\), pH 6.7, 9 mM SDS, 6 mM 2-mercaptoethanol, 15% glycerol, 0.01% bromphenol blue) was added and the sample boiled for 2 min. Dissociated protein A-Sepharose was removed by centrifugation and a portion (15–25 \(\mu\)l) of the supernatant of each sample subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 10% gel. The dried gel was developed by autoradiography.

**Assay of CaM Kinase II Activity**—CaM kinase II activity was assayed by modification of previously described methods (Waldman et al., 1989; MacNicol et al., 1990; MacNicol and Schulman, 1992; Jefferson et al., 1991). Islets were washed and counted to polymer tubes (400/tube) as described above. Islets were then preincubated in 500 \(\mu\)l of basal KRB medium (3 mM glucose) for 10 min at 37°C. Incubations were initiated by the removal of the bathing medium and replacement with KRB medium containing basal or stimulatory concentrations of glucose, 8–28 mM, or 40 mM KCl. Incubations were continued for 0–20 min at 37°C in a shaker water bath. To terminate the incubation, the islets were briefly (5 s) sedimented by centrifugation and the bathing medium removed and collected for the assay of insulin content. Ice-cold homogenization buffer (20 mM Tris-HCl, pH 7.5, 0.5 mM EGTA, 1.0 mM EDTA, 2.0 mM dithiothreitol, 10 mM sodium pyrophosphate, 0.4 mM ammonium molybdate, 100 \(\mu\)g/ml leupeptin, 200 \(\mu\)l) was immediately added to the islets and the sedimentation process repeated. The buffer supernatant was discarded. Fresh homogenization buffer (75 \(\mu\)l) was then added and the islets lysed by sonication (10 pulses, setting 5, 30% duty cycle; Branson Ultrasonics, Danbury, CT). The resulting lysate was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by autoradiography. The protein concentration of the homogenate ranged from 1.5 to 2.2 mg/ml.

CaM kinase II activity was assayed in a reaction mixture containing 50 mM PIPES, pH 7.0, 10 mM MgCl\(_2\), 0.1 mg/ml bovine serum albumin (BSA), 9 M urea, 10 units/ml of the reaction (V, 50 nM ATP, 1 mM Mg\(^{2+}\) activity, 40 Ci/mmol), and either 0.5 mM CaCl\(_2\), 5 \(\mu\)g/ml calmodulin for Ca\(^{2+}\) -stimulated activity or 0.9 mM EGTA for Ca\(^{2+}\)-independent activity. Total reaction volume was 50 \(\mu\)l. The assay was initiated by
the addition of 10 µl of islet homogenate and continued for 30 s at 30 °C. The reaction was terminated by the addition of ice-cold trichloroacetic acid (25 µl, 15%). Tubes were placed on ice for 20 min to precipitate large proteins, which were then sedimented by centrifugation for 1 min at 12,000 × g (Sorvall Microspin). Thirty-five µl of the resulting supernatant was spotted onto 5-cm by 2-cm strips of phosphocellulose paper (Whatman P-81). Strips were washed 5 times in 500 ml of distilled H2O, dried at 110 °C for 15 min, and 32P incorporation into autocamtide-2 determined by Cerenkov radiation (Beckman Instruments). Initial experiments were performed to established conditions for linearity of activity with respect to protein concentration and time. Total CaM kinase II activity in islet homogenates by this method was calculated as approximately 3 nmol of P, incorporated per min/mg of protein.

Autocamtide-2 phosphorylation has been reported as being highly selective for CaM kinase II (Hanson et al., 1989). That this is true for the β-cell was demonstrated by incubating RIN1N5F homogenate with peptide in the absence and presence of activators of CaM kinase II (Ca2+/calmodulin), protein kinase A (cAMP, 1 µM), and protein kinase C (12-O-tetradecanoylphorbol-13-acetate, 1 µM). Phosphate incorporation into autocamtide-2 was increased 70.0 ± 4.9-fold relative to control by the addition of Ca2+/calmodulin but was not significantly increased in the presence of cAMP or 12-O-tetradecanoylphorbol-13-acetate (2.4 ± 0.1- and 1.5 ± 0.3-fold, relative to control, respectively).

In the described experiments, 32P incorporation into autocamtide-2 in the absence of Ca2+/calmodulin (autonomous CaM kinase II activity) is expressed as a percentage of incorporation in the presence of these cofactors (Ca2+/calmodulin-dependent CaM kinase II activity).

**Insulin Secretion**—For static secretion experiments, islets were counted (20/tube) into 12 × 75-mm siliconized borosilicate tubes and preincubated for 30 min at 37 °C with gentle shaking in KRB basal medium containing 3 mM glucose and 0.1% bovine serum albumin (200 µl) under an atmosphere of oxygen/CO2 (95:5%). The medium was replaced with fresh KRB basal medium alone or supplemented with stimulatory concentrations of glucose (8, 11, 17, or 28 mM) and the incubation continued for a further 30 min. The incubation was terminated by the removal of the medium. Insulin content of incubation media was determined by a double antibody radioimmunossay (Morgan and Lazarow, 1963).

**Protein Determination**—Protein concentration was estimated by the method of Bradford (1976) using bovine serum albumin as standard.

**Statistical Treatment of Data**—Statistical significance was assessed by Student's t test.

**RESULTS**

**Autophosphorylation of CaM Kinase II**—In vitro studies in neuronal tissues have established that the activation of CaM kinase II is accompanied by enzyme autophosphorylation and the resultant generation of autonomous kinase activity (Hanson and Schulman, 1992). Other studies have extended these observations to demonstrate that this is a valid determinant of enzyme activation in situ (Godrick et al., 1988; Fukunaga et al., 1989; MacNicol et al., 1990; Jeffrey et al., 1991; Ocorr and Schulman, 1991). CaM kinase II autophosphorylation occurs in PC12 cells in response to bradykinin (MacNicol et al., 1990) and in both PC12 and GH3 cells in response to depolarizing concentrations of K+ (MacNicol et al., 1990; Jeffrey et al., 1991). In the current study, an immunoprecipitation procedure using polyclonal anti-CaM kinase IIα antibodies was employed to determine whether the phosphorylation status of CaM kinase II is increased in islets exposed to stimulatory concentrations of the insulin secretagogues, K+ (40 mM) and glucose (28 mM). Such concentrations of K+ induce β-cell depolarization and subsequent influx of Ca2+, which results in a marked, but transient, increased insulin secretion response (Henquin and Lambert, 1974). As such, K+ is thought to mimic the cellular effects of glucose to open voltage-dependent Ca2+ channels. It was reasoned that such an increase in intracellular concentration of Ca2+ should be sufficient to promote the activation of CaM kinase II and was therefore used as a positive control with which the effects of glucose could be compared.

Islets were prelabeled with [32P]orthophosphoric acid as described under "Experimental Procedures" and subsequently stimulated by K+ (40 mM) or glucose (28 mM). Control incubations using KRB basal medium (3 mM glucose, 5 mM K+) were conducted in parallel. After stimulation for 2.5 min, CaM kinase II was immunoprecipitated from islet homogenates and subjected to electrophoresis and autoradiography. As demonstrated in Fig. 1, a phosphoprotein of M, ~54,000, consistent with that expected of the α-subunit of CaM kinase II (CaM kinase IIα) (Lin et al., 1987), was immunoprecipitated by these procedures. Radioactive phosphate (32P) incorporation into this protein was increased by stimulation of the islets with either 40 mM K+ or 28 mM glucose as demonstrated by the increased intensity of this band by autoradiography. Quantification of 32P incorporation by densitometry (Optium Imager using BIOMED software) demonstrated that K+ (40 mM) and glucose (28 mM) increased phosphate incorporation into CaM kinase II by 175 ± 15 and 189 ± 19%, respectively. These data are consistent with secretagogue-induced autophosphorylation of CaM kinase II in islets.

**Generation of Autonomous CaM Kinase II Activity**—A hallmark of autophosphorylated CaM kinase II is the possession of Ca2+/calmodulin-independent (autonomous) kinase activity (Schulman and Hanson, 1993). Therefore, in order to confirm that K+ - and glucose-induced enzyme phosphorylation was the result of enzyme autophosphorylation, the ability of these secretagogues to induce autonomous enzyme activity in islets was assessed. Autonomous CaM kinase II activity was assayed in islet homogenates using a synthetic peptide, autocamtide-2, incorporating the autophosphorylation sequence (RQETVD) of the α-subunit of rat brain CaM kinase II (Hanson et al., 1989).

**Potassium-induced Increase in Autonomous CaM Kinase II Activity**—The effects of K+ on CaM kinase II activity were studied over a time period in which K+-induced insulin secretion occurs (0–20 min) (Henquin and Lambert, 1974). As illustrated in Fig. 2, K+ (40 mM) induced a rapid and marked increase in the proportion of islet CaM kinase II in the autonomous form. By 2.5 min, autonomous CaM kinase II
Activity-The principal objective of this study was to determine the result of cell depolarization. These data demonstrate that homogenates remained constant. This activation is likely due to increased Ca\(^{2+}\) influx as described, the total amount of CaM kinase II activity in islet homogenates remained constant. These data demonstrate that K\(^{+}\) stimulates CaM kinase II activity in isolated islets, and this activation is likely due to increased Ca\(^{2+}\) influx as the result of cell depolarization.

Glucose-induced Increase in Autonomic CaM Kinase II Activity-The principal objective of this study was to determine whether glucose activates CaM kinase II in isolated islets. Using conditions established in the previous experiments, the ability of glucose to promote the generation of autonomous CaM kinase II activity was assessed. Glucose at a concentration that maximally stimulates insulin secretion from isolated islets (28 mM) induced a marked increase in autonomous CaM kinase II activity (Fig. 3A). In basal conditions, autonomous CaM kinase II activity represented 4.2 ± 1.6% of activity achieved in the presence of Ca\(^{2+}\) and calmodulin; this value did not vary significantly over the time period studied (0–20 min). In a manner similar to K\(^{+}\), glucose induced a rapid increase in autonomous CaM kinase II activity, which, at peak stimulation at 2.5 min, was 11.0 ± 0.9% of Ca\(^{2+}\)/calmodulin-dependent activity (2.9 ± 0.2-fold induction over 3 mM glucose control, n = 4). This stimulation was similar qualitatively to the stimulation of enzyme phosphorylation by glucose (Fig. 1) and autonomous CaM kinase II activity induced by depolarizing concentrations of K\(^{+}\) (Fig. 2). As was the case in K\(^{+}\)-stimulated islets, autonomous activity in glucose-stimulated islets remained elevated relative to control over 5–10 min (Fig. 3A). Autonomous CaM kinase II activity in these islets was not significantly different from control at 20 min. Cumulative glucose (28 mM)-induced insulin secretion, monitored in these incubations, increased steadily throughout the period of the study relative to control (3 mM glucose) (Fig. 3B).

Correlation of Glucose-induced Autonomous CaM Kinase II Activity and Insulin Secretion-To further characterize the effect of glucose to activate CaM kinase II, islets were incubated at the optimal time point (2.5 min) in medium containing increasing concentrations of the nutrient secretagogue (3–28 mM). Glucose dose dependently stimulated the production of autonomous CaM kinase II activity (Fig. 4A). The threshold of activation occurred between 8 and 11 mM glucose, and, at a concentration considered to be near maximal for glucose (28 mM) (Wollheim and Sharp, 1981), activation achieved was 2.68 ± 0.15-fold of control. This sigmoidal-like relationship correlated very closely with glucose-induced insulin secretion from isolated islets (Fig. 4B). Using values at 28 mM glucose as an approximation of maximal activation, the concentrations of glucose required to produce half-maximal increases were 14 and 17 mM for insulin release and CaM kinase II activation, respectively.

Effect of Mannohexulose-To further determine whether the activation of CaM kinase II by glucose was dependent on the metabolism of glucose, the ability of mannoheptulose, which is known to suppress glucose oxidation by the inhibition of glucokinase (Ashcroft et al., 1970), to prevent this effect was studied. In these experiments, glucose (17 mM) induced...
a 1.6 ± 0.2-fold activation of CaM kinase II relative to control (3 mM glucose) as determined by the appearance of autonomous kinase activity. Mannoheptulose (25 mM), when added in addition to glucose (17 mM), completely prevented glucose-induced activation of CaM kinase II; under these conditions, autonomous CaM kinase II activity was 68 ± 3% of control (3 mM glucose alone). These data suggest that glucose-induced activation of CaM kinase II is absolutely dependent on its metabolism and, therefore, support a specific effect of glucose. Furthermore, since insulin secretion induced by glucose is similarly obliterated by co-incubation with mannoheptulose (Ashcroft et al., 1970), the mechanisms in the regulation of secretion and activation of CaM kinase II likely involve the same components.

**DISCUSSION**

CaM kinase II has long been implicated in the regulation of insulin secretion, but a definitive evaluation of this hypothesis has been hindered by the lack of information concerning the function and regulation of this enzyme in the β-cell. In particular, the endogenous substrate(s) for CaM kinase II in islets is (are) not known. In other pharmacological approaches, some putative inhibitors of CaM kinase II have proven not to have desired selectivity. Moreover, until recently, there have not been methods of sufficient sensitivity to detect the activation of islet CaM kinase II in situ in response to insulin secretagogues. The recent surge of information regarding the regulation of CaM kinase II by autophosphorylation and generation of autonomous CaM kinase II activity has provided insight into possible cellular functions of the enzyme and has further allowed an experimental means for the assessment of its activation in intact cells.

The principal observation of this study was that glucose induced the increased phosphorylation of an α-like subunit of CaM kinase II and the generation of autonomous CaM kinase II activity in isolated islets. These observations provide the first clear evidence that glucose activates CaM kinase II in isolated islets. The increased phosphorylation of CaM kinase II is thought to represent enzyme autophosphorylation since: 1) autonomous kinase activity is a hallmark characteristic of CaM kinase II phosphorylated at the “autonomy” site (e.g. Thr-286 in the α-subunit); and 2) the effect of glucose to increase enzyme phosphorylation and the generation of autonomous activity was quantitatively similar and was mimicked closely by stimulatory K+.* Depolarization K+ has previously been shown to induce autophosphorylation of CaM kinase II in PC12 (MacNicol et al., 1990) and GH3 cells (Jefferson et al., 1991). A definitive demonstration of CaM kinase II autophosphorylation in response to glucose would require the identification, by phosphopeptide analysis, of the phosphorylation at a site that corresponds to the autonomy site on rat brain kinase. This was not attempted in this study because of the low amount of material and subsequent signal generated from isolated islets.

That the activation of CaM kinase II occurs in the β-cell of the islet is suggested in preliminary studies that demonstrate that depolarizing K+ and glyceraldehyde induce similar enzyme autophosphorylation in the clonal β-cell line, RINm5F (Landt, 1992). Furthermore, the immunoprecipitation of CaM kinase II further provides immunological evidence for the presence of this enzyme in isolated islets (this study) and β-cells (Landt, 1992) and complements enzymatic evidence reported previously. The precise molecular structure and isoenzymic form of CaM kinase II possessed by the β-cell of the islet has yet to be determined.

The activation of CaM kinase II by glucose and K+ as measured by the generation of autonomous CaM kinase II activity was essentially identical. Thus, the maximal effects of both secretagogues were quantitatively similar (approximately 3-fold in each case) and were achieved after the same exposure time (2.5–5 min). Furthermore, autonomous activity persisted in glucose- and K+-treated islets for a period of at least 10 min. These observations suggest that the mechanism by which these secretagogues activate CaM kinase II involve similar or identical components and are consistent with the currently proposed mechanism of glucose-induced insulin secretion. Depolarization of the β-cell by elevated extracellular concentrations of K+ promotes Ca2+ influx through the opening of L-type Ca2+ channels (Prentki and Matschinsky, 1987). Similarly, glucose is thought to depolarize the cell as the result of its metabolism and subsequent closing of KATP channels (Ashcroft, 1988; Cook et al., 1988). That glucose metabolism is required for the activation of CaM kinase II was demonstrated by the ability of the glucokinase inhibitor, mannoheptulose, to prevent glucose generation of autonomous activity.

A striking observation from this study was the close correlation in islets of the concentration dependence of glucose-induced insulin secretion and CaM kinase II activity. The threshold of activation was estimated at between 8 and 11 mM in each case, and the glucose concentrations required to elicit a half-maximal stimulation (14–17 mM) were similar. Such a close correlation suggests that similar biochemical mechanisms lead to the activation of enzyme and secretion but further argues for a role of CaM kinase II in the mediation of glucose-induced insulin secretion. A temporal correlation of CaM kinase II activation with insulin secretion is, however, less obvious. The rapid activation of CaM kinase II by glucose (maximum at 2.5 min) correlates with the first phase of glucose-induced insulin secretion, which is transient and also peaks approximately 2.5–5 min after initial exposure of the islet to glucose (Easom et al., 1990). A tentative assignment of CaM kinase II to the regulation of the first phase of secretion is further supported in this study by the essentially identical activation of this enzyme by K+. Depolarization K+ (Henquin and Lambert, 1974) and other secretagogues that result in transient influx of Ca2+ such as tolbutamide (Henquin, 1980) and the Ca2+ ionophore A23187 (Zawalich et al., 1985) elicit a transient secretion response reminiscent of the first phase of glucose-induced insulin secretion. The interpretation that CaM kinase II may function to mediate the first phase secretion is, however, difficult to reconcile with the results of a recent report that demonstrated that the CaM kinase II inhibitor, KN-62, failed to inhibit Ca2+-induced insulin secretion from detergent- or electro-permeabilized β-cells (HIT) (Li et al., 1992). Ca2+ induces a transient insulin secretion response in permeabilized islets resembling first phase secretion and induces the phosphorylation of a 54,000 molecular weight protein likely to be CaM kinase II (Jones et al., 1992). We have since confirmed this observation in electro-permeabilized islets indicating that this result is not due to unique characteristics of HIT cells, although there remains the possibility that insulin secretion is fundamentally altered in the permeabilized β-cell.

As an alternate possibility, the persistence of autonomous CaM kinase II activity beyond the period of first phase secretion could be active in the initiation and/or propagation of the second phase of secretion. Such a role could be mediated by the proposed cross-talk of CaM kinase II with other cellular pathways, such as a protein kinase C pathway (MacNicol and

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3 R. M. Wenham, L. C. Craig, and R. A. Easom, unpublished observations.
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fully acknowledge the excellent technical assistance of Eric Babb.

anti-CaM kinase

insulin secretion. These data suggest an important role of CaM kinase I1 in the glucose regulation of P-cell function related to insulin secretion.

In summary, the data presented in this study clearly demonstrate that glucose activates CaM kinase I1 in isolated rat islets and that the extent of activation correlates closely with insulin secretion. These data suggest an important role of CaM kinase I1 in the glucose regulation of β-cell function related to insulin secretion.

Acknowledgments—We thank Dr. Paul Kelly for the provision of anti-CaM kinase I1 antibodies and for helpful discussion. We gratefully acknowledge the excellent technical assistance of Eric Balbi.

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