Biochemical Pharmacology, Vol. 52, pp. 793–799, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
PII S0006-2952(96)00391-7

Modulation of Glyceraldehyde 3-Phosphate Dehydrogenase Activity in Isolated Pancreatic Islets

*Suzanne G. Laychock**

Department of Pharmacology and Toxicology, State University of New York at Buffalo, School of Medicine and Biomedical Sciences, Buffalo, NY 14214, U.S.A.

ABSTRACT. Culture of isolated rat islets at either 5.5 or 11 mM glucose for up to 6 days was associated with significant time-dependent increases in glyceraldehyde 3-phosphate dehydrogenase (G3PD) activity of islet homogenates compared with freshly isolated islet G3PD activity. In addition, after 6 days of culture of islets at 11 mM glucose, there was a significant increase in G3PD activity compared with the enzyme activity of islets cultured at 5.5 mM glucose. Culture of islets at 5.5 mM glucose for 2 days in the presence of forskolin, 3-isobutylmethylxanthine (IBMX), and 8-bromo-cyclic AMP also significantly increased G3PD activity compared with control islets, although there was no change in enzyme activity after only 1 day of culture with forskolin. Treatment with forskolin was associated with an increase in the V_{max} of G3PD, but no change was observed in the apparent K_m with NAD. IBMX and 8-bromo-cyclic AMP also increased G3PD activity in islets cultured at 11 mM glucose for 2 days. 8-Bromo-cyclic AMP did not affect or inhibit G3PD activity when added directly to islet homogenates. Islets cultured with 8-bromo-cyclic GMP for 2 days at 5.5 or 11 mM glucose did not show changes in G3PD activity. Increases in G3PD activity did not correlate with significant changes in islet glucose utilization. Thus, G3PD activity is modulated by the duration of glucose stimulation in cultured islets, and cyclic AMP may mediate changes in G3PD activity in islet cells. BIOCHEM PHARMACOL 52;5:793–799, 1996.

KEY WORDS. cyclic AMP; forskolin; carbohydrate

Glyceraldehyde phosphate is a glycolytic intermediate and potent insulin secretagogue from pancreatic islets [1, 2]. Recent evidence suggests that the oxidation of glyceraldehyde 3-phosphate by G3PD† plays an important role in the production of NADH which increases the production of ATP in β-cells [3]. The glucose-stimulated increase in ATP closes the ATP-sensitive K^+ channel, leading to β-cell depolarization and opening of voltage-dependent Ca^{2+} channels which trigger insulin secretion [4]. Glyceraldehyde phosphate appears to stimulate the early phase of insulin secretion which requires a minimal supply of cytosolic energy [2]. The observation that an inhibitor of G3PD activity, iodoacetate, completely prevents the glucose-induced closure of the ATP-dependent K^+ channel and insulin secretion [1, 2] suggests that NADH is a critical intermediate in glucose-stimulated insulin release.

Isolated pancreatic islets cultured at 11 mM glucose for up to 6 days have been reported to have increased basal insulin release and reduced glucose sensitivity with regard to insulin secretion [5]. *In vivo* studies have also described elevated basal insulin secretion and reduced glucose sensitivity of islets from rats rendered hyperglycemic for prolonged periods [6, 7]. In addition, it has been reported that adult rats fed a high carbohydrate diet during the neonatal period are hyperinsulinemic, they have reduced glucose sensitivity in the glucose tolerance test, and their isolated islets have increased G3PD activity [8, 9]. In this study, cultured islets were investigated to determine whether G3PD activity was sensitive to glucose stimulation and other cell stimuli over the long term.

MATERIALS AND METHODS

Materials

Collagenase type P was obtained from Boehringer Mannheim (Indianapolis, IN). D-[5-^{3}H]Glucose was from American Radiolabeled Chemicals, Inc. (St. Louis, MO). All other reagents were reagent grade and purchased from the Sigma Chemical Co. (St. Louis, MO). Rat insulin radioimmunoassay standard was a gift from the Eli Lilly Co. (Indianapolis, IN).

Tissue Preparation and Experimental Design

Pancreatic islets were isolated from male Sprague–Dawley rats (225–280 g) using a collagenase digestion of the pan-

* Corresponding author: Dr. S. Laychock, 102 Farber Hall, Department of Pharmacology and Toxicology, SUNY at Buffalo School of Medicine, Buffalo, NY 14214. Tel. (716) 829-2808; FAX (716) 829-2801; E-mail: slaychoc@ubmed.buffalo.edu

† *Abbreviations:* cAMP, adenosine 3′,5′-cyclic monophosphate; G3PD, glyceraldehyde 3-phosphate dehydrogenase; cGMP, guanosine 3′,5′-cyclic monophosphate; IBMX, 3-isobutyl-1-methylxanthine; and KRB, Krebs Ringer bicarbonate.

Received 26 October 1995; accepted 12 April 1996.

creas, as described previously [5]. Isolated islets were either homogenized immediately for determination of enzyme activity, or they were placed in primary tissue culture as described previously [5]. The culture medium, CMRL-1066, contained 2 mM glutamine, 10% heat-inactivated fetal bovine serum, penicillin (100 U/mL), streptomycin (100 μg/mL), and either 5.5 or 11 mM glucose. The islets were cultured in a humidified atmosphere of 5% CO_2–95% air at 35° for up to 6 days. The culture medium was replaced every other day.

Following culture, the islets were transferred to plastic microfuge tubes. The culture medium was removed, and KRB buffer (pH 7.4) containing 16 mM HEPES, 0.01% bovine serum albumin, and 5.5 or 11 mM glucose to match the primary culture glucose concentration of each group of islets was added. The islets were incubated at 37° in an atmosphere of 95% O_2–5% CO_2 for 1 hr. Then, the islets were microfuged for 15 sec, the KRB buffer was removed, and the islets were used for enzyme determination as described below.

Insulin Determinations

The content of insulin in the CMRL-1066 medium following islet culture for 2 days was determined by radioimmunoassay, as described previously, using rat insulin as a standard [5]. Insulin values for CMRL-1066 in the absence of islets (blank) were subtracted from the islet values. Insulin release is expressed as total insulin released during the culture period per islet.

G3PD Assay

The determination of the G3PD activity was carried out by monitoring the reduction of NAD^+ to NADH, as described previously [9]. Isolated islets were placed in microfuge tubes and washed with homogenization buffer containing HEPES (100 mM), pH 7.5, and dithiothreitol (2 mM). Islets (25 per preparation) were homogenized by sonication for 10 sec in 86 μL of homogenization buffer. The homogenate was microfuged for 30 sec, and the supernatant was used for the enzyme assay and protein determination [10]. The resulting supernatant was assayed for G3PD activity with buffer containing (mM): triethanolamine (50), pH 7.6, arsenate (50), glyceraldehyde 3-phosphate (100 μg/mL), and reduced glutathione (2.4). Buffer together with added homogenate (1–3 μg protein) was incubated in a water bath for 5 min at 37°, after which a baseline fluorimetric reading was obtained at 347 nm excitation and 448 nm emission. Then, NAD^+ (250 μM) was added to start the reaction, and the linear change in fluorescence was recorded for up to 5 min. Data are expressed as the change in NADH per microgram protein with time.

To determine the K_m and V_{max} for NAD dependence of homogenate G3PD, seven concentrations of NAD were used (0.62 to 39 μM) in the presence of glyceraldehyde 3-phosphate (100 μg/mL), with a reaction time of 1 min, with all other conditions as described above. Hanes' analysis was used to determine kinetic parameters for a single substrate.

Glucose Utilization

Islet utilization of D-[5-^{3}H]glucose was determined essentially as described previously, by quantitating the conversion of D-[5-^{3}H]glucose to ^3H_2O [11]. Islets were incubated in 0.1 mL KRB buffer containing D-[5-^{3}H]glucose (17 mM; 1 μCi/sample) for 90 min, at 37°. ^3H_2O recovery was used to determine glucose utilization based upon the glucose specific activity per sample. Blank values for samples devoid of islets were subtracted from islet glucose utilization values. The protein content of the islets was determined.

Statistical Analysis

Data are shown as means ± SEM. Significant differences between data groups were determined by Student's *t*-test (paired) or one-way ANOVA combined with the Duncan/Newman–Keuls multiple comparison test. Values of P less than 0.05 were accepted as significant.

RESULTS

Islets freshly isolated (fresh) had significantly lower G3PD levels than islets cultured for 2 or 6 days, but not 1 day, at a basal glucose concentration of 5.5 mM (Fig. 1). When islets were cultured at 5.5 mM glucose for 2 days and the culture medium (10 mL) was changed every 6–12 hr in order to remove/dilute accumulating metabolites or other islet cell products, the G3PD activity of islet homogenates (258 ± 24 ng NADH/μg protein/min) was similar to basal G3PD activity of homogenates of islets cultured for 2 days in 2 mL culture medium without a medium change (Figs. 1 and 2). Culturing islets at a submaximal insulin secretagogic concentration of glucose (11 mM) for 2 days did not increase G3PD activity significantly over islets cultured at 5.5 mM glucose, although the G3PD activity was significantly higher than basal levels in homogenates of islets cultured at 11 mM glucose for 6 days (Fig. 1). The G3PD activity of homogenates of fresh islets incubated with 11 mM glucose for 1 hr was similar to the basal control enzyme activity (data not shown).

Islets were also cultured in the presence of an adenylyl cyclase stimulus, forskolin, as well as a phosphodiesterase inhibitor, IBMX, to determine if the augmentation of cyclic AMP levels in islet cells might modulate G3PD activity. When islets were cultured at basal glucose levels (5.5 mM glucose) for 2 days in the presence of forskolin (1 μM), there was a significant increase in G3PD activity of more than 50% in islet homogenates (Fig. 2). This coincided with an increase in insulin release with forskolin (4.0 ± 0.4 pmol/islet) which was five times higher ($P < 0.01$) than basal release at 5.5 mM glucose alone (0.8 ± 0.03 pmol/islet). Forskolin did not elicit a significant change in G3PD

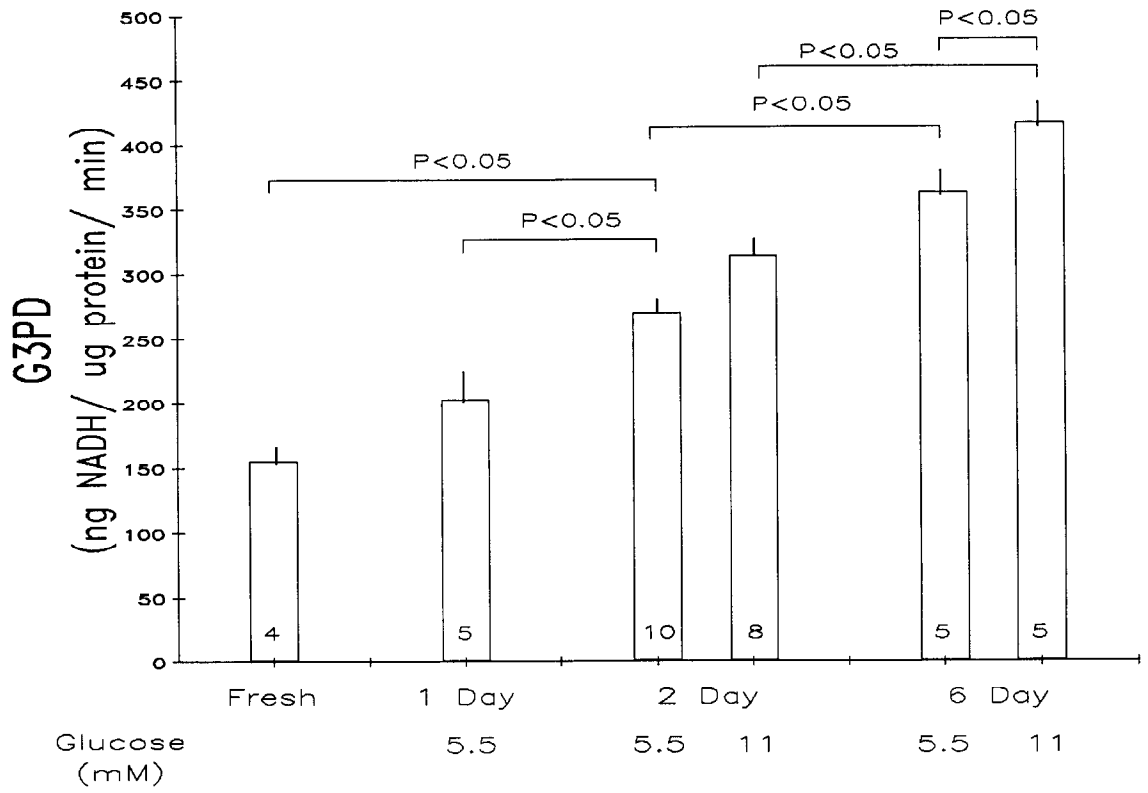

FIG. 1. G3PD activity in freshly isolated (fresh) and cultured pancreatic islets. Islet homogenates were prepared from fresh islets, or islets cultured in CMRL-1066 containing 5.5 or 11 mM glucose for 1, 2 or 6 days. G3PD activity is expressed as the mean ± SEM for the number of determinations shown at the base of each bar. Significant differences were determined by one-way ANOVA and a multiple comparison test.

activity after only 1 day of culture, although there was a trend toward higher activity levels (Fig. 2). Similarly, the addition of IBMX to the islets during culture at basal glucose levels for 2 days resulted in increased G3PD activity in islet homogenates (Fig. 2). Moreover, homogenates of islets cultured with 11 mM glucose and IBMX (0.2 mM) had significantly higher G3PD activity than control islets in the presence of the glucose stimulus alone (Fig. 3).

The kinetic parameters of G3PD activity were determined for NAD following culture for 2 days in the absence or presence of forskolin. As indicated in Table 1, forskolin did not have a significant effect on the apparent K_m for NAD, although the V_{max} was increased significantly following islet culture in the presence of the stimulus.

8-Bromo-cAMP (5 mM) also stimulated the activity of G3PD in homogenates of islets cultured with 5.5 mM glucose (Fig. 2) or 11 mM glucose (Fig. 3) for 2 days. When glucose (11 mM) together with 8-bromo-cAMP (5 mM) was added to fresh islets incubated in KRB buffer for 60 min, the activity of G3PD in the islet homogenates was not different from the basal G3PD activity of islets incubated at 5.5 mM glucose (data not shown). The cAMP-elevating agent glucagon (1 μM) together with 11 mM glucose in islet culture for 2 days did not result in significantly elevated activity of G3PD in islet homogenates compared with 11 mM glucose-treated control islets (Fig. 3). In contrast to 8-bromo-cAMP, 8-bromo-cGMP (5 mM) did not significantly affect G3PD activity of islets cultured at 5.5 or 11 mM glucose (Figs. 2 and 3).

When 8-bromo-cAMP (5 mM) was added directly to fresh islet homogenate for 5 min prior to the fluorimetric determination of NADH, G3PD activity was reduced by 54% compared with basal values (data not shown). However, subsequent studies showed that 5′-ADP (5 mM) and 8-bromo-cGMP (5 mM) also reduced G3PD activity in islet homogenates by approximately 50%. A lower concentration of 8-bromo-cAMP (0.1 mM) did not affect G3PD activity in fresh islet homogenates. Thus, nucleotides inhibited G3PD activity in homogenates in a nonspecific manner, unlike the stimulation of G3PD activity observed during culture of the islets in the presence of 8-bromo-cAMP.

Glucose utilization was determined by quantitating the conversion of D-[5-^{3}H]glucose to ^3H_2O, since H_2O is generated after G3PD-catalyzed conversion of glyceraldehyde to 1,3-diphosphoglycerate and just prior to the generation of phospho-*enol*-pyruvate. However, compared with glucose utilization for control islets cultured for 2 days at 5.5 mM glucose (253 ± 30 pmol glucose utilized/μg protein; N = 3), none of the following treatments produced significant differences: islets cultured at 11 mM glucose for 2 days (231 ± 28 pmol glucose utilized/μg protein; N = 3), islets cultured for 2 days at 5.5 mM glucose in the presence of forskolin (1 μM) (239 ± 18 pmol glucose utilized/μg protein) or the

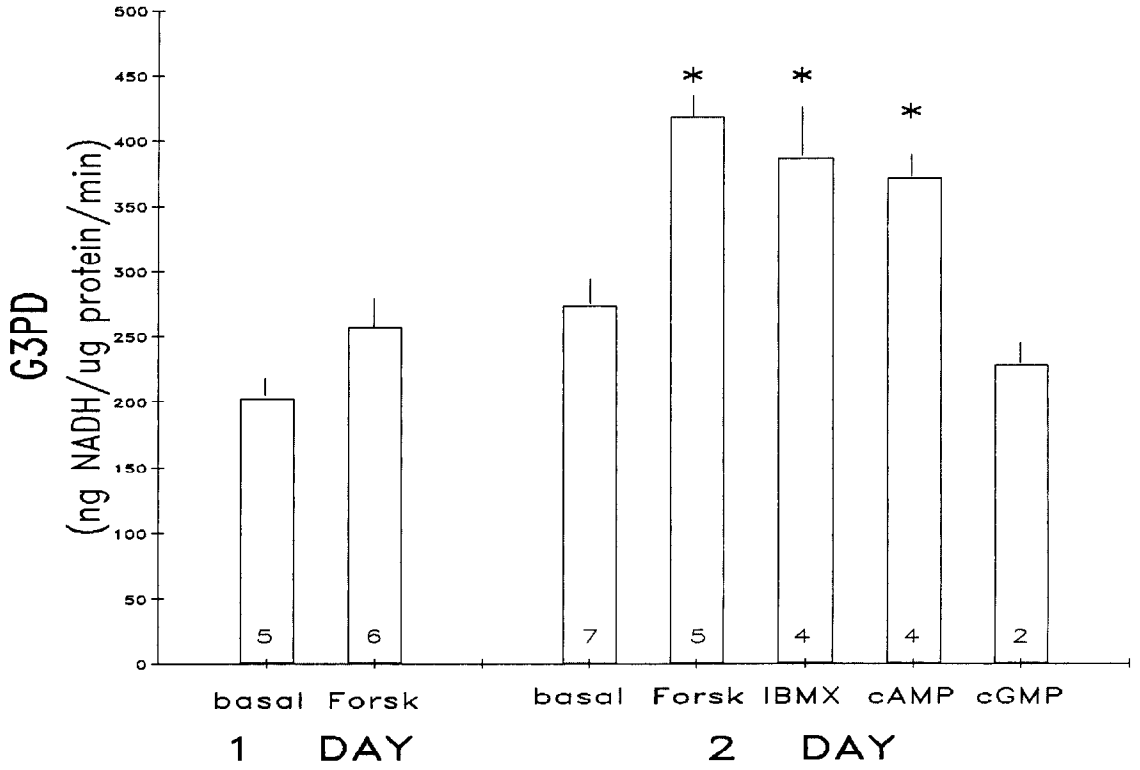

FIG. 2. G3PD activity in islets cultured for 1 or 2 days with cAMP-elevating agents. Islets were cultured in CMRL-1066 containing 5.5 mM glucose in the absence (basal) or presence of forskolin (Forsk; 1 μM), IBMX (0.2 mM), 8-bromo-cAMP (cAMP; 5 mM), or 8-bromo-cGMP (cGMP; 5 mM). Values are the means ± SEM for the number of determinations indicated at the base of each bar except for cGMP where the value is shown as the mean ± range. Key: (*) significantly different ($P < 0.05$) from basal values of similarly cultured islets, as determined by one-way ANOVA and a multiple comparison test.

presence of IBMX (0.2 mM) (253 ± 9 pmol glucose utilized/μg protein; N = 3), or islets cultured for 2 days at 11 mM glucose in the presence of forskolin (1 μM) (208 ± 11 pmol glucose utilized/μg protein; N = 3) or the presence of IBMX (0.2 mM) (234 ± 17 pmol glucose utilized/μg protein; N = 3).

Islet protein content was also determined. Whereas fresh islets contained a slightly higher (34%) concentration of protein per islet than the other cultured islet preparations, there were no significant differences in the protein content between any of the cultured islet treatment groups (Table 2).

DISCUSSION

G3PD activity is increased in a time-dependent manner in homogenates of islets cultured for up to 1 week in 5.5 or 11 mM glucose, although after 6 days the level of activity is higher following continuous stimulation with 11 mM glucose. An increase in G3PD activity during a 7-day culture of islets from fetal rats has also been reported and ascribed to the functional maturation of the islet cells [12]. The explanation for why the G3PD activity is elevated at a non-secretagogic concentration of glucose during culture of islets from adult rats is not obvious, except that culture conditions, including serum constituents, may have contributed to the activation. The increase may also be the response to release from normal inhibitory influences exerted *in vivo*. It does not appear that accumulation of cell metabolites or hormones (insulin, glucagon, somatostatin) released from the islets contributed to the activation since a similar profile of G3PD activity was observed even when the culture medium of the islets was replaced with fresh medium every 6–12 hr for 2 days. In addition, the increase in G3PD activity is unique to this cultured islet model, since it has been reported previously that the activity of 5′-nucleotidase does not increase in islets cultured for 6 days at 11 mM glucose, and the activity of Na^+-K^+-ATPase declines with continuous glucose stimulation [5]. Moreover, differences in islet protein levels do not appear to account for the changes observed in G3PD activity, since protein levels did not change during the course of culture, although fresh islets had slightly elevated protein levels compared with cultured islets.

The induction of enzymes such as G3PD in cultured islets may be an important consideration in the culture of islets for later human or animal transplantation [13, 14]. The secretion of insulin from pancreatic islets in response to the primary stimulus glucose and other secretagogues such as glyceraldehyde and 2-ketoisocaproic acid appears to be mediated by changes in the ATP/ADP ratio, and it also appears that the generation of NADH in the β-cell is im-

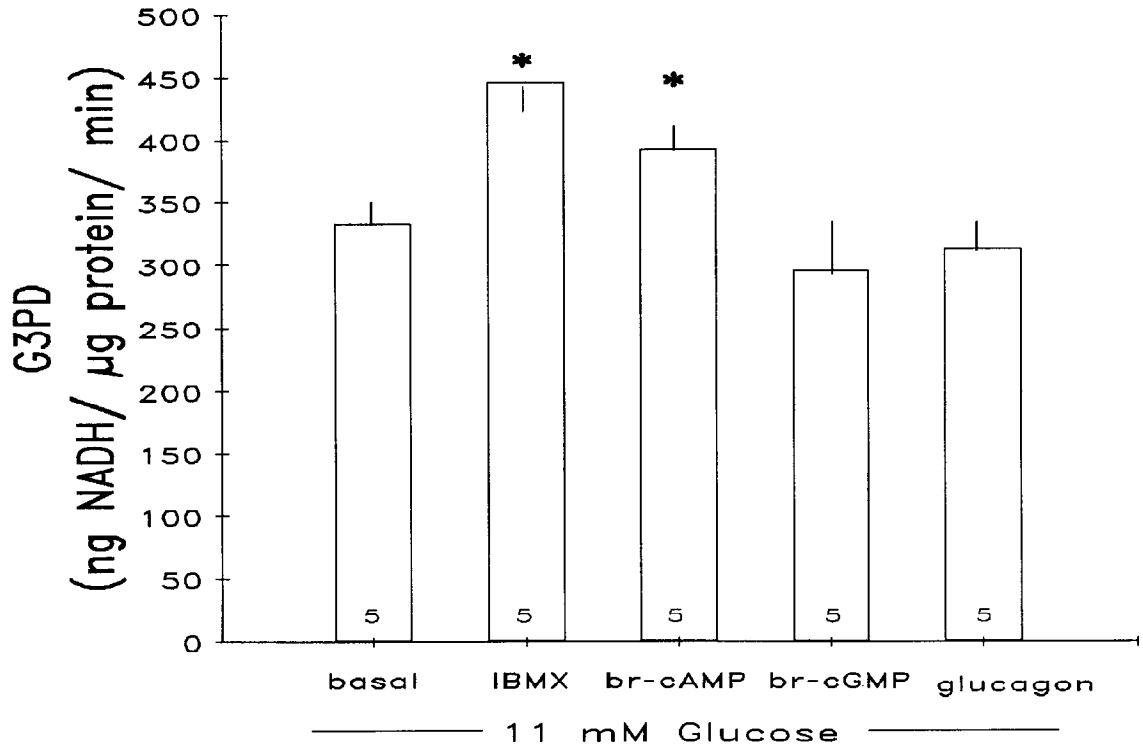

FIG. 3. G3PD activity in islets cultured with elevated glucose levels and cyclic nucleotide modulating agents. Islets were cultured in CMRL-1066 containing 11 mM glucose in the absence (basal) and presence of IBMX (0.2 mM), 8-bromo-cAMP (br-cAMP; 5 mM), 8-bromo-cGMP (br-cGMP; 5 mM), and glucagon (1 µM). Values are the means ± SEM for the number of determinations shown at the base of each bar. Key: (*) significantly different ($P < 0.05$) from basal values, as determined by one-way ANOVA and a multiple comparison test.

portant for ATP generation and insulin release since impaired synthesis of NADH correlates with reduced glucose-sensitive insulin secretion [15]. G3PD is an important enzyme in the metabolism of glyceraldehyde and the generation of NADH in the β-cell, and it has been proposed to be a key step in the regulation of mitochondrial ATP generation [3]. In islets cultured for 6 days at 11 mM glucose, not only is the glucose sensitivity of insulin secretion reduced, but the ability of the cells to generate ATP in response to glucose is compromised [5, 16]. Although it is not known at which step glucose desensitization affects metabolism to reduce ATP generation, it is possible that it is at a mitochondrial step and that the increase in cellular G3PD reflects a compensatory increase in an enzyme important for ATP generation. Alternatively, MacDonald [17] has provided evidence that formation of the unmetabolizable glycerate 1-phosphate in islets through G3PD may effect changes in the NADH/NAD ratio, affecting the ability of cells to reoxidize cytosolic NADH, and resulting in toxic effects. It may be that the loss of insulin secretory responsivity to glucose in cultured islets is related to inappropriate G3PD metabolism of glyceraldehyde.

TABLE 1. Kinetic analysis of islet G3PD

	Islet G3PD	
Treatment	K_m of NAD (µM)	V_{max} (ng NADH/min/µg protein)
Control	9.9 ± 0.7	67.2 ± 6.3
Forskolin	8.1 ± 1.6	108.9 ± 9.1*

Islets were cultured for 2 days in CMRL-1066 containing 5.5 mM glucose in the absence (control) or presence of forskolin (1 µM). The islets were washed and homogenized, and the activity of G3PD was determined from the formation of NADH during 1 min. Values, derived from Hanes' analysis of data, are the means ± SEM from four independent paired determinations per group.

* $P < 0.01$ compared with control V_{max} values, as determined by Student's t-test (paired).

The islet model used in the present study is one that has been characterized as glucose desensitized, having elevated basal insulin release and showing a progressive reduction in glucose sensitive insulin secretion during 6 days of continuous glucose stimulation in culture [5]. In addition, a previous study demonstrated that G3PD activity was increased by approximately 30% in homogenates of islets that were desensitized to glucose stimulation following a high carbohydrate diet *in vivo* and had elevated basal insulin secretion [9]. In those studies, islets were isolated from adult rats that were rendered hyperinsulinemic and insulin resistant at an early age by feeding a diet high in carbohydrate calories during the neonatal period [8]. It is interesting that islets desensitized to glucose stimulation from both *in vivo* and *in vitro* models display a similar degree of enhanced G3PD activity. In addition, glucose induces a gradual change in G3PD activity which leads to a significant enhancement of

TABLE 2. Islet protein content

Treatment	Protein (μg)/islet	N
Fresh	0.47 + 0.03*	8
1 day 5.5 G	0.35 ± 0.08	3
2 days, 5.5 G	0.33 ± 0.03	5
6 days, 5.5 G	0.35 ± 0.02	6
2 days, 5.5 G, IBMX	0.29 ± 0.05	3
2 days, 11 G	0.33 ± 0.04	3
6 days, 11 G	0.35 ± 0.02	6
2 days, 11 G, IBMX	0.30 ± 0.04	7
2 days, 5.5 G, Forskolin	0.37 ± 0.05	3
2 days, 5.5 G, 8-bromo-cAMP	0.35 ± 0.04	4

Protein content of islet homogenates used in G3PD activity assays was determined, and the results were normalized per islet. Treatment groups were fresh islets, or islets cultured at 5.5 or 11 mM glucose for 1, 2 or 6 days, in the absence or presence of IBMX (0.2 mM), forskolin (1 μM), or 8-bromo-cAMP (5 mM). Values are the means ± SEM for the number of independent determinations (N).

* $P < 0.05$ versus other treatment groups, as determined by one-way ANOVA.

activity only after at least 6 days of continuous stimulation. It is not known if this change in G3PD is due to transcriptional regulation, but glucose has been reported to induce transcriptional changes in another enzyme important for β-cell glucose metabolism, acetyl-CoA carboxylase [18]. Perhaps an increase in metabolism through G3PD contributes to the increase in basal insulin release noted in both the *in vivo* and *in vitro* islet β-cell desensitization models.

Although cAMP is an important second messenger in β-cells which modulates insulin secretion, and glucose induces increases in cAMP generation in isolated islets [19], cAMP is not an acute mediator of glucose metabolism [20]. Rather, it appears that cAMP functions in the β-cell to induce acute changes in protein kinase A, protein phosphorylation, and voltage-sensitive Ca^{2+} channel activity as well as other events important in potentiating insulin secretion [20]. The long-term effects of cAMP may also include the stimulation of the insulin promoter [21]. In the present study, several approaches were undertaken to determine if cAMP was likely to be an intermediate in the glucose stimulation of G3PD activity. Forskolin, an adenylyl cyclase activating agent, was added to cultured islets to increase cAMP generation. Forskolin induced a time-dependent change in G3PD activity which was significant by day 2 of stimulation. Similarly, inhibition of phosphodiesterase activity by IBMX [19] during islet culture at low or elevated glucose concentrations resulted in increased G3PD activity of islet homogenates similar to the increases in activity observed with forskolin pretreatment. The increased V_{max} for NAD stimulation of G3PD in islets treated with forskolin suggests that the enzyme activity is enhanced as a result of the drug treatment, although there was no effect on the apparent K_m. The K_m of NAD for G3PD in this study is similar to the value (<10 μM) reported previously for pancreatic islets [17]. It has been reported that incubation of cells of an insulin-producing cell line, β-TC1, with forskolin or IBMX resulted in changes in intracellular cAMP levels as well as cell-cycle related gene expression, including c-*fos* and c-*jun* gene expression and insulin promoter activity [22]. It is possible that the elevation of cAMP levels in cultured islet cells also regulates the expression and activity of G3PD. Activation of the enzyme has also been reported to be indirect through regulation of a cytosolic protein that activates G3PD and lowers the apparent K_m for NAD [23]. However, in the present studies no significant change in the apparent K_m for NAD was observed. G3PD in rabbit muscle has been reported to serve as a substrate for Ca^{2+}/calmodulin-dependent protein kinase II [24], and in pancreatic islets it is possible that the enzyme is regulated through a phosphorylation-mediated event.

The addition of a cell permeant and phosphodiesterase resistant cAMP analog, 8-bromo-cAMP, to the cultured islets for 2 days at a concentration previously demonstrated to potentiate insulin secretion [25] also resulted in the stimulation of G3PD activity in islet homogenates. The increases in G3PD activity were similar between glucose, IBMX, forskolin, and 8-bromo-cAMP stimulation. In contrast, 8-bromo-cGMP did not evoke a change in G3PD activity even after 2 days of culture. In addition, the changes in G3PD activity observed after culture with 8-bromo-cAMP appear to have been time dependent since this cyclic nucleotide did not stimulate G3PD activity following a 1-hr exposure of the islets to the agent, and 8-bromo-cAMP did not stimulate G3PD directly in islet homogenates. Unfortunately, an attempt to inhibit adenylyl cyclase activity and G3PD activation by IBMX over the long term with the α_2-adrenoceptor agonist clonidine was unsuccessful (data not shown), probably due to the down-regulation of α_2-adrenoceptors during continuous stimulation [26]. Similarly, continuous stimulation with glucagon for 2 days (data not shown) did not result in any change in G3PD in islet homogenates, probably also as a result of receptor down-regulation [27].

Although G3PD activity was increased by glucose and various drug treatments in this study, glucose utilization by islets cultured with elevated glucose in the absence or presence of forskolin or IBMX did not show significant changes in glucose utilization. Perhaps the maximal glucose concentration (17 mM) used in the analysis precludes the identification of small changes in glucose utilization. On the other hand, G3PD is only one of several regulatory enzymes in the glycolytic pathway, and changes in other glucose-metabolizing enzyme activities may overshadow changes in G3PD alone.

In summary, G3PD is an enzyme which has, heretofore, been assumed to be unregulated in most cells. However, this study describes an increase in G3PD activity in cell homogenates resulting from the long-term stimulation of the intact cell with a physiological stimulus for the β-cell as well as agents that increase the production and intracellular concentration of cAMP. Since increased G3PD activity in islet homogenates has also been observed in animals previously stimulated long-term with glucose-supplemented diets

[9], or in cultured fetal islet cells [12], it is possible that the activity and perhaps level of G3PD or a regulatory cytosolic protein are stably altered in response to continuous cell stimulation. G3PD has been shown to be autophosphorylated in rabbit skeletal muscle [28]. Future studies will determine whether glucose and cAMP alter the transcription of mRNA for G3PD in β-cells or change enzyme activity through phosphorylation-mediated steps.

The technical assistance of Swaroopa Rani Vadlamudi, Jill Platten, and Amy Bauer is greatly appreciated. These studies were supported by NIH Grant DK25705.

References

1. MacDonald MJ and Fahien LA, Glyceraldehyde phosphate and methyl esters of succinic acid. Two "new" potent insulin secretagogues. *Diabetes* **37:** 997–999, 1988.
2. MacDonald M and Fahien LA, Insulin release in pancreatic islets by a glycolytic and a Krebs cycle intermediate: Contrasting patterns of glyceraldehyde phosphate and succinate. *Arch Biochem Biophys* **279:** 104–108, 1990.
3. Dukes ID, McIntyre MS, Mertz RJ, Philipson LH, Roe MW, Spencer B and Worley JF III, Dependence on NADH produced during glycolysis for β-cell glucose signaling. *J Biol Chem* **269:** 10979–10982, 1994.
4. Laychock SG, Glucose metabolism, second messengers and insulin secretion. *Life Sci* **47:** 2307–2316, 1990.
5. Xia M and Laychock SG, Insulin secretion, *myo*-inositol transport, and Na^+-K^+-ATPase in glucose-desensitized rat islets. *Diabetes* **42:** 1392–1400, 1993.
6. Leahy JL, Cooper HE, Deal DA and Weir GC, Chronic hyperglycemia is associated with impaired glucose influence on insulin secretion. *J Clin Invest* **77:** 908–915, 1986.
7. Leahy JL and Weir GC, B-cell dysfunction in hyperglycemic rat models: Recovery of glucose-induced insulin secretion with lowering of the ambient glucose level. *Diabetologia* **34:** 640–647, 1991.
8. Vadlamudi S, Kalhan SC and Patel MS, Persistence of metabolic consequences in the progeny of rats fed a HC formula in their early postnatal life. *Am J Physiol* **269:** E731–E738, 1995.
9. Laychock SG, Vadlamudi S and Patel MS, Neonatal rat dietary carbohydrate affects pancreatic islet insulin secretion in adults and progeny. *Am J Physiol* **269:** E739–E744, 1995.
10. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
11. Laychock SG, Effects of cyclic guanosine 3′,5′-monophosphate on glucose utilization in isolated islets of Langerhans. *Endocrinology* **120:** 517–524, 1987.
12. Sener A, Welsh M, Welsh N, Hellerstrom C and Malaisse WJ, Maturation of fetal rat islet cells *in vitro* during tissue culture is associated with increased mitochondrial function. *Diabetes Res* **13:** 157–161, 1990.
13. London NJ and Donnelly PK, Techniques of pancreas and islet transplantation. *Baillieres Clin Gastroenterol* **8:** 517–532, 1994.
14. Korsgren O, Andersson A and Sandler S, Pretreatment of fetal porcine pancreas in culture with nicotinamide accelerates reversal of diabetes after transplantation to nude mice. *Surgery* **113:** 205–214, 1993.
15. Newgard CB and McGarry JD, Metabolic coupling factors in pancreatic β-cell signal transduction. *Annu Rev Biochem* **64:** 689–719, 1995.
16. Xia M, Hokin LE and Laychock SG, Rat pancreatic islets cultured at hyperglycemic glucose concentrations have altered phosphoinositide turnover, inositol phosphate production, and ATP levels. *Endocrine* **2:** 741–747, 1994.
17. MacDonald MJ, Does glyceraldehyde enter pancreatic islet metabolism via both the triokinase and the glyceraldehyde phosphate dehydrogenase reactions? A study of these enzymes in islets. *Arch Biochem Biophys* **270:** 15–22, 1989.
18. Brun T, Roche E, Kim K-H and Prentki M, Glucose regulates acetyl-CoA carboxylase gene expression in a pancreatic β-cell line (INS-1). *J Biol Chem* **268:** 18905–18911, 1993.
19. Laychock SG, Evidence for guanosine 3′,5′-monophosphate as a putative mediator of insulin secretion from isolated rat islets. *Endocrinology* **108:** 1197–1205, 1981.
20. Malaisse WJ, Regulation of insulin release by the intracellular mediators cyclic AMP, Ca^{2+}, inositol 1,4,5-trisphosphate, and diacylglycerol. In: *Insulin* (Eds. Cuatrecasas P and Jacobs S), pp. 113–124, Springer, New York, 1990.
21. Maldonato A, Renold AE, Sharp GWG and Cerasi E, Glucose-induced proinsulin biosynthesis: Role of islet cyclic AMP. *Diabetes* **26:** 538–545, 1977.
22. Lavergne C, Breant B and Rosselin G, Modulation of growth-related gene expression and growth inhibition by cyclic adenosine 3′,5′-monophosphate-elevating agents in the insulin-producing cell line βTC1. *Endocrinology* **131:** 2351–2356, 1992.
23. Aithal HN, Walsh-Reitz MM and Toback FG, Regulation of glyceraldehyde-3-phosphate dehydrogenase by a cytosolic protein. *Am J Physiol* **249:** C111–C116, 1985.
24. Ashmarina LI, Louzenko SE, Severin SE Jr, Muronetz VI and Nagradova NK, Phosphorylation of D-glyceraldehyde-3-phosphate dehydrogenase by Ca^{2+}/calmodulin-dependent protein kinase II. *FEBS Lett* **231:** 413–416, 1988.
25. Laychock SG, Mediation of insulin release by cGMP and cAMP in a starved animal model. *Mol Cell Endocrinol* **32:** 157–170, 1983.
26. Jones SB, Leone SL and Bylund DB, Desensitization of the *alpha*-2 adrenergic receptor in HT29 and opossum kidney cell lines. *J Pharmacol Exp Ther* **254:** 294–300, 1990.
27. Murphy GJ, Hruby VJ, Trivedi D, Wakelam JO and Houslay MD, The rapid desensitization of glucagon-stimulated adenylate cyclase is a cyclic AMP-independent process that can be mimicked by hormones which stimulate inositol phospholipid metabolism. *Biochem J* **243:** 39–46, 1987.
28. Kawamoto RM and Caswell AH, Autophosphorylation of glyceraldehydephosphate dehydrogenase and phosphorylation of protein from skeletal muscle microsomes. *Biochemistry* **25:** 657–661, 1986.