Perspectives in Diabetes

The Succinate Mechanism of Insulin Release

Leonard A. Fahien[1] and Michael J. MacDonald[2]

Nutrient secretagogues can increase the production of succinyl-CoA in rat pancreatic islets. When succinate esters are the secretagogue, succinyl-CoA can be generated via the succinate thiokinase reaction. Other secretagogues can increase production of succinyl-CoA secondary to increasing α-ketoglutarate production by glutamate dehydrogenase or mitochondrial aspartate aminotransferase followed by the α-ketoglutarate dehydrogenase reaction. Although secretagogues can increase the production of succinyl-CoA, they do not increase the level of this metabolite until after they decrease the level of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). This suggests that the generated succinyl-CoA initially reacts with acetoacetate to yield acetoacetyl-CoA plus succinate in the succinyl-CoA-acetoacetate transferase reaction. This would be followed by acetoacetyl-CoA reacting with acetyl-CoA to generate HMG-CoA in the HMG-CoA synthetase reaction. HMG-CoA will then be reduced by NADPH to mevalonate in the HMG-CoA reductase reaction and/or cleaved to acetoacetate plus acetyl-CoA by HMG cleavage enzyme. Succinate derived from either exogenous succinate esters or generated by succinyl-CoA-acetoacetate transferase is metabolized to malate followed by the malic enzyme reaction. Increased production of NADPH by the latter reaction then increases reduction of HMG-CoA and accounts for the decrease in the level of HMG-CoA produced by secretagogues. Pyruvate carboxylation catalyzed by pyruvate carboxylase will supply oxaloacetate to mitochondrial aspartate aminotransferase. This would enable this aminotransferase to supply α-ketoglutarate to the α-ketoglutarate dehydrogenase complex and would, in part, account for secretagogues increasing the islet level of succinyl-CoA after they decrease the level of HMG-CoA. Mevalonate could be a trigger of insulin release as a result of its ability to alter membrane proteins and/or cytosolic Ca^{2+}. This is consistent with the fact that insulin secretagogues decrease the level of the mevalonate precursor HMG-CoA. In addition, inhibitors of HMG-CoA reductase interfere with insulin release and this inhibition can be reversed by mevalonate. *Diabetes* 51:2669–2676, 2002

From the [1]Department of Pharmacology, University of Wisconsin Medical School, Madison, Wisconsin; and the [2]Childrens Diabetes Center, University of Wisconsin Medical School, Madison, Wisconsin.

Address correspondence and reprint requests to Leonard A. Fahien, University of Wisconsin Medical School, Department of Pharmacology, 301 SMI, 1300 University Ave., Madison, WI 53706. E-mail: lafahien@facstaff.wisc.edu.

Received for publication 7 August 2001 and accepted in revised form 27 February 2002.

AcAc, acetoacetate; Ac-CoA, acetyl-CoA; AcAc-CoA, acetoacetyl-CoA; BCH, aminobicyclo (2.2.1) heptane carboxylic acid; GABA, γ-aminobutyric acid; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; SAT, succinyl-CoA-acetoacetate transferase.

We (1–4) found that 10 mmol/l levels of exogenous methylesters of succinate (which are converted into succinate in the cell) are almost as potent in promoting insulin release as the most potent insulin secretagogue glucose. This effect was specific in that 10 mmol/l levels of malate and α-ketoglutarate and esters of fumarate or citrate did not promote insulin release (3,4). We believe that succinate has unique insulinotropic properties because, as shown in Scheme 1 (Fig.1), it is the only nutrient secretagogue that can react with one enzyme, succinate dehydrogenase (reaction 6 of Scheme 1), which is a direct source of metabolic energy, a second enzyme, succinate thiokinase, which can generate succinyl-CoA (reaction 1 of Scheme 1), and a third enzyme, succinyl-CoA-acetoacetate transferase (SAT) (reaction 2 of Scheme 1), which can react with succinyl-CoA to increase the production of acetoacetyl-CoA[1] (AcAc-CoA). AcAc-CoA, can in turn be utilized via the combined 3-hydroxy-3 methylglutaryl CoA (HMG-CoA) synthetase and HMG-CoA reductase reactions to produce mevalonate (reactions 3 and 4 of Scheme 1). We propose that mevalonate or one of its metabolites is a signal of insulin release.

In islets, the succinate thiokinase reaction with succinate can consume either GTP or ATP (5). However, this cost in metabolic energy would be more than compensated for when succinate esters are the secretagogues, because a high cellular level of succinate would also increase flux through succinate dehydrogenase. In this reaction, two molecules of ATP are generated per molecule of succinate oxidized.

In the sequence of reactions described above, pyruvate via the pyruvate dehydrogenase reaction (reaction 10 of Scheme 1) plus the oxidation of fatty acids and some amino acids could be sources of acetyl-CoA (Ac-CoA) for HMG-CoA synthetase (6). The acetoacetate (AcAc) required for the SAT reaction would be provided for by both HMG-CoA cleavage enzyme (reaction 5 of Scheme 1) and 3-hydroxybutyrate dehydrogenase (reaction 14 of Scheme 1). The AcAc-CoA utilized by HMG-CoA synthetase could be produced by both SAT and ketothiolase (reaction 11 of Scheme 1). The metabolism of succinate itself can produce Ac-CoA and AcAc-CoA (7,8). In rat islet mitochondria, fumarate produced by oxidation of succinate by succinate dehydrogenase is converted into malate by fumarase (reaction 7 of Scheme 1). Malate then enters the cytosol, where it is converted into pyruvate by malic enzyme (reaction 8 of Scheme 1), followed by pyruvate

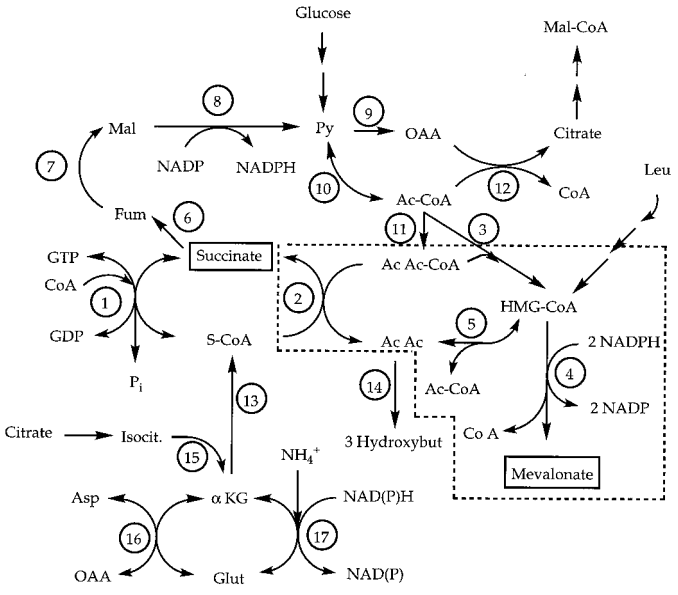

FIG. 1. Scheme 1: Interaction between succinate and HMG-CoA metabolism. This scheme shows how a high mitochondrial level of succinate (as would be present when succinate esters are added) could increase the level of succinyl-CoA (S-CoA) by reversing the succinate thiokinase reaction (reaction 1). Succinyl-CoA could then divert acetoacetate (AcAc) from the 3-hydroxybutyrate dehydrogenase (reaction 14) and to the succinyl-CoA-acetoacetate transferase reaction (reaction 2) so that succinate would be regenerated and acetoacetyl-CoA (AcAc-CoA) would be produced. AcAc-CoA would then react with acetyl-CoA (Ac-CoA) in the HMG-CoA synthetase reaction (reaction 3) to produce HMG-CoA. The latter would then be reduced to mevalonate by HMG-CoA reductase (reaction 4) and/or cleaved to Ac-CoA plus AcAc by HMG-CoA cleavage enzyme (reaction 5). Scheme 1 also shows how nutrient secretagogues, which increase production of α-ketoglutarate (αKG) by mitochondrial aspartate aminotransferase (reaction 16), glutamate dehydrogenase (reaction 17), or NAD-isocitrate dehydrogenase (reaction 15), can enhance production of succinyl-CoA by the α-ketoglutarate dehydrogenase complex (reaction 13). The generated succinyl-CoA would then be utilized to produce mevalonate and succinate as described above for the case when succinate is the secretagogue. The generated succinate, as exogenous succinate, would be converted into malate (Mal) by the combined succinate dehydrogenase-fumarase reactions (reactions 6 and 7, respectively). Malate would then leave the mitochondria and enter the cytosol where it would be converted into pyruvate (Py) by malic enzyme (reaction 8). The generated pyruvate then enters the mitochondria where it is converted into acetyl-CoA (Ac-CoA) by the pyruvate dehydrogenase complex (reaction 10). Ac-CoA is utilized to produce AcAc-CoA in the ketothiolase reaction (reaction 11), and Ac-CoA plus AcAc-CoA then provide HMG-CoA synthetase with its substrates. Pyruvate is also converted into oxaloacetate by pyruvate carboxylase (reaction 9), which supplies citrate synthase (reaction 12) and mitochondrial aspartate aminotransferase with substrate. Also shown is the production of HMG-CoA by the metabolism of leucine (Leu). The basic reactions for the synthesis of mevalonate, the proposed signal of insulin release, are enclosed by the dashed lines. Additional abbreviations are glutamate (Glut), fumarate (Fum), 3-hydroxybutyrate (3-hydroxybut), and malonyl-CoA (Mal-CoA).

entering the mitochondria, where it is converted into Ac-CoA and oxaloacetate by pyruvate dehydrogenase (reaction 10 of Scheme 1) and pyruvate carboxylase (reaction 9 of Scheme 1), respectively. The generated Ac-CoA plus AcAc-CoA produced from Ac-CoA by ketothiolase (reaction 11 of Scheme 1) would then be utilized as substrates by HMG-CoA synthetase.

Succinate metabolism would also enhance insulin release as a result of increased NADPH production by malic enzyme. This, in turn, would increase the production of mevalonate by HMG-CoA reductase. The importance of malic enzyme is indicated by the fact that succinate is not a secretagogue in mouse islets that, unlike rat islets, do not possess malic enzyme activity (9) (M.J.M., unpublished observations).

According to our proposed mechanism, which we refer to as the succinate mechanism, a basic requirement for insulin release would be the generation of mevalonate. Thus, the insulinotropic action of succinate and other nutrient secretagogues could be mediated by an increase in flux through the reactions enclosed in Scheme 1. These reactions are SAT, HMG-CoA synthetase, HMG-CoA reductase, and HMG-CoA cleavage enzyme (reactions 2–5 of Scheme 1). The net of these four reactions (starting with the SAT reaction with succinyl-CoA) is succinyl-CoA + HMG-CoA + two NADPH + two H^+ + H_2O → succinate + two CoA + mevalonate + two NADP. When succinate esters are the secretagogues, the succinyl-CoA that reacts with SAT would be produced by succinate thiokinase. The α-ketoglutarate dehydrogenase complex (reaction 13 of Scheme 1) would generate succinyl-CoA in the case of many other nutrient secretagogues. In this sequence of reactions, HMG-CoA is consumed, while mevalonate is generated. This is consistent with the fact that an early event after administration of insulin secretagogues is a marked decrease in the level of HMG-CoA (10,11). In addition to mevalonate, succinate is another net product of this sequence of reactions. Therefore, when succinate esters are the secretagogue or when other secretagogues generate succinate via this reaction sequence, succinate metabolism could lead to a buildup of oxaloacetate by pyruvate carboxylase, as previously described. This could enhance the activity of citrate synthase (reaction 12 of Scheme 1) and mitochondrial aspartate aminotransferase (reaction 16 of Scheme 1). Consequently, α-ketoglutarate production by this aminotransferase and isocitrate dehydrogenase (reaction 15 of scheme 1) could be increased, which would increase succinyl-CoA production by the α-ketoglutarate dehydrogenase complex (reaction 13 of Scheme 1). This could, in part, explain why nutrient secretagogues, after decreasing the level of HMG-CoA, increase the level of succinyl-CoA (10,11). In addition, increased citrate production could result in increased utilization of citrate for the production of malonyl-CoA (Scheme 1). This could account for the fact that after decreasing the level of HMG-CoA, nutrient secretagogues also increase the level of malonyl-CoA (10,11).

The succinate mechanism is also consistent with the fact that we (L.A.F. and M.J.M., unpublished observations) found that the specific activity of SAT, with AcAc as a substrate, was quite high in rat islet mitochondrial fractions, and others (12–17) have found that islets have the capacity to oxidize ketone bodies. This indicates that in islet mitochondria, the level of SAT is sufficiently high for it to play the role that we propose.

According to the succinate mechanism, flux through aminotransferases would not be essential for insulin release promoted by succinate esters, but flux through succinate dehydrogenase would be essential for the insulinotropic action of essentially all secretagogues. Consequently, we found that in rat islets, the insulinotropic actions of both glucose and methylsuccinate was completely blocked by the potent succinate dehydrogenase inhibitor 3-nitropropionate (1 mmol/l). However, even 10 mmol/l levels of the aminotransferase inhibitor aminooxy-

acetate had no effect on insulin release promoted by succinate esters but almost completely inhibited glucose-stimulated insulin release (4).

ROLE OF MEVALONATE

According to the succinate mechanism, production of mevalonate by the reactions enclosed in Scheme 1 results in insulin release. This concept is supported by the fact that before insulin secretagogues promote insulin release, they decrease the islet level of the mevalonate precursor HMG-CoA (10,11). Furthermore, it is known that intact pancreatic islets synthesize and metabolize mevalonate (18,19). In addition, lipid-soluble HMG-CoA reductase inhibitors, such as lovastatin and simvastatin, inhibit glucose-promoted insulin release from rat islets, and this can be prevented by mevalonate (18–20).

Triggering of insulin release by mevalonate could be related to the known ability of mevalonate to alter membrane proteins and/or the cytosolic level of Ca^{2+}. For example, G-proteins require isoprenylation for their association with membranes, and many GTP-binding proteins are modified by mevalonate-dependent isoprenylation (18,19). In islets, lovastatin decreases protein isoprenylation and produces accumulations in the cytosol of low–molecular weight GTP-binding proteins. However, adding mevalonate totally prevented these effects (18).

In addition, simvastatin inhibits the first phase increase in oscillations of cytoplasmic Ca^{2+} induced by glucose in β-cells, reversibly inhibits β-cell L-type Ca^{2+} channels, and inhibits the increase in cytosolic Ca^{2+} produced by L-arginine and KCI (20).

ROLE OF GLUTAMATE DEHYDROGENASE AND MITOCHONDRIAL ASPARTATE AMINOTRANSFERASE

According to the above discussion, succinate and other nutrient secretagogues enhance mevalonate production and insulin release by increasing flux through the reactions enclosed in Scheme 1. As shown in the following section, in the case of many nutrient secretagogues, the succinyl-CoA required to increase flux through this pathway is generated as a result of the secretagogue associating with and thereby enhancing α-ketoglutarate production by either glutamate dehydrogenase or mitochondrial aspartate amino transferase (reactions 16 and 17 of Scheme 1) (21–24). This would be followed by increased production of succinyl-CoA by the α-ketoglutarate dehydrogenase complex.

Glutamate dehydrogenase and mitochondrial aspartate aminotransferase could be more efficient in supplying the α-ketoglutarate dehydrogenase complex with α-ketoglutarate than other mitochondrial enzymes that generate α-ketoglutarate. This is because, according to our results obtained with a system of pure enzymes (25–28) as well as results with intact mitochondria obtained by others (29), glutamate dehydrogenase and mitochondrial aspartate aminotransferase can form a complex with the α-ketoglutarate dehydrogenase complex. Consequently, via substrate channeling α-ketoglutarate bound to glutamate dehydrogenase or aspartate aminotransferase can be a better substrate than free α-ketoglutarate for the α-ketoglutarate dehydrogenase complex (25–29).

Glucose cannot directly enhance the activity of either glutamate dehydrogenase or mitochondrial aspartate aminotransferase. However, according to the succinate mechanism, glucose is an ideal secretagogue because it can be metabolized to pyruvate, which can be utilized to generate Ac-CoA via the pyruvate dehydrogenase reaction (reaction 10 of Scheme 1). Ac-CoA plus AcAc-CoA generated from Ac-CoA via ketothiolase (reaction 11 of Scheme 1) can then supply HMG-CoA synthetase with its substrates. Pyruvate can also be utilized in the pyruvate carboxylase reaction (reaction 9 of Scheme 1) to generate oxaloacetate. α-Ketoglutarate production by mitochondrial aspartate aminotransferase can then be enhanced as a result of this enzyme transaminating oxaloacetate with glutamate.

COMPATIBILITY OF SECRETAGOGUES WITH SUCCINATE MECHANISM

According to the above sections, nutrient secretagogues increase mevalonate production secondary to increasing succinyl-CoA production followed by the reactions enclosed in Scheme 1. In the following discussion, we will determine whether the succinate mechanism can explain how known and proposed secretagogues can promote insulin release.

Phenylpyruvate. Phenylpyruvate is insulinotropic (30, 31). According to the succinate mechanism, this could be because phenylpyruvate can enhance production of α-ketoglutarate by mitochondrial aspartate aminotransferase as a result of associating with this enzyme followed by transaminating with glutamate (21). Furthermore, although γ-aminobutyric acid (GABA) alone has no effect on insulin release, it enhances the insulinotropic effect of phenylpyruvate (30). This would be consistent with the succinate mechanism because, when phenylpyruvate plus glutamate are transaminated by mitochondrial aspartate aminotransferase, GABA could facilitate a bypass of the generated α-ketoglutarate away from the α-ketoglutarate dehydrogenase complex and toward GABA aminotransferase to yield succinate semialdehyde. The latter can then be directly converted to succinate by succinate semialdehyde dehydrogenase. Succinate thiokinase could then convert succinate into succinyl-CoA, which via the reactions enclosed in Scheme 1, could be utilized to generate mevalonate. This "bypass" mechanism would be enhanced by the high affinity GABA aminotransferase has for succinate semialdehyde dehydrogenase (32) and the considerably higher level of GABA aminotransferase and succinate semialdehyde dehydrogenase present in islet mitochondria than in the mitochondria of other peripheral organs (33). This system of reactions is also of interest because GABA is generated by glutamate decarboxylase, a known β-cell antigen in insulin-dependent diabetes (34).

Glutamate. It has been proposed that glutamate is the primary messenger of insulin release (35). However, this is not the case in rat islets, where we (36) found that although glucose (16.7 mmol/l), methylsuccinate (10 mmol/l), leucine (10 mmol/l), and α-ketoisocaproate (10 mmol/l) markedly enhanced insulin release, none of these secretagogues increased the intracellular level of glutamate over its basal level of 21–25 nmol/mg protein. We also found that glutamine (10 mmol/l) did not promote insulin release, even though it increased the intracellular level of glutamate to 10-fold over basal levels. Therefore, as pre-

viously shown (3,23,24), these results demonstrate that although glutamine provides ample glutamate to function as a putative signal and ample NH_4^+ for the generation of glutamate by glutamate dehydrogenase, increasing the intracellular level of glutamate with glutamine alone does not promote insulin release. In addition, we (36) found that although the combination of leucine (10 mmol/l) plus glutamine (10 mmol/l) decreased the level of glutamate to 138 nmol/mg protein from its value of 256 nmol/mg protein with glutamine alone, it markedly increased insulin release over that produced by leucine alone. This is consistent with previous results (23,24) that demonstrated that the combination of leucine plus glutamine increases glutamate utilization by glutamate dehydrogenase more than that produced by either alone. This could be because leucine plus glutamate functions synergistically in increasing α-ketoglutarate production by glutamate dehydrogenase (37). According to the succinate mechanism, this increase in α-ketoglutarate, and ultimately succinyl-CoA and mevalonate, production would be responsible for the observation that the combination of leucine plus glutamine increases insulin release more than that produced by leucine alone (23,24).

Leucine. Leucine and its nonmetabolized analog 2 amino (2.2.1) heptane carboxylic acid (BCH) are the only amino acids that by themselves can promote insulin release, and they are also the only ones that can allosterically activate glutamate deamination by glutamate dehydrogenase (3,22–24). Furthermore, metabolites that can increase glutamate deamination by glutamate dehydrogenase can also enhance leucine-induced insulin release, and leucine-induced insulin release can be decreased by metabolites that lead to the inhibition of glutamate deamination (3).

According to the succinate mechanism, leucine would enhance insulin release because, as mentioned above, increased generation of α-ketoglutarate by glutamate dehydrogenase would lead to enhanced production of mevalonate and succinate via the reactions enclosed in Scheme 1. However, as also shown in Scheme 1, the metabolism of leucine to HMG-CoA and ultimately mevalonate might also contribute to the insulinotropic action of leucine. Consequently, leucine is not a secretagogue in glucose-treated islets, which have a decreased level of branch chain ketoacid dehydrogenase, unless glutamine is also added (38,39). However, BCH alone that is not metabolized can promote insulin release. This could be because BCH, unlike leucine, can activate glutaminase (40). This indicates that if leucine metabolism is absent, as is the case with glucose-treated islets or when BCH is the secretagogue, then glutamate is also required for insulin release. Glutamate would be produced by glutaminase reacting with exogenous glutamine when leucine is the secretagogue. When BCH is the secretagogue, glutamate would be produced by glutaminase reacting with endogenous glutamine.

In the absence of leucine or BCH, glutamine neither promotes insulin release nor markedly increases glutamate dehydrogenase activity (23,24,36). This could be because glutamate is the most abundant amino acid in islets, where its level is at least 1–2.5 mmol/l (36,41). In the absence of BCH or leucine, the K_m of glutamate in the glutamate dehydrogenase reaction is 0.2–0.5 mmol/l. Thus, the addition of glutamine might fail to increase flux through glutamate dehydrogenase because this enzyme is already saturated with glutamate. However, the K_m of glutamate in the mitochondrial aspartate aminotransferase is high, ~18 mmol/l (25). Therefore, in islet mitochondria in the absence of leucine or BCH, glutamine by increasing glutamate levels markedly increases flux through this aminotransferase. This results in the production of a large amount of α-ketoglutarate (23,24). This is because in islet mitochondria the level of aspartate aminotransferase is quite high and considerably higher than that of glutamate dehydrogenase (L.A.F. and M.J.M., unpublished observations). The high level of α-ketoglutarate does not cause insulin release, possibly because high levels of α-ketoglutarate inhibit glutaminase, glutamate dehydrogenase, and mitochondrial aspartate aminotransferase (29,37). Consequently, when the level of α-ketoglutarate is high, glutamine cannot produce additional flux through the α-ketoglutarate dehydrogenases complex and the enzymes represented in Scheme 1. In addition, α-ketoglutarate inhibits ketothiolase (42). This would decrease the ability of ketothiolase to produce AcAc-CoA for HMG-CoA synthetase, which in turn would decrease mevalonate production.

Leucine and BCH increase the K_m of glutamate, the K_i of α-ketoglutarate, and the V_{max} in the glutamate dehydrogenase reaction (37). The increase in the V_{max} and K_m of glutamate increases α-ketoglutarate production by glutamate dehydrogenase and as a result increases insulin release (23,24). The increase in the K_i of α-ketoglutarate would decrease inhibition of glutamate dehydrogenase by α-ketoglutarate. However, in islet mitochondria there is considerably less total glutamate dehydrogenase than aspartate aminotransferase activity, and leucine and BCH do not protect mitochondrial aspartate aminotransferase against inhibition by α-ketoglutarate (3,37). Therefore, the enhanced production of α-ketoglutarate by glutamate dehydrogenase, mediated by the combination of leucine plus glutamine, results in enhanced inhibition by α-ketoglutarate of the more abundant mitochondrial aspartate aminotransferase. This results in the level of α-ketoglutarate being lower in the presence of both leucine and glutamine than in the presence of glutamine alone (23,24). This favors insulin release by relieving ketothiolase of inhibition by α-ketoglutarate and thus permits ketothiolase to play its role in generating mevalonate, thereby promoting insulin release. The concept that these activators in the presence of glutamine enhance insulin release by favoring glutamate dehydrogenase over aspartate aminotransferase activity is consistent with our observation that leucine-glutamine–promoted insulin release is not inhibited but slightly enhanced by the aminotransferase inhibitor aminooxyacetate (4).

α-Ketoisocaproate. α-Ketoisocaproate can also promote insulin release (43). According to the succinate mechanism, its insulinotropic action could result from its catabolism to HMG-CoA plus its transamination to leucine, followed by leucine activating glutamate dehydrogenase (22–24).

Glucose. The metabolism of glucose to pyruvate, followed by the transport of pyruvate into mitochondria and the pyruvate dehydrogenase and pyruvate carboxylase reactions, is apparently essential in glucose-stimulated insulin

release. Consequently, we found that *1*) the rates of the pyruvate carboxylase and pyruvate dehydrogenase reactions in islets are roughly equal and directly proportional to the level of extracellular glucose and thus insulin secretion (44), *2*) treated islets that are deficient in both pyruvate carboxylase and pyruvate dehydrogenase activity do not release insulin when supplied with glucose (38), and *3*) the level of pyruvate carboxylase activity in islets is high and approximately the same as in gluconeogenic organs, such as liver and kidney (45).

According to the succinate mechanism, the Ac-CoA generated by the pyruvate dehydrogenase complex and the oxaloacetate produced by pyruvate carboxylase would be important for mevalonate production and glucose-stimulated insulin release. This is because Ac-CoA produced by the former would be utilized by HMG-CoA synthetase, while the oxaloacetate generated by the latter would be utilized by the combined mitochondrial aspartate aminotransferase-α-ketoglutarate dehydrogenase reactions for the production of succinyl-CoA. Support for the concept that glucose increases mitochondrial aspartate aminotransferase activity and that this increase is essential for glucose-stimulated insulin release is derived from experiments that demonstrated that in rat islets, the increase in the level of α-ketoglutarate (46) and insulin release produced by glucose was almost completely inhibited by the aminotransferase inhibitor aminooxyacetate (4).

Citrate. The Ac-CoA and oxaloacetate obtained from glucose metabolism could also be utilized by citrate synthase (reaction 12 of Scheme 1) to produce citrate. Consequently, glucose can increase the level of citrate in islets, and this increase coincides with the initiation of insulin release (47).

According to the succinate mechanism, increased generation of citrate could enhance insulin release because in mitochondria, citrate is a source of succinyl-CoA via the aconitase-isocitrate dehydrogenase-α-ketoglutarate dehydrogenase reactions. Furthermore, conversion of α-ketoglutarate generated by NAD-isocitrate dehydrogenase (reaction 15 of Scheme 1) into succinyl-CoA could be enhanced as a result of the ability of this enzyme to form a complex with the α-ketoglutarate dehydrogenase complex (48). In addition, mitochondrial citrate can be transported into the cytosol, where it is utilized by ATP-citrate lyase for oxaloacetate production. As discussed in a subsequent section, an increase in the level of oxaloacetate in the cytosol can favor insulin release by enhancing the metabolism of glucose to pyruvate. The importance of these interactions is indicated by the fact that the level of ATP-citrate lyase is high in islets (49) and that glucose-stimulated insulin release is inhibited by both the ATP-citrate lyase inhibitor hydroxycitrate (50) and butylmalonate, an inhibitor of both di- and tricarboxylate acid transport (51). However, compared with succinate esters, exogenous citrate and its esters alone are not potent secretagogues (4,46,47).

Long-chain Acyl-CoA. According to previous work with HIT cells and islets (10,11), the Ac-CoA produced in the cytosol with oxaloacetate in the ATP-citrate lyase reaction is utilized by Ac-CoA carboxylase to produce malonyl-CoA before glucose-stimulated insulin release. Malonyl-CoA is then utilized for the synthesis of long-chain acyl-CoA and inhibits entry of long-chain acyl-CoA into the mitochondria. It was proposed that either the long-chain acyl-CoA or triglycerides derived from long-chain acyl-CoA would then in some way trigger insulin release. However, in other experiments it was found that markedly decreasing the intracellular level of malonyl-CoA or long-chain acyl-CoA esters had no effect on glucose-stimulated insulin release (52).

REGULATORY FEATURES OF THE SUCCINATE MECHANISM

In this section, we will summarize the key roles played by some other enzymes in glucose-stimulated insulin release and how this is consistent with the succinate mechanism.

Glyceraldehyde-3-phosphate dehydrogenase and cytosolic malate dehydrogenase. Glyceraldehyde promotes insulin release (1). Furthermore, glucose-stimulated insulin release is inhibited by both iodoacetamide, an inhibitor of glyceraldehyde-3-phosphate dehydrogenase (reaction 1 of Scheme 2, Fig. 2), and NaF, an inhibitor of enolase (reaction 6 of Scheme 2) (1,53–56). This indicates that glucose-stimulated insulin release requires both the metabolism of glucose to glyceraldehyde-3-phosphate and further metabolism of the latter. According to the succinate mechanism, conversion of glucose to pyruvate via glycolysis enables pyruvate to be utilized for the production of mevalonate via the combined pyruvate carboxylase-mitochondria aspartate aminotransferase-α-ketoglutarate dehydrogenase (reactions 9, 16, and 13 of Scheme 1) reactions plus the reactions enclosed in Scheme 1.

Cytosolic malate dehydrogenase (reaction 2 of Scheme 2) could also play an important role in insulin release by promoting glycolysis. This is because by reducing oxaloacetate with NADH, this enzyme would resupply NAD to glyceraldehyde-3-phosphate dehydrogenase and prevent potent product inhibition of this latter enzyme by NADH (57). In islets, cytosolic malate dehydrogenase could be the major enzyme that plays this role. This is because it is present in high levels (51) and because it removes hydrogen from the A side instead of the B side (Scheme 3, Fig. 3) of the pyridine ring of NADH (58). According to previous results (58), this can permit cytosolic malate dehydrogenase to associate with the complex between NADH and a second dehydrogenase, such as glyceraldehyde-3-phosphate dehydrogenase, which has B-sided specificity. The NADH in this ternary complex can then be directly transferred to malate dehydrogenase. Consequently, NADH would be protected from interception by competing dehydrogenases because it would not have to dissociate from glyceraldehyde-3-phosphate dehydrogenase and diffuse to cytosolic malate dehydrogenase (58). In islets, the level of lactate dehydrogenase might be too low for this enzyme to play a major role in converting NADH produced by glyceraldehyde-3-phosphate dehydrogenase back to NAD (59,60). Cytosolic glycerol-3-phosphate dehydrogenase also may not be capable of playing a major role because it has B-sided specificity for NADH (58). Also, we (61) found that at the physiological pH of the cytosol of 7.1 (62) cytosolic glycerol-3-phosphate dehydrogenase in rat islet cytosolic fractions was considerably more active with NADPH than with NADH.

Cytosolic glycerol-3-phosphate dehydrogenase. Since,

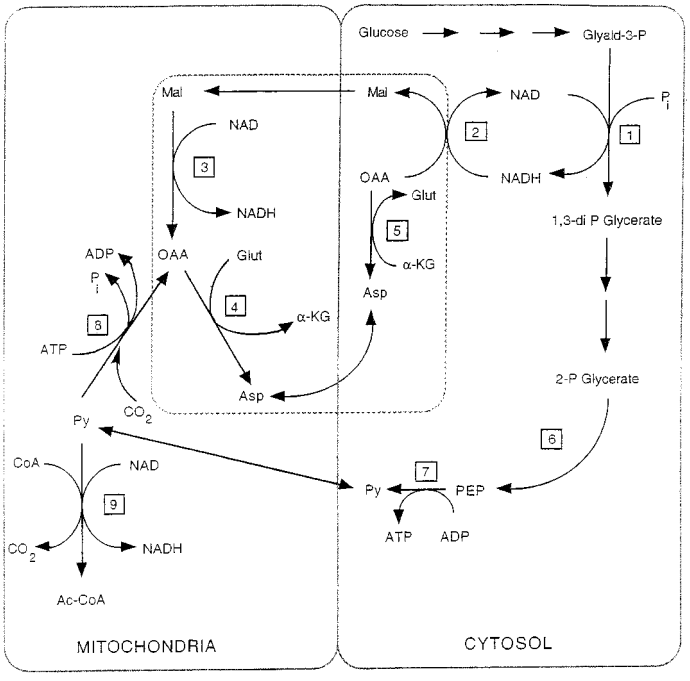

FIG. 2. Scheme 2: Interaction between glycolysis and mitochondrial reactions. This scheme shows an abbreviated glycolytic pathway where glucose is converted into pyruvate. As part of this scheme, cytosolic malate dehydrogenase (reaction 2) reacts with oxaloacetate (OAA) plus the NADH generated by glyceraldehyde-3-phosphate dehydrogenase (reaction 1) to supply malate (Mal) to the mitochondria and to relieve glyceraldehyde-3-phosphate dehydrogenase of its potent product inhibition by NADH. The latter allows glycolysis to proceed. Malate that enters the mitochondria is oxidized to oxaloacetate (OAA) by mitochondrial malate dehydrogenase (reaction 3). Oxaloacetate then transaminates with glutamate (Glut) to yield α-ketoglutarate (α-KG) plus aspartate (Asp) in a reaction catalyzed by mitochondrial aspartate aminotransferase (reaction 4). The generated aspartate enters the cytosol where it is utilized by cytosolic aspartate aminotransferase (reaction 5) to generate oxaloacetate for cytosolic malate dehydrogenase. This completes the shuttle enclosed in solid lines and is referred to as the malate-aspartate shuttle. The generated α-ketoglutarate is then utilized for the production of succinyl-CoA as shown in Scheme 1. Glucose can also enhance oxaloacetate and ultimately α-ketoglutarate and succinyl-CoA production in mitochondria because pyruvate (Py) derived from glucose and generated by pyruvate kinase (reaction 7) can enter the mitochondria where about one-half of it is converted into oxaloacetate by pyruvate carboxylase (reaction 8). The remaining half of the glucose-derived pyruvate that enters the mitochondria is converted into acetyl-CoA (Ac-CoA) via the pyruvate dehydrogenase complex (reaction 9). As shown in Scheme 1, Ac-CoA can then be utilized to produce acetoacetyl-CoA (AcAc-CoA) via the ketothiolase reaction. Ac-CoA plus AcAc-CoA are then used as substrates for 3-hydroxy-3-methylglutaryl-CoA synthetase to ultimately generate mevalonate. In rat islets both the glycolytic pathway and the malate-aspartate shuttle are required for glucose stimulated insulin release. Consequently, the latter can be almost completely eliminated by inhibitors of glyceraldehyde-3-phosphate dehydrogenase, inhibitors of enolase (reaction 6), aminotransferase inhibitors, and inhibitors of the transport of dicarboxylic acids through the mitochondria membrane.

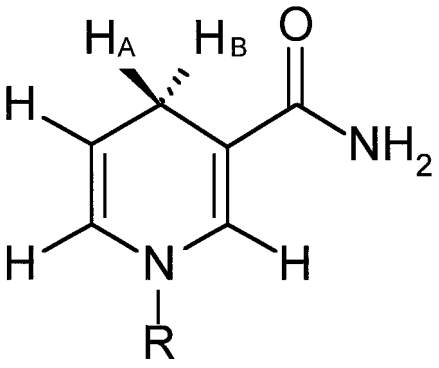

FIG. 3. Scheme 3: Nicotinamide moiety of NADH. Scheme 3 shows the nicotinamide moiety of NADH, which has hydrogen on the A side (H_A) in the front of the nicotinamide plane and hydrogen on the B side (H_B) behind the nicotinamide plane. Dehydrogenases with A-sided specificity remove hydrogen from the A-side while dehydrogenases with B-sided specificity remove hydrogen from the B-side. This can enable A-sided dehydrogenases to transfer NADH directly to B-sided dehydrogenases and vice versa according to the reaction: E_A − NADH + E_B (↔ E_A − NADH − E_B ↔ E_A + E_B − NADH. However, there will not be a transfer of NADH between dehydrogenases that have the same chiral specificity with respect to NADH. An additional factor that can facilitate direct transfers of NADH between dehydrogenases of opposite chiral specificity is that A-sided dehydrogenases can have almost a completely negative charge in the surface region surrounding the active site cleft of the dehydrogenase. On the other hand, the surface region of B-sided dehydrogenase can be almost completely positively charged (58).

in islets, cytosolic glycerol-3-phosphate dehydrogenase can be more reactive with NADPH than with NADH, this enzyme could be a source of NADP for malic enzyme and cytosolic NADP-isocitrate dehydrogenase. The latter two are by far the most abundant NADP-linked dehydrogenases in rat islet cytosol, since very little glucose is metabolized via the hexose monophosphate pathway (44,45,63,64). Production of NADP would be important in the islet cytosol, because then citrate can be utilized in the combined aconitase-cytosolic NADP-isocitrate dehydrogenase-cytosolic aspartate aminotransferase reactions to generate the oxaloacetate, which is reduced by cytosolic malate dehydrogenase. In addition, malic enzyme could provide HMG-CoA reductase with NADPH and could also generate the pyruvate, which could be utilized for oxaloacetate, Ac-CoA, and ultimately mevalonate production (Scheme 1).

Mitochondrial glycerol-3-phosphate dehdyrogenase. Islets from mutant mice that lack mitochondrial glycerol-3-phosphate dehydrogenase activity have normal glucose-stimulated insulin release (56,65). This is consistent with the succinate mechanism, which does not require an intact glycerol-3-phosphate shuttle for glucose-stimulated insulin release.

Some physiological aspects of succinate mechanism. An insulin deficiency can result in decreased entry of glucose into cells and therefore less utilization of glucose for the production of oxaloacetate. Because of the latter, less Ac-CoA is utilized by citrate synthase and more is utilized for the production of the ketone bodies AcAc and 3-hydroxybutyrate, as shown in Scheme 1. The major site of production of ketone bodies is the liver. From the liver, they are transported in the blood to peripheral tissues, where they can be a major source of metabolic fuel and can increase the level of Ac-CoA, glutamate, and Krebs cycle metabolites. These are the same effects that can be produced by insulin (62,66,67), which, in addition to increasing glucose transport, increases pyruvate dehydrogenase activity. Thus, ketosis, which is a physiological response to insulin deprivation during starvation, is equivalent in metabolic effects to the action of insulin. Ketone bodies can bypass the block in glucose transport caused by a lack of insulin by utilizing the monocarboxylate carrier. Ketone bodies also bypass the inhibition of pyruvate dehydrogenase induced by insulin deficiency by providing an alternative source of mitochondrial Ac-CoA (62,66,67).

According to the succinate mechanism, ketone bodies

are utilized in islets to generate mevalonate, a trigger of insulin release. However, to prevent ketone bodies from promoting insulin release during starvation, they are not insulinotropic (12–17) by themselves. They are only effective if there is a source of succinyl-CoA to enable AcAc-CoA and ultimately mevalonate to be produced. Succinyl-CoA is generated as part of the action of glucose and other insulin secretagogues.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grant DK-28348, the Oscar C. Rennebohm Foundation, and the Robert Wood Johnson Family Trust (to M.J.M.).

The authors thank Dr. Henry A. Lardy for helpful discussion.

REFERENCES

1. MacDonald MJ, 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
2. MacDonald MJ: Elusive proximal signals of β-cells for insulin secretion. *Diabetes* 29:1461–1466, 1990
3. Fahien LA, MacDonald MJ, Kmiotek EH, Mertz RJ, Fahien CM: Regulation of insulin release by factors that also modify glutamate dehydrogenase. *J Biol Chem* 263:13510–13514, 1988
4. MacDonald MJ, Fahien LA, Mertz RJ, Rana R: Effect of esters of succinic acid and other citric acid cycle intermediates on insulin release and inositol phosphate formation by pancreatic islets. *Arch Biochem Biophys* 269:400–406, 1989
5. Kowluru A: Adenine and guanine nucleotide-specific succinyl-CoA synthetases in the clonal beta-cell mitochondria: implications in beta-cell high energy phosphate metabolism in relation to physiological insulin secretion. *Diabetologia* 44:89–94, 2001
6. Hellerstrom C: Effects of carbohydrates on the oxygen consumption of isolated pancreatic islets of mice. *Endocrinology* 18:105–112, 1967
7. MacDonald MJ: Metabolism of the insulin secretagogue methyl succinate by pancreatic islets. *Arch Biochem Biophys* 300:201–205, 1993
8. Malaisse WJ, Sener A: Metabolic effects and fate of succinate esters in pancreatic islets. *Am J Physiol* 264:434–439, 1993
9. MacDonald MJ, Marshall LK: Survey of normal appearing mouse strain which lacks malic enzyme and NAD^+–linked glycerol phosphate dehydrogenase: normal pancreatic beta cell function, but abnormal metabolite pattern in skeletal muscle. *Mol Cell Biochem* 220:117–125, 2001
10. Corkey BE, Glennon MC, Chen KS, Deeney JT, Matschinsky FM, Prentki M: A role for malonyl-CoA in glucose-stimulated insulin secretion from clonal pancreatic β-cells. *J Biol Chem* 264:21608–21612, 1989
11. Prentki M, Vischer S, Glennon MC, Regazzi R, Deeney JT, Corkey BE: Malonyl-CoA and long chain acyl-CoA esters as metabolic coupling factors in nutrient-induced insulin secretion. *J Biol Chem* 267:5802–5803, 1992
12. Dean PM, Matthews EK, Sakamoto Y: Pancreatic islet cells: effects of monosaccharides, glycolytic intermediates and metabolic inhibitors on membrane potential and electrical activity. *J Physiol (Lond)* 246:459–478, 1975
13. Metzger P, Franken P, Balasse E: Permissive role of glucose on the insulinotropic effect of ketone bodies in vivo. *Horm Metab Res* 5:313–315, 1973
14. Biden TJ, Taylor KW: Effects of ketone bodies on insulin release and islet-cell metabolism in the rat. *Biochem J* 212:371–377, 1983
15. Malaisse WJ, Lebrun P, Yaylali B, Camara J, Valverde I, Sener A: Ketone bodies and islet function: ^{45}Ca handling, insulin synthesis, and release. *Am J Physiol* 259:E117–E122, 1990
16. Malaisse WJ, Lebrun P, Rasschaert J, Blachier F, Yilmaz T, Sener A: Ketone bodies and islet function: Rb handling and metabolic data. *Am J Physiol* 259:E123–E130, 1990
17. Berne C: The metabolism of lipids in mouse pancreatic islets: the oxidation of fatty acids and ketone bodies. *Biochem J* 152:661–666, 1975
18. Metz SA, Rabaglia, M., Stock TB, Kowluru A: Modulation of insulin secretion from normal rat islets by inhibitors of the post-translational modifications of GTP-binding proteins. *Biochem J* 295:31–40, 1993
19. Li G, Regazzi R, Roche E, Wollheim CB: Blockade of mevalonate production by lovastatin attenuates bombesin and vasopressin potentiation of nutrient-induced insulin secretion in HIT-T15 cells: probable involvement of small GTP-binding proteins. *Biochem J* 289:379–385, 1993
20. Yada T, Nakata M, Shiraishi T, Kakei M: Inhibition by simvastatin, but not pravastatin of glucose-induced cytosolic Ca^{2+} signaling and insulin secretion due to blockade of L. type Ca^{2+} channels in rat islet beta-cells. *Br J Pharmacol* 126:1205–1213, 1999
21. Miller JE, Litwack G: Purification, properties and identity of liver mitochondrial tyrosine aminotransferase. *J Biol Chem* 246:3234–3240, 1971
22. Sener A, Malaisse-Lagae F, Malaisse WJ: Stimulation of pancreatic islet metabolism and insulin release by a nonmetabolizable amino acid. *Proc Natl Acad Sci U S A* 78:5460–5464, 1981
23. Malaisse-Lagae F, Sener A, Garcia-Morales P, Valverde I, Malaisse WJ: The stimulus-secretion coupling of amino acid-induced insulin release I. *J Biol Chem* 257:3754–3758, 1982
24. Malaisse WJ, Sener A, Malaisse-Lagae F, Welsh M, Matthews DE, Bier DM, Hellerstrom C: The stimulus-secretion coupling of amino acid-induced insulin release II. *J Biol Chem* 257:8231–8232, 1982
25. Fahien LA, Kmiotek EH, MacDonald MJ, Fibich B, Mandic M: Regulation of malate dehydrogenase activity by glutamate, citrate, α-ketoglutarate, and multienzyme interaction. *J Biol Chem* 263:10687–10697, 1988
26. Fahien LA, Teller JK: Glutamate-malate metabolism in liver mitochondria: a model constructed on the basis of mitochondria levels of enzymes, specificity, dissociation constants and stoichiometry of hetero enzyme complexes. *J Biol Chem* 267:10411–10422, 1992
27. Fahien LA, MacDonald MJ, Teller JK, Fibich B, Fahien CM: Kinetic advantages of hetero-enzyme complexes with glutamate dehydrogenase and the α-ketoglutarate dehydrogenase complex. *J Biol Chem* 264:12303–12312, 1989
28. Fahien LA, Chobanian MC: Kinetic advantages of multienzyme complexes involving aminotransferases. In *Channeling in Intermediary Metabolism*. Agius L, Sherratt HAS, Eds. London, Portland Press, 1997, p. 219–236
29. Smith BC, Clotfelter LA, Cheung JT, LaNoue KR: Differences in α-oxoglutarate dehydrogenase regulation in liver and kidney. *Biochem J* 284:819–826, 1992
30. Sorenson RL, Garry DG, Brelje TC: Structural and functional considerations of GABA in islets of Langerhans: β-cells and nerves (Review Article). *Diabetes* 40:1365–1374, 1991
31. Lenzen S, Rustenback T, Panten U: Transamination of 3-phenylpyruvate in pancreatic β-cell mitochondria. *J Biol Chem* 259:2043–2046, 1983
32. Hearl WG, Churchich JE: Interactions between 4-aminobutyrate aminotransferase and succinate semialdehyde dehydrogenase, two mitochondrial enzymes. *J Biol Chem* 259:11459–11463, 1984
33. Garry DJ, Coulher HD, McIntree TJ, Wu JY, Sorenson RL: Immunoreactive GABA transaminase within the pancreatic islet is localized in mitochondria in the β-cell. *J Histochem Cytochem* 35:831–836, 1987
34. Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olesen MI, Camilli PD: Identification of the 64K antoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature (Lond)* 347:151–156, 1990
35. Maechler P, Wollheim CB: Mitochondrial glutamate as a messenger in glucose-induced insulin exocytosis. *Nature* 402:685–689, 1999
36. MacDonald MJ, Fahien LA: Glutamate is not a messenger in insulin secretion. *J Biol Chem* 275:34025–34027, 2000
37. Fahien LA, Teller JK, MacDonald MJ, Fahien CM: Regulation of glutamate dehydrogenase by Mg^{2+} and magnification of leucine activation by Mg^{2+}. *Mol Pharmacol* 37:943–949, 1990
38. MacDonald MJ, Kaysen JH, Moran SM, Pomije CE: Pyruvate dehydrogenase and pyruvate carboxylase: sites of pretranslational regulation by glucose of glucose-induced insulin release in islets. *J Biol Chem* 266: 22392–22397, 1991
39. MacDonald MJ, McKenzie DI, Kaysen JH, Walker TN, Moran SM, Fahien LA, Towle HC: Glucose regulates leucine-induced insulin release and the expression of the branch chain keto-acid dehydrogenase E1 subunit gene in pancreatic islets. *J Biol Chem* 260:1335–1341, 1991
40. Zaleski J, Wilson DF, Ericinska M: β-2-aminobicyclo-(2.2.1)-heptane-2-carboxylic acid: a new activator of glutaminase in intact rat liver mitochondria. *J Biol Chem* 261:14091–14094, 1986
41. Dannielson A, Hellman B, Idahl L-A: Levels of α-ketoglutarate and glutamate in stimulated pancreatic β-cells. *Horm Metab Res* 2:28–31, 1970
42. Gehring H, Lynen F: Thiolase. *The Enzymes* 7:391–405, 1972
43. Lambert N, Lars-Åke I: α-Ketoisocaproate is not a true substrate for ATP production by pancreatic β-cell mitochondria. *Diabetes* 47:339–344, 1998
44. MacDonald MJ: Estimates of glycolysis, pyruvate (de) carboxylation, pentose phosphate pathway, and methyl succinate metabolism in incapacitated pancreatic islets. *Arch Biochem Biophys* 305:205–214, 1993

45. MacDonald MJ: Feasibility of a mitochondrial pyruvate malate shuttle in pancreatic islets. *J Biol Chem* 270:20051–20058, 1995
46. Alarcon C, Schultz V, Deeney JT, Corkey BE, Rhodes J: Succinate as a preferential metabolic stimulus-coupling signal for glucose-induced proinsulin biosynthesis translation (Abstract). *Diabetes* 49 (Suppl. 1):A252, 2000
47. Farfari S, Schulz V, Corkey B, Prentki M: Glucose-regulated anaplerosis and cataplerosis in pancreatic β-cells: possible implication of a pyruvate/citrate shuttle in insulin secretion. *Diabetes* 49:718–726, 2000
48. Porpaczy Z, Sumegi B, Alkonyi I: Interaction between NAD-dependent isocitrate dehydrogenase, α-ketoglutarate dehydrogenase complex and NADH: ubiquinone oxidoreductase. *J Biol Chem* 262:9509–9514, 1987
49. Berne C: Nicotinamide dinucleotide phosphate-converting enzymes and adenosine triphosphate citrate lyase in some tissues and organs of New Zealand obese mice with special reference to the enzyme pattern of the pancreatic islets. *J Histochem Cytochem* 23:660–665, 1975
50. Chen S, Ogawa A, Ohneda M, Unger RH, Foster DW, McGarry JD: More direct evidence for a malonyl-CoA-carnitine palmitoyltransferase I interaction as a key event in pancreatic β-cell signaling. *Diabetes* 43:878–883, 1994
51. MacDonald MJ: Evidence for the malate-aspartate shuttle in pancreatic islets. *Arch Biochem Biophys* 213:643–649, 1982
52. Antinozzi PA, Segall L, Prentki M, McGarry JD, Newgard CB: Molecular or pharmacologic perturbation of the link between glucose and lipid metabolism is without effect on glucose-stimulated insulin secretion: a reevaluation of the long-chain acyl-CoA hypothesis. *J Biol Chem* 273:16146–16154, 1999
53. Malaisse WJ, Kawazu S, Hercuelz A, Hutton JC, Sommers G: The stimulus secretion coupling of glucose-induced insulin release. *Arch Biochem Biophys* 194:49–62, 1979
54. Sener A, Kawazu S, Hutton JC, Boschere AC, DeVis G, Somers C, Herchuelz A, Malaisse WJ: The stimulus-secretion coupling of glucose-induced insulin release effect of exogenous pyruvate on islet function. *Biochem J* 176:217–232, 1978
55. Mertz RJ, Worley JF, Spencer B, Johnson JH, Dukes IR: Activation of stimulus-secretion coupling in pancreatic β-cells by specific products of glucose metabolism. *J Biol Chem* 271:4838–4845, 1996
56. Eto K, Tsubamoto Y, Terauchi Y, Sugiyama T, Kishimoto T, Takahashi N, Yamauchi N, Kubota N, Murayama S, Alawa T, Akanuma Y, Aizaua S, Kasai H, Yazaki Y, Kadowaki T: Role of NADH shuttle system in glucose-induced activation of mitochondria metabolism and insulin secretion. *Science* 283:981–985, 1999
57. Velick SF, Furfine C: Glyceraldehyde-3-phosphate dehydrogenase. In *The Enzymes*. Vol. 7, 2nd ed. Boyer PD, Lardy H, Myrback K, Eds. New York, Academic Press, 1963, p. 243–273
58. Srivastava D. Bernhard SA: Enzyme-enzyme interactions and the regulation of metabolic reaction pathways. *Curr Topics in Cell Regul* 28:1–68, 1986
59. Sekine N, Vincenzo C, Ragazzi R, Brown LJ, Gine E, Tamarit-Rodriguez J, Givotti M, Sandrine M, MacDonald MJ, Wellheim CB, Rutter GA: Low lactate dehydrogenase and high mitochondrial glycerol phosphate dehydrogenase in pancreatic β-cell. *J Biol Chem* 269:4895–4902, 1994
60. Hassan J, Rasschaert J, Nadi AB, Leclerq-Meyer V, Sener A, Malaisse WJ: Relevance of lactate dehydrogenase activity to the control of oxidative glycolysis in pancreatic islet β-cells. *Arch Biochem Biophys* 327:260–264, 1996
61. Prabhakar P, Laboy JI, Wang J, Budker T, Din ZZ, Chobanian M, Fahien LA: Effect of NADH-X on cytosolic glycerol-3-phosphate dehydrogenase. *Arch Biochem Biophys* 360:195–205, 1998
62. Sato K, Kashiwaya Y, Keon CA, Tsuchia N, King M, Radda G, Chance B, Clarke K, Veech R: Insulin, ketone bodies and mitochondrial energy transduction. *FASEB* 9:651–658, 1995
63. Ashcroft SJH, Christie MR: Effects of glucose on the cytosolic ratio of reduced/oxidized nicotinamide-adenine dinucleotide phosphate in rat islets of Langerhaus. *Biochem J* 184:697–700, 1979
64. Ashcroft SJH, Weerasinghe LCC, Basset JM, Randle PD: The pentose cycle and insulin release in mouse pancreatic islets. *Biochem J* 126:525–532, 1972
65. Ravier MA, Eto K, Jonkers FC, Nequin M, Kadowaki T, Henquin JC: The oscillatory behavior of pancreatic islets from mice with mitochondrial glycerol-3-phosphate dehydrogenase knockout. *J Biol Chem* 275:1587–1593, 2000
66. Veech RL, Chance B, Kashiwaya Y, Lardy H, Cahill GF Jr: Ketone bodies, potential therapeutic uses. *Life* 51:241–247, 2001
67. Kashiwaya Y, Sato K, Tsuchiya S, Thomas S, Fell DA, Veech RL, Passonneau JV: Control of glucose utilization in working perfused rat heart. *J Biol Chem* 269:25502–25514, 1994