Biochem. J. (1990) 267, 527–530 (Printed in Great Britain)

Glucose-induced activation of pyruvate dehydrogenase in isolated rat pancreatic islets

James G. McCORMACK,*‡ Eugenio A. LONGO† and Barbara E. CORKEY†
*Department of Biochemistry, University of Leeds, Leeds LS2 9JT, U.K., and †Division of Diabetes and Metabolism, Boston University Medical Center, Boston, MA 02118, U.S.A.

1. Rat pancreatic islets were isolated and then maintained in culture for 2–4 days before being incubated in groups of 100 in the presence of different glucose (0–20 mM) or $CaCl_2$ (1.2–4.2 mM) concentrations, or with uncoupler. 2. Increases in extracellular glucose concentration resulted in increases in the amount of active, non-phosphorylated, pyruvate dehydrogenase in the islets, with half-maximal effects around 5–6 mM-glucose. Increasing extracellular glucose from 3 to 20 mM resulted in a 4–6-fold activation of pyruvate dehydrogenase within 2 min. 3. The total enzyme activity was unchanged, and averaged 0.4 m-unit/100 islets at 37 °C. 4. These changes in active pyruvate dehydrogenase were broadly similar to changes in insulin secretion by the islets. 5. Increasing extracellular Ca^{2+} or adding uncoupler also activated pyruvate dehydrogenase to a similar degree, but only the former was associated with increased insulin secretion.

INTRODUCTION

The stimulation of insulin secretion from mammalian pancreatic β-cells which is caused by increases in the extracellular concentration of glucose is mediated by increases in the cytosolic concentration of Ca^{2+} within the sub-micromolar range, and involves the enhanced metabolism and oxidation of the glucose (for reviews see Hedeskov, 1980; Meglasson & Matschinsky, 1986; Prentki & Matschinsky, 1987; Matschinsky *et al.*, 1989). However, it is not yet clear how these two responses are related and integrated.

The major committed step in carbohydrate catabolism in mammalian tissues is catalysed by the multienzyme complex pyruvate dehydrogenase (PDH) (Randle, 1986; Sugden *et al.*, 1989). This step is highly regulated, and principally by factors which control the amounts of active, non-phosphorylated, PDH (PDH_a) through their effects on the activities of the interconverting PDH_a kinase and PDH phosphate phosphatase (Randle, 1986). Hence the MgATP-dependent kinase is inhibited by pyruvate and ADP, and activated by increases in the concentration ratios of NADH/NAD^+ and acetyl-CoA/CoA (Randle, 1986). On the other hand, the phosphatase is subject to control by non-metabolites, and is activated by Mg^{2+} and also by Ca^{2+} (Denton *et al.*, 1972), which thus makes PDH one of three key regulatory oxidative dehydrogenases which are exclusively found within the mitochondrial matrix in mammalian tissues and which can be activated by increases in the concentration of Ca^{2+} within the approximate range 0.05–2 μM (Denton & McCormack, 1985; Hansford, 1985; McCormack *et al.*, 1989*a*). The other two Ca^{2+}-sensitive intramitochondrial enzymes are in the citric acid cycle and are the NAD^+-linked isocitrate dehydrogenase (EC 1.1.1.41) and the 2-oxoglutarate dehydrogenase complex (Denton *et al.*, 1978; McCormack & Denton, 1979); Ca^{2+} activates these two enzymes by causing marked decreases in their respective substrate K_m values for *threo*-D_s-isocitrate and 2-oxoglutarate. These other two enzymes can also be activated by decreases in the NADH/NAD^+ and ATP/ADP ratios, but Ca^{2+} can further activate all three of these dehydrogenases while these key ratios are maintained or even increased (see Denton & McCormack, 1985).

There is now good evidence that, in rat heart (McCormack & Denton, 1984) and liver (Assimacopoulos-Jeannet *et al.*, 1986), hormones which act by causing increases in cytosolic Ca^{2+} also, as a result, bring about increases in intramitochondrial Ca^{2+} and hence the activation of these enzymes. Thus the stimulation by Ca^{2+} of energy-utilizing events in the cytosol such as contraction or secretion is balanced by Ca^{2+}-mediated increases in oxidative metabolism and subsequent ATP production in the mitochondrial matrix, and hence energy homoeostasis can be achieved at times of increased demand. Such a mechanism could also explain how increased oxidative phosphorylation could be sustained without decreasing, or even increasing, the key NADH/NAD^+ or ATP/ADP ratios (see Unitt *et al.*, 1989).

Thus, in stimulated islets, either or both stimulated glucose metabolism (e.g. increasing pyruvate) or increased cytosolic Ca^{2+} might be expected to lead to increases in PDH_a. There is indeed already evidence that both acetyl-CoA (Corkey *et al.*, 1989) and NAD(P)H production are enhanced and that ATP/ADP levels are elevated (Matschinsky *et al.*, 1989) during such conditions of increased substrate oxidation. We have therefore now examined the effects of increases in the extracellular concentration of glucose on the amounts of PDH_a in isolated rat pancreatic islets. A small part of the present work has been previously reported briefly as a meeting abstract (McCormack *et al.*, 1989*b*).

MATERIALS AND METHODS

Islets were picked by hand under a dissecting microscope after collagenase digestion of pancreata as described by Lacy & Kostinovsky (1967). Groups of islets (10–100) were then transferred to Petri dishes containing 2 ml of RPMI 1640 (Gibco) tissue-culture medium containing 10 mM-glucose and 10% (v/v) foetal-calf serum (Hyclone), and placed in an incubator (37 °C) under O_2/CO_2 (19:1) for 2–4 days. This procedure allowed stabilization of the islets after isolation so that good and reproducible increases in insulin release were obtained in response to increased extracellular glucose.

Preliminary experiments (results not shown) revealed that 100 islets were suitable for each incubation sample and would enable

Abbreviations used: PDH, the pyruvate dehydrogenase complex; PDH_a, the active, non-phosphorylated, form of PDH.

‡ To whom reprint requests should be addressed.

PDH to be measured adequately. After a preincubation (20–30 min with 3 mM-glucose), groups of 100 islets were therefore incubated in 200 μl of oxygenated (O_2/CO_2, 19:1) Krebs bicarbonate buffer (Krebs & Henseleit, 1932), which contained 1.2 mM-$CaCl_2$ with further additions as indicated, at 37 °C for the times indicated in Figure and Table legends.

After incubation, the islets were harvested by centrifugation (5 s, 6000 *g*), and insulin released during the incubations was assayed in 150 μl of the supernatant by using the Micromedic insulin-radioimmunoassay kit (ICN Micromedic Systems, Horsham, PA, U.S.A.). The pellets were freeze-dried by spinning for 1 h in a Speedvac concentrator and then stored at −70 °C. The pellets were then extracted at 0–4 °C into 50μl of 100 mM-KH_2PO_4 (pH 7.1) containing 2 mM-EDTA, 1 mM-dithiothreitol, 0.1% (v/v) Triton X-100 and also 50 μl of rat serum/ml (McCormack & Denton, 1989), by passage up and down (about 10 times) in a 100 μl micro-syringe before being frozen in liquid N_2.

After thawing, the samples were spun at 10000 *g* for 1 min, and PDH_a activity in the supernatants was assayed at 37 °C in 0.6 ml of buffer containing the appropriate substrates (McCormack & Denton, 1989), by monitoring the production of acetylated *p*-(*p*-aminoazo)benzenesulphonic acid by decreases in the A_{460} as has been fully described previously (McCormack & Denton, 1989); this is dependent on acetyl-CoA production from pyruvate via PDH and the activity of added arylamine acetyltransferase. Total PDH activity was assayed as described above after converting all of the PDH phosphate in the sample into PDH_a with purified (and PDH-free) PDH phosphate phosphatase as described by McCormack & Denton (1989). Glutamine dehydrogenase activity was also assayed as an alternative marker for mitochondria which was easier to assay (Martin *et al.*, 1972). It should be noted that attempts were also made to use the much more sensitive (about 10 times) fluorimetric assay for PDH described by Solomon & Stansbie (1984), which exploits the acetylation of Cresyl Violet acetate by using a similar approach to that described above. However, although qualitatively similar results could be obtained with this technique, great problems were experienced with quantification and reproducibility with this method, and virtually every sample had to be calibrated separately.

A unit of enzyme activity is defined as that which catalyses the conversion of 1 μmol of substrate/min at 37 °C. Statistical significance was assessed by an unpaired Student's *t*-test. All biochemicals and chemicals used were of the highest grade commercially available and were obtained from the sources given above or listed previously (McCormack, 1989).

RESULTS

Fig. 1 (■) shows that the isolated islets exhibited increases in insulin release in response to increases in the extracellular glucose concentration, indicating that this system was viable to assess the effects of extracellular glucose on islet PDH_a content. Fig. 1 (●) shows that increases in extracellular glucose produced clear increases in islet PDH_a content, with half-maximal effects around 5–6 mM-glucose; this dose–response curve thus correlates very well with that for insulin release (Fig. 1).

Fig. 2 (■) shows the time course of increased insulin secretion after an increase in extracellular glucose concentration from 3 mM to 20 mM, and Fig. 2 (●) shows the corresponding PDH_a data; the maximal PDH_a response actually appears to precede the maximal secretion response.

Table 1 summarizes some of these data and also shows that increases in PDH_a of a similar order of magnitude could also be achieved in the presence of 3 mM-glucose by increasing the extracellular concentration of $CaCl_2$ from 1.2 mM to 4.2 mM. In addition, Table 1 also shows that the addition of uncoupler can also activate PDH, presumably in this instance by decreasing the cellular ATP/ADP ratio. Table 1 also shows the corresponding insulin-release data.

The total activity of islet PDH was not changed by any of the treatments used in the present study, and was 401 ± 24 μ-units/

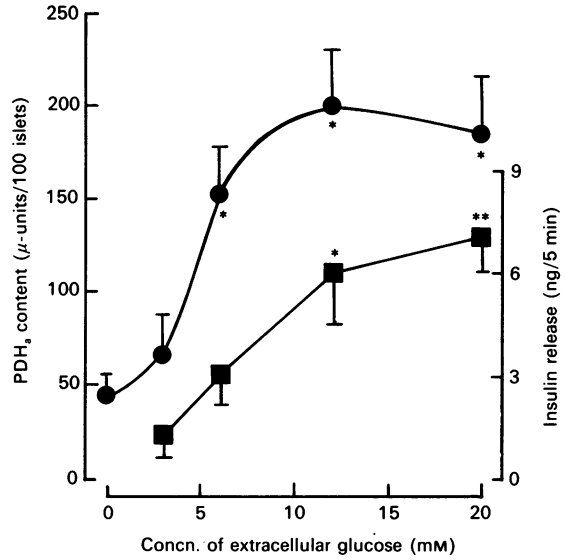

Fig. 1. Effects of increases in the extracellular concentration of glucose on the PDH_a content of, and insulin secretion by, isolated rat pancreatic islets

Groups of 100 islets were incubated for 5 min as described in the Materials and methods section and with the concentrations of glucose shown. Insulin secretion (■) and islet PDH_a content (●) were then measured as also described in the Materials and methods section. Each point and error bar represents the mean ± S.E.M. for four separate groups of 100 islets: $*P \leqslant 0.05$ or $**P \leqslant 0.01$ versus the appropriate control value at 3 mM-glucose.

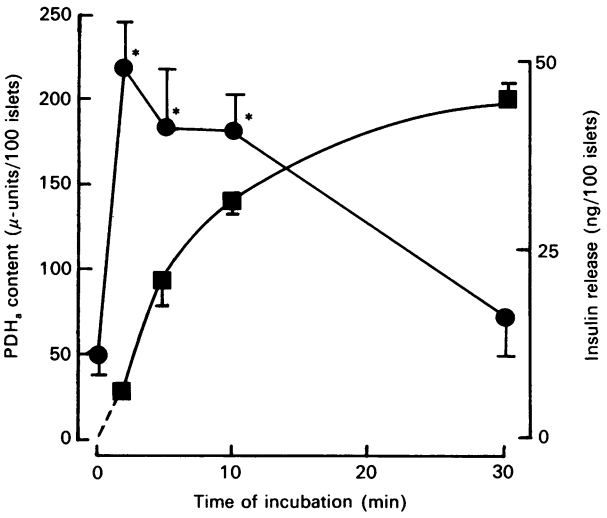

Fig. 2. Time courses for the effects of increasing extracellular glucose from 3 to 20 mM on the PDH_a content of, and insulin secretion by, isolated rat pancreatic islets

Groups of 100 islets were preincubated (20–30 min) in the initial presence of 3 mM-glucose as described in the Materials and methods section, and then the glucose concentration was raised to 20 mM for the times shown. Values are expressed as in Fig. 1, and, for the PDH_a measurements alone, $*P \leqslant 0.001$ versus the 0 min value.

Table 1. Effects of increases in the extracellular concentration of glucose or of Ca^{2+}, and of uncoupler, on islet PDH_a content and insulin release

Islets were incubated for 5 min at 37 °C as described in the Materials and methods section and with further additions to the media as shown. Results are means ± S.E.M. for the numbers of observations in parentheses; $*P \leq 0.01$ versus control. Abbreviation: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

Additions to incubations	PDH_a content (μ-units/100 islets)	Insulin release (ng/100 islets)
3 mM-glucose (control)	50 ± 13 (8)	4.9 ± 1.3 (4)
20 mM-glucose	166 ± 25* (8)	24.2 ± 0.1* (4)
3 mM-glucose plus an extra 3 mM-$CaCl_2$	154 ± 32* (4)	13.1 ± 1.3* (4)
2 μM-FCCP (no substrate added)	143 ± 28* (4)	1.8 ± 1.2 (4)

100 islets (mean ± S.E.M. for 16 observations). The activity of glutamate dehydrogenase was also not affected, and was 10.4 ± 0.7 (16) m-units/100 islets; this enzyme would be a useful and easier-to-assay alternative to total PDH measurement for quantifying changes in PDH_a with respect to mitochondrial content (see Martin *et al.*, 1972).

DISCUSSION

Note that for this discussion we have assumed that all of the reported changes occurred in the β-cells of the islets; this is based on the fact that they constitute most the islets and respond to glucose in a stimulatory manner (see, e.g., Hedeskov, 1980; Meglasson & Matschinsky, 1986; Prentki & Matschinsky, 1987).

The only previous measurement of PDH activity in pancreatic islets to our knowledge is that by Paxton *et al.* (1988), also on the rat. These authors reported a value of 5.09 pmol/min per islet (at 37 °C) for total PDH activity (equivalent to about 0.5 m-unit/100 islets), which agrees with the present estimate of around 0.4 m-unit/100 islets. By using the conversion parameters listed by Paxton *et al.* (1988), this latter value would correspond to approx. 1.1 units/g wet wt. of islet tissue, or 28.5 m-units/mg of mitochondrial protein. The analogous values for heart (e.g. McCormack & Denton, 1984) or liver (e.g. Assimacopoulos-Jeannet *et al.*, 1986) would respectively be about 5 or 2 units/g wet wt. and about 120 or 50 m-units/mg of mitochondrial protein. Therefore the values for the islet are perhaps rather lower than expected, given that the β-cells have among the highest mitochondrial content of all mammalian tissues (e.g. Montague, 1983).

The earlier work by Paxton *et al.* (1988) reported that almost all of the PDH existed in the active form (94%). In the present work, however, under control incubation conditions (3 mM-glucose, 1.2 mM-$CaCl_2$), only approx. 16% of the enzyme was in the active form. Moreover, this was acutely raised to about 50% in the presence of a maximally stimulating glucose concentration (12 or 20 mM). The concentration-dependency of this activation was thus similar to that for the insulin-secretory function of the tissue, and the time course for the activation actually preceded the insulin response (Fig. 2). It is therefore tempting to speculate that this enzyme activation, given the importance of PDH in carbohydrate metabolism (see the Introduction), may be an important part of the response of the β-cell to increased extracellular glucose, both in terms of maintaining energy homoeostasis (as discussed in the Introduction), and perhaps also as a part of the secretory signalling mechanism itself. Such possibilities clearly warrant further study of this phenomenon.

Pyruvate dehydrogenase can be activated by increases in Ca^{2+} or pyruvate concentration, or by decreases in the concentration ratios of ATP/ADP or NADH/NAD^+ or acetyl-CoA/CoA (Denton *et al.*, 1975). It is likely that, as in other mammalian tissues (Denton *et al.*, 1975; Randle, 1986), increases in pyruvate concentration would lead to increases in PDH_a in pancreatic islets. The effects of increased glucose concentration on pyruvate and lactate production by islets are as yet unclear [see Meglasson & Matschinsky (1986) for a full discussion of this].

However, it is clear that the oxidation of glucose in islets occurs primarily in the tricarboxylic acid cycle (Meglasson & Matschinsky, 1986). We have argued previously that the regulation of this enzyme and the other two key dehydrogenases (see the Introduction) by Ca^{2+} allows a means whereby external agents such as hormones, or in this case increased glucose, could increase oxidative flux, and hence ATP production, without the need to decrease the above key concentration ratios (see, e.g., Denton & McCormack, 1985; Corkey *et al.*, 1988*a*,*b*). Moreover, this would occur at times of increased ATP utilization, as increased cytosolic Ca^{2+} generally stimulates energy-requiring events in this cell compartment such as secretion in the islet (Denton & McCormack, 1985). Certainly, in the islet there is evidence that increased extracellular glucose causes sustained increases in ATP/ADP and NAD(P)H/$NAD(P)^+$ (e.g. Prentki & Matschinsky, 1987; Matschinsky *et al.*, 1989) and acetyl-CoA/CoA (Corkey *et al.*, 1989) ratios. This could be consistent with either the Ca^{2+} and/or pyruvate hypotheses. However, the succinyl-CoA/CoA ratio also rises, which would be consistent with a concomitant activation of 2-oxoglutarate dehydrogenase (Corkey *et al.*, 1989); this latter observation would be more difficult to reconcile with a mechanism based on pyruvate only.

Increased glucose has been shown directly to cause increases in cytosolic Ca^{2+} concentration in isolated islet cells (Rorsman *et al.*, 1984; Rorsman & Abrahamsson, 1985). It is also known that increased extracellular Ca^{2+} can promote secretion in a solely Ca^{2+}-dependent manner (e.g. Hellman & Gylfe, 1986), and the data in Table 1 of the present paper indicate that increasing extracellular Ca^{2+} activates PDH; however, this Table also shows that depleting ATP by uncoupler could also activate the enzyme (but did not promote secretion) and, as noted above, increased pyruvate would also lead to activation. At present it is thought that the glucose-induced increase in cytosolic Ca^{2+} arises from inhibition of plasma-membrane ATP-sensitive K^+ channels by a metabolism-derived increase in ATP/ADP ratio and then subsequent opening of voltage-dependent Ca^{2+} channels after depolarization (e.g. Ashcroft, 1988); this would clearly be at variance with a Ca^{2+}-dependent activation of PDH_a, which would be a consequence rather than a cause of the elevated cytosolic Ca^{2+}. However, Corkey *et al.* (1988*a*) have argued that a transient fall in ATP/ADP owing to initial glucose phosphorylation may allow internal Ca^{2+} to rise by depressing the activity of membrane Ca^{2+}-ATPases. Such a transient fall in ATP/ADP could also, of course, play a role in activating PDH (see Unitt *et al.*, 1989). Clearly it remains an intriguing question as to whether the activation of PDH is a consequence of, or a cause of, the increases in cytosolic Ca^{2+} and/or of enhanced glucose metabolism; further study is required to explain this interesting and potentially very important observation.

We thank the M.R.C. and Lister Institute (U.K.) and the N.I.H. (ROI-DK 35914) and Upjohn Co. (U.S.A.) for financial support, and Professor R. M. Denton (University of Bristol) and Dr. M. Prentki (University of Geneva) for much helpful advice and discussion. J.G.McC. is a Lister Institute Research Fellow.

REFERENCES

Ashcroft, F. M. (1988) Annu. Rev. Neurosci. **11**, 97–118

Assimacopoulos-Jeannet, F., McCormack, J. G. & Jeanrenaud, B. (1986) J. Biol. Chem. **261**, 8799–8804

Corkey, B. E., Deeney, J. T., Glennon, M. C., Matschinsky, F. M. & Prentki, M. (1988*a*) J. Biol. Chem. **263**, 4247–4253

Corkey, B. E., Tornheim, K., Deeney, J. T., Glennon, M. C., Parker, J. C., Matschinsky, F. M., Ruderman, N. B. & Prentki, M. (1988*b*) J. Biol. Chem. **263**, 4254–4258

Corkey, B. E., Glennon, M. C., Chen, K., Deeney, J. T., Matschinsky, F. M. & Prentki, M. (1989) J. Biol. Chem. **264**, 21608–21612

Denton, R. M. & McCormack, J. G. (1985) Am. J. Physiol. **249**, E543–E554

Denton, R. M., Randle, P. J. & Martin, B. R. (1972) Biochem. J. **128**, 161–163

Denton, R. M., Randle, P. J., Bridges, B. J., Cooper, R. H., Kerbey, A. L., Pask, H. T., Severson, D. L., Stansbie, D. & Whitehouse, S. (1975) Mol. Cell. Biochem. **9**, 27–53

Denton, R. M., Richards, D. A. & Chin, J. G. (1978) Biochem. J. **176**, 899–906

Hansford, R. G. (1985) Rev. Physiol. Biochem. Pharmacol. **103**, 1–72

Hedeskov, C. J. (1980) Physiol. Rev. **16**, 442–509

Hellman, B. & Gylfe, E. (1986) in Calcium and Cell Function, vol. 6 (Cheung, W. Y., ed.), pp. 253–326, Academic Press, New York

Krebs, H. A. & Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. **210**, 33–66

Lacy, P. E. & Kostinovsky, M. (1967) Diabetes **16**, 35–39

Martin, B. R., Denton, R. M., Pask, H. T. & Randle, P. J. (1972) Biochem. J. **129**, 763–773

Matschinsky, F. M., Corkey, B. E., Prentki, M., Meglasson, M. D., Erecinska, M., Shimizu, T., Ghosh, A. & Parker, J. (1989) in Diabetes 1988 (Larkins, R., Zimmet, P. & Chisholm, D., eds.), pp. 17–26, Elsevier, Amsterdam

McCormack, J. G. (1989) Biochem. J. **264**, 167–174

McCormack, J. G. & Denton, R. M. (1979) Biochem. J. **180**, 533–544

McCormack, J. G. & Denton, R. M. (1984) Biochem. J. **218**, 235–247

McCormack, J. G. & Denton, R. M. (1989) Methods Enzymol. **174**, 95–118

McCormack, J. G., Browne, H. M. & Dawes, N. J. (1989*a*) Biochim. Biophys. Acta **973**, 420–427

McCormack, J. G., Longo, E. A. & Corkey, B. E. (1989*b*) Diabetologia **32**, p516A

Meglasson, M. D. & Matschinsky, F. M. (1986) Diabetes Metab. Rev. **2**, 163–214

Montague, W. (1983) Diabetes and the Endocrine Pancreas: A Biochemical Approach, Croom Helm, London

Paxton, R., Harris, R. A., Sener, A. & Malaisse, W. J. (1988) Horm. Metab. Res. **20**, 317–322

Prentki, M. & Matschinsky, F. M. (1987) Physiol. Rev. **67**, 1185–1248

Randle, P. J. (1986) Biochem. Soc. Trans. **14**, 799–806

Rorsman, P. & Abrahamsson, H. (1985) Acta Physiol. Scand. **125**, 639–647

Rorsman, P., Abrahamsson, H., Gylfe, E. & Hellman, B. (1984) FEBS Lett. **170**, 196–200

Solomon, M. & Stansbie, D. (1984) Anal. Biochem. **141**, 337–343

Sugden, M. C., Holness, M. J. & Palmer, T. N. (1989) Biochem. J. **263**, 313–323

Unitt, J. F., McCormack, J. G., Reid, D., MacLachlan, L. K. & England, P. J. (1989) Biochem. J. **262**, 293–301

Received 17 October 1989/15 January 1990; accepted 20 February 1990