Increase in CO_3H^- influx and cellular pH in glucose-stimulated pancreatic islets

Michel Deleers, Philippe Lebrun and Willy J. Malaisse

Laboratories of Experimental Medicine and Pharmacology, Brussels Free University, Brussels, B-1000, Belgium

Received 17 February 1983

When rat islets are preincubated with fluorescein diacetate and examined in a spectrofluorometer, the intracellular pH rapidly increases by 0.21 pH units in response to a rise from 1.7 – 16.8 mM glucose. This coincides with a marked increase in $^{14}CO_3H^-$ net uptake by the islets, suggesting that the glucose-induced increase in H^+ generation rate is compensated for by stimulation of CO_3H^-/Cl^- exchange.

Pancreatic islet *Intracellular pH* *Bicarbonate*

1. INTRODUCTION

The release of insulin evoked by glucose and other nutrient secretagogues tightly depends on the capacity of these agents to augment catabolic and oxidative fluxes in pancreatic islet cells [1]. The coupling of nutrient metabolism to more distal events in the secretory sequence, such as the remodelling of ionic fluxes across the plasma membrane, is thought to represent a multifactorial process [2] including changes in the generation rate of reducing equivalents (NAD(P)H), high-energy phosphate intermediates (ATP) and protons (H^+). The participation of protons in the coupling process is supported by the finding that glucose increases the net output of H^+ from the islets [2], and the knowledge that ionic fluxes, as well as the bioelectrical and secretory activity of the islet cells, are affected by changes in extracellular or intracellular pH [3–9]. The increased generation of H^+, resulting from the metabolism of glucose, may participate to the nutrient-induced decrease in both K^+ and Ca^{2+} outflow from the islet cells [10]. For instance, H^+ may compete with Ca^{2+} for an ionophoretic process of Na^+/Ca^{2+} or Na^+/H^+ countertransport [11]. It is indeed currently believed that the extrusion of Ca^{2+} from the islet cells is mediated, for its major part, by Na^+/Ca^{2+} countertransport and that the glucose-induced inhibition of Ca^{2+} outflow is attributable to inhibition of such a countertransport process [12].

No direct evidence is so far available, however, to support the view that glucose lowers the cytoscolic pH as apparently implied by these concepts. On the contrary, when the intracellular pH was measured in islets exposed to 4,4-dimethyloxazolidine-2,4-dione, a trend towards an apparent increase in cellular pH was usually observed at high glucose concentrations [2,9]. This suggests that the nutrient-induced increase in H^+ generation is efficiently compensated, e.g., by stimulation of either Na^+/H^+ countertransport or a process of exchange between influent CO_3H^- and effluent Cl^- coupled to the intracellular conversion of CO_3H^- and H^+ to CO_2 and H_2O [13]. The postulated participation of such a process of CO_3H^-/Cl^- exchange to the extrusion of protons is compatible with the finding that glucose augments the outflow of Cl^- from the islets [14]. The present work provides direct evidence that glucose stimulates CO_3H^- inflow and increases intracellular pH in pancreatic islets.

2. MATERIALS AND METHODS

All experiments were performed with pancreatic islets removed from albino rats [15].

For measuring $^{14}CO_3H^-$ uptake, groups of 10

Published by Elsevier Science Publishers
00145793/83/0000–0000/$3.00

islets each were preincubated for 30 min at 37°C in 50 μl of a salt-balanced medium (Na^+ 139, K^+ 5, Ca^{2+} 0 or 1, Mg^{2+} 0 or 1, Cl^- 120–124 mM and HCO_3^- 24 mM) buffered with Hepes–NaOH (10 mM; pH 7.3), containing bovine albumin (5 mg/ml), glucose (1.7 mM) and sucrose (1 mM), equilibrated against a mixture of CO_2 (5%) and O_2 (95%), and layered in a microcentrifuge tube on 175 μl of oil (Versilube, F50, General Electric). An aliquot (50 μl) of the same medium enriched with $^{14}CO_3HNa$ (25 μCi/ml), [6,6′(*n*)-^{3}H]sucrose (17 μCi/ml), and, when required, glucose was then added to the preincubation medium. After incubation for zero to 15 min at 37°C, the islets were pelleted at the bottom of the microcentrifuge tube (45 s, 13000 × *g*). There was a delay of 60–90 s between the time at which the radioactive medium was added to each tube (time zero of incubation) and the time at which the islets were centrifuged into the layer of oil. The tip of each tube was removed with a scalpel and examined for its radioactive content by liquid scintillation. The apparent space of distribution of $^{14}CO_3H^-$ in excess of the [^{3}H]-sucrose space was expressed as nl/islet taking into account the radioactivity of the incubation medium at time zero. In these experiments, the $^{14}CO_3H^-$ content of the incubation medium decreased with time. Over 0–15 min incubation, the [^{3}H]sucrose space of distribution averaged 0.76 ± 0.03 nl/islet (n = 175).

For measuring intracellular pH, groups of 10 large islets each were preincubated for 30 min at 37°C in 1.0 ml Hanks solution containing bovine albumin (2 mg/ml), glucose (1.7 mM) and fluorescein diacetate (50 μM) and equilibrated against CO_2 and O_2 (5/95, v/v), the islets were then washed twice with 1.0 ml of our usual bicarbonate-buffered medium [16], which contained bovine albumin (5 mg/ml), glucose (1.7 mM), penicillin (0.15 mg/ml) and streptomycin (0.13 mg/ml). One or two large islets were then placed on a platinum ring in a quartz cuvette containing 2 ml of the same bicarbonate-buffered medium. The quartz cuvette was placed in an Aminco SPF500 spectrofluorometer (American Instrument Co., Urbane IL). After 10 min incubation at the low glucose concentration, an aliquot (0.2 ml) of the same medium was added to the cuvette to either increase [glucose] from 1.7–16.7 mM or maintain low [glucose]. At 2 min intervals, the fluorescence intensity was recorded at 520 nm after excitation at 490 and 435 nm, respectively [17]. The changes in pH were judged by reference to a calibration curve established in a citrate–phosphate buffer [17]. If the absolute pH were to be judged by reference to the same calibration curve, the initial apparent pH of the islet cells (time zero of incubation) would be close to 6.3.

All results are presented as the mean (± SEM) together with the number of individual determinations (n).

3. RESULTS

The apparent distribution space of $^{14}CO_3H^-$ was higher in the presence of both Ca^{2+} and Mg^{2+} than

Table 1

$^{14}CO_3H^-$ distribution space in pancreatic islets

Ca^{2+} (mM)	Mg^{2+} (mM)	Incubation (min)	$^{14}CO_3H^-$ distribution space (ml/islet)	
			Glucose 1.7 mM	Glucose 16.7 mM
1.0	1.0	10	12.10 ± 2.22 (10)	34.09 ± 4.17 (10)
–	1.0	10	4.28 ± 1.30 (10)	8.93 ± 1.57 (10)
1.0	–	10	5.16 ± 1.33 (10)	14.40 ± 3.08 (10)
–	–	10	1.14 ± 0.24 (10)	4.32 ± 2.12 (10)
–	–	0	0.69 ± 0.22 (30)	1.73 ± 0.26 (47)
–	–	3	1.74 ± 0.50 (29)	3.24 ± 0.58 (43)
–	–	6	1.68 ± 0.52 (30)	2.48 ± 0.76 (39)
–	–	10	2.07 ± 0.40 (88)	4.77 ± 0.72 (107)
–	–	15	2.52 ± 0.66 (29)	5.59 ± 1.42 (46)

in the sole presence of only one of these divalent cations and, even more so, than in the absence of both Ca^{2+} and Mg^{2+} (table 1). Under the latter experimental conditions, the apparent distribution space of $^{14}CO_3H^-$ increased with the length of incubation, whether at low or high glucose concentration. Glucose (16.7 mM) increased the net uptake of $^{14}CO_3H^-$. Such an effect of glucose was observed both in the presence and absence of Ca^{2+} and/or Mg^{2+}. It was already detected after the shortest time of exposure to high concentration of glucose (time 'zero' of incubation; i.e., after <60–90 s exposure to the high [sugar]). As judged from the data summarized in table 1, a rise in [glucose] from 1.7–16.7 mM increased $^{14}CO_3H^-$ net uptake by 124 ± 23% (degree of freedom, 549; $P< 0.001$) above the mean paired basal value. Fig. 1 illustrates the dose–action relationship for the effect of glucose upon $^{14}CO_3H^-$ net uptake.

The ratio in fluorescence intensity of islets preincubated with fluorescein diacetate and incubated at low [glucose] (1.7 mM), as taken at 520 nm after excitation at 435 nm and 490 nm ($I_{490/435}$), increased progressively during incubation, tending towards an asymptotic value (fig. 2). A rise in [glucose] from 1.7–16.7 mM caused a rapid and sustained increase in the fluorescence ratio, suggesting an increase in intracellular pH. After 7–9 min exposure to the high concentration of glucose, the glucose-induced increment in fluorescence ratio corresponded to an apparent mean increase in pH of 0.21.

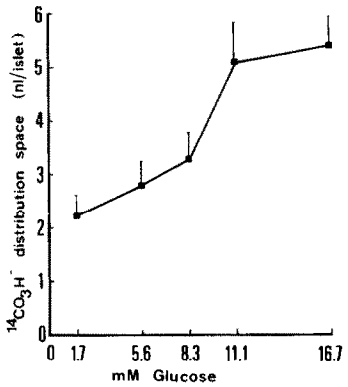

Fig. 1. Effect of increasing concentrations of glucose upon the apparent distribution space of $^{14}CO_3H^-$ in pancreatic islets incubated for 10 min in the presence of $^{14}CO_3H^-$ but absence of Ca^{2+} and Mg^{2+}. Mean values (± SEM) refer to 44–45 individual determinations.

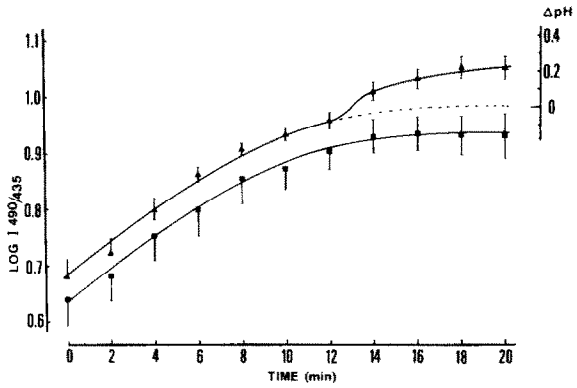

Fig. 2. Time course for the changes in the ratio of fluoroscence intensity in islets prelabelled with fluorescein diacetate, as taken at 520 nm after excitation at 435 and 490 nm, respectively: (■—■) islets exposed throughout to a medium containing 1.7 mM glucose; (▲—▲) [glucose] was raised from 1.7–16.7 mM at 11 min incubation. Mean values (± SEM) refer to 9–11 individual expt. In the right scale, the change in cellular pH (ΔpH) was estimated by reference to a standard curve established in a citrate–phosphate buffer.

4. DISCUSSION

The apparent distribution space of $^{14}CO_3H^-$ in islets incubated at normal extracellular [Mg^{2+}] and [Ca^{2+}] largely exceeded the intracellular water space ($\leqslant 3$ nl, see [18]). The apparent distribution space of $^{14}CO_3H^-$ was decreased when Mg^{2+} and/or Ca^{2+} were removed from the incubation medium, suggesting that the deposition of the carbonate salts of these divalent cations accounted in part for the high distribution space of $^{14}CO_3H^-$. Glucose caused a rapid, dose-related and sustained increase in $^{14}CO_3H^-$ net uptake. The time course for the response to glucose suggests that such an increase corresponds, in part at least, to stimulation of $^{14}CO_3H^-$ entry into the islet cells. Therefore, our results support the concept that glucose stimulates a process of CO_3H^-/Cl^- exchange. Such a stimulation may participate, possibly in association with other modalities of H^+ extrusion and/or buffering, to a glucose-induced increase in intracellular pH.

The experiments performed with islets preincubated in the presence of fluorescein diacetate in-

deed suggest that glucose increases the pH of the islet cells. Our measurements do not inform, however, on a possible heterogeneity in the pH of distinct subcellular compartments and in their response to glucose stimulation. The progressive increase in fluorescence ratio seen in the control performed throughout at low [glucose] (fig. 2) could be due, inter alia, to a leakage of fluorescein from the islet cells into the alkaline incubation medium. If glucose were to affect the latter process, the apparent increase in intracellular pH seen in response to a rise in glucose concentration could conceivably be attributed to an accelerated release of fluorescein from the islet cells. To the extent that our measurements reflect a true increase in intracellular pH, they suggest that the increase in H^+ production rate associated with stimulation of glucose metabolism is not solely compensated for but actually overcome by other mechanisms controlling the content and extrusion of protons from the islet cells.

An increase in intracellular pH is not an uncommon feature of cell activation [19,20]. Although the present findings may impose a reevaluation of the precise role of H^+ as a coupling factor between metabolic and ionic events in glucose-stimulated islets, they remain compatible with the view that a remodelling of H^+ fluxes is involved in the stimulus–secretion coupling for glucose-induced insulin release.

ACKNOWLEDGEMENTS

This work was supported in part by grants from the Belgian Foundation for Scientific Medical Research and Belgian Ministry of Scientific Policy. The authors are grateful to M. Mahy for technical assistance and C. Demesmaeker for secretarial help. P.L. is a Research Fellow of the Belgian National Foundation for Scientific Research.

REFERENCES

[1] Malaisse, W.J., Sener, A., Herchuelz, A. and Hutton, J.C. (1979) Metabolism 28, 373–386.
[2] Malaisse, W.J., Hutton, J.C., Kawazu, S., Herchuelz, A., Valverde, I. and Sener, A. (1979) Diabetologia 16, 331–341.
[3] Malaisse, W.J., Malaisse-Lagae, F. and Brisson, G. (1971) Horm. Metab. Res. 3, 65–70.
[4] Carpinelli, A.R., Sener, A., Herchuelz, A. and Malaisse, W.J. (1980) Metabolism 29, 540–546.
[5] Hutton, J.C., Sener, A., Herchuelz, A., Valverde, I., Boschero, A.C. and Malaisse, W.J. (1980) Horm. Metab. Res. 12, 294–299.
[6] Henquin, J.C. (1981) Mol. Cell. Endocrinol. 21, 119–138.
[7] Tarvin, J.T., Sachs, G. and Pace, C.S. (1981) Am. J. Physiol. 240, C127–C134.
[8] Smith, J.S. and Pace, C.S. (1983) Diabetes 32, 61–66.
[9] Lebrun, P., Malaisse, W.J. and Herchuelz, A. (1982) Biochim. Biophys. Acta 72, 357–365.
[10] Malaisse, W.J., Herchuelz, A. and Sener, A. (1980) Life Sci. 26, 1367–1371.
[11] Anjaneyulu, K., Anjaneyulu, R. and Malaisse, W.J. (1980) J. Inorg. Biochem. 13, 178–188.
[12] Herchuelz, A., Sener, A. and Malaisse, W.J. (1980) J. Membr. Biol. 57, 1–12.
[13] Roos, A. and Boron, W.F. (1981) Physiol. Rev. 61, 296–434.
[14] Sehlin, J. (1978) Am. J. Physiol. 235, E501–E508.
[15] Lacy, P.E. and Kostianovsky, M. (1967) Diabetes 16, 35–39.
[16] Malaisse, W.J., Brisson, G. and Malaisse-Lagae, F. (1970) J. Lab. Clin. Med. 76, 895–902.
[17] Slavik, J. (1982) FEBS Lett. 140, 22–26.
[18] Malaisse, W.J., Boschero, A.C., Kawazu, S. and Hutton, J.C. (1978) Pflügers Arch. 373, 237–242.
[19] Nuccitelli, R. and Heiple, J.M. (1982) in: Intracellular pH: Its measurement, regulation and utilization in cellular functions (Nuccitelli, R. and Deamer, D. eds) pp. 567–586, Alan R. Liss, New York.
[20] Gerson, D.F., Kiefer, M. and Eufe, W. (1982) Science 216, 1009–1010.