Effects of glucose on insulin release and ^{86}Rb permeability in cultured neonatal and adult rat islets

A. Carlos Boschero, Donatella Tombaccini and Illani Atwater

Laboratory of Cell Biology and Genetics, NIDDK, Bldg 8/Rm 403, National Institutes of Health, Bethesda, MD 20892, USA

Glucose-induced insulin release and modifications in ^{86}Rb outflow were studied in cultured neonatal and adult rat islets. The dose-response curve for neonatal islets was steeper than for adult islets and the maximal response was clearly shifted towards lower glucose concentrations. In neonatal islets, glucose-induced insulin release was inhibited by the Ca^{2+}-channel blocker, nifedipine. In the absence of glucose, the ^{86}Rb outflow from neonatal islets was lower than from adult islets. Also, the glucose-induced reduction in ^{86}Rb outflow was less pronounced in neonatal islets. Altered K^+ permeability in the B-cell membrane could explain the change in glucose sensitivity of neonatal islets.

Insulin release; ^{86}Rb$^+$ efflux; K^+ permeability; Glucose; (B-cell, Islets of Langerhans, Rat)

1. INTRODUCTION

Insulin secretion in response to glucose has consistently been reported to be lower in fetal and neonatal islets than in adult islets [1–5]. Similarly, other substrates which must be metabolized to induce insulin release, like glyceraldehyde and leucine, also show a reduced response [2,4,5]. Since theophylline strongly potentiates glucose-induced insulin secretion, the mechanism of exocytosis seems to be operative in these immature islets [4,5]. Furthermore, the range of glucose concentrations over which the islets respond (glucose sensitivity) has been reported to be narrowed in neonatal islets [2,3]. The nature of these abnormalities is not yet fully elucidated. Metabolic 'defects' have been proposed as the underlying disorder [2,6], however, the stimulus coupling between metabolism of the fuels and the K^+ channels may also be affected [7]. Here, we have compared the glucose responsiveness of neonatal and adult rat islets cultured under the same conditions. The

Correspondence address: A.-C. Boschero, Laboratory of Cell Biology and Genetics, NIDDK, Bldg 8/Rm 403, National Institutes of Health, Bethesda, MD 20892, USA

range of glucose concentrations to which the neonatal islets respond is reduced. Also, we have observed that ^{86}Rb efflux from neonatal islets in the absence of glucose is lower than in adult islets and less affected by glucose.

2. MATERIALS AND METHODS

2.1. Animals
Wistar rats were obtained from the NIH animal facility. Neonatal rats were less than 36 h old and adult rats were 3 months old. Islets were obtained as described from neonatal rats [8,9] and adult rats [10].

2.2. Tissue culture
The medium for culture was composed of EMEM (Biofluids, Rockville, MD) with 10% fetal calf serum (Biofluids). The explant fragments from 10–12 neonatal animals were distributed in 4 plastic 100-mm culture dishes (Falcon 3003) with 10 ml culture medium and maintained at 37°C in an atmosphere of 5% CO_2. After 24 h the medium was changed and the fragments transferred to new culture dishes. Culture dishes and medium were renewed every 48 h. The islets obtained from adult rats were maintained in identical medium. The medium was changed every 48 h. Neonatal and adult islets were kept in culture for 7–9 days before use.

2.3. Experimental protocol
To measure insulin release, groups of neonatal islets were transferred to 10-mm plastic dishes 2 days before the experi-

ment. The culture medium was then discarded and the islets were washed with a bicarbonate-buffered solution containing (mM): 115 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 24 $NaHCO_3$, supplemented with 3 mg/ml of bovine serum albumin (Armour Pharmaceuticals, Eastbourne, England), preincubated for 60 min in identical medium and finally incubated in 1 ml for another period of 60 min at 37°C and equilibrated with a mixture of 95% O_2/5% CO_2. At the end of the incubation period, an aliquot of the medium was drawn for insulin measurements and the insulin content of the islets was extracted with 2 ml acid-ethanol. The same procedure was followed for adult islets, with the exception that the experiments were done with groups of 5 islets each, transferred to the incubation vials immediately before use. Insulin release during incubation for 60 min was expressed as a percentage of the islet insulin content at the end of incubation.

The method used to measure ^{86}Rb efflux and insulin release from perifused islets has been described [11,12]. In this case, the insulin was expressed as pg/islet per min, ^{86}Rb efflux being expressed as fractional outflow rate (% of instantaneous islet content per min).

Nifedipine was dissolved in dimethyl sulfoxide. The same amount of solvent was present in the control media (1 μl/ml). Immunoreactive insulin from the incubation media, perifused samples and extracts was measured as in [13] using pork insulin as a standard. All results are expressed as means ± SE together with the number of individual experiments (n). The statistical significance of differences between mean experimental and control data was assessed by Student's t-test.

3. RESULTS

Fig.1 illustrates the stimulatory effects of glucose, theophylline and glyceraldehyde on neonatal rat islets kept in culture for 9 days. The basal insulin release measured during 1 h of incubation in the absence of glucose averaged 0.54% of the total insulin content of the islets. Glucose (16.7 mM) and glyceraldehyde (10 mM) stimulated insulin release 2.1-fold ($p < 0.05$, in both cases). Glucose (5.6 mM) did not significantly increase secretion over basal value. However, addition of 10 mM theophylline in the presence of 5.6 mM glucose increased insulin release 3.0-fold over basal ($p < 0.01$).

The insulin response to various concentrations of glucose of neonatal and adult islets is compared in fig.2. Both types of islets show a sigmoidal response to glucose. The threshold for glucose-stimulated release was shifted slightly towards higher concentrations of glucose for the adult islets. Peak release occurred at a lower concentration of glucose in the neonatal than in the adult islets, namely at 11.1 and 16.7 mM, respectively. It is clear that the dose-response curve for neonatal
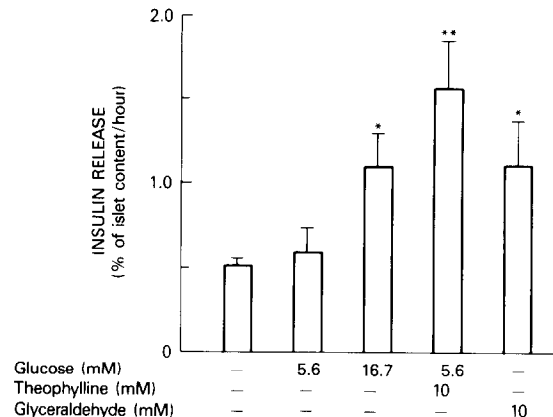
Fig.1. Effects of glucose, theophylline and glyceraldehyde on insulin release from neonatal rat islets. After 7–9 days in culture, the islets were incubated for 1 h in a medium containing the indicated concentration of glucose, theophylline or glyceraldehyde. Values are means ± SE for 5–11 batches of islets. Statistical differences are related to basal values measured in the absence of glucose (* $p < 0.05$ and ** $p < 0.01$).

islets is steeper than for adult islets, the half-maximal response corresponding to about 7.3 and 11.9 mM for neonatal and adult islets, respectively. In both cases, peak release corresponded to just over 1% per h of the total insulin content.
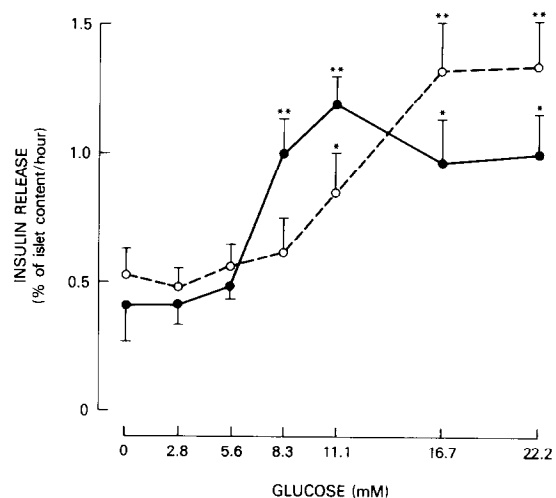
Fig.2. Insulin release induced by various concentrations of glucose in neonatal (●——●) and adult (○---○) rat islets. Values are means ± SE for 5–17 batches of islets incubated for 1 h. Statistical differences are related to the respective basal value at 2.8 mM glucose (* $p < 0.05$ and ** $p < 0.01$).
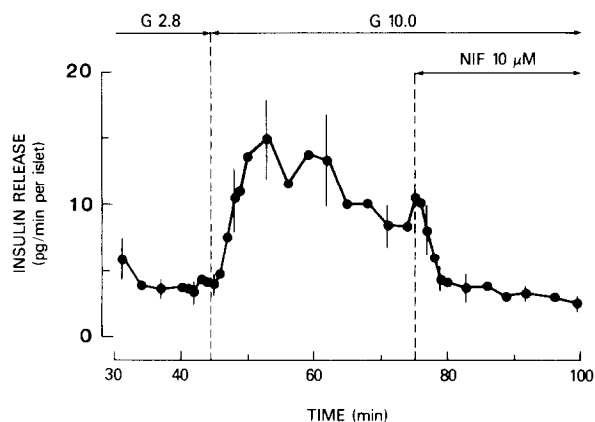
Fig.3. Effect of glucose and nifedipine on insulin release from neonatal rat islets. The perifusate contained 2.8 mM glucose (G) from the onset of perifusion until min 44. Glucose was increased to 10 mM at min 45 and 10 μM nifedipine (NIF) was added at min 75 in the continued presence of 10 mM glucose, as indicated over the figure. Batches of 200–250 islets were perifused in each experiment; values are means ± SE of 4 experiments.
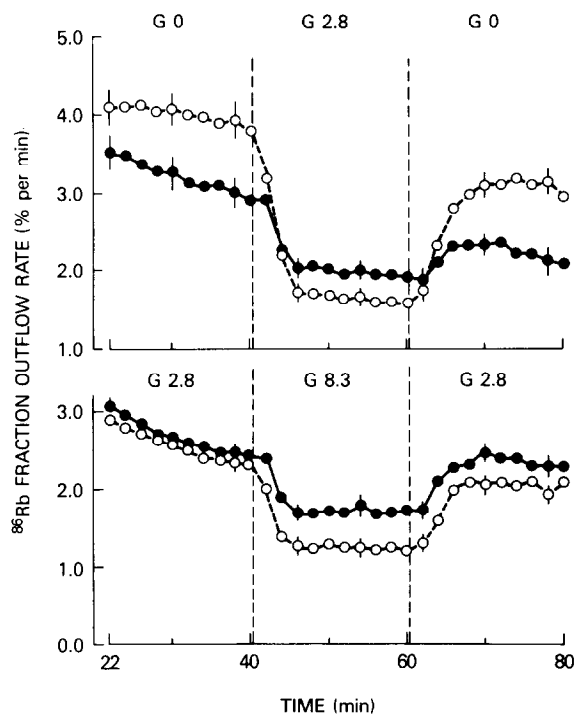

Fig.4. Effect of glucose at 2.8 mM (A) and 8.3 mM (B) on ^{86}Rb fractional outflow rate from neonatal (●) and adult (○) rat islets. Values are means ± SE of 4 experiments.

Insulin release measured in perifused neonatal islets is illustrated in fig.3. An increase in glucose concentration from 2.8 to 10 mM rapidly augmented the secretion of insulin, peak secretion being reached within 9 min and then slowly decreasing with time. The mean insulin release during the control period in the presence of 2.8 mM glucose (min 31–44) averaged 4.15 ± 0.7 pg/islet per min (n = 4). During exposure to 10 mM glucose (min 45–75), insulin secretion averaged 10.2 ± 1.4 pg/islet per min (n = 4) (p < 0.01), representing an increase of about 2.5-fold over basal. Nifedipine inhibited the glucose-induced insulin release; the mean value (min 76–100) was 4.65 ± 0.8 pg/islet per min (n = 4), not significantly different from the control period (p > 0.3). Preliminary experiments indicate that another Ca^{2+}-channel blocker, ω-conotoxin, did not inhibit insulin release from neonatal islets (not shown).

Fig.4 shows the effect of different glucose concentrations on ^{86}Rb efflux from perifused neonatal and adult islets. In the absence of glucose, the ^{86}Rb efflux is lower than from adult islets (fig.4A). Mean values (min 30–40) averaged 3.06 ± 0.05 and 3.93 ± 0.06% per min, respectively (n = 4) (p < 0.025). The addition of 2.8 mM glucose reduced the ^{86}Rb efflux 36% from neonatal and 58% from adult islets; average values (min 50–60) were 1.97 ± 0.02% per min (p < 0.01) and 1.65 ± 0.02% per min (p < 0.001), respectively.

Fig.4B shows that increasing glucose concentration from 2.8 to 8.3 mM further inhibits ^{86}Rb efflux. The inhibition is greater in adult than in neonatal islets. The mean values (min 50–60) averaged 1.73 ± 0.01 and 1.25 ± 0.02 (n = 4) % per min for neonatal and adult islets, respectively.

4. DISCUSSION

The present results indicate that neonatal rat islets, maintained in culture for 7–9 days, respond to glucose, glyceraldehyde and theophylline (fig.1). Basal insulin release (60 min) corresponds to approx. 0.5% of the total insulin content in the islets. Maximum release, induced by glucose, is about 2.5-times the basal value, comparable to that obtained by other investigators using fetal [5,7] or neonatal rat islets [2,7]. The glucose-induced insulin release is inhibited by nifedipine in neonatal islets (fig.3), as in adult islets [14], in-

dicating that these islets contain dihydropyridine-sensitive Ca^{2+} channels and that glucose-induced insulin secretion is dependent on Ca^{2+} entry. This is in contrast to recent observations in fetal rat islets (21 days), showing that glucose failed to increase $^{45}Ca^{2+}$ uptake [1] and that glucose-induced insulin release in fetal islets was not blocked by verapamil [7].

Insulin release from neonatal islets was lower than from freshly isolated adult islets [2]. However, when adult islets were maintained in culture, glucose-induced insulin release was also reduced. This may be related to the composition of the culture medium which only contained 5.6 mM glucose [8]. In spite of the lower secretion by adult islets in culture, the sigmoidal response to glucose was preserved (fig.2). In neonatal islets, cultured under identical conditions, the values of half-maximal and maximal stimulation were clearly shifted to the lower glucose concentrations. Similar results, showing a narrowed response to glucose, were reported in freshly isolated neonatal islets [2], isolated neonatal B-cells [15] and fetal pancreas [3].

In the absence of glucose, the ^{86}Rb efflux is lower in neonatal than in adult islets (fig.4A). Furthermore, the glucose-induced reduction in ^{86}Rb efflux is smaller in neonatal islets (fig.4A,B). Recently, Ammon et al. [1] failed to detect any reduction in ^{86}Rb efflux when increasing glucose from 3 to 16.7 mM in collagenase-isolated islets from 21-day fetal rats. These results indicate that in these immature islets, the K^+ channels (whose activity mainly determines the Rb^+ efflux) show an effective low density and/or an altered behavior.

Two of the K^+ channels identified in neonatal and adult isolated B-cells are the ATP-blockable (sulfonylurea-blockable) K^+-channel [16–18] and the Ca^{2+}-activated (voltage-gated) K^+-channel [19,20]. Both have been observed to be inhibited by glucose in adult rat B-cells [21]. Recent observations have shown that the K^+-[ATP] channel is insensitive to glucose in B-cells from fetal rats, although it can be inhibited by the sulfonylurea, tolbutamide [22]. However, glipizide, another sulfonylurea also reported to inhibit K^+-[ATP] channels, does not increase insulin secretion from fetal B-cells [23]. On the other hand, diazoxide, reported to stimulate K^+-[ATP] channels, inhibits glucose-induced insulin release in fetal rat islets

maintained in culture [5]. The K^+-[ATP] channel is regulated by cell metabolism [18,24]. It may be that the alteration in glucose sensing is due to a change in metabolism [2,6] or to a functional alteration of the K^+-[ATP] channel, as recently suggested [7]. On the other hand, cytosolic Ca^{2+} handling in neonatal islets may be altered, and this may, in turn, affect the activity of the K^+-[Ca^{2+}] channel.

In conclusion, it is proposed that the decreased range of glucose sensitivity observed in neonatal islets is due to altered K^+ permeability of the B-cell membrane. This may be due to alterations in the K^+ channels themselves, or in the cell metabolism which controls their activity.

REFERENCES

[1] Ammon, H.P.T., Fahmy, A., Mark, M., Strölin, W. and Wahl, M.A. (1985) J. Physiol. 358, 365–372.
[2] Grill, V., Lake, W. and Freinkel, N. (1981) Diabetes 30, 56–63.
[3] Kervran, A. and Randon, J. (1980) Diabetes 29, 673–678.
[4] Lambert, A.E., Kanazawa, Y., Burr, I.M., Orci, L. and Renold, A.E. (1971) Ann. NY Acad. Sci. 185, 232–244.
[5] Mourmeaux, J.L., Remacle, C. and Henquin, J.C. (1985) Mol. Cell. Endocrinol. 39, 237–246.
[6] Ågren, A., Andersson, A. and Hellerström, C. (1976) FEBS Lett. 71, 185–188.
[7] Hole, R.L., Pian-Smith, M.C.M. and Sharp, G.W.G. (1988) Am. J. Physiol. 254, E167–E174.
[8] Hellerström, C., Lewis, N.J., Borg, H., Johnson, R. and Freinkel, N. (1979) Diabetes 28, 769–776.
[9] Hegre, O.D., Marshall, S., Schulte, B.A., Hickey, G.E., Williams, F., Sorenson, R.L. and Serie, J.R. (1983) In Vitro 19, 611–620.
[10] Lacy, P.E. and Kostianovsky, M. (1967) Diabetes 16, 35–39.
[11] Boschero, A.C. and Malaisse, W.J. (1979) Am. J. Physiol. 236, E139–E146.
[12] Herchuelz, A. and Malaisse, W.J. (1978) J. Physiol. 283, 409–424.
[13] Scott, A.M., Atwater, I. and Rojas, E. (1981) Diabetologia 21, 470–475.
[14] Malaisse, W.J. and Boschero, A.C. (1977) Hormone Res. 8, 203–209.
[15] Nakhooda, A.F. and Marliss, E.B. (1977) in: Pancreatic Beta Cell Culture (Wasielewski, E.V. and Chick, W.L. eds) pp.135–142, Excerpta Medica, Amsterdam.
[16] Cook, D.L. and Hales, C.N. (1984) Nature 311, 271–273.
[17] Rorsman, P. and Trube, G. (1985) Pflügers Arch. 405, 305–309.

[18] Ashcroft, F.M., Harrison, D.E. and Ashcroft, S.J.H. (1986) in: Biophysics of the Pancreatic B-Cell (Atwater, I. et al. eds) pp.53–62, 211, Plenum, New York.

[19] Cook, D.L., Ikeuchi, M. and Fujimoto, W.Y. (1984) Nature 311, 269–271.

[20] Findlay, I., Dunne, M.J. and Petersen, O.H. (1985) J. Membrane Biol. 88, 165–172.

[21] Atwater, I., Li, M.X., Rojas, E. and Stutzin, A. (1988) Biophys. J. 53, 145a.

[22] Welsh, M., Berggren, P.-O. and Rorsman, P. (1987) Diabetologia 30, 596a.

[23] Peterson, C.M., Miller, N., Walker, L. and Formby, B. (1986) Diabetes Care 9, 556–557.

[24] Misler, S., Falke, L.C., Gillis, K. and McDaniel, M.L. (1986) Proc. Natl. Acad. Sci. USA 83, 7119–7123.