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THE EFFECT OF GLUCOSE ON INSULIN RELEASE AND ION MOVEMENTS IN ISOLATED PANCREATIC ISLETS OF RATS IN OLD AGE

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SUMMARY

1. The effect of glucose on $^{86}Rb^+$ efflux, $^{45}Ca^{2+}$ net uptake and insulin secretion of pancreatic islets from 3- and 24-month-old rats was studied.

2. Raising the glucose concentration from 3 to 5·6 and 16·7 mM had no effect on $^{86}Rb^+$ efflux from islets of 24-month-old male rats whereas that from 24-month-old female rats was decreased.

3. At 16·7 mM-glucose, net uptake of $^{45}Ca^{2+}$ was significantly diminished in islets of 24-month-old rats compared to islets of 3-month-old rats.

4. In the presence of 16·7 mM-glucose, islets of 24-month-old rats exhibited only 60–70 % of the insulin release obtained with islets from 3-month-old rats.

5. Neither net uptake of $^{45}Ca^{2+}$ nor insulin secretion appear to differ between the sexes.

6. These data suggest that the decreased insulin secretory response to glucose during old age is due, at least in part, to inadequate inhibition of K^+ efflux and diminished net uptake of Ca^{2+}.

INTRODUCTION

During old age, hyperglycemia and/or deterioration of glucose tolerance have been reported to occur in rats (Gommers & De Gasparo, 1972; Gommers & Genne, 1975; Bracho-Romero & Reaven, 1977) and in man (O'Sullivan, Mahan, Freedlender & Williams, 1971; Sandberg, Yoshimine, Maeda, Symons & Zavodnick, 1973; Dudl & Ensinck, 1977; Defronzo, 1979). A major factor which may contribute to this phenomenon has been suggested to be a diminished insulin secretory response of the pancreatic B cell (Reaven, Gold & Reaven, 1979; Lipson, Bobrycki, Bush, Tietjen & Yoon, 1981; Molina, Premdas & Lipson, 1985).

One of the first steps of stimulus–secretion coupling is thought to be inhibition of K^+ efflux from B cells by glucose and subsequent influx of Ca^{2+} through the voltage-dependent Ca^{2+} channels. Elevation of cytosolic free Ca^{2+} concentration provides a link with the insulin exocytotic process (Wollheim & Sharp, 1981).

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The aim of the present study was to investigate whether or not the effect of glucose on $^{86}Rb^+$ efflux and net $^{45}Ca^{2+}$ uptake of pancreatic islets from 24-month-old rats differs from that observed in islets of 3-month-old rats, which are usually used for studies on insulin release and ion movements.

METHODS

Animals

Adult (240–260 g body weight, 3-month-old) and 2-year-old (female, 250–290 g; male, 420–480 g) Wistar rats were obtained from a local source. The animals had free access to tap water and a standard diet (Altromin 1324, Altromin-Futterwerk, Lage, F.R.G.). Room temperature was maintained at 22 °C with a light–dark rhythm of 12 h.

Chemicals

Collagenase (196 u/mg) was obtained from Worthington Biochemicals, Freehold, NJ, U.S.A.; bovine serum albumin was purchased from Behringwerke AG, Marburg, F.R.G. $^{45}CaCl_2$, $^{86}RbCl$ and [6,6-^{3}H]sucrose were supplied by Amersham-Buchler (Braunschweig, F.R.G.); insulin radioimmunoassay kits by Isotopen Dienst West, Dreieich, F.R.G. Silicone oil Versilube F 50 was purchased from General Electronic Company, Waterford, U.S.A. and D-glucose from Serva (München, F.R.G.). All other chemicals and reagents of analytical grade were obtained from E. Merck, Darmstadt, F.R.G.

Preparation and incubation of islets

The rats were anaesthetized with ether and the pancreatic islets were prepared using a collagenase technique (Lacy & Kostianovsky, 1967).

The ionic composition of the medium used was as follows (mM): NaCl, 122; KCl, 4·7; $CaCl_2$, 2·5; $MgCl_2$, 1·1; $NaHCO_3$, 20. The buffer was gassed with 95% O_2–5% CO_2 and supplemented with 0·5% (w/v) bovine serum albumin. The pH was adjusted to 7·4 in all experiments. $NaHCO_3$ was replaced by 10 mM-HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid) and gassed with ambient air for uptake studies and measurement of insulin release.

Because of the large difference in body weight and the difference in the effect of glucose on $^{86}Rb^+$ efflux rate (see below) between sexes, islets of male and female 24-month-old rats were investigated separately. No sex differences were observed in 3-month-old rats (data not shown). Data presented for islets of these adult rats are means of equal numbers of experiments on males and females.

Measurement of $^{86}Rb^+$ efflux

Measurement of $^{86}Rb^+$ efflux has been shown to be a suitable model for determining K^+ efflux (Henquin, 1980*a*). $^{86}Rb^+$ efflux was measured as described previously (Ammon, Fahmy, Mark, Strölin & Wahl, 1985). Briefly, groups of forty islets were first incubated for 90 min in Krebs–Ringer buffer containing $^{86}RbCl$ (450–680 mCi/mmol specific activity). After washing, islets were placed into perifusion chambers (0·3 ml) to which the perifusate was conveyed at 37 °C and a flow rate of 1·1 ml/min. After an equilibration period of 20 min, $^{86}Rb^+$ appearing in the effluent fractions (collected at 2 min intervals) was determined. For each collection interval the fractional efflux of radioactivity has been calculated. Since this method determines the percentage release of $^{86}Rb^+$ from pancreatic islets at each time point, $^{86}Rb^+$ efflux data are independent of islet size.

Assay of $^{45}Ca^{2+}$ net uptake

Uptake of $^{45}Ca^{2+}$ has been studied in excess of [^{3}H]sucrose which is restricted to the extracellular space according to the method described by Henquin (1980*b*).

After pre-incubation at 37 °C for 30 min in Krebs–Ringer buffer containing 3 mM-glucose, batches of ten islets were placed in polypropylene microfuge tubes together with 0·05 ml Krebs–Ringer HEPES buffer. At zero time 0·1 ml of medium, enriched with $^{45}CaCl_2$ and substances to be tested, was added. Both tubes and media were prewarmed before starting the incubation. After incubation for 5 and 30 min, islets were separated from the surrounding medium by centrifugation through a layer of silicone oil (0·15 ml) into 0·05 ml KOH (3 M). The bottom of the tube containing the remainder of the islets was cut and transferred into scintillation vials. $^{45}Ca^{2+}$ content was

determined after disappearance of luminescence by liquid scintillation counting. After subtraction of blanks, correction for label in the extracellular space and comparison with internal standards, data were calculated and expressed as pmol Ca^{2+}.

Assay of insulin

The incubation procedure for measuring insulin secretion has been described previously in detail (Ammon, Amm, Eujen, Hoppe, Trier & Verspohl, 1984). Insulin released into the medium was measured in duplicate by double-antibody radioimmunoassay using the insulin reagent kit mentioned above.

In order to avoid distortion of the data by the different islet sizes of 3- and 24-month-old rats, results obtained from $^{45}Ca^{2+}$ uptake and insulin secretion were calculated on the basis of islet protein content. In each experiment islet protein content was determined by the method of Bradford (1976).

Evaluation of data

Student's *t* test for unpaired data was used for statistical evolution of the data. Values are given as means ±S.E.

RESULTS

Insulin secretion

As shown in Fig. 1, islets of 24-month-old rats showed a significantly diminished insulin secretory response in the presence of 16·7 mM-glucose compared with islets of 3-month-old rats. Basal insulin release was also slightly reduced. Although insulin release in islets of 24-month-old male rats appeared to be slightly lower than in islets of 24-month-old female rats, the difference was not statistically significant. No differences between the sexes were detected in islets of 3-month-old animals (data not shown).

$^{86}Rb^+$ efflux

(1) *Effect of 3 mM-glucose.* When islets of 3-month-old rats were perifused in the presence of 3 mM-glucose throughout the experiment (controls), $^{86}Rb^+$ efflux rate slowly and regularly declined with time (Fig. 2, left panel). Islets of 24-month-old female (middle panel) and 24-month-old male (right panel) rats exhibited a similar efflux pattern to that seen with islets of adult rats, but although the rate of decline was greater there was no significant difference between them.

(2) *Effect of 5·6 mM-glucose.* When the glucose concentration was raised from 3 to 5·6 mM, between the 40th and 70th minute of perifusion, islets of 3-month-old rats showed a marked and rapid decrease in $^{86}Rb^+$ efflux rate to a lower stable level. The effect of glucose was found to be fully reversible when the basal medium (70th–90th minute period) was re-employed (Fig. 3, left panel).

Islets of 24-month-old female rats (middle panel) also exhibited a decrease in $^{86}Rb^+$ efflux rate. However, this change was smaller than in islets of 3-month-old rats. Islets of 24-month-old male rats did not show any decrease in $^{86}Rb^+$ efflux rate after raising the glucose concentration from 3 to 5·6 mM.

(3) *Effect of 16·7 mM-glucose.* Employing an insulin stimulatory concentration of 16·7 mM-glucose during the period between the 40th and 70th minute in islets of 3-month-old rats evoked a different efflux pattern to that obtained using 5·6 mM-glucose. A rapid initial reduction in $^{86}Rb^+$ efflux rate was followed by a transient increase. Subsequently the rate of $^{86}Rb^+$ efflux decreased again. The effect of glucose

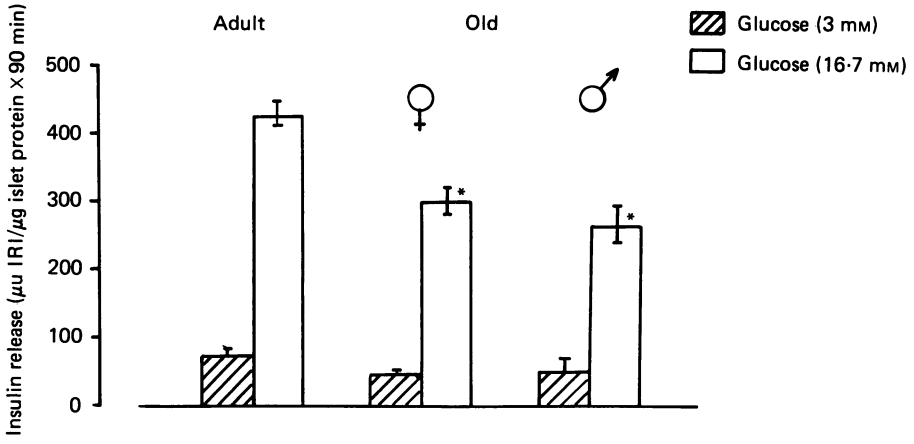

Fig. 1. Effect of glucose on insulin release from islets of adult (3 month) and old (24 month) rats. Five islets of adult or old rats were incubated for 90 min in Krebs–Ringer HEPES buffer supplemented with 0·5 % (w/v) bovine serum albumin and in the presence of 3- or 16·7 mM-glucose at 37 °C in a shaking water-bath. Values are means ± S.E. of thirteen to twenty-three observations. * $P < 0.001$ *versus* control with adult rats. IRI = immunoreactive insulin.

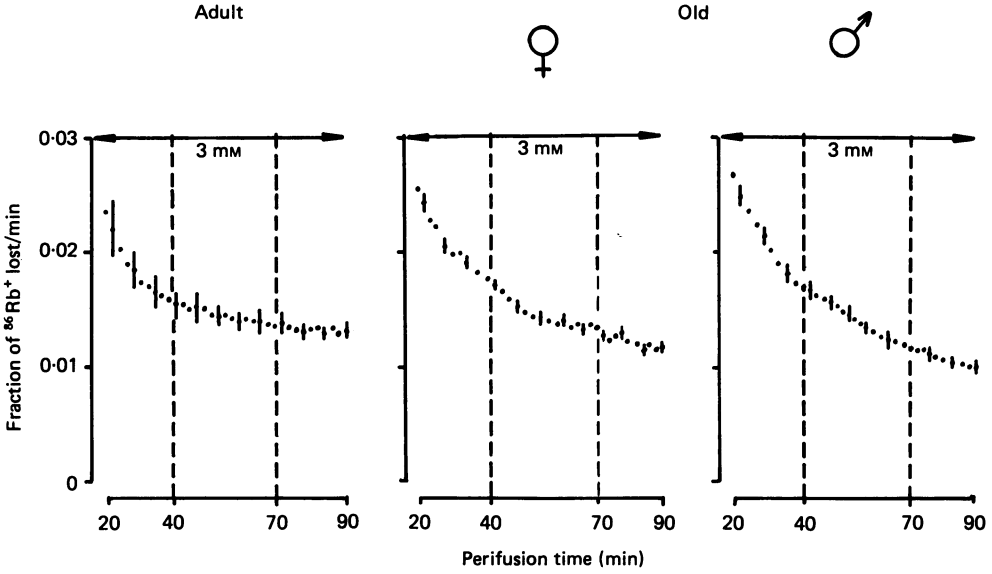

Fig. 2. Effect of 3 mM-glucose on $^{86}Rb^+$ efflux rate from perifused islets of adult (3 month) and old (24 month) rats. Glucose concentration was 3 mM throughout the experiment. Values are means ± S.E. of four experiments. The data are expressed as the fractional efflux rate. Dead time of the system (1·4 min) has not been considered. For details see Methods.

was fully reversible on return to 3 mM-glucose (Fig. 4, left panel). In islets of 24-month-old female rats, elevation of glucose concentration from 3 to 16·7 mM now caused a marked decrease in $^{86}Rb^+$ efflux rate (middle panel), similar to that observed in islets of 3-month-old rats at 5·6 mM-glucose. But in contrast to islets of 3-month-old rats (16·7 mM-glucose) no biphasic efflux pattern was observed. In addition, the effect

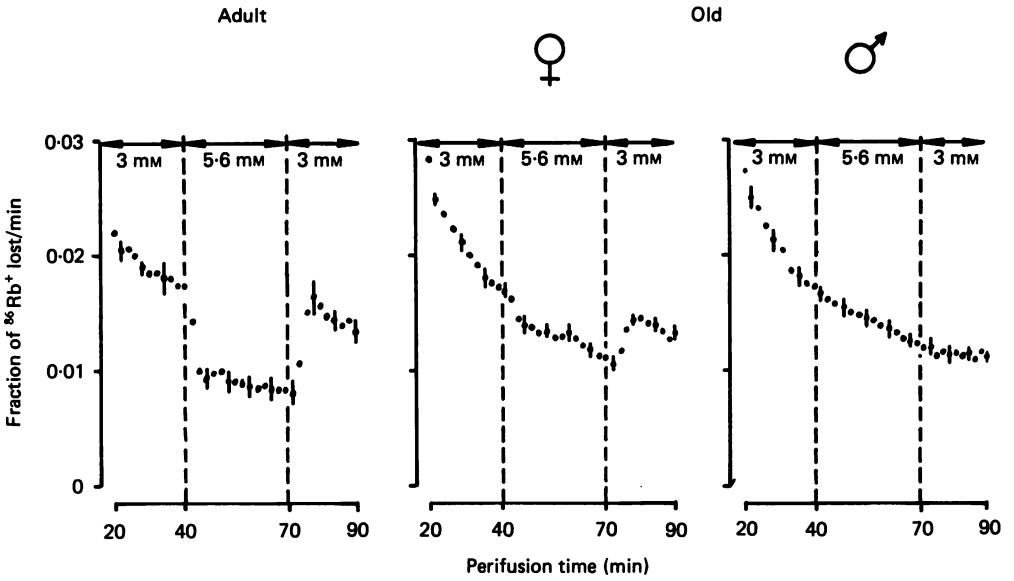

Fig. 3. Effect of 5·6 mM-glucose on $^{86}Rb^+$ efflux rate from perifused islets of adult (3 month) and old (24 month) rats. Glucose concentration was 3 mM (20th–40th minute and 70th–90th minute) and 5·6 mM (40th–70th minute). Values are means ± S.E. of four experiments. Data are expressed as the fractional efflux rate, exclusive of dead space.

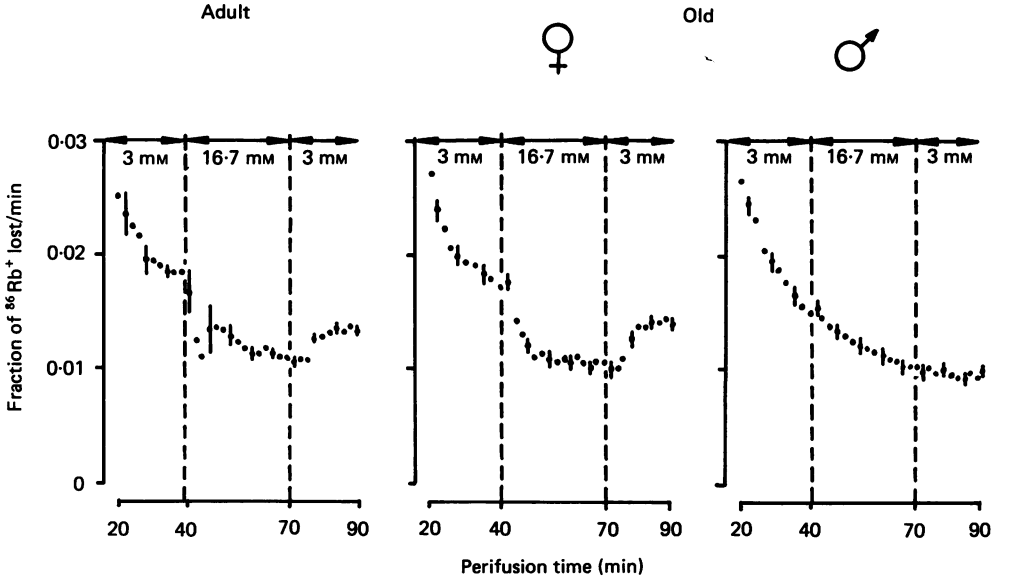

Fig. 4. Effect of 16·7 mM-glucose on $^{86}Rb^+$ efflux rate from perifused islets of adult (3 month) and old (24 month) rats. Glucose concentration was 3 mM (20th–40th minute and 70th–90th minute) and 16·7 mM (40th–70th minute). Values are means ± S.E. of four experiments. Data are expressed as the fractional efflux rate, exclusive of dead space.

of glucose was somewhat delayed. Thus the nadir of $^{86}Rb^+$ efflux in islets of female 24-month-old rats was reached in the 5th fraction after changing the medium (Figs. 3 and 4, middle panels), whereas it occurred in the 3rd fraction in the 3-month-old group employing 16·7 mM- or 5·6 mM-glucose respectively (Figs. 3 and 4, left panels). The effects of these increased concentrations of glucose were completely

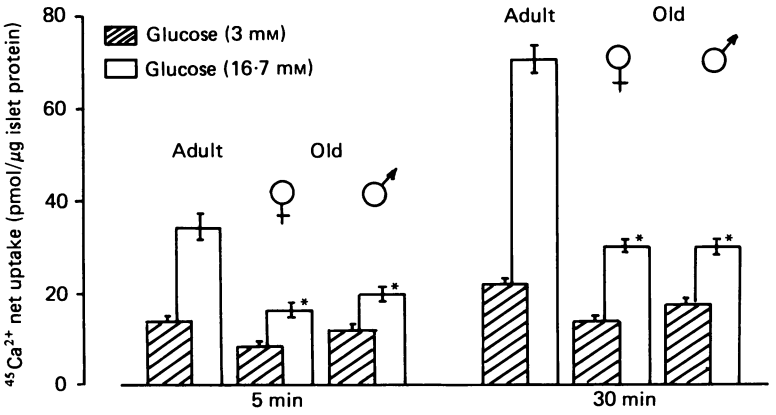

Fig. 5. Effect of glucose on $^{45}Ca^{2+}$ net uptake in islets of adult (3 month) and old (24 month) rats. Batches of ten islets each were incubated for 5 or 30 min in Krebs–Ringer HEPES buffer supplemented with 0·5% (w/v) bovine serum albumin and in the presence of 3 or 16·7 mM-glucose at 37 °C in a shaking water-bath. $^{45}CaCl_2$ specific activity was 5 mCi/mmol. For details see Methods. Values are expressed as pmol Ca^{2+}/μg islet protein. Values are means ± S.E. of fourteen observations. * $P < 0.001$ *versus* control with adult rats.

reversible on return to 3 mM-glucose. Islets of 24-month-old male rats (Figs. 3 and 4, right panels) did not show any change in $^{86}Rb^+$ efflux rate when the glucose concentration was raised, as had been observed with 5·6 mM-glucose (Fig. 3, right panel).

$^{45}Ca^{2+}$ net uptake

$^{45}Ca^{2+}$ net uptake was determined after a short (5 min) as well as a longer (30 min) incubation period. After 5 and 30 min of incubation, glucose-induced (16·7 mM) $^{45}Ca^{2+}$ net uptake in islets of 24-month-old rats of either sex was significantly decreased when compared to islets of 3-month-old rats. In the presence of 3 mM-glucose, which represents basal uptake, $^{45}Ca^{2+}$ uptake was also somewhat lower in islets of 24-month-old rats (Fig. 5).

As with insulin secretion, there were no differences in $^{45}Ca^{2+}$ net uptake between the sexes in islets of 24-month-old rats.

DISCUSSION

Our data show that, in agreement with the results of others (Reaven & Reaven, 1980; Lipson, Bush, Tietjen & Yoon, 1981; Molina *et al.* 1985), insulin release from islets of 24-month-old rats in response to 16·7 mM-glucose was diminished when compared with islets of 3-month-old rats.

According to observations reported by Reaven *et al.* (1979), islets of rats contain a larger number of B cells and of insulin secretory granules per B cell in old age. In this case the diminished insulin secretion of individual B cells could well be more profound than the results of this study suggest.

Our data also show that in old age the action of glucose on $^{86}Rb^+$ efflux from pancreatic islets is different compared with islets of 3-month-old rats. Thus, the inhibitory action of glucose on $^{86}Rb^+$ efflux is absent in islets of 24-month-old males and is less marked in 24-month-old females, suggesting that the capacity of the B cell to produce inhibition of K^+ efflux in response to glucose is decreased in old age. Since depolarization of the pancreatic B cell is thought to be due to inhibition of K^+ efflux (Henquin & Meissner, 1984), it is possible that in islets of 24-month-old rats glucose causes inadequate depolarization of the B cell membrane with subsequent inadequate uptake of Ca^{2+} through the voltage-dependent Ca^{2+} channel. Our observations indicating that $^{45}Ca^{2+}$ net uptake in the presence of a high glucose concentration was far less in islets of 24-month-old rats than it was at 3 months old, support this view.

It is interesting to note that in islets of 24-month-old male rats, glucose still promotes insulin release and $^{45}Ca^{2+}$ net uptake without having any effect on $^{86}Rb^+$ efflux. This suggests that inhibition of K^+ efflux is not the only way in which glucose initiates Ca^{2+} uptake and secretion of insulin. Previous studies with islets of old rats have shown that glucose metabolism through glycolysis and/or the pentose-phosphate shunt is depressed (Reaven & Reaven, 1980; Ammon *et al.* 1984). Since metabolism of glucose is a prerequisite for inhibition of K^+ efflux (Henquin, 1980*c*) and ultimately insulin secretion (Ashcroft, 1980), one could hypothesize that deficient glucose metabolism in old age is responsible for the failure of glucose to inhibit $^{86}Rb^+$ efflux adequately.

Recently we have demonstrated that intracellular glutathione is closely related to stimulus–secretion coupling at the step of Ca^{2+} uptake through voltage-dependent Ca^{2+} channels (Ammon & Mark, 1985). Interestingly, in old-aged rats glucose-induced elevation of glutathione and of the ratio of reduced to oxidized glutathione is significantly depressed compared with that from 3-month-old rats (Ammon *et al.* 1984).

We conclude that decreased glucose metabolism may lead to a diminished inhibition of K^+ efflux rate by glucose in old age in the rat. Consequently Ca^{2+} uptake through voltage-dependent Ca^{2+} channels is decreased. However, other factors related to Ca^{2+} uptake may also be changed under these conditions.

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