Rapid Papers

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Defective Calcium Handling and Insulin Release in Islets from Diabetic Chinese Hamsters

By EBERHARD G. SIEGEL,* CLAES B. WOLLHEIM,* GEOFFREY W. G. SHARP,†
LIESELOTTE HERBERG‡ and ALBERT E. RENOLD*

*Institut de Biochimie clinique, University of Geneva, 1211 Geneva 4, Switzerland, and

‡Diabetes Research Institute, Düsseldorf, West Germany

(Received 11 December 1978)

In pancreatic islets from normal Chinese hamsters preloaded with $^{45}\text{Ca}^{2+}$, glucose-induced biphasic insulin release was associated with increased $^{45}\text{Ca}^{2+}$ efflux; islets from diabetic hamsters showed decreased insulin release and no increase in $^{45}\text{Ca}^{2+}$ efflux. The lack of stimulated $^{45}\text{Ca}^{2+}$ efflux persisted even when glucose-induced insulin release was potentiated by 3-isobutyl-1-methylxanthine. Since glucose-stimulated $^{45}\text{Ca}^{2+}$ uptake by diabetic islets was not impaired, a defect in intracellular Ca^{2+} handling may be involved in the defective insulin release of the diabetic Chinese hamster.

Previous studies have demonstrated metabolic derangements in several sublines of Chinese hamsters similar to those observed in non-obese diabetic humans (Renold & Burr, 1970; Gerritsen & Blanks, 1974). A defect in insulin secretion has been suggested, since the decreased plasma insulin in severely diabetic Chinese hamsters (Gerritsen & Blanks, 1974; Like *et al.*, 1974) is associated with only a moderate decrease in pancreatic insulin content (Frankel *et al.*, 1974; Grodsky *et al.*, 1975). Data obtained with the perfused pancreas (Frankel *et al.*, 1974) support this view. Decreased cyclic AMP response to glucose has been suggested as a possible cause of the defect (Rabinovitch *et al.*, 1976). Since glucose-induced insulin secretion is thought to be mediated by increased intracellular Ca^{2+} (Malaisse *et al.*, 1978; Wollheim *et al.*, 1978), the present study was undertaken to investigate whether defective Ca^{2+} handling in pancreatic islets of diabetic Chinese hamsters might be responsible for their impaired insulin release.

† Present address: Department of Physiology, Tufts University School of Medicine, Boston, MA 02111, U.S.A.

Materials and Methods

Adult male and female non-ketonic Chinese hamsters (two diabetic hamsters had shown an intermittent slight ketonuria) originating from the same colony were obtained from Thomae G.m.b.H., Biberach, West Germany, and L. Herberg, Diabetes Research Institute, Düsseldorf, West Germany. The body weight ranged from 28 to 46 g. At death, in the fed state, all diabetic animals tested had +++ glucosuria ($>2\text{ g/dl}$, determined with Lilly Testape). For further characterization see Table 1.

Collagenase-isolated islets were maintained for 45–47 h in tissue-culture medium 199, also containing 1.8 mM-CaCl_2 and 8.3 mM-glucose (Wollheim *et al.*, 1977). In parallel isolations, the average yield was 120 islets from a non-diabetic and 60 from a diabetic pancreas. Islets used for $^{45}\text{Ca}^{2+}$ -efflux studies were maintained in the presence of $^{45}\text{CaCl}_2$ (specific radioactivity approx. $54\text{ }\mu\text{Ci}/\mu\text{mol}$). $^{45}\text{Ca}^{2+}$ uptake over 5 min (Wollheim *et al.*, 1977) and total exchangeable calcium, as measured by $^{45}\text{Ca}^{2+}$ content after 46 h in tissue culture (Kikuchi *et al.*, 1978), were determined by oil centrifugation methods.

Table 1. Characteristics of the Chinese hamsters used in the study

Except for the age, data are given as means $\pm$ S.E.M., for the numbers of observations in parentheses. Insulinogenic index is defined as plasma insulin ($\mu\text{units/ml}$) divided by plasma glucose (mg/dl); 1 unit is equivalent to 24 mg of insulin.

	Normals	Diabetics	P
Age (months)	18–22	15–22	
Plasma glucose (mg/dl)	113 ± 13 (10)	468 ± 18 (24)	<0.001
Plasma insulin ($\mu\text{units/ml}$)	157 ± 20 (7)	81 ± 8 (15)	<0.001
Insulinogenic index	1.39 ± 0.18 (7)	0.17 ± 0.02 (15)	<0.001
Islet insulin (ng/islet)	13.00 ± 0.92 (5)	9.00 ± 0.73 (5)	<0.02
Protein content (ng/islet)	231 ± 34 (5)	179 ± 40 (7)	>0.30
Calcium content (pmol/islet)	14.9 ± 3.2 (7)	12.4 ± 2.9 (8)	>0.60

For measurement of $^{45}\text{Ca}^{2+}$ efflux and dynamic insulin release 40 islets per chamber were perfused with Krebs-Ringer bicarbonate buffer (Krebs & Henseleit, 1932) containing 1.0 mM- CaCl_2 and 0.5% dialysed bovine serum albumin (Kikuchi *et al.*, 1978). Islet insulin was extracted in acid ethanol (ethanol/water/HCl, 150:47:3, by vol.). Immunoreactive insulin was determined by radioimmunoassay (Herbert *et al.*, 1965), by using a pig insulin standard. $^{45}\text{Ca}^{2+}$ efflux was measured by liquid-scintillation spectrometry by using Instagel (Kikuchi *et al.*, 1978), and plasma glucose by a glucose oxidase method (Boehringer test kit no. 124036). Islet protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

The sources of the materials used have been described previously (Wollheim *et al.*, 1978), except for the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (Sigma Chemical Co., St. Louis, MO, U.S.A.). Data given as means $\pm$ S.E.M. were analysed by Student's unpaired *t* test.

Results

Islet contents of protein, insulin and calcium

In islets from diabetic animals the contents of protein, insulin and calcium were 23, 31 and 17%

lower respectively than in islets from non-diabetic animals. Only the insulin content was significantly lower (see Table 1). The diabetic islets thus showed a tendency to be smaller.

$^{45}\text{Ca}^{2+}$ uptake

Basal (2.8 mM-glucose) $^{45}\text{Ca}^{2+}$ uptake by the diabetic islets was 0.86 ± 0.11 pmol/5 min per islet ($n=10$) and that by non-diabetic islets 1.12 ± 0.07 ($n=9$). This decreased uptake of 23% was not significant ($P>0.05$). Glucose (16.7 mM) significantly stimulated $^{45}\text{Ca}^{2+}$ uptake in both the non-diabetic (2.84 ± 0.26 pmol/5 min per islet, $n=9$) and the diabetic islets (2.09 ± 0.29 , $n=7$). Again, the 26% difference was not significant ($P>0.05$).

Insulin release and $^{45}\text{Ca}^{2+}$ efflux from perfused islets

Basal insulin release in the diabetic islets was 23% lower (6.0 ± 1.0 pg/min, $n=14$), but again did not differ significantly from the non-diabetics (7.8 ± 0.8 pg/min, $n=14$, $P>0.20$). In addition, there was no significant difference in the gradually declining basal $^{45}\text{Ca}^{2+}$ efflux (2.8 mM-glucose) during the entire perfusion (Fig. 1).

In the non-diabetic islets 16.7 mM-glucose caused biphasic insulin release (Fig. 1a). The

Fig. 1. $^{45}\text{Ca}^{2+}$ efflux and insulin release from islets of (a) normal and (b) diabetic Chinese hamsters. Islets preloaded with $^{45}\text{CaCl}_2$ for 46 h were perfused for 46 min with 2.8 mM-glucose and then stimulated (arrow) for 44 min with 16.7 mM-glucose with (Δ ; $n=5$) or without ($\bullet$; $n=5$) 1 mM-3-isobutyl-1-methylxanthine. A control group with 2.8 mM-glucose throughout was also included ($\circ$; $n=4$). The mean basal $^{45}\text{Ca}^{2+}$ efflux during 41–46 min (64 ± 4 c.p.m./min and 61 ± 7 c.p.m./min for non-diabetic and diabetic islets respectively) was set at 100% and the subsequent values are expressed as percentages of these means. Data are shown as means $\pm$ S.E.M., for the numbers of experiments in parentheses.

rise started 1 min after exposure, and the peak was reached at 3 min; a nadir at 6 min was followed by a progressively increasing second phase. The first phase of insulin release was paralleled by an increase in $^{45}\text{Ca}^{2+}$ efflux, whereas the second phase was not. The effects of 16.7 mM-glucose were enhanced by 3-isobutyl-1-methylxanthine, which caused a 2-fold increase in the two phases of insulin release. $^{45}\text{Ca}^{2+}$ efflux was also enhanced.

In the diabetic islets, 16.7 mM-glucose caused only a small increase of insulin secretion, with no obvious biphasic pattern (Fig. 1b). The total incremental insulin release over the whole stimulation period was decreased by 78% compared with normal islets (223 ± 62 pg compared with 1003 ± 210 pg, $P < 0.005$). No increase in $^{45}\text{Ca}^{2+}$ efflux was observed. The addition of 3-isobutyl-1-methylxanthine caused an almost 8-fold increase of insulin release relative to that with 16.7 mM-glucose alone. The total incremental insulin release was 1893 ± 449 pg, compared with 2154 ± 399 for the non-diabetics ($P > 0.50$). However, no first phase could be seen. This marked insulin release was accompanied by only a small and non-significant increase in $^{45}\text{Ca}^{2+}$ efflux. In diabetic islets, 1 mM-3-isobutyl-1-methylxanthine at low glucose (2.8 mM) elicited only a small insulin release over the 44 min stimulation period (150 ± 27 pg per islet, $n = 5$) without any change in $^{45}\text{Ca}^{2+}$ efflux, whereas in non-diabetic islets, the significantly higher insulin release (447 ± 114 , $n = 5$) was accompanied by an increase in $^{45}\text{Ca}^{2+}$ efflux.

Discussion

The severity of the diabetic syndrome in the animals used here and in a previous study (Rabinovitch *et al.*, 1976) appears to be similar to that in the ketonuric groups studied by others (Gerritsen & Blanks, 1974; Like *et al.*, 1974), as judged by the decrease in plasma insulin and the insulinogenic index. The lack of ketonuria could be due to the low dietary fat content of 4 g/100 g (Grodsky *et al.*, 1975).

The islets from the non-diabetic hamsters responded to glucose stimulation with a typical biphasic pattern of insulin release, which resembles that of the perfused pancreas (Frankel *et al.*, 1974). The defect in insulin release from freshly isolated islets of diabetic hamsters (Rabinovitch *et al.*, 1976) persisted even after 2 days of maintenance in tissue culture. The decreased islet insulin content is not a sufficient explanation of the defect, since glucose-stimulated insulin release was only 2.6% of the initial content, compared with 8.5% for normal islets.

The phosphodiesterase inhibitors theophylline (Frankel *et al.*, 1974) and 3-isobutyl-1-methylxanthine (Rabinovitch *et al.*, 1976) have been shown to enhance glucose-induced insulin release in diabetic hamsters. In the present study, the 3-isobutyl-1-methylxanthine-induced enhancement resulted in an overall insulin

release similar to that of control islets, although the first phase was not restored. In the diabetic-hamster islets, insulin release in response to glucose or glucose and 3-isobutyl-1-methylxanthine was not accompanied by a significant increase in $^{45}\text{Ca}^{2+}$ efflux. A failure of glucose to stimulate $^{45}\text{Ca}^{2+}$ efflux has been reported for islets from diabetic (*db/db*) mice (Gylfe *et al.*, 1978). Since the $^{45}\text{Ca}^{2+}$ efflux is thought to reflect increased cytosolic Ca^{2+} concentrations (Malaisse *et al.*, 1978; Wollheim *et al.*, 1978), and as total $^{45}\text{Ca}^{2+}$ content and basal $^{45}\text{Ca}^{2+}$ -efflux rates were similar in the two types of islets, a defect in the ability to increase cytosolic Ca^{2+} is suggested.

Glucose may raise cytosolic Ca^{2+} concentration either by stimulating Ca^{2+} uptake from the extracellular fluid (Malaisse *et al.*, 1978; Wollheim *et al.*, 1977), or by acting on intracellular calcium, for instance by inhibiting efflux across the plasma membrane (Malaisse *et al.*, 1978; Kikuchi *et al.*, 1978; Gylfe *et al.*, 1978; Frankel *et al.*, 1978), by inhibiting the sequestration of Ca^{2+} (Sugden & Ashcroft, 1978), or by causing mobilization of stored calcium (Malaisse *et al.*, 1978; Wollheim *et al.*, 1978). As the stimulation of $^{45}\text{Ca}^{2+}$ uptake by glucose was similar in diabetic and normal islets, a defect in the utilization of intracellular calcium appears to be involved. The lack of first-phase insulin release even when glucose and 3-isobutyl-1-methylxanthine were added together supports this theory, since the first phase has been shown to be dependent mainly on intracellular Ca^{2+} (Wollheim *et al.*, 1978; Kikuchi *et al.*, 1979). The dissociation of insulin release and $^{45}\text{Ca}^{2+}$ efflux in the diabetic islets in response to 3-isobutyl-1-methylxanthine and glucose raises the possibility that increased cellular cyclic AMP concentrations do not increase insulin release only by dislocation of intracellular Ca^{2+} , but may act to sensitize the release machinery.

In certain types of human diabetes the impairment of insulin release mainly affects the first phase (Cerasi *et al.*, 1972). To elucidate whether this is due to a defect in Ca^{2+} handling similar to that shown in the present study for the Chinese hamster, studies on human pancreatic tissue are required.

We are grateful to Miss D. Cassard and Mrs. T. Cuche for their skilled technical assistance, as well as to Mr. R. Cuche for excellent animal care. This work was supported by the Swiss National Science Foundation (grant no. 3.774.076 SR).

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