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Metabolism of Cold-Stored Pancreatic Islets

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Summary. A previous study showed that the ability of glucose to stimulate insulin release was retained in islets stored at 8 °C for one week provided that glucose was present in a high concentration in the storage medium. The metabolic properties of islets stored in the cold have now been further explored in an attempt to clarify the protective effect of glucose. During storage in the cold the islet formation of ^3H_2O from (5−^{3}H) glucose and oxygen consumption were only a few per cent of that of fresh islets whereas the uptake of $^{86}Rb^+$ was 20–48%. Rewarming the cold-stored islets to 37 °C after one week of cold-storage restored the $^{86}Rb^+$ uptake, the formation of ^3H_2O and $^{14}CO_2$ from labelled glucose and oxygen consumption to 75, 80, 60 and 40% respectively of fresh islet levels. The results emphasize the usefulness of cold-storage for preservation of functionally intact isolated islets.

Key words: Pancreatic islets, cold-storage, culture, glucose utilization, oxygen consumption, $^{86}Rb^+$-uptake, metabolism.

Methods for preservation of functionally intact pancreatic islets may be of great importance for future treatment of diabetes mellitus in allowing transplantation of large amounts of islets. It was evident from a previous study [1] that glucose-stimulated insulin release decreased when the microdissected islets were stored for one week at 37 °C, but that functional integrity could be maintained by storage at 8 °C. Successful maintenance of glucose-stimulated release, required the presence of glucose at a high concentration in the storage medium. The purpose of the present work was to study the effect of temperature reduction on islet metabolic activity. We have therefore measured the islet production of ^3H_2O and $^{14}CO_2$ from labelled glucose, the uptake of $^{86}Rb^+$ and consumption of oxygen before, during and after cold storage.

Materials and Methods

Chemicals

Reagents of analytical grade and deionized water were used throughout. The chemicals and their sources were as follows. N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid (Hepes) and bovine serum albumin (fraction V) from Sigma Chemical Co., St. Louis, Mo., USA; tissue Culture Medium 199 (TCM 199, 10X concentrated) from Statens bakteriologiska laboratorium, Stockholm, Sweden; benzylpenicillin from AB Kabi, Stockholm, Sweden; gentamicin from Schering Corp., Kenilworth, N. J., USA; crystalline mouse insulin from Novo A/S, Copenhagen, Denmark; ^{125}I-insulin from Farbwerke Hoechst A. G., Frankfurt/M., FRG; ^{86}RbC1, (6,6'-^{3}H)-sucrose, D-(U-^{14}C)glucose and D-(5-^{3}H)-glucose from The Radiochemical Centre, Amersham, England.

Media

The basal medium consisted of modified TCM 199 with Hanks' salts containing 25 mmol/l Hepes, but lacking phenol red and glucose. This medium was supplemented with 1 mg/ml bovine serum albumin and 1–40 mmol/l glucose. All storage media contained 50 μg/ml benzylpenicillin, and the media used for oxygen consumption studies also contained 50 μg/ml gentamicin. Albumin was excluded from the incubation media when measuring ^3H_2O or $^{14}CO_2$ production and $^{86}Rb^+$ uptake.

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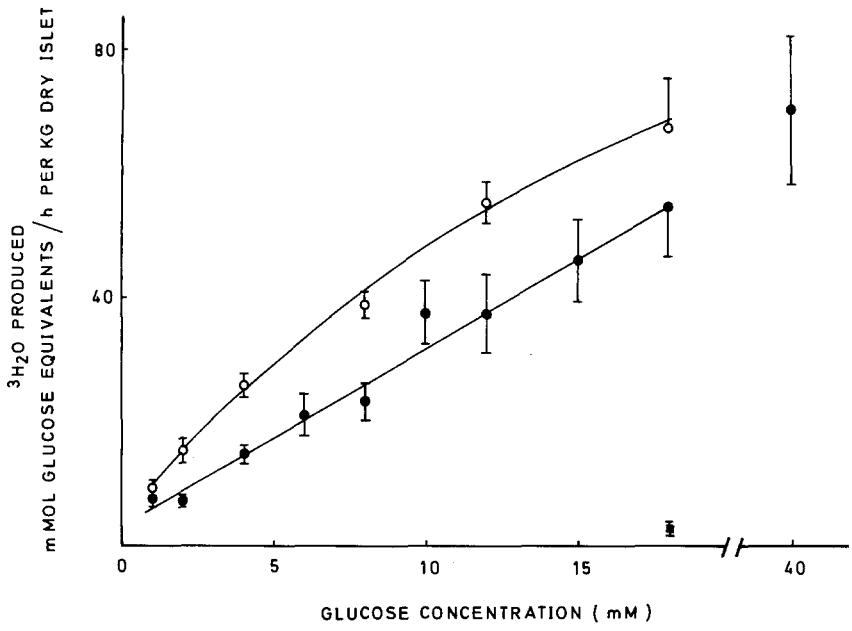

Fig. 1. Production of ^3H_2O from D-(5-^{3}H)glucose in fresh islets and in islets stored for 1 week at 8 °C. The results are expressed as mmoles of glucose equivalents metabolized/h/kg dry islet. Each point represents the mean value ± SEM for 4–9 experiments (animals). Fresh islets (○) were first preincubated for 60 min at 37 °C in 1 ml medium containing 1 mmol/l glucose and then incubated for 60 min at the same temperature in 15 µl medium with 1–40 mmol/l D-(5-^{3}H)glucose (0.25–10.0 Ci/mol). Islets stored at 8 °C (●) were incubated for 4 hours at 37 °C in medium containing 18 mmol/l glucose prior to the preincubation and incubation periods as above. In one series of experiments the production of ^3H_2O from stored islets was measured during 3–5 hours at 8 °C without preceding incubations at 37 °C (■)

Table 1. Production of $^{14}CO_2$ from D-(U-^{14}C)glucose in fresh islets and in islets stored for 1 week at 8 °C

Type of islets	Glucose concentration (mmol/l)		
	1	18	Difference
Fresh islets	1.1 ± 0.1 (10)	26.2 ± 3.5 (10)	25.1 ± 3.5[a] (10)
Cold-stored islets	1.2 ± 0.2 (12)	15.4 ± 1.4 (11)	14.3 ± 1.5[a] (11)

The results are expressed as mmoles of glucose equivalents metabolized/h/kg dry islet and given as mean values ± SEM for the number of experiments (animals) given within parentheses. Fresh islets were first preincubated for 60 min at 37 °C in 1 ml medium containing 1 mmol/l glucose and incubated for 60 min at the same temperature in 100 µl medium with 1 (17.1 Ci/mol) or 18 mmol/l (1.0 Ci/mol) D-(U-^{14}C) glucose. Islets previously stored at 8 °C were kept for 4 hours at 37 °C in a medium containing 18 mmol/l glucose prior to the preincubation and incubation periods as above

[a] $P < 0.001$

Animals and Microdissection of Islets

Male obese-hyperglycaemic mice (gene symbol ob/ob) 8–10 months old, were taken from the Umeå colony [2]. After fasting overnight, the animals were killed by decapitation under ether anaesthesia and fresh pancreatic islets were microdissected [3] from the pancreas at room temperature in medium containing 1 mmol/l glucose.

Storage, Incubation and Weighing of Islets

Storage of islets was performed for 7 days at 8 °C in a medium containing 18 mmol/l glucose as described previously [1]. For studies of oxygen consumption, islets were stored at 8 °C for 5–7 days. Islets stored in the cold were usually incubated for 4 hours at 37 °C in storage medium before further incubation periods. ^3H_2O production, ^{86}Rb uptake and O_2 consumption were also studied in the cold without a prior period at 37 °C. Measurements of ^3H_2O and $^{14}CO_2$ production were preceded by a preincubation period as described in the legends to Figure 1 and Table 1, and the O_2 consumption studies were preceded by an equilibration period. The incubation procedures, stated in the legends to the Tables and Figures, were similar to those previously described for measurement of the rates of $^{14}CO_2$ production [4], ^3H_2O production [5], ^{86}Rb uptake [6] and O_2 consumption [7]. After incubation, the islets were placed on pieces of aluminium foil and gently freed of as much contaminating fluid as possible with the aid of a micropipette. After freeze-drying overnight (−40 °C, 0.1 Pa), the islets were weighed on a quartz fibre balance [8].

Analytical Procedures

The methods for determining $^{14}CO_2$ [4] and ^3H_2O production [5], ^{86}Rb uptake [6] and O_2 consumption [7] have been described previously.

Statistics

Statistical significance was estimated from the difference between paired test and control data using the two-tailed Student distribution.

Results

The islet production of ^3H_2O from (5-^{3}H)glucose is shown in Figure 1. In fresh islets ^3H_2O formation increased from 9.6 to 67.3 mmol glucose equivalents/h/kg dry islet between 1 and 18 mmol/l glucose. The ^3H_2O production at 8 °C in 18 mmol/l glucose was measured after storage at this temperature for one week and found to be only 3.0 mmol glucose equivalents/h/kg dry islet. Warming the cold-stored islets for 4 hours at 37 °C restored ^3H_2O formation at 18 mmol/l glucose to about 80% that of fresh islets.

The glucose concentration dependency was similar to that of fresh islets.

Table 1 shows the islet production of $^{14}CO_2$ from D-(U-^{14}C)glucose. Similar rates of $^{14}CO_2$ production were noted both in fresh and cold stored islets at 1 mmol/l glucose, whereas at 18 mmol/l glucose the $^{14}CO_2$ production of cold stored islets was reduced to about 60% that of fresh islets.

Oxygen consumption data are presented in Table 2. Fresh islets incubated with 18 mmol/l glucose consumed 20.4 l O_2/h/kg dry islet at 37 °C. Storage for 5–7 days at 8 °C reduced oxygen consumption at 37 °C to 40% of that of fresh islets. At 8 °C fresh islets consumed only 7% of the consumption at 37 °C. This figure was further reduced to 4% after storage in the cold.

Figure 2 shows the islet uptake of $^{86}Rb^+$ as a function of time. Maximal levels of rubidium uptake were reached between 60 and 120 min in fresh islets at 37 °C. The 120 min level represents a 52 fold accumulation. After 4 hours or 1 week of cold-storage, ^{86}Rb-uptake levels were 48% and 20% that of fresh islets respectively. Warming the islets stored in the cold for 1 week to 37 °C restored the $^{86}Rb^+$-uptake to 75% of the fresh islet levels.

Discussion

In a previous study a procedure was established for keeping pancreatic islets functionally intact for long periods of time [1]. After one week at 8 °C followed by a 4-hour period at 37 °C glucose stimulated insulin release to the same extent as in freshly dissected islets. Despite the low temperature a high glucose concentration in the storage medium was mandatory for preservation of function. The protective effect of glucose was probably not a result of an interaction with transport carriers in the B-cell membrane, since high concentrations of 3-0-methyl glucose, which shares a common transport mechanism with glucose [9], did not maintain B-cell function (not shown).

Cold stored islets have now been further characterized with respect to the role of glucose during storage. The formation of ^3H_2O from D-(5-^{3}H)glucose was used as a measure of the combined flux through the glycolytic and phosphogluconate pathways [10], and the formation of $^{14}CO_2$ from D-(U-^{14}C)glucose was used as an indicator of the aerobic degradation of glucose. The total oxidative metabolism of the pancreatic islets was studied by measuring oxygen consumption with an automatic electromagnetic respirometer [7], and $^{86}Rb^+$ was used as a functional analogue to study the islet ability to concentrate K^+ [6]. Since all measurements were performed in TCM 199, it is of interest to compare the control data obtained with fresh islets at 37 °C to previously published observations in less complex media. As might be expected, the present rate of $^{14}CO_2$ formation was almost 50% lower or reduced by 20–25 mmol glucose equivalents/hour/kg dry islet when compared to islets incubated with 18 mmol/l glucose as the only substrate [11]. A similar comparison of the formation of ^3H_2O revealed a reduction of the same magnitude from about 95 [5] to about 70 mmol glucose equivalents metabolized/hour/kg dry islet. Another difference in the ^3H_2O production at various glucose con-

Table 2. Oxygen consumption of fresh islets and islets stored for 5–7 days at 8 °C

Type of islets	Temperature °C	
	8	37
Fresh islets	1.4 ± 0.1	20.4 ± 3.2
Cold stored islets	0.8 ± 0.2	7.7 ± 2.2

The results are expressed as l O_2/h/kg dry islet under normal conditions (NTP) and given as mean values of 5 expermiments ± SEM. Islets were placed in respiration chambers containing 20–30 µl medium supplemented with 18 mmol/l glucose. The chambers were placed in an automatic electromagnetic respirometer (7) and after an equilibration period of 2–5 hours the mean oxygen consumption during 3 hours at 37 °C or 5 hours at 8 °C was determined

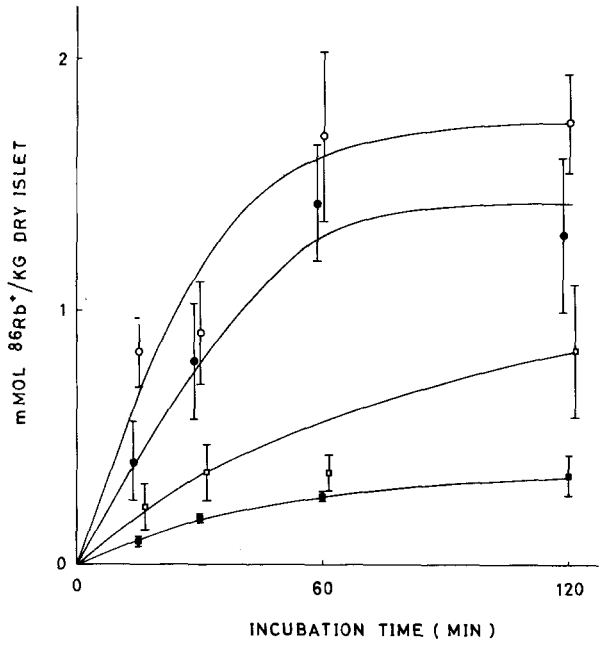

Fig. 2. Uptake of Rb^+ with time in fresh islets (○, □) and in islets stored for 1 week at 8 °C (●, ■). Rb^+ values in excess of the sucrose space are given as the mean values ± SEM for 3–4 experiments (animals). The islets were first incubated for 4 h in medium containing 18 mmol/l glucose, and then incubated for 15, 30, 60 or 120 min in the same type of medium supplemented with 28 µmol/l ^{86}RbCl (710 Ci/mol) and 100 µmol/l (6,6'-^{3}H)sucrose (150 Ci/mol). The temperature during the incubation periods was either 37 °C (○, ●) or 8 °C (□, ■)

centrations was the lack of an apparent sigmoidal dose-response relationship. Since this relationship has been found to depend on oxygen tension [5], use of ambient air as gas phase instead of 95% O_2/5% CO_2 may explain the absence of a sigmoidal relationship. However, it should be noted that a clearly sigmoidal dose-response relationship has been found for glucose-stimulated insulin release under identical conditions both in fresh [12] and cold-stored islets [1]. Oxygen consumption was almost 2.5 times greater in TCM 199 than has previously been observed with Krebs-Ringer-phosphate buffer [13]. A control experiment with phosphate buffer indicated that this discrepancy could largely be explained by the different media. Uptake of $^{86}Rb^+$ was the only measured variable which did not differ much from previously published data [6] suggesting that there is a characteristic ionic equilibrium for functioning pancreatic B-cells.

After storage at 8 °C and a 4 hour rewarming period at 37 °C the $^{86}Rb^+$ uptake, ^3H_2O formation, $^{14}CO_2$ formation and oxygen consumption of the islets were maintained at 75, 80, 60 and 40% of the fresh islet levels. The recoveries of below 100% may indicate decreased viability. However, such a decrease cannot be large since glucose-stimulated insulin release was fully maintained after cold-storage [1]. The total aerobic metabolism was drastically reduced after cold-storage, whereas that of glucose was less affected. Since glucose may stimulate insulin secretion by being metabolized via the glycolytic pathway [11, 14–16] it is particularly interesting to note that glycolysis was less affected by cold-storage than aerobic breakdown of glucose. The preservation of glucose-stimulated insulin release after cold-storage is thus consistent with the good maintenance of ^3H_2O production.

The metabolism and $^{86}Rb^+$-accumulating ability of islets were studied at 8 °C. Whereas the ability to accumulate $^{86}Rb^+$ severalfold was largely retained, the islet formation of ^3H_2O from (5-^{3}H)glucose and the oxygen consumption were reduced to only a few per cent of the values at 37 °C. Thus, it appears that metabolism, although strongly reduced, can maintain $^{86}Rb^+$ pumping into the islet cells at 8 °C.

A high concentration of glucose is necessary to maintain the B-cell sensitivity to glucose as stimulator of insulin release whether the islets have been stored at 8 °C [1] or cultured at 37 °C [12, 17]. This observation together with the present data lend some support to the suggestion that the protective effect of glucose is due to interaction with a specific glucoreceptor in the B-cells rather than its function as a metabolic fuel [17].

The present studies suggest that storage at 8 °C is an useful technique for preserving glucose-stimulated insulin release from isolated pancreatic islets by reversibly decreasing cellular metabolism.

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