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LONG-TERM EFFECT OF 2-DEOXY-2-FLUOROGLUCOSE ON MAINTENANCE IN CULTURE OF THE NEONATAL B CELL OF RAT

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This report describes a beneficial role for 2-deoxy-2-fluoroglucose(2-dfg) in promoting survival of neonatal B cells of the rat. Culturing of islets in medium with 5.5 mM glucose abolished the glucose responsiveness of B cells after 7 days of culture. By contrast, the 2-dfg supplemented culture maintained glucose-induced insulin release, and the recovery of insulin in B cells at day 7 at levels 4 to 7-fold higher than at day 0. Slightly enhanced insulin biosynthesis was observed in those B cells. In addition, a selective deletion of fibroblasts was caused by the addition of the fluoroglucose.

INTRODUCTION

In the last decade preservation of islet cells in culture has attracted a great number of investigators. However, the survival or growth of islets in culture was greatly limited by the fact that fibroblasts, which more rapidly proliferate than do endocrine cells, overgrow islets so as to inhibit their further growth. Several additional approaches have been devised to reduce the number of fibroblasts in culture (1-3). Recently, we demontrated that 2-deoxyglucose is effective to delete fibroblasts, and to promote a greater survival of B cell in culture (4,5). At present time, the mechanism by which these effects were produced remains unexplained. Therefore, we designed to examine the long-term effect of 2-dfg, with a closer structural resemblance to D-glucose as compared to 2-deoxyglucose, on maintenance in culture of neonatal B cells of the rat.

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MATERIAL AND METHODS

Reagents

All reagents used were of analytical grade. Collagenase (type I) and bovine insulin were purchased from Sigma Chemical Co.. 2-Deoxy-2-fluoroglucose (2-dfg) was from Calbiochem-Behring Co.. Bovine serum albumin (BSA, fraction V) was from Armour Pharmaceutical Co.. Medium 199 was from Grand Island Biological Co..Fetal bovine serum was from Flow Laboratories. Rat insulin was from Novo Industri. L-[4,5-^{3}H]Leucine (52-85 μCi/mmol) was from Radiochemical Center. Insulin RIA kit was from Dainabot Co..Cyclic AMP kit was from Yamasa Shoyu Co.. Other reagents were the products of Wako Junyaku Co..

Monolayer cell culture of neonatal rat pancreas

Pancreatic monolayer cell culture of the neonatal rat was according to a modification of the method of Lambert et al.(6)as described in our previous report(7). Briefly, the pancreases of 3-4 day old Sprague-Dawley rats were removed, and minced using a sterile technique. The pancreatic tissue was treated with a mixture of trypsin and collagenase in calcium- and magnesium-free phosphate buffer saline, pH 7.4, containing 2.8 mM glucose at 37 °C. Isolated cells were diluted with medium 199 containing 10 % heat-inactivated fetal bovine serum and 16.7 mM glucose, and plated in culture dishes (Falcon Plastics 3003, Becton and Dickinson Co.). About 20 hr after the initial plating at 16.7 mM glucose, cells were collected, diluted with the same medium to 1 x 10^6 cells/ml, and then planted in new culture dishes. After a 24-hr culture, the cultures were continued in media with 5.5 mM glucose or further supplemented with 1 mM 2-dfg for 7 days. The culture media were renewed at 1-2 day intervals.

Incubation

The monolayer culture was incubated in 300 μl of Krebs-Henseleit bicarbonate buffer (KHBB) containing 0.5 % BSA, 5 μCi of L-[^{3}H]leucine and 3 mM theophylline in the presence of 16.7 mM glucose or in its absence for 2 hr at 37 °C. After incubation, the medium was removed, and 500 μl of acid ethanol was added to culture dishes, which were kept at - 20 °C overnight.

Separation of (pro)insulin

The acid ethanol extract was mixed with bovine insulin and evaporated to dryness under N_2 gass. The fraction of (pro)insulin was separated on a Sephadex G-50 column (0.9 x 60 cm) with 1 M acetic acid. The amount of [^{3}H]leucine incorporated into (pro)-insulin was quantified by summing the radioactivities making up (pro)insulin after subtraction of the background.

Measurement of insulin in cells and medium

The recovery of insulin in cells and medium was measured by a double-antibody radioimmunoassay using rat insulin as standard.

Measurement of cAMP in cells and medium

The recovery of cAMP in cells and medium was measured by cAMP assay kit. The samples for assay of cell cAMP was extracted with 1.2 ml of 6 % trichloroacetic acid, and then washed with ethyl ether several times. All samples for cAMP assay were liophylized, and then stored at - 70 °C just before assay.

Measurement of protein concentration

The concentration of cell protein, precipitable with 6 % trichloroacetic acid was determined by the method of Lowry et al. (8).

Determination of statistical significance

Student's t test was used to determine statistical significance.

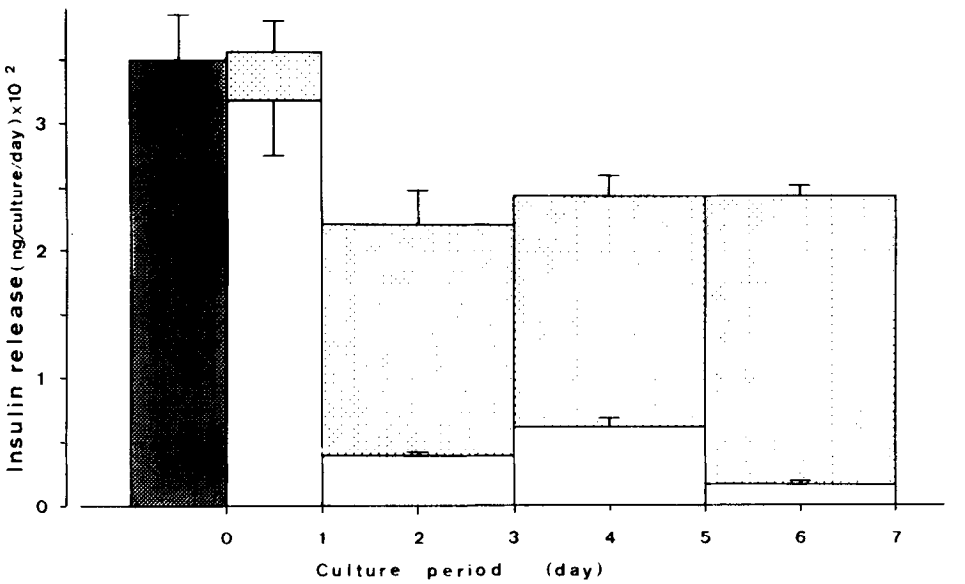

Fig. 1.
The culture of monolayer islets was maintained in media with 5.5 mM glucose alone (☐) or further supplemented with 1 mM 2-dfg (▨) for 7 days. The results are expressed as the mean ± SEM of 3 experiments.

RESULTS

The culture of islets in monolayer culture in medium with 5.5 mM glucose reduced the recovery of insulin in medium to 5 % of the initial level (day 0:350 ± 35 ng/culture) at the end of culture. By contrast, the medium supplemented with 1 mM 2-dfg maintained the recovery of insulin in medium throughout the culture study period at level slightly lower than the initial (day 0)(Fig. 1). The amounts of glucose-induced insulin release and biosynthesis in B cells at day 0 were 39.0 ± 0.8 ng/culture/hr, and 5647 ± 502 cpm/culture/hr, respectively. A 90 % drop of insulin release was observed in B cells after 7 days of culture with basal medium, whereas the 2-dfg supplemented culture enhanced insulin release 4-fold. In addition, the fluorosugar prevented the decrease of insulin biosynthesis in B cells cultured in basal medium, yielding similar level of [^{3}H]leucine incorporation into (pro)insulin as the initial (Fig. 2).

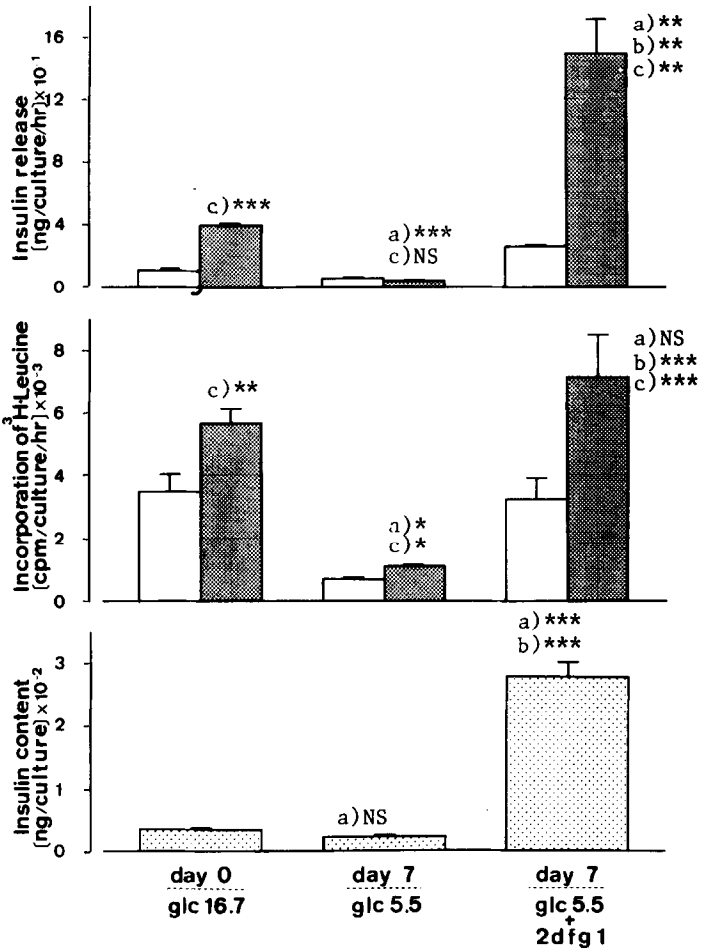

Fig. 2.
Insulin release and biosynthesis were determined by incubation of cultures with (▩) or without (□) 16.7 mM glucose in the presence of 3 mM theophylline for 2hr at 37 °C. The results are expressed as the mean ± SEM of 3 experiments. a) compared with the stimulating level at day 0, b) compared with the stimulating level of glc 5.5 culture at day 7, c) compared with each basal level in the same culture. * $P<0.02$ ** $P<0.01$ *** $P<0.001$.

Table 1 summarizes the data on the recovery of cAMP in cells and incubation medium at day 0 or 7. The recovery of cAMP in cells after 7 days of culture in basal medium remained at the initial level, whereas it was increased by the addition of 2-dfg (1.8-fold). Furthermore, a significant increase of either basal or stimulating levels of cAMP release was seen in B cells cultured in medium with 2-dfg. As depicted in Fig. 3, the 2-dfg supplemented culture prevented the deterioration of islet cells cultured in basal medium, and selectively deleted fibroblasts at the end of culture.

Table 1

Effects of the 2-dfg supplemented culture on the recovery of cAMP in cells and incubation medium

Culture (day)	Culture condition (mM)	cAMP release (f mol/μg.protein/hr) glc 0	cAMP release (f mol/μg.protein/hr) glc 16.7	cAMP content (f mol/μg.protein)	Protein (μg/culture)
0	glc 16.7	4.37 ± 0.71	5.67 ± 0.36	6.01 ± 0.91	119
7	glc 5.5	4.85 ± 0.26	6.43 ± 0.17	6.46 ± 0.41	111
	glc 5.5 + 2-dfg 1.0	27.87 ± 3.07 a)* b)*	53.65 ± 0.83 a)** b)** c)*	10.86 ± 0.49 a)# b)*	34.8

The results are expressed as the mean ± SEM of 3 experiments. a) compared with glc 16.7 culture at day 0, b) compared with glc 5.5 culture at day 7, and c) compared with the basal level. # $P < 0.02$ * $P < 0.01$, ** $P < 0.001$.

DISCUSSION

In the present study, we demonstrated the superiority of the 2-dfg supplemented medium in promoting a greater survival of B cells in culture. The low, physiological concentration of glucose abolished the glucose sensitivity of B cells after 7 days of culture, and rapidly deteriorated clusters of monolayer islets. This effect is likely to be mainly due to the rapid growth of fibroblasts established along with endocrine cells. The mechanical crowding effect of fibroblasts may produce a poor nutritional condition upon the islet cells by their quantitative consumption of glucose and other nutrients. On the other hand, several workers indicated an important role for vitamines or nicotinamide in stimulating the growth of fetal or neonatal B cells in culture (9,10). However, it was clear that the commonly used medium 199 containing 5.5 mM glucose, further supplemented with 2-dfg is effective to promote survival and to stimulate growth of neonatal B cells, and in addition causes a selective deletion of fibroblasts. Furthermore, the addition of 2-dfg enhanced the basal or stimulating levels of cAMP release. This agreed well with a greater response of insulin release. Similarly, enhanced accumulation of cAMP in

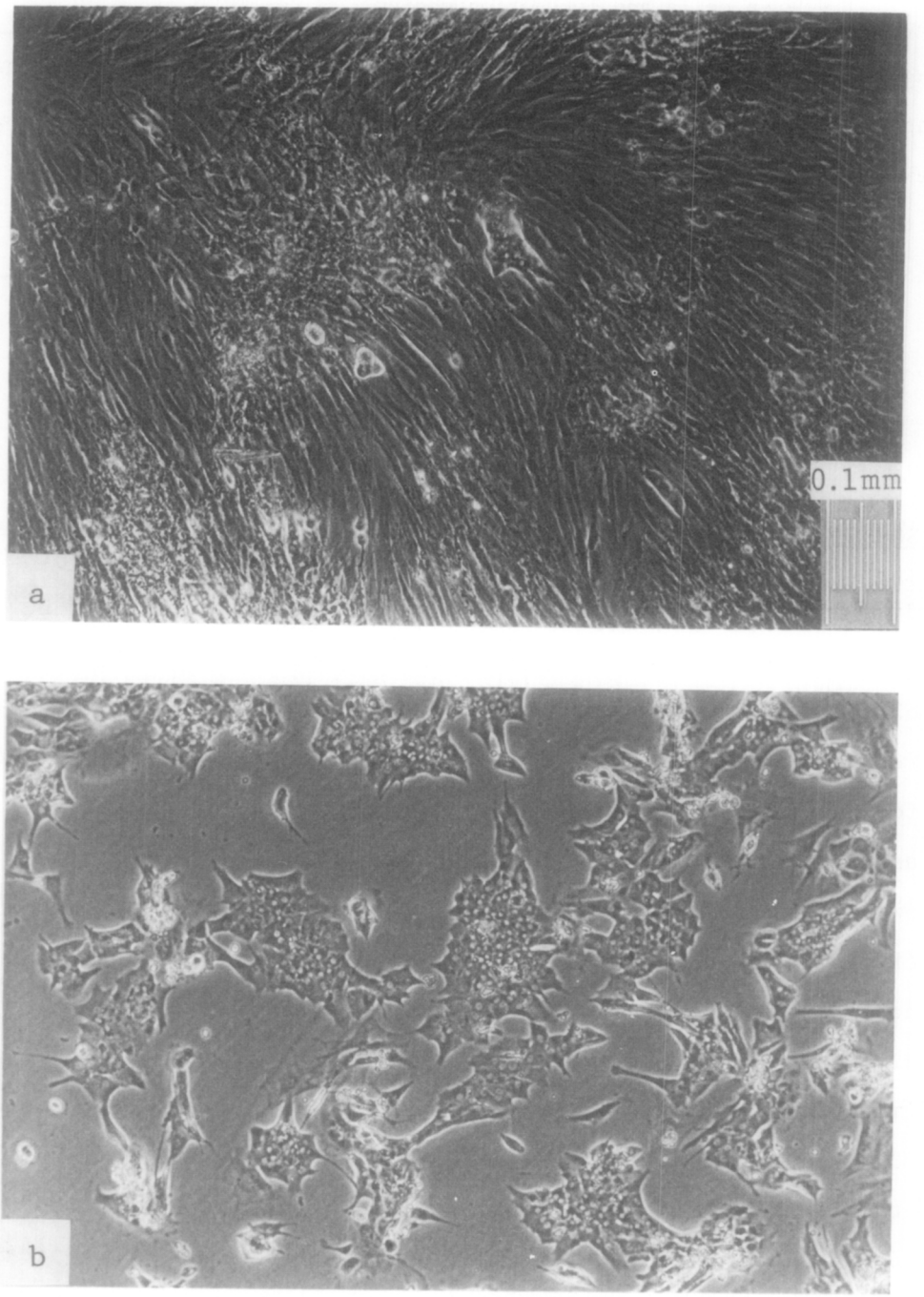

Fig. 3.
Frames show clusters of islet cells observed by a phase-contrast microscope (x 100), maintained for 7 days in media with 5.5 mM glucose (a) or further supplemented with 1 mM 2dfg (b).

cells may promote the proliferation of B cells in culture (11). Yoshida et al.(12) observed that the addition of glucosamine is beneficial to the in vitro function of B cells, although the amino-sugar did not delete fibroblasts. These findings suggest another intrinsically important role(s) for this fluorosugar. Since three hexoses noted above are inhibitors of either glycolysis or the glycosylation of glycoproteins (13-17), it seems conceivable that those hexoses may alter the constituents of glycoproteins in cells so as to affect the activity of surface enzymes including adenylate cyclase(18). Overall,the present findings may provide a possible key to clarify the proliferation process of the neonatal B cell in culture. The molecular details of 2-dfg and the functional significance of the carbohydrate chains in cells will need further investigations.

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