Long-term Effect of pH on B-Cell Function in Isolated Islets of Langerhans in Tissue Culture

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Summary. Collagenase isolated mouse pancreatic islets were maintained in tissue culture for up to 5 months in a culture medium buffered with Hepes and the pH varying between 6.8 and 7.6. The amount of insulin released into the medium and the insulin response to glucose and glucose plus theophylline were measured during the culture period. It was found that islets cultured at pH 7.2 maintained the ability to release insulin into the medium for at least 5 months, which was longer than islets cultured at the other pH values. During the first weeks, the islets cultured at pH 7.6 had a higher response to both glucose and glucose plus theophylline than islets cultured at the other pH values, but later they lost their insulin releasing ability.

Key words: Pancreatic islets, tissue culture, insulin release, pH-values, free-floating.

The maintenance of islets of Langerhans in a functional state for extended periods of time would be a valuable tool for collecting material for transplantation and for studying the effect of various hormones and metabolites on islet function *in vitro*.

There have been several reports dealing with long-term survival of isolated pancreatic islets in tissue culture [5, 11, 12, 18, 20, 21] and a number of factors affecting the survival and function of the islets have been studied, e. g. glucose [1], media [3] and sera [8, 17], but little attention has been paid to the effect of the pH of the culture medium. In tissue culture of pancreatic islets, the pH is normally maintained in the range of 7.2 to 7.4 by using a culture medium buffered with sodium bicarbonate in a CO₂-incubator. Utilization of Hepes (N-2-hydroxyethyl-

piperazine-N'-2-ethanesulphonic acid) as an alternative buffer to bicarbonate in tissue culture has become common; it does not require a CO₂-enriched atmosphere and it is possible to vary the pH in the culture medium.

In this report we describe the effect of pH in the culture medium on insulin secretion from isolated mouse pancreatic islets in free floating culture, a recently described technique [3, 8] which permits survival and function of isolated islets for many months [18].

Materials and Methods

Tissue Culture

Isolated islets were obtained by a collagenase method [4] from over-night fasted male NMRI mice, with a body weight of 25–30 g. Briefly, pancreases from 2 animals were cut into small pieces, which were suspended in 4 ml of Hank's Balanced Salt Solution, HBSS, with 25 mmol/l Hepes adjusted to pH 7.4 (Flow Laboratories) containing 2 mg/ml collagenase (Type I, Sigma Chemical Company) in a glass scintillation vial. After shaking the suspension in a water bath at 37° C and 250 rpm (reciprocating) for 20 min, the digested tissue was washed with cold HBSS and the islets were collected with the aid of a braking pipette and transferred to plastic Petri dishes (NUNC, Denmark) with 5 ml culture medium (100 islets per dish). The Petri dishes are made of a type of plastic which does not permit attachment of the islets. The culture medium consisted of RPMI 1640 with 20 mmol/l Hepes (Flow Laboratories) supplemented with 4.1 mmol/l NaHCO₃, 10000 U/ml penicillin, 100 mg/l streptomycin and 10% non-heat-inactivated newborn calf serum (Gibco Bio-cult). The glucose concentration of this culture medium was 11 mmol/l. Before addition of serum, the medium was kept for 3 h at 37° C; the pH was then adjusted with HCl or NaOH (1 mol/l) so that it was at the desired value after addition of serum. A small increase in pH could be observed throughout the culture period, probably caused by escape of CO₂, but this did not exceed 0.1 pH-unit. Cultures were incubated at 37° C in a humidified atmosphere of air and the culture medium was changed every second or third day and once a week glucose stimulated insulin release was studied in a short-term experiment.

Fig. 1. Immunoreactive insulin released into the medium during culture of mouse islets at different pH-values as shown at the bottom of each bar. Each bar represents the mean $\pm$ SEM of 4-9 medium samples taken during the indicated period

Insulin Release

Accumulation of insulin in the culture medium was followed by determination of the insulin content in a sample taken at each medium change. For the weekly studies of short-term insulin secretion, the islets were incubated in small Petri dishes in HBSS with 25 mmol/l Hepes at pH 7.4 (unless otherwise stated) containing 5.5 mmol/l glucose and 10% calf serum, final volume 2 ml, for 2 h; subsequently the medium was changed to 22 mmol/l glucose for 1 h, and then to 22 mmol/l glucose with 5 mmol/l theophylline. Finally, the islets were transferred to new culture medium (RPMI 1640) with 10% calf serum.

The samples from the culture medium and the secretory medium were stored at -20°C prior to insulin assay. Insulin was determined by radioimmunoassay using the double antibody technique of Hales and Randle [9] and a crystalline mouse insulin as standard (NOVO).

Results

The effect of different pH-values in the culture medium on release of insulin into the medium is illustrated in Figure 1. During the first weeks of culture, the insulin release was independent of pH from 7.2 to 7.6. Already, after one month in culture, the insulin release from islets kept at pH 6.8 was minimal and the islets became smaller and gradually disappeared. After two months only a small amount of insulin was released from islets kept at pH 7.6 and 7.4, but the islets were still present. Those kept at pH 7.0 showed a decrease after 3-4 months and those at pH 7.2 were only slightly decreased after 5 months.

Glucose-induced insulin release, in the absence of theophylline, from islets cultured at different pH-values is shown in Figure 2. Glucose-induced insulin release was very much affected by pH. In the first weeks of culture, the islets were stimulated to the highest insulin release when cultured at pH 7.6, but long-term culture at this pH reduced insulin secretion and after 2 months of culture the islets did not respond to glucose. The basal insulin release, i. e. in 5.5 mmol/l glucose during the same experimental conditions, was found to be between 0.1 and 0.5 ng insulin per ten islets and 1 hour for all cultures. Culture of islets at pH 6.8 also reduced the glucose-induced insulin release markedly. Islets cultured at pH 7.2 maintained their insulin-releasing ability; after 4-5 months of culture the glucose-stimulated insulin release was 70% of that of the first week, but only 40% of the maximal response, which was reached after one month in culture.

Insulin release, induced with both glucose and theophylline (Figure 3), was dependent on the pH in a similar way to insulin release induced with glucose alone. Islets cultured at pH 7.2 were still able to secrete insulin after 5 months of culture to the same extent as islets cultured for one week. However, during the first 50 days of culture at pH 7.2 there was an increase in the insulin releasing capacity in short-term stimulations, followed by a gradual decrease.

In order to see whether variation in pH influences glucose-induced insulin release in short-term experi-

Fig. 2. Immunoreactive insulin release induced by 22 mmol/l glucose from mouse islets cultured in RPMI 1640 at different pH-values, as shown at the bottom of each bar. Each bar represents the mean $\pm$ SEM of 2-3 experiments in each period

Fig. 3. Immunoreactive insulin release induced by 22 mmol/l glucose and 5 mmol/l theophylline from mouse islets cultured in RPMI 1640 at different pH-values, as shown at the bottom of each bar. Each bar represents the mean $\pm$ SEM of 2-3 experiments in each period

ments, islets cultured at pH 7.2 were subjected to glucose and glucose plus theophylline at different pH-values (Table 1). It is seen that the pH-value during short-term stimulation had only a minor influence on the insulin release.

Since presence of non-heat-inactivated calf serum

results in loss of insulin by degradation [18], the amount of insulin given in Figure 1 are all underestimates. The degrading capacity varied only little in the present pH-interval (data not shown). In the 1 hour release experiments (Figure 2, 3 and Table 1) the loss of insulin due to degradation is estimated to be less than 10%.

Table 1. Effect of pH in short-term incubations

pH value in short-term incubations	Insulin secretion (ng/10 islets $\times$ h)	
	22 mmol/l glucose	22 mmol/l glucose + 5 mmol/l theophylline
6.5	3.1 $\pm$ 0.5	10.8 $\pm$ 1.3
7.0	3.5 $\pm$ 0.6	15.9 $\pm$ 0.7
7.5	2.7 $\pm$ 0.2	13.0 $\pm$ 0.4
8.0	2.8 $\pm$ 0.2	11.9 $\pm$ 0.7

Immunoreactive insulin release induced by 22 mmol/l glucose or 22 mmol/l glucose plus 5 mmol/l theophylline at different pH-values from mouse islets cultured in RPMI 1640 at pH 7.2 and 10% calf serum for 15–30 days. Mean $\pm$ SEM of 4 experiments are given

Discussion

Long-term culture of isolated islets from different species in different types of culture systems, such as perfusion in filter units [12] or capillary culture units [21], culture on cellophane in culture chambers [11] or in Microtest II trays [20], have been reported. The Petri dish method developed by Andersson et al. [2, 4, 5] for tissue culture of pancreatic islets, for metabolic and functional studies, permits simple handling of the cultures and direct observation of the islets.

For transplantation purposes the maintenance of islets in a functional state for extended periods of time would be convenient in order to collect enough of the required tissue type, since the yield of islets from human tissue is variable [5]. The method recently described by Andersson [3], with islets in free floating culture in Petri dishes, seems very useful for storage of isolated islets in a functional state for a long time. Some of the factors affecting the survival and function of cultured islets have been investigated [3, 18], but the method still needs to be optimized. The presence of serum is necessary for a good survival and a normal insulin response to glucose [2, 3, 13]. Attempts to keep islets functional in culture in the absence of serum have been done with some success with microdissected islets from ob/ob mice [6], but only when the glucose concentration of the culture medium was markedly elevated. Culture in the absence of serum prevents the islets from attaching to the bottom of the Petri dishes [6]; this seems favourable, since the islets then do not migrate to form a monolayer consisting partly of fibroblasts. However, by adding homologous serum [8] or by using bacteriological Petri dishes [3, 22] islets can be kept free floating with little functional change for several months [18].

Recent studies [3, 18] have shown that the RPMI 1640 medium is superior to other commercially

available media in maintaining the glucose-stimulated insulin secretion for prolonged periods [18]. This medium contains higher concentrations of glucose (11 mmol/l), phosphate, and vitamins of the B-group than the commonly used Parker 199 medium.

In order to optimize further the conditions for maintaining isolated islets in a functional state in the free floating culture system we have studied the influence of pH in the culture medium. It has earlier been shown that non-transformed cells are especially sensitive to even small deviations from their optimum pH [7]. The culture conditions commonly used with a bicarbonate buffered medium and incubating in a CO₂-incubator, do not allow easy variation in the pH. By using an organic buffer as an alternative to bicarbonate, it is possible to vary the pH in the culture medium. In some studies, Hepes was found to be superior to bicarbonate/CO₂ with respect to maximal cell density and viability of a number of cell systems [19]. In this study we have used Hepes as buffer, but we have not excluded bicarbonate totally, since it is well known that bicarbonate is an essential cell nutrient [16] and it has been shown that bicarbonate is important for the glucose-stimulated insulin release [10].

The present studies indicate that the substitution of bicarbonate in part with Hepes changes neither the function of the islets nor the length of the culture period with good survival; the islets cultured at the optimum pH (7.2) release insulin into the medium during culture and respond to glucose loads just as well as the islets cultured in a bicarbonate buffered medium [18].

In earlier studies on freshly isolated islets Malaisse and Malaisse-Lagae [15] and Lernmark [14] found a reduction in the glucose-induced insulin release at low pH-values (5.0–6.3) and a maximal release at pH-values of 7.4–8.0, which could be due to an alteration in the calcium influx into the beta-cell [15]. Similarly, Ziegler et al. [23] reported a higher insulin release from islets cultured for 2 weeks at pH 8.0 than at pH 7.3. These results are in agreement with the insulin release during the first week of culture (Figure 1). However, the short-term effect of pH on islets cultured for 2 to 4 weeks revealed only minor differences in the glucose-induced insulin release (Table 1), and long-term exposure to various pH resulted in loss of islets when pH is below 7.0 and loss of the insulin releasing capacity when pH is higher than 7.4. The best preservation of beta-cell function was found at pH 7.2.

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