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Preservation studies using acinar cell cultures of the pancreas: stimulation of amylase/lipase release before and after hypoxic stress

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Abstract In clinical pancreas transplantation, postischemic (i.e. postpreservation) transplant pancreatitis is a major problem in some cases but also an interesting model of pancreatitis. In this study, the effect of simulated organ preservation of isolated acinar cells was evaluated as regards enzyme release (basic and cerulein-stimulated), using common preservation solutions as the incubation media. Primary pancreas acinar cell cultures were isolated from the pancreas of male Wistar rats using the modified method of Amsterdam and Jamieson [1]. After resting the cells in culture flasks for 1 week, monolayer cultures were obtained. The basic enzyme release of amylase and lipase was measured as well as the effect of stimulation with cerulein (10^{-8} M) and the effect of replacing the medium (without changing the consistence or temperature of the medium). In a second step, the cltures were incubated under conditions of cold hypoxia for 6 h (4 °C, $P O_2 < 0.1$ mm Hg) using Krebs-Henseleit solution (KH), Euro-Collins solution (EC), HTK solution of Bretschneider (HTK) or University of Wisconsin solution (UW) as the incubation solution. After 6 h, the media were changed to warm normoxic KH, and a second stimulation test with cerulein was performed. The native microstructure of the cultures was observed as well. Enzyme release was elevated by a factor of 5 by stimulating the acinar cells with cerulein as well as by changing the medium in the experiments prior to the hypoxic incubation. After hypoxic incubation and change to KH, the morphology of the cultures was excellent, while the basic enzyme release was on a very low level, no matter which preservation solution was used during cold hypoxia. Stimulation with cerulein caused only minimal elevation of enzyme release during an observation period of 60 min. These observations show that cold storage in preservation solution provides maintenance of the cell morphology and sufficient down-regulation of enzyme release of pancreatic acinar cells. Thus, acinar cells alone do not seem to be the pacemaker of pancreatitis after organ preservation. The presented experimental model will be the subject of extended evaluation in the future.

Key words Preservation studies
Pancreas · Acinus cells

Introduction

Due to its special structure and function, the exocrine pancreas is very sensitive to shock, ischemia, direct injury, or drugs. Thus, severe shock may be followed by partial or total necrosis of the organ, presenting as severe pancreatitis with shock, fever, pain, and exocrine pancreas insufficiency.

A special form of postischemic pancreatitis is known from pancreas transplantation, which represents a major problem in some cases as well as an interesting model of pancreatitis. In this form, the process of harvesting, preservation, and reperfusion injury leads to inflammation and necrosis, so that treatment and prophylaxis can be performed before the beginning of the pathomechanisms. This fact has played a major role mainly in the development of modern preservation solutions.

Since cell culture studies are used more and more to evaluate preservation solutions and their components on a preclinical level, in this study the effect of stimulated organ preservation of isolated acinar cells was studied as regards enzyme release (basci and cerulein-stimulated).

Materials and methods

Cell preparation

Primary pancreas acinar cell cultures were isolated from the pancreas of male Wistar-rats using the modified method of Amsterdam and Jamieson [1, 4].

After killing the Wistar rat, the pancreas was removed and trimmed of fat. A volume of the first type of digestion solution was injected into the parenchyma. This solution is based on collagenase I and soybean trypsin inhibitor, which are dissolved in a medium containing phosphate and HEPES buffer as well as Eagle's essential medium, glucose, glutamine, and sodium pyruvate, glutamate, and fumarate. After the sequential addition of EGTA and hyaluronidase, the pancreas tissue dispersed and could be passed several times through a 19-gauge needle. After final filter passage and centrifugation, the cell pellet was seeded into culture flasks coated with collagen.

During storage and conditioning of monolayers, the cells were stored in the incubator at 37 °C under a special culture medium normally recommended for the storage of whole acini. It contains Ham's F-12, Dulbecco's MEM, HEPES, albumin, soybean trypsin inhibitor, ascorbic acid, EGF, ITS (insulin, transferrin, selenium), penstrep, and amphotericin B.

Experiments

After resting the cells in culture flasks for 1 week, monolayer cultures were obtained. The basic enzyme release of amylase and lipase was measured as well as the effect of stimulation with cerulein (10^{-8} M) [2] and the effect of replacing the medium (without changing the consistency or temperature of the medium). After 6 h, the media were changed to warm normoxic Krebs-Henseleit solution (KH), and a second stimulation test with cerulein was performed. The native microstructure of the cultures was observed as well. The same procedures were repeated using common preservation solutions [Euro-Collins (EC), HTK solution of Bretschneider and University of Wisconsin (UW; (Belzer) solution] as incubation medium in the cold hypoxic phase. Reoxygenation and rewarming was performed in KH as well.

Results

By isolating and treating acinus cells as described, it was possible to achieve monolayer cultures, which were not confluent. The cells could be identified as acinus cells by the maximal release of amylase and lipase during the first 2 weeks. Amylase and lipase release rose up to 3000–5000 IU/l during the first day after isolation, while after 2 weeks the lipase activity was always very low. Enzyme release was elevated by a factor of 5 by stimulating the acinar cells with cerulein as well as by changing the medium in the experiments prior to hypoxic incubation (Fig. 1 a). After hypoxic incubation and change to warm

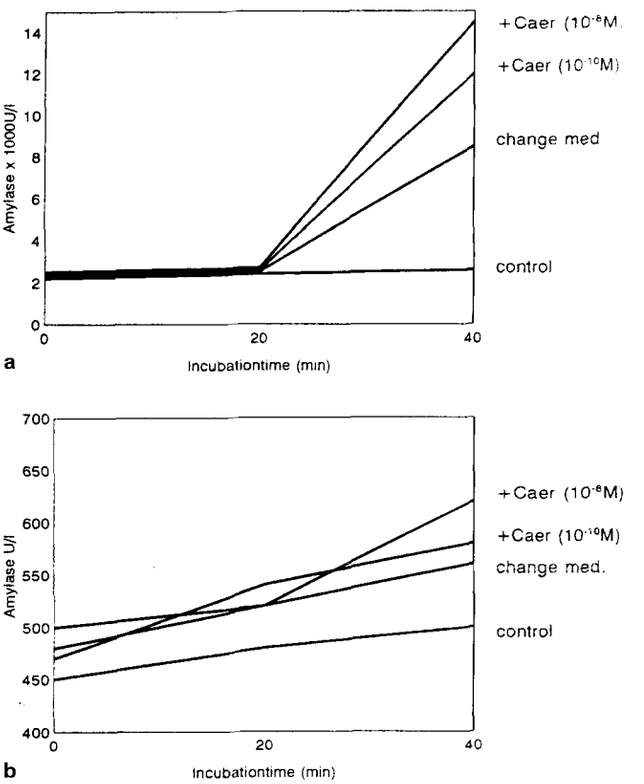

Fig. 1 a, b Amylase release by cultured pancreas acinus cells after stimulation with cerulein (*Caer*) before (**a**) and after (**b**) simulated organ preservation (mean values from 40 incubations of five different pancreas preparations)

normoxic KH, the morphology of the cultures was not significantly altered, while the basic enzyme release was on a very low level, no matter which preservation solution was used during cold hypoxia. Stimulation with cerulein caused only a minimal elevation of enzyme release during an observation period of 60 min, while the controls remained unchanged; a change of medium resulted in no significant release of enzymes. The outcome of the stimulation test after cold hypoxic incubation in the above-mentioned preservation media was exactly as in KH.

Discussion

Since it can be demonstrated that cell culture simulation experiments form a valuable model for studing liver preservation [5] in this paper the first results of preservation simulations in pancreas cell cultures were obtained in a model experiment. The observation that in native monolayers of primary cultured pancreatic acinus cells, high enzyme release can be stimulated by cerulein but not by cold storage may be carefully interpreted as an efficient effect of the cold storage itself. However, it may be that the sensitivity to cerulein decreases during the conditioning of monolayer cultures. There is no doubt that the cold storage solutions are able to maintain unaltered cell morphology. Also, the cells of the monolayer do not accumulate trypan blue and thus probably represent a selected cell population which is extremely resistant to hypoxic cell stress when preserved well and in a cold environment, e.g., cultured hepatocytes are much more sensitive to hypoxia and reoxygenation [5].

Under these conditions, the conclusion may be drawn that acinus cells of a transplanted pancreas are preserved, so well that they are unable to act as the pacemaker of pancreatitis and necrosis. Concerning pathological situations like the postischemic phase of shock or organ transplantation, there are valid data which indicate that the first step of postischemic injury is paced by the immigration of great amounts of white blood cells into the parenchyma of the organ [3]. The experimental model must be evaluated further.

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