

# Adrenergic Lipolysis in Immobilized and Perfused Adipocytes

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## Summary

The method of cellular immobilization and perfusion was applied to adipocytes. The lipolytic effect of isoprenaline, whose action is produced as a result of receptor-drug interaction, was followed. An agarose solution kept at at 37 °C was mixed 1:1 with the cell suspension. Thereafter, adipocytes were immobilized in the agarose threads. The lipolytic effect of 0.1 ml of isoprenaline ( $1 \times 10^{-4}$  mol/l), that was rapidly introduced to the cell perfusion inlet in a non-recirculating system, was monitored by assessing glycerol production. The immobilized and perfused adipocytes exhibited significant lipolytic activity. After reaching the maximum effect, 0.1 ml of propranolol ( $1 \times 10^{-3}$  mol/l) that was applied to the bioreactor inlet, abolished the isoprenaline effect. The present data demonstrate the potential applicability of immobilized perfused adipocytes for various kinds of studies.

## Key words

Immobilized adipocytes – Isoprenaline – Propranolol

## Introduction

In the previous articles of this issue, a detailed description of the metabolism of both exogenous and endogenous compounds by isolated liver cells in a small laboratory bioreactor has been presented (Farghali *et al.* 1994, Kameníková *et al.* 1994, Hynie *et al.* 1994). This experimental cellular model (where the isolated hepatocytes were immobilized in agarose) enabled the perfusion of cells and consequently the follow up of cell functions for a long period of time (Foxall *et al.* 1984).

It was found that the utilization of this small scale laboratory bioreactor is advantageous over other cellular models. This system made it possible to obtain information which could hardly be found by using other cellular models, such as in isolated cellular suspensions, and the data are much more informative. These improved conditions which enable an excellent cellular performance has stimulated us to apply the procedure of cellular immobilization and perfusion to another type of cells, namely, adipocytes. According to the available literature, the perfusion of immobilized adipocytes has not been reported previously. Potentially, the preparation of fat cell bioreactor

broadens the application of cellular perfusion. Among other possible applications, the present study deals with the lipolytic effect of different drugs, whose action is produced as a result of receptor-drug interaction.

## Methods

The preparation of adipocyte bioreactor consists of two steps.

### 1. Preparation of the fat cells

The method of Rodbell (1964), with some modifications, was applied for the isolation of fat cells. Male Wistar rats (body weight of 200–250 g) which had free access to a commercial diet and water were used for fat cell isolation. Adipocytes were prepared from the rat epididymal adipose tissues. A medium consisting of Krebs-Ringer bicarbonate buffer (pH = 7.4) containing 1 % bovine serum albumin and glucose (1.04 g/l) was used. The enzyme collagenase was added to 5 ml medium at the concentrations of 1–2 mg/g adipose tissue. After the addition of collagenase, the

adipose tissue was incubated in the forementioned medium under the atmosphere of carbogen for either 60 or 30 min, respectively. The collagenase concentrations that were utilized in the present study are much lower than those used by other authors (Rodbell 1964, Allen *et al.* 1973). After the incubation period, fat cells were separated from the connective and non-digested tissues by filtration through a nylon mesh. The isolated fat cells were washed 3 times with glucose-free Krebs-Ringer phosphate buffer containing 1 % bovine serum albumin. The reason for glucose exclusion is its interference with the assay of glycerol which was used as an indicator for the degree of lipolysis in this study.

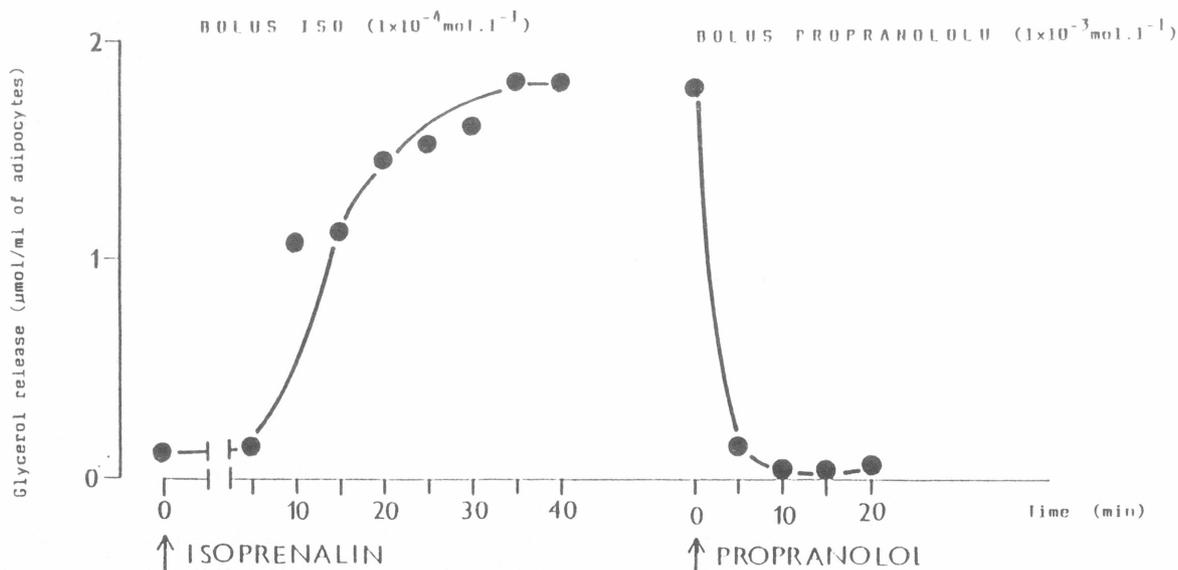
It is noteworthy to mention that adipocyte isolation and handling must be performed in either siliconized glass or plastic containers. To demonstrate the good functional activity of adipocytes, the response of the isolated adipocytes to the lipolytic effect of isoprenaline was evaluated.

In pilot experiments, we have demonstrated that the selected collagenase concentrations of 1 and 2 mg/g adipose tissue, under the forementioned conditions, produced a good yield of isolated fat cells. The functional activity of the cell harvest that was obtained by both collagenase concentrations, was assessed by the degree of lipolysis produced by isoprenaline ( $1 \times 10^{-6}$  mol/l), the phosphodiesterase inhibitor, theophylline ( $2 \times 10^{-4}$  mol/l) and the potentiation of the lipolytic effect of isoprenaline by theophylline. There were no significant differences by using either concentration of collagenase.

## 2. Immobilization and perfusion of the isolated adipocytes

In preliminary experiments, the immobilization of adipocytes in agarose threads was carried out in a similar way as described earlier for hepatocytes (Farghali *et al.* 1994). The agarose solution kept at  $37^\circ\text{C}$  was mixed 1:1 with a suspension of fat cells (usually 5 ml). The mixture was immobilized in the agarose threads by extruding the agarose-cell mixture through cooled Chemflur teflon TFE tubing (0.5 mm internal diameter) into a cooled medium in the bioreactor container. Cooling of the medium used for threading of the adipocytes resulted in the production of immobilized cells with minimum functional activity as assessed by the isoprenaline lipolytic effect. The low functional activity of adipocytes prepared under the forementioned conditions in the bioreactor model may be due to several factors (Lech and Calvert 1966, Vaughan and Steinberg 1965). For example, it is known that the fat cells are very sensitive to the decrease in temperature. Cooling of fat cells halts the lipolytic process in general not only because of increasing the rigidity of the phospholipid cellular membrane but also due to the overall effect on the adenylate cyclase system.

Glycerol concentration in the incubation or in the perfusion media of the adipocytes was measured according to Lambert and Neish (1950).



**Fig. 1**

The effect of isoprenaline and propranolol on glycerol release from immobilized perfused rat epididymal adipocytes. Both isoprenaline ( $1 \times 10^{-4}$  mol/l) and propranolol ( $1 \times 10^{-3}$  mol/l) were added as a bolus.

## Results and Discussion

To prepare well functioning immobilized and perfused adipocytes, extra cooling of the cells is not recommended. It seems that minimum cooling which permits threading (gelling) of the agarose-cell mixture is convenient for well functioning adipocytes. In fact, the agarose-cell mixture was extruded in the bioreactor tube at the laboratory temperature (about 22 °C) when the agarose containing the adipocytes was gelled and the immobilized cells were carefully packed to a final volume of 8 ml. The immobilized cells were perfused in a non-recirculating fashion with glucose-free Krebs-Ringer phosphate buffer containing 1 % bovine serum albumin at a rate of 1 ml/min. The outflow from the bioreactor was used for the assay of glycerol. After 1–2 h period of stabilization, samples of the medium were collected before isoprenaline administration and at 5 min intervals after its application. The latter was introduced at the inflow inlet of the bioreactor as a bolus of 0.1 ml (Isoprenaline at concentration  $1 \times 10^{-4}$  mol/l). Fig. 1 illustrates significant lipolytic activity in the bioreactor adipocytes which were responding to lipolytic stimulation by isoprenaline. The maximum lipolytic effect was obtained 30–35 min after isoprenaline application. The relatively long lag period after which maximum lipolysis occurs is apparently due to the dead volume of the bioreactor and due to the diffusion of isoprenaline in the gel matrix before reaching cell receptor sites. Moreover, the observed

delay in the response to isoprenaline might be, in part, an essential feature of the lipolytic response *per se*.

At the peak of the isoprenaline effect, it was necessary to test its  $\beta$ -adrenergic specificity. Therefore, 0.1 ml of propranolol ( $1 \times 10^{-3}$  mol/l) was applied to the bioreactor in the form of a bolus. As shown in Fig. 1, 10 min after propranolol addition, the isoprenaline lipolytic effect was completely abolished which indicates the specificity of the observed effect.

In addition to the adipocyte functional activity in the bioreactor model, we were interested in observing the histological appearance of the cells after their immobilization in agarose. Light microscopy demonstrated cells containing neutral lipids and other fat cells with high metabolic activity, as revealed by their low lipid content.

In conclusion we were able, for the first time, to prepare functioning adipocytes in a bioreactor model. The present data demonstrate the potential applicability of immobilized perfused adipocytes for various kinds of studies. More work has to be done to find out the optimum conditions for adipocyte functions in a small bioreactor.

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## Reprint Requests

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