notes on methodology

Characterization of the multiple-chamber perifused fat cell system

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Summary A multiple-chamber perifused fat cell system is described. Six chambers containing fat cells were perifused in parallel with buffer. Perifusate was collected for assay of glycerol as an index of lipolytic rates and cells in each chamber can be taken for analysis of biochemical intermediates. The system is so designed that drugs can be infused into the buffer and equally distributed in each chamber or can be individually infused into the buffer to one chamber, allowing for six different conditions to be tested in the same population of fat cells. The time and distribution characteristics of infused material are described. Time relationships are described for isoproterenol and glycerol release and for cyclic AMP levels in the fat cells, and the dose–response relationship between isoproterenol and glycerol release is shown.—**Allen, D. O., K. J. Long, and J. T. Majors.** Characterization of the multiple-chamber perifused fat cell system. *J. Lipid Res.* 1979. **20:** 1036–1040.

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The study of hormonal control of the lipolytic process was greatly facilitated by development of the isolated fat cell preparation (1). These isolated cells could be incubated in buffered solutions and the rates of lipolysis could be quantitated by the accumulation of glycerol and/or free fatty acids. Additionally, intermediate biochemical reactions such as elevations of cyclic AMP levels and increase in protein kinase activity could be determined in these flask-incubated cells (2). However, this technique suffered several disadvantages. The accumulation of materials such as free fatty acids (3, 4) and other agents (5, 6) exerts an inhibitory influence on the lipolytic process, and the accumulated free fatty acids result (6, 7) in altered pH of the buffered solution. Also, changes in lipolytic activity over short periods of time cannot be assessed by this technique. Generally, times greater than half an hour are necessary for adequate accumulation of the products of the lipolytic process.

Many of these disadvantages of the flask incubation technique were overcome by the development of the perifused fat cell system (8). In this technique cells are placed in a column through which appropriate buffer is pumped, thus perifusing the fat cells. Collection of the perifusate from the column and the assay of glycerol allowed for the continuous monitoring of lipolysis using time periods as short as 15 sec. This technique has been used to study the effect of a number of drugs and hormones on lipolytic activity.

The perifused fat cell system, however, suffered from the limitations that samples of adipose cells could not be taken for the determination of biochemical intermediates such as cyclic AMP levels and only one condition could be studied at one time. The present report describes a multiple-chambered perifused fat cell system, the use of which allows for determination of lipolytic activity under several conditions and the determination of biochemical intermediates from a single population of cells.

METHODS

Isolated fat cells were prepared from fed, Sprague-Dawley rats (180–220 g) by the method of Lech and Calvert (9). The cells were washed with Krebs-Ringer bicarbonate and suspended in the same buffer. An aliquot of the cell suspension was transferred to each of the six chambers of the apparatus shown in Fig. 1. This apparatus consisted of six parallel plastic columns into which buffer was pumped. Both ends of the columns were plugged with fibrous filter material to form a cell chamber with a volume of approximately 3.0 ml. A Krebs-Ringer bicarbonate buffer (pH 7.4) containing 1% (w/v) bovine serum albumin maintained at 37°C in a constant temperature water bath was pumped into the tops of the columns at a flow rate of 2.5 ml/min per chamber.

The flow of the buffer through the cell chambers produced a constant mixing of the fat cell suspension. An injection port was situated at the top of each column in such a way that a hypodermic needle could be inserted and through which drugs could be infused at a constant rate (0.1 ml/min). Another injection port was located in the common tubing just before the splitting of the flow for each of the chambers. A mixing chamber was situated just downstream from the port, which assured adequate mixing of the infused drug with the buffer and thereby

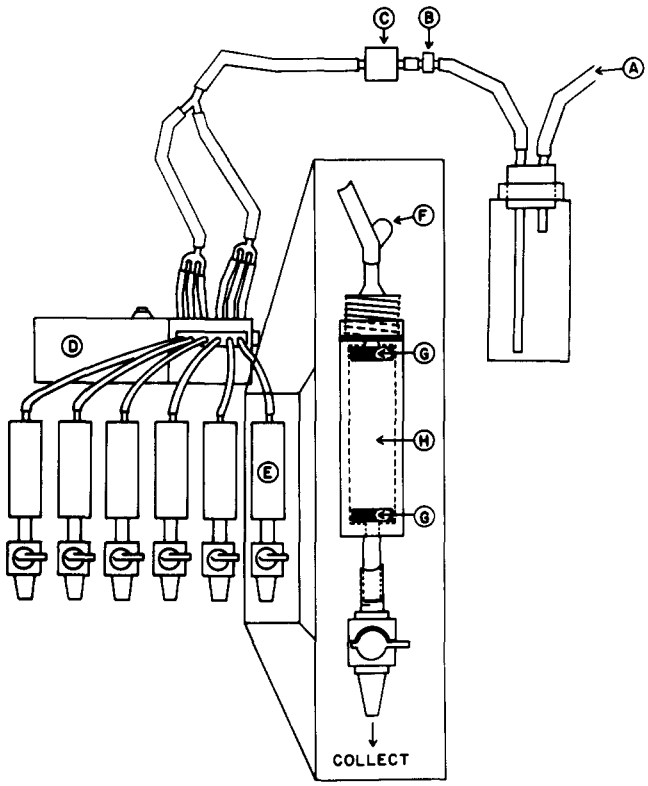

Fig. 1. Apparatus for multichamber perifused fat cell system. *A*, flow of 95% O_2/5% CO_2; *B*, injection port; *C*, mixing chamber; *D*, multichannel pump; *E*, cell chamber; *F*, injection port of individual column; *G*, fibrous filter plugs; *H*, cell chamber.

equal distribution of the compound to each of the six chambers. The mixing chamber contained a magnetic stir bar which was kept in motion by a magnetic stirrer. All drug concentrations refer to the final concentration of the material reaching the cells in the perifusing buffer.

The perifusate was collected from the bottom of each column for a known period of time and the concentration of materials was determined. In other experiments the columns were disconnected at appropriate times and the entire content of the chamber was placed in 15-ml centrifuge tubes. For the determination of cyclic AMP levels, each centrifuge tube contained sufficient trichloroacetic acid to give a final concentration of 5%. Even when a column was disconnected, flow through that channel was maintained, thus leaving unaltered the flow through the remaining columns and through the total system.

The rate of lipolysis was quantitated by determining the amount of glycerol which appeared in the perifusate over a known period of time. Glycerol was determined by the method of Chernick (10). The cyclic AMP content of the fat cells was determined by the radioimunoassay of Steiner et al. (11).

RESULTS

A series of experiments was conducted to determine the flow and distribution characteristics of buffer and drugs through the apparatus. Chambers without fat cells and containing filter plugs were used. A solution of Blue Dextran 2000 was infused into the perifusing buffer at a point just before the mixing chamber (Point *A*, **Fig. 1**). The perifusate from each of the six chambers was collected for 30 sec at 2-min intervals and the concentration of dye was determined at 600 nm in a spectrophotometer. Following a 2-min lag period, dye rapidly appeared in the perifusate reaching steady-state values by 8 min after the start of the infusion (data not shown).

In another series of experiments, Blue Dextran 2000 was infused at the rate of 0.1 ml/min into each of the six chambers through the injection port located just above the entrance to the chambers (Point *F*). At 1-min intervals, one of the chambers was disconnected from the perfusion system and the entire contents of the chamber were transferred to a centrifuge tube following which the absorbance was determined (**Fig. 2**). By 1 min, substantial amounts of the dye had entered the chamber and by 4 min equilibration had been reached. The concentration of the dye in the chambers remained constant for at least 1 more min.

The dose–response curve characteristics for isoproterenol on lipolysis were determined in this system

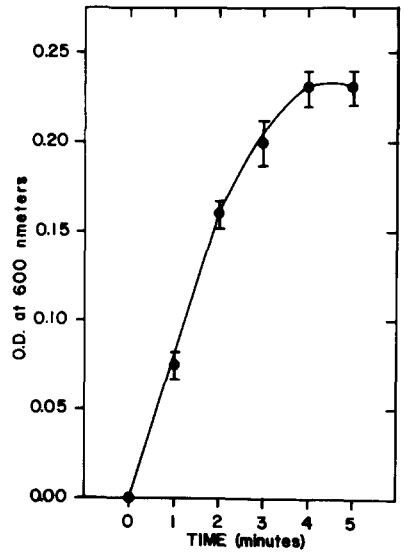

Fig. 2. Time course of appearance of Blue Dextran 2000 in the chambers of six-chamber perifusion system. Blue Dextran 2000 was infused into each chamber at point *F* (see Fig. 1). At appropriate times the entire contents of a chamber were collected and treated as described in Methods. Results are expressed as the mean and SEM of five experiments. Zero time is the time the dye infusion was started. O.D., optical density (absorbance).

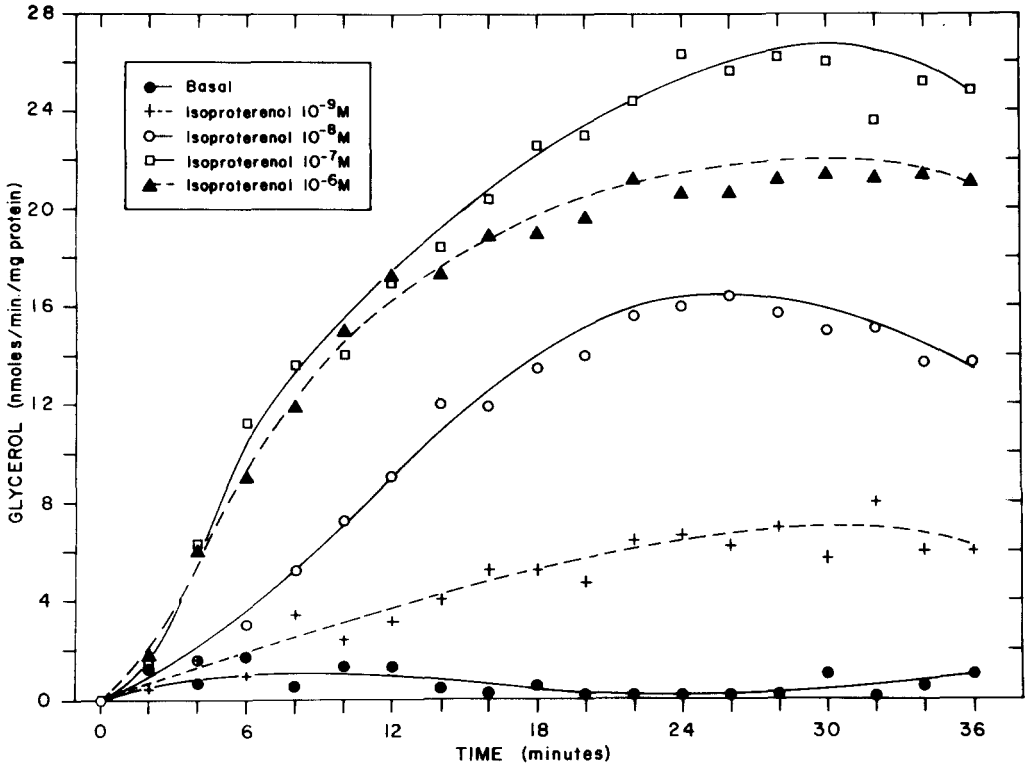

Fig. 3. Time course of the lipolytic response to various concentrations of isoproterenol. Different concentrations of isoproterenol were infused into the respective injection ports of the individual columns (see point *F*, Fig. 1). Samples of perifusate were collected and analysis for glycerol was as described in Methods. Results are expressed as the means of five experiments. Zero time is the time the isoproterenol infusion was started.

(**Fig. 3**). Different concentrations of isoproterenol were infused into the different chambers at a point just before the entrance to the chamber. Perifusate was collected at 2-min intervals and glycerol concentration was determined. Concentrations as low as 10^{-9} M isoproterenol produced a significant increase in the rate of glycerol output. Concentrations of 10^{-8} and 10^{-7} M isoproterenol resulted in even greater increases in rates of lipolysis. Maximum lipolytic rates at all concentrations were reached approximately 26 min after the start of the catecholamine infusion.

The time course for isoproterenol-stimulated increases in cyclic AMP was determined in a series of five experiments (**Fig. 4**). Isoproterenol (10^{-6} M) was infused into the injection port just anterior to the mixing chamber. At appropriate times each of the chambers was removed from the perifusion system and the entire contents were rapidly transferred to a centrifuge tube containing sufficient trichloroacetic acid to result in a final concentration of 5%. Cyclic AMP levels were then determined as described in Methods. To eliminate the lag period found in the dye infusion experiment, zero time was taken as 1 min after the start of the isoproterenol infusion. Significant increases in cyclic AMP levels were seen by 1 min with further increases occurring at 2 min and maximal values obtained by 3 min. Levels at 4 and 5 min remained at a maximum.

DISCUSSION

Development of the perifused fat cell system has facilitated the study of hormone-stimulated lipolysis (12, 13). Repetitive samples of perifusate can be collected and rates of lipolysis can be assessed over very short time intervals. The system, however, suffers the disadvantage that multiple concentrations of agent cannot be studied in the same population of cells nor can tissue samples be taken for biochemical analysis. Attempts to use several single channels simultaneously were unsuccessful. Variations in pump speeds and infusion rates resulted in nonuniform delivery of drugs.

The development of the multichamber perifused fat cell system overcomes these disadvantages. The simultaneous perifusion of six samples of fat cell allows for uniform drug delivery and time studies

or for comparison of the effect of six different conditions on the same cell population. The system is so designed that one or more of the samples can be taken for analysis of biochemical events within the cells. This was not possible with the single-channel system.

For the present system to be of value, it was necessary to characterize it in terms of distribution of perifusate to the various chambers and in terms of the times for equilibration of materials with the cell population. Additionally, it was necessary to show that cell populations could be taken for biochemical analysis.

Characterization of the six-chambered perifused fat cell system showed that there was a rapid appearance in the perifusate of materials infused into the perifusing medium. There was an equal distribution of the dye to each of the six columns. At equilibrium the concentration of dye in each perifusate varied by less than 5%. The mixing chamber shown in Fig. 1 is essential to this equal distribution of the compounds. Similar types of experiments conducted without a mixing chamber demonstrated greater than a 25% deviation in the concentration of dye in the different channels (data not shown).

Equilibrium was established more rapidly within the cell chambers than in perifusate. Taking into account the 1-min lag time for the drug to reach the chambers (0 time taken as 1 min after the start of the infusion), there is a very rapid equilibration of the dye within the chambers with maximum concentration being achieved at 4 min. Within 1 min the dye concentration had reached greater than 30% of its final concentration.

As noted previously (8), there is a time lag between the introduction of the hormonal stimulant and maximum rates of lipolysis. A similar lag period was noted in the present study. This time delay was not a function of equilibration of the drug with the cells as equilibrium was established within 4 min while maximum lipolytic activity was not seen until 26 min after the introduction of isoproterenol. The time to reach maximum lipolytic activity appeared to be unrelated to the concentration of isoproterenol used. The dose–response relationship for isoproterenol seen in this system was similar to that observed in the more traditional flask incubation method (14).

Cyclic AMP levels increased in the fat cells over a 3-min period following the start of the isoproterenol infusion. This time most likely represents the response time of the cells to a maximum stimulating concentration of isoproterenol, for a supermaximum concentration of 10^{-6} M isoproterenol was used. As seen previously, the material equilibrates rapidly in

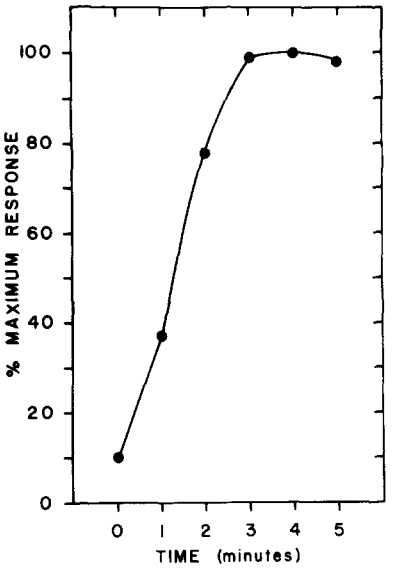

Fig. 4. Time course for the accumulation of cyclic AMP following an infusion of isoproterenol. Isoproterenol (10^{-6} M) was infused at point *B* and the contents of the chambers were taken at various times for determinants of cyclic AMP content. Results are expressed as the means of five experiments. Zero time was the time 1 min after isoproterenol infusion was started (lag time).

the chambers with greater than 30% of the maximum of concentration occuring at 1 min. This means that the maximum stimulating concentration of isoproterenol (10^{-7} M) was reached in the cell chambers prior to the 1-min time period. Therefore, the time response for cyclic AMP represents the time required for the cells to respond and was not a function of the mechanics of the system.

It appears, therefore, that the multichamber perifused fat cell system can be used to collect multiple tissue samples for biochemical analysis, thus providing an advantage over the single-channel perifused fat cell system. The system also is useful in determining the response of the same population of cells to multiple concentrations of hormone or to varying conditions. These are advantages over the single-chambered perifused fat cell system in which different conditions had to be determined in different cell populations and no samples could be taken for biochemical analysis.

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