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ANTILIPOLYTIC ACTION OF INSULIN IN THE PERIFUSED FAT CELL SYSTEM*

DONALD O. ALLEN and ELIZABETH A. GARDNER

Department of Pharmacology, University of South Carolina, School of Medicine, Columbia, SC 29208, and College of Science, Rochester Institute of Technology, Rochester, NY 14623, U.S.A.

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Abstract—The antilipolytic action of insulin was investigated using the perifused fat cell system. Epinephrine (10^{-5} M) and glucagon (5×10^{-6} M) both stimulated lipolysis by at least 6-fold, and insulin inhibited both responses. The time course and the magnitude of the antilipolytic action of insulin were determined in the perifused fat cell system. At a concentration of 100 μU/ml, insulin inhibited epinephrine-stimulated lipolysis approximately 50 percent, with a half-time response of 4–5 min. The antilipolytic action of insulin persisted for at least 45 min. following the termination of insulin infusion. This prolonged phase of the action of insulin could be terminated by treatment of the cells with trypsin. These results suggest that only small percentage of insulin receptors is occupied during maximum lipolytic activity and that in adipose tissue many spare receptors exist for insulin.

A great deal of attention has been accorded to the mechanism by which insulin regulates metabolism in a variety of tissues. One particularly useful model is the isolated fat cell preparation which has been used to study insulin effects on glucose utilization [1, 2] and lipolytic activity [3, 4]. This system also has been used to examine the binding of insulin to specific cell surface receptors and the kinetics of that binding [5–7].

Very little work has been done on correlating the kinetics of insulin binding to adipocytes with the physiological action of insulin. This relationship was examined by Gliemann *et al.* [7] for the lipogenic action of insulin. However, no correlation was drawn between insulin binding and the antilipolytic action of this hormone. This was due particularly to the difficulty of studying the rates at which lipolytic activities changed following the addition of insulin, a difficulty which was overcome by the development of the perifused fat cell system [8]. Using this technique, it is possible to measure lipolytic rates over very short periods of time (less than 1 minute) and to examine the rates at which changes in lipolysis occur following the addition of hormones or the removal of these materials from the system [9].

The purpose of this study was to examine the antilipolytic effect of insulin in the perifused fat cell system and to correlate the action of insulin with existing data on the binding of this material to the fat cells.

METHODS

Isolated fat cells were prepared from the epididymal fat pads of fed Cox Holtzmann rats (weighing from 150 to 220 g) according to the methods of Lech and Calvert [10]. Isolated fat cells were perifused as described previously [8]. Briefly, 1 ml of packed cells was placed in a water-jacketed plastic column maintained at 37°. Krebs–Ringer bicarbonate buffer (pH 7.4) containing 1% (w/w) albumin was pumped into the top of the column at a flow rate of 2.5 ml/min. The buffer was maintained at 37° under an atmosphere of 95% oxygen–5% CO_2. The fat cells were perifused for a 10-min equilibration period after which several samples were taken over the next 10 min for the determination of basal rates of lipolysis. Each agent to be tested was infused into the cell chamber by means of an injection port located at the top of the column. Infused material reached 90% of maximum concentration in the perifusate, leaving the column in 2 min [8]. All concentrations of hormones are expressed as the concentration of material reaching the fat cells. The perfusate was collected at the bottom of the column for 1-min intervals.

The concentration of glycerol in the perfusate was determined by the fluorometric method of Chernick [11]. Protein content of the isolated fat cells was determined by the method of Lowry *et al.* [12].

Bovine serum albumin (fraction V) and *l*-epinephrine were purchased from the Sigma Chemical Co. (St. Louis, MO). Crystalline porcine insulin (24 U/mg) and glucagon were supplied by Eli Lilly & Co. (Indianapolis, IN).

RESULTS

An infusion of epinephrine (10^{-5} M) into the perifused fat cell system resulted in a large increase on lipolytic activity, with maximum rates occurring in 30 min. While maintaining the infusion of epinephrine for the remainder of the experiment, insulin (100 μU/ml) was also infused for 25 min. Insulin reduced the lipolytic activity by about 50 percent. Maximum inhibition was observed 20 min after the start of the insulin infusion. Half-time for the inhibitory effect was calculated at 5 min. Following termination of the insulin infusion (but in the presence of epinephrine), the inhibition of lipolysis continued for the duration of the experiment (45 min). A summary of four experiments of this type is shown in

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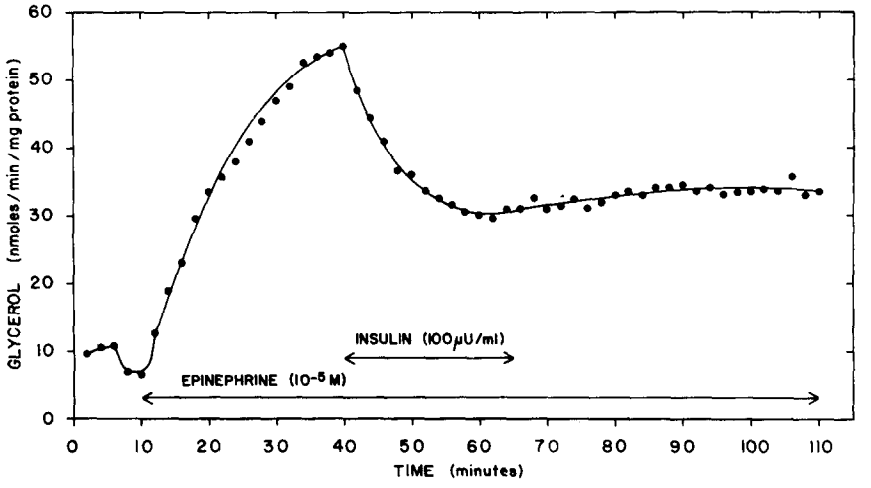

Fig. 1. Antilipolytic action of insulin (100 μU/ml) in the perifused fat cell system. Epinephrine and insulin were infused during the times indicated by the arrows. Results are expressed as the means of four experiments.

Fig. 1. Five similar experiments were conducted using a lower concentration of insulin (10 μU/ml), and similar results were seen. Following the termination of the insulin infusion, the lipolytic activity remained at the reduced value and failed to return to pre-insulin values (Fig. 2).

In five experiments in which glucagon (5×10^{-6} M) was used as the lipolytic agent, insulin was also found to be antilipolytic. The infusion of glucagon resulted in a lipolytic response which reached maximum values in approximately 25 min. Infusion of insulin (100 μU/ml) resulted in a 50 percent reduction in the lipolytic response and reached maximum inhibitory levels approximately 15 min after the start of the infusion. Half-time for the antilipolytic effect of insulin was approximately 4 min. As seen in the other experiments, the inhibitory action of insulin was prolonged following the termination of the insulin infusion. The antilipolytic action of the hormone persisted for the duration of the experiment, a period of 45 min (Fig. 3).

In a series of six experiments, an attempt was made to overcome the prolonged inhibitory influence of insulin by using two lipolytic stimuli. The antilipolytic effect of insulin on epinephrine-stimulated lipolysis was established over a 30 min period of time following which the insulin infusion was terminated but the epinephrine infusion continued. Ten minutes

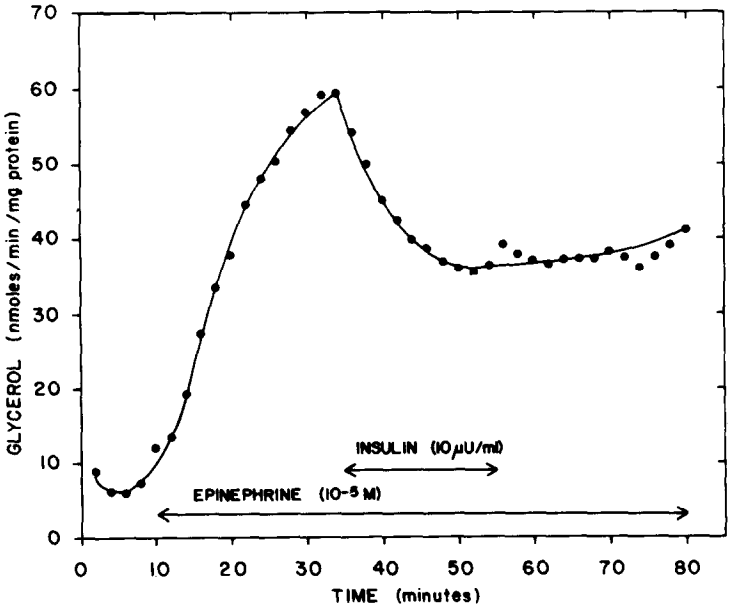

Fig. 2. Antilipolytic action of insulin (10 μU/ml) in the perifused fat cell system. Epinephrine and insulin were infused during the times indicated by the arrows. Results are expressed as the means of five experiments.

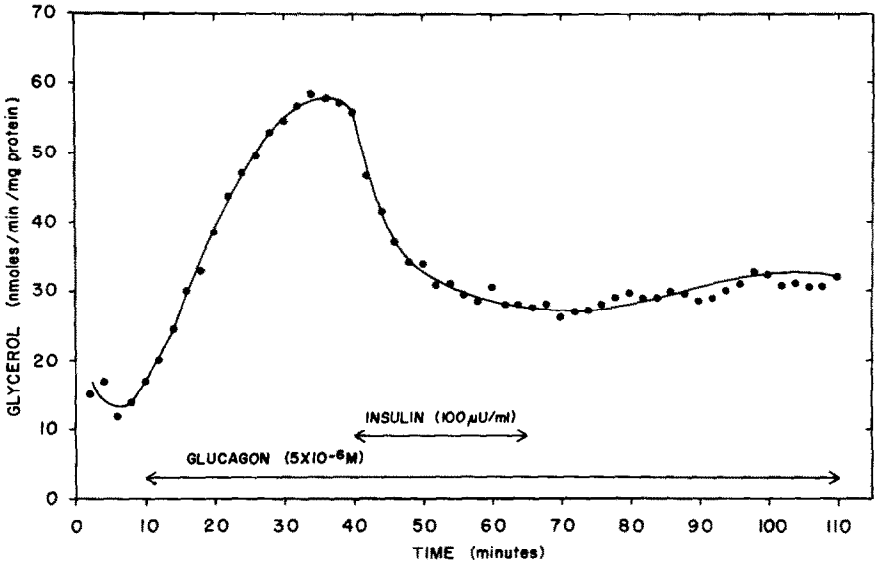

Fig. 3. Antilipolytic action of insulin on glucagon-stimulated lipolysis in the perifused fat cell system. Glucagon and insulin were infused during the times indicated by the arrows. Results are expressed as the means of five experiments.

later an infusion of glucagon (5×10^{-6} M) was started so that the cells were exposed to both epinephrine and glucagon. Even under these conditions of dual stimulation the prolonged antilipolytic action of insulin could not be overcome (Fig. 4).

In an attempt to terminate the prolonged antilipolytic action of insulin, cells were exposed to trypsin following an exposure to insulin and in the presence of epinephrine. An infusion of epinephrine (10^{-6} M) was begun and insulin (10 μU/ml) was added 20 min later for a 20-min time period. As before, the lipolytic activity failed to return to pre-insulin values over the next 15 min. At that time, trypsin (500 μU/ml) was infused for 25 min during which lipolytic activities remained depressed. Following termination of the trypsin infusion (but still in the presence of epinephrine), lipolytic activity increased and returned toward pre-insulin values (Fig. 5).

DISCUSSION

Many investigators have studied the mechanisms by which insulin regulates metabolism in a variety of tissues. Particular emphasis has been placed on the regulation of adipose tissue metabolism, with studies focusing on the binding of insulin to membrane receptors [5–7] and the regulation of metabolic events such as increased glucose uptake [1, 2], and increased lipogenesis [7, 13].

Owing to the methological difficulties in assessing

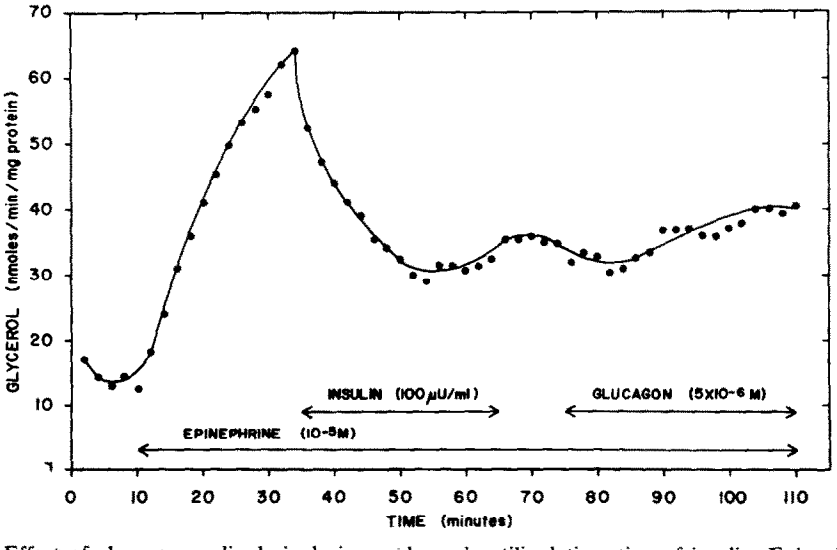

Fig. 4. Effect of glucagon on lipolysis during prolonged antilipolytic action of insulin. Epinephrine, insulin and glucagon were infused during the times indicated by the arrows. Results are expressed as the means of six experiments.

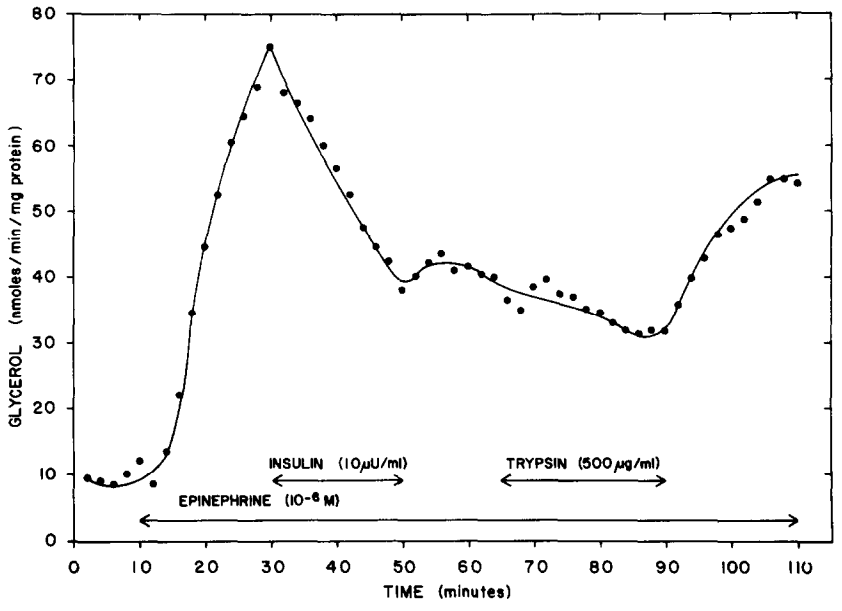

Fig. 5. Effect of trypsin treatment on the prolonged antilipolytic response to insulin. Epinephrine, insulin and trypsin were infused during the times indicated by the arrows. Results are expressed as the means of five experiments.

rates of change in lipolysis, very little work has been done describing the rates at which insulin alters hormone-stimulated lipolysis in adipose tissue. The development of the perifused fat cell system has allowed for assessment of lipolytic activity over very short periods of time [8, 9], and has provided a system in which the rates of change of lipolysis can be determined following the addition or removal of insulin.

As described previously [14], glucagon and epinephrine are able to stimulate the lipolytic process in the perifused fat cell system. Insulin at a concentration of 100 μU/ml strongly reduces the lipolytic activity in the presence of either of these hormones.

Studying the binding of radiolabeled insulin to intact fat cells, Gammeltoft and Gliemann [6] and Kono and Barham [4] have demonstrated a group of insulin receptors with a dissociation constant of approximately 3×10^{-9} M. In studying the relation between insulin binding and insulin-induced lipogenesis, Gliemann *et al.* [7] suggested that the first step in the action of insulin on lipid synthesis from glucose is the interaction with these receptors. It was further suggested that occupancy of the receptor was the rate-limiting factor when low concentrations of insulin were used. However, at higher insulin concentrations, some other step become the rate-limiting factor and, although additional receptors were occupied, no further increase in lipogenesis was observed. Similar conclusions were reached by Kono and Barham [4] who observed that concentrations of insulin which occupied only a small proportion of the binding site on fat cells stimulated glucose oxidation maximaly.

If many spare receptors exist, as proposed, than at high insulin concentrations maximum metabolic responses should occur at a time prior to equilibrium of binding. Such a relationship exists for the effects of insulin on lipid synthesis [7], and the present results suggest that the same relationship holds for the antilipolytic effect of insulin. Following the infusion of insulin (100 μU/ml), the time to one-half of the maximum response is between 4 and 5 min. This is about one-half of the value of $T_{1/2}$ for binding at this concentration of insulin predicted by the equation of Gammelgoft and Gliemann [6].

At lower concentrations of insulin, the $T_{1/2}$ for antilipolytic activity was approximately 8 min. This value is also somewhat less than the value for $T_{1/2}$ binding predicted by the equation of Gammeltoft and Gliemann and considerably shorter than the experimentally determined half-time for equilibrium of binding at this concentration [7].

It would appear, therefore, that under these conditions maximum antilipolytic response can occur at a time prior to equilibrium of binding. We interpret this to mean that only a small proportion of the total receptor population needs to be occupied by insulin molecules in order to exert maximum antilipolytic activity.

Using the hypothesis of Gammeltoft and Gliemann [6] that maximal effects of insulin are obtained when only a small portion of the receptors are occupied, it can be predicted that the metabolic effect of insulin would be maintained following the removal of the hormone from the incubation medium. Consistent with this hypothesis is the observation of Gliemann *et al.* [7] that a prolonged effect of insulin on glucose utilization occurred following the removal of insulin from the incubation medium. In other studies, Gliemann [15] demonstrated that the prolonged effect of insulin on glucose conversion to lipids persisted for as long as 70 min after the removal of insulin from the incubation medium. This prolongation of response was present even after the addition of an excess amount of insulin antibody. In addition, Solomon and Duckworth [16] have demonstrated that prior exposure of fat cells

to insulin results in a reduction in the lipolytic response to epinephrine.

At the concentrations used in the present study (10 or 100 μU/ml), insulin had a prolonged antilipolytic effect in the perifused fat cell system. This antilipolytic effect of insulin persisted for as long as 45 min after the termination of the insulin infusion. The inhibitory influence of insulin could not be overcome by adding glucagon to the perifusion medium in addition to the existing perifusion with epinephrine. The prolonged inhibitory influence of insulin was not dependent on the hormonal stimulant used. The prolonged effect was seen when either glucagon or epinephrine was used as the lipolytic stimulant. These data lend support to the hypothesis of Gliemann *et al.* [7] and Kono and Barham [4] that only a small proportion of the receptors must be occupied to exert a maximum metabolic effect in adipose tissue. Thus, during the washing period molecules of insulin continuously dissociated from their receptor and were removed from the system. Because occupancy of a small proportion of the receptors was sufficient to maintain the antilipolytic action of insulin, the action of insulin persisted for the period of time during which much of the hormone was removed from the system.

This prolonged antilipolytic action of insulin could be terminated by treatment of the perifused fat cells with trypsin. Based on the work of Kono and Barham [4 17], these results could be interpreted to mean that trypsin was proteolytically removing the insulin receptor and its associated insulin molecule from the fat cell, thereby terminating the action of the complex. Alternatively, it must be considered that the trypsin destroyed whatever insulin might have been attached to the adipose cell receptors.

It appears that unbound insulin was not responsible for the prolonged action of the hormone. It follows that insulin molecules attached to the receptors are in such configuration that they do not react with antibodies to the insulin molecules. Antibodies to insulin failed to reverse the prolonged effect of insulin on glucose utilization [7] or the prolonged antilipolytic action of insulin in the perifused fat cell system (E. A. Miller and D. O. Allen, unpublished observations). If insulin was in an unbound state, the antibody should terminate its action.

Recent results suggest that insulin may be internalized and possibly exert an action within the cell [18]. The result with trypsin suggest that the prolonged antilipolytic action of insulin was not the result of internalization of the molecules, but rather was an action on the exterior of the cell.

In summary, it can be concluded that the model suggested by Gliemann *et al.* [7] for the action of insulin on lipid synthesis also applies to the antilipolytic action of insulin. According to this model, the binding of insulin to specific receptors represents the first step in the action of insulin. However, many receptors exist, and the occupancy of only a small portion of the total receptors is needed to exert maximum antilipolytic activity. The existence of these spare receptors and their occupancy by insulin are sufficient to explain the prolonged antilipolytic action of insulin following the removal of the hormone from the perifusing solution.

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