Fat Cell Adenylate Cyclase Activation by Sheep β-Lipotropic Hormone[1] (36215)

M. Lis,[2] C. Gilardeau, and M. Chrétien

Protein and Polypeptide Hormone Laboratory, Institut de Recherches Cliniques de Montréal, Montréal, Quebec, Canada

It is now well established that 3′, 5′-cyclic adenosine monophosphate (cAMP) serves as a mediator in the activation of hormone-sensitive triglyceride lipase in adipose tissue (1). The majority of lipolytic hormones were found to act presumably through the activation of adenylate cyclase system in plasma membranes as, for example, the catecholamines (2), ACTH (3), glucagon, and TSH (4).

Sheep β-lipotropic hormone (β-LPH) was isolated and characterized by Li *et al.* (5). The structural relation to β-MSH and ACTH led us to believe that the mechanism of action in the fat tissue could be of similar nature to that of ACTH. In the following study we tried to elucidate if the action of sheep β-LPH preparation on fat tissue could be also classified as adenylate cyclase dependent.

Material and Methods. Animals. 250 g COBS® (Charles River) male rats were fed by Purina Chow and tap water *ad libitum.* Epididymal fat pads were removed immediately after decapitation. In each experiment the fat pads of 4–5 rats were pooled before cell isolation.

Three kilogram male New Zealand white rabbits were utilized in experiments on rabbit isolated fat cells. Epididymal fat pads were removed under Nembutal anesthesia. Pooled tissue of three rabbits was utilized in each experiment.

Incubation and adenylate cyclase determination. We used a modified method based on those previously described (6, 7). Adipose tissue (5 g) cut in 50 mg fragments was incubated in about 4 ml of Krebs-Ringer bicarbonate buffer containing 0.5 m*M* of sodium pyruvate (Baker) and 10 μCi of adenine-8-^{14}C (New England Nuclear, specific activity of 50.7 mCi/m*M*) at 38° with 95% O_2 and 5% CO_2 as gas phase. After one hour of incubation, 10 ml of Krebs-Ringer bicarbonate buffer containing 4% of bovine serum albumin (Fraction V, Pentex) and 30 mg of collagenase (Worthington) were added. Following an additional 60 min of incubation, the isolated cells were treated as proposed in the original Rodbell procedure (8). Uniformity of the cell suspension was measured in microhematocrit capillaries. Cell volume values were calibrated by their triglyceride content. Incubation with hormones were performed in 1 ml volumes of Krebs-Ringer bicarbonate buffer containing pyruvate, 2% albumin and 10 m*M* of theophylline. The reaction was terminated by the transfer of incubated medium and the cells into test tubes containing 1 ml of recovery mixture and by immersion of the tubes into a boiling water bath for 4 min. The recovery mixture contained "carrier" ATP (Sigma), cAMP (Sigma) and 0.5 μCi/ml of tritiated cAMP (New England Nuclear, specific activity 24.1 Ci/m*M*). Precipitate was then extracted with 2 ml of hexane and after centrifugation 3 fractions were obtained: a hexane phase, a water phase, and a precipitate. Fifty microliters of the water phase was then chromatographed on silica gel impregnated glass fiber paper (Gelman ITLC-type SG) in the system *n*-butanol: isopropanol: NH_4OH (7:2:1) (7). Spots corresponding to cAMP were visualized in UV light, transferred into counting vials and counted in a Packard 3-channel liquid scintillation counter in 15 ml of toluene containing 5 g PPO and 50 mg POPOP/liter. Because of the absence of significant quenching in the system as judged by

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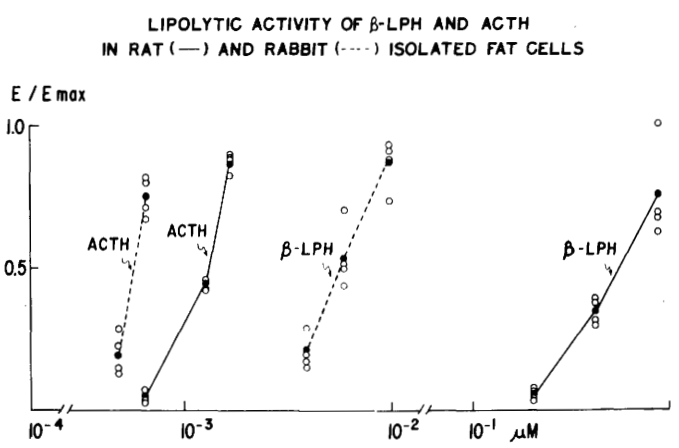

FIG. 1. Lipolytic activity was determined as a glycerol release from isolated fat cells into the incubation medium. *E max* is the maximal response of given cell preparation as determined by the lipolytic effect of 0.1 μg/ml ACTH. *E* is the lipolytic effect of given concentration. Concentration in μ*M* is on logarithmic scale.

Rabbit cells were incubated for 2 hr, rat cells for 1 hr. Open circles are the individual responses, filled circles are the means at each dose level.

external standard ratio the results are expressed as cpm. Recovery of tritiated cAMP varied between 90–100%.

In some incubations, EGTA [ethylene bis (oxyethylenenitrilo)] tetraacetic acid (Baker) was added to bring the final concentration to the values indicated in each particular experiment. Every incubation was done in duplicate and every experiment was repeated at least twice to assure good reproducibility. Results of only one experiment are presented.

Lipolytic assay. Lipolytic assay was essentially the same as the previous procedure but the preincubation with ^{14}C-adenine was omitted, incubations contained 4% albumin and no theophylline. Reaction was terminated by cooling in a ice bath, and glycerol was determined in the media. The results are expressed as a ratio of glycerol released into the medium (E) to the glycerol concentration considered as a maximal response (E max). The maximal response was determined in each assay using 0.1 μg/ml of ACTH.

Hormones tested. Synthetic ACTH 1-24 (Cortrosyn®, Organon) and β-LPH prepared in our laboratory (5) were used.

Analytical procedures. Triglycerides were determined by the method of Laurell (9) and glycerol by a semiautomatic modification of the enzymatic methods previously described (10, 11).

Results. We first determined the lipolytic dose-response relationship of β-LPH in our system of isolated fat cells and compared it to ACTH. The comparisons in both rat and rabbit epididymal fat cells are shown in Fig. 1. The maximal and half-maximal doses for β-LPH and ACTH were determined for rabbit and rat tissues. These doses were later used for adenylate cyclase stimulating activity. Results are shown in Fig. 2 for rat tissue and in Fig. 3 for rabbit tissue. We also verified in rat adipose cells the effect of EGTA (at different concentrations, 0.5–4 m*M*) and we found out (Fig. 4) that the β-LPH stimulation of adenylate cyclase activity seems to be calcium dependent.

Since (Fig. 4) 4 m*M* EGTA completely inhibited the stimulation of adenylate cyclase by β-LPH, we verified its effects when adding it at different times of incubation. Results revealed (Fig. 5) that the stimulation of adenylate cyclase activity is inhibited as soon as EGTA is added to the medium.

Discussion. β-LPH, as ACTH, stimulates the adenylate cyclase system in adipose tissue. However, the lipolytic activity of these two hormones shows some species difference when incubated with rabbit and rat adipose tissue. The maximal stimulation of rat adenylate cyclase was reached in our system after 10 min of incubation and fell rapidly thereafter. In rabbits we observed continuous increase up to

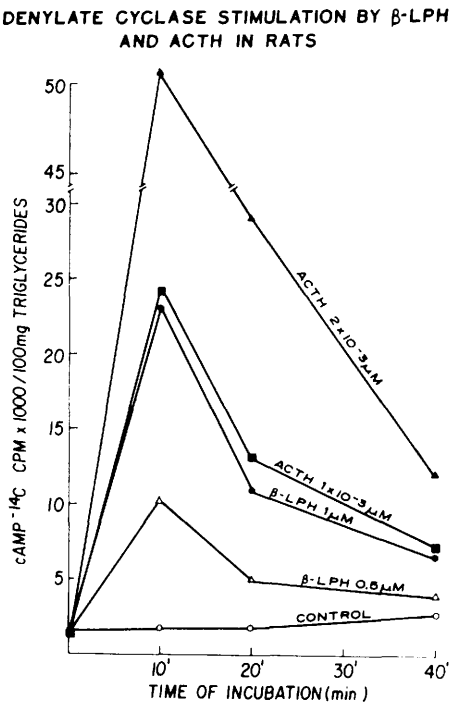

FIG. 2. Adenylate cyclase activity is determined as ^{14}C-cAMP cpm in isolated fat cells labeled previously with ^{14}C-adenine. Cpm determined in the incubation were adjusted to 100 mg of triglycerides of adipose cells. Only one experiment is presented, each point being the mean of duplicate incubations. The ACTH and β-LPH doses were chosen from the log dose-response relationship as shown in Fig. 1. The doses giving maximal and half-maximal lipolytic responses were used.

the 40 min as shown in Fig. 3. We previously observed that in rabbit isolated cells the maximal lipolytic response is usually obtained after 2 hr of incubation. In rat cells maximal response is obtained in 1 hr. It is possible that this difference just reflects different timing of the adenylate cyclase system activation.

When equipotent lipolytic doses of ACTH and β-LPH were used for adenylate cyclase stimulation, ACTH was found to be more active in rat cells while β-LPH was found to be more active in the rabbit cells preparation.

This difference is difficult to interpret by present knowledge. There is a possibility of some collateral regulatory mechanism of lipase activation not yet described and different in species utilized. However, this will require separate and more detailed study.

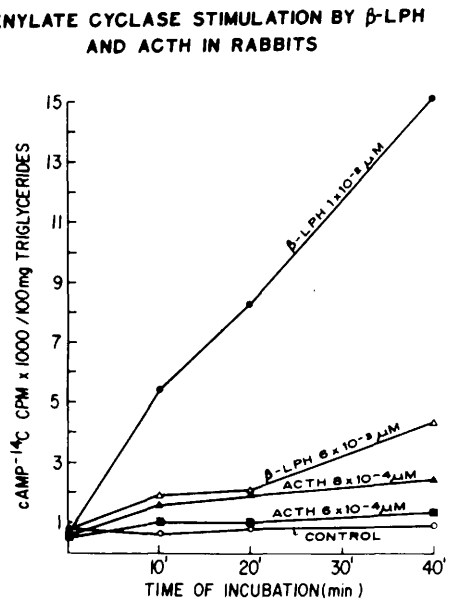

FIG. 3. Experimental conditions are the same as in Fig. 2. The ACTH and β-LPH doses were determined on rabbit isolated cells by the same method as in Fig. 2.

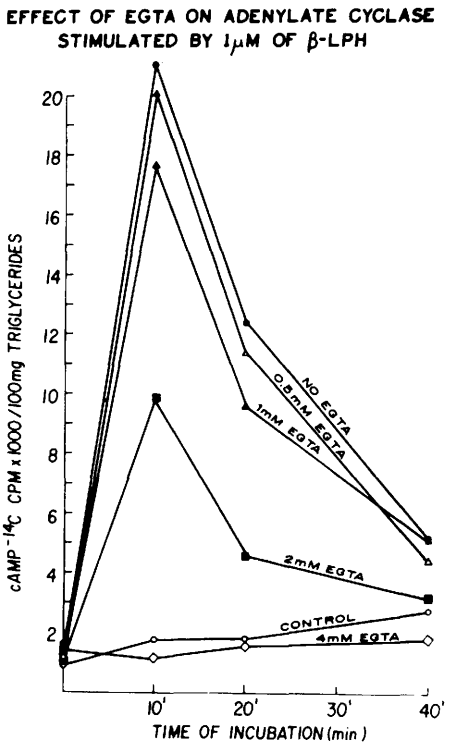

FIG. 4. Experimental conditions are the same as in Fig. 2. Rat isolated fat cells were used in incubations containing different concentrations of EGTA.

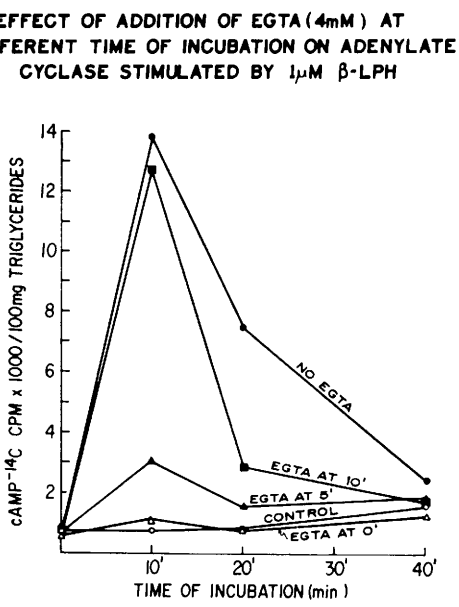

FIG. 5. Experimental conditions are the same as in Fig. 2. 4 m*M* concentration of EGTA was added to the incubated rat fat cells at 0, 5, and 10 min.

While this study was in progress Braun *et al.* (12) published, in abstract form, that β-LPH stimulated the adenylate cyclase in rabbit adipose cell ghosts but did not stimulate it in an identical preparation of cells from rat adipose tissue. However, there were no details about doses or procedures utilized and we cannot compare their results with our own experiments. We have previously shown (13) that calcium is necessary for the lipolytic activity of β-LPH. The present results revealed that calcium is essential for the stimulation of the adenylate cyclase system. Calcium dependence of adenylate cyclase activation by ACTH was previously reported (14–16). In this respect the receptor site for β-LPH behaves like the receptor site for ACTH.

These experiments do not completely rule out the possibility of phosphodiesterase involvement as the 10 m*M* theophylline does not provide 100% inhibition of phosphodiesterase. However, so far no lipolytic hormone was reported to directly affect phosphodiesterase activity. Consequently we may consider the accumulation of ^{14}C-cAMP as adenylate cyclase dependent.

Summary. Sheep β-LPH stimulated adenylate cyclase of isolated rat and rabbit adipose cells. Two doses of β-LPH were compared with two lipolytically equipotent doses of synthetic ACTH. ACTH was found to be more active in stimulating adenylate cyclase of rat fat cells while β-LPH was more potent in rabbit cells. β-LPH activation of adenylate cyclase was blocked when EGTA was added to the incubations. Differences in adenylate cyclase system in rats and rabbits were also observed.

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