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ACTH-DEPENDENT PROCESS OF LIPASE EXCRETION BY RAT PANCREATIC ACINAR CELLS

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Abstract

Modulator effect of adrenocorticotropic hormone (ACTH) on the process of lipase excretion by rat pancreatic acinar cells was studied in the model in vivo and in vitro experiments. Before the experiment an investigation protocol was drawn up which included all necessary conditions and phases mandatory to study the impact of endogenously active substances of hormonal nature upon diffrent integrative systems of the mammal organism. The protocol included a number of cumpalsory conditions, which we believe should be followed by the researchers while studying different aspects of endocrine system. It's about corticosterone and transcortin, released concretely in rats, which are endowed with circadian and seasonal rhythm. Synacthen (Germany), a synthetic adrenocorticotropic hormone analog, was used in the trial. Lipase activity was determined by means of diagnostic Lipase Activity Assay Kit (Spain). As revealed in in vivo experiment i.e. intravenous infusion of Synachten in a dose of $6x10^5$ mol/L, the first peak of lipase activity in serum was observed in the 10-15th minutes while the second peak occured in the 20-30th minutes.

During in vitro experiments (in the conditions of bile duct drainage with Synachten introduction) lipase activity peak in pancreas was determined in 20th minute. During in vitro experiments (on the pancreatic cell culture in the conditions of Synacthen introduction into the cultural environment) release of lipase from acinar cells was observed at quite a wide range of injected hormone concentration -10^{-10}- 10^{-15}Ml. The conducted researches found out that synthetic adrenocorticotropic hormone analog in therapeutic dose under both in vivo and in vitro conditions produces stimulating effect on the process of lipase release by the cells. Due to in vitro studies (on the pancreatic cell culture) it was determined that synthetic ACTH analog in a dose a magnitude higher than therapeutic dose, i.e. 10^{-15}mol provides a significant release from the cultured cells.

Thus, in the experiment conducted on rats, a new 'side' effect of adrenocorticotropic hormone was revealed, in which excretory apparatus of the pancreas with enhanced release of lipase by acinar cells presents as a target.

Keywords: ACTH, pancreas, acinar cells, stimulation, lipase activity.

Introduction

It is a long-established fact that basic function of ACTH is to regulate production of hormones of adrenal cortex. Stimulating effect of lipase activity in adipose tissue is known to be an auxiliary or "side" function of ACTH [*Hardley M, Haskell-Luevano C, 1999; Solomon S, 1999; Reaux-Le Goazigo A et al., 2011*]. No concrete data on the ACTH impact on the processes of lipase excretion by pancreatic acinar cells was revealed available in the literature. The fact which is worth attention is the one according to which the structure of lipase localized in the adipose tissue is similar to the one localized in the pancreatic acinar cells. We have assumed that in mammal

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organism ACTH has modulator effect on the excretion of lipase localized in the cells of pancreatic acinar apparatus.

While conducting experiments, we took into consideration some criteria, associated with metabolic and rhythmic peculiarities of glucocorticoid functioning in mammal organism. Adrenal glucocorticoids are known to be presented mostly by corticosterone. It is a long-established fact that adrenal glucocorticoids are prone at least to circadian and seasonal rhythm [*Yakovlev W, Shustov , 1989; Kriegsfeld L et al., 2002; La Fleur S, 2003; Hastings M et al., 2007; Cowan M et al., 2017*]. Circadian rhythms of glucocorticoids in rats differ considerably in early morning hours. The level of hormone starts increasing only after noon [*Ader R, 1969; Dunn J et al., 1972; Moore H, 1972; Bellinger L et al., 1975; Takahashi K et al., 1979; Levine R et al 1980; Ulrich-Lai Y et al., 2006*]. Approximate analog circadian rhythm is observed in ACTH when its lowest concentrations in the rats are also recorded in the early morning hours [*Ungar F, Halberg F, 1962; Halberg F, 1964; Romanov Yu, Tabolin W, 1982; Eckel Mahan K et al.,2012; Kalsbeek A et al., 2012*]. Besides circadian rhythms, seasonal rhythm is pathognomonic for glucocorticoids as well. Thereby, the lowest concentrations of corticosterone in serum of the rats was observed in autumn [*Golicov P.P., 1968*]. As known, glucocorticoids circulating in the blood occur in three conditions - circulating free, transcortinol and γ-globuline bound [*Golikov P.P., 1968*]. Moreover, the most active in application points and realized effect are circulating in blood free glucocorticoids (basically cortisol in human and corticosterone in rats).

All the mentioned peculiarities of glucocorticoid hormones were taken into consideration while conducting the experiment. That is primarily about in vivo experiments in which in the conditions of Synacthen introduction, mediated effect of endogenous corticotropin on the processes of lipase activity of pancreatic acinar cells of rats is not excluded. That is why in vivo experiments, i.e. in the conditions of synthetic ACTH analog injection to the animals, were conducted within the period of the least daily concentrations of corticosterone and ACTH (i.e. at 9 in the morning) taking into account not only circadian but seasonal rhythms. Thus, experiments were carried out in autumn, since among all the seasons of the year, the lowest concentrations of corticosterone are observed in serum of rats in this particular period [*Golikov P, 1988; Lowenberg M et al., 2008; Maghnie M et al., 2013*].

In our experimental research, it was compulsory to consider both circadian and seasonal rhythm, glucocorticoids are endowed with, as ACTH injection to rats is known to induce significant reduction of corticosterone-binding property of transcortin. It should be also noted, that the choice of laboratory animals for the experiment was conditioned by the highest corticosterone-binding ability of transcortin in rats and as a result, low concentrations of free corticosterone are detected in the serum of this laboratory animals. It is a well-established fact that circulating in blood optimal and/or high concentrations of namely free corticosterone have high biological activity over the target cells, while glucocorticoids (corticosterone, cortisol) bound to transcortin are metabolically not active [*Golikov P, 1988; Lowenberg M et al., 2008; Maghnie M et al., 2013*].

MATERIAL AND METHODS

Laboratory animals used in the experiment were white male rats weighing 150-170*g*. The experiment was conducted in the early morning hours (9-10 o'clock) in autumn. Synthetic ACTH analog, used in the study was "Synacthen" (produced by Sigma-tau Arzneimittel Gmbh, Munich, Germany) presented by N-terminal section of peptide chain (1-24), similar in different animals and human, with biological activity only i.e. deprived of any immunologic peculiarity. Lipase activity was determined by means of diagnostic Lipase Activity Assay Kit (produced by Bio Systems S.A., Barcelona, Spain) and expressed in *mol/L*.

In vivo experiment. White rats (n=20) were intravenously injected with ACTH ($6x10^{-5}$ *mol/L*) up to the terminal concentration of 10^{-9} mol/L. Lipase activity was measured in serum before and certain periods of time after the introduction of hormone.

In vitro experiment. Perfused pancreas was chosen to be a possible target for ACTH activity.

Pancreas (n=20) was perfused through the bile duct with further introduction of ACTH ($6x10^{-5}$ *mol/L*). The organ was extracted and placed in the same solution. Afterwards, lipase activity in the solution was measured at regular intervals.

Cell culture trial. Rat pancreatic cells were harvested. Collagenase solution (1.4 *mg/ml* in RPMI-1640 environment) was introduced through the bile duct. The pancreas was then extracted and incubated in the same solution at 37^0C for 30 minutes. Cells were disaggregated by cautious cell-suspension. Cell concentration was brought to $0.5x10^6$ *cell/ml* with the addition of RPMI-1640 environment, containing 10% fetal bovine serum [*Chung C et al., 2010; Clardy S et al., 2015*]. The cell suspension ready for cultivation was divided into 9 groups of 10 samples each. After an hourly preliminary cultivation ACTH solution ($6x10^{-5}$ *mol/L*) was added up to terminal concentration of $0\text{-}10^{-5}$*mol/L*. Then, after 20-minute incubation lipase activity in the cultural environment was measured.

Results and discussion

The results of in vivo experiments revealed, that first peak of lipase activity in the serum of experimental animals was determined in the 10-15th minutes, while the second one was registered in the 20-30th minutes (Fig. 1).

In vivo experiment. Serum. First peak of lipase activity was recorded in the 10-15th minutes, while the second one was determined in the 20-30th minutes.

During in vitro experiments lipase activity peak in pancreas in the conditions of synthetic ACTH analog perfusion into the pancreas through the bile duct was registered in the 20th minute only (Fig. 2).

In vivo experiment. Lipase activity peak in pancreas was determined in the 20th minute.

We assume, that the first peak of lipase activity in the serum, i.e. *in vivo*, is probably conditioned by mediated influence of corticosteroids on the processes of lipase activity concretely in adipose tissue. Corticosteroids are well known to make a modulator effect on adipocytes localized in the adipose depots manifesting particularly in lipase activation. That's why such kind of mechanism is probably launched in the conditions of our experiment either, i.e. high synthesis of glucocorticoids

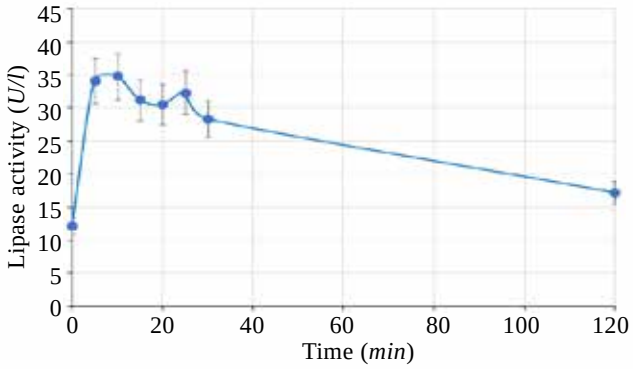

***Figure 1**. Lipase activity in the serum in the conditions of synthetic ACTH analog dose introduction.*

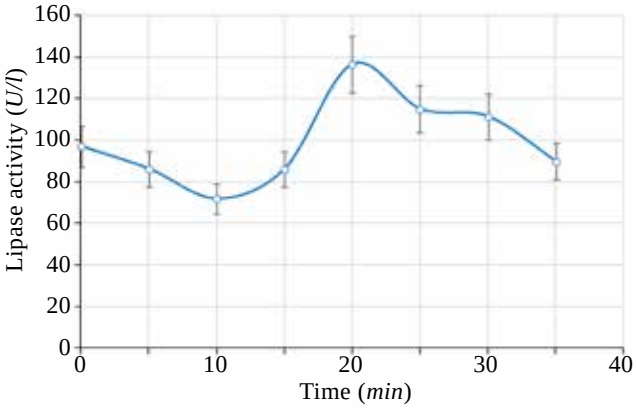

***Figure 2.** Lipase activity in pancreas in the conditions of bile duct drainage with the introduction of therapeutic dose of synthetic ACTH analog.*

on the introduction of synthetic analog occurs due to which lipase activity in adipose tissue and subsequently in the serum increases significantly. At the current stage of *in vivo* experiment, direct effect of glucocorticoids on lipase activity of pancreatic acinar cells is not excluded.

It should be noted that revealed effect (high lipase activity in the serum of rats) was observed while considering circadian and seasonal rhythms of adrenal glucocorticoids in rats, i.e. in the experiments conducted in the autumn season and early morning hours of the day when according to the laboratory results, the lowest concentrations of corticosterone are detected in the blood of rats.

It's quite remarkable that no similar effect was observed *in vitro* in profusion of adrenal gland with a single introduction of therapeutic dose of synthetic ACTH analog into bile duct in 10-15th minute. Wherein, lipase activity in adrenal gland was possible to be recorded much later - in 20th minute.

Thus, based on the comparison of *in vitro* and *in*

vivo results obtained in our experimentations the following conclusion can be made. Therapeutic dose of ACTH produces direct stimulating effect on the processes of lipase secretion by pancreatic acinar cell, since in vitro i.e. in the conditions of pancreas perfusion, direct stimulating effect of corticosteroids over the pancreatic lipase activation is excluded. Indirect confirmation of our assumption is different time intervals of lipase activity, observed during in vitro and in vivo experiments. the second peak of lipase activity increase in blood, observed in 25th minute, which nearly coincides with the lipase activity of pancreas (with 5-minute cease) is probably conditioned by the enzyme release into blood specifically from pancreas, and not from adipose depots.

In a special series of conducted experimentations on the culture of pancreatic cells with the introduction of synthetic ACTH analog into cultural environment, promotion of lipase release by acinar cells was observed at a wide range of hormone concentration. Wherein, lipase activity was noted to be directly dependent on the concentration of hormone. It should be noted that authentic increase in lipase release by isolated acinar cells occurred even at the lowest limit range of acting concentrations of hormone (10^{-10} *mol/L*), i.e. the concentration a magnitude higher that its therapeutic dose of 10^{-11} *mol/L* (Fig. 3).

In vitro experiments. Concentration dependence. Direct dependence between lipase activity and ACTH concentrations is observed at 10^{-10} -10^{-15}*M* range of ACTH concentrations. Thus, our studies determined, that Synacthen, synthetic ACTH analog, has a dose-dependent stimulating effect on the processes of lipase secretion in the pancreatic excretory apparatus. Since the structure of Synacthen is presented by the N-terminal section of peptide chain (1-24) which is analog to native ACTH, the conclusion can be made that ACTH endogenously produced in mammal organism due to its biologically active peptic chain (1-24) directly participates in the process of lipase release by pancreatic acinar cells.

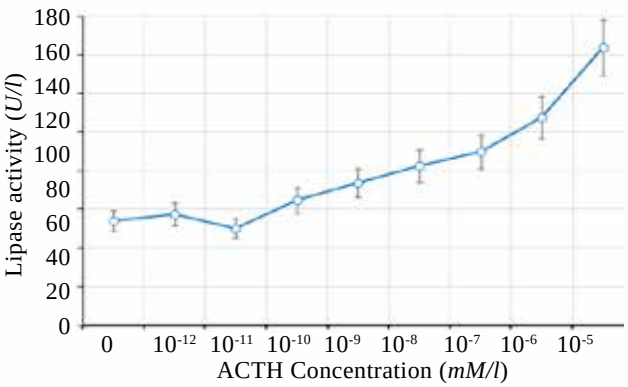

FIGURE 3. *Lipase activity in the culture of pancreatic cell on the introduction of synthetic ACTH analog into cultural environment.*

It should be noted that functional purpose of ACTH on the periphery is not exclusively limited to its stimulating effect on secretory cells of adrenal cortex and adipocytes. Another fact which worth attention is that stimulating effect ACTH spreads over the cells of pancreatic incretory apparatus as well, which was earnestly demonstrated in vitro and in vivo by a number of researches on rats, mice and rabbits [*Lebovitz H, Pooler K, 1967; Malaisse W et al., 1967; Sussman K, Vaughan G, 1967; Curry D, Bennet L, 1973; Flores L et al., 1998; Shpakov A et al., 2012*]. In this regard, the studies of a number of authors are of considerable interest. Model *in vitro* experiments conducted by the authors using human and rat β-cells imperatively proved direct stimulating effect of physiologic concentrations of ACTH, accompanied by enhanced insulin synthesis. The experiment was not limited to establishing this fact only, which by itself is of a great scientific significance, but a goal was set to find out fine mechanisms underlying the basis of direct stimulating effect of ACTH on isolated β-cells i.e. to reveal the mechanisms involved namely in the origin of receptors. In this regard, we assume scientific methodological approach for the solution of the problem is justified [*Al-Majed H, 2004; Briscoe C et al., 2006; Maragliano R et al., 2015*]. Therefore, the authors followed the long-established principle, involved in the mechanisms of ACTH stimulating the process of glucocorticoid production in adrenal glands. The point is that ACTH produces its effect on the secretory cells of adrenal cortex due to the melanocortin-2 (MC2-R) presence on the surface of the cells. Conducted correct and strictly targeted researches revealed that β-cells of Langerhans islets are also endowed with melanocortin-2 receptors. Thus,

both in the adrenal glands and in the pancreas ACTH effect is accordingly realized as a result of direct ACTH impact on adrenocorticocytes and insulinocytes in accordance with a single receptor mechanism, due to the single melanocortin-2 receptor which is present on the surface of both secretory cells and is involved in both cases. Therefore, based on the above-mentioned literature data and the results of our research, the following assumption can be made that secretory processes occurring in the pancreatic incretory - excretory apparatus such as insulin synthesis and lipase activity are also ACTH-dependent.

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