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INSULIN EFFECTS ON DNA INTERNUCLEOSOMAL FRAGMENTATION AND PARP-1 ACTIVITY IN RAT LIVER AND THYMUS CELL NUCLEI

A.V. MARGARYAN

Yerevan State University, Faculty of Biology, Department of Biophysics,
E-mail: ashotmarg2004@gmail.com

Tissue homeostasis is maintained by a dynamic balance between cell proliferation and cell death which is mainly executed in the form of apoptosis in normal physiological conditions. In this work the effect of insulin was studied, as a strong survival factor, on the internucleosomal DNA fragmentation as a widely accepted hallmark of apoptosis. The isolated rat liver and thymocyte nuclei were examined after 24 hours of in vivo hormone administration. Enzymatic activity changes of poly(ADP-rybose) polymerase-1 (PARP-1) were evaluated as playing a pivotal role in apoptosis. The results obtained indicate that in 24 hours of insulin action, thymocytes revealed a delay in apoptotic DNA fragmentation. In contrast, liver nuclei displayed an increased fragmentation intensity. Insulin had a notable effect on PARP-1 only in thymocyte nuclei, where it significantly lowered the enzyme's basal activity.

Insulin – PARP-1 activity – DNA fragmentation

Հյուսվածքային հոմեոստազը պահպանվում է բջիջների աճի և մահվան դինամիկ հավասարակշռության շնորհիվ: Հայտնի է, որ բջջի ֆիզիոլոգիական մահը հիմնականում իրականանում է ապոպտիկ ճանապարհով: Ներկայացված աշխատանքում ուսումնասիրվել է գոյատևման հզոր գործոն՝ ինսուլինի, ազդեցությունը ԴՆԹ-ի ինտերնուկլեոսոմային ֆրագմենտավորման և պոլի(ԱԿՖ-ռիբոզ) պոլիմերազ-1-ի (ՊԱՌՊ-1) ակտիվության վրա, քանի որ վերջիններս ապոպտոզի բնորոշ հատկանիշներ են:

Ստացված արդյունքները ցույց են տալիս, որ ինսուլինի in vivo ազդեցությունից 24 ժ հետո, առնետի թիմոցիտների մեկուսացված կորիզներում ԴՆԹ-ի ապոպտիկ ֆրագմենտավորումը դանդաղում է, ի հակադրություն լարդի կորիզների, որոնց մոտ դիտվում է ֆրագմենտավորման ինտենսիվության աճ: ՊԱՌՊ-1-ի ակտիվության վրա ինսուլինը դրսևորում է նկատելի ազդեցություն միայն թիմոցիտների կորիզներում՝ զգալիորեն իջեցնելով ֆերմենտի բազային ակտիվությունը:

Ինսուլին — ՊԱՌՊ-1-ի ակտիվություն — ԴՆԹ-ի ֆրագմենտավորում

Гомеостаз тканей осуществляется путем динамического баланса между клеточной пролиферацией и гибелью, которая в нормальных физиологических состояниях в основном протекает в форме апоптоза. В настоящей работе нами было исследовано действие инсулина, мощного фактора выживания, на интернуклеосомальную фрагментацию ДНК и активность поли(АДФ-рибоз) полимеразы-1 (ПАРП-1), которые являются основными признаками апоптоза. Наши данные показали, что in vivo воздействие инсулина через 24 ч приводит к замедлению интернуклеосомальной фрагментации ДНК в ядрах тимоцитов

крыс, в то время как в ядрах клеток печени наблюдается увеличение интенсивности фрагментации ДНК. Инсулин проявляет заметное воздействие на активность ПАРП-1 только в ядрах тимоцитов, значительно снижая базовую активность фермента.

Инсулин – активность ПАРП-1 – фрагментация ДНК

Tissue homeostasis is a major physiological incidence that ensures a dynamic balance between cell proliferation and cell death in the maintenance and regulation of normal tissue morphology and function. Tissue maintenance is a continuous process by which progenitor cells are recruited to differentiate into specific cell types and unwanted cells are eliminated without affecting neighboring cells. In normal physiological conditions the prevalent mode of cell death in most tissues is apoptosis which is precisely regulated by the interplay of various hormones and paracrine factors, that modulate tissue and cell-specific responses relevant to proliferation and apoptosis [1,2].

A well known endogenous factor displaying such regulatory functions is the peptide hormone insulin which plays a pivotal role in control of basic cellular processes such as carbohydrate, fat and protein metabolism. Recent studies demonstrated that in addition to its well known functions insulin can also regulate other key biological processes e.g. cell survival, apoptosis, proliferation and cell cycle [3,4]. It was shown that insulin along with insulin-like growth factor (IGF) is capable of preventing apoptosis in various cells and cell types by acting as a survival factor [5].

In last two decades it was shown that poly(ADP-ribose) (PAR) metabolism plays a vital role in a wide range of biological structures and processes, including DNA repair and maintenance of genomic stability, transcriptional regulation, apoptosis and etc. The major enzyme in PAR metabolism in the nuclei is the founding member of poly(ADP-ribose) polymerase (PARP) family: PARP-1. This enzyme uses NAD^+ as a substrate to perform post-translational modification of various nuclear proteins including itself. Being activated by DNA strand breaks and inhibited by ATP, PARP-1establishes a molecular link between DNA damage, energy status and chromatin modification in cells. This enzyme also plays a key role in cell's decision to commit apoptosis by depleting its NAD^+ and ATP pools, controlling chromatin accessibility and apoptotic endonuclease activity which is required for normal nuclear degradation [6-8].

It is well known that thymus and liver though in different extent have a prominent role in formation of immunological reactions. Coming from this we were interested to study how insulin affects the basic biochemical processes that contribute to apoptosis, necrosis or cell survival. In present paper we examined DNA oligonucleosomal fragmentation and PARP-1 activity after 24 hours of insulin administration in rat liver and thymocyte nuclei.

Materials and methods. All the chemicals and reagents were purchased from Sigma. Outbred, 6 weeks old, white, male rats (120 grams weight) were used throughout experiments. Insulin was administrated peritoneal 2 units per 100 gram animal weight in water solution after 15 minutes of glucose injection (to be approx. 0.12% in blood fluid). In 24 hours of insulin injection the rats were decapitated under light ether anesthesia. Nuclei of liver and thymus cells were isolated by the method of [9]. Sucrose solutions buffered with 25 mM Tris (pH 7.5) containing 150 mM NaCl, 60 mM KCl, 15 mM spermine and 5 mM spermidine were used. DNA preparation and electrophoresis procedures were done by methods described elsewhere [10].

PARP-1 activity assay was based on chemical quantitation on NAD^+ in PARP-1 buffer [11]. The assay was modified for NAD^+ detection in isolated nuclei by us. Briefly, 2000 µl of isolated nuclear suspensions containing 1000 µg DNA were incubated with $CaCl_2$, $MgCl_2$ (final concentrations of 1 and 6 mM respectively) and 0.5 mM NAD^+ for 10 minutes. The reaction was stopped by removal of nuclei from reaction mixture by centrifugation at 13000 g for 2 minutes.

Supernatant was aliquoted to 700 µl in new test tubes to perform NAD^+ quantitation. NAD^+ content in aliquots was determined by sequential addition of 2 M KOH, 20% acetophenone (in EtOH) and 88% formic acid yielding final concentrations of these agents according to original assay. PARP-1 assay buffer was used as a reference containing 0.5 mM NAD^+ and all aforementioned reagents with corresponding final concentrations. NAD+ quantity was measured by the absorbance of NAD^+ reaction product at wavelength 378 nm. The activity of PARP-1 was defined as the reduction of NAD^+ content in the samples which was determined by subtracting the absorbance of test samples from the reference.

Results and Discussion. To investigate the effects of insulin on the final "point of no return" phase of apoptosis i.e.DNA internucleosomal fragmentation, we assessed DNA cleavage in thymocyte and liver nuclei in cell free system of naked nuclei isolated from control and insulin injected animals. The results show that in control samples, oligonucleosomal DNA fragmentation in thymocyte nuclei was apparent after 6 hours of nuclei incubation in isolation media and increased further 24 hours. In contrast, isolated thymocyte nuclei from insulin treated animals revealed no DNA fragmentation after 6 hour incubation, but the intensity of fragmentation was not altered in further incubation periods (Fig. 1).

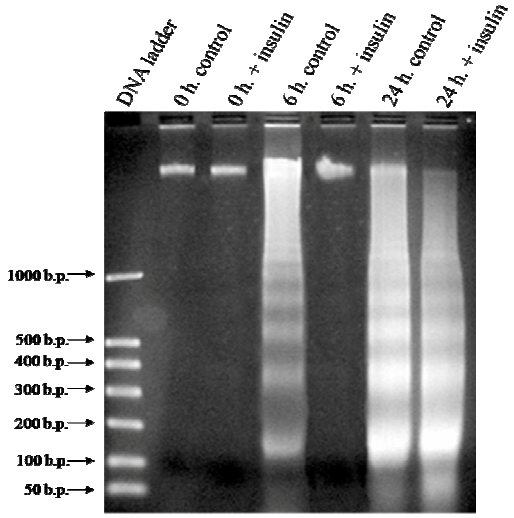

Fig. 1. DNA oligonucleosomal fragmentation in thymocyte nuclei from control and insulin treated (24 hours) animals after 0, 6 and 24 hours of incubation.

It is known that thymus expresses no insulin receptors in contrast to liver cell which are one of the main insulin target cells along with myocytes and adipocytes in the body [12]. However thymus widely displays insulin like growth factor receptors (IGFR) which have high homology with insulin receptors (IR) [13]. Recent works have shown that insulin, though with less affinity, can bind to IGFR and activate its downstream signaling cascade, which in turn results in cell proliferation, survival and activation of antiapoptotic mechanisms. Our results that show a delay in DNA internucleosomal fragmentation after insulin action are in good agreement with these data.

Effect of insulin on liver nuclei was evaluated by assessing the intensity of DNA fragmentation induced by Ca^{2+} and Mg^{2+} ions after 0 and 60 minutes of incubation with cations. Data presented in Fig. 2 shows no visible DNA degradation in the samples

derived from the nuclei immediately after isolation procedure (0 hours of incubation) from control and insulin injected animals. However, marked differences in DNA fragmentation intensity were observed in the samples incubated in the presence of divalent ions. It is obvious that insulin enhanced internucleosomal DNA fragmentation. We suppose that in liver cells that largely express IR, insulin affects metabolic pathways responsible for elevation of intracellular Ca^{2+} content which in turn, causes the activation of cation-dependent apoptotic endonucleases.

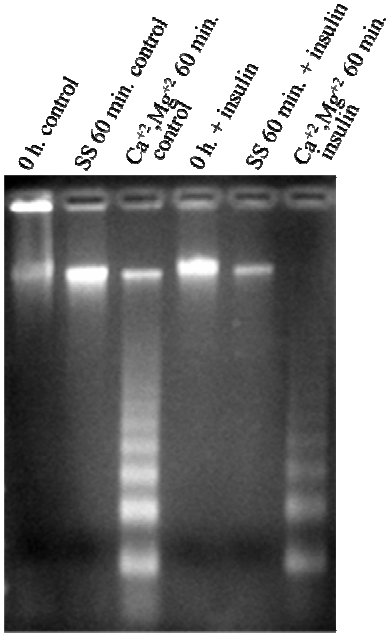

Fig. 2. DNA oligonucleosomal fragmentation in liver nuclei induced by Ca^{2+} and Mg^{2+} ions from control and insulin treated (24 hours) animals after 0 and 60 minutes of incubation.

*SS – spermine + spermidine.

To examine whether the above mentioned differences in internucleosomal DNA fragmentation had any notable impact on PARP-1, we assessed the changes of enzyme activity after 24 hours of in vivo insulin treatment. The results obtained from these experiments indicate that insulin did not affect enzyme activity in liver cell nuclei (Fig. 3).

However we observed that insulin significantly suppressed PARP-1 activity in thymocyte nuclei (Fig. 4).

It is well known that basal activity of PARP-1 strongly depends on tissue and cell context. Our data support the results of other investigators that revealed high PARP-1 activity in lymphoid organs [14]. Inactivation of PARP-1 under insulin action in thymocytes nuclei can be caused by PARP-1 dissociation from DNA [15] and may prevent intracellular NAD^+ and ATP pools depletion in thymocytes thus enhancing cell survival.

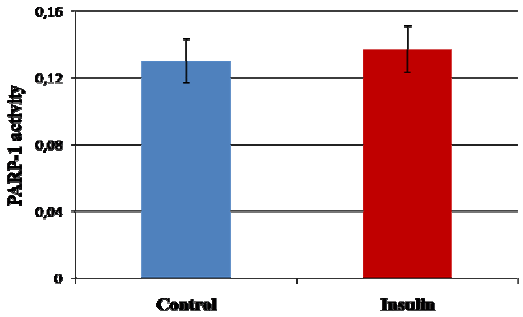

Fig. 3. PARP-1 activity (relative units) of liver nuclei of control and insulin treated animals.

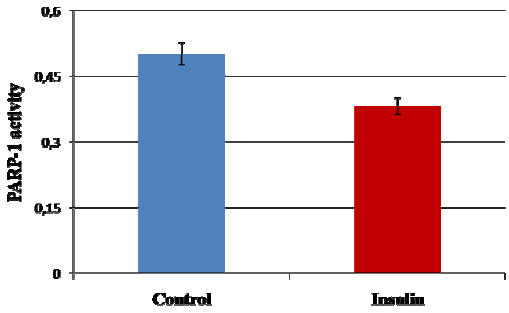

Fig. 4. PARP-1 activity (relative units) of thymocyte nuclei of control and insulin treated animals.

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