BIOPHYSICS AND BIOCHEMISTRY

Effect of Delta-Sleep-Inducing Peptide on Expression of Heat Shock Protein 70 kDa in K562 Cells

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For evaluation of the stress-protective influence of delta-sleep inducing peptide we studied its effects on the system of heat-shock proteins in immune cells using the method of flow cytometry. The peptide affected the expression of heat-shock protein 70 kDa in cultured human myeloleukemia K562 cells. Delta-sleep-inducing peptide reduces accumulation of intracellular heat shock proteins 70 kDa in cells cultured under conditions of high density.

Key Words: *delta-sleep-inducing peptide; stress-heat shock proteins; myeloleukemia; K562*

Delta-sleep-inducing peptide (DSIP) was isolated from the cerebral venous blood of rabbits as a possible endogenous sleep factor, but further analysis showed that the stress-protective and adaptogenic effects of DSIP on the organism of humans and animals predominate in a wide spectrum of its physiological effects [6]. Despite recent progress in the understanding of the biochemical mechanisms of the effects of this peptide, molecular and cellular events constituting the basis of its polyfunctional biological activity were practically never studied.

The effects of the peptide on the state of the nervous system under normal and stress conditions are best studied. Administration of DSIP to experimental animals modulates the level of neurotransmitters [2], hormones and neuropeptides [3], and activities of brain enzymes [1]. These changes are most pronounced under stress conditions. Preliminary treatment with DSIP prevents excessive activation of the hypothalamic—pituitary—adrenal system caused by immobilization of the experimental animals [4]. At the same time, the effect of the peptide on the immune system are less studied, despite the fact that this system also plays an important role in the formation of organism's resistance to various stress factors [8]. In light of this we studied direct effect of DSIP on immune cells. To this end we analyzed the effect of this peptide on the system of protective heat shock proteins (HSP), *i.e.* used a parameter directly reflecting cell reaction to stress.

It is known that adaptation of the organism to stress factors is associated with enhanced production of special protective proteins HSP [7]. These proteins play an extremely important role in the maintenance of vital functions of cells acting as chaperones and antiapoptotic factors [10] and are very important molecules under stress conditions. At the same time, the release of HSP on the cell surface and into extracellular space is a factor stimulating adaptation [9]. We studied possible regulatory role of DSIP in the functioning of the classical stress-protective system, in particular, HSP70.

In the present study we evaluated the effect of DSIP on intracellular concentration of HSP70 and apoptosis process in K562 cells and on the expression of HSP70 on the cell surface.

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MATERIALS AND METHODS

K562 cells were grown in complete nutrient medium on the basis of RPMI-1640 supplemented with 10% FCS, 0.2 $NaHCO_3$, and 100 μg/ml lincomycin (all reagents were from Sigma) at 37°C in a humid atmosphere with 5% CO_2.

Before the experiment, all cultures at the end of the log-phase of their grown were seeded into 96-well plates (cell concentration in wells was 2.5×10^6 per 1 ml medium) and incubated for 16 h in the absence (control) or presence of 10^{-6} M DSIP.

The expression of HSP70 in the extracellular space and on the cell surface was evaluated using the method of flow cytometry allowing analysis of the content of certain substances in individual cells. For evaluation of HSP70, standard indirect immunofluorescent staining of cells with antibodies against HSP70 (primary antibodies, clone BRM22, mouse IgG1; Sigma) and FITC-labeled Fab-fragments of rabbit antimouse-IgG antibodies (secondary antibodies; Sigma) were used. Intracellular content of HSP70 was measured after perforation of fixed cells (2% paraformaldehyde in phosphate buffered saline at 37°C for 20 min) with 0.5% Triton X-100 in phosphate buffered saline for 1 h followed by their staining with primary and secondary antibodies. The content of HSP70 on the cell surface was measured after staining of viable cells with primary and secondary antibodies at 0°C in a buffer containing 0.05% sodium azide. Measurements were performed on a Epics-XL flow cytofluorometer (Coulter). At least 5000 cells in each micropreparation were analyzed.

The percent of apoptotic cells in samples was determined using standard cytometric analysis of intracellular DNA content using a fluorescent dye propidium iodide (PI). To this end, the samples were fixed in cold (4°C) ethanol for 60 min, washed 2 times, and transferred into phosphate buffered saline containing 1 μg/ml PI and 20 U/ml RNase.

The data obtained on flow cytofluorometer were processed using WinMDI 2.8 software. The significance of differences was evaluated using two-way Student's *t* test.

RESULTS

After 16-h incubation of cells under conditions of increased density in culture, the intracellular level of HSP70 in the control increased by 70%, while in the presence of 10^{-6} M DSIP by only 44% (Table 1). Under these conditions, DSIP had practically no effect on the apoptosis process (Table 2), which suggests that DSIP produces no cytotoxic effect on K562 cells.

TABLE 1. Effect of DSIP on Intracellular HSP70 Content in K562 Cells after 16-h Incubation under Conditions of Increased Cell Density in Culture *In Vitro* (n=6; $M\pm S_E$)

Incubation conditions	HSP70, %
Control (0 h incubation)	176.40±9.09
16-h incubation	
without DSIP (control)	300.1±11.6
with DSIP	253.80±16.24*

Note. *p<0.05 compared to 16-h incubation without DSIP.

TABLE 2. Effect of DSIP on Percent of Apoptotic K562 Cells after 16-h Incubation under Conditions of Increased Cell Density in Culture *In Vitro* (n=6; $M\pm S_E$)

Incubation conditions	Content of apoptotic cells, %
Control (0 h incubation)	5.42±0.37
16-h incubation	
without DSIP (control)	6.80±0.51
with DSIP	6.21±0.34

The decrease in intracellular content of HSP70 can be explained by the effect of the peptide on transcription factors or on the process of HSP biosynthesis. There are no published data on specific inhibitors of HSP70 biosynthesis. Moreover, attempts to inhibit expression of this protein with antisense oligonucleotides lead to paradoxical increase in the intracellular content of HSP70 (probably via a compensatory mechanism) or to cell death.

At the same time, the decrease in HSP70 content in cells observed in our experiments can be related to translocation of these proteins onto the outer cell surface or their secretion into the extracellular space. In humans and animals, the release of this protein into extracellular space stimulates adaptive processes [9]. For instance, exposure of HSP70 on the cell membrane and their presence in the extracellular medium promotes activation of NK cells [5]. However, our preliminary experiments on evaluation of the effect of DSIP on the expression HSP70 on the surface of K562 cells revealed no stimulating effect of DSIP on translocation of HSP70 on the cell surface, which probably confirms the possibility of DSIP-induced secretion of these proteins in the applied model. Thus, the decrease in the content of HSP70 in K562 cells under the effect of DSIP can be a result of induction of HSP70 exocytosis by this protein.

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