Insulin-Like Growth Factor-1 and Insulin Inhibit Caspase-3/7 Activation Initiated by Interleukin-1β in the Rat Eye Retinal Neurons

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Proinflammatory cytokine IL-1β specifically stimulates caspase-3/7 *in vitro* in RGC-5 rat eye retinal neurons. Insulin and insulin-like growth factor-1 and their combination inhibit caspase-3/7 activation in these cells, induced by removal of the serum from culture medium and/or by IL-1β treatment.

Key Words: *insulin-like growth factor-1; interleukin-1β; tumor necrosis factor-α; diabetic retinopathy; caspase-3/7*

Interleukin-1β is a proinflammatory and proapoptotic protein. It plays the key role in the development of neurodegenerative diseases, one of which is diabetic retinopathy, degeneration of the eye retina characterized by rapid loss of retinal neurons as a result of their apoptotic death. Retinal neurons die by apoptosis at the initial stages of diabetes in humans and in rats [3,7,12]. In addition, some characteristics of diabetic retinopathy are intrinsic of a chronic inflammatory disease. High levels of proinflammatory cytokines (IL-1β, IL-6, and IL-8) are found in the vitreous humor of patients with proliferative diabetic retinopathy and in the retinas of rats with streptozotocin-induced diabetes [2,5,14]. Serum levels of TNF-α cytokine, its soluble receptor, and IL-1β increase in patients suffering from diabetes [6,10,11]. During reperfusion ischemia IL-1β induces retinal neuron apoptosis [15].

It was experimentally shown *in vitro* that incubation of bovine eye retinal capillary endotheliocytes with IL-1β stimulates cell apoptosis by 70% and activates caspase-3 by 40% [9]. On the other hand, the involvement of IL-1β and other cytokines in the pathogenesis of diabetic retinopathy remains not quite clear.

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We studied the effect of IL-1β on survival and death of RGC-5 eye retinal ganglionic neurons *in vitro*.

MATERIALS AND METHODS

The studies were carried out on RGC-5 cell culture, a clone of psi2E1A virus-transformed rat eye retinal cells isolated on day 1 postpartum. Stimulation of caspase-3/7 by serum-free medium and its inhibition by insulin and insulin-like growth factor-1 (IGF-1) were studied by inoculating RGC-5 cells at a density of 1.8×10^5 cell/cm^2 in 96-well plates (Cornig Incorporated) and incubation (24 h) in DMEM (Cellgro, Mediatech) with 10% FCS (common medium). The cells were then washed in PBS and fresh medium (50 μl per well), or serum-free medium with 10 nM insulin, or 10 ng/ml IGF-1, or insulin+IGF-1 was added to the wells. Medium with 10% FCS was added to control cultures. The cells were then cultured (4 h) and activation of caspase-3/7 was measured using Apo-ONE™ Homogeneous Caspase-3/7 Assay Kit (Promega). Activity of caspase-3/7 was evaluated by cleavage of caspase-3/7-specific fluorogenic substrate (rhodamine 110; Z-DEVD-R110) on a Wallac 1420 multi-series counter (Perkin Elmer) at stimulation wavelength λ=485 nm and emission wavelength λ=530 nm.

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In order to evaluate caspase-3/7 stimulation by cytokines IL-1β, IL-6, and TNF-α, RGC-5 cells were incubated in DMEM with 10% FCS (20 h), washed in PBS, and 50 μl medium with 10% serum containing 5, 15, or 50 ng/ml of each cytokine was added. After incubation during the next 4 h, activity of caspase-3/7 was measured.

The data are presented as the means of 3 repeated experiments normalized by caspase-3/7 activity in medium with 10% serum.

Statistical analysis for comparing the values in different experimental groups was carried out using One-way ANOVA and Tukey's Multiple Comparison Test software.

RESULTS

Activity of caspase-3/7 increased sharply after RGC-5 cell culturing in serum-free medium in comparison with enzyme activity in cells growing in medium with the serum. Addition of insulin and IGF-1 to serum-free medium led to inhibition of caspase-3/7 stimulation (Fig. 1). Treatment with a combination of insulin and IGF-1 also suppressed activity of caspase-3/7. Differences between caspase-3/7 activation in serum-free medium and in medium with insulin or IGF-1 or both are statistically significant. On the other hand, insulin, IGF-1, or their combination inhibited activity of caspase-3/7 virtually similarly.

Our results indicate that insulin, IGF-1, and/or their combination are important factors of cell survival protecting the RGC-5 eye retinal neurons from apoptosis. Our results confirm the results of a previous study [4] according to which insulin and IGF-1 in physiological concentrations protect R28 rat eye retinal neurons from apoptosis and inhibit caspase-3 stimulation.

In order to detect the effects of cytokines involved in inflammatory processes on stimulation of caspase-3/7 in eye retinal cells *in vitro*, RGC-5 cells were incubated in common medium with serum with 5, 15, or 50 ng/ml IL-6, TNF-α, or IL-1β. It was found that IL-1β (but not IL-6 or TNF-α) highly specifically stimulated caspase-3/7. The maximum effect of IL-1β manifested at a concentration of 5 ng/ml, while the effects of 15 and 50 ng/ml on capsase-3/7 stimulation were no higher than that of the lower dose (Fig. 2). No statistically significant differences in the effects of IL-6 and TNF-α on stimulation of caspase-3/7 in RGC-5 cells were detected. This experiment confirms the hypothesis according to which proinflammatory cytokine IL-1β stimulates caspase-3/7 and hence, is involved in apoptosis induction in the eye retinal neurons. Our data are in line with the previous reports [1] that IL-1β is toxic and stimulates caspase-3/7 in the eye retinal neurons.

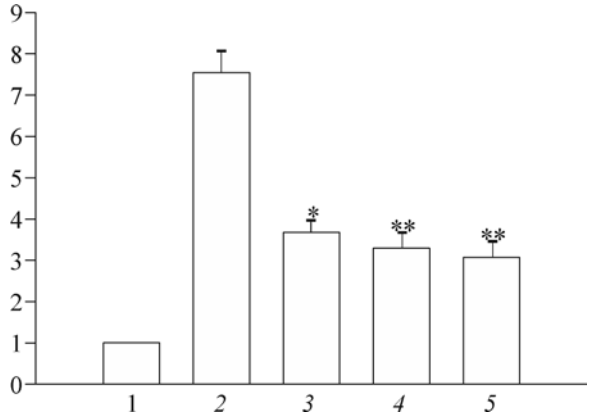

Fig. 1. Activity of caspase-3/7 induced in RGC-5 cells cultured in serum-free medium. *1*) control (with serum in culture medium); *2*) without sera in culture medium; *3*) with 10 nM insulin; *4*) 10 ng/ml IGF-1; *5*) insulin+IGF-1. *p<0.01, **p<0.001 compared to *2*.

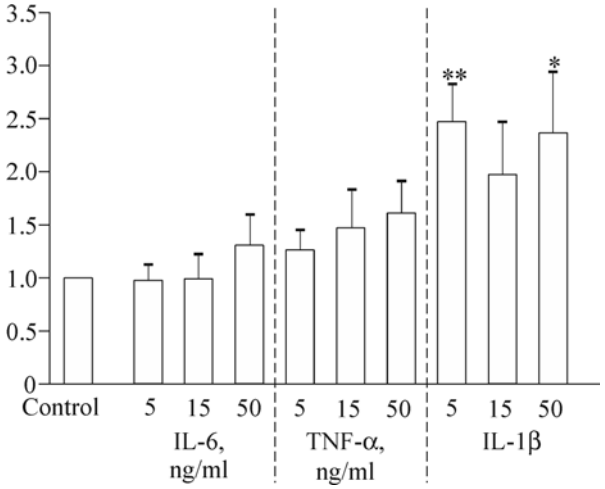

Fig. 2. Activity of caspase-3/7 in RGC-5 cells cultured with serum containing IL-6, TNF-α, or IL-1β. *p<0.05, **p<0.01 compared to control (medium with serum).

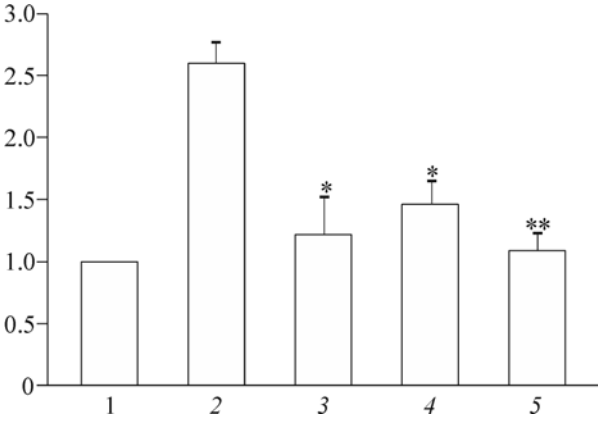

Fig. 3. Activity of caspase-3/7 in RGC-5 cells cultured in medium with serum containing 5 ng/ml IL-1β. *1*) common culture medium with serum; *2*) with IL-1β; *3*) with IL-1β+10 nm insulin; *4*) with IL-1β+10 ng/ml IGF-1; *5*) with IL-1β+10 nM insulin+10 ng/ml IGF-1. *p<0.05, **p<0.01 compared to *2*.

Physiological concentrations of insulin and IGF-1 and/or their combination reduce significantly activity of caspase-3/7 induced in the presence of 5 ng/ml IL-1β in RGC-5 cells, the differences being statistically significant. On the other hand, the effects of insulin, IGF-1, and their combination on caspase-3/7 activity were similar (Fig. 3). Since insulin and its receptors are present in high amounts in the retina, particularly in the inner plexiform layer mainly formed from the neuronal axons [8], and insulin receptor is present in high amounts in the rat retina and exhibits stable tyrosine kinase activity [13], presumably, insulin and IGF-1 protect the retina *in vivo*.

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