MODULATION OF SODIUM TRANSPORT DURING INTERFERON-α2b-INDUCED DIFFERENTIATION IN HUMAN NEUROBLASTOMA CELLS

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Introduction

Over the past 20 years, neural-immune interactions are a matter of topical interest in modern neurobiological investigations. Recent findings indicate that the central nervous and immune systems are intimately linked. These two systems use the common signals molecules (hormones, neurotransmitters, neuropeptides, cytokines), and common receptors for the bidirectional communication and regulation. In this respect, studies of the neurotropic activity of cytokines released by activated immune cells have been of fundamental importance. According to modern concepts, interferons (IFNs) are one of the key polyfunctional cytokines providing integrative activity of the neural and immune systems (Blalock, 1989; Wrona D., 2006).

IFNs are inducible proteins that regulate resistance to viral infection, enhance innate and acquired immune responses, modulate cellular metabolism, and control cell growth and differentiation. After binding to high-affinity receptor on the cell surface, IFNs initiate a signaling cascade through signaling proteins. Cellular actions are mediated through specific IFN-stimulated genes (ISGs), which underlie a variety of cellular responses (Borden et al., 2007).

IFN-α was originally considered as a cytokine with antiviral, immunomodulating, and antiproliferative properties. Current studies indicate that IFN-α affects the central nervous system activity. It was demonstrated that IFN-α administration results in an alteration of the electrophysiological activity of brain regions participating in pain suppression mechanisms and temperature and food-intake regulation. Some of the reports indicate that IFN-α modulates neuronal activity via opiate receptors (for review, see Dafny; Yang., 2005). It was shown that IFN-α2b is involved in regulation of neurogenesis (Rozhmanova et al., 2004). These findings suggest that IFN-α is one of the regulatory mediators, which links the central nervous and the immune systems.

Since the disturbance of this interaction underlies many disorders of the central nervous system, the problem of neural-immune interactions is not only of a theoretical importance. Therefore, both experimental and clinical aspects of this interaction are now intensively investigated.

At present, recombinant IFN-α is widely used for treatment of a number of viral and oncological diseases, in particular neuroblastoma (Bruchelt et al., 1990; Gutterman, 1994). The molecular mechanisms of IFN antiproliferative activity are still obscure. The processes of cell growth and division depend significantly on the concentration gradients of Na^+, K^+, and Ca^{2+} ions (Vereninov; Marakhova, 1986). Therefore, the antiproliferative effect of IFN-α2b can be related to the dysfunction of the ion-transporting systems. Taking into account the above arguments, we focused our attention on the changes in transport of sodium ions, which accompany interferon-α2b-induced differentiation of human neuroblastoma cells.

Morphological differentiation of IMR 32 cells

Human neuroblastoma IMR 32 cell line is a classic model for the investigation of neuronal differentiation induced by different factors, in particular, cytokines. In our experiments we used human recombinant IFN-α2b (laferon). IMR 32 cells were cultured during twenty-two hours in the modified media containing 600 IU/ml IFN-α2b. As criteria of morphological differentiation, we used generally accepted parameters for nerve cells, such as a decrease in the proliferative activity of the cells, an increase in the total length of the neuritis, and a change in the projective area of soma.

At the end of the first day of culturing, the differences between control cells and those placed in the modified media were observed (Rozhmanova et al., 2000). Statistical data of changes in cell density demonstrate that IFN-α2b inhibited cell proliferation and induced differentiation in 50% of the cultured IMR 32 cells. At the same time, the mean value of the projective area of cell soma in differentiated cells was by 56% larger than that of the control cells. Mean values of the total neurite length did not significantly differ from the analogous parameter in the control group. A significant difference in the length of processes was observed 48 h after IFN-α2b had been applied.

Along with suppression of the proliferative activity and changes in the morphological parameters, the enhancement of protein synthesis in IMR 32 cells was noted. 22 h after IFN-α2b addition both total protein content in the cell and protein content in the membrane fraction were, on average, 2.3 times those in the control cells. The present results show that IFN-α2b controls the proliferation and induces differentiation of IMR 32 cells.

Effect of IFN-α2b on the Na^+ influx

The role of sodium ions transport mechanisms in cell proliferation is still unknown. However, there is increasing evidence that they are involved in regulation of the cell cycle (Arcangeli; Becchetti, 2006). The study of these mechanisms is very important for understanding of certain regulatory pathways, which modulate the transition between proliferative and nonproliferative states. Therefore, we focused our attention on the changes of inward and outward sodium fluxes in IMR 32 cells, which accompany cell differentiation (Rozhmanova et al., 2000, 2001).

Early changes of the Na^+ influx. Using corresponding extensively known techniques, we studied the effects of short-lasting (to 1 h) and long-lasting (22 h) action of IFNα2b on the $^{22}Na^+$ influx and activity of the Na^+, K^+-ATPase in IMR 32 cells (Rozhmanova, 2001).

Our experimental results convincingly demonstrated that IFN-α2b modulates $^{22}Na^+$ influx in IMR 32 cells at the resting potential. It was demonstrated that after 20 min IFN-α2b application the $^{22}Na^+$ influx in neuroblastoma cells was reduced by 20%, in comparison with the control cells, and then gradually recovered.

Veratrine increases probability of opening of sodium channels, while the scorpion toxin from *Leiurus quinquestriatus* inhibits their inactivation in neuroblastoma cells (Catterall, 1975). In the presence of these neurotoxins, in 20 min following the onset of IFN-α2b application, the $^{22}Na^+$ influx increased by about 50% in the control cells and by 70 % in the cells affected by IFN-α2b. A blocker of voltage-operated sodium channels, tetrodotoxin (TTX, $4 \cdot 10^{-7}$ M), completely blocked the $^{22}Na^+$ influx in the control cells and only by about 75% in the IFN-α2b-treated cells.

These findings allow one to conclude that IFN-α2b modulates the transport of sodium ions through the voltage-operated TTX-sensitive sodium channels. Our data are in agreement with electrophysiological investigations, which demonstrated that IFN-α2b alters characteristics of such channels in undifferentiated IMR 32 cells (Kucher et al., 2000).

Delayed changes of the Na^+ influx. In the cells cultured for 22 h in the medium containing 600 IU/ml IFN-α2b, the $^{22}Na^+$ influx in the absence of neurotoxins was by 65% larger, and in the presence of neurotoxins it was by 220% larger then that observed in the control (undifferentiated) cells. TTX completely blocked $^{22}Na^+$ influx through modified with neurotoxins sodium channels in undifferentiated cells, while the $^{22}Na^+$ influx dropped only by 50% upon the action of TTX in differentiated cells. As these findings indicate the fact that the long-lasting action of IFN-α2b causes the appearance of voltage-operated TTX-insensitive sodium channels in the plasma membrane of the IMR 32 cells. The role of Na^+ channels in cell differentiation has not been entirely established. However, there are evidences that regulation of the expression and function of sodium channels play important role during cell differentiation (Gomez et al., 1993; Tabb et al., 1994).

Effect of IFN-α2b on Na^+, K^+-ATPase activity

Na^+, K^+-ATPase maintains the ion asymmetry in both nonexcitable and excitable cells and is involved in the control of different cell processes, including cell growth, division, and differentiation. An important condition for realization of the entire cycle of cell reproduction is activation of the Na^+, K^+-ATPase system, which accompanies transitions of the cell into G_1 phase (Vereninov; Marakhova, 1986). Therefore, we could suggest that IFN-α2b changes Na^+, K^+-ATPase activity during IMR 32 cells differentiation.

The activity of Na^+, K^+-ATPase was estimated in a microsomal fraction obtained from undifferentiated (control) or differentiated IMR 32 cells (Rozhmanova et al., 2000). In undifferentiated cells Na^+, K^+-ATPase activity decreased by half within 5 min of IFN-α2b addition. Following 22 h culturing in the IFN-α2b-containing medium, in differentiated cells, this activity recovered to 80% of its level in undifferentiated cells.

Evaluating the data obtained in our study, we can conclude that IFN-α2b induced differentiation of human neuroblastoma cells IMR 32. This effect is associ-

ated with modulation of sodium ions transport through voltage-operated sodium channels and Na^+, K^+-ATPase activity. The principles learned from *in vitro* studies are very important in our understanding of IFN-α2b neurotropic actions and can be used to evaluate its antiproliferative effect *i*n *vivo*.

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