Protective Effect of Biolan during Ischemic Damages to Cultured Cerebellar Granular Cells

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 2, pp. 162-164, February, 2002
Original article submitted October 5, 2001

Biolan containing carnosine and delta sleep-inducing peptide produced a protective effect on cerebellar granular cells from 7-day-old rats exposed in culture to ischemia (oxygen and glucose deprivation) and cytotoxic influence of glutamate. These results indicate that Biolan holds much promise for correction of ischemic brain damages in clinical practice.

Key Words: *cultured nerve cells; ischemia; Biolan; neuroprotection*

The use of regulatory peptides and their synthetic analogues in clinical practice attracted much recent attention. Food additive Biolan (Yupiter Company, St. Petersburg; registration certificate No. 001774. R.643.06.2000, 22.06.2000, Russian Ministry of Health) contains a complex of amino acids and natural peptides, including carnosine and delta sleep-inducing peptide (DSIP).

Clinical observations showed that Biolan modulates functional activity of the central nervous system: it improves selective attention during information perception, enhances memory consolidation, affects adaptive capacities of the brain, and increases its resistance to stress. Biolan improves the state of patients with organic pathology of the nervous system, including insult, neuroinfections, multiple sclerosis, encephalopathy, autism, and brain traumas [1,4], which suggests that this preparation possesses neuroprotective activity. However, the protective effects of Biolan on brain neurons were not studied.

Dissociated cultures of various brain cells are widely used for elucidation of mechanisms of ischemic damages to central neurons. As differentiated from *in vivo* observations, this experimental model allows direct evaluation of the effects of various compounds with neuroprotective activity on living neurons at the cellular level.

Here we studied the neuroprotective effects of Biolan on dissociated cultures of rat cerebellar granular cells (CGC) exposed to ischemia or cytotoxic influence of glutamate. According to current concepts, glutamate-induced hyperactivation of postsynaptic receptors plays an important role in the pathogenesis of ischemic damages to central neurons.

MATERIALS AND METHODS

Experiments were performed on cultured CGC from 7-day-old Wistar rats [6]. On days 7-8 *in vitro* the cultures were washed 2 times with a control saline (143.4 mM NaCl, 25.0 mM KCl, 2.0 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 10.0 mM glucose, and 5.0 mM HEPES, pH 7.4), preincubated in the same saline in the presence or absence of Biolan for 30 min, and exposed to 60-90-min ischemia. Ischemia was induced by oxygen and glucose deprivation (OGD). To this end, the cells were maintained in a sealed box containing glucose-free deoxygenated saline and filled with argon (35.5°C, 1.0-1.5 h) in the presence or absence of Biolan in the same concentrations). After OGD the cultures were incubated in a control saline with or without Biolan at 35.5°C

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0007-4888/02/1332-0136$27.00

for 4-5 h under normoxic conditions. In some experimental series carnosine or DSIP were added together with Biolan.

Experiments with glutamate were carried out according to the same scheme. Control saline contained 138.2 mM NaCl, 25.0 mM KCl, 2.3 mM $CaCl_2$, 0.3 mM Na_2HPO_4, 12.0 mM $NaHCO_3$, and 10.0 mM glucose (pH 7.4). The cells were exposed to the cytotoxic effect of 25 μM glutamate for 15 min.

During the last 2-2.5 h of the postischemic or postglutamate period CGC were incubated with 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 0.5 mg/ml), lyophilized in dimethylsulfoxide, and assayed spectrophotometrically (MTT test for cell survival). In some experiments with glutamate neuronal viability was estimated morphometrically. To this end, the cultures were stained with cresyl violet after termination of the postglutamate period. Dead neurons (pyknotic nuclei) were counted in 10 fields (×400).

The results were analyzed by Microsoft Excel software. The data are presented as arithmetic mean± standard error.

RESULTS

Incubation in the control saline was not accompanied by appreciable destruction of cultured neurons. Treatment with 25 μM glutamate decreased cell survival compared to the control (Fig. 1). The count of undamaged neurons in these cultures was 25.1±2.0%. In the incubation medium containing Biolan the survival of cultured cells and the count of undamaged neurons increased (Fig. 1). A direct correlation was found between the results of biochemical and morphometrical assays. In further experiments CGC survival was estimated only by the MTT test.

CGC survival in cultures exposed to OGD in the absence of Biolan was lower than in the control (Fig. 2). Biolan in concentrations of 10, 50, and 100 μg/ml markedly increased cell survival under ischemic conditions (Fig. 2). It should be emphasized that Biolan in the lowest dose was most effective (similarly to experiments with glutamate). Moreover, in a special experimental series with OGD Biolan in a concentration of 0.5 μg/ml produced the most pronounced protective effects (data not shown).

These results indicate that Biolan acts as a potent neuroprotector during ischemic and cytotoxic damages to cultured neurons. The preparation in low doses produces the most pronounced protective effects.

Biolan contains natural oligopeptides carnosine and DSIP possessing neuroprotective activity. Carnosine and related natural peptides present in small amounts in mammalian olfactory bulb and brain glial cells [2,9] increase rat survival after global cerebral ischemia [5,12], reduce zinc and copper neurotoxicity [10], block the toxic effect of N-methyl-D-aspartate, and prevent oxidative stress-induced destruction of CGC [2,3,7]. DSIP decreases xanthine oxidase activity, inhibits lipid peroxidation in the brain after cold stress [8], and improves the resistance to emotional stress in rats with damaged limbic structures [11]. We compared the neuroprotective effects of Biolan, carnosine, and DSIP on cultured cells exposed to ischemia. Biolan in a dose of 10 μg/ml produced the most pronounced protective effects and increased the survival of cultured neurons by 30.8±5.5% compared to the control. DSIP in concentrations comparable to those

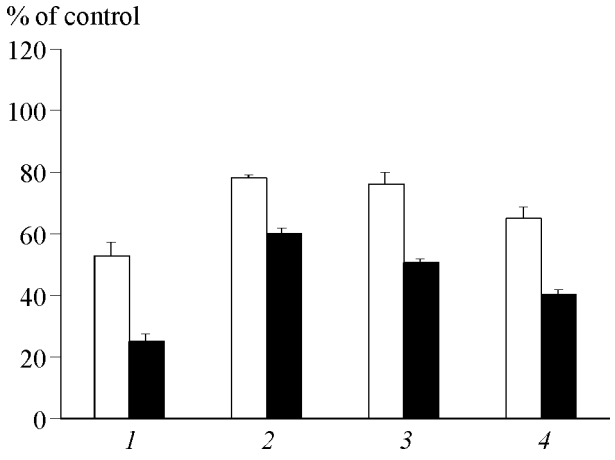

Fig. 1. Survival of cultured cerebellar granular cells exposed to the neurocytotoxic effect of glutamate. MTT test (light bars) and morphometry (dark bars). *1*) glutamate (25 μM); glutamate and Biolan in concentrations of 10 (*2*), 50 (*3*), and 300 μg/ml (*4*). Here and in Fig. 2: $p<0.05$ compared to group *1*.

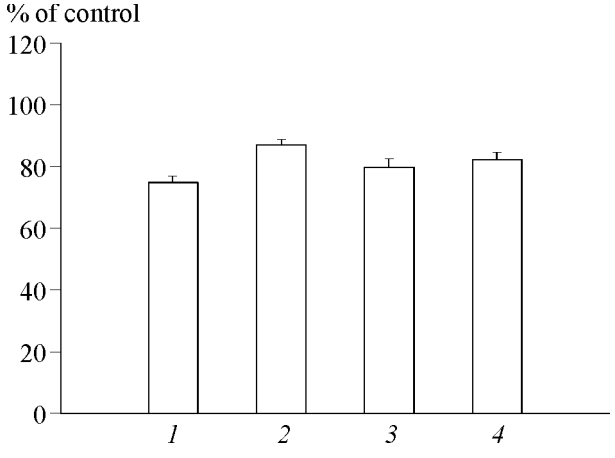

Fig. 2. Survival of cultured cerebellar granular cells during ischemia (MTT test): ischemia (*1*); ischemia and Biolan in concentrations of 10 (*2*), 50 (*3*), and 100 μg/ml (*4*).

present in Biolan (1-10 μg/ml) increased cell survival by 12.5±2.1% compared to the control. Carnosine was ineffective under these conditions: cell survival was 11.5% below the control. Moreover, carnosine potentiated the adverse effects of OGD. These data suggest that carnosine and DSIP, components of Biolan, form a complex compound and produce potent neuroprotective effects. However, carnosine in a higher concentration (2260 μg/ml) displayed high protective activity and increased neuronal survival by 33.1% compared to the control. It should be emphasized that death of central neurons under ischemic conditions, and especially, in the postischemic period is associated with overproduction of reactive oxygen species [13]. It can be hypothesized that the neuroprotective properties of Biolan are primarily associated with antioxidant activity of its components.

Our results and clinical observations indicate that Biolan holds much promise for the correction of ischemic brain damages in clinical practice.

This work was supported by the Russian Foundation for Basic Research (grant No. 01-04-48915).

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