Effect of Ethanol on the Growth and Differentiation of Spinal Motoneurons and Possibility of Correcting This Effect *In Vitro*

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We studied the effect of ethanol on the growth and differentiation of spinal cells in 14-day chick embryos *in vitro* and the possibility of correcting the destructive changes with dolivin (antioxidant). Ethanol blocked migration of glial cells and growth of axons. Addition of dolivin into the nutrient medium together with ethanol prevented the effect of ethanol and stimulated axon growth and migration of glial cells.

Key Words: *tissue culture; spine; axon; ethanol; dolivin*

Ethanol exerts an appreciable effect on the growth and differentiation of cultured neurons. In the culture of fetal rat neocortical neurons ethanol decreased the number of viable cells due to inhibition of cell proliferation and accumulation of neurons in the state of apoptosis [8]. Ethanol delayed cell development and accelerated neuronal death in the culture of cerebellar granular cells [13]; the earliest changes in the culture of rat hippocampus were decrease in the length and number of dendrites and number of synapses [15]. Ethanol intoxication inhibited DNA and protein synthesis in cultured astrocytes, which was associated with accumulation of cells in G_0/G_1 phases of the cell cycle [6].

Antioxidants (most often vitamin E as the natural antioxidant [1,7]) are usually applied to prevent oxidative stress induced by free radicals. Hypoxia, playing an important role in the formation of embryonal alcohol syndrome [3] is the cause of neuronal death during ethanol intoxication.

We studied the effect of ethanol on the growth and differentiation of neurons and glial cells in chick embryo spinal cultures and the possibility of preventing destructive changes with antioxidant dolivin.

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MATERIALS AND METHODS

The study was carried out on spinal explants from 14-day chick embryos at early stages of culturing (24 h, 48 h, 3, 5, 7 days). The explants were cultured in Maximov chambers at 37°C. Three series of cultures, 120-130 explants per series, were studied. In series I the explants were cultured in standard nutrient medium [2], in series II in nutrient medium with ethanol (100 ml/liter) [6], and in series III with ethanol and dolivin (10^{-5} M). Dolivin contains two mutually supplementing and potentiating components: baker's yeast autolysate and effective antihypoxant hypoxene (olifene), as well as amino acids, vitamins of B group, PP, E, and H, trace elements, and polysaccharides.

Neuronal growth index (NGI) in cultures was evaluated as described previously [9]. NGI was estimated by evaluating the density of axon distribution and length (in μ at 6×12.5 magnification) in the growth zone of each explant. The density was multiplied by the length and the results were averaged for estimating NGI. The cultures were examined under phase-contrast microscope (Ienaval). The data were processed using Student—Fisher test.

RESULTS

Intensive migration of glial cells into the growth zone with the formation of the substrate for further growth

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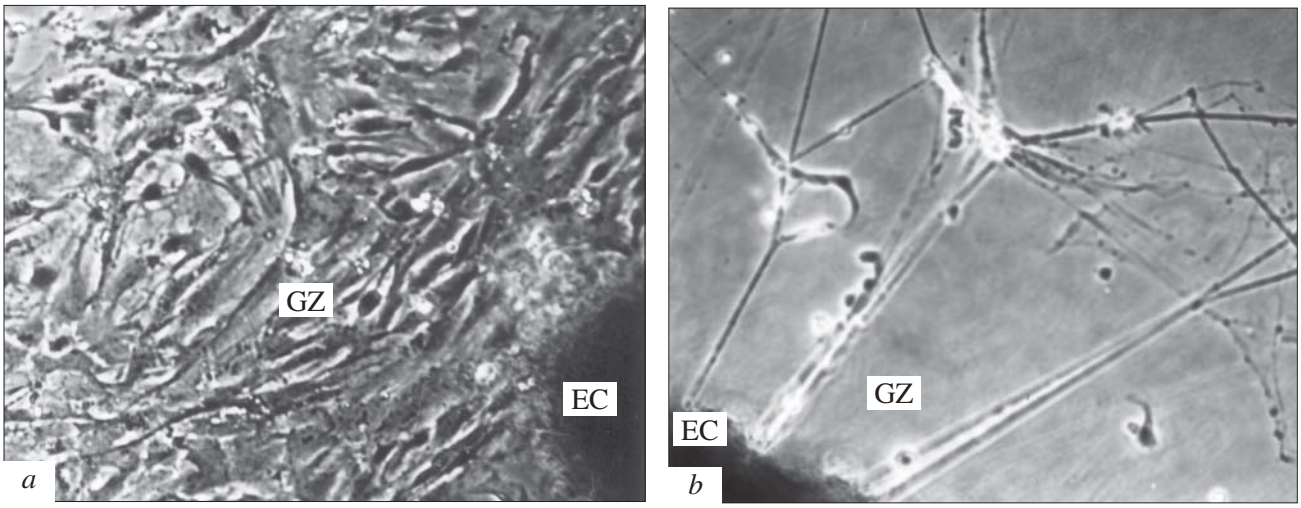

Fig. 1. Active growth of glial cells (*a*) and axons (*b*) in spinal explant growth zone in control cultures. *a*) 24-h culture; *b*) 3-day culture; ×70. Here and in Fig. 2: EC: explant; GZ: growth zone. Phase contrast microscopy.

of axons was observed in the control cultures after 24 h. Contacting glial cells (mainly plasmatic astrocytes) formed glial membranes covering a large area of the substrate (Fig. 1, *a*). Fibrous astrocytes with numerous processes forming reticular structures were also seen in the growth zone.

Two types of axons grew into the growth zone from the spinal explants: linearly growing axons forming numerous collaterals characteristic of motoneurons of the ventral spinal horn and fine axons of associative neurons, which grew from the explants, formed loops in the growth zone and grew back into the explant (Fig. 1, *b*) [2,12]. We previously explained it by the effect of motoneurons which, when situated in the explant, served as the targets for axons of associative neurons and regulated the direction of their growth [2].

Published data [14] and our findings [4] indicate that culturing of spinal explants leads to restoration of neuroglial relations in the growth zone and to intense growth of axons of motoneurons and associative neurons.

Addition of ethanol into nutrient medium inhibited the development of growth zone and formation of cell-cell contacts. Few axons and glial cells were detected in the majority of explants of this series (Fig. 2, *a*).

A stimulating effect was observed in cultures grown in the presence of ethanol and dolivin. Active growth of axons was diffuse (Fig. 2, *b*) or they formed cords (Fig. 2, *c*). Axons and their collaterals intensely grew from the terminals of the cords. Active migration of glial cells was observed in the growth zone; some cells were located along growing fibers and their collaterals.

Special attention was paid to growth zone formation during culturing. Quantitative analysis showed that culturing in standard nutrient medium yielded 38% explants with well-developed growth zone (from the total number of cultures). After addition of ethanol into nutrient medium the growth zone contained few axons and glial cells; the percent of these explants was 9% ($p<0.01$). After addition of dolivin together with ethanol into nutrient medium the number of cultures with well-developed growth zone, containing intensely growing axons and glial cells subsided from the explant, reached 50% ($p<0.01$).

For evaluation of the intensity of neurite growth under conditions of simultaneous effects of ethanol and dolivin, the neurite growth index was determined. The results of quantitative analysis confirmed dolivin capacity to inhibit the cytotoxic effect of ethanol. Both parameters (distribution density of axons and their length) increased significantly in cultures treated with ethanol and dolivin simultaneously (Table 1).

Hence, ethanol inhibits migration of glial cells and growth of axons in chick embryo spinal cultures, which is in line with the data on *in vivo* inhibition of

TABLE 1. Effects of Ethanol and Dolivin on Distribution Density, Length of Axons, and Neurite Growth Index ($M\pm m$)

Parameter	Control	Ethanol	Ethanol+dolivin
Distribution density of axons	6.0±0.4	4.0±9.5*	$7\pm1^{++}$
Axon length	302±35	187±19**	$408\pm25^{+}$
Neurite growth index	1812±261	748±144	$2856\pm548^{+}$

Note. $^{*}p<0.01$, $^{**}p<0.05$ compared to the control; $^{+}p<0.01$, $^{++}p<0.05$ compared to ethanol.

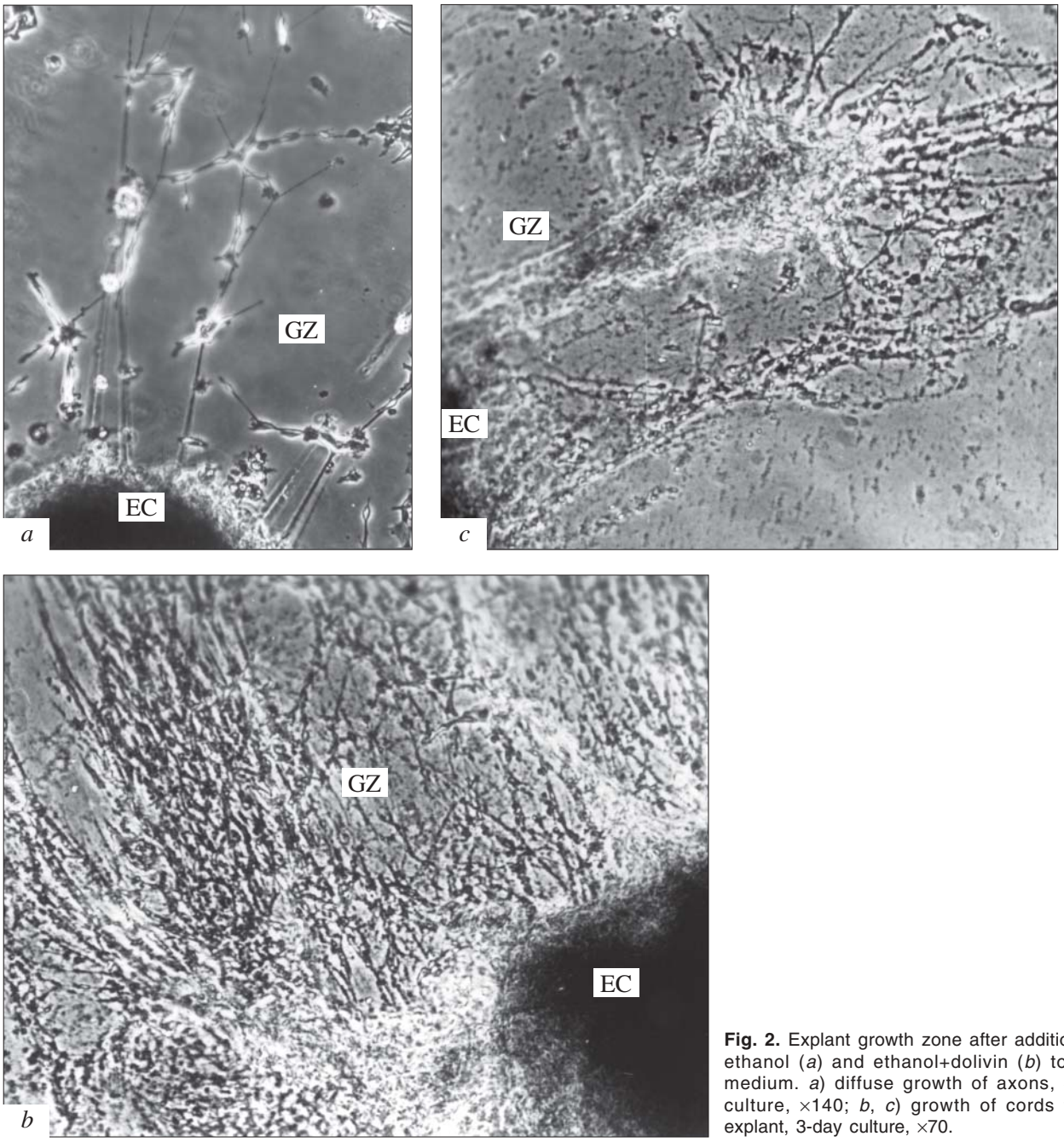

Fig. 2. Explant growth zone after addition of ethanol (*a*) and ethanol+dolivin (*b*) to the medium. *a*) diffuse growth of axons, 48-h culture, ×140; *b*, *c*) growth of cords from explant, 3-day culture, ×70.

CNS development under the effect of ethanol [6,10, 11]. Presumably, neurons in the spinal explant die under the effect of ethanol, including death via apoptosis [8,11]. Ethanol produced a destructive effect on neuroglial cells, as a result of which the neurotrophic factor characteristic of these cells did not promote neuronal survival [5,6]. Addition of dolivin into the nutrient medium prevented the cytotoxic effect of ethanol and stimulated the growth of axons and migration of neuroglial cells. Dolivin containing hypoxene produces antiradical and antioxidant effects. Incorporation of the thiosulfate group in the structure of hypoxene determines its neuroprotective effect, which manifests in protection of cell membranes and mitochondria from destructive effects of free radicals forming during ethanol-induced LPO.

REFERENCES

1. N. V. Gorbunov and A. N. Erin, *Byull. Eksp. Biol. Med.*, **111**, No. 5, 477-478 (1991).
2. D. P. Museridze and I. K. Svanidze, *Ontogenez*, **3**, 313-316 (1984).
3. E. N. Popova, *Arkh. Anat., Gistol. Embriol.*, **44**, No. 3, 5-10 (1988).

4. I. K. Svanidze and D. P. Museridze, *Byull. Eksp. Biol. Med.*, **116**, No. 12, 650-652 (1993).
5. D. L. Davies and W. E. Cox, *Brain Res.*, **547**, No. 1, 53-61 (1991).
6. C. Guerri, R. Saez, M. Sancho-Tello, *et al.*, *J. Neurochem. Res.*, **15**, No. 5, 559-565 (1990).
7. M. B. Heaton, J. J. Mitchell, and M. Paiva, *Alcohol Clin. Exp. Res.*, **24**, No. 4, 512-518 (2000).
8. J. S. Jacobs and M. W. Miller, *J. Neurocytol.*, **30**, No. 5, 391-401 (2002).
9. G. E. Landreth and B. W. Agraniff, *Brain Res.*, **161**, 39-53 (1979).
10. J. Luo and M. B. Miller, *Brain Res. Brain Res Rev.*, **27**, No. 2, 157-167 (1998).
11. J. K. Robinson and R. D. Mair, *Neurosci.*, **106**, 623-633 (1992).
12. H. M. Sobkowicz, R. W. Guillery, and M. B. Bornstein, *J. Comp. Naurol.*, **32**, 365-395 (1968).
13. N. Srivastava and A. Vernadakis, *J. Dev. Neurosci.*, **13**, No. 3, 529-537 (1995).
14. H. Tanaka and K. Obata, *Dev. Brain Res.*, **4**, No. 1, 313-321 (1982).
15. P. A. Yanny and T. A. Lindsley, Brain Res. Dev. Brain Res., **120**, No. 2, 233-243 (2000).