EFFECT OF NERVE GROWTH FACTOR AND BENACTYZINE ON SURVIVAL AND METABOLISM OF SPINAL GANGLIA IN CULTURE

S. N. Olenev and L. D. Shklyaeva

UDC 612.822-085.2

Spinal ganglia of a 9-day chick embryo were cultured by the "floating raft" method in ordinary medium (control) and in medium containing benactyzine (100 μg/ml) or nerve growth factor (50 μg/ml). Under the influence of benactyzine the number of surviving neurons, most of which contained monoamine oxidase, increased, as also did NAD-diaphorase, lactate dehydrogenase, and isocitrate dehydrogenase activity. Under the influence of nerve growth factor the number of nerve cells with acetylcholinesterase increased, NAD-diaphorase activity rose, lactate dehydrogenase activity became maximal, malate dehydrogenase activity increased a little, but succinate dehydrogenase activity was depressed.

KEY WORDS: nerve growth factor; benactyzine; spinal ganglia; enzyme activity.

Neurotropic chemical substances can modify the metabolism of nerve and accessory cells. The dehydrogenases and diaphorases – enzymes connected with the production and consumption of energy – are essential indices of the overall metabolism of cells. The value of cytophotometry as a method of quantitative estimation of enzyme activity rests on a firm basis [1]. Through the use of an equimolar concentration of substrates in incubation media and choice of incubation time, it is possible to compare the activities of different enzymes and to represent the whole process of glycolytic metabolism as a single scheme. Data on changes in metabolism of neurons of the spinal ganglia in culture under the influence of nerve growth factor and of benactyzine, obtained by means of this technique, are described below.

EXPERIMENTAL METHOD

Spinal ganglia of 9-day chick embryos were grown by the "floating raft" method in medium consisting of Eagle's MEM (in tablet form from Microbiol. Assoc., Bethesda), 10% horse serum, 0.4% glucose, and 0.4 unit/ml insulin. From the 5th through the 15th day the culture medium contained nontoxic doses of benactyzine (100 μg/ml) or nerve growth factor (50 μg/ml). The nerve growth factor (NGF) was obtained by the method described previously [2]. On the 15th day of the experiment the cultures, with or without (control) benactyzine and NGF, were stained to detect the enzymes. Photometry was carried out by the plug method on the MTsFV-1 instrument (LOMO) at a wavelength of 470 nm and with a probe 8 μ in diameter.

EXPERIMENTAL RESULTS

Neurons distinguished by their large size and high NAD-diaphorase and dehydrogenase activity were clearly detectable in the control. The neurons differed from one another in their enzyme activity. The greatest number of neurons was detected by combined staining for monoamine oxidase and cholinesterase and also by staining for NAD-diaphorase. Activity of the other enzymes was found in some of the cells (Table 1). In cultures treated by the same histochemical method, the number of neurons differed by not more than 1.5-2 times.

Under the influence of benactyzine the character of growth of the explants showed little difference from that in the control. Neurons were arranged in the center of the explant; in the peripheral zone of growth, formed by bundles of nerve fibers, migration of fibroblasts and Schwann cells was inhibited a little. Counting showed (Table 2) that the number of surviving neurons was 1.6 times greater ($P < 0.01$), the number of neurons with

Morphological Group, Central Research Laboratory, Leningrad Pediatric Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 2, pp. 212-215, February, 1978. Original article submitted April 20, 1977.

0007-4888/78/8502-0215$07.50

TABLE 1. Relative Enzyme Activity of Neurons in Control Cultures and under the Influence of Benactyzine and NGF (M ± m)

Enzyme	Control	Addition of benactyzine	Addition of NGF
NAD-diaphorase	1,53±0,07 (100)	2,27±0,07 (100)	2,32±0,09 (43)
Glucose-6-phosphate dehydrogenase (NADP)	0,68±0,02 (54)	0,43±0,02 (28)	0,37±0,02 (57)
6-Phosphogluconate dehydrogenase (NADP)	0,47±0,03 (50)	0,38±0,03 (53)	0,26±0,01 (50)
α-Glycerophosphate dehydrogenase (NAD)	0,12±0,03 (2,5)	0,06±0,03 (13)	0,02±0,02 (14)
Pyruvate dehydrogenase (NAD)	0,02±0,01 (2,6)	0,00 (2,1)	0,00 (1,0)
Lactate dehydrogenase (NAD)	0,42±0,02 (12)	0,65±0,03 (42)	1,08±0,07 (70)
Succinate dehydrogenase (-)	0,29±0,01 (14,5)	0,12±0,01 (7,5)	0,12±0,01 (0)
Isocitrate dehydrogenase (NAD)	0,45±0,02 (69)	0,73±0,01 (77)	0,80±0,01 (37)
Malate dehydrogenase (NAD)	0,25±0,03 (27)	0,51±0,03 (30)	0,68±0,06 (50)
β-Hydroxybutyrate dehydrogenase (NAD)	0,40±0,03 (8)	0,42±0,02 (6)	0,35±0,01 (21)

Legend. Percentage of reacting neurons given in parentheses; n > 30.

TABLE 2. Number of Neurons in One Spinal Ganglion in Culture and Percentage of Neurons with Monoamine Oxidase and Cholinesterase in Control and under the Influence of Benactyzine and NGF

	Control	Addition of benactyzine	Addition of NGF
Number of neurons (M ± m)	172±19	268±28	164±24
Cells with monoamine oxidase (MAO):			
Type A^{mao+++}	6	24	5
Type B^{mao+++}	29 } 35	44 } 68	23 } 28
Cells with acetylcholinesterase (CH):			
Type A^{ch+++}	23	9	13
Type B^{ch+++}	24 } 47	16 } 25	49 } 62
Cells containing both acetylcholinesterase and monoamine oxidase:			
Type $A^{mao+ch+}$	17	5	8
Type $C^{mao+ch+}$	0,5 } 17,5	0 } 5	0,4 } 8,4

Legend: A) largest neurons, B) medium-sized neurons, C) small neurons; +++) high enzyme activity, +) low enzyme activity.

monoamine oxidase was almost doubled, and there was also a significant increase in the activity of NAD-diaphorase ($P < 0.001$) and lactate, isocitrate, and malate dehydrogenases. The number of neurons containing lactate dehydrogenase was significantly increased. Activity of NAD-diaphorase and lactate dehydrogenase was completely absent in the satellite and Schwann cells, which migrated less than in the control.

Under the influence of NGF considerable growth of the processes of the neurons of the spinal ganglia took place and migration and development of satellites and Schwann cells were activated. Counting showed that compared with the control the number of surviving cells was not significantly changed, but the number of cells with acetylcholinesterase activity was almost doubled. Cytophotometry showed a marked increase in activity of lactate dehydrogenase and also (in some neurons) of NAD-diaphorase. Meanwhile the activity of enzymes of the pentose cycle ($P < 0.001$) and succinate dehydrogenase fell. During the action of NGF NAD-diaphorase activity in the satellites and Schwann cells, located between the neurons and their processes in the central zone of the explant, increased considerably. Migrating cells in the zone of growth differed from the satellites in the central zone by the presence of several enzymes (α-glycerophosphate, succinate, glucose-6-phosphate, and lactate dehydrogenases, NAD-diaphorase).

The data on metabolism in the control cultures and under the influence of benactyzine and NGF are illustrated schematically in Fig. 1, in which the thickness of the arrows is proportional to optical density multiplied by the fraction (% of reacting neurons/100). It will be clear from Fig. 1 that an increase in energy consumption under the influence of benactyzine and NGF (in the reaction of splitting of NADH by NAD-diaphorase) is accompanied by activation of lactate dehydrogenase (forming NADH). The depression of succinate and pyruvatedehydrogenases under these circumstances will be noted. The data on the effect of benactyzine on metabolism are in agreement with Laborit's conclusions [7] regarding the activating effects of atropine on glycolysis and the Krebs' cycle.

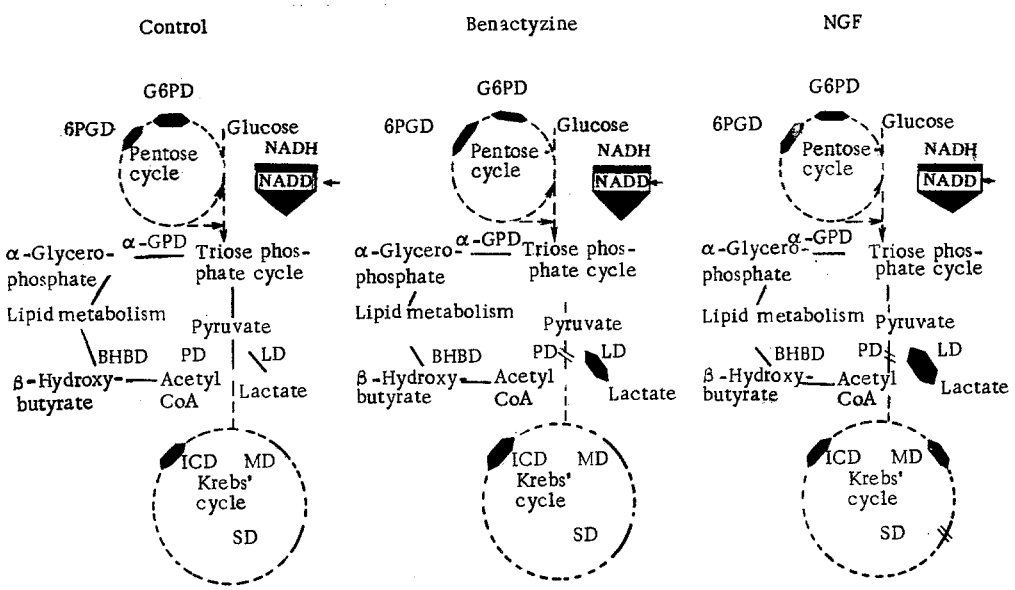

Fig. 1. Scheme representing metabolism of nerve cells in control culture and under influence of benactyzine (100 μg/ml) and NGF (50 μg/ml). Thickness of arrows is proportional to enzyme activity in neurons (optical density × % of reacting neurons/100). BHBD) β-hydroxybutyrate dehydrogenase, G6PD) glucose-6-phosphate dehydrogenase, 6PGD) 6-phosphogluconate dehydrogenase, α-GPD) α-glycerophosphate dehydrogenase, ICD) isocitrate dehydrogenase, LD) lactate dehydrogenase, MD) malate dehydrogenase, NADD) NAD-diaphorase, PD) pyruvate dehydrogenase, SD) succinate dehydrogenase.

Under the influence of benactyzine most neurons contained monoamine oxidase, whereas under the influence of NGF most contained acetylcholinesterase. It can tentatively be suggested that the addition of benactyzine, which blocks M-cholinergic receptors, to the culture medium facilitates survival of cells which have no M-cholinergic receptors [4], i.e., neurons of the spinal ganglion which contain monoamine oxidase, unlike neurons containing cholinesterase, have no M-cholinergic receptors.

The data indicating an increase in the number of surviving neurons in the spinal ganglion in medium containing benactyzine can be compared with the observed increase [5] in survival of neurons of the spinal ganglia under the influence of an increased K^+ concentration, when they are in a state of depolarization. If it is remembered that the ganglion of the 9-day chick embryo contains about 400 nerve cells, during culture for 2 weeks in the presence of benactyzine about 67% of the initially explanted neurons will survive, and 68% of them contain monoamine oxidase. In the control culture no such number of cells with monoamine oxidase was observed. Presumably this can be regarded as evidence of the possible transformation of neurons from acetylcholinesterase-containing into monoamine oxidase-containing, which is accompanied by a parallel change in glycolytic metabolism.

LITERATURE CITED

1. I. M. Buikis, Histochemistry of Dehydrogenases of the Developing Spinal Cord [in Russian], Riga (1975).
2. G. V. Konovalov, S. N. Olenev, and V. M. Pleskov, Tsitologiya, No. 10, 1268 (1974).
3. H. Laborit, Metabolic and Pharmacological Bases of Neurophysiology [in Russian], Moscow (1974).
4. S. N. Olenev, in: Textbook of Nerve Tissue Culture [in Russian], Moscow (1976), pp. 47-88.
5. B. S. Scott and K. C. Fischer, Exp. Neurol., 27, 16 (1970).