CHANGES IN THE VELOCITY OF AXOPLASMIC PROTEIN TRANSPORT ALONG AXONS OF MOTOR AND SENSORY NEURONS DURING INTENSIVE GROWTH OF THE RAT SCIATIC NERVE

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UDC 612.815.1].2.015.348 : 612.66

KEY WORDS: axoplasmic protein transport; axons of sensory and motor neurons; ontogeny; rats.

The transport of materials and structures along axons of neurons is an essential feature of the cytophysiology of nerve cells [2, 5, 7]. In the differentiated neurons of adult animals the quantity of substance, in particular protein, transported from the perikaryon into the axons and catabolized in them evidently correlates with the total weight of cytoplasm of dendrites, axons, and terminals. In the period of intensive growth and functional maturation of axons, changes may be expected in the parameters of these transport processes. The quantity of material transported along the axons depends on the carrying capacity of the transport system if the velocity of axoplasmic transport remains unchanged. Data cited by several workers indicate that both these ways may be used [4, 5, 9]. Nevertheless, the problem as a whole requires further study.

The aim of the present investigation was to compare the velocities of the axoplasmic flow of slow and fast protein fractions transported along axons of sensory and motor neurons of rats in the period of marked growth and maturation of the axons of these neurons.

EXPERIMENTAL METHOD

Male albino rats aged 2 weeks (weighing 10-15 g) and 4 weeks (weighing 40-60 g) were used. During this age interval a significant increase in length of the somatic nerve and an increase in the diameter of the nerve fibers are observed and are accompanied by marked changes in the protein content in these axons [1, 6]. By using a micromanipulator, under pentobarbital anesthesia, a microinjection of ^{14}C-glycine (specific activity 84 Ci/mmole) was given into the 5th lumbar spinal ganglion (in a dose of 2-4 μCi) or into the anterior horn of the spinal cord of the same segment (in a dose of 5-8 μCi).

At fixed time intervals after microinjection of the amino acid, a fragment of spinal cord (L5), the ventral and dorsal roots of the corresponding level, and the sciatic nerve as far as its division into tibial and peroneal branches, were removed on the side of injection. The roots and nerve were cut with a razor into segments 3 mm long. The origin of ventral root L5 in the case of analysis of transport along motor fibers or the 5th lumbar spinal ganglion in the case of analysis of transport along sensory fibers was chosen as the zero point ("0"). The spinal ganglia and fragments of nerves and spinal cords were placed in individual counting flasks. The tissues were solubilized by the method described in [3]. Radioactivity was determined in Bray's dioxan scintillator (4 g PPO, 200 mg POPOP, 60 g naphthalene, 100 ml methanol, 20 ml ethylene-glycol, and 1 liter dioxan) on a Multimat (Intertechnique, France) liquid scintillation counter.

The results were expressed in conventional units:

$$\frac{\text{cpm/min/3-mm segment}}{\text{cpm/min ganglion or segment of spinal cord}} = 100$$

The mean velocity of protein transport was calculated from the position of the radioactivity front [5].

EXPERIMENTAL RESULTS

Results giving some idea of the velocity of transport of fast and slow protein fractions in the central and peripheral axons of sensory neurons of rats aged 2 and 4 weeks are illustrated in Figs. 1 and 2. In the

Department of Biology and Department of Pharmacology, N. I. Pirogov Second Moscow Medical Institute.
(Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 1, pp. 59-61, January, 1981. Original article submitted March 6, 1980.

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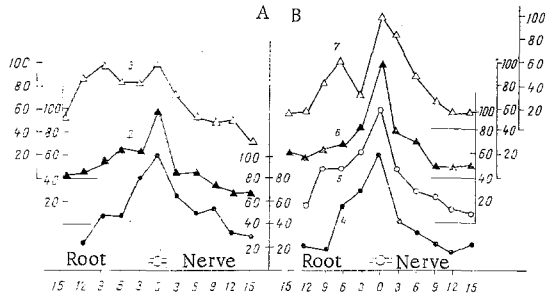

Fig. 1. Transport of slow (A) and fast (B) fractions of labeled protein along central and peripheral axons of sensory neurons. Abscissa, distance (in mm) from ganglion; ordinate, radioactivity (in conventional units). 1) Animals aged 2 weeks, killed one day after microinjection; 2) animals aged 4 weeks, killed two days after injection, 3) animals aged 4 weeks killed after four days; 4) animals aged two weeks killed 1 h after microinjection; 5) animals aged 2 weeks killed after 2 h; 6) animals aged 4 weeks killed after 1 h; 7) animals aged 4 weeks killed after 2 h.

TABLE 1. Velocity of Axoplasmic Flow (in mm/day) in Sensory and Motor Fibers of Rats (M ± m)

Test object	Age of animals, weeks	
	2	4
Slow fraction:		
in central sensory fibers	4,5±0,27	3,75±0,35
in peripheral sensory fibers	4,5±0,3 $P = 0,23$	4,1±0,25
in motor fibers	5,25±0,31	3,45±0,23*
Fast fraction:		
in central sensory fibers	126±14,07	144±23,34
in peripheral sensory fibers	126±12,1 $P = 0,36$	144±16,34
in motor fibers	99±13,12	$P = 0,079$ 198±18,9*

*$P < 0.05$ compared with group of rats aged 2 weeks.

animals aged 4 weeks the absolute velocity of axoplasmic flow of the slow fraction was lower, and of the fast fraction higher than in animals aged 2 weeks. However, the differences were not significant (Table 1).

Regarding the motor fibers of the sciatic nerve (Figs. 2 and 3, Table 1) a significant decrease ($P = 0.009$) was found in the velocity of axoplasmic flow of the slow labeled protein fraction, accompanied by a significant (approximately twofold, $P = 0.001$) increased in the velocity of axoplasmic flow of the fast fraction in 4-week-old rats compared with animals aged 2 weeks.

In the calculations done in this investigation the time interval from injection of the labeled amino acid to the appearance of the first labeled proteins in the perikarya of the neurons of the anterior horns of the spinal cord of spinal ganglion was not taken into account. The calculated values of velocities of axoplasmic flow of the slow and, in particular, of the fast protein fractions obtained in this investigation were thus underestimated. There are indications that the maximum of labeled polypeptides in neuron cytoplasm is found 20-30 min after introduction of radioactive amino acid into the cell [3]. Allowing for these data, the actual velocity of the axoplasmic flow of the fast fraction would differ by more than 10% from its calculated value.

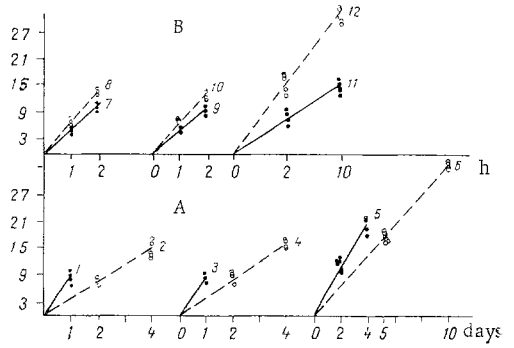

Fig. 2

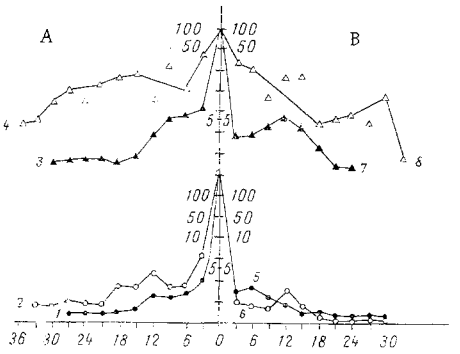

Fig. 3

Fig. 2. Velocity of transport of slow (A) and fast (B) fractions of labeled protein along sensory and motor fibers of sciatic nerve of rats aged 2 and 4 weeks. Abscissa, time between microinjection and sacrifice of animals; ordinate, distance from bodies of neurons (in mm). Sensory fibers: 1, 7) central axons of animals aged 2 weeks; 2, 8) central axons of animals aged 4 weeks; 3, 9) peripheral axons of animals aged 2 weeks; 4, 10) peripheral axons of animals aged 4 weeks. Motor fibers: 5, 11) animals aged 2 weeks; 6, 12) animals aged 4 weeks.

Fig. 3. Transport of slow (A) and fast (B) fractions of labeled protein along axons of motor neurons. Abscissa, distance (in mm) from anterior horns of spinal cord; ordinate, radioactivity (in conventional units). 1) Animals aged 2 weeks killed 2 days after microinjection; 2) animals aged 2 weeks killed after 4 days; 3) animals aged 4 weeks killed after 5 days; 4) animals aged 4 weeks killed after 10 days; 5) animals aged 2 weeks killed 2 h after microinjection; 6) animals aged 2 weeks killed after 4 h; 7) animals aged 4 weeks killed after 2 h; 8) animals aged 4 weeks killed after 4 h.

The results of the present investigation agree with those obtained by other workers [4, 10] who observed an increase in the velocity of axoplasmic flow of the fast protein fraction during postnatal development of mammals. The data obtained by several workers also point to a decrease in the velocity of axoplasmic flow of the slow fraction with age [3, 4, 9, 10].

In the present investigation changes in the rate of flow were found for axons of motor nerve cells. Regarding axons of sensory neurons, in this case only a tendency could be found for the rate of axoplasmic flow of proteins to change, in the same direction. The slow and fast fractions include proteins differing in localization in the structures of the axons and terminals and in their functional role. A progressive decrease in the rates of growth in early postnatal ontogeny explains the decrease in the velocity of axoplasmic flow of the slow fraction, a considerable volume of which consists of material of the structures of the axons and endings themselves [2, 5, 7]. Components of the fast fraction mainly ensure adequate functioning of the excitable membrane and of the synaptic apparatus in the mature nerve cell [2, 5, 7]. It therefore seems logical that the velocity of expolasmic flow of this fraction should increase in adult animals.

The data given in Fig. 1 and Table 1 indicate that the velocities of axoplasmic flow of both protein fractions in the peripheral and central axons of sensory neurons of the spinal ganglion are comparable. Some workers [9], however, describe a higher velocity of axoplasmic flow of the slow fraction in peripheral axons of sensory neurons in adult rats. The disagreement between our own results and those obtained by these workers may perhaps be attributable to differences in the age of the rats used in the experiments.

In the present investigation no significant differences in the velocity of axoplasmic flow of the slow and fast protein fractions along sensory and motor fibers of the sciatic nerve could be found for rats of the two age groups studied (Table 1), in agreement with results obtained by other workers.

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PRODUCTION OF PURE LINES OF THYMUS STROMAL FIBROBLASTS AND DISCRIMINATION OF FIBROBLASTS AND EPITHELIUM IN THYMUS CULTURES

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UDC 612.438-085.23

KEY WORDS: thymus; stromal fibroblasts; pure lines of fibroblasts.

Thymic humoral factors (thymosins) influencing differentiation of T lymphocytes are produced, it is supposed, not by thymocytes but by stromal cells of the thymus, but which stromal cells has not yet been established. It has been shown [6, 7, 8, 9] that thymosin is found in culture media of thymus explants. However, all types of stromal cells of the thymus, including macrophages, fibroblasts, and epithelium, are present in such cultures [3, 4, 7].

Accordingly the investigation described below was undertaken to determine precise criteria for discrimination in vitro between epithelial cells of the thymus and its stromal fibroblasts and to obtain pure cultures of cells of each of these types.

EXPERIMENTAL METHOD

Thymus cells of guinea pigs aged 3-4 weeks were used for explantation. The cells were isolated either mechanically or by trypsinization. In the first case the thymus was teased apart by needles in medium 199, after which the cell suspension was pipeted. In the second case the thymus was cut into fragments measuring 0.5-1 mm and agitated on a magnetic mixer for 30 min at room temperature. The medium with the liberated cells was separated and the residual fragments of tissue were covered with 0.5% trypsin and agitated on the mixer for a further 30 min. The suspensions thus obtained were sedimented by centrifugation, resuspended in medium, and filtered through four layers of Kapron. Suspensions prepared by all the methods mentioned above consisted of isolated cells, of which over 99% were thymocytes. The cells were explanted by the method in [3, 4] in plastic flasks (area of the bottom 25 cm^2), into each of which 1×10^5-5×10^5 cells were introduced, or in glass flasks (area of the bottom 40 cm^2), into each of which 1×10^6-3×10^6 cells were introduced. The cells were cultured in medium 199 with 20% embryonic calf serum. The medium was changed once a week. Some cultures were repeatedly subcultured, the first time after 2-3 weeks, and thereafter once a week. During subculture the cells were washed with medium 199, treated with 0.25% trypsin solution, and the detached cells were transferred to flasks with twice the previous area. Living cultures, and also cultures stained by the PAS and by Giemsa's methods, and after the indirect immunofluorescence test with antibodies against fibronectin (isolated from serum obtained by immunizing rabbits with electrophoretically pure fibronectin) and with antifibroblastic serum (prepared by immunizing rabbits with bone marrow fibroblasts grown in cultures [1, 2]), were used for morphological investigation. The antifibroblastic serum also was used for the complement-dependent cytotoxic test. For this purpose cultures were treated with antiserum (30 min at room temperature) 4 h after primary explantation or after routine subculture, complement was added (for 60 min at 37°C), and the cultures were then washed, covered with medium, and cultured for 12 days. The colony formation

Laboratory of Immunomorphology, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 1, pp. 61-64, January, 1981. Original article submitted February 20, 1980.

