Dynamics of Morphological Changes in the Trigeminal Ganglion Neurons in Compression Injury of the Rat Maxillary Nerve

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The dynamics of reparative processes in rat trigeminal ganglion neurons was studied during the early posttraumatic period; somatic location and interactions of neurons with glial cells were evaluated. The neurons and their nucleoli were enlarged during this period, proliferation of glial cell and transcription activity of chromatin increased. The location of neuronal bodies belonging to the maxillary nerve was determined by the phenomenon of chromatolysis. The neurons belonging to this nerve were located under the node capsule.

Key Words: *compression injury; rat; trigeminal ganglion; neurons; glia*

Changes in neurons during the posttraumatic period provide effective regeneration of the peripheral nerve and recovery of innervated tissue functioning [8]. The data on morphological changes in Gasser's ganglia in response to facial skeletal bone injuries are scanty and contradictory [7].

A stable trend to an increase in the incidence and severity of traumatic injuries to the middle facial zone is observed at present [3,6], but studies of the morphological aspects of this problem in humans are difficult. Therefore, most studies are carried out on laboratory models.

Despite numerous studies of changes in the protoneurons after damage to their processes, there is still no universal opinion on the pattern and outcome of neuronal reaction to injury. It seems that the contradictory data and difficulties in their comparative analysis are explained by differences in experimental treatment and periods of analysis of the material [4]. The time course of reparative process, dynamic alternation and continuity of various cell forms, their biological and more narrow medical significance remain unknown up to the present time [5].

Schwann's cells analogs located in the spinal nodes (satellite cells) are involved in the survival of damaged sensory neurons. Surrounding the neurons, they protect them from exposure to neurotoxins, synthesize and release neurotrophic factors, and phagocytose fragments of degenerating neurons [9-12].

We studied the dynamics of reparative processes in the rat trigeminal ganglion neurons during the early posttraumatic period, evaluated the somatotopic location and the interactions of neurons with satellite cells. During this period, enlargement of neurons and their nucleoli, proliferation of glial cells, and increase in chromatin transcription activity are observed in the absence of apparent neurological symptoms.

MATERIALS AND METHODS

The study was carried out on 40 outbred male albino rats (4-6 months, 200-300 g), 30 of these were used in experiments and 10 were controls. After infliction of compression injury to the middle zone

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of the facial part of the head, the trigeminal ganglion cells were examined. The animals were sacrificed on days 1, 3, 7, 14, 21 after the injury in accordance with "Regulations on Handling Laboratory Animals" (Supplement to Order of the Ministry of Health of the USSR No. 755 of August 12, 1977). The material was collected directly after sacrifice.

The material was stained after Nissl, with hematoxylin and eosin, and by routine methods for electron microscopy. Micropreparations were examined under a BIMAM P-11 microscope using Nikon Microflex AFX-DX camera (magnification 200).

Cytokaryometrical analysis of cells was carried out using a MOB-1-16 micrometer ocular; cell number per unit area, cell diameter and area, and nuclear area were evaluated, and cell and nucleus volume were calculated by the formula $V=0.523 D^3$ [2].

The data were processed using Microsoft Excel and Statistica 6.0 software. Mann—Whitney nonparametric test was used. The results were considered significant at $p<0.005$.

RESULTS

The trigeminal ganglion neurons of control rats were represented by pseudounipolar neurons. According to morphometric data, the cells constituted 3 groups by their size: 1) large cells of 32.3-50 μ; 6% of all cells; 2) medium-sized cells of 17.6±28.3 μ in size (52%); and 3) cells 10.5-16.7 μ in size (42%). The neurocyte nuclei (round and oval) had 1-2 large well-discernible nucleoli.

The Nissl substance was heterogeneous. Large lumps, filling the entire perinuclear cytoplasm, were seen in almost all neurons, the bulk of the substance being dispersed. The neuron capsule contained neuroglia satellite cells. The mean number of these cells per neuron was 4.5±1.3. The neurons were unevenly distributed in a ganglion. The bulk of neurons were under the ganglionic capsule (small and medium-sized cells), while large neurons were located closer to the periphery of the sensory radicle. The neurons formed small groups in the central part of the ganglion; these groups were close to each other, and bundles of nerve fibrils passed between these groups.

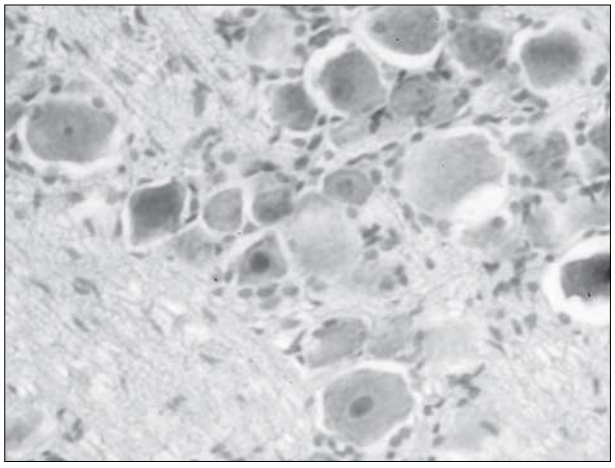

Fig. 1. Reaction of trigeminal ganglion neurocytes (day 1 after injury), ×200. Hematoxylin and eosin staining.

After the injury, the neurons and other elements of the ganglion underwent reactive and degenerative changes.

On day 1 after the trauma the histoarchitecture of the ganglion was retained, the nuclei of some cells were shifted, with clearly discernible contour. In parallel with thus, there were groups of cells with blurred nuclei, and in some cells nuclear pyknosis started. Nissl substance in the cytoplasm of the rest cells was finely granular with even distribution of the grains (Fig. 1). Slight proliferation of the neuroglia was noted. The number of gliocytes virtually did not change. Analysis of the structure of trigeminal ganglionic cells showed initial destructive changes in the trigeminal ganglion and related nerve on day 1. Dislocation of the nucleus, increased content of chromatin, and its slight condensation under the nuclear membrane were seen in the neurocytes.

Three days after injury, the nuclei retained clear-cut contour in some neurons. The contour of Nissl substance was well discernible and it was evenly distributed in the entire cytoplasm. Cells with sharply

TABLE 1. Quantitative Changes in Cells with and without Nuclei

Parameter	Control (n=10)	Day of analysis				
		1 (n=10)	3 (n=10)	7 (n=10)	14 (n=10)	21 (n=10)
Number of nuclear cells per μ²	28.5±1.6	21.3±1.1	20.1±1.3	16.4±0.8	15.2±1.8	17.1±0.7
Number of anuclear cells per μ²		16.6±0.9	28.4±1.5	30.2±1.7	34.2±0.9	22.6±1.2
Proportion of nuclear to anuclear cells		1.3:1	1:1.4	1:1.8	1:2.2	1:1.3

Note. $p<0.05$.

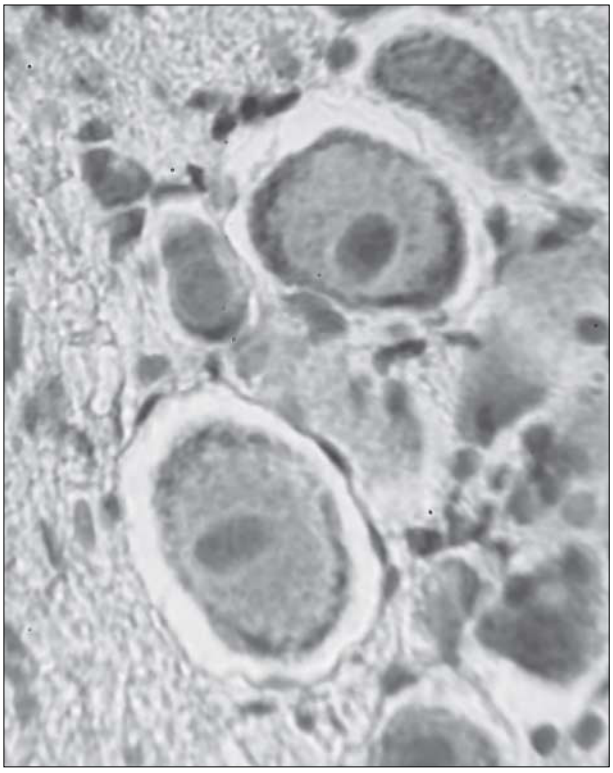

Fig. 2. Chromatin condensation under cell membrane and nuclear ectopy, ×200. Hematoxylin and eosin staining.

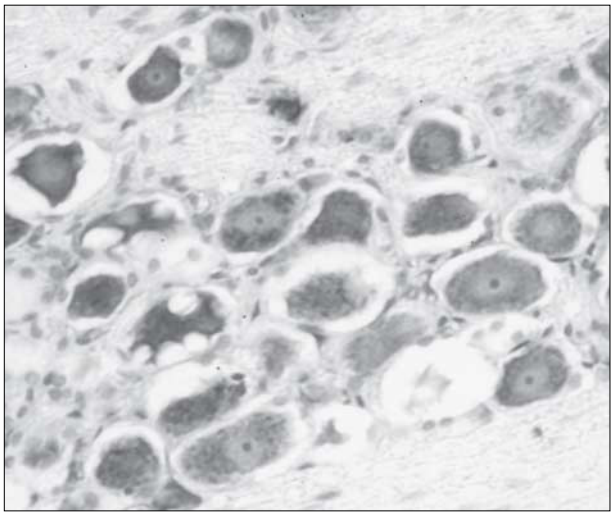

Fig. 3. Cell pyknosis (day 14 of experiment), ×200. Nissl staining.

pronounced perineuronal gaps and proliferation of the glia elements were detected. Focal proliferation (increased number) of the glia elements was seen.

Study of the preparations by optic microscopy showed the emergence of signs of axonal reaction (chromatin condensation under cell membrane) in parallel with central chromatolysis and nuclear ectopy 1 week after the injury in the majority of neurons (Fig. 2).

Focal glial reaction was observed on day 7 of the experiment. Proliferation of glial cells predominated around cells without nuclei and with diffuse chromatolysis, their number per neuron increased.

Fourteen days after the trauma the neuronal reaction was more pronounced in comparison with the previous periods. Severe destructive changes of the pyknosis type were seen in the majority of neurons, diffuse chromatolysis persisting in some cells (Fig. 3). Neurons with the structure close to the norm were located in the center of the ganglion. Nerve cells with total chromatolysis and poorly visualized contours of the nuclei and nucleoli were often seen during this period.

Changes in the satellite cells were diffuse and focal, their number increasing in comparison with the previous period.

On day 21, hyperchromatosis of the nuclei, which were located in the center of the cells, was detected. Neurocytes with diffuse chromatolysis were seen during this period. Despite partial elimination of nerve cells, the majority of surviving neurons exhibited signs of regeneration, consisting in their hypertrophy and restoration of the lumpy and granular form of chromatophilic substance.

Diffuse increase in the number of satellite cells was observed. The number of gliocytes per neuron reached 10.6±0.8.

It was found that rat trigeminal ganglion was similar by its structure to other sensory ganglia. The greater part of neurons were located under the ganglionic capsule, the center of the ganglion was represented by groups of neurons, divided by nerve fibrils [5].

The neurons belonging to the involved branch of the trigeminal ganglion were detected by chromatolysis developing in compression of the infraorbital nerve. It was found that the localization of neuron bodies belonging to the infraorbital nerve is clearly somatotopic, which is seen by the location of these cells under the ganglionic capsule.

Staged morphogenesis of the traumatic process in the trigeminal ganglion was detected. Alteration

TABLE 2. Dynamics of Quantitative Changes in Perineuronal Glia

Control	Day 1	Day 3	Day 7	Day 14	Day 21
4.5±1.3	4.7±1.1	4.8±1.2	5.6±1.5	6.2±1.2	10.6±0.8

Note. $p<0.05$.

with compensation and degeneration phenomena were observed at the initial stages, while the final periods of experiment were characterized by regeneration. The intensity of the detected changes in the peripheral nervous system depended on the period of experiment.

The compensatory changes most often manifested by increase of the cell size (Table 1). The main morphometric parameters of the neurons with structural disorders of varying severity differed greatly. More severe changes were associated with enlargement of the nuclear volume from 338.5±1.9 μ^3 (control) to 595.8±2.5 μ^3 at the end of the study.

The predominant type of degenerative changes at the initial stage was shrinkage of the neurons, associated with decrease of their size and deformation of the contour, intensive staining of the cytoplasm, deformation and hyperchromatosis of the nuclei. Pronounced vacuolation of the cytoplasm was much more rare.

Virtually no reaction of the glia was seen at the beginning of experiment (on days 1-3; Table 2), because active metabolic processes in the respective neurons were maintained at the expense of the cellular own resources [1].

The number of glial satellite cells increased between days 3 and 7; this was caused by exhaustion of the neuron resource.

Presumably, the increase in the number of satellites starting from week 2 of experiment resulted from increased trophic requirements of the neurons during active intracellular regeneration, characterized not only by plastic processes, but also by normalization of the hemodynamics in the ganglion.

On the other hand, we cannot assert that the cellular regeneration processes were over by the end of the experiment, which could be seen from enlarged size of the neurons and proliferation of glia elements [13].

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