CHANGES IN RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE IN THE TRIGEMINAL NERVE IN KERATITIS FOLLOWING BURNS

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KEY WORDS: trigeminal nerve; retrograde peroxidase transport; keratitis following burns.

Application of highly purified horseradish peroxidase (HRP) to peripheral branches of afferent neurons leads to the appearance of the marker in the perikaryon [6]. HRP is taken up by the axon, with the formation of endosomes [8], which are carried by retrograde transport mechanisms into the neuron body. Both the assimilation and the axial flow of HRP have been shown to depend on temperature, on the action of inhibitors of metabolism [4, 9, 10], and on various other factors.

After exposure of the cornea to a high temperature both direct injury to HRP transport processes in afferent neurons and a subsequent effect on retrograde HRP transport may be expected as a result of developing inflammation of the cornea — burn-induced keratitis. It has been shown [3] that changes in the intensity of the inflammatory response in burn-induced keratitis take place in stages.

The aim of this investigation was to compare the time course of labeling neurons of the trigeminal ganglion with HRP at different stages of burn-induced keratitis.

EXPERIMENTAL METHOD

Experimental were carried out on 45 noninbred male albino rats weighing 210-270 g. A circular incision 0.05 mm in depth, 0.1 mm wide, and 4 mm in diameter was made by means of a calibrated applicator on the cornea of the rats, anesthetized with pentobarbital (30 mg/kg, intraperitoneally). A 50% aqueous solution of HRP was applied in a dose of 10 µl to the region of the incision. To prevent the solution from leaking and to ensure its uniform distribution over the whole perimeter, a glass ring slightly larger in diameter than the incision was applied to the cornea. The duration of exposure to HRP was 30 min. Next, without removing the glass ring, HRP was carefully washed away with warm (32°C) physiological saline.

The cornea was burned for 2 sec with a thermocautery, with electronic temperature stabilization, heated to 90°C (diameter 3 mm). The burn and the incision were so arranged that the latter surrounded the area of the burn and lay outside it.

Incision of the cornea and application of HRP were carried out during successive stages of keratitis: in the reactive stage 1 h after burning of the cornea, in the dystrophic stage on the 6th day, and in the regenerative stage on the 21st day. The number of animals in each series was 9. To study the effect of the burn on the phase of endocytosis, HRP was injected into 9 animals 1 h before burning. Under pentobarbital anesthesia (50 mg/kg) rats of each series were perfused, 12, 24, and 48 h after injection of HRP, through the left ventricle with a solution of polyglucin and heparin, followed by a fixing solution consisting of a 0.5% solution of paraformaldehyde and a 1.25% solution of glutaraldehyde in 0.1 M Na-phosphate buffer, pH 7.4. After fixation, the dissected trigeminal ganglion was washed with 30% sucrose solution in phosphate buffer to remove the fixative. Serial sections 40 µ thick, cut on a freezing microtome, were stained by the method in [7] with 3,3'-diaminobenzidine tetrahydrochloride (from Chemapol, Czechoslovakia).

The numerical data were subjected to statistical analysis by Student's test.

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TABLE 1. Number of Labeled Trigeminal Ganglion Cells at Different Stages of Keratitis (M ± m)

Time of HRP transport, h	Reactive stage		Dystrophic stage	Stage of regeneration	Control (without burning)
	application of HRP 1 h before burning	application of HRP 1 h after burning	application of HRP on 6th day after burning	application of HRP on 21st day after burning	
12	11.7±1.3*	0	0	16.3±2.7*	46.6±6.1
24	37.0±1.9*	23.6±2.8*	16.3±3.5*	31.7±4.3*	132.7±21.0
48	42.3±2.7*	38.6±3.8*	28.3±5.0*	37.6±6.4*	174.7±14.0

Legend. *P < 0.05 compared with control.

EXPERIMENTAL RESULTS

Examination of serial sections showed that peroxidase-active cells (PAC) were present throughout the volume of the ophthalmic subdivisions of the trigeminal ganglion. The degree of optical density of the PAC, recorded visually, varied from weak to intensely stained. All cells with a positive reaction for HRP were counted. The results are given in Table 1.

Application of HRP to the cornea before and after burning differed in its effect on the time of appearance of the enzyme in the perikarya of the trigeminal ganglion neurons and on the number of labeled cells. If HRP was applied 1 h after burning, no labeled cells were found in the trigeminal ganglion 12 h, and they were fewer in number 24 and 48 h, after application of HRP than at the corresponding times in the experiment in which HRP was applied 1 h before burning. The differences in the results of experiments in which HRP was applied before and after burning show that a high temperature, causing destructive changes in the neuron, evidently affects the uptake of the enzyme, which is slowed or stopped, and also inhibits transport of HRP already assimilated.

By the 6th day, in the dystrophic stage of keratitis, inflammation of the cornea is intensified and the zone involved by it is enlarged [3]. At this time a further decrease is found in the number of HRP-labeled neurons in the trigeminal ganglion, more marked 48 h after application of HRP. The number of neurons palely stained by the chromogen was increased. Consequently, in this phase of keratitis retrograde HRP transport was disturbed even more.

On the 21st day, in the regenerative stage of burn-induced keratitis, the number of PAC in the trigeminal ganglion was increased. This increase was not yet significant, nor did it reach the control level. The increase in the number of HRP-containing neurons only 12 h after its application in this stage indicates activation of HRP uptake and transport in the nerve cells. The small difference in the number of PAC between groups of animals killed 24 and 48 h after application of HRP can evidently be regarded as evidence that there exists a limited number of neurons capable of transporting HRP, and in about the same morphological and functional state. Most cells were deeply stained. This intensification of retrograde transport is evidently associated with the development of reparative processes in the eye and regeneration of nerve endings.

The region of thermal injury in burns has been shown [1, 2] to be wider than the zone of direct contact between the hot surface and the cornea, and the interval of 1 mm was sufficient, when the burn was inflicted by this method, to avoid direct morphological destruction of neurons in the region of application of HRP. However, because of the considerable branching of the corneal nerve fibers [12], some branches of a neuron lying in the zone of the burn were damaged and the time course of labeling of the perikarya of the trigeminal ganglion cells reflected regeneration processes of the neuron. Meanwhile the general response of the nerve cells did not correspond exactly to the picture of the damaging action of the burn on nerve tissue only. The absence of cell labeling 12 h after application of HRP in the group of animals with burns, whereas labeled cells were present in the control, their appearance after 24 h, and the further increase in their number after 48 h indicate that some cells remain capable of retrograde transport of materials but more slowly. Even greater inhibition of the retrograde axial flow was observed when HRP was applied on the 6th day during the period of development of dystrophic changes in the cornea, evidently due to involvement of the neurons in the dynamics of the inflammatory process.

The investigation described above thus revealed a damaging action of thermal burns on retrograde axonal transport. The time course of HRP labeling of trigeminal ganglion neurons

coincides with the sequence of stages of keratitis, evidence that processes of retrograde transport and the functional state of the tissue are interconnected.

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PREVENTION OF CATECHOLAMINE-INDUCED LESIONS OF THE ENDOTHELIUM OF THE RABBIT AORTA BY ALPHA- AND BETA-ADRENORECEPTOR ANTAGONISTS AND LITHIUM HYDROXYBUTYRATE

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Injury to the endothelium and the associated increased permeability of the intima of arteries play a leading role in the initial stages of atherogenesis [7]. Catecholamines, the principal mediator of stress, cause damage to the endothelium of the perfused rabbit aorta and increase its permeability for low-density lipoproteins [4]. Experimental lesions of the myocardium induced by catecholamines and isoproterenol, starting with pycnosis of the cardiomyocyte nuclei and ending with myocytolysis, are known [5, 8]. Lesions of the cardiomyocytes, but not of endothelial cells, have been found in investigations of the perfused heart [8]. Beta-adrenoreceptor antagonists can prevent catecholamine-induced damage to cardiomyocytes [5]. Lithium salts, which inhibit adenylate cyclase [6], like beta-adrenoreceptors, prevent the development of stress-induced myocardial lesions [3].

The authors have studied the action of lithium hydroxybutyrate on endothelial lesions induced by catecholamines in the perfused rabbit aorta and have compared the effects of lithium hydroxybutyrate with those of adrenoreceptors antagonists.

EXPERIMENTAL METHOD

Experiments were carried out on 47 male chinchilla rabbits weighing 3-4 kg. Under pentobarbital anesthesia with artificial ventilation of the lungs the thoracic and abdominal portions of the aorta were mobilized, all lateral branches were ligated, and the aorta was divided into four segments of equal length, which were perfused simultaneously. Lung segments served as the control; the rest were perfused with the test substances in different concentration. Perfusion was carried out under standard conditions [1]. The perfusion fluid was medium 199

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