

# CHANGES IN THE MICROCIRCULATION IN RATS WITH EXPERIMENTAL TRIGEMINAL NEURALGIA

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In previous experiments on rats with a pain syndrome of spinal origin (PSSO), caused by the creation of a generator of pathologically enhanced excitation (GPEE) [6, 11] in the dorsal horns of the spinal cord, it was shown that the stage of a marked pain syndrome is accompanied by the development of disturbances in the mesenteric microcirculation [7]. It was not clear whether these disturbances are generalized or whether they appear only in zones close in their innervation to a GPEE, localized in the spinal cord at the lumbar level.

To solve these problems an investigation was carried out in which trigeminal neuralgia (GN), induced by a GPEE in the caudal nucleus of the spinal tract of the trigeminal nerve, was chosen as the model.

## EXPERIMENTAL METHOD

The investigation was conducted on 37 male rats. Experimental TN was produced in the rats by the method described previously [8], by the creation of a GPEE in the caudal nucleus of the spinal tract of the trigeminal nerve (Fig. 1a). It was accompanied by the appearance of characteristic features of trigeminal neuralgia in man [2, 3]. The animals were divided into groups: group 1 (10 rats) consisted of animals with TN evoked by the creation of a GPEE by microinjection of 1 MLD of tetanus toxin (TT) for rats in 1  $\mu$ l into the nucleus (development of the syndrome and the state of the microcirculation were assessed 4-6 h after the injection of TT); group 2 (16 rats) were animals with TN induced by the creation of a GPEE by microinjection of 0.25 MLD in 1  $\mu$ l into the nucleus (the microcirculation of these animals was studied after 1 and 4 days). Each experimental group of rats was compared with a group of control animals undergoing a mock operation, and receiving, instead of TT, an injection of 0.9% sodium chloride solution in the same volume into the nucleus. Altogether 11 rats were subjected to the mock operation. The mesenteric microcirculation was studied in rats anesthetized with pentobarbital (5 mg/100 g). After investigation of the microcirculation the percentage of degranulated mast cell was determined after fixation of an area of the mesentery in 96° alcohol, and staining with 1% toluidine blue. The venular permeability for colloidal carbon particles was determined by the "labeled vessels" method [1]. The intensity of the stress reaction in the animals was determined by measuring changes in the weight of the thymus, spleen, and adrenals. For comparison of the data, the weights of the organs were expressed per 100 g body weight.

## EXPERIMENTAL RESULTS

Between 2 and 3 h after injection of 1 MLD TT the rats began to scratch a particular part of the snout with the corresponding hind limb on the side of injection of the toxin. In intervals between scratchings the rats were quiet. With the course of time (4-6 h after injection of TT) the episodes of scratching increased in frequency and intensity and were accompanied by a cry. Traces of vocalization and scratching activity during the development of the attack of pain are illustrated in Fig. 1b. Just

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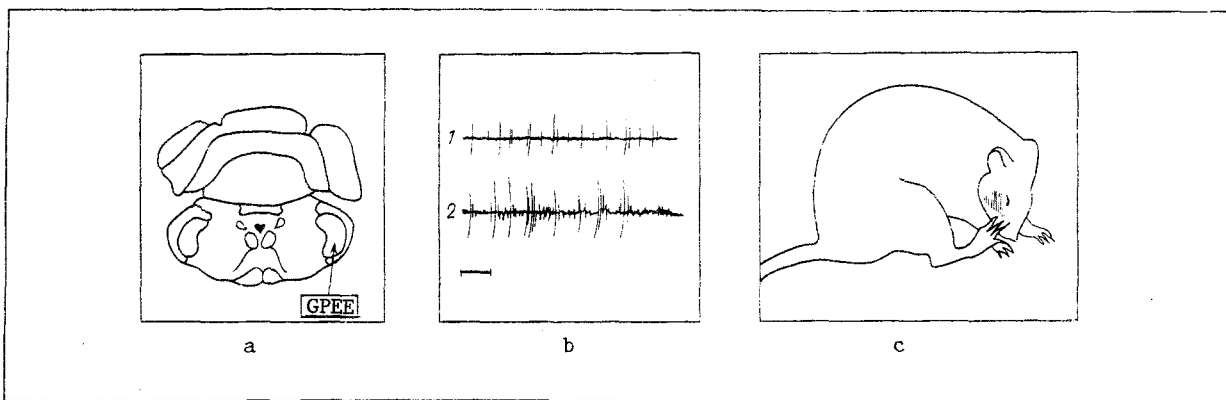


Fig. 1. Model of trigeminal neuralgia caused by creation of a GPEE in caudal nucleus of trigeminal nerve: a) diagram of frontal brain section passing through caudal nucleus, in which GPEE was created; b) trace of vocalization (1) and scratching movements (2) during attack of pain; c) typical posture of animal with trigeminal neuralgia during an attack (scratching zone is shaded).

as previously, the attacks were paroxysmal in character, but they were now easily provoked by stimulation applied to the scratching zone (the "trigger zone"). They could be accompanied by tactile stimulation of this zone which varied in strength, and could even be weak. The scratching zone increased in size and the skin of this zone was injured as a result of scratching, and the hair cover was removed (Fig. 1c). At this stage of maximal development of TN the animals were taken for investigation of their microcirculatory system.

If a smaller dose (0.25 MLD) was used the pain syndrome had the same characteristic features, but the increase in the intensity of the symptoms of TN in these animals took place more slowly. The syndrome remained at a relatively high level of intensity for 4-10 days. The body weight of the rats 4-6 h after injection of 1 MLD TT (group 1) was unchanged. After injection of 0.25 MLD of TT (group 2), animals in which the pain syndrome lasted a long time (1-4 days) showed a decrease of their body weight (17-20 g daily), whereas in animals undergoing the mock operation, there was a daily increase of body weight of on average 10 g. Changes in weight of the thymus and spleen were not observed in the rats in any of the series of experiments. Hypertrophy of the adrenals was found in the rats of group 2. The weight of these organs was as follows:  $9.8 \pm 0.2$  mg/100 g after 1 day and  $13.0 \pm 1.2$  mg/100 g after 4 days (in animals undergoing the mock operation  $7.1 \pm 1.6$  mg/100 g) respectively.

Thus a stress reaction developed in the experimental animals 24 h after injection of 0.25 MLD of TT, and was due to the development of a pain syndrome, which intensified toward the 4th day.

Biomicroscopic investigations showed that as early as 4-6 h after injection of 1 MLD TT, the blood flow in the venules was slowed in the rat mesentery, aggregates of erythrocytes appeared in the capillaries and venules, and processes of plasmotization and stasis spread. Solitary foci of extravasation of erythrocytes appeared close to the venules. On biomicroscopy of rats receiving 0.25 MLD of TT the same disturbances of the microcirculation of the blood were observed as in the animals of group 1. In rats with various degrees of severity of the syndrome, 24 h after injection of TT the disturbances of the microcirculation were identical. Changes in the microhemodynamics in the mesentery were identical after 1 and 4 days. Thus aggravation of the disturbances of the microcirculation were not observed in the course of development of TN induced by injection of 0.25 MLD TT.

The venular permeability for colloidal carbon particles was greater in rats after injection of 1 MLD TT than in animals undergoing the mock operation, as regards both extent and intensity of labeling. An increase in venular permeability also was observed in rats receiving 0.25 MLD TT compared with rats undergoing the mock operation; this process, moreover, did not increase in severity, but actually decreased with an increase in the time after injection of TT (Table 1). In rats with more marked manifestations of the pain syndrome, disturbances of venular permeability were more severe as regards both extent and intensity of labeling.

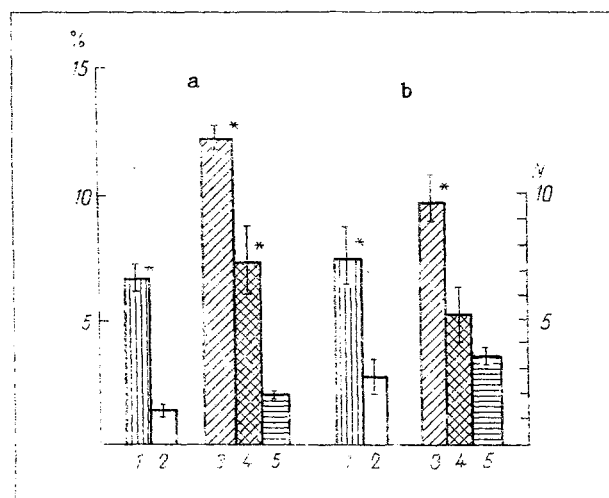


Fig. 2. Degranulation of mast cells (a) and contraction of walls of lymphatic microvessels (b) in experimental trigeminal neuralgia. Ordinate (a), % of degranulated mast cells; ordinate (b), number of contractions of walls of lymphatic microvessels (in 1 min). Details of series of experiments: 1) 4-6 h after injection of 1 MLD TT into caudal nucleus; 2) 4-6 h after injection of 0.9% sodium chloride into caudal nucleus; 3) 24 h after injection of 0.25 MLD TT into caudal nucleus; 4) 4 days after injection of 0.25 MLD TT into caudal nucleus; 5) 1-4 days after injection of 0.9% sodium chloride into caudal nucleus; \*p < 0.05 significant difference from control in same series.

TABLE 1. Permeability of Venular Mesenteric Microvessels in Rats with Experimental Trigeminal Neuralgia

Experimental conditions (n denotes number of animals)	Extent of spread disturbances			Intensity of disturbances				
	number of mesenteric fenestrae, in % of total number examined			number of rats with different degrees of labeling, in % of total number				
	with-out label	1-10 vessels labeled	more than 10 vessels labeled	0	I	II	III	IV
4-6 h after injection of 1 MLD TT into caudal nucleus (n = 10)	66*	24*	10*	0*	100*	100*	70*	40
24 h after injection of 0.25 MLD TT into caudal nucleus (n = 10)	56*	29*	15*	0*	100*	100*	100*	100*
4 days after injection of 0.25 MLD TT into caudal nucleus (n = 8)	76*	22*	4	20	80	60	0	0
4-6 h after injection of 0.9% sodium chloride into caudal nucleus (n = 5)	96	4	0	80	20	20	0	0
1-4 days after injection of 0.9% sodium chloride into caudal nucleus (n = 6)	94	4	2	60	40	40	0	0

Legend. \*p < 0.05 denotes differences from corresponding control are statistically significant.

The study of the morphological state and function of the mast cells showed that in animals receiving 0.25 or 1 MLD TT, the number of degranulated mast cells is at the above-mentioned times of the investigation was significantly increased (Fig. 2a). In rats receiving TT in a dose of 0.25 MLD and with a severe course of the pain syndrome, the percentage of degranulated mast cells was twice as high as in animals with a mild pain syndrome. A significant increase in the number of contractions of the walls of the lymphatic microvessels was observed 4-6 h after injection of 1 MLD TT and 24 h after injection of 0.25 MLD TT in the mesentery of the rats (Fig. 2b), evidence of stimulation of the drainage function. This adaptive reaction was evidently aimed at removing macromolecules from the interstitial spaces. No such adaptive reaction could be observed 4 days after injection of 0.25 MLD TT, probably due to a relative reduction of venular permeability.

Thus disturbances of the microcirculation of the blood appeared in the mesentery of animals with TN, just as in the mesentery of rats with a PSSO [7], venular permeability for colloidal carbon particles was increased, and so also was the intensity of mast cell degranulation. These results are evidence that the changes described above are characteristic of pain syndromes of central origin. The uniform changes observed in the microcirculation in the mesentery of rats with pain syndromes induced by creation of a GPPE in different parts of the nociceptive system (for PSSO at the level of the spinal cord, for TN at the level of the medulla) are evidence that disturbances of the microcirculation are generalized and do not depend on the location of the GPPE.

Relative normalization of the state of components of the microcirculatory system was clearly revealed in animals with a less severe general course of the pain syndrome. This could be seen particularly clearly in relation to the reduction of venular permeability and mast cell degranulation.

Thus the severity of the disturbance in the microcirculatory system and the manifestation of features of adaptation could be used as prognostic criteria for predicting the course of the pain syndrome. Our own results are in agreement with other data [4] showing that in patients with TN changes take place in the hemostasis system: generalization of increased platelet aggregation, hypercoagulation, etc. The disturbances of the central hemodynamics and microcirculation found in pain syndromes of neurogenic character probably arise as a result of a disturbance of activity of the autonomic nervous system [5, 6, 9-11].

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