Effects of Ischemia on the Development of a Deafferentation Pain Syndrome and on Microcirculatory Disturbances in Rats

I. V. Mironova, M. P. Gorizontova, and V. K. Reshetnyak

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In a group of rats with transected sciatic nerve, ischemia of the operated limb produced by femoral artery ligation was found to result in an accelerated onset and increased severity of autotomy as compared to a similar group of rats without ischemia. Biomicroscopic examination of the mesenteric microcirculation showed that the ischemia also intensified disturbances of the terminal blood flow, made the venules more permeable, and increased the percentage of degranulated mast cells. The possible mechanisms by which ischemia promotes the development of chronic pain syndromes are discussed.

Key Words: *chronic pain syndrome; microcirculation; ischemia*

We showed previously that the development of chronic pain syndromes is accompanied by generalized microcirculatory disorders [1,3,5]. However, disturbances of the local blood flow and microcirculation may themselves lead to pain; examples are the ischemic pains arising in angina pectoris, infarction of internal organs, and other conditions in which the local blood flow in some part of the body is limited because of arteriolar constriction or for another reason. Also, patients with a phantom limb pain syndrome very often have a history of diseases associated with vascular disorders of the lower limbs (endarteritis obliterans, thrombophlebitis, diabetes mellitus, etc.) [4]. The linkage between disorders of local circulation and pain syndromes may be mediated via activation of histamine, kinins, substance P, prostaglandins, or other agents that promote microcirculatory disturbances while at the same time acting as pain transmitters. Local ischemia of the spinal cord in rats has been shown [7] to result in a chronic pain syndrome manifested clinically in allodynia and autotomy.

The considerations outlined above led us to address the question of what role circulatory disorders in a limb might play in initiating the development of a deafferentation pain syndrome (DPS).

MATERIALS AND METHODS

A total of 44 male Wistar rats were used. To produce a DPS, the left sciatic nerve was transected as described previously [5], and the pain syndrome was considered to have developed if the phenomenon of autotomy was observed in the operated limb. The severity of autotomy was evaluated by adding up points using an arbitrary scoring scale, in which separation of the distal half of the claw in digits 1 through 5 was assigned 1 point; removal of the proximal half of one or more claws

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow. (Presented by G. N. Kryzhanovskii, Member of the Russian Academy of Medical Sciences)

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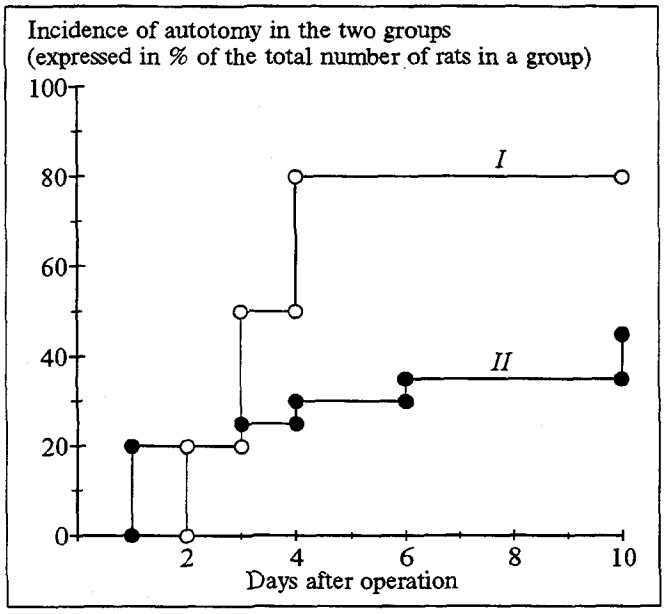

Fig. 1. Effect of lower limb ischemia on the incidence of autotomy in rats with DPS. Here and in Fig. 2: *I*) group with transected sciatic nerve and ligated femoral artery; *II*) group with transected sciatic nerve.

(up to the phalanx), 2 points; removal of the distal phalanx from each digit, 4 points; removal of the proximal phalanx from each digit, 6 points; removal of skin from the foot, 2 points; removal of muscles from the foot, 4 points; and removal of the bone, 6 points.

To make the left hind limb ischemic, the femoral artery was tied with two ligatures placed above the origin of the muscle branches from that artery. Observations showed that the macroscopically visible signs of dystrophy in the ischemic limb (its volume, color of its skin, and thinning of its claws) had cleared and the limb's skin temperature had returned to normal 8-10 days after the operation; a collateral circulation had probably developed by that time.

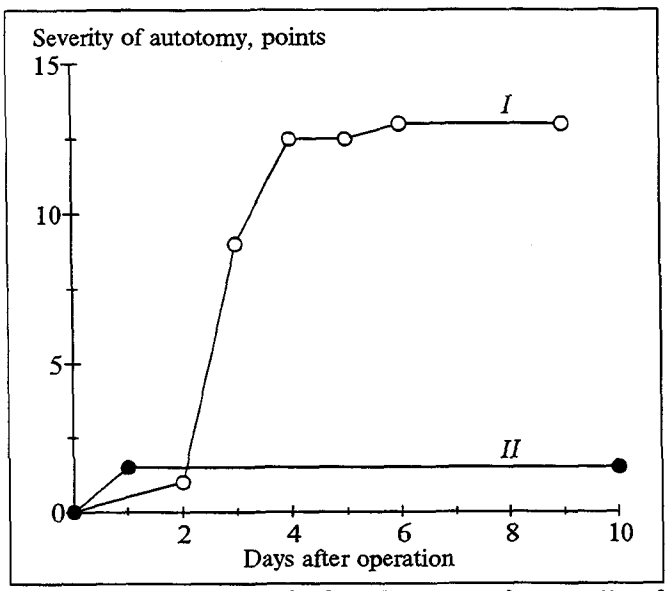

Fig. 2. Effect of lower limb ischemia on the severity of autotomy in rats with DPS.

To evaluate the state of the terminal blood flow, biomicroscopy of the mesentery was performed in all rats under Nembutal anesthesia (5 mg/100 g body weight). Venular permeability and the morphology and function of mast cells in the mesentery were assessed using previously described procedures [2].

The 44 test rats were divided into three groups: in group I (n=15) the left sciatic nerve was transected and the left femoral artery was ligated; in group II (n=15), only the left sciatic nerve was transected; in group III (n=14), only the left femoral artery was ligated. The rats were observed during 10 days after surgery. The study was conducted in November and December, and all operations and biomicroscopic examinations were performed before noon. The animals were maintained on a standard diet in the vivarium, both before and after the operation. The results were treated statistically using Student's t test.

RESULTS

In group I, the incidence of autotomy was 20% on the 2nd postoperative day, 50% on the 3rd, and 80% on the 4th, remaining at this level until the end of the observation period (day 10). In group II, it amounted to 21% on day 2, 27% on day 3, 32% on day 4, and to 47% on day 10 (Fig. 1).

The autotomy in group I was significantly more severe than in group II, the mean scores in these two groups being 10.3±0.2 and 1.2±0.4, respectively (Fig. 2).

In group III, the proportion of rats showing autotomy was 7% on day 1 and only 14% on day 10, and the autotomy was much less severe than in group I (mean score=1) and was observed for only 1 or 2 days.

Thus, the three groups could be arranged in the order I>II>III in terms of decreasing incidence and severity of autotomy.

Upon mesenteric biomicroscopy, slowed venular blood flow, leukocyte adherence to venular walls, erythrocyte aggregation in capillaries and venules, plasmacytosis, and stasis were more frequently observed in group I than in group II (particularly erythrocyte aggregation in venules and stasis) and were rarely (if at all) seen in group III. Impaired venular permeability was also observed most frequently in group I and least frequently in group III (Fig. 3).

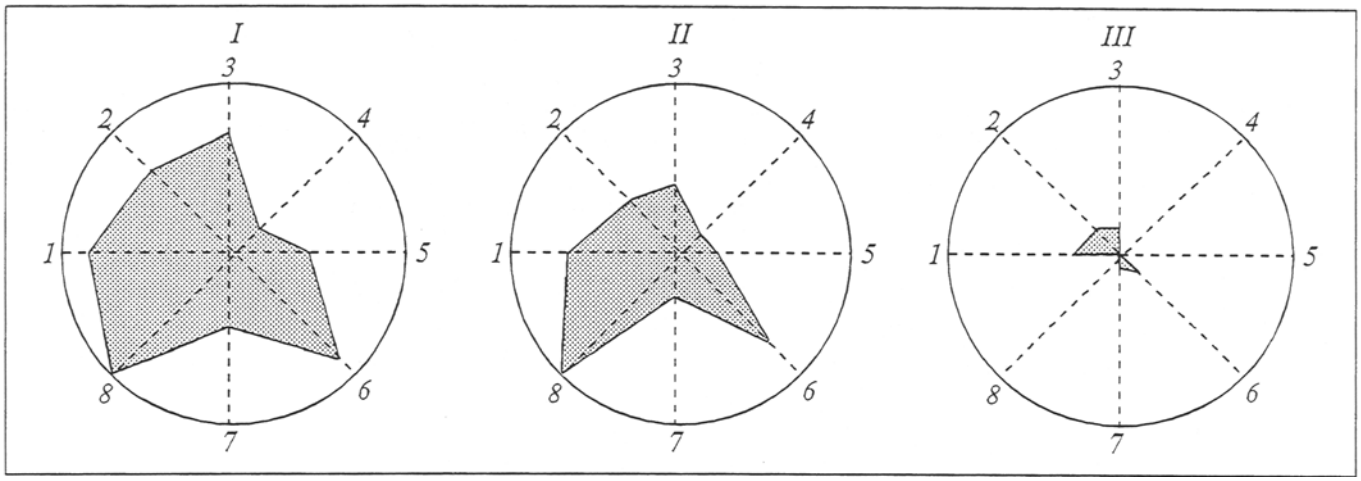

Fig. 3. Impact of ischemia and sciatic nerve transection on mesenteric microcirculation. *I*, *II*, and *III*: groups of rats. The eight radii of these circular diagrams each represent the percentage of rats with a particular symptom in the respective groups, as follows: *1*) slowed venular blood flow; *2*) erythrocyte aggregation in capillaries; *3*) erythrocyte aggregation in venules; *4*) plasmacytosis in vessels; *5*) stasis; *6*) leukocyte adherence to venular walls; *7*) prevalence of impaired permeability in venules; *8*) severity of impairment in venular permeability.

The percentage of degranulated mesenteric mast cells was significantly higher in group I than in group II (8.6±0.5 *vs* 5.8±0.4; $p<0.001$); in group III, the percentage of such cells was significantly lower than in the other two groups (1.8±0.3%; $p<0.001$).

Thus, the restriction of blood flow in the major artery of the hind limb (group III), which led to dystrophic changes there that persisted until a collateral circulation developed on days 8-10, resulted in a slightly marked autotomy of short duration in only 14% of the rats, without causing significant changes in either the terminal blood flow, venular permeability, or the morphofunctional state of mast cells in the mesentery. In group II rats (those with transected sciatic nerve), both the incidence and severity of autotomy and the microcirculatory disturbances were similar to those observed by us earlier for such animals [1]. In group I (rats with transected sciatic nerve and ligated femoral artery), the incidence and severity of autotomy were much higher than in group II (Figs. 1 and 2), and the disturbances of the terminal blood flow, impairment of venular permeability, and degranulation of mast cells were also more frequent and pronounced. It should be stressed that the autotomies and microcirculatory disorders were all observed during the first 8-10 days after femoral artery ligation, i.e., in the period when dystrophic disturbances were progressing in the limb and the collateral circulation there was still inadequate.

It may therefore be stated that preliminary ischemia of the limb contributed to the development of a DPS. Here, the following mechanisms may be involved.

It has been shown that when a limb is made ischemic for a short time by ligation of the femoral artery, neurons of the dorsal spinal cord horns are activated through excitation of the NMDA receptors [6]. The development of ischemia is accompanied by increased lipid peroxidation, activation of the kallikrein-kinin system and of prostaglandin synthesis, and by mast cell degranulation, which results in sensitization of nociceptors. These processes are implicated in the development of generalized microcirculatory disorders and promote the emergence of a generator of pathologically enhanced excitation and of a chronic pain syndrome.

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