MODIFICATION OF THE TIME COURSE OF ASEPTIC INFLAMMATION BY SODIUM HYDROXYBUTYRATE

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γ-Hydroxybutyric acid (GHBA), a metabolite of the central inhibitory GABA-ergic system, can significantly increase the resistance of the body cells to hypoxia, by modifying the level of metabolism [5, 7].

The aim of the present investigation was to study the possibility of modifying the time course of cellular reactions in an inflammatory focus using GHBA, on the assumption that disturbance of the microcirculation and the development of hypoxia constitute the key stages in the pathogenesis of the inflammatory process, and also taking account of the broad spectrum of clinical application of sodium hydroxybutyrate.

EXPERIMENTAL METHOD

Inflammation was induced by insertion of a sterile celloidin wafer measuring 1.5 mm into the subcutaneous connective tissue of rats. GHBA (100 mg/kg) was injected 30 min before and 2 and 4 h after insertion of the wafer. To assess the time course of the cellular reactions in the inflammatory focus a morphometric method was used, by means of which the thickness of the cellular barrier, the density of neutrophils, macrophages, and undifferentiated and mature fibroblasts inside and outside the cellular barrier (peripheral zone), the number of layers of fibroblasts in the capsule [3], the concentration and average index of mast cell degranulation (ADMC — by the formula of Astaldy and Verga), and also the number of active vessels, were determined under standard conditions. The content of collagen in the fibroblastic capsule was estimated by means of an ocular grid in sections stained with picrofuchsine by Van Gieson's method.

EXPERIMENTAL RESULTS

Injection of GHBA before the beginning and during the first few hours of the course of inflammation significantly modified the reaction of the microvascular bed and of cells responsible for the realization of inflammation at all stages of this process. In animals receiving GHBA, ADMC in the focus of inflammation was higher throughout the period of observation (2.2 ± 0.12 conventional unit, compared with 1.7 ± 0.1 conventional unit in rats of the control group; p < 0.05), evidence of the more intensive secretory activity of these cells. Meanwhile, during the leukocytic phase (1st day) their number in the experimental rats was only half of that in rats of the control group (14.6 ± 2.2 and 27.6 ± 4.4/mm² respectively; p < 0.05). Similar changes also were observed in activity of the microvascular bed. It will be clear from Fig. 1 that on the first day of inflammation the number of active capillaries in the experimental animals was 4-6 times less (p < 0.01). One cause of the reduced vascular activity in animals receiving GHBA is evidently the smaller number of mast cells, which regulate vascular permeability. Despite this, the degree of leukocytic infiltration of the inflammatory focus in rats receiving GHBA was actually higher than in animals of the control group: the number of neutrophils in the peripheral zone of the focus was 2.4 times greater (p < 0.05; Fig. 1), suggesting more active migration of neutrophils through the vessel wall.
Fig. 1. Time course of number of blood-filled vessels (1, 2), of density of neutrophils in leukocytic barrier (3, 4), and of peripheral zone (5, 6) of inflammatory focus during leukocytic phase in animals of control group (1, 3, 5), and animals receiving GHBA (2, 4, 6). Abscissa, time of observation (in days); ordinate: a) number of vessels per 50,000 μ² and number of neutrophils in leukocytic barrier per 1000 μ², b) number of neutrophils in peripheral zone of inflammatory focus, per μ². Diagram below graphs: duration of leukocytic (black part) and macrophagal (dotted part) phases of inflammation.

Fig. 2. Time course of density of macrophages in cellular barrier (1, 2) and collagen content in capsule (3, 4) in animals of control group (1, 3) and animals receiving GHBA (2, 4). Abscissa, time of observation (in days); ordinate: I) number of macrophages per 1000 μ², II) collagen content (in %).

Fig. 3. Time course of number of blood-filled vessels in inflammatory focus (5, 6), of number of layers of fibroblasts (1, 2), of density of these cells in capsule (3, 4) in animals of control group (C: 1, 3, 5) and in animals receiving GHBA (G: 2, 4, 6). Abscissa, time of observation (in days); ordinate: I) number of fibroblasts per 1000 μ² and number of layers of these cells in capsule, II) number of blood-filled vessels per 50,000 μ². Below – duration of phases of inflammation: black part – leukocytic phase; obliquely shaded part – macrophagal phase; dotted part – fibroblastic phase.

However, the maximal density of neutrophils in the leukocytic barrier was the same in animals of the two groups. Moreover, in rats receiving GHBA it was achieved 12 h later (Fig. 1). It is interesting to note that the density of macrophages in the leukocytic barrier during this period (1st day) when GHBA was given was only half as great (p < 0.05; Fig. 2), although their density in the peripheral zone was the same in rats of the two groups. It can be concluded from these findings that migration of neutrophils and macrophages from the peripheral zone of the inflammatory focus toward the foreign body was slowed. The reasons for this phenomenon must evidently be sought not in cells with high ability to migrate from the vessels, but in the state of the intercellular substance in which they move about. One of the mechanisms facilitating migration of the cells in the inflammatory focus is activation of hyaluronidase. According to data in [1], GABA reduces the activity of this enzyme. It is also possible that GHBA, a GABA metabolite, may have a similar action, and may thus help to maintain the high viscosity of the ground substance of the connective tissue of the inflammatory focus. Another cause of delayed cell migration may be diminution of inflammatory edema, as shown by the decrease in activity of the microcirculatory bed under the influence of GHBA (Fig. 1). It is important to note that the duration of the leukocytic phase (the 1st day) in rats receiving GHBA was twice as long as for rats of the control group (12 h). This evidently is due to the ability of GHBA to inhibit lipid peroxidation and to reduce damage to mitochondria under hypoxic conditions [4], and consequently, to prolong the life of the cells.

During the macrophagal phase the intensity of the vascular reaction in rats of both groups was almost equal. As Fig. 1 shows, after 2 days of inflammation the number of active vessels in animals of the control group fell sharply, whereas in animals receiving GHBA it remained at its previous level. It is interesting to note that the number of mast cells at this