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/mm^2 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.

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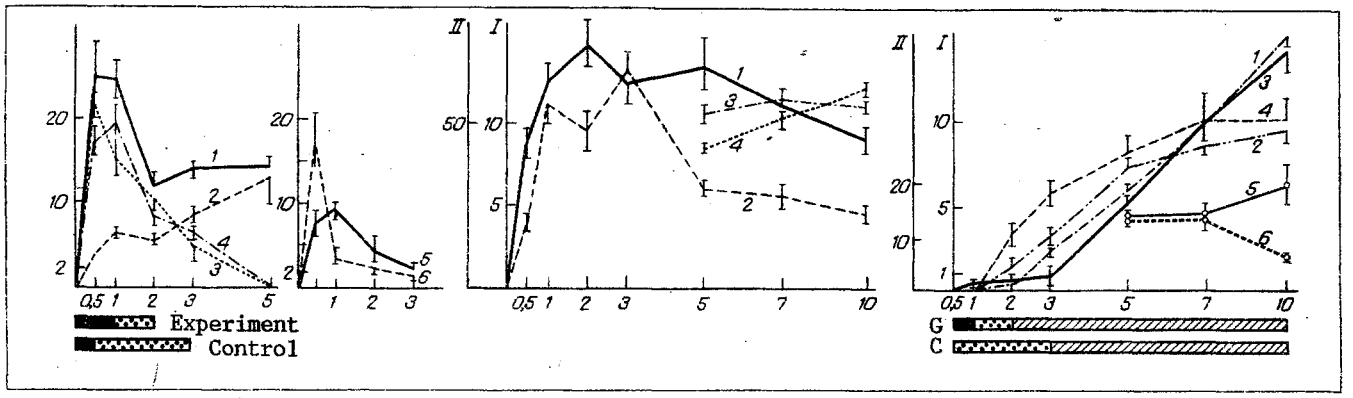

Fig. 1 Fig. 2 Fig. 3

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 μ^2 and number of neutrophils in leukocytic barrier per 1000 μ^2, b) number of neutrophils in peripheral zone of inflammatory focus, per μ^2. 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 μ^2, 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 μ^2 and number of layers of these cells in capsule, II) number of blood-filled vessels per 50,000 μ^2. 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

period in animals of the two groups did not differ significantly (40.0 ± 11.9 and 23.4 ± 4.4/mm2). The density of macrophages in the cellular barrier of animals of the experimental group was one-third lower ($p < 0.01$; Fig. 2). It must be emphasized that the duration of the macrophagal phase in rats receiving GHBA also was significantly shorter (Fig. 3).

The response of the fibroblasts during inflammation fell into several consecutive stages: proliferation, differentiation, and collagen synthesis. The proliferative activity of the fibroblasts was estimated by measuring the density of undifferentiated fibroblasts in the peripheral zone of the inflammatory focus [2]. The maximal density of these cells in animals of the control group was observed on the 2nd day when it was 11 ± 0.7, whereas in animals receiving GHBA it reached a maximum on the 3rd day, at 6.6 ± 0.5 cells/5000 μ^2 ($p < 0.01$), evidence of inhibition of the proliferative activity of the fibroblasts under the influence of GHBA. Despite this fact, migration of the fibroblasts toward the foreign body and their orientation in parallel rows and, consequently, the differentiation of these cells, took place at the same rate in rats of the two groups. The time course of the number of layers of fibroblasts and of the density of these cells in the capsule until the 7th day of the inflammatory process revealed no statistically significant differences (Fig. 3). Meanwhile, the synthetic activity of the fibroblasts in rats receiving GHBA at this stage of inflammation was reduced: after 5 days of inflammation the collagen content in the capsule of the experimental rats was 25% less than in the control animals ($p < 0.05$; Fig. 2). On the basis of these results two possible mechanisms of regulation of collagen production can be discussed. One of them consists of stimulation of this process by macrophages, either by contact with fibroblasts or by the formation of collagen breakdown products, stimulating the synthesis of this protein [6]. Shortening the duration of the macrophagal phase and reduction of the density of macrophages in the cellular barrier throughout the period of inflammation of animals receiving GHBA suggests that a weaker effect of the macrophages is possible under these conditions, aimed at stimulating collagen synthesis. Another factor is the well-known stimulating action of mast cells on collagen production, for in the period of intensive synthetic activity of the fibroblasts these cells are concentrated near the developing capsule. The present investigation showed that the largest number of mast cells in animals of the control group near the foreign body was observed on the 3rd-5th day of inflammation, but in animals receiving GHBA it was observed on the 5th-7th day (34.8 ± 13.8 and 39.2 ± 11.6/mm^2 respectively).

In the late stages of formation of the fibroblastic capsule, starting with the 7th day, stabilization of the density of mature fibroblasts in the peripheral zone and in the fibroblastic capsule, and also stabilization of the number of layers of fibroblasts and collagen in the capsule was observed in the experimental animals, whereas in animals of the control group these parameters continued to rise ($p < 0.01$; Figs. 2 and 3). These same rules also were observed in the dynamics of active vessels. Starting with the 7th day, in animals receiving GHBA, the number of blood-filled vessels was reduced ($p < 0.05$), whereas in rats of the control group, this parameter remained at its previous high level (Fig. 3). Weakening of the response of the vessels in the inflammatory focus, stabilization of the number of layers of fibroblasts and of the density of these cells in the capsule and peripheral zone, and also of the collagen content in the capsule toward the 10th day of inflammation in animals receiving GHBA, are evidence of earlier resolution of the inflammatory process.

Thus the inflammatory process, when accompanied by administration of sodium hydroxybutyrate, is characterized by weakening of the response of the microcirculatory bed, by a decrease in the density of macrophagal infiltration, by shortening of the duration of the macrophagal phase, and by the earlier resolution of the inflammatory process as a whole.

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