Effect of Glycine on the Microcirculation in Rat Mesenteric Vessels

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Model experiments on biomicroscopy of mesenteric microvessels in laboratory rats were performed to evaluate the effect of natural metabolites (*e.g.*, amino acid glycine) on the microcirculation. The effect of glycine was determined from a change in the diameter of arterioles. Application of glycine (0.1 ml, 1 M) to the mesenteric surface was followed by arteriolar dilation (by 50-80%). Histamine-induced disturbances in the microcirculation were not observed after preapplication of glycine. Under these conditions, pretreatment with histamine was accompanied by reversible changes. Our results suggest that the natural metabolite glycine has a prophylactic and therapeutic effect on microcirculatory disturbances, which are induced by inflammatory-and-allergic mediator histamine.

Key Words: *microcirculation; mesenteric arterioles; glycine; histamine; biomicroscopy*

The microcirculation has an important role in physiology and pathology. Hence, it may be considered as a specific functional system for oxygen supply to tissues [8,14]. Microcirculatory disturbances are followed by the development of ischemia and cascade of interrelated pathological processes, including erythrocyte aggregation, thrombosis, thromboembolic diseases, infarctions, and other complications. The incidence of these disorders constantly increases [12].

Microcirculatory disturbances have a general pathological role, which includes the involvement into the pathogenesis of septic or endotoxic shock. The number of intestinal villi with normal microvessels decreases sharply under the influence of endotoxins. A change in properties of the mucosal layer and decrease in the rate of villus microcirculation may occur even in normal blood pressure [9,10]. Microcirculatory dysfunction causes tissue hypoxia and deficiency of vital functions. These changes are observed in patients with the critical stage of sepsis [7].

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A study of regulatory mechanisms and development of potent drugs to improve blood supply to organs (*i.e.*, function of microvessels) are the urgent problems. Much attention is paid to natural metabolites, including the amino acid glycine. These substances may be used in combination therapy for the diseases associated with tissue ischemia, shock, and other disturbances. Published data show that glycine has a normalizing effect on various structures and functions of the organism [15]. Previous studies revealed that glycine causes pial arteriolar dilation in rats [4]. Glycine probably has a systemic effect, which is confirmed by the distribution of fluorodeoxyglucose (positron emission tomography and computer tomography, PET-CT) [6].

Here we studied the effect of glycine on the microcirculation in intestinal vessels. A biomicroscopic study was performed to valuate the influence of this amino acid on rat mesenteric vessels. The microcirculatory bed of rat mesentery serves as a convenient intravital model to study a variety of pathological processes, including ischemia, thrombosis, erythrocyte aggregation, abnormal permeability, vasomotor response, inflammation, and other microcirculatory disturbances. Moreover, this ap-

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proach may be used for the evaluation of physiologically active substances and pharmaceutical products [1-3]. The molecular mechanisms for action of glycine were studied on the model of histamine-induced microcirculatory disturbances.

MATERIALS AND METHODS

Experiments were performed on 30 male Wistar rats weighing 180-200 g. The animals were anesthetized with intraperitoneal injection of chloral hydrate in a dose of 400 mg/kg. The microcirculation was studied in mesenteric microvessels. A biomicroscopic study was conducted on a Docuval microscope (Carl Zeiss). Experiments were performed on a temperature-controlled table for anesthetized animals. The rat was placed on a table in the lateral position. A small cut was made to open the abdominal cavity. The mesentery was thoroughly removed and straightened (without tension) under a light guide. This device provides light transmission during a microscopic study. Optimal humidity of the mesentery was maintained by application of physiological saline (38°C).

A solution of the amino acid glycine (1 M, 0.1 ml) was applied to the mesenteric surface. The final dose of glycine was 40 mg/kg. Control animals were treated with physiological saline. Function of state microcirculatory bed was determined by means of visual monitoring with a Philips digital camera. The interval varied from several seconds to 3 min. The examination was performed with a surveillance lens (×10) and immersion lens (×30), which allowed us to obtain the images of microvessels at a 100-300-fold magnification. The microcirculation in mesenteric vessels was evaluated from the reaction of arterioles with a diameter of 20-200 m. This parameter serves as major criterion for blood flow in vessels. The diameter of arterioles was measured by a graduated scale (16-m mark). This scale was projected onto the image of microvessels.

Microcirculatory disturbances were induced by application of 0.1% histamine solution (0.1-0.3 ml). The treatment was followed by sludge, stasis, thrombosis, and other signs of microcirculatory disorders. These changes became irreversible with an increase in the dose of histamine to 0.5 ml. Blood flow in the examined region did not return to normal. The solution of glycine (1 M) was applied after histamine treatment. We studied the effect of glycine on histamine-induced disturbances in the microcirculation. Physiological saline served as the control.

RESULTS

Dilation of arterioles (diameter 30-50 m) was observed 10-15 sec after application of glycine and persisted for 5-10 min (Fig. 1).

Treatment with histamine (standard test) was followed by arteriolar dilation and stasis, which reached maximum by the 3rd minute. The signs of stasis were irreversible in the follow-up period. The typical dynamics of stasis was manifested in a decrease in blood flow rate and thrombosis. The observed changes were followed by circulatory arrest in microvessels. A biomicroscopic study revealed the so-called "lightening" of microvessels, which was related to the reduction of blood filling (Fig. 2).

Under these conditions, application of glycine in the same doses was accompanied by the imme-

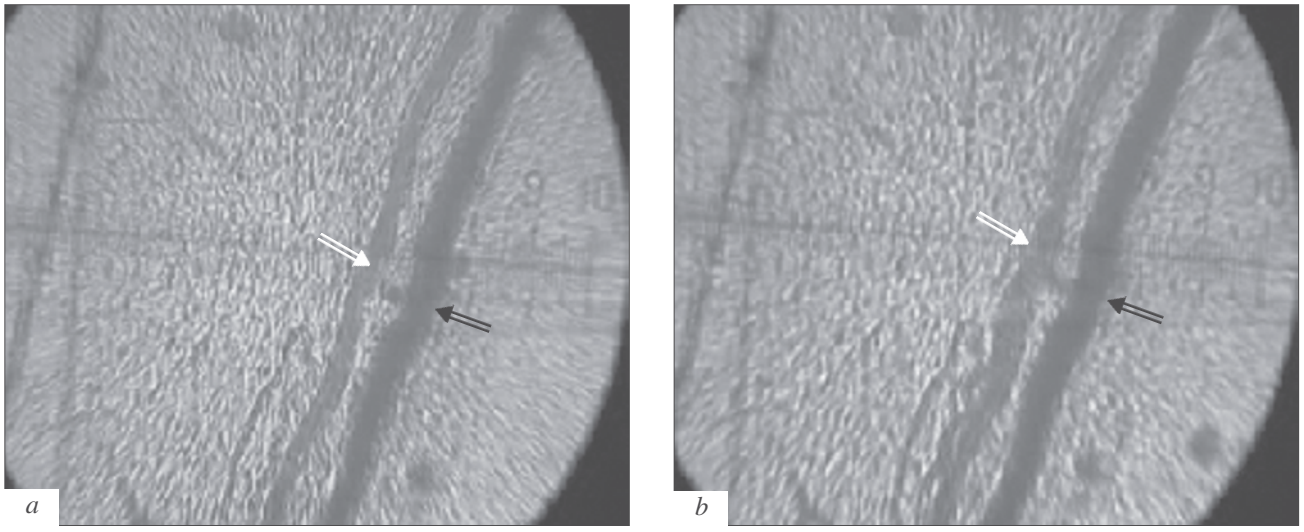

Fig. 1. Dilatory effect of glycine on mesenteric arterioles (×100). Initial state of microvessels (*a*); arteriolar dilation after glycine application (*b*). Light arrow, arteriole; dark arrow, venule.

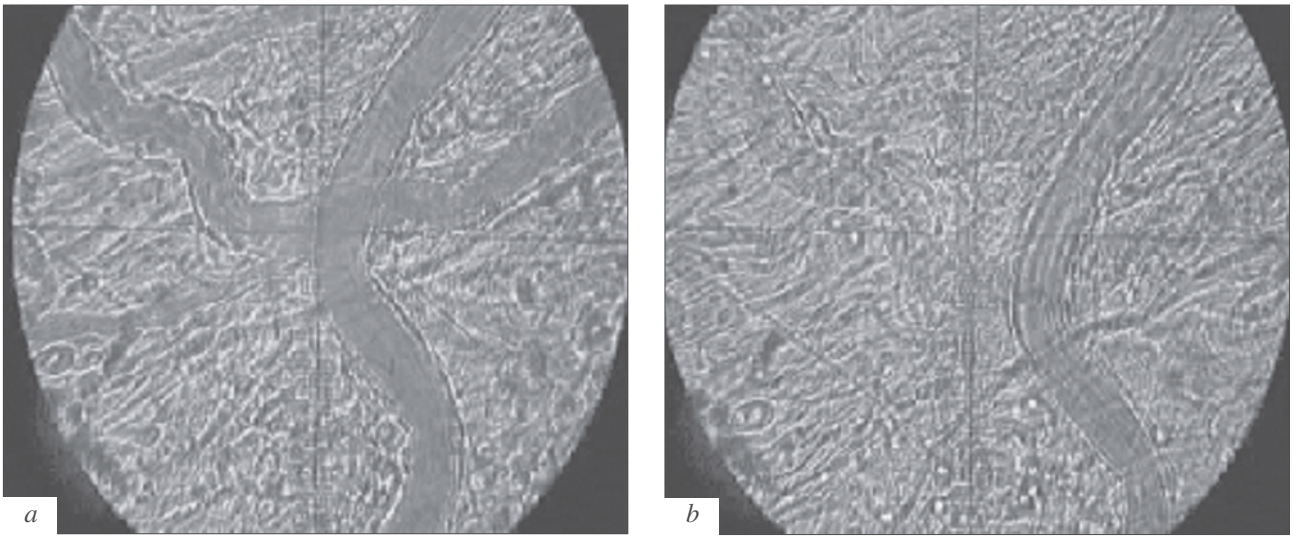

Fig. 2. Histamine-induced microcirculatory disturbances. Lightening of arterioles and stasis (×300). Initial state of microvessels (*a*); effect of lightening and stasis after glycine application (*b*).

diate restoration of blood flow ("at the needle tip", after 3-5 sec). This effect developed before the cessation of glycine treatment (Fig. 3). Glycine in the lower concentration (0.1 M, final dose 4 mg/kg) had a similar, but less pronounced effect.

Administration of histamine after pretreatment with glycine did not cause microcirculatory disturbances. The effect was achieved after repeated treatment or application of histamine at higher doses (by 2-3 times).

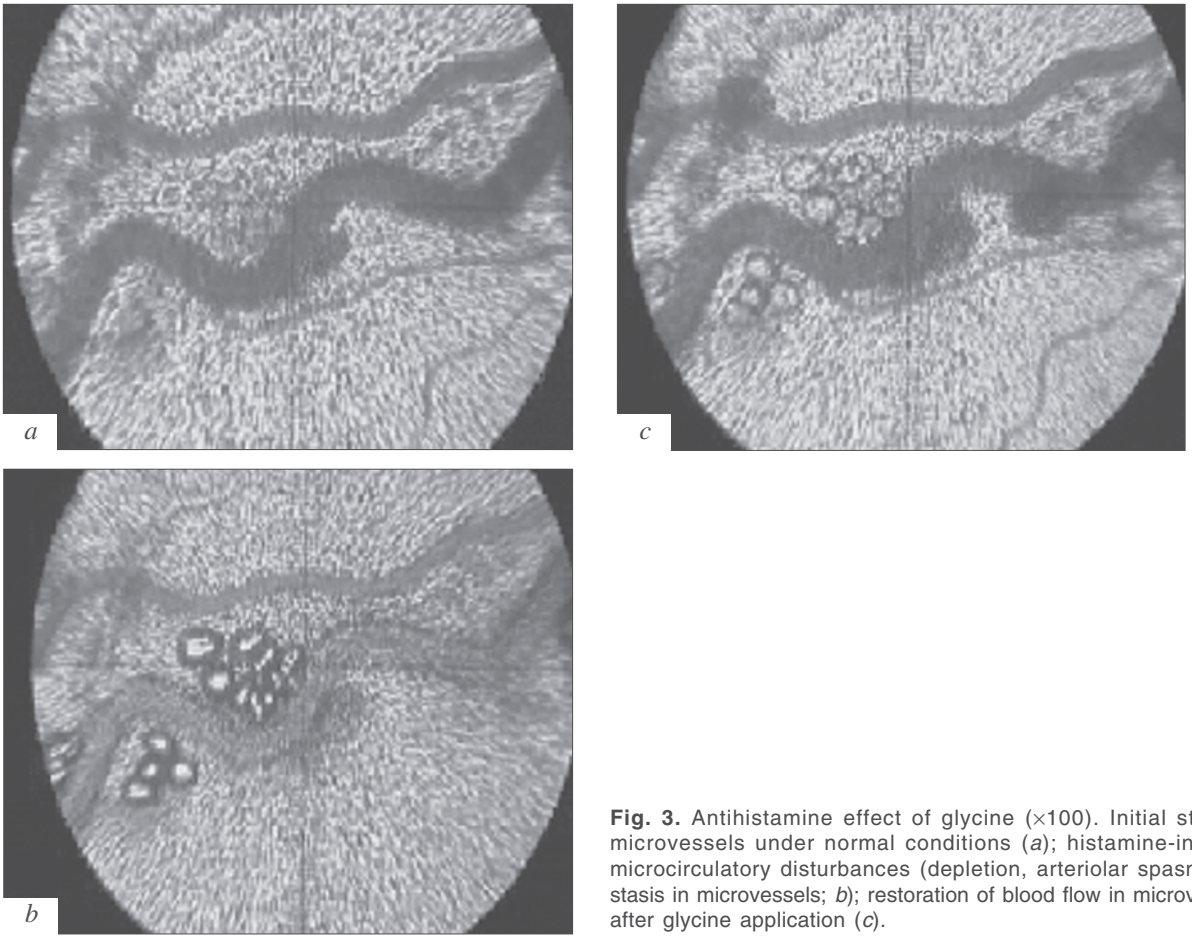

Fig. 3. Antihistamine effect of glycine (×100). Initial state of microvessels under normal conditions (*a*); histamine-induced microcirculatory disturbances (depletion, arteriolar spasm, and stasis in microvessels; *b*); restoration of blood flow in microvessels after glycine application (*c*).

These data show that glycine causes vasodilation of mesenteric arterioles. Application of glycine after the development of histamine-induced disturbances in the microcirculation was followed by blood flow restoration. Moreover, the effect of histamine was not observed after pretreatment with glycine. Hence, glycine prevents the development of microcirculatory disturbances under the influence of histamine. The antihistamine effect of this amino acid holds promise for clinical practice.

The stimulatory effect of amino acids on intestinal microcirculation was reported previously. Combined treatment with L-arginine and vasopressor drugs was effective in the early stage of endotoxemia [11].

The microcirculation has an important role in the maintenance of barrier function of the intestinal tract and development of nonspecific resistance to infection. Comparative experiments on gnotobiotic models showed that the microflora serves as an important stimulatory factor, which maintains the reactivity of microvessels and microcirculation in the mucosal layer [5]. This property provides a protective role of the mucosal layer (*e.g.*, in the intestine). Pathogenic microorganisms, including enteropathogenic *Escherichia coli*, cause microcirculatory disturbances during inflammation. It should be emphasized that the effect of lightening in abacterial rats is observed after treatment with histamine at higher doses (as compared to normal animals) [13].

The role of direct biomicroscopic examination is not diminished in the epoch of instrumental approaches (*e.g.*, indirect methods) to study the microcirculation. This valid and objective method allows us to evaluate the degree of microcirculatory disturbances. It is used for a comparative study and validation of other indirect methods (in the difficult or doubtful interpretation of data). Our experiments showed that products of natural metabolites (*e.g.*, glycine) prevent histamine-induced disturbances in the microcirculation in rat mesenteric microvessels. They have a normalizing effect on the microcirculation in the early stage of stasis and microcirculatory dysfunction. The data suggest that glycine has an antihistamine effect. The action of glycine is probably associated with blockade of histamine receptors, activation of tissue histaminase, and interaction with histamine at the molecular level. These mechanisms require further investigations. An important finding is that glycine has a normalizing effect and prevents microcirculatory disturbances, which develop in response to treatment with inflammatory-and-allergic mediator histamine. The mechanisms for antihistamine activity of glycine should be evaluated in further experiments.

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