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# Assessment of Microcirculatory Effects of Glycine by Intravital Microscopy in Rats

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*Abstract*— **Experimental studies using laboratory animal models have shown a potential vasoactive effect of natural metabolites such as glycine. The present study used intravital microscopy in laboratory rat models to study the microcirculation in the brain pial and mesentery vessels.**

**To investigate the pial microvasculature, a stereotaxis-like animal fixing device was used. The intravital microscopy unit consisted of a binocular microscope equipped with a digital photo-video camera, processor, monitor and printer. Using reflected light, a special contact lens with an amplified focus depth provided high-resolution images of nontransparent tissue objects that typically have insufficient light exposure.**

**Glycine had a vasodilatory effect on microvessels in the rat brain and mesenterium. The diameter of pial arterioles increased after glycine application especially markedly (up to 250% of initial size). These changes were not observed when physiological saline was used. Even a very small amount of glycine (a drop on the needle) was sufficient to stop the early stages of histamine-induced blood stasis development in 3–5 s in mesenterial microvessels.**

**The vasodilatory effect of glycine on the pial microcirculation correlates with its reported positive therapeutic effect in cerebral ischemic stroke. The ability of glycine to avoid or prevent histamine-induced microcirculatory alterations in mesenterial microvessels may have potential clinical applications.**

## I. Introduction

Animal-based models are increasingly being used to elucidate microcirculatory dysfunction, such as the pathogenesis of ischemic lesions that underlie severe disease (e.g., ischemic stroke). These models are also used to perform preclinical assessments of new microcirculatory pharmacological preparations. Frequently, such studies employ noninvasive methods of investigation, focusing on locations such as the skin folds, auricles, various skinny or mucous membranes, etc. However, the restricted localization of the superficially located microvessels accessible for microscopy prevents the modeling or investigation of microcirculatory processes in remote organs and tissues. Furthermore, data obtained in distant vessels do not correlate with the microcirculation status in other organs.

Indirect evaluation methods such as laser Doppler flowmetry also have been applied for microcirculatory research [1]. However, these methods have distinct disadvantages, including the need for decoding programs for the vascular phenomena based on spectral characteristics of the registered parameters, the potential for data misinterpretation, and the substantial time required for data analysis. Moreover, indirect measurements of the local microcirculation and microcirculatory bed density do not exclude microcirculation intensification by arteriovenous shunting and bypass of the natural microcirculation. Therefore, data obtained by indirect means must be correlated with direct microscopy results [2].

Direct microscopic investigation is one of the most reliable and demonstrative approaches for study of the microcirculation. Invasive intravital imaging techniques offer direct visualization of microcirculatory disturbances and of the microcirculation in organs and tissues [3],[4]. These methods are widely used for experimental purposes, such as the study of blood circulation in the liver, mesentery, or brain. In practice, intravital microscopy consists of a biological part (the object of investigation), the microscope system itself, microvessel selection, and photometry using quantitative parameters characterizing the microcirculatory bed status and its changes in dynamics.

Substantial experimental evidences indicate that free glycine may help protect tissues against injuries such as ischemia, hypoxia, and reperfusion [5]. Glycine as a medical preparation is being produced in tablet form and according to accepted therapeutic practice is given sublingually. An absorption from sublingual region of oral mucosa as well as signaling action through the receptor zone in that area is considered as a primary way of action of the preparation on cerebral microvessels in human situation.

A positive clinical effect of glycine treatment has been observed in patients with ischemic stroke [6] or with tissue alterations due to ischemia in the liver, kidney, or skeletal muscles [7],[8],[9]. However, the precise mechanisms of these effects are not completely understood. Therefore, the purpose of the present study was to evaluate the microcirculatory effect of glycine, using intravital microscopy of the microvessels in the rat brain and mesentery.

## II. Materials and Methods

A cranial window of 2×4 mm was drilled to expose the dura mater. Because removal of the dura matter provokes

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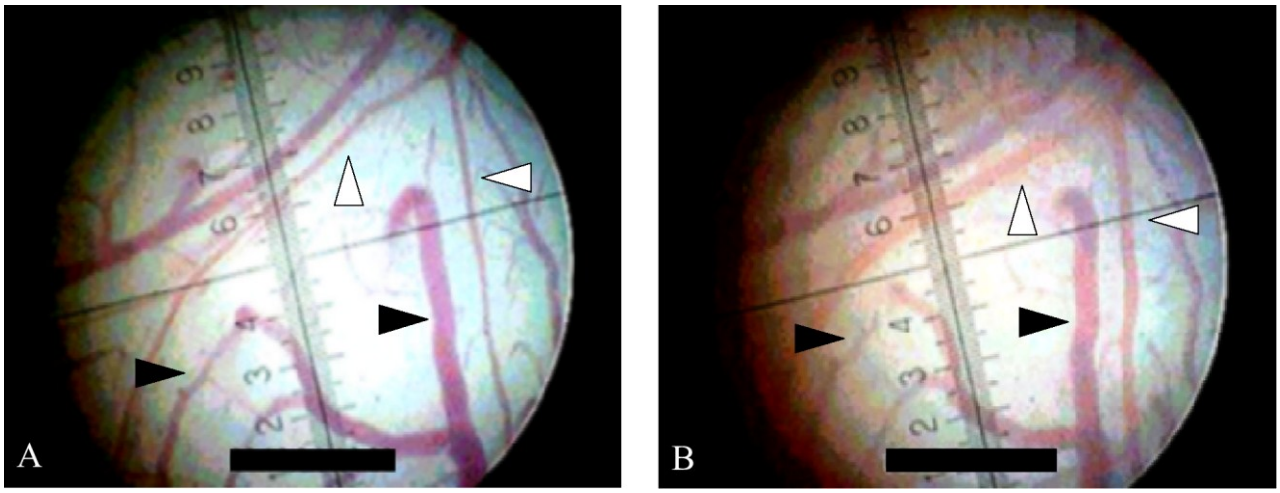


Figure 1. Vasodilatiry effect of glycine the pial microcirculation: A. arterioles (empty arrows) and venules (black arrows) before glycine application; B. increasing of arterioles' diameter (empty arrows) and non changing of venules' diameter (black arrows) 2 min after glycine application.

cranial decompression followed by temporary vessel dilatation, microscopy was started after this effect was over. Microscopy of the pial microvessel through intact dura mater is also possible. Use of special contact objectives provided a greater depth of focus (about 100 μm) and a higher resolution, which helped to overcome the problem of insufficient light exposure of the nontransparent tissues by using reflected light to illuminate the objects. As a result, high-quality microscopic microvessel images were obtained.

For easy local application of the studied preparations and solutions, only a small needle puncture in the membrane is needed, which reduces the risk of side reactions in the microvessels [10]. A volume of 0.1 mL of glycine (1 M) dissolved in saline was applied on the parietal cortex via the cranial window. The final dose of glycine was 40 mg/kg. In the control experiment, pure saline without glycine was applied.

To observe the mesentery vessels, the animals were immobilized in a side position. After performing a midline laparotomy, an intestinal loop was extracted and the mesentery was gently spread over a glass window. To prevent the mesentery from drying out, it was continuously moistened with warm saline. To model alterations in the mesentery microcirculation, 0.1–0.3 mL of histamine solution (0.1%) was applied locally. To test the effect of glycine, 0.1 M glycine was added directly to the mesenterial membrane surface.

The microcirculatory bed status was evaluated by visual monitoring followed by photo-video registration for 3 min. Images were obtained without damaging the membranes using a contact objective (10×). The vessel diameters were measured using a graded scale. ScopePhoto software was also employed. This software uses an incorporated calibrated screen ruler, which permitted measurement of the microvessel diameters in μm.

## III. Results

Visual monitoring revealed that glycine had a vasodilatatory effect on the pial and mesentery microvessels.

### *A. Pial Micorovessels*

Application of glycine solution dilated the arterioles by 150–250%, depending on the initial size of the intact vessel, within 1–3 min of glycine application (Fig. 1).

The amplitude of vessel dilatation was inversely proportional to the initial diameter size. Hence, the diameter of 50 μm arterioles increased by 200%; and those of 80 μm arterioles by 150% (Fig. 2). The microvessel diameter size returned to the initial level 10–20 min after a single glycine application. Repeated applications of glycine produced similar increases in the vessel diameter. These changes were not observed when pure physiological saline was used instead of glycine. The diameter of the venules remained unchanged after glycine (Fig. 1) or physiological saline application.

### *B. Mesentery Microvessels*

The effect of histamine was manifested by various stages of blood stasis. Microscopically it was manifested in the "intravascular brightening" (Fig. 3B), wherein the vessels appeared illuminated, which can be explained by the decreased blood cell filling of the microvessels. Increasing the histamine dosage to 0.5 mL elicited an irreversible

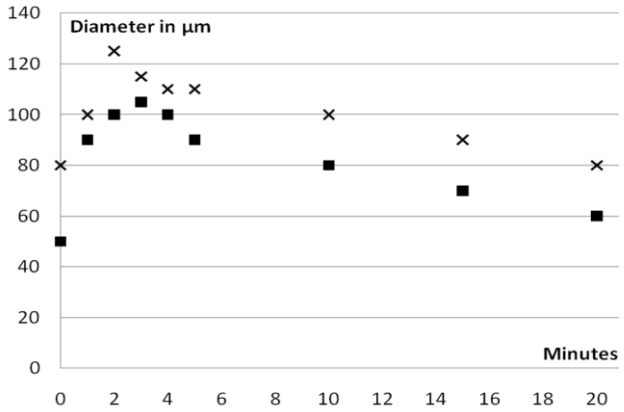


Figure 2. Typical variations of arterioles diameter after glycine application (× - arteriole of 80 μm, ■ - arteriole of 50 μm)

alteration of the microcirculation such as stasis in the affected arterioles.

The application of even a very small dose of glycine (a drop on the needle) at the early stage of blood stasis stopped stasis development in 3–5 s. After application of a full volume of glycine (0.1 mL), the microcirculation was completely normalized (Fig. 3C). A similar effect was observed at lower concentrations of glycine. Conversely, histamine applied at the same dose against the background of previously applied glycine did not induce microcirculatory alterations. To reach this effect, the histamine dose had to be increased 2–3 times or applied repeatedly.

## IV. Discussion

The results of the present study demonstrated the vasoactive action of glycine on microvessels in the brain and mesentery of rats. The difference in the dilatation reaction between arteriole and venule is due to more developed smooth muscle layer in the arteriole wall. That is why a vessel tone in vascular system depends mainly on arteriole reactivity to vasoactive preparations.

Our findings suggest that the local glycine concentration positively influences the regional microcirculation, supporting the expediency of using glycine as a neuroprotector for patients with ischemic stroke or other cerebral disorders associated with microcirculatory dysfunction.

The observed vasodilatatory effect of glycine may be explained by several mechanisms. One of them possibly includes an interaction with extrasynaptic NMDA receptors recently known to be involved in many important physiological functions [11]. Some molecular mechanisms of glycine action can include an interaction with NDMA receptors, as well as facilitating the interaction of glutamat with its receptors. Glycine is a known activator of endothelial nitric oxide (NO) synthase, which leads to an increase in NO production and smooth muscle cell (SMC) relaxation in the vessel wall. Thus, glycine improves NO-dependent blood supply to the rat kidney [12].

The protective action of glycine may be associated with the cellular compartmentalization of calcium ions [7]. Alternatively, the microcirculatory improvement may be related to the direct effect of glycine as an inhibitory neurotransmitter [9].

Under in vivo conditions, the molecular-level activities of glycine may be realized through a combination of several of these mechanisms, with their relative prevalence being determined by the metabolic activities of the neurons and glial cells. Recently, the effects of glycine on the microcirculation and its potential anti-ischemic activity on induced ischemic alterations in the brain have been examined using natural and computer modeling based on in vitro functional imaging techniques [13],[14]. In the case of the mesenteric arteriols, glycine application preceded or followed by histamine treatment successfully avoided and reversed the histamine-induced microcirculatory alterations. This blocking effect of glycine suggests a possible “antihistamine” effect of glycine, which may have promising medical impact. The mechanism of such an activity remains to be elucidated, but may include a blocking effect on histamine receptors, activation of tissue histamineases, or other molecular-level interactions with histamine. Such possibilities warrant further investigation.

Other amino acids also reportedly show stimulatory effects on the intestinal microcirculation. The results from the present study are consistent with an observed positive effect of L-arginine in combination with vasopressive preparations for intestinal microcirculation improvement during the early stages of endotoxemia [15]. The microcirculation plays an important role in the maintenance of the intestinal tract barrier function and nonspecific resistance to infection. We previously showed that microflora help to maintain microcirculatory function and microvessel reactivity in the mucous membranes. Pathogenic microorganisms, such as enteropathogenic *Escherichia coli*, induce alterations in the microcirculation as part of the inflammation process, as indicated by comparative studies of the intestinal tract and lungs in gnotobiotic and conventional animals [16],[17]. Of note, a higher dose of histamine was required to obtain the "intravascular brightening" in the mesentery microvessels of gnotobiotic rats compared to conventional animals [17]. The capacity of glycine to prevent some histamine-induced microcirculatory alterations is of prospective clinical interest, particularly as histamine is one of the most potent inflammatory and allergy mediators known.

## V. Conclusion

Direct intravital microscopy approaches, combined with recent innovations in optics, electronics, imaging, etc., are a

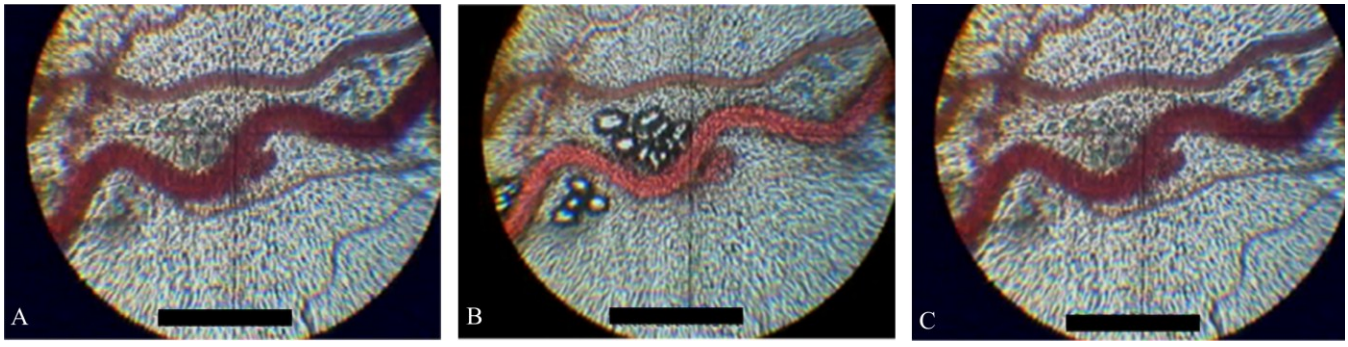


Figure 3. Vasodilatory effect of glycine on the mesentery microcirculation after histamine application
A. before histamine application; B. histamine-induced stasis; C. immediate restoration of microcirculation after glycine application.

potential approach in microcirculation research and for the testing of vasoactive properties of new candidate pharmacological preparations and substances [13]. Despite the intense development and implementation of other experimental methods, the direct microscopic approach remains a reliable method for objectively assessing microcirculatory alterations. The approach is important for correlation purposes as well as for validation and comparative studies using new methods (especially indirect ones) where data interpretation can be difficult [18].

Examination of the microcirculation in the cerebral pial vessels and intestinal mesentery of laboratory rats served as a suitable and informative biomodel for assessing the features of the microcirculation and the vasoactive potential of the studied preparations (i.e., glycine vs. saline). Glycine exerted a vasodilatatory influence on both the pial and mesenteric arterioles of rats, and blocked or prevented histamine-induced mesenteric arteriole microcirculation alterations, including stasis development. Experimental data showing the ability of glycine to improve the microcirculation and blood supply correlates well with clinical observations of its positive therapeutic effect when used in a complex treatment for early stage cerebral ischemic stroke [6]. Further research is needed to better understand the mechanisms of this natural metabolite.

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