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Full paper

L-Citrulline dilates rat retinal arterioles via nitric oxide- and prostaglandin-dependent pathways in vivo

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ABSTRACT

L-Citrulline is an effective precursor of L-arginine produced by the L-citrulline/L-arginine cycle, and it exerts beneficial effects on the cardiovascular system by supporting enhanced nitric oxide (NO) production. NO dilates retinal blood vessels via the cyclooxygenase-mediated pathway. The purpose of this study was to examine the effects of L-citrulline on retinal circulation and to investigate the potential involvement of NO and prostaglandins in L-citrulline-induced responses in rats. L-Citrulline (10–300 μg kg^{-1} min^{-1}, i.v.) increased the diameter of retinal arterioles without significantly changing mean blood pressure, heart rate, and fundus blood flow. The vasodilator response of retinal arterioles to L-citrulline was significantly diminished following treatment with N^G-nitro-L-arginine methyl ester (30 mg/kg, i.v.), an NO synthase inhibitor, or indomethacin (5 mg/kg, i.v.), a cyclooxygenase inhibitor. In addition, α-methyl-DL-aspartic acid (147 mg/kg, i.v.), an inhibitor of argininosuccinate synthase, the rate-limiting enzyme for the recycling of L-citrulline to L-arginine, diminished the L-citrulline-induced retinal vasodilation. These results suggest that both NO- and prostaglandin-dependent pathways contribute to the L-citrulline-induced vasodilation of rat retinal arterioles. The L-citrulline/L-arginine recycling pathway may have more importance in regulating vascular tone in retinal blood vessels than in peripheral resistance vessels.

1. Introduction

The vascular endothelium plays a crucial role in regulating retinal blood flow, and it performs this role via several vasodilators, including nitric oxide (NO), prostanoids (e.g., prostaglandin I_2), and endothelium-derived hyperpolarizing factor (EDHF) (1–4). In the vascular endothelium, NO is produced by endothelial NO synthase (eNOS), which uses L-arginine as a substrate and produces L-citrulline as a by-product. The L-citrulline to L-arginine recycling pathway consists of two enzymes, argininosuccinate synthase and argininosuccinate lyase, which are present in endothelial cells and other cell types (5,6). In endothelial cells, plasmalemmal caveolae—the site of the L-citrulline/L-arginine recycling pathway—may be the principal source of L-arginine (7,8).

L-Citrulline is an α-amino acid isolated from watermelon juice, and it is beneficial to the cardiovascular system (9). In isolated blood vessel preparations, L-citrulline induced endothelium-dependent relaxation by enhancing the release of NO; this NO release could be due to the recycling of L-citrulline to L-arginine (10). Furthermore, L-citrulline supplementation exhibits several beneficial effects on the cardiovascular system (11–14). However, the effect of L-citrulline on retinal blood vessels and the importance of the L-citrulline/L-arginine recycling pathway for the regulation of retinal circulation remain to be elucidated.

Retinal blood vessels anatomically and functionally resemble cerebral blood vessels (3,16), and they have different characteristics compared to blood vessels in other peripheral circulatory beds. In most vascular beds, NO dilates blood vessels by activating soluble guanylyl cyclase and elevating intracellular cGMP levels. However, in rat retinal vasculature, NO preferentially stimulates the cyclooxygenase-1/cAMP-mediated pathway (17). Furthermore, in diabetic conditions, retinal and systemic vascular endothelial cells

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display differential susceptibility to hyperglycemia, because the endothelium-dependent vasodilatory mechanisms of retinal arterioles are more vulnerable than those of peripheral resistance vessels to the effects of hyperglycemia (18,19). Thus, the importance of the L-citrulline/L-arginine recycling pathway in regulating vascular tone may differ between retinal and peripheral vasculature.

To test this hypothesis, we examined 1) the effects of intravenously administered L-citrulline on the diameter of retinal blood vessels and blood pressure, and 2) how N^G-nitro-L-arginine methyl ester (L-NAME), a non-selective inhibitor of NOS, indomethacin, a non-selective inhibitor of cyclooxygenase, and α-methyl-DL-aspartic acid (α-MDLA), a selective inhibitor of argininosuccinate synthetase, affect the responses to L-citrulline in rats.

2. Materials and methods

2.1. Animals

Twenty six male Wistar rats (8- to 10-week-old) were maintained in a room with constant temperature (22 ± 2 °C), constant humidity (55 ± 5%) and a 12-h light/dark cycle, and they were allowed free access to standard rat chow and tap water. All animal procedures were performed in accordance with the Association for Research in Vision and Ophthalmology Statement on the Use of Animals in Ophthalmic and Vision Research and the Regulations for the Care and Use of Laboratory Animals adopted by the Institutional Animal Care and Use Committee at Kitasato University.

2.2. Experimental procedures

The experimental procedures used in this study were described previously (20,21). Briefly, rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). After disappearance of the corneal reflex, each animal was placed on a heating pad. A tracheotomy was performed for artificial ventilation. Catheters were inserted into the femoral and jugular veins for drug administration. The left femoral artery was cannulated to measure arterial pressure, which was recorded on a thermal pen recorder (WT-645G, Nihon Kohden, Tokyo, Japan) via a pressure transducer (DX-360, Nihon Kohden) and a preamplifier (AP-610G, Nihon Kohden). Heart rate was measured using a cardiotachometer (AT-601G, Nihon Kohden) triggered by the blood pressure pulse. Mean arterial pressure and heart rate were digitized at 1 Hz (15BXW-H4; Dacs Giken, Okayama, Japan) and stored on the hard disk of a personal computer. To minimize the influence of nerve activity and capture fundus images at the same angle throughout the experiment, rats were treated with tetrodotoxin (50 μg/kg, i.v.) to prevent eye movement under artificial ventilation with room air (the stroke volume, 10 mL/kg; the frequency, 80 strokes/min) using a rodent respirator (SN-480-7, Shinano, Tokyo, Japan). Blood pressure and heart rate were decreased after treatment with tetrodotoxin; therefore, methoxamine was continuously injected into the jugular vein at a constant rate using a syringe pump (Model 1140-001, Harvard Apparatus, South Natick, MA, USA) to maintain adequate systemic circulation. Treatment with L-NAME, indomethacin, or α-MDLA was performed just before starting the methoxamine infusion. The dose of methoxamine was ~40 μg kg^{-1} min^{-1} (~5 μg kg^{-1} min^{-1} for L-NAME-treated rats).

In the first series of experiments, we examined the effects of intravenous infusion of L-citrulline (10–300 μg kg^{-1} min^{-1}, i.v.) on the diameter of retinal arterioles, blood pressure and heart rate (n = 5). L-Citrulline solution was infused into the femoral vein using a syringe pump (Harvard Apparatus), and the infusion rate was sequentially increased in a stepwise fashion. For comparison, saline was infused with the same procedure (n = 3). To examine the possible involvement of NO and prostaglandins in L-citrulline-induced responses, L-citrulline infusion was performed as mentioned above in rats treated with L-NAME (30 mg/kg, i.v., n = 5) or indomethacin (5 mg/kg, i.v., n = 5). The doses of L-NAME and indomethacin were chosen based on previous reports from our lab (17,18,22).

In the second series of experiments, we examined L-citrulline-induced responses after treatment with α-MDLA (147 mg/kg, n = 4) or its vehicle (n = 4) to determine the role of L-citrulline/L-arginine recycling pathway in the L-citrulline-induced vasodilation of retinal arterioles. α-MDLA was reported to selectively inhibit argininosuccinate synthetase (23). The dose of α-MDLA was chosen based on a previous study showing its effectiveness in vivo (23).

2.3. Fundus photography and retinal arteriolar diameter measurement

The techniques used for fundus photography and retinal arteriolar diameter measurement were previously described (18,19,21,22,24,25). Briefly, hydroxyethylcellulose (SCOPISOL 15®, Senju Pharmaceutical, Osaka, Japan) was dropped onto the cornea of rats to prevent drying of the eye. The optic disc was centered and focused in the field of view. Sodium fluorescein (10% solution, 0.8 mL/kg) and brilliant blue 6B (5% solution, 0.8 mL/kg) were injected into the right femoral vein to enhance blood vessel contrast. Fundus images were captured using a digital camera (Finepix S3 pro; Fuji Photo Film, Tokyo, Japan) equipped with a bore scope-type objective lens for small animals (Model 01; Scalar, Tokyo, Japan), and they were stored on the hard disk of a laboratory computer system. A region (120 × 240 μm) including a retinal arteriole in the fundus image (2820 × 4230 μm) was selected for analysis, and the diameter of blood vessel in the same region was measured throughout the experiment.

2.4. Measurement of fundus blood flow

Fundus blood flow was measured as reported previously (21,26). Briefly, a non-contact probe for blood flow measurement (outer diameter, 0.5 mm) was placed along the bore scope-type objective lens at an angle of approximately 30°. Blood flow in the region including the optic nerve head (fundus blood flow) was assessed using laser Doppler blood-flowmetry (Omega Flow FLO-N1, Omegawave, Tokyo, Japan). The fundus was monitored using the fundus camera prior to the start of the experiment to confirm the region of fundus to be illuminated by the laser. Fundus blood flow was digitized at 1 Hz using 15BXW-H4 (Dacs Giken) and recorded on an ink-writing recorder (R-62, Rikadenki Kogyo, Tokyo, Japan). The digitized blood flow data and fundus images were stored on the hard disk of a laboratory computer system.

2.5. Drugs

L-Citrulline was produced from KYOWA HAKKO BIO CO., LTD (Ibaraki, Japan). Indomethacin, L-NAME, methoxamine and α-MDLA were obtained from Sigma–Aldrich (St. Louis, MO, USA), and tetrodotoxin was obtained from Nacalai Tesque (Kyoto, Japan). Indomethacin was dissolved in 0.24% Na_2CO_3, and all other drugs were dissolved in saline.

2.6. Data analyses

The diameter of the retinal arteriole, mean arterial pressure, and heart rate were expressed as percentages of the baseline values (mean values of data obtained at the time from −2 min to 0 min)