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Cellular mechanisms underlying Hyperin-induced relaxation of rat basilar artery

Yi-Fei Fan, Zhi-Wu Chen *, Yan Guo, Qi-Hai Wang, Biao Song

Department of Pharmacology, Anhui Medical University, Hefei, Anhui 230032, China

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ABSTRACT

Background and aim: Hyperin, a flavonol compound extracted from the Chinese herb *Abelmoschus manihot* L. Medic, is reported to exert protective actions in cerebral ischemic injury. The specific aim of the present study was to study the relaxation of Hyperin in rat isolated basilar artery and identify the underlying cellular mechanisms.
Methods: Rat isolated basilar artery segments were cannulated and perfused while being superfused with PSS solution. Vessel images were recorded by video microscopy and diameters measured. Membrane potential was recorded using glass microelectrodes to evaluate the basilar artery smooth muscle cell hyperpolarization.
Results: Perfusion of Hyperin (1~100 μM) elicited a concentration-dependent relaxation of basilar artery segments preconstricted with 0.1 μM U46619. The response was significantly inhibited by the removal of the endothelium. Hyperin also elicited marked and concentration-dependent hyperpolarization of smooth muscle cells. 30 μM nitro-L-arginine (an inhibitor of nitric oxide synthase) and indomethacin (an inhibitor of cyclooxygenase), partially inhibited Hyperin-induced relaxation and hyperpolarization leaving an attenuated, but significant, endothelium-dependent relaxation and hyperpolarization. This remaining effect was almost completely blocked by 1 mM tetraethylammonium (an inhibitor of Ca^{2+}-activated K^+ channels), or by 100 μM DL-propargylglycine, an inhibitor of cystathionine-γ-lyase (a synthase of the endogenous H_2S).
Conclusion: These findings show that Hyperin produces significant hyperpolarization in rat basilar artery smooth muscle cells and relaxation through both endothelium-dependent and endothelium-independent mechanisms. The underlying mechanisms appeared to be multi-factorial involving nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF). Our data further suggest that endogenous H_2S is a component of the EDHF-mediated hyperpolarization and relaxation to Hyperin.

1. Introduction

The vascular endothelium is well known to release endothelium-derived relaxing factors in response to a number of agonists and physical stimuli that mediate relaxation. These factors include nitric oxide (NO), prostacyclin (PGI_2), and several factors collectively called endothelium-derived hyperpolarizing factors (EDHFs) [1].

EDHF is often functionally defined as the endothelial dependent relaxation that remains in the presence of combined inhibition of NO synthase (NOS) and cyclooxygenase [2]. Mechanistically, EDHF affects relaxation via the opening of arterial smooth muscle cell K^+ channels which causes hyperpolarization [3]. The actual chemical identity of EDHF may differ between species, vascular diameter, and vascular beds [4]. Specifically, EDHF-mediated relaxations

* Corresponding author. Tel.: +86 551 5161133.
E-mail address: aychenzhiwu@yahoo.com.cn (Z.-W. Chen).

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have been reported to involve the release by the endothelium of factors including H_2O_2 [5–7] and epoxyeicosatrienoic acids (EETs) [8]. Alternatively, the hyperpolarization may be mediated via direct coupling of endothelial and smooth muscle cells through myoendothelial gap junctions and may not require generation of a factor, per se [9].

Compared to peripheral vessels (for example, mesenteric and coronary) relatively little is known regarding the specifics of EDHF in cerebral arteries. Importantly, it has been suggested that EDHF is a regulator of the cerebral blood flow during physiological states and may become even more relevant in pathological conditions such as ischemia or traumatic brain injury [10]. It has been reported that EDHF-mediated relaxation in rat cerebral arteries does not involve EET or H_2O_2, [11] leading some to propose that EDHF in cerebral vessels may be unique [12].

Recently it was reported that some flavone compounds, native or synthetic, can relax vessels via an apparent EDHF-mediated mechanism [13,14]. Hyperin (structure shown in Fig. 1), a flavonol extracted from the Chinese herb *Abelmoschus manihot* L. Medic, found mainly in the south of China, is reported to have protective effects against cerebral ischemic injury.

For example, in rats, it reduces the degree of cerebral edema, attenuates the effects of free radicals and exerts protective effects against cognitive impairment during global cerebral ischemia reperfusion injury [15]. Importantly, Hyperin reduced the resistance of rat cerebral vessels and increased the rat cerebral blood flow, suggesting that Hyperin may relax cerebral arteries [16]. Consistent with this, our previous studies showed that Hyperin causes endothelium-dependent dilation in rat abdominal aorta [17]. Therefore, in this study, we tested the hypothesis that Hyperin can mediate vasorelaxation in the basilar artery (BA), the main artery supplying the cerebellum, brain stem and other encephalic regions, and whether its effects involved an EDHF component.

A second aim of the study was to investigate the chemical identity of EDHF in rat BA. H_2S, a newly recognized signaling molecule, has received an increasing attention as an endogenous vasodilator in a number of tissues (for example, aorta and mesenteric arteries) and is believed to protect the heart from ischemia/reperfusion damage [18]. Furthermore, H_2S-induced endothelium-derived relaxation in rat mesenteric artery was shown to be partly inhibited by the blockade of calcium-activated potassium (K_{Ca}) channels [19]. Thus, on the basis of these characteristics of H_2S, we also aimed to study the possible role of H_2S in Hyperin-mediated relaxation in rat BA.

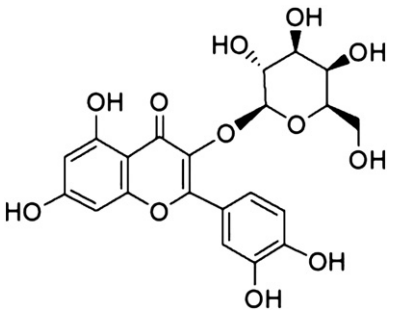

Fig. 1. Structure of Hyperin.

2. Methods

2.1. Drugs and solutions

Hyperin, a yellow powder, mp 230–231 °C, purity >98%, was provided by Anhui Institute of Medical Science (Heifei, China). 9,11-dideoxy-11α, 9α-epoxymethano-prostaglandin F2α(U46619), nitro-L-arginine (L-NA), indomethacin (Indo), DL-propargylglycine (PPG) and tetraethylammonium (TEA) were obtained from Sigma (St. Louis, MO, USA). Phosphate saline solution (PSS) comprised the following (mM): NaCl 118, KCl 3.4, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11.1) and was bubbled with 95% O_2 and 5% CO_2. The pH of the PSS solution was adjusted to 7.4 with NaOH and the solution was oxygenated during the incubation period.

2.2. Animals

Male Sprague–Dawley rats (SD rats, 250–350 g body wt, aged 6–8 weeks) were obtained from the Experimental Animal Center of Anhui Medical University. All studies and surgical procedures were performed according to the regulations stipulated by Anhui Medical University Animal Care Committee which follows the protocols outlined in *The Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH publication NO. 85–23, revised 1996).

2.3. Vessel experiments

Rats were anesthetized with 20% urethane by peritoneal injection and killed by decapitation. The brain was rapidly removed and placed in chilled physiological salt solution (PSS). The BA was then carefully dissected from the brain and placed in a vessel chamber. Micropipettes were inserted into each end of the BA segment and secured as previously described [19]. The vessel was bathed with 37 °C PSS equilibrated with a gas mixture of 95% O_2 and 5% CO_2. The vessel chamber was placed on the stage of a stereoscopic microscope. The BA was pressurized to 85 mm Hg by a column of PSS that was raised to the appropriate height above the vessel [20,21]. Luminal flow was adjusted to 150 μl/min by setting the inflow and outflow rate. 0.1 μM U46619 was added to the superfusate until reproducible contractions were obtained. Each BA preparation was magnified (×100), imaged using a digital camera (Nikon), and displayed on a computer screen. Changes in diameter were continually measured using E-rule software, either online or after the recording was replayed.

BA dilations were expressed as the percentage of the maximum diameter (% Dmax) using the following equation:

$$\textbf{Relaxation}\ (\%) = (\mathbf{D_x} - \mathbf{D_{min}}) / (\mathbf{D_{max}} - \mathbf{D_{min}})$$

Where D_x is the diameter after luminal administration of either Hyperin or other reagents, D_{min} is the diameter after the addition of the 0.1 μM U46619 (precontraction), and D_{max} is the maximum diameter for 1 h at 85 mm Hg, which was obtained after 1 h equilibrium.