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Mechanism of vasorelaxation induced by eupatorin in the rats aortic ring

Mun Fei Yam [a,b], Chu Shan Tan [b,*], Mariam Ahmad [b], Ruan Shibao [a,*]

[a] College of Pharmacy, Fujian University of Traditional Chinese Medicine, 1 Qiuyang Road, Shangjie, Minhou, Fuzhou 350122, Fujian, China
[b] School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Pulau Pinang, Malaysia

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ABSTRACT

Previous studies demonstrated that eupatorin content in *Orthosiphon stamineus* fractions correlated with their vasorelaxation activity. Even with previous studies, there is still very little information on the vasorelaxation effect of eupatorin, and not many scientific studies had been carried out. Therefore, the present study was designed to investigate the vasorelaxation activity and mechanism of action of eupatorin. The vasorelaxation activity and the underlying mechanisms of eupatorin was evaluated on thoracic aortic rings isolated from Sprague Dawley rats. Eupatorin caused the relaxation of aortic rings pre-contracted with phenylephrine with and without endothelium (pD2 = 6.66 ± 0.13, E_{MAX} = 99.72 ± 6.39%; pD2 = 6.10 ± 0.22, E_{MAX} = 65.78 ± 8.01%), and also the relaxation of endothelium-intact aortic rings pre-contracted with potassium chloride (pD2 = 6.20 ± 0.30, E_{MAX} = 71.89 ± 12.25%). In the presence of Nω-nitro-l-arginine methyl ester (pD2 < 4.60, E_{MAX} = 24.91 ± 6.39%), methylene blue (pD2 = 6.05 ± 0.38, E_{MAX} = 66.79 ± 9.69%), ODQ (pD25.84 ± 0.32, E_{MAX} = 60.47 ± 9.6%), indomethacin (pD2 = 6.27 ± 0.21, E_{MAX} = 76.03 ± 9.45%), tetraethylammonium (pD2 = 6.09 ± 0.35, E_{MAX} = 69.35 ± 11.31%), 4-aminopyridine (pD2 = 6.34 ± 0.12, E_{MAX} = 76 ± 6.1%), barium chloride (pD2 = 6.47 ± 0.14, E_{MAX} = 79.61 ± 10.02%), atropine (pD2 = 6.36 ± 0.29, E_{MAX} = 86.47 ± 12.95%) and propranolol (pD2 = 6.49 ± 0.26, E_{MAX} = 83.2 ± 12.01%), relaxation stimulated by eupatorin was significantly reduced. Eupatorin was also found to be active in reducing Ca^{2+} release from sarcoplasmic reticulum and in blocking calcium channels. The present study demonstrates the vasorelaxation effect of eupatorin involving NO/sGC/cGMP and indomethacin pathways, calcium and potassium channels, and muscarinic and beta-adrenergic receptors.

1. Introduction

Orthosiphon stamineus is a medicinal plant that is widely used in Southeast Asia, especially in Malaysia for the treatment of hypertension and kidney stone. In clinical studies, it was been tested as an additional regimen for antihypertensive treatment (Trimarco et al., 2012). Our previous results showed that the chloroform fraction of *O. stamineus* possessed excellent vasodilatory effects on the isolated rat aorta *in vitro* model (unpublished data). The most noteworthy is that the eupatorin (Fig. 1) present in the chloroform fraction amounted to a total of 46 μg/mg of fraction. Moreover, statistical analysis from the previous study also revealed that the amount of eupatorin in the fractions correlated with the vasodilation effect of the fractions. Xu and colleagues suggested that flavonoids are required to have 5-OH (i), 7-OH (ii), 4′-OH (iii), C2=C3 (iv) and C4=O (v) in order to possess good vasorelaxant effects (Xu et al., 2007). Considering the chemical structure, as eupatorin fulfilled criteria (iv) and (v), we hypothesized that eupatorin may be one of the active ingredient of *O. stamineus* contributing to vasorelaxatory activity. Therefore, we designed the present study to investigate the vasodilatory activity and mechanism of action of eupatorin.

2. Materials and methods

2.1. Materials and chemicals

Acetylcholine (Ach), nifedipine and phenylephrine (PE) were purchased from Acros Organics (Belgium). Nω-nitro-l-arginine methyl ester (L-NAME), indomethacin, tetraethylammonium (TEA), barium chloride ($BaCl_2$), glibenclamide, 1*H*-[1,2,4]Oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ), atropine, eupatorin (purity ≥ 97%) and propranolol were purchased from Sigma aldrich (USA). 4-aminopyridine was purchased from Merck (Germany). Ethylene glycol tetraacetic acid (EGTA) was purchased from

* Corresponding authors.
E-mail addresses: weazley90@hotmail.com (C.S. Tan), 1554850002@qq.com (R. Shibao).

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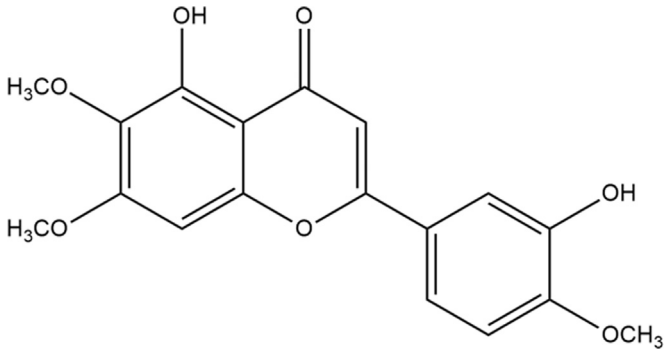

Fig. 1. The chemical structure of eupatorin.

Calbiochem (Germany). Methylene blue was purchased from Promedipharm Sdn. Bhd. (Malaysia).

2.2. Animals

Healthy adult male Sprague-Dawley rats (250–300 g) were used in this experiment. The animals were kept under a 12/12 h light/dark cycle and allowed free access to food and water. The investigation conforms to the Guide for the Care and Use of Laboratory Animal by the Fujian University of Tradition Chinese Medicine.

2.3. Aortic ring preparation and vasorelaxation study

A male Sprague-Dawley rat, weighing 200–240 g, was used in this experiment. An empty Petri dish filled with Krebs-Henseleit

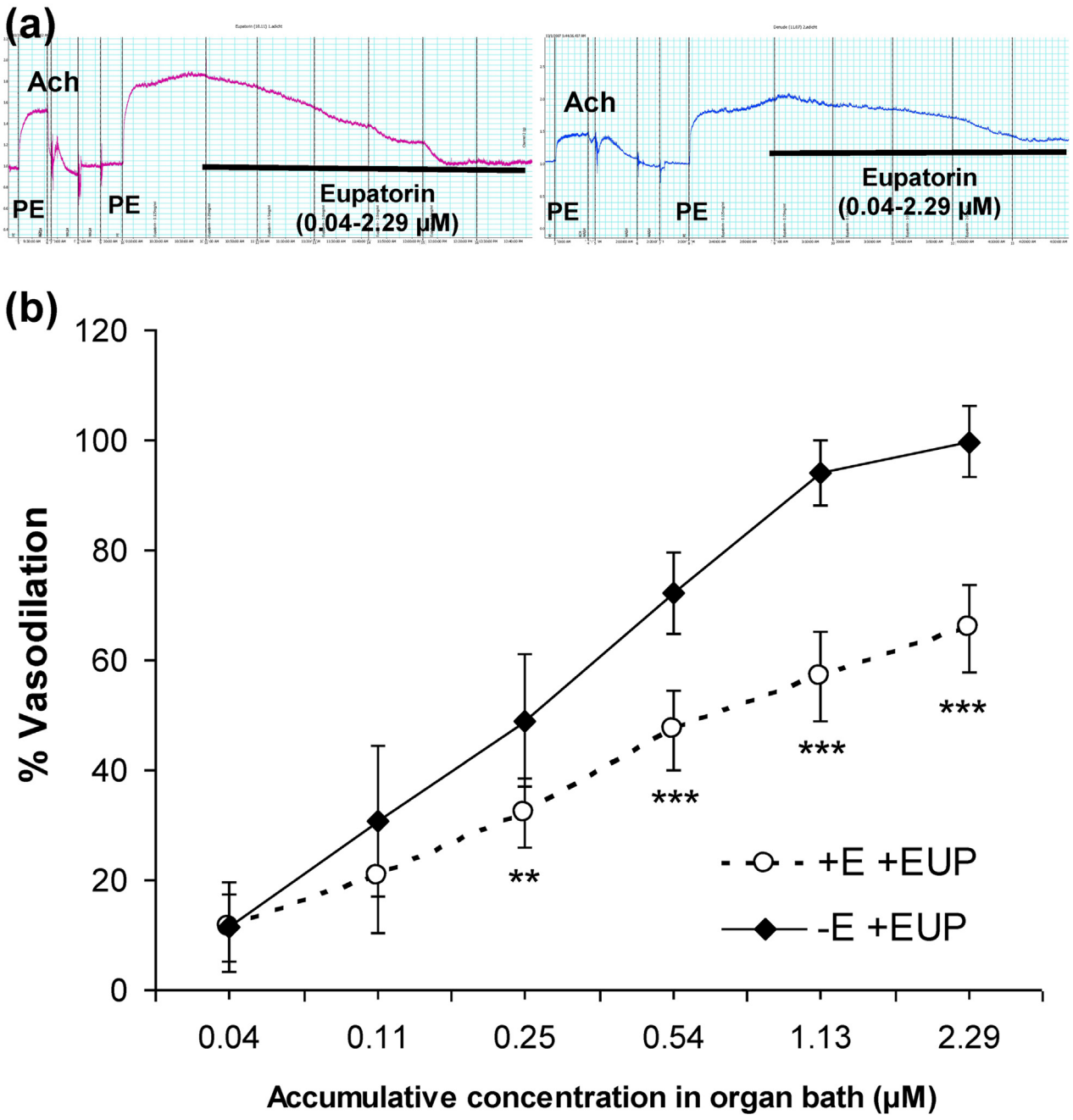

Fig. 2. Original isometric force recordings (a) and vasorelaxation effect (b) of eupatorin in endothelium-intact and denuded rat aortic rings pre-constricted by PE (N=8). ** and *** indicate significant at $P<0.01$ and $P<0.001$, respectively as compare endothelium-intact aortic ring group.

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