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Vascular dilation by paeonol — A mechanism study

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ABSTRACT

The goal of this study was to investigate the mechanism underlaying the vasodilatory effect of paeonol, a major active element from the root bark of Chinese herbs *Paeonia suffruticosa* Andr. and *Cynanchum paniculatum* (Bunge) Kitagawa. Paeonol relaxed isolated rat aorta rings by 95.6% while the 10^{-6} M forskolin-induced vasodilatation used as 100%. The EC_{50} of vasodilatation by paeonol is 2.9×10^{-4} M. Although paeonol exerted endothelium-independent relaxation, L-NAME treatment inhibited paeonol-induced vasodilation of endothelium intact rings, while indomethacin did not. Both L-NAME and ODQ did not affect paeonol relaxation in the rings without endothelium. In addition, paeonol markedly elevated NO generation in cultured endothelial cells. Pre-treatment of propranolol, glibenclamide, TEA and $BaCl_2$ did not affect paeonol relaxation of endothelium removed rings. On the other hand, pre-treated of rings (without endothelium) with paeonol markedly blocked vasoconstriction induced by AngII, $PGF_{2\alpha}$, 5-HT, dopamine, vasopressin, endothelin-1 and PE. The paeonol incubation also significantly attenuated KCl-induced contraction which mainly depended on Ca^{2+} influx. In Ca^{2+}-free medium (containing 10^{-4} M of EGTA and 60 mM of KCl), paeonol suppressed the contraction curve of $CaCl_2$. In addition, paeonol also inhibited contraction by PE in Ca^{2+} free solution (containing 10^{-4} M of EGTA) which mainly relied on intracellular Ca^{2+} release. Whole-cell patch-clamp experiment showed that paeonol shifted the *I–V* curve and the peak value of calcium currents was significantly inhibited. In conclusion, our study suggested that voltage-dependent and receptor-operated Ca^{2+} channel, as well as intracellular Ca^{2+} release were all inhibited by paeonol. An intracellular Ca^{2+} regulatory mechanism may be responsible to potent vasodilatory effect of paeonol.

1. Introduction

Paeonol (2′-hydroxy-4′-methoxyacetophenone, Scheme 1) is a main element extracted from the Chinese herbs *Paeonia suffruticosa* Andr. and *Cynanchum paniculatum* (Bunge) Kitagawa. Ancient Chinese medicine has recorded various effects of the herbs on human sickness such as reducing heat, promoting blood flow, eliminating stasis, relieving pain stopping cough and using as diuresis to alleviate edema. For example, Mudanpi, a Chinese medicine prepared from the root bark of *Paeonia suffruticosa* Andr, (Scheme 1) has been used traditionally to promote the blood flow and remove blood stasis (Zhang, 1988). Furthermore, experiments carried out on cultured neonatal rat ventricular cells showed that extracts of Mudanpi decreased action potential amplitude and shortened action potential duration (Ma and Li, 1986).

The pharmacological active content of Mudanpi can be separated into lipid-soluble and water-soluble fractions. Its lipid-soluble fraction contains paeonol, volatile oil, sterol alkaloid, paeoniflorigenone, 6-hydroxycoumarin. The components in aqueous extract are: Polysaccharide, Paeonoside, Apiopaeonoside, Paeoniflorin, Benzoylpaeoniflorin, and Oxypaeoniflorin (Yan et al., 1996). The polysaccharide extracted from Mudanpi can improve the utilization of glucose in peripheral tissues, and whole body sensitivity to insulin (Wang et al., 2001) and Paeoniflorin effects mainly on digestive system and central nervous system (Wu et al., 2002; Hong et al., 2005). Paeonol is one of the major active element abundance in Mudanpi. Accumulating evidence has shown the pharmacological activity of the agent especially on cardiovascular system. In animal studies, paeonol has been shown to possess many of the actions of the Mudanpi, besides of anti-ischemia reperfusion injury (Zhang and Zhang, 1994), it also used to lowering blood pressure (Yan et al., 1996), inhibiting platelet aggregation scavenging oxygen free radical and anti-artherosclerosis by regulating blood fat, improving hemorheology, anti-lipid peroxidation and inhibiting smooth muscle cell proliferation (Dai et al., 1999, 2000, 2001). The mechanism for some of paeonol's activity may be related to inhibition of certain ion channels. It has been reported

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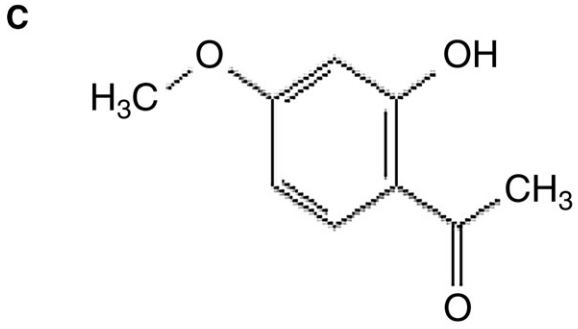

Scheme 1. (A) The plants of Mudanpi (*Paeonia suffruticosa* Andr.). (B) Decoction pieces of Mudanpi. (C) The chemical structure of paeonol.

that paeonol can inhibit delayed outward K^+ current (IK), the Na^+ current (INa), and Ca^{2+} currents (T- and L-type) (Hu and Shi, 1994). Therefore, interfering ion channel currents may play a role in the protection by paeonol against myocardial injury (Zhang et al., 2003).

Despite the pharmacological studies on paeonol so far, the effect of paeonol on vasculature has never been characterized. In the present study, we systemically evaluated vasodilatory effect of paeonol on isolated rat aorta ring and vascular smooth muscle cells and explored action mechanism underlying vascular effect of paeonol.

2. Methods

2.1. Reagents

Paeonol ($C_9H_{10}O_3$; Scheme 1) injection was purchased from Tianzhen Pharmaceuticals Company (Ningbo, China; Batch No. 20060901; molecular weight 166.18). Acetylcholine (Ach), phenylephrine (PE), NG-nitro-L-arginine methyl ester (L-NAME),1H-[1,2,4]-oxadiazole-[4,3-a] -quinoxalin-1-one (ODQ), indomethacin, propranolol,glibenclamide, tetraethylammonium (TEA), $BaCl_2$, angiotensin II (Ang II), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$),5-hydroxytryptamine (5-HT), dopamine, vasopressin, endothelin-1 (ET-1 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ethyleneglycol bis (2-aminoethyl ether) tetraacetic acid (EGTA) and other inorganic salt were all purchased from Sinopharm Chemical Reagent Co., Ltd, (Batch no. F20060620).

Ach, PE, L-NAME, TEA, propranolol, AngII, $PGF_{2\alpha}$, 5-HT, dopamine, vasopressin, ET-1 were dissolved in distilled water; Stock solutions of indomethacin, glibenclamide, ODQ were dissolved in DMSO; working solutions were made in Krebs solution (pH 7.4, containing (mmol/L) NaCl 118, KCl 4.7, $MgSO_4$ 1.1, KH_2PO_4 1.2, $CaCl_2$ 1.5, $NaHCO_3$ 25 and glucose 10). Control experiments had demonstrated that the highest DMSO level (1:400) had no effect on vascular tone.

2.2. Animals and vascular ring preparation

Adult, male Sprague–Dawley rats weighing 250 to 300 g were purchased from Shanghai Experimental Animal Center of Academia Sinica and used for all experiments. Animals were handled and cared for in compliance with the *Guide for the Care and Use of Laboratory Animals* (Shanghai University of Tradition Chinese Medicine).

The thoracic aortas were rapidly and carefully moved after rat euthanasia by cervical dislocation, and then carefully dissected in ice-cold Krebs solution. The aortae were removed free of connective tissue and fat, and then made into rings of approximately 3 mm width. All dissecting procedures were done with extreme care to protect the endothelium from inadvertent damage. In some aortic rings, the endothelial layer was mechanically removed by gently rubbing the luminal surface of the aortic ring with wooden tooth pick. Endothelial integrity or functional removal was verified by the presence or absence of the relaxant response to Ach respectively. Thus, endothelium was considered intact while relaxation reach 15%–20% by 10^{-7} M of Ach; 60% by 10^{-6} M of Ach; and 80% by 10^{-5} M of Ach in PE-pre-contracted aorta ring. When endothelium was fully removed, <1% relaxation to Ach should be recorded.

2.3. Record of isometric vascular tone

The aortic rings were suspended by means of two L-shape stainless-steel wires inserted into lumen in a tissue bath containing Krebs solution [pH 7.4, containing (mM) NaCl 118, KCl 4.7, $MgSO_4$ 1.1, KH_2PO_4 1.2, $CaCl_2$ 1.5, $NaHCO_3$ 25 and glucose 10] at 37 °C, while continuously bubbled with 95% O_2–5% CO_2. The baseline load placed on the aortic rings was 2.0 g, and the changes in isometric tension were recorded using a force-displacement transducer connected to computer with professional software (PowerLab blood vessel functional examination system, AD Instruments Company, Australia). After equilibrating for 1 h, the aortic rings were contracted twice with 60 mM KCl to obtain maximal response, then the rings were washed every 20 min with Krebs solution until the tension returned to the basal level. The relaxant response curve to Ach (10^{-8} M–10^{-5} M) was performed in endothelium intact and endothelium removed aortic