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Vasodilative effect of perillaldehyde on isolated rat aorta

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Abstract

The vasodilative effect of perillaldehyde, one of the major oil components in *Perilla frutescens* BRITTON, was studied using isolated rat aorta. Perillaldehyde at final concentrations of 0.01 to 1 mM showed dose-dependent relaxation of the aorta contracted by treatment with prostaglandin $F_{2\alpha}$ or norepinephrine. Neither the presence of N^G-nitro-L-arginine methyl ester nor removal of the aortic endothelium affected the vasodilatation, suggesting that perillaldehyde exerts a direct effect on vascular smooth muscle cells. The vasodilative effect of perillaldehyde was not inhibited by pretreatment with a β-adrenergic receptor blocker (propranolol), an inhibitor of phosphodiesterase (theophylline), a delayed rectifier K^+ channel blocker (tetraethylammonium chloride), or an ATP-sensitive K^+ channel blocker (glibenclamide). However, perillaldehyde showed contrasting effects on vasodilatation of the aorta contracted by an influx of extracellular Ca^{2+} – perillaldehyde caused little vasodilatation on the aorta contracted by the Ca^{2+} ionophore A23187, while it inhibited the vasoconstriction induced by treatment with high-concentration K^+, which dominantly opened the voltage-dependent Ca^{2+} channel. These results suggest that the vasodilative effect of perillaldehyde is derived from blocking the Ca^{2+} channels.

Keywords: Perillaldehyde; Rat thoracic aorta; Vasodilator effect; Voltage-dependent Ca^{2+} channel; Ca^{2+} channel blockade; *Perilla frutescens* BRITTON

Introduction

Perilla frutescens BRITTON (Perilla) has been used as an ingredient in Chinese medicine because of its diuretic, sedative, detoxic, and antibacterial actions. These actions were found in the ether, ethanol and water extracts from its leaves. Rosmarinic acid, isolated as the major polyphenol composition from Perilla leaves, was reported to have anti-inflammatory and anti-allergic characteristics (Gracza et al., 1985; Okuda et al., 1986). Perillaldehyde was found as a component of the major volatile extracts in Perilla leaves, and the essential oil containing perillaldehyde is reported to be one of the most effective ingredients of Chinese medicine (Ito et al., 1999). The sedative activity in Perilla is mainly due to

Abbreviations: $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; NE, norepinephrine; L-NAME, N^G-nitro-L-arginine methyl ester; TEA, tetraethylammonium chloride; NO, nitric oxide.

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the presence of both perillaldehyde and stigmasterol, and the anti-fungal activity is mainly due to perillaldehyde (Honda et al., 1986). Recently, the water extract of Perilla leaves was reported to induce NO production in cultured murine vascular smooth muscle cells (Makino et al., 2002). An aldehyde component in Chinese medicine, Cinnamomi cortex, was reported to cause vasodilatation in the aorta isolated from spontaneously hypertensive rat (Kasahara et al., 2002). These results led us to examine the vasodilative effect of perillaldehyde. In this study, we found that perillaldehyde acts as a vasodilator on the aorta contracted by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or norepinephrine (NE), thus indicating the possibility of a novel function of perillaldehyde as a Ca^{2+} channel blocker.

Materials and methods

Materials

Perillaldehyde was purchased from Nakalai tesque, Inc., Kyoto, Japan. $PGF_{2\alpha}$, NE, N^G-nitro-L-arginine methyl ester (L-NAME), acetylcholine and the inhibitors used in this study from Wako Pure Chemical Industr., Osaka, Japan.

Preparation of rat aorta

Wistar male rats weighting 350–400 g were anesthetized (50 mg/kg i.p. pentobarbital) and killed by cutting their abdominal aorta. Fats and connective tissues were removed from a section of the thoracic aorta, and 3-mm-wide aortic rings were prepared. For an endothelium-free aorta, the endothelial lining of each ring was removed by pressing the ring and rolling it gently onto a filter paper a few times. The removal of the endothelium was confirmed by the absence of acetylcholine-induced relaxation (Furchgott and Zawadzki, 1980). Experimental protocols met the "Guidelines for Animal Experimentation" approved by the Japanese Association of Laboratory Animal Science and the Japanese Pharmacological Society.

Vasodilative effect of perillaldehyde on isolated aortic rings

The aortic rings were mounted on steel hooks in a Magnus chamber (Kishimoto UC-5TD, Kyoto, Japan). One end of the aorta was attached to a force–displacement transducer (Kishimoto UM-203) so that its isometric contraction could be recorded (NEC RECHI HORIZ-8 K, Tokyo, Japan). The baths were filled with 5 ml of Krebs solution with the following composition (mM): NaCl 120, KCl 4.7, $NaHCO_3$ 25.0, KH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2$ 2.5, and glucose 10.0. The solution was maintained at 37 °C and bubbled continuously with 5% CO_2 in O_2 at pH 7.4. The rings were equilibrated for 40 min at an initial resting tension of 1 g. During this time, the Krebs solution in the bath was replaced every 15 min. $PGF_{2\alpha}$ (6 μM) or NE (1 μM) was added to the bath to contract the aortic strips. After the contraction reached a plateau, perillaldehyde was added to the bath in cumulatively increasing doses of 0.1 μM–1 mM. L-NAME (0.1 mM) or various inhibitors (1 mM propranolol, 0.1 mM theophylline, 5 mM tetraethylammonium chloride (TEA), and 10 μM glibenclamide) were pre-treated for 60 and 10 min, respectively, and then the vasodilative effect of perillaldehyde was examined on the endothelium-intact aorta. Each inhibitor alone at the concentrations used had no effect on vasoconstriction.

The addition of 6 μM A23187 to the Krebs solution was used to cause an influx of extracellular Ca^{2+} and induce vasoconstriction. After the contraction reached a plateau, perillaldehyde was added in cumulatively increasing doses of 0.1 μM–1 mM. As for the vasoconstriction achieved with a high concentration of K^+, the aortic rings were suspended in Ca^{2+}-depleted Krebs solution containing 60 mM KCl for 10 min, and 1 mM perillaldehyde or 1 μM verapamil was added to the solution. Then, Ca^{2+}-induced contractions of the aorta rings were elicited by the cumulative addition of $CaCl_2$ at final concentrations of 10 μM–3 mM to the bath. The resulting contractile tension was measured and expressed as a percentage of the maximum tension obtained in the control experiment.

Statistics

Data were presented as mean with standard error. Statistical comparisons were made using Student's *t*-test and repeated measures ANOVA. The level of statistical significance was defined as $p < 0.05$.

Results

Perillaldehyde inhibited the $PGF_{2\alpha}$- or NE-induced contraction of rat aortic rings

One end of the aorta with endothelium was attached to a force–displacement transducer in a Magnus chamber to record its isometric contraction. The addition of $PGF_{2\alpha}$ produced sustained contraction in the aorta. After the rings achieved a stable contractile tension, perillaldehyde was added to the chamber in cumulatively increasing doses of 0.1 μM–1 mM at final concentrations (Fig. 1A). Fig. 1B shows that perillaldehyde caused a dose-dependent vasodilatation