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Hypotensive and vasorelaxant effects of (E) – Methyl isoeugenol: A naturally occurring food flavour

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ABSTRACT

Application of naturally occurring (E) – methyl isoeugenol (MIE) as food flavour has been widely accepted despite the growing concerns over cardiovascular issue. Hence, we sought to investigate hypotensive property of MIE and the involvement of central and/or peripheral mechanism (s). Variation in mean arterial pressure (MAP), heart rate (HR), systolic blood pressure (SBP), baroreflex sensitivity of normotensive rats and vascular reactivity were recorded. MIE (1.11, 2.25 or 4.50 mg/kg, iv) elicited dose-related decrease in MAP (−16.9 ± 1.13; −19.0 ± 4.18 or −27.2 ± 3.65 mm Hg, respectively) and an increase in HR (17.4 ± 1.79; 24.4 ± 5.11 or 29.9 ± 6.62 bmp, respectively). MIE 25 or 50 mg/kg (p.o) reduced the SBP (−13.6 ± 4.18 or −16.6 ± 5.60 mm Hg, respectively) without altering baroreflex sensitivity. The hypotensive effect of MIE remained unaltered by WAY100635 (antagonist of 5-HT1A) and L-NAME (NO synthase inhibitor). Intracerebroventricular injection of MIE did not change MAP. MIE elicited endothelium independent vasorelaxation (endothelium-intact vessels, Emax 92.5 ± 1.75%; Endothelium-denuded vessels, Emax 91.4 ± 2.79%). MIE blocked $CaCl_2$ or BAY K8644 (L-type voltage gated calcium channel activator)-induced vascular contractions. Our findings showed evidence of hypotensive and vasorelaxation effects of MIE with involvement of calcium channel.

1. Introduction

Natural flavour represents a growing demand within the food industry. Biotechnological techniques are being employed to produce aromatic chemicals (Benz and Muheim, 1996). Meanwhile, an increase in the demand for food products made of natural ingredients (functional food, food supplements, herbal teas and food flavours) has been associated the consumer perception that being natural is equivalent to safety (Ávila et al., 2009). (E) – methyl isoeugenol – MIE otherwise known as 1,2-dimethoxy-4-(prop-1-en-1-yl) benzene is a major phenylpropanoid derivative (93.9%) in the essential oils of *Pimenta pseudocaryophyllus* leaf (Paula et al., 2011). As an aromatic compound without record of health hazards, there are increasing interest in its application as a food flavour. According to Smith (2012), flavour is considered as configurations of sapid, odorous and textural properties of foods. Interestingly, flavours or food additives are primarily considered inert in several cases.

The category of botanical flavour like alkenylbenzene (Rietjens et al., 2005) constitute two different chemical classes: the allylbenzenes (with a 2,3-double bond) such as eugenol, eugenol methyl ether, estragole or myristicin which are genotoxic carcinogens in rodents (Stanfill et al., 2003; Hasheminejad and Caldwell, 1994) and propenylbenzenes (with a 1,2-double bond) such as isoeugenol or anethole which are non-genotoxic. Other flavour-related compounds, including coumarin and pulegone, also exhibit potentially harmful effects. Coumarin, a benzopyrone compound, has been demonstrated to elicit hepatotoxicity in animals and has been prohibited for use as a food additive in the US since 1956 (Hazleton et al., 1956; CFRT, 2006). As a propenylbenzenes, MIE is considered non-genotoxic. Although MIE as a naturally occurring flavour has been consumed for years, continuous worry about cardiovascular effect of MIE can be traced to the previous studies that demonstrated a vascular function of methyl eugenol (derivative of MIE) and an inhibition of voltage-dependent and receptor-operated channels (Lima et al., 2000). Additionally, a reduction in arterial blood pressure of dogs and an increase in blood flow following intravenous injections of eugenol (Sticht and Smith, 1971) support the assumption that MIE could possess cardiovascular property. The proper functioning of cardiovascular

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system is vital to human health. Thus, an in – depth understanding of cardiovascular effect of MIE and its mechanisms of action are critical to an effective and safe application of MIE in food industry. The control of blood pressure is sometimes dependent upon the integration of multiple cardiovascular regulatory systems (Thomas and Steven, 2008; Abrams, 1988; Scher, 1989; John, 2007).

Functional and structural alterations in resistance arteries have been associated with a long-lasting high blood pressure and cardiovascular complications (Sukumaran et al., 2013). The evaluation of arterial blood pressure, heart rate, systolic blood pressure, baroreflex sensitivity and vascular tone in preclinical studies are vital cardiovascular parameters towards the discovery of compound with potential cardiac and vascular function. Meanwhile, alterations in these parameters could be mediated centrally or peripherally. Centrally acting cardiovascular drugs are considered to target I_1-imidazoline receptors and/or α_2-adrenoceptors within the rostral ventrolateral medulla (RVLM) towards the control of sympathetic outflow and blood pressure (Bui et al., 1992; Guyenet, 1997; Haxhiu et al., 1994). It has been established experimentally that oral administration of losartan antagonized pressor effects elicited by intravenous treatment with Ang II, but did not antagonize the pressor effects of intracerebroventricular treatment of Ang II (Bui et al., 1992). The endothelium-derived vasodilators, adenosine triphosphate (ATP), bradykinin, stress, mechanical stimuli, ion channels (K^+ and Ca^{2+}) among other signaling proteins are capable of mediating vascular tone, regional blood flow and arterial blood pressure (Sukumaran et al., 2013; Alfredo et al., 2005; Calder et al., 1993). Hence, unravelling the cardiovascular mechanism of psychoactive compound like MIE poses some challenges in determining the involvement of peripheral mediators. In this study, we specifically sought to evaluate hypotensive property and participation of CNS, and peripheral components of the cardiovascular action of MIE.

2. Material and methods

2.1. Drugs and treatment

(E) – methyl isoeugenol – MIE [1,2-dimethoxy-4-(prop-1-en-1-yl)benzene: Sigma–Aldrich, St. Louis, MO, USA], Tween 80 (Polyoxyethylenesorbitan monooleate, Synth, Diadema, SP, Brazil), halothane (Cristália, Itapira, SP, Brazil), urethane (Sigma–Aldrich, St. Louis, MO, USA), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP; Sigma–Aldrich, St. Louis, MO, USA), N-{2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl}-N-2-pyridinylcyclo-hexanecarboxamide (WAY100635; Sigma–Aldrich, St. Louis, MO, USA), N^G-nitro-l-arginine methyl ester (L-NAME; Sigma–Aldrich, St. Louis, MO, USA), muscimol (Sigma–Aldrich, St. Louis, MO, USA), norepinephrine (NOR; União Química, Brazil), tetraethylammonium (TEA; Sigma–Aldrich, St. Louis, MO, USA), ketamine (Cristália, Itapira, SP, Brazil), BAY K8644, phenylephrine – PhE, Acetylcholine – ACh, sodium nitroprusside (Sigma–Aldrich, St. Louis, MO, USA) were used in the present study. A mixture of Tween 80 (2%) and Saline was used as a vehicle to dissolve drugs or treat the control groups. Animals received 0.1 mL of drug solution or vehicle per 0.3 kg body weight (0.33 mL/kg) intravenously, 0.2 mL per 0.2 kg body weight. (1.0 mL/kg) orally or volume of 2.0 μL through intracerebroventricular microinjection.

2.2. Animals

Experimental subjects were adult male Wistar normotensive rats (290 ± 40 g) provided by the central animal house, Federal University of Goiás. Animals were kept for acclimatization under 23 ± 2 °C (12 h light–dark cycles) with access to standard diet and water *ad libitum*. Experiments were carefully conducted by a trained researcher to minimize animaĺs pain or distress in compliance with the experimental protocol (number # 172/09) as approved by the Ethical Committee of the Federal University of Goiás and in agreement with the relevant national and international laws (Kilkenny et al., 2010).

2.3. Catheterization of trachea femoral venous and artery

Rats were anaesthetized with halothane (2–3% in 100% O_2, i.p); the skin fur of the animal was shaved with sterilized surgical tools within inguinal region while on the supine position. This was followed by an incision and subsequent isolation of the femoral vein and artery with fine tip forceps. A small cut was made with a sharp pointed scissors through the vein and artery. The small flap of these vessels was lifted up to permit insertion of a fine plastic polyethylene catheter (1.5 mm external diameter). Injections of the drugs were realized through the vein catheter while the artery catheter was connected for the recording of arterial pressure and heart rate. Trachea was isolated and catheterized to facilitate the removal of secretions and prevents respiratory distress of the animal. Maintenance of the anesthesia was realized with urethane (1.2 g/kg body weight.). 50 i.u./mL of heparin was used in this procedure to prevent blood clotting.

2.4. Recording of mean arterial pressure and heart rate

The animals were mounted prone on a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA) with the incisor bar 11 mm below the interaural line. The blood pressure was register through the arterial catheter connected to a pressure transducer attached to a bridge amplifier. An analog-to-digital converter (PowerLab System ADInstruments Inc., Colorado Springs, CO, USA) was used for continuous recording of the pulsatile pressure. The mean arterial pressure (MAP) and heart rate (HR) were determined through the pulsatile signal with Chart software (version 7.3.1. ADInstruments Inc., Colorado Springs, CO, USA). Animal were placed on the thermostatically controlled heated table to maintain the body temperature at 37 ± 0.5 °C.

2.5. Indirect measurement of systolic blood pressure (SBP)

Animals were acclimatized for about 5–6 h in the laboratory at room temperature. SBP and HR were measured with a tail-cuff sphygmomanometer with mercury (Harvard apparatus, USA) in conscious rats pre-warmed at approximately 37 °c. This is a non-invasive and indirect method of measuring SBP. The apparatus set up include a restrainer, a tail cuff containing latex tube and a dual-channel recorder. The conscious normotensive rats were exposed to this apparatus for acclimatization. Tail-cuff was placed on the rat tail and moved till the sensor detects pulses. We used a plethysmo-graphic device as a sensor for pulse detection after 5 days of oral administration of vehicle (1 mL/kg) or MIE (12.5, 25 or 50 mg/kg). A mean of at least 3 readings was taking as systolic blood pressure of each animal. Note, measurement of the basal SBP was carried out before oral administration.

2.6. Investigation of baroreflex sensitivity

Increasing doses of baroreflex activators [phenylephrine (0.30, 0.45 and 0.60 μg/kg) and sodium nitroprusside (3.0, 4.5 and 6.0 μg/kg)] were infused through the venous catheter to test baroreflex sensitivity of rats previously treated with vehicle (p.o) or MIE (25 mg/kg, p.o). The Peak changes in MAP and/or HR after phenylephrine or sodium nitroprusside injections were recorded. The values of baroreflex index were subsequently calculated.

2.7. Intracerebroventricular microinjections of MIE

Rats were anesthetized with ketamine (70 mg/kg, i.p.) and xylazine (30 mg/kg, i.p.) and mounted prone in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA) with the tooth bar fixed at 3.3 mm below the interaural line; the skull was surgically exposed (craniotomy), and a stainless steel guide cannula was implanted intracerebroventricularly – icv (1.2 mm posterior, 1.5 mm lateral, 4.0 mm ventral from the bregma). Stereotaxic coordinates for the implantation of the guide cannula were selected from the brain atlas (Xavier et al., 2009; Paxinos and Watson, 2007). Cannula was fixed to the skull with dental acrylic cement anchored to a stainless steel screw. A tightly fitted mandrel was kept inside the guide cannula to avoid its occlusion. After five days of recovery, animals were anesthetized with 2% halothane in O_2 and a polyethylene catheter was implanted into the femoral artery for blood pressure recording. The catheter was secured in the position with threading and passed under the skin to be extruded on the dorsum of the animal. The animals were kept for 24 h to recover. ICV injection was performed using a 10 mL syringe (Hamilton, Reno, NV, USA). After 20 min of HR and MAP baseline recording, animals received icv injection (2 μL each) of vehicle (2% Ethanol), MIE (0.7, 2.0 and 6.0 μmol) or muscimol 4 mM (a GABA A agonist). Microinjection of 2% Evans Blue dye (Sigma–Aldrich) was also performed at the end of the experiment for histological study.

2.8. Histological determination of the microinjection sites

At the end of the experiments, the rats were transcardially perfused with NaCl 0.15M, followed by a 10% formaldehyde solution (500 mL; Sigma–Aldrich). The brains were removed, post-fixed in the same formaldehyde solution and cryoprotected in a 30% sucrose solution. Subsequently, the brains were dissected into 40 μm-thick coronal sections with a cryostat (CM1900, Leica, Germany) and stained with 1% neutral red. The correctness of microinjection needles placement was verified by analyzing serial sections according to the rat brain atlas of Paxinos and Watson (2007).