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The role of the central thromboxane A_2 in cardiovascular effects of a phospholipase A_2 activator melittin administrated intracerebroventricularly in normotensive conscious rats

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Abstract

The current study was designed to determine the cardiovascular effect of centrally administrated melittin, a phospholipase A_2 (PLA_2) activator, and the mediation of central thromboxane A_2 (TXA_2) and its receptors in normotensive conscious rats. Studies were performed in normotensive male Sprague Dawley rats injected intracerebroventricularly (i.c.v.) with melittin. Melittin (1.5, 3.0, 6.0 μg/5.0 μl; i.c.v.) caused dose- and time-dependent increases in mean arterial pressure (MAP) and decrease in heart rate (HR). Maximal effects were observed 5–10 min after 3.0 μg dose of melittin. In order to test the mediation of central TXA_2 and its central receptors in the cardiovascular effect of melittin, the rats were pretreated with furegrelate (500.0 μg; i.c.v.), a TXA_2 synthesis inhibitor, and SQ-29548 (8.0 μg; i.c.v.), a TXA_2 receptor antagonist, 15 min prior to melittin (3.0 μg). Furegrelate or SQ-29548 partially inhibited the pressor effect and bradycardia elicited by melittin.

In conclusion, our findings show that centrally administered melittin increases MAP and decreases HR in conscious rats. Moreover, according to our findings, central TXA_2 and its receptors may in part mediate melittin-induced cardiovascular effects.

Keywords: Brain phospholipase A_2; Melittin; Mean arterial pressure; Heart rate; Thromboxane A_2; Intracerebroventricular

1. Introduction

The phospholipase A_2 (PLA_2) form an expanding superfamily of enzymes that specifically hydrolyze the acyl ester bond at the *sn*-2 position of glycerol in membrane phospholipids to produce free fatty acids and lysophospholipids (Akhlaq and Lloyd, 2004). PLA_2 was detected in central nervous system (Molloy et al., 1998) and it was observed that it provides the precursors for many of the lipid mediators involved in normal brain function and neuroinflammatory pathophysiological processes (Akhlaq and Lloyd, 2004). Melittin is a polypeptide component of bee venom that leads to an increase in arachidonic acid release and subsequently in prostaglandin synthesis by activating PLA_2 (Hassid and Levine, 1977). Prostaglandins (PGs) have various roles on central regulation of cardiovascular system and these effects of PGs are mostly variable. Thromboxane A_2 (TXA_2), which is a biologically active PG (Narumiya et al., 1999; Wolfe, 1982) synthesized in the central nervous system (Kong et al., 1991; Sirko et al., 1989; Wolfe, 1982) has an important role on the regulation of cardiovascular and endocrine function (Armstead et al., 1988; Brooks et al., 1986; Murakami

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et al., 1998; Murakami et al., 2002; Tong et al., 1998; Yamaguchi et al., 1998; Yalcin and Savci, 2004). Previously we reported that intracerebroventricularly injected TXA_2 causes an increase in blood pressure by activating central TXA_2 receptors in normotensive (Yalcin et al., 2005a) and hypotensive rats (Yalcin and Savci, 2004) and also that central TXA_2 is involved in central regulation of blood pressure especially in hypotensive conditions (Yalcin et al., 2005b). Recently, it was reported that PLA_2 (Yokotani et al., 2000) and TXA_2 (Murakami et al., 2002) activated central sympathoadrenomedullary outflow and thus increased plasma adrenalin and noradrenalin. Furegrelate, a TXA_2 synthase inhibitor, completely abolished the increase of adrenalin but not that of noradrenalin, induced by melittin (Yokotani et al., 2000).

Considering these previous reports, the present study aimed to determine the cardiovascular effect of melittin, a PLA_2 activator, and mediation of central TXA_2 and its central receptors on the effect of melittin in normotensive conscious rats.

2. Methods

2.1. Animals

Fifty adult male Sprague Dawley rats (250–300 g) (Experimental Animals Breeding and Research Center, Uludag University, Bursa, Turkey) were used in the experiments. Rats were housed five per cage, in controlled conditions of temperature (20–24 °C), humidity (60–70%), lighting (12 h light/dark cycle) and provided with food and water ad libitum. The surgical and experimental protocols were approved by the Animal Care and Use Committee of Uludag University and were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (http://oacu.od.nih.gov/regs/guide/guide1.htm).

2.2. Surgical procedures

Under ether anesthesia, the left common carotid artery of rats was cannulated with PE 60 tubing filled with heparinised saline (100 U/ml). For intracerebroventricular (i.c.v.) injection of drugs, a burr hole was drilled through the skull 1.5 mm lateral to midline and 1.0 mm posterior to bregma. A 22-gauge stainless steel hypodermic tubing was directed through the hole towards the lateral ventricle. The cannula was lowered 4.2 mm below the surface of the skull and was fixed to the skull with acrylic cement. After surgery, the rats were placed in individual cages and allowed to recover from anesthesia for 4–5 h. During this period, the rats remained calm and without evidence of pain.

2.3. Blood pressure and heart rate recording

After the recovery period, the arterial cannula was connected to a volumetric pressure transducer (BPT 300, BIOPAC Systems Inc., CA, USA). Blood pressure and heart rate of rats were recorded and analyzed using the MP35 system and AcqKnowledge software (BIOPAC Systems Inc., CA, USA). The blood pressure is reported as mean arterial pressure (MAP) (mm Hg) and heart rate (HR) is expressed as beats/min.

2.4. Experimental protocol

In order to determine the cardiovascular effect of melittin, a PLA_2 activator, it was injected i.c.v. at doses of 1.5, 3.0 and 6.0 μg/5 μl in normotensive rats and MAP and HR changes were recorded during 1 h after treatment. In order to test the mediation of central TXA_2 and its central receptors in the effect of melittin, TXA_2 synthesis inhibitor furegrelate (500.0 μg; i.c.v.) and TXA_2 receptor antagonist SQ-29548 (8.0 μg; i.c.v.) were administrated 15 min before melittin (3.0 μg; i.c.v.) injection, and MAP and HR values were recorded. Furegrelate attenuate the increase in TXA_2 at the hypothalamus induced by hemorrhage (Yalcin et al., 2005b). I.c.v. pretreatment with SQ-29548 completely block MAP and HR responses to U-46619, a TXA_2 analog, in normotensive (Yalcin et al., 2005a) and hypotensive rats (Yalcin and Savci, 2004).

2.5. Drugs

Melittin and furegrelate were purchased from Sigma–Aldrich Co. (Deisenhofen, Germany) and were dissolved in 0.9% saline. SQ-29548 was obtained from Cayman Chemical (Ann Arbor, MI, USA) and was dissolved in saline containing 5% dimethylsulfoxide (DMSO).

2.6. Intracerebroventricular injection of drugs

I.c.v. injections were made with hand-made injection cannula (28 gauge stainless steel tubing; made by Murat Yalcin) connected to a polyethylene tubing, which was filled with saline or saline containing the desired dose of the drug of interest in a 10 μl microsyringe. The injection cannula was inserted through the guide cannula. Drugs were then infused slowly within 60 s. The volume of vehicle or drug-containing vehicle was 5 μl.

2.7. Statistics

Data are presented as mean ± SEM. Repeated-measures analysis of variance (RM-ANOVA; two way) was performed for groups. Dunnett's test was applied