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β-eudesmol, an oxygenized sesquiterpene, affects efferent adrenal sympathetic nerve activity via transient receptor potential ankyrin 1 in rats

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ABSTRACT

The autonomic nervous system innervates various peripheral tissue functions. Various external stimuli affect autonomic nerve activity, however, there is little information about the involvement of sensory receptors in the responses. The TRPA1 is a calcium-permeable non-selective cation channel which plays a crucial role in the susceptibility to various stimuli. β-Eudesmol, an oxygenated sesquiterpene found in hop essential oil and beer, activates the TRPA1. Intragastric administration of β-eudesmol decreased efferent adrenal sympathetic nerve activity (ASNA) in rats, whereas subcutaneous administration did not. ASNA suppression by β-eudesmol was not observed in TRPA1 knockout rats. The β-eudesmol derived ASNA suppression was partially, but significantly, eliminated by subdiaphragmatic vagotomy in rats, suggesting the afferent vagal nerve from the gastrointestinal tract to the brain is involved in the effect of β-eudesmol on ASNA. Our results indicate that β-eudesmol suppresses ASNA, partly through TRPA1 and the afferent vagus nerve. These findings introduce the physiological significance of the TRPA1 in the control of ASNA.

1. Introduction

Homeostasis is maintained by functions of the endocrine and autonomic nervous systems. The autonomic nervous system is comprised of the sympathetic and the parasympathetic nerves. The autonomic nerves innervate peripheral tissues and organs. Environmental stimuli, both chemical and physical, affect autonomic nerve activity, and although the effects of external stimuli have been widely reported [1], there is very little information on the sensory mechanisms involved in the alterations in autonomic nerve activities. TRPA1 is mainly expressed in sensory nerves and plays an important role in the susceptibility to various noxious stimuli, such as chemical, mechanical, and cold stimuli [2]. It is also involved in the pain sensitivity of inflammation [3], suggesting that development of a TRPA1 antagonist is important in pharmaceutical development. Many foods contain plant secondary metabolites which activate TRPA1, such as allyl isothiocyanate in horseradish [4]. Although considered to be noxious stimuli when used in excess, use of these TRPA1 activators have been empirically preferred as food ingredients. The physiological significance of TRPA1 activators in foods has not been well elucidated, therefore, we have investigated their effects on autonomic nerve activity.

β-Eudesmol, a sesquiterpenoid which accumulates in the essential oil of a particular hop cultivar, has been reported to contribute to the spicy sensation of beer [5] and activates TRPA1 [6]. Oral-administration of β-eudesmol elevated rat gastric vagal nerve activity (GVNA) via TRPA1 [7], however, the effects on other efferent autonomic nerves have not been elucidated. Previously reported efferent autonomic nerve responses have differed when different agents have been administered, suggesting that further comprehensive studies are required to understand the effect of β-eudesmol on autonomic nerve activity and the involvement of the TRPA1 on the autonomic nerve control mechanism. In this report, we investigated the effect of β-eudesmol on efferent adrenal sympathetic nerve activity (ASNA), which we used to represent sympathetic nerve activity as it reflects sympathetic adrenomedullary axis activation, which is stimulated by the stress response system [8]. TRPA1 knockout rats were utilized to confirm the involvement of the TRPA1 in the effects of β-eudesmol.

2. Experimental procedures

2.1. Materials

β-Eudesmol was purchased from Wako Pure Chemical Industries (Osaka, Japan). Thioperamide and carboxymethylcellulose were purchased from Sigma-Aldrich (MO, USA) and Calbiochem (MA, USA), respectively.

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2.2. Animal and diets

Male Wistar rats, weighing 300–350 g, were used in the experiments. TRPA1 knockout rats were generated by zinc finger nuclease technology [7,9]. To measure autonomic nerve activity, rats were housed in a room maintained at 24 ± 1 °C and illuminated for 12 h (07:00 to 19:00 h) every day. Food (type MF; Oriental Yeast Co., Tokyo) and water were freely available. Rats were adapted to the environment for at least 1 week prior to the experiment. The study was conducted in accordance with the guidelines for animal care, handling, and termination from Kirin Company (Approval Number: YO11-00063, 00094), and ANBAS Corporation (Approval Number: ANBAS_00206, 00212, 00253, 00300, 00340, 00441), which are in line with international and Japanese guidelines of animal care and welfare.

2.3. In vivo measurements of autonomic nerve activities

Briefly, under anesthesia (1 g/kg urethane, intraperitoneal (i.p.)), a polyethylene catheter was inserted into the left femoral vein for intravenous (i.v.)-injection. The depth of anesthesia was monitored by the paw pinch method, as described previously [10]. The rats were then cannulated intratracheally, and fixed in a stereotaxic apparatus while body temperature was maintained at 37.0–37.5°C using a heating pad. β-Eudesmol (5 ppb in 0.5% carboxymethylcellulose solution) was administered at 1 ml/300 g body weight. To record ASNA or afferent GVNA, rats was fixed, facing upward, in a stereotaxic apparatus; the distal end of the adrenal sympathetic nerve or afferent gastric vagal nerve was then ligated and connected to a pair of silver wire electrodes. The recording electrodes were immersed in a pool of liquid paraffin oil or a mixture of warm Vaseline and liquid paraffin oil to prevent dehydration and for electrical insulation, respectively. The rat was allowed to stabilize for 30–60 min after the nerves were placed on the recording electrodes. Electrical changes in ASNA or afferent GVNA were amplified, filtered, monitored on an oscilloscope and converted to standard pulses by a window discriminator. Counted discharge rate was sampled with a Power-Lab analog-to-digital converter, and stored on hard disk for off-line analysis. The nerve activity data were analyzed using an average firing frequency value (pulses / 5 s) every 5 min: the normalization interval to calculate the average experimental period was 5 min. For gastric ligation, between the stomach and the duodenum was ligated with a thread. For subdiaphragmatic vagotomy, the stomach and lower esophagus were exposed. The stomach was retracted and the nerve bundles of anterior and posterior vagi were dissected away from the esophagus and transected. Control rats received a sham-operation without nerve transection [11].

2.4. Measurement of adrenaline level in rat plasma

Plasma was collected from the jugular vein under anesthesia (1 g/kg urethane, intraperitoneal (i.p.)). Plasma samples were immediately frozen in liquid nitrogen and stored at −80 °C until use. Adrenaline was measured using the Adrenaline (Epinephrine) ELISA Kit (DRG International, Inc., New Jersey). In accordance with the manufacturer's instructions, EDTA blood collection tubes (Becton, Dickinson and Co., Tokyo, Japan) were used to collect plasma.

2.5. Statistical analysis

Statistical differences were analyzed by the Mann-Whitney *U* test for comparison between two groups, the Kruskal Wallis test followed by Steel-Dwass for comparison of multiple groups, and ANOVA with Bonferroni correction for comparisons of a value in the same group. P values < 0.05 were considered statistically significant

3. Results

3.1. β-Eudesmol decreased efferent adrenal sympathetic nerve activity

Changes in the ASNA were measured after intragastric administration of β-eudesmol to rats. Representative recordings of ASNA during the electrophysiological experiments are shown in Fig. 1B: there was a slight decrease in ASNA when the control vehicle solution (0.5% carboxymethylcellulose) was intragastrically administered; the activity then gradually increased. Administration of β-eudesmol (5 ppb, 16.7 ng/kg body weight) caused a gradual decrease in ASNA (Fig. 1C). ASNA was significantly decreased 40 min after β-eudesmol administration when compared with the control group. In addition, the average ASNA was also significantly decreased in the β-eudesmol group (72.0% of the control group) (Fig. 1D). Plasma adrenaline levels gradually decreased during experiment periods. Whereas significant difference between the groups was not observed, adrenaline levels were significantly lower at 60 and 120 min after β-eudesmol administration compared with before and 15 min after administration. No significant decreases in adrenaline levels were observed in control rats in the same experimental period (Fig. 1E).

To identify the mechanism of action, β-eudesmol was administered to rats after ligation of the stomach at the pylorus to prevent the flow of β-eudesmol beyond the stomach area. ASNA was suppressed after intragastric administration of β-eudesmol in pylorus-ligated rats (Fig. 2A, B), however, subcutaneous administration of β-eudesmol did not decrease ASNA (Fig. 2C, D). These results suggest that the stomach may be critical for the suppression of ASNA after β-eudesmol administration.

To confirm whether the afferent vagal nerve from the gastrointestinal tract is involved in the effect of β-eudesmol on ASNA, changes in afferent gastric vagal nerve activities (GVNA) after β-eudesmol administration were measured. Intragastric administration of β-eudesmol significantly enhanced afferent GVNA (Fig. 3), suggesting that the afferent vagal nerve from the gastrointestinal tract may be involved in the β-eudesmol-derived ASNA decrease. In order to confirm the involvement of the afferent vagal nerve, we examined the effect of subdiaphragmatic vagotomy: β-eudesmol-derived ASNA suppression was partly inhibited by subdiaphragmatic vagotomy (Fig. 3). The mean ASNA value for the sham operation-β-eudesmol group was 79.5% lower than the sham operation control group, and the mean ASNA for the vagotomy β-eudesmol group was 89.4% lower than the vagotomy control group (Fig. 3).

Thioperamide, an inhibitor of the histamine H_3 receptor, suppressed the action of scent stimulation on ASNA (13), therefore, we investigated the effect of intravenous administration of thioperamide on the suppressive action of β-eudesmol. As seen in Fig. 4, thioperamide completely eliminated the suppressive action of β-eudesmol.

3.2. TRPA1 involvement in the suppressive action of β-eudesmol on ASNA

Our previous study showed that β-eudesmol activated TRPA1 [6], therefore we investigated whether the TRPA1 was involved in the suppressive action of β-eudesmol. To confirm the involvement of TRPA1, we examined the effect of β-eudesmol on ASNA in TRPA1 knockout rats: β-eudesmol suppression of ASNA was not observed in TRPA1 knockout rats (Fig. 5), suggesting that the TRPA1 is involved in the suppressive action of β-eudesmol on ASNA.

4. Discussion

Our findings suggest that, in rats, β-eudesmol has a suppressive effect on ASNA and the critical site for the suppressive action is the stomach; the vagal afferent nerve, histaminergic H_3 receptor and TRPA1 are also implicated in the mechanism of the suppressive action of β-eudesmol.