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G-protein coupled estrogen receptor-mediated non-genomic facilitatory effect of estrogen on cooling-induced reduction of skin blood flow in mice

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ABSTRACT

An enhanced vasoconstrictor activity of cutaneous arteries participates in the reduction of skin blood flow induced by cooling stimulation. Raynaud's phenomenon, which is characterized by intense cooling-induced constriction of cutaneous arteries, is more common in women during the period from menarche to menopause. We thus investigated the effect of 17β-estradiol (E2) on cooling-induced reduction of plantar skin blood flow (PSBF) in mouse in vivo. Ovariectomized female ddY mice, anaesthetized with pentobarbital, were treated with tetrodotoxin for eliminating the sympathetic nerve tone and artificially ventilated. The PSBF was measured by laser Doppler flowmetry. Cooling air temperature around the foot from 25 to 20, 15, or 10 °C decreased the PSBF in a temperature-dependent manner, which was suppressed by the specific α_{2C}-adrenoceptor antagonist MK-912. When E2 was intravenously administered as a bolus followed by a constant infusion for 10 min just before the cooling stimulation, the cooling-induced reduction of PSBF was facilitated by E2 in a dose-dependent manner. The facilitatory effect of E2 was not induced after the treatment with MK-912. Similar facilitatory effect was induced by an intravenous application of G-1, an agonist of G protein-coupled estrogen receptor (GPER, also termed GPR30). Moreover, the facilitatory effect of E2 was abolished by the GPER antagonist G15. These results suggest that acute administration of E2 leads to the facilitation of cooling-induced, α_{2C}-adrenoceptor-mediated reduction of skin blood flow via the activation of the non-genomic estrogen receptor GPER.

1. Introduction

Cutaneous vasoconstriction in response to cooling is a physiological response to protect body from heat loss, being mediated not only by a reflex increase in sympathetic tone, but also by a locally enhanced cutaneous vasoconstriction (Vanhoutte, 1980). The latter local response is suggested to involve the augmentation of α_2-adrenoceptor reactivity during cooling in in vitro (Flavahan et al., 1985, Harker et al., 1990 and Vanhoutte et al., 1985) and in vivo studies (Ekenvall et al., 1988 and Freedman et al., 1992). Especially, the translocation of α_{2C}-adrenoceptors from the Golgi compartment to the plasma membrane during cooling is likely to be essential for the response (Chotani et al., 2000; Bailey et al., 2004 and Jeyaraj et al., 2001).

Raynaud's disease, which is characterized by episodic vasospasm of the fingers and toes to cold exposure, is more common in women during the period from menarche to menopause (Block and Sequeira, 2001). α_2-Adrenoceptors are likely involved in the disease, since the vasospasm is prevented by α_2-adrenoceptor antagonists and the mRNA level for α_{2C}-acrenoceptors is higher in cutaneous arteries of female than male (McNeill et al., 1999). In isolated male mouse tail arteries pre-incubated with 17β-estradiol (E2) for 24 h, increased expression of α_{2C}-adrenoceptors and enhanced constrictor response to the α_2-adrenoceptor agonist UK-14304 at 28 °C, but not at 37 °C, have been shown (Eid et al., 2007). Estrogen is, thus, suggested to be involved in the increased activity of cold-induced, α_{2C}-adrenoceptor-mediated vasoconstriction via its genomic action.

In addition to genomic action, growing evidence also supports non-genomic action of estrogen. The genomic action involves two estrogen receptors, ERα and ERβ, which function as transcription factors (Nilsson et al., 2001). In contrast, the non-genomic action occurs much faster than the genomic action through, at least in part, membrane-associated ERα and its truncated isoforms (Nilsson et al., 2001). In addition, several estrogenic responses have been shown to be mediated through G protein-coupled estrogen receptor (GPER, also termed GPR30) (Nilsson et al., 2011). The GPER agonist G-1 causes vascular relaxation (Broughton et al., 2010; Haas et al., 2009; Lindsey et al., 2009; Meyer et al., 2010; Yu et al., 2014), which is suggested to involve the release of nitric oxide from endothelial cells (Broughton et al., 2010; Lindsey et al., 2014) and the activation of adenylyl cyclase in vascular smooth muscle cells (Lindsey et al., 2014; Yu et al., 2014).

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In addition, G-1 reduces serum-stimulated proliferation of vascular smooth muscle cells (Haas et al., 2009) and induces apoptosis of vascular smooth muscle cells via activation of phosphoinositide 3-kinase and inhibition of adenylyl cyclase (Ding et al., 2009). Thus, GPER could couple to both G_s and $G_{i/o}$ in vascular smooth muscle cells, which may depend on the site, type, or condition of blood vessels.

The present study aimed to elucidate whether the non-genomic action of estrogen contributes to cold-induced skin vasoconstriction in vivo. We explored acute effects of estrogen on the reduction of plantar skin blood flow (PSBF) induced by local cooling in ovariectomized female mice.

2. Materials and methods

2.1. Animals

Female ddy mice (SLC, Hamamatsu, Japan) were housed in a 12 h light-dark cycle, with food and water available ad libitum, and treated as approved by the Institutional Animal Care and Use Committee and according to the Guidelines for Animal Experiments established by the Japanese Pharmacological Society.

Female mice were ovariectomized at 4 week-old and experimented at 8–12 week-old to minimize the influence of endogenous estrogen. The bilateral removal of ovaries was achieved via a dorsal approach through two small lateral skin incisions under the anesthesia with intraperitoneal (i.p.) administration of pentobarbital sodium (50 mg/kg). The ovaries were pulled out through the incision and severed with scissors. After each excision surgery, incisions were appropriately sutured. We confirmed that increased body weight and marked atrophy of uterus were induced in ovariectomized female mice compared with sham-operated mice as has been reported earlier (Shimomura et al., 2002).

2.2. Experimental procedures

The neuronal reflex through the sympathetic efferent is well developed in cutaneous circulation (Johnson et al., 1986), which makes it difficult to obtain stable measurement of skin blood flow in vivo. For the stable measurement of skin blood flow, we used the treatment with tetrodotoxin, a voltage-dependent Na^+ channel blocker, which eliminates the sympathetic nerve tone (Koganezawa et al., 2006; Honda et al., 2007; Sahara et al., 2013). The PSBF in tetrodotoxin-treated mice was measured as described previously (Honda et al., 2007). Briefly, the ovariectomized mice were anesthetized with intraperitoneal (i.p.) administration of pentobarbital sodium (75 mg/kg), and two polyethylene tubes were inserted in the right femoral vein for drug administration and in the right carotid artery for measurement of mean arterial blood pressure and heart rate. After intravenous (i.v.) administration of tetrodotoxin (30 μg/kg), the mice were mechanically ventilated with air using a rodent ventilator (SN-480-7; Shinano, Tokyo, Japan) at a stroke volume of 0.2 ml per 10 g body weight and a rate of 85 strokes per min. A laser Doppler flow probe (NS type; Omega Wave, Tokyo, Japan) was then set to the position about 5-mm apart from the center of the plantar surface of the left foot to measure PSBF with a non-contact laser Doppler flow meter (ALF 2100; Advance, Tokyo, Japan). The right foot served as the control. The blood flow was expressed as arbitrary perfusion units (PU). The skin temperature of the plantar surface was measured using a thermosensor (AW-601H, Nihon Kohden, Tokyo, Japan). Data were stored and analyzed on a Macintosh computer with an AD converter (Lab Stack; Keisoku Giken, Tokyo, Japan).

The cooling apparatus for the mouse foot was constructed in our laboratory as described previously (Honda et al., 2007; Sahara et al., 2013). A rubber tube (a 25 ml plastic syringe) was coiled around the apparatus and water was perfused in the tube by a roller pump (PA-12; Cole Parner Instrument, Chicago, IL, U.S.A.). The temperature in the apparatus was continuously monitored with a thermosensor (SXB-54; Techno-Seven, Yokohama, Japan), and regulated by changing the temperature of the perfusing water. The left foot was placed in the apparatus to apply local cooling. The temperature and humidity of the laboratory were maintained at 24 ± 2 °C and 55 ± 10%, respectively.

2.3. Drugs

The following drugs were used: tetrodotoxin and clonidine hydrochloride (Wako, Osaka, Japan); 17β-estradiol (E2) and MK-912 ((2S-trans)−1,3,4,5′,6,6′,7,12b-Octahydro-1′,3′-dimethyl-spiro(2H-benzofuro[2,3-*a*]quinolizine-2,4′(1′H)-pyrimidin)−2′(3′H)-one hydrochloride; Sigma-Aldrich, St Louis, MO, USA); G-1 (Calbiochem, Darmstadt, Germany); and G15 (Cayman Chemical, Ann Arbor, MI, USA). Tetrodotoxin was dissolved in distilled water. E2, G-1, and G15 were dissolved in dimethyl sulfoxide (DMSO; Wako Pure Chemical Industries, Osaka, Japan) and then diluted with saline (final DMSO concentration < 2%). The other drugs were dissolved in saline. E2 was administered as an intravenous bolus dose (0.33 ml/kg) followed by a continuous infusion at a rate of 0.01 ml/min. The other drugs were intravenously administered as a bolus injection of 0.01 ml per 10 g body weight. G-1 and G15 was administered 3 and 15 min before the cooling stimulation, respectively. The appropriate vehicle controls showed no apparent effect.

2.4. Statistical analysis

All data are expressed as mean ± S.E.M and *n* shows the number of animals. The statistical significance was evaluated by use of Student's paired *t*-test or William's multiple comparison test. P values less than 0.05 were considered significant.

3. Results

3.1. Cooling-induced reduction of PSBF

Fig. 1A shows the changes in the heart rate, mean arterial blood pressure, and PSBF induced by local cooling of the left foot in ovariectomized female mice. When the air temperature in the apparatus was lowered from 25 to 10 °C, the PSBF of the left foot decreased and reached a plateau around after 10 min. In contrast, the heart rate or mean arterial blood pressure, or the PSBF of the right foot did not change during the cooling. When the temperature in the apparatus was returned to 25 °C, the PSBF of the left foot recovered to the basal level within 15 min. Although the cooling-induced reduction of PSBF showed temperature-dependency, the amplitude of the responses was highly variable between individuals (Fig. 1B). In the present study, therefore, the cooling condition to either 10 or 15 °C was chosen in each experiment to obtain comparable control responses.

Our previous study showed that cooling-induced reduction of PSBF primarily results from increased reactivity of α_{2C}-adrenoceptors in male mice (Honda et al., 2007). We thus first investigated the involvement of α_{2C}-adrenoceptors in the cooling-induced response in ovariectomized female mice. The specific α_{2C}-adrenoceptor antagonist MK-912 (30 μg/kg, i.v.) per se had no effect on the mean arterial blood pressure, but caused small, but significant, increases in the heart rate and PSBF (Table 1). MK-912 (30 μg/kg, i.v.) significantly suppressed the cooling-induced reduction of PSBF (Fig. 2). We previously confirmed that this dose of MK-912 had no effect on the pressor response to phenylephrine (5 μg/kg, i.v.), an α_1-adrenoceptor agonist, or clonidine (3 μg/kg, i.v.), an α_2-adrenoceptor agonist, in mice (Honda et al., 2007)..

3.2. Effect of estrogen on the cooling-induced response

The effect of exogenous E2 on the cooling-induced reduction of