Short Communication

Forced Exercise-Induced Flushing of Tail Skin in Ovariectomized Mice, as a New Experimental Model of Menopausal Hot Flushes

Hideki Shuto¹, Atsushi Yamauchi¹, Munehiko Ikeda¹, Yoshio Sohda¹, Ayako Koga¹, Kohji Tominaga¹, Takashi Egawa¹, and Yasufumi Kataoka¹,*

¹Department of Pharmaceutical Care and Health Sciences, Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan

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Abstract. Hot flushes are the most common complaint of menopausal women. In the present study, a new animal model of hot flushes was established. Tail skin temperature was measured with a thermo tracer after mice were subjected to a forced exercise task using a motor driven treadmill. In ovariectomized mice, forced exercise for 10 min was most effective in increasing tail skin temperature over that of sham-operated mice. This elevation was blocked by estradiol replacement (1 mg/kg per week for 3 weeks), suggesting that our model simulates menopausal hot flushes.

Keywords: ovariectomy, forced exercise, menopausal flushing

The menopausal hot flush is a bothersome symptom occurring in more than 75% of climacteric women (1). Manifesting as a transient increase in skin temperature and sweating hinders daily activity. Hot flushes have been linked to a transient disruption of the thermoregulatory mechanism that activates a heat-loss response including increased peripheral blood flow (2). Because hot flushes vary in duration, frequency, intensity, and duration of an individual flush, quantitative assessment of the disorder can be difficult (2). Flushing of the tail skin in ovariectomized (OVX) animals is known to be a good parameter of menopausal hot flushes, although the spontaneous appearance of flushing is irregular. Experimental manipulations including treatment with drugs trigger flushing in OVX-animals (3, 4). After morphine withdrawal with naloxone, a marked rise in tail skin temperature and an increase in heart rate appeared in OVX-rats (5). We previously demonstrated that nifedipine elevated tail skin temperature and that nifedipine-induced flushing was aggravated in mice with ovariectomy (6). This aggravation was blocked by estradiol replacement (6). In addition to drug-induced flushing, Rogers and Sheriff recently demonstrated that ovariectomy decreased hindlimb vascular conductance during graded mild-intensity treadmill locomotion in rats, this vascular modulation being reversed by estrogen replacement (7). Estrogen deficiency produces an abnormality of vascular tonus and/or insufficient autoregulation of the local vasculature. Therefore, we hypothesized that OVX-animals do not readily recover from forced exercise-increased peripheral vascular conductance. In the present study, we investigated changes in tail skin temperature before and after forced exercise on a motor-driven treadmill in OVX-mice as an experimental model of menopausal flushing.

Female ICR mice weighing 25–30 g were used (KyuDo Co., Ltd., Kumamoto). The mice were maintained on a 12-h light/dark schedule (lights on 7:00 a.m.) at a temperature of 24 ± 1°C with free access to food and water. All the procedures involving experimental animals adhered to the law (No. 105) and notification (No. 6) of the Japanese Government and were approved by the Laboratory Animal Care and Use Committee of Fukuoka University.

Mice underwent a bilateral ovariectomy or sham-operation under sodium pentobarbital anesthesia (50 mg/kg, i.p.) (6). Vehicle (sesame oil) and estradiol valerate (1.0 mg/kg) (Pelanin Depot; Mochida Pharmaceutical, Tokyo) were injected into the thigh muscle in a volume of 0.1 ml/100 g body weight once a week for 3 weeks starting 7 days after the operation. Twenty-eight days post-surgery, mice were subjected to the following
experiment in a room maintained at a temperature of 25 ± 1°C. The body weight in sham-mice, OVX-mice, and estradiol-treated OVX-mice were 31.0 ± 0.2, 34.0 ± 0.4, and 33.8 ± 0.2 g, respectively.

Tail skin temperature was measured according to a procedure described previously (6). Mice were restrained in a holder in a conscious state and the tail skin temperature was measured at the dorsal surface of the tail about 1 cm from its base with a thermo tracer (TH5108ME; NEC San-ei, Tokyo) for 15 min. The data were stored in 1-min blocks and analyzed with the Thermal Image processing program (TH51-701, NEC San-ei) and Remote Control program (TH51-723, NEC San-ei). Two hours after the basal tail skin temperature was measured, mice were forced to run (15 m/min) on a motor driven treadmill (MK-680S; Muromachi Kikai, Tokyo) for a period of 5, 10, or 20 min and the running time was measured. After termination of the forced exercise, tail skin temperature was measured for 15 min. Changes in tail skin temperature were assessed using $\Delta TST$. $\Delta TST = (\text{tail skin temperature in each 1-min block after the forced exercise}) - (\text{average basal tail skin temperature for the period from 1 to 6 min}).$

Values are expressed as the means ± S.E.M. Statistical analysis was performed using the two-way analysis of variance (ANOVA) followed by the Tukey-Kramer test. A value of $P<0.05$ was considered to be statistically significant. The intraobserver or interobserver variation was <5% in each experiment.

As shown in Fig. 1A, OVX-mice subjected to the forced exercise (10 min) showed rapid and marked increases in tail skin temperature, with a return to the basal level within 7 – 8 min. Meanwhile, sham-operated (sham) mice showed only slight increases in the early stage after forced exercise. Based on these time-courses of $\Delta TST$ in sham- and OVX-mice (Fig. 1A), we evaluated the effect of forced exercise on tail skin temperature by accumulating $\Delta TST$ for the period from 1 to 6 min after the forced exercise (total $\Delta TST$). Forced exercise for 10 and 20 min produced a marked increase in the total $\Delta TST$ of OVX-mice compared to the sham-mice (Fig. 1B). The total $\Delta TST$ of sham-mice increased with the amount of time the animals were forced to exercise. The difference in total $\Delta TST$ between OVX- and sham-mice was the greatest (9.73 ± 1.06°C) after a 10-min period of forced exercise. The total running time of OVX-mice in each period was the same as that of sham-mice (inset of Fig. 1B). When OVX-mice were treated with estradiol valerate (1.0 mg/kg) once a week for 3 weeks, forced exercise-induced increases in total $\Delta TST$ were markedly lowered by 81.3 ± 11.9% (Fig. 2A). In sham-mice, similar estradiol treatment did not influence the total $\Delta TST$ and the running time after and during the forced exercise (10 or 20 min), respectively (data not shown). Representative thermograms show flushing of the tail skin in OVX-mice and estradiol-treated OVX-mice at 2 min after a 10-min period of forced exercise (Fig. 2B). There were no differences in running time between OVX-mice and estradiol-treated OVX-mice (inset of Fig. 2A).

In the present study, OVX-mice but not sham-mice showed a marked elevation in tail skin temperature at the early stage (1 to 6 min) after treadmill locomotion (15 m/min) for 10 min and this elevation was reversed by estrogen replacement. We propose that forced
exercise-induced flushing of tail skin in OVX-mice is a conventional and useful experimental model of menopausal flushing. Changes in hindlimb vascular conductance during treadmill locomotion in OVX-rats were suggested to be positively associated with the production of endothelial nitric oxide (NO) (7). Chronic exercise increased the gene expression of endothelial NO synthase in canine aortic endothelial cells (8). Delp and Laughlin reported that levels of endothelial NO synthase protein in the aortas of rats increased after exercise training (9). Li et al. reported that basal NO generation is important in control of the cutaneous thermoregulatory microcirculation by ameliorating the arteriovenous anastomosis tone (10). These evidences together with the present findings suggest that the flushing of tail skin in OVX-rats is attributable to an insufficient vascular recovery from the vasodilatory response to forced exercise. Our previous findings demonstrated that ovariectomy significantly elevated the rise in tail skin temperature induced by nifedipine at a dose having no influence on blood pressure and that this event was blocked by estradiol replacement (6). Since estrogen promotes vascular relaxation and inhibits vascular contraction, the net result is a decrease in vascular resistance (11). It is, therefore, likely that estrogen deficiency leads to an abnormality of vascular tonus and/or insufficient autoregulation of the local vasculature. The protective effect of estrogen replacement in the present study may be related to an improvement of the abnormal local vascular tonus and autoregulation. On the other hand, calcitonin gene-related peptide (CGRP), a vasodilator neuropeptide is known to participate in the occurrence of menopausal hot flushes (12, 13). Recently, Noguchi et al. reported that ovariectomy not only potentiated CGRP-induced elevation of skin temperature and arterial vasorelaxation but also induced a lower concentration of endogenous CGRP in plasma and up-regulation of arterial CGRP receptors and that 17β-estradiol inhibited the CGRP-related responses in OVX-rats (14). Further studies are needed to clarify the CGRP-related mechanism in the present experimental model, forced exercise-induced flushing of tail skin in OVX-mice. Among sham-mice, OVX-mice, and estrogen-treated OVX-mice, there were no differences in the total running time during forced exercise (5, 10, and 20 min). This is consistent with the finding that changes in estrogen levels did not affect motor activity in rats (15). Therefore, the possibility that differences in forced exercise-induced flushing of the tail skin are due to changes in treadmill locomotion after estrogen withdrawal and/or estrogen replacement could be excluded. In OVX-mice, flushing is detectable in the tail skin (3, 4), although experimental interventions are required for the induction of flushing. The present study demonstrated that forced exercise-induced flushing of tail skin in OVX-mice is a potentially useful experimental model of menopausal flushing.

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**References**