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Ovariectomy aggravates nifedipine-induced flushing of tail skin in mice

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Abstract

Flushing is one of the most common vasodilation-related adverse effects associated with Ca^{2+} channel antagonist treatment. This symptom is known to occur more frequently in women than men. The present study aimed at investigating the effect of ovariectomy on nifedipine-induced flushing in mice. Ovariectomy markedly increased the tail skin temperature, a parameter of skin flushing, induced by nifedipine at a dose showing no influence on blood pressure. This event was blocked by estradiol replacement. Estrogen withdrawal is, therefore, included in the risk factors for nifedipine-induced flushing and this risk is lessened by estrogen replacement.

Keywords: Ca^{2+} channel antagonist; Nifedipine; Ovariectomy; Flushing; Menopause; Tail skin temperature

1. Introduction

The menopausal hot flush is one of the most bothersome symptoms occurring in more than 75% of climacteric women (Dennerstein, 1996; Freedman, 2001). This symptom manifests itself as a transient increase in skin temperature and sweating and sometimes hinders the activity of daily life. Hot flushes have been linked to a transient disturbance of the thermoregulatory mechanism that activates a heat-loss response including increased peripheral blood flow (Stearns et al., 2002).

Dihydropyridine Ca^{2+} channel antagonists are widely used to lower blood pressure in hypertension by vasodilating the peripheral vasculature. However, the usefulness of dihydropyridines such as nifedipine is limited by the occurrence of adverse effects often due to a loss of vascular control in the periphery. These effects include headache, flushing, dizziness and palpitations, and usually occur within the first 14 days of treatment. They are dose-related and associated with a rapid rise in drug plasma concentration (Kleinbloesem et al., 1987). The incidence of adverse effects is lower with slow-release formulations such as nifedipine retard. However, the adverse effects still cause early withdrawal from therapy in a significant number of patients (Morley, 1989).

Flushing associated with Ca^{2+} channel antagonist treatment in women is sometimes similar to menopausal hot flushes (Stearns et al., 2002). Moreover, earlier studies showed that the incidence of flushing induced by Ca^{2+} channel antagonists is high in middle-aged women (Kubota et al., 1995; Kim et al., 2000). These observations suggest that the menopause may be a risk factor for Ca^{2+} channel antagonist-induced flushing. We previously presented evidence suggesting that climacterium is included in the risk factors for contrast medium-induced pulmonary reactions and immunosuppressant-induced neurotoxicity (Tominaga et al., 2001a,b, 2002). Recently, Kobayashi et al. (2000) demonstrated that elevation of the tail skin temperature is a good parameter of skin flushing in rats. To test whether Ca^{2+} channel antagonist-induced flushing is exaggerated in the menopause, we investigated the effect of nifedipine on tail skin temperature in ovariectomized mice, as a menopause/climacterium model.

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2. Materials and methods

2.1. Animals

Female ICR mice weighing 25–30 g were purchased from Kyudo (Kumamoto, Japan). 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.

2.2. Drugs

Nifedipine and estradiol valerate (Pelanin Depot, 10 mg/ml/ampule) were purchased from Wako (Osaka, Japan) and Mochida Pharmaceutical (Tokyo, Japan), respectively. Nifedipine was first dissolved in ethanol and diluted with saline immediately before use. The vehicle solution for nifedipine consisted of ethanol and saline.

2.3. Ovariectomy and estradiol treatment

Mice underwent bilateral ovariectomy or sham operation under sodium pentobarbital anesthesia (50 mg/kg, i.p.). Seven days after the operation, the ovariectomized mice were randomly divided into two groups; ovariectomized group receiving vehicle (sesame oil) and estradiol/ovariectomized group receiving estradiol valerate (1.0 mg/kg). Vehicle and estradiol 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. Sham-operated mice were treated with the vehicle from 7 days after the sham operation. Twenty-eight days after the operation, the animals were used for the following experiments.

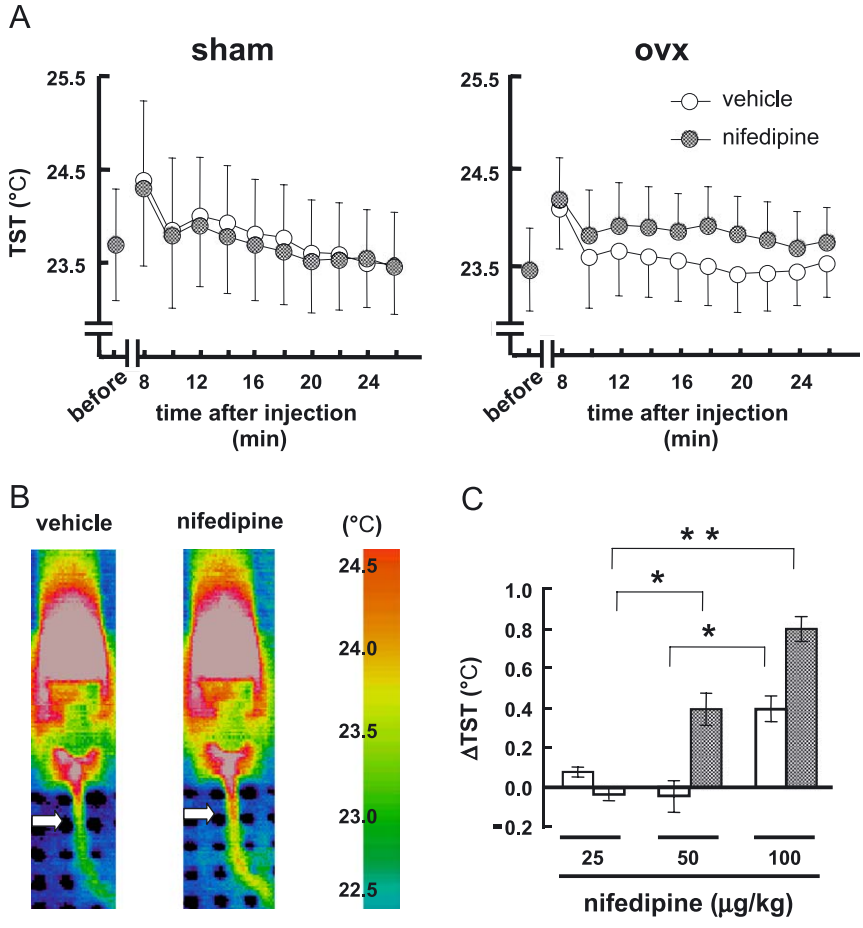

Fig. 1. Effect of treatment with nifedipine on the tail skin temperature of sham-operated (sham) and ovariectomized (ovx) mice. (A) Time course of tail skin temperature (TST) after injection of the vehicle and nifedipine (50 μg/kg) in sham (left) and ovx mice (right). Values are the means ± S.E.M. for 11 to 16 animals. (B) Representative thermograms showing flushing induced by nifedipine (50 μg/kg) in ovx mice. Thermograms were taken 15 min after the injection of the vehicle (left) and 50 μg/kg nifedipine (right). (C) Dose-dependent effect of nifedipine on ΔTST of sham and ovx mice. ΔTST was calculated as follows, ΔTST=(the average TST from 10 min to 25 min after injection of nifedipine) − (the basal TST during the same period after injection of the vehicle). Values are the means ± S.E.M. for 7 to 10 animals. * and ** denote $P<0.05$ and $P<0.01$, respectively.

2.4. Effect of nifedipine on the tail skin temperature in mice

Mice were restrained in a holder in a conscious state. After a 10-min period of adaptation, vehicle (0.1 ml/100 g body weight) was administered i.p. to mice 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, Japan) for 30 min. Subsequently, nifedipine (25–100 μg/kg) was administered i.p. and the tail skin temperature was measured for the subsequent 30 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). Throughout the recording period, the room temperature was maintained at 24 ± 1 °C. Blood pressure in conscious mice was measured by the tail-cuff method (Model MK-2000, Muromachi Kikai, Tokyo, Japan) every 5 min during the experiments.

2.5. Statistical analysis

Statistical analysis was performed using the unpaired Student's *t*-test or one-factor analysis of variance (ANOVA) followed by Scheffe's *F*-test. A value of $P < 0.05$ was considered significant. The intraobserver or interobserver variation was < 5% in each experiment.

3. Results

3.1. Effect of nifedipine on tail skin temperature in sham-operated and ovariectomized mice

As shown in Fig. 1A, there were no differences in tail skin temperature before each injection between sham-operated and ovariectomized mice (23.7 ± 0.60 vs. 23.4 ± 0.44 °C, respectively). In sham-operated mice receiving a vehicle injection, the procedure of injection and restraint in the holder induced a transient increase in tail skin temperature. This increased tail skin temperature returned to its basal level within 10 min, and remained unchanged from 10 to 25 min after vehicle injection (Fig. 1A). The basal tail skin temperature was obtained by calculating the mean tail skin temperature in the period between 10 and 25 min after injection of vehicle in each mouse. Nifedipine at 50 μg/kg significantly elevated tail skin temperature in ovariectomized mice but not in sham-operated mice (Fig. 1A). Fig. 1B shows the representative thermograms of flushing taken 15 min after injection of vehicle and 50 μg/kg nifedipine in ovariectomized mice.

The dose-dependent effect of nifedipine on tail skin temperature was determined by using ΔTST. This index was calculated as follows: ΔTST=(the average tail skin temperature in the period between 10 and 25 min after injection of nifedipine) − (the basal tail skin temperature during the same period after injection of the vehicle). In sham-operated mice, ΔTST did not change after the administration of 25 or 50 μg/kg nifedipine, and increased significantly after the administration of 100 μg/kg nifedipine (Fig. 1C). In contrast, in ovariectomized mice, the administration of nifedipine (25, 50, and 100 μg/kg) induced an increase in ΔTST in a dose-dependent manner (Fig. 1C). In both sham-operated and ovariectomized mice, vehicle and nifedipine had no effect on blood pressure at any time in the period lasting 10 to 25 min after injection. The values were as follows: sham-operated mice (121.8 ± 4.1, 127.8 ± 3.6, and 121.8 ± 3.4 mmHg for vehicle, 50 μg/kg nifedipine and 100 μg/kg nifedipine, respectively), ovariectomized mice (115.2 ± 5.3, 117.7 ± 6.3, and 113.7 ± 6.0 mm Hg for vehicle, 50 μg/kg nifedipine and 100 μg/kg nifedipine, respectively).

3.2. Effect of estradiol replacement on nifedipine-increased ΔTST

Nifedipine at 50 μg/kg significantly increased ΔTST to 0.392 ± 0.092 °C in ovariectomized mice compared with sham-operated mice (− 0.047 ± 0.080 °C) (Fig. 2A). How-

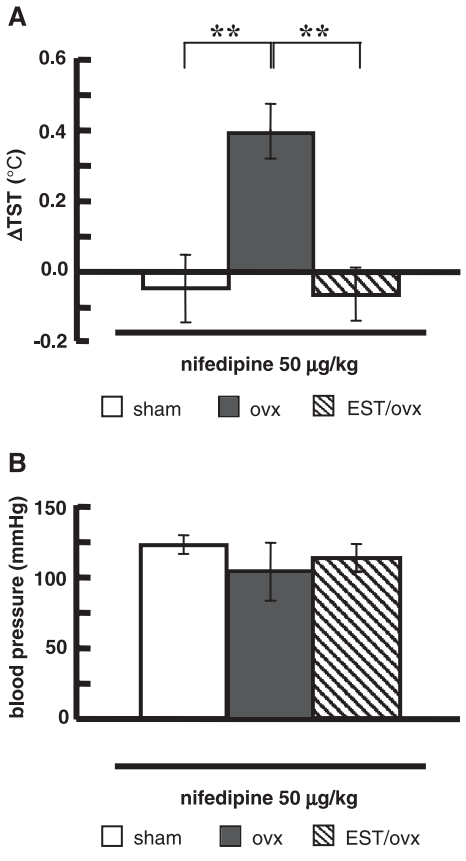

Fig. 2. Tail skin temperature change (ΔTST, A) and blood pressure (B) induced by nifedipine (50 μg/kg) in sham (open bar), ovx (gray bar) mice, and ovx mice treated with estradiol (1 mg/kg) once a week for 3 weeks (EST/ovx, hatched bar). Values represent the means ± S.E.M. for 9 to 16 animals. $^{**}P < 0.01$, significant difference between nifedipine treatment in ovx mice and that in sham mice.

ever, this effect of nifedipine was completely reversed by estradiol treatment (-0.066 ± 0.073 °C). There was no significant difference in blood pressure among the three groups (Fig. 2B).

4. Discussion

In the present study, 100 μg/kg nifedipine significantly elevated tail skin temperature in sham-operated mice, suggesting that changes in tail skin temperature are attributable to an elevation of local blood flow induced by nifedipine. This notion is supported by evidence that nifedipine-induced flushing is related to a direct effect of nifedipine on the local vasculature but not to systemic effects such as fluid overload (Dougall and McLay, 1996). Nifedipine at 50 μg/kg elevated tail skin temperature in ovariectomized mice but not in sham-operated mice and the elevation of tail skin temperature induced by 100 μg/kg nifedipine in ovariectomized mice was much higher than that in sham-operated mice. Estrogen replacement almost reversed the aggravation of nifedipine-elevated tail skin temperature due to ovariectomy. It is noteworthy that the effects of nifedipine at the doses used in the present study on systemic blood pressure were not affected by ovariectomy. These observations indicate that estrogen deficiency accelerates the increased local blood flow due to vasodilator effects of nifedipine on the local, rather than systemic, vasculature. Since estrogen promotes vascular relaxation and inhibits vascular contraction, the net result decreases vascular resistance (Austin, 2000). These effects may contribute to the reduced incidence of cardiovascular diseases in pre- and post-menopausal women receiving estrogen replacement therapy (Austin, 2000). It is, therefore, likely that estrogen deficiency leads to an abnormality of vascular tonus and/or insufficient autoregulation of the local vasculature. This pathophysiology may be responsible for the acceleration of nifedipine-increased local blood flow in ovariectomized mice.

A prospective study in Korea showed that Ca^{2+} channel antagonist-induced flushing was more frequent in women (Kim et al., 2000). Prescription-event monitoring of the four Ca^{2+} channel antagonists (diltiazem, nicardipine, isradipine, and amlodipine) showed that flushing, headache and dizziness were much more frequent in female than male patients and that 85% of patients who complained of flushing in the first 6 months were female. Furthermore, the incidence of flushing was high in the middle-aged patients (Kubota et al., 1995). It is thus suggested that Ca^{2+} channel antagonist-induced flushing tends to be aggravated in menopausal women. However, the efficacy of nifedipine at lowering the blood pressure is not influenced by estrogen withdrawal. This aspect is strongly supported by the present experimental evidence. As nifedipine-induced flushing may occur in men, it is suggested that factors other than the menopause are also important.

In light of these findings, the possibility that estrogen lack is included in the risk factors for vasodilation-related flushing induced by Ca^{2+} channel antagonists should be considered.

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