Influences of Ovariectomy and Continuous Replacement of 17β-Estradiol on the Tail Skin Temperature and Behavior in the Forced Swimming Test in Rats

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ABSTRACT The effect of ovariectomy and continuous subcutaneous replacement of 17β-estradiol was examined in female Wistar rats. Tail skin temperature significantly increased in ovariectomized rats 6 days after ovariectomy, and the elevated level was sustained until 21 days after ovariectomy. 17β-Estradiol at doses of 0.3 and 1.0 µg/body/day suppressed the increases in tail skin temperature. In the forced swimming test, the ovariectomized control rats showed significantly prolonged immobility time in comparison with sham-ovariectomized rats 14 days after ovariectomy. The duration of immobility of ovariectomized rats treated with 17β-estradiol (0.3, 1.0, 3.0 µg/body/day) or maprotiline (0.6 mg/body/day) was significantly shorter than that of ovariectomized control rats.

Keywords: Ovariectomy, Tail skin temperature, Forced swimming test

Female Wistar rats (Charles River Japan, Tokyo), 9–10 weeks of age, body weight of 183–261 g at the beginning of the study, were used. Animals were housed (5–6 rats per group) in an air-conditioned room at 22.5±2.5°C with 50±10% humidity and given normal pellet food (CE-2; Nihon Clea, Tokyo) and tap water ad libitum. 17β-Estradiol and maprotiline hydrochloride (Ciba-Geigy, Basle, Switzerland) were dissolved in propylene glycol (Wako, Osaka) and administered continuously to the animals by subcutaneously implanted Alzet osmotic mini-pumps (model 2002, lot. 041302; Alza, Palo Alto, CA, USA). Rats were bilaterally ovariectomized under pentobarbital anesthesia in the investigation of tail skin temperature and the forced swimming test. In the investigation of tail skin temperature, osmotic mini-pumps containing 17β-estradiol (0.3, 1.0 µg/body/day) were implanted subcutaneously 7 days after ovariectomy. In examining the forced swimming test, osmotic mini-pumps containing 17β-estradiol (0.3, 1.0, 3.0, 10.0 µg /body/day) or maprotiline (0.6 mg/body/day) were implanted subcutaneously immediately after ovariectomy. Pumps containing only propylene glycol were given to ovariectomized control group and sham-operated rats.

Postmenopausal women often suffer from symptoms called postmenopausal syndrome. These symptoms consist of hot flush, a sudden hot feeling in the upper body, and mental symptoms such as depression, irritation and insomnia (1, 2). In humans, estrogen treatment is thought to be effective in alleviating hot flush (1–3) and has reported to have a positive effect in reversing depression (4). Mechanisms for the symptoms of postmenopausal syndrome have not yet been clarified. However, those symptoms are known to be related to the decrease of the serum 17β-estradiol (E2) level (2, 3). It was recently reported that ovariectomy transiently increases the tail skin temperature in rats (5). Since hot flushes are associated with an acute rise in skin temperature (6, 7), the increase of tail skin temperature in rats might represent hot flush in humans. In addition, using the tail suspension test, one of the models of depression, in ovariectomized mice, it was shown that E2 could significantly reverse the effect of ovariectomy (8). In this study, we examined the effect of E2 on the correlate of hot flush in humans, the increase in tail skin temperature following ovariectomy, and the antidepressant-like action of E2 using the forced swimming test.

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Estrous cycles and body weight of animals were examined every morning for 14 or 21 days after ovariectomy. Tail skin temperature was measured by a small digital thermometer (D616; Techno Seven, Tokyo) at 3, 6, 10, 14, 21 days after ovariectomy (at 13:00 each day). The thermometer was attached to the surface of the tail 2 cm from the hip of the animal with surgical tapes. Rats could freely move around during the measurement of tail skin temperature. The forced swimming test was performed according to the method reported by Porsolt et al. (9). The 13 day post-ovariectomy rats were placed individually in plastic cylinders (height: 40 cm; diameter: 18 cm; Sample, Tokyo (10)) containing 18 cm water at 25 ± 1°C; and 15 min later, they were removed to a 30°C drying room for 15 min. The next day the rats were placed in the cylinders again and immobility time was measured for 5 min. A rat was judged to be immobile according to the criteria of Naito et al. (10). The Mann-Whitney U-test was used in the behavioral test and Dunnett's test was used to analyze body weights and tail skin temperature.

To determine the effectiveness of ovariectomy, we examined estrous cycles of rats by checking their vaginal smears. Vaginal smears of ovariectomized (OVX) control rats treated with propylene glycol showed diestrus figures from 3 to 21 days after ovariectomy. The E2-treated OVX rats showed estrus figures from 2-3 days after ovariectomy until the end of the study. Sham-operated rats had regular estrous cycles. The body weight of ovariectomized rats is known to increase following ovariectomy (11). To determine if E2 was effective at reversing this increase, the body weight was measured (Fig. 1a). At 10 days post-ovariectomy, the body weight of OVX control rats (268.5±4.9 g) was significantly greater than that of sham-operated rats (246.6±3.3 g, P < 0.01). At 21 days post-ovariectomy, the OVX control rats continued to show significantly greater body weights than sham-operated rats. E2 treatment (0.3 µg/body/day) suppressed the observed increase in body weight. The suppression by this low dose of E2 was observed at 10 days post-ovariectomy (P < 0.05). E2 at a higher concentration (1.0 µg/body/day) was more effective at suppressing the increased body weight observed in OVX control rats. The tail skin temperature was measured to determine if treatment was effective at reversing the increase in tail skin temperature observed following ovariectomy (Fig. 1b). Three days after ovariectomy, the tail skin temperature of OVX control rats (25.6±0.2°C) was not significantly different from that of the sham-operated group (25.4±0.1°C). However, at 6 days after ovariectomy, the OVX control rats showed significantly higher tail skin temperatures than sham-operated rats (26.5±0.1°C vs 25.6±0.1°C, P < 0.01). The OVX control rats continued to show significantly higher tail skin temperatures until the end of the study period. Ovariectomized rats treated with low (0.3 µg/body/day) or high (1.0 µg/body/day) doses of E2 had a tail skin temperature significantly lower than that of the OVX control. While the pathogenesis of postmenopausal hot flush is still poorly understood, one
Hypothesis is that a perimenopausal decline in circulating E2 levels may increase central norepinephrine and LH-RH secretion and produce a downward setting of the central thermostat, resulting in a hot flush (12). Chen et al. suggested that an increase of the calcitonin gene-related peptide (CGRP) level might lead to an occurrence of hot flushes (5).

Human menopausal symptoms are transiently observed and especially, mental symptoms occur during the perimenopausal period. Based on these results, we performed the behavioral test at 14 days after ovariectomy. Vaginal smears of OVX control rats and maprotiline-administered OVX rats showed diestrus figures from 2–3 to 14 days after ovariectomy. E2-treated OVX rats showed estrus figures from 2–3 days after ovariectomy (and pump implantation) until the end of the study. Sham-operated rats had regular estrous cycles. There were no significant differences in body weight among the groups at the beginning of the study. At the end of the study, body weight of rats in the OVX control group were 290.2 ± 3.1 g, which was significantly higher than 258.2 ± 3.6 g for the sham-operated group. All E2-treated OVX rats showed a decrease in body weight in comparison with OVX control rats. The body weight of maprotiline-treated OVX rats was 281.4 ± 6.4 g, which was not significantly different from that of the OVX control rats. We examined the immobility time in this model to determine if E2 had a positive effect similar to that of the typical antidepressant maprotiline. The immobility time of OVX control rats was 185.6 ± 6.5 sec, which was longer than the 154.0 ± 9.6 sec for sham-operated rats (Fig. 2). Maprotiline treatment after ovariectomy significantly decreased the immobility time to 157.5 ± 8.1 sec. E2 administered continuously significantly reduced the immobility time at the doses of 0.3, 1.0 and 3.0 µg/body/day to 160.9 ± 9.2, 155.1 ± 10.9 and 154.3 ± 6.2 sec, respectively. E2 at 10 µg/body/day tended to decrease the duration of immobility, but there was no significant difference, compared with the value of ovariectomized control rats. E2 showed a significant antidepressive effect at 0.3, 1.0 and 3.0 µg/body/day. However, the dose-dependency at these doses was not clear. This result was similar to the result of Bernardi et al. (8), in which there was no observable dose-dependency. Butcher et al. have reported that E2 administration produces a physiological E2 level at 1.0–2.0 µg/body/day (13). Since the rats used in the present study and the study of Butcher et al. were of equivalent body weight, it might be possible that the doses of 0.3–3.0 µg/body/day might produce a physiological E2 level and that a dose of 10 µg/body/day of E2 might result in a non-physiological level. This suggests that E2 may show an antidepressive action only when it is in the physiological range of serum levels. E2 shares some biochemical properties that typical antidepressants possess; for example, β-receptor down...
regulation (14) and inhibition of monoamine oxidase (MAO) activity (15).

As mentioned above, ovariectomy produced changes in tail skin temperature and a depressive state similar to that observed in postmenopausal women. Furthermore, these changes were ameliorated by E2 administration.

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