Effects of the Japanese herbal medicine *Keishi-bukuryo-gan* and 17β-estradiol on calcitonin gene-related peptide-induced elevation of skin temperature in ovariectomized rats

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Abstract

The effects of a Japanese herbal medicine, *Keishi-bukuryo-gan*, and 17β-estradiol on calcitonin gene-related peptide (CGRP)-induced elevation of skin temperature were investigated in ovariectomized (OVX) rats. 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, suggesting that lowered CGRP in plasma due to ovarian hormone deficiency increases the number of CGRP receptors and consequently amplifies the stimulatory effects of CGRP to elevate skin temperature. Oral *Keishi-bukuryo-gan* (100–1000 mg/kg, once a day for 7 days) restored a series of CGRP-related responses observed in OVX rats by normalizing plasma CGRP levels in a dose-dependent manner as effectively as s.c. injection. 17β-estradiol (0.010 mg/kg, once a day for 7 days). However, *Keishi-bukuryo-gan* did not affect the lower concentration of plasma estradiol and the decreased uterine weight due to ovariectomy, although the hormone replacement of 17β-estradiol restored them. These results suggest that *Keishi-bukuryo-gan*, which does not confer estrogen activity on plasma, may be useful for the treatment of hot flashes in patients for whom estrogen replacement therapy is contraindicated, as well as menopausal women.


Introduction

Hot flashes are the most common symptom of the climacteric and are reported by the vast majority of menopausal women (Reber & Spitzer 1987). The symptom is subjectively experienced as a sensation of internal heat, spreading upward from the chest to the neck and face, and profuse sweating often occurs in these areas (Freedman 2001). Although hot flashes accompany the estrogen withdrawal that occurs at menopause, the mechanism underlying the symptom has not been clarified. Recently, it was reported that the plasma level of calcitonin gene-related peptide (CGRP), a vasodilator neuropeptide (Brain et al. 1985, Wanaka et al. 1987), in menopausal women rapidly increases during hot flashes (Chen et al. 1993, Valentini et al. 1996, Wyon et al. 2000). This is supported by our recent finding that i.v. injection of CGRP elevates skin temperature in ovariectomized (OVX) rats more than in sham-operated (Noguchi et al. 2002). In addition, an increase in blood flow caused by CGRP-induced vasodilation and an up-regulation of CGRP receptors following the lower concentration of plasma CGRP are observed in OVX rats. On the basis of these results, we speculated that ovarian hormone deficiency increases the number of CGRP receptors and consequently amplifies the stimulatory effects of CGRP to elevate skin temperature (Noguchi et al. 2002). If the series of the CGRP-related responses described above were restored by estrogen replacement, our hypothesis would be strongly supported. Therefore, the first objective of the present study was to investigate the effect of 17β-estradiol, which is a potent estrogen, on the CGRP-related responses in OVX rats.

*Keishi-bukuryo-gan* is a traditional herbal medicine called ‘Kampo medicine’ in Japan. This remedy for menopausal symptoms has been approved by the Ministry of Health, Labor and Welfare in Japan. To date, there are several studies demonstrating that *Keishi-bukuryo-gan* ameliorates menopausal hot flashes (Mochimaru et al. 1984, Tanaka 2001). However, whether the CGRP-related mechanism is involved or not in the anti-hot flashes effect of *Keishi-bukuryo-gan* has not been investigated. Therefore, the second objective of the present study was to investigate the effects of *Keishi-bukuryo-gan* on CGRP-induced elevation of skin temperature and a series of the CGRP-related responses in OVX rats.
Materials and Methods

Animals

Ten-week-old female Sprague–Dawley rats weighing 200–250 g were purchased from Charles River Laboratories (Yokohama, Japan). The animals were allowed free access to water and standard laboratory food, and housed in stainless steel cages at a temperature of 23 ± 2 °C, relative humidity 55 ± 10% and a 12 h light:12 h darkness cycle, with lights on from 0700 to 1900 h daily.

The rats were anesthetized with an i.p. injection of sodium pentobarbital (50 mg/kg) and bilaterally ovariectomized or sham–operated as controls.

All experimental procedures were performed according to the ‘Guidelines for the Care and use of Laboratory Animals’ approved by the Laboratory Animal Committee of Tsumura & Co.

Drugs and reagents

The Keishi-bukuryo-gan (TJ-25) used in the present study, composed of equal parts of Cinnamomum cortex, Paeoniae radix, Moutan cortex, Hoelen and Persicae semen, was obtained from Tsumura Co. Ltd (Tokyo, Japan), in the form of a dried powder extract.

Rat αCGRP was purchased from Peptide Institute (Osaka, Japan). 125I–CGRP (specific activity 2000 Ci/mmol) was purchased from Peninsula Laboratories (San Carlos, CA, USA). Prostaglandin F2α (PGF2α), urethane, α-chloralose and bacitracin were purchased from Sigma Chemical (St Louis, MO, USA). 17β-Estradiol and aprotonin were purchased from Wako Pure Chemical Industries (Osaka, Japan). Sodium pentobarbital was purchased from Dinabot Laboratories (North Chicago, IL, USA). Other reagents used for analysis were the highest purity commercially available.

Measurement of CGRP-induced elevation of skin temperature

Keishi-bukuryo-gan (100, 300 or 1000 mg/10 ml per kg, p.o., n=7 or 8 in each group) suspended in distilled water or 17β-estradiol (0·010 mg/ml per kg, s.c., n=7) dissolved in olive oil was administered for 7 days (once a day) from the 2nd week after ovariectomy. Distilled water (10 mg/kg, p.o., n=8) as control or Keishi-bukuryo-gan (1000 mg/10 ml per kg, p.o., n=8) was administered to sham–operated rats for 7 days according to the same schedule. Measurement of skin temperature was carried out on the day following the final administration according to a procedure described previously (Noguchi et al. 2002). In brief, rats were anesthetized with i.p. co-injection of urethane (0·75 g/kg) and α-chloralose (0·06 g/kg), and then two thermistor probes (SXN-54; Technol Seven Co., Yokohama, Japan) were taped to the plantar faces of both hind feet. Forty minutes later, the mean temperature was automatically measured at 5 min intervals throughout the experiment. Data were recorded by a K932 recording device (Technol Seven Co.). αCGRP (10 μg/kg, i.v.) dissolved in saline was injected after the basal temperature was stable. The area under the temperature curve (AUC) following the injection of αCGRP was calculated using PAG–CP (Pharmacokinetic Analysis and Graphics for Clinical Pharmacology) analysis (Medical Research AS Medica, Osaka, Japan).

Measurement of CGRP-induced relaxation of PGF2α-induced vasoconstriction in isolated mesenteric vascular beds

In another set of experiments, distilled water (10 ml/kg, p.o., n=11), Keishi-bukuryo-gan (1000 mg/10 ml per kg, p.o., n=13) or 17β-estradiol (0·010 mg/ml per kg, s.c., n=12) was administered to OVX rats, and distilled water (10 ml/kg, p.o., n=9) to sham–operated rats according to the schedule described in the section ‘Measurement of CGRP-induced elevation of skin temperature’. Changes in the resistance of the mesenteric vascular bed obtained from drug-treated rats were evaluated on the day following the final administration according to a procedure described previously (Noguchi et al. 2002). In brief, all rats were anesthetized with pentobarbital (50 mg/kg, i.p.). A vascular bed including small arteries was isolated, placed in a water-jacketed organ bath maintained at 37 °C and perfused at a constant rate of 5 ml/min using a peristaltic pump (AC-2110; Atto Co., Tokyo, Japan) with a modified Krebs solution gassed with 5% CO2 in O2. The modified Krebs solution was composed of (in mM): NaCl 120·0, KCl 5·0, MgSO4 1·2, NaHCO3 25·0, CaCl2 2·4 and glucose 5·0. Changes in the perfusion pressure were measured with a pressure transducer (San–ei Co., Tokyo, Japan) and recorded on a polygraph (Rika Denki Co., Tokyo, Japan). After the basal pressure was stable, 10 μM PGF2α was continuously perfused to elevate the pressure by vasoconstriction of the vascular bed. After the PGF2α-induced vasoconstriction reached a plateau, αCGRP (30 pmol/10·0 μl) dissolved in saline was added to the perfusate. The relaxation rate induced by CGRP was expressed as a percentage of the maximum pressure obtained by the PGF2α-induced constriction.

CGRP receptor-binding assay in isolated mesenteric vascular beds

In another set of experiments, Keishi-bukuryo-gan (1000 mg/10 ml per kg, p.o., n=8) or 17β-estradiol (0·010 mg/ml per kg, s.c., n=8) was administered to OVX rats according to the schedule described in the section ‘Measurement of CGRP-induced elevation of skin temperature’. Distilled water (10 ml/kg, p.o.) was also administered to sham–operated rats (n=8) and OVX rats (n=8) in the same schedule. Plasma membranes of mesenteric arteries from all rats were prepared on the day
following the final administration according to a procedure described previously (Noguchi et al. 2002). The pooled mesenteric vascular beds of two rats in each group were used for the determination. In brief, the two mesenteric vascular beds isolated from decapitated rats were placed in cold 0·25 M sucrose. The mesenteric arteries obtained by removing fatty tissue, lymph nodes and veins from the vascular beds were homogenized in 0·25 M sucrose with a Polytron homogenizer (Kinematica Inc., Cincinnatti, OH, USA) at setting 7 for 30 s. The crude membrane fraction (pellet) was obtained by centrifugation at 100 000 g at 4 °C for 30 min. The fraction was suspended in 5·0 ml 0·25 M sucrose and layered on 5·0 ml 29% sucrose solution. After centrifugation at 100 000 g for 120 min, the plasma membrane fraction (the middle layer) was collected. The membrane suspension was washed with 0·25 M sucrose and centrifuged at 100 000 g for 30 min. The sediment was suspended in 50 mM Tris–HCl buffer, pH 7·4, containing 5 mM MgCl₂ and 0·25 M sucrose. The concentration of membrane protein was determined by the method of Lowry et al. (1951). Finally, the concentration of the membrane suspension was adjusted to 1·0 mg/ml with the buffer.

The CGRP receptor-binding assay was performed as described previously (Noguchi et al. 2002). In brief, the membrane suspension (20–25 μg protein) was incubated with 125I-CGRP (final concentration 50 pM) in 100 μl 50 mM Tris–HCl buffer, pH 7·4, containing 5 mM MgCl₂, 2 mM EGTA, 1·0% BSA, 2 mM bacitracin, 200 kIU/ml aprotinin, 50 mM NaCl and 0·25 M sucrose. After incubation at 23 °C for 1 h, the incubation medium was passed through a GF/B filter (Whatman International Ltd, Maidstone, Kent, UK) that had been pre-soaked in polyethylenimine for 1 h. Receptor–ligand complexes on the filter were rinsed three times with 3 ml ice-cold 200 kIU/ml aprotinin, 50 mM NaCl and 0·25 M sucrose. Radioactivity on the filter was counted by a COBRA II autogamma counting system (Packard, Meriden, CT, USA). Non-specific binding was determined by adding 0·5 μM unlabeled CGRP instead of the labeled peptide.

Measurement of plasma concentration of CGRP

To determine the plasma CGRP level, Keishi-bukuryo-gan (1000 mg/10 ml per kg, p.o., n=7) or 17β-estradiol (0·010 mg/ml per kg, s.c., n=7) was administered to OVX rats according to the schedule described in the section ‘Measurement of CGRP-induced elevation of skin temperature’. Distilled water (10 ml/kg, p.o.) was also administered to sham-operated rats (n=7) and OVX rats (n=8) on the same schedule. On the day following the final administration, all rats were anesthetized with diethyl ether, and then blood (approximately 6 ml) was collected from the abdominal aorta in a polypropylene tube containing 6·0 mg EDTA-2Na. The blood was centrifuged at 1500 g at 4 °C for 15 min. The plasma obtained was stored at −80 °C until estradiol assay. Plasma concentration of estradiol was measured using a 125I-estradiol RIA kit (Diagnostic Products Co., Los Angeles, CA, USA).

The uterus was removed and weighed after exsanguination on the day of experiment.

Statistical analysis

All values are represented as the mean ± S.E.M. The statistical significance was evaluated by a one-way ANOVA followed by Dunnett’s test. The significance level was accepted at P<0·05.

Results

Effects of Keishi-bukuryo-gan and 17β-estradiol on CGRP-induced elevation of skin temperature

In all groups, skin temperature was maximally elevated 40–50 min after the injection of CGRP (10 μg/kg, i.v.), and thereafter it recovered to the basal levels by 120 min. The degree of changes in skin temperature elevated by CGRP was calculated as AUC in each group. Figure 1 shows the changes in each group as a percentage of AUC.
in the control group; when the AUC of the water-treated control group of the sham-operated rats was expressed as 100%, ovariectomy significantly enhanced CGRP-induced elevation of skin temperature (210% of control group, \( P < 0.01 \)). In OVX rats, Keishi-bukuryo-gan (100–1000 mg/kg, p.o.) inhibited the elevation of skin temperature in a dose-dependent manner. A significant inhibition was observed at a dose of 1000 mg/kg Keishi-bukuryo-gan (\( P < 0.05 \)). 17\(^{\beta}\)-estradiol (0.010 mg/kg, s.c.) significantly inhibited the CGRP-induced vasorelaxation (\( P < 0.01 \)).

On the other hand, Keishi-bukuryo-gan (1000 mg/kg, p.o.) did not affect CGRP-induced elevation of skin temperature in sham-operated rats.

Effects of Keishi-bukuryo-gan and 17\(^{\beta}\)-estradiol on CGRP-induced relaxation of PGF\(_{2\alpha}\)-induced vasoconstriction in isolated mesenteric vascular beds

Continuous perfusion of PGF\(_{2\alpha}\) (10 \(\mu\)M) constricted the isolated mesenteric vascular beds obtained from sham-operated and OVX rats, and the constriction reached a plateau. No significant differences were observed in the degree of PGF\(_{2\alpha}\)-induced constriction in vascular beds between sham-operated and OVX rats. As shown in Fig. 2, CGRP (30 pmol) relaxed the PGF\(_{2\alpha}\)-induced constriction in the vascular bed isolated from sham-operated rats. The vasorelaxation effect, however, was significantly greater in OVX rats than in sham-operated rats (\( P < 0.01 \)). In OVX rats, Keishi-bukuryo-gan (1000 mg/kg, p.o.) and 17\(^{\beta}\)-estradiol (0.010 mg/kg, s.c.) significantly inhibited the PGF\(_{2\alpha}\)-induced vasorelaxation (\( P < 0.01 \)).

Effects of Keishi-bukuryo-gan and 17\(^{\beta}\)-estradiol on \(^{125}\)I-CGRP binding in isolated mesenteric arteries

The results of specific binding of \(^{125}\)I-CGRP to CGRP receptors in mesenteric arteries of OVX and sham-operated rats are shown in Fig. 3. The receptor binding (2.28 fmol/mg protein) significantly (\( P < 0.01 \)) increased in OVX rats over that (0.83 fmol/mg protein) in sham-operated rats. Keishi-bukuryo-gan (1000 mg/kg, p.o., \( P < 0.05 \)) and 17\(^{\beta}\)-estradiol (0.010 mg/kg, s.c., \( P < 0.01 \)) significantly inhibited the ovariectomy-induced increase in the receptor binding.

Effects of Keishi-bukuryo-gan and 17\(^{\beta}\)-estradiol on plasma concentration of CGRP

Effects of Keishi-bukuryo-gan and 17\(^{\beta}\)-estradiol on plasma concentration of CGRP in plasma of OVX rats are
shown in Fig. 4. The CGRP level (6.38 ± 0.39 pmol/l) in OVX rats was significantly lower (P<0.05) than that (13.34 ± 1.41 pmol/l) in sham-operated rats. The ovariectomy-induced lower level was restored (P<0.05) to sham-operated level by treating with Keishi-bukuryo-gan (1000 mg/kg, p.o.) or 17β-estradiol (0.010 mg/kg, s.c.).

**Effects of Keishi-bukuryo-gan and 17β-estradiol on plasma concentration of estradiol and uterine weight**

The plasma concentration of estradiol and the uterine weight in OVX and sham-operated rats are shown in Table 1. The estradiol level in OVX rats was significantly lower (P<0.05) than that in sham-operated rats. The lower estradiol level was restored (P<0.05) to sham-operated level by treating with Keishi-bukuryo-gan (1000 mg/kg, p.o.) or 17β-estradiol (0.010 mg/kg, s.c.).

Uterine weight also significantly (P<0.01) decreased in OVX rats over that in sham-operated rats. The decreased tissue weight was recovered (P<0.01) by supplying 17β-estradiol, but it was not affected by Keishi-bukuryo-gan.

**Discussion**

We have demonstrated in a previous study (Noguchi et al. 2002) that CGRP-induced elevation of skin temperature is antagonized by pretreatment with CGRP1 receptor antagonist CGRP8–37, suggesting that the elevation is due to the response through CGRP1 receptors. On the other hand, it has been demonstrated that the mesenteric vascular bed is densely innervated by CGRP-containing nerves (Ishida-Yamamoto & Tohyama 1989) and the main CGRP receptor type mediating relaxation in the mesenteric arteries is also a CGRP1 receptor (Han et al. 1990, Lei et al. 1994). These suggest that mesenteric vascular beds including small arteries, for which preparation is comparatively easy, are useful materials to examine the changes in CGRP1 receptors and the mediated responses, in place of skin arteries for which preparation is rather difficult. Therefore, we investigated the changes in CGRP-induced relaxation and the number of CGRP receptors by using mesenteric arteries.

In the present study, we demonstrated that ovariectomy potentiated not only CGRP-induced elevation of skin temperature but also CGRP-induced arterial relaxation, which agrees with the results of our previous study.
maximal number of binding sites (Bmax) of 125I-CGRP to previous Scatchard analysis, which demonstrated that the increased in OVX rats. This also supports the results of aing to mesenteric arterial CGRP receptors significantlyabolished by ovariectomy, because it may be synthesizedin plasma under the condition of estrogen deficiency inOVX rats. However, the plasma estradiol is not completelysuppressed in OVX, whereas the C19 steroids isophi17estradiol, which are ovarian hormones, stimulateCGRP peptide synthesis in dorsal root ganglia neurons byincreasing CGRP mRNA (Gangula et al. 2000a). Theneuronal CGRP is transported towards the perivascularnerve endings and released into the circulation (Zaidi et al.1985, Holzer 1992). Circulating levels of CGRP in rats andhumans have been reported to increase with pregnancyand decrease at postpartum (Stevenson et al. 1986,Saggese et al. 1990, Gangula et al. 2000b). Valentini etal. (1996) have reported that plasma CGRP levels in postmenopausal women are lower than in fertile women. Thesefindings also suggest that ovarian hormones regulateplasma CGRP levels. Taken together, the present resultsuggest that ovarian hormone deficiency may decrease theCGRP level in plasma and consequently may increase thenumber of CGRP receptors, i.e. up-regulation of thereceptors to maintain vascular adaptation. Because skin temperature is regulated by regional blood flow (Randet al. 1965, Kronenberg 1994), the elevation of skin temperature potentiated by CGRP in OVX rats is thoughttobe mediated by the blood flow increase following thevasodilation by this peptide of the up-regulated arterialCGRP receptors in the skin. This hypothesis is stronglysupported by the results of the supply of 17β-estradiol,which is a potent estrogen, to OVX rats; the hormone replacement clearly inhibited ovariectomy-induced ele-varation of skin temperature with the decrease of plasmaCGRP level, up-regulation of CGRP receptors andpotentiation of vasorelaxation.

(Noguchi et al. 2002). In addition, the 125I-CGRP bindingto mesenteric arterial CGRP receptors significantlyincreased in OVX rats. This also supports the results ofa previous Scatchard analysis, which demonstrated thatthe maximal number of binding sites (Bmax) of 125I-CGRpto arterial CGRP receptors was higher in OVX rats than in sham-operated rats without altering the dissociation constant (Kd) value (Noguchi et al. 2002). The increase in the number of CGRP receptors suggested by the increases of 125I-CGRP binding rate and Bmax was always accompanied by lower levels (10−40 pg/ml, a quarter the amount of the sham-operated level) of endogenous CGRP in the plasma under the condition of estrogen deficiency in OVX rats. However, the plasma estradiol is not completely abolished by ovariectomy, because it may be synthesized from C19 steroids in a reaction catalyzed by aromatase in extraovular tissues, such as adipose tissue and skin fibroblasts, in the OVX condition (Simpson et al. 1994, Bulun et al. 2000). It has been reported that both estrogen and progesterone, which are ovarian hormones, stimulate CGRP peptide synthesis in dorsal root ganglia neurons by increasing CGRP mRNA (Gangula et al. 2000a). The neuronal CGRP is transported towards the perivascular nerve endings and released into the circulation (Zaidi et al. 1985, Holzer 1992). Circulating levels of CGRP in rats and humans have been reported to increase with pregnancy and decrease at postpartum (Stevenson et al. 1986, Saggese et al. 1990, Gangula et al. 2000b). Valentini et al. (1996) have reported that plasma CGRP levels in postmenopausal women are lower than in fertile women. These findings also suggest that ovarian hormones regulate plasma CGRP levels. Taken together, the present results suggest that ovarian hormone deficiency may decrease the CGRP level in plasma and consequently may increase the number of CGRP receptors, i.e. up-regulation of the receptors to maintain vascular adaptation. Because skin temperature is regulated by regional blood flow (Rand et al. 1965, Kronenberg 1994), the elevation of skin temperature potentiated by CGRP in OVX rats is thought to be mediated by the blood flow increase following the vasodilation by this peptide of the up-regulated arterial CGRP receptors in the skin. This hypothesis is strongly supported by the results of the supply of 17β-estradiol, which is a potent estrogen, to OVX rats; the hormone replacement clearly inhibited ovariectomy-induced elevation of skin temperature with the decrease of plasma CGRP level, up-regulation of CGRP receptors and potentiation of vasorelaxation.

**Table 1** Effects of Keishi-bukuryo-gan and 17β-estradiol on concentration of plasma estradiol and uterine weight in OVX rats. Keishi-bukuryo-gan (1000 mg/kg, p.o., n=7) or 17β-estradiol (0.010 mg/kg, s.c., n=7) was administered for 7 days (once a day) from the 2nd week after ovariectomY. Distilled water wasadministered to sham-operated (n=7) and OVX (n=8) rats for7 days according to the same schedule. Measurement was carriedout on the day following the final administration.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma estradiol (pg/ml)</th>
<th>Uterine weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operation</td>
<td>43.59 ± 6.23</td>
<td>368 ± 19</td>
</tr>
<tr>
<td>OVX</td>
<td>10.40 ± 3.36*</td>
<td>91 ± 4**</td>
</tr>
<tr>
<td>OVX + Keishi-bukuryo-gan</td>
<td>9.02 ± 2.91*</td>
<td>92 ± 5**</td>
</tr>
<tr>
<td>OVX + 17β-estradiol</td>
<td>58.60 ± 14.53††</td>
<td>357 ± 14††</td>
</tr>
</tbody>
</table>

Each value is expressed as the mean ± S.E.M. Significance with Dunnett’s test following a one-way ANOVA (plasma estradiol: F(3,25)=9.556, P<0.001 or uterine weight: F(3,25)=60.529, P<0.001) is indicated as *P<0.05 and **P<0.01 vs sham-operated group, or ††P<0.01 vs OVX group.
(Kuiper et al. 1998, Diel et al. 2001), that activate estrogen-controlled functions by activating transcription via estrogen receptors (ERs). In addition, raloxifin, which is known as a synthetic selective ER modulator (SERM), exhibits anti-estrogenicity in the breast and uterus, but acts as an agonist in bone and liver (Jisa et al. 2001). Recently, a phytoestrogen genistein derivative 6-carboxymethyl genistein was reported to possess the same selectivity as raloxifin in tissues derived from OVX rats (Somjen et al. 2002). If such phytoestrogen-like or SERM-like substances are contained in Keishi-bukuryo-gan, the estrogen-like activity to release CGRP from the perivascular nerve endings into the circulation may be induced without restoring the decreased plasma estrogen level in OVX rats. To date, we have isolated and identified many ingredients including paeoniflorin, oxypaeoniflorin, benzoypaeoniflorin, albiflorin, paconol, amygdalin, cinnamic acid, cinnamic aldehyde and penta-O-gallonyl-β-D-glucopyranose from the extract of Keishi-bukuryo-gan (Usuki 1987). However, there have been no reports investigating potential phytoestrogen-like and SERM-like properties of each ingredient. Recently, we have reported that Keishi-bukuryo-gan, which is extracted from a blend of five galenicals, does not activate the transcription in estrogen-dependent human breast cancer cell line MCF-7, which expresses ERα abundantly (Shizaki et al. 1999). This finding suggests that Keishi-bukuryo-gan may not have an ERα-like effect. However, we cannot rule out the possibility that Keishi-bukuryo-gan has phytoestrogen- and SERM-like effects, because ERβ has been shown to have different tissue distribution and different ligand binding specificity from ERα (Kuiper et al. 1997, 1998, Watanabe et al. 1997). Keishi-bukuryo-gan, which did not affect plasma estrogen activity and uterine weight, may have tissue specificity compared with supplemental estrogen that is considered to affect all estrogen-target tissues including uterine with increasing plasma estrogen levels. Therefore, it is important to characterize the estrogenic properties of Keishi-bukuryo-gan in future studies. In addition, Keishi-bukuryo-gan did not affect CGRP-induced elevation of skin temperature in sham-operated rats. This fact suggests that this herbal medicine may act only under the condition of hormonal imbalance in ovarioctomy. We also have to investigate the mechanism in future studies.

In conclusion, Keishi-bukuryo-gan and 17β-estradiol inhibited the potentiation of CGRP-induced elevation of skin temperature in OVX rats. The reversible results due to estrogen replacement in a series of the CGRP-related responses strongly support our previous hypothesis that ovarian hormone deficiency induces up-regulation of CGRP receptors due to the lowered level of plasma estrogen and consequently amplifies the ability of CGRP to elevate skin temperature. The present study also suggests that estrogen might be useful as hormone replacement therapy for menopausal hot flashes. In addition, Keishi-bukuryo-gan, which does not confer estrogen activity on plasma, may be useful for women experiencing hot flashes with specific contraindications, such as a history of estrogen-dependent cancer (Loprinzi et al. 2001), as well as menopausal women (Reber & Spitzer 1987, Freedman 2001).

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