Characteristics of contractile activity in the renal artery of ovariectomized rats

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Received December 25, 2007; Accepted January 10, 2008

Abstract

The incidence of cardiovascular disease is markedly lower in cycling, pre-menopausal women and post-menopausal women receiving estrogen than in men or untreated post-menopausal women. Clinical studies demonstrate a protective role of estrogen in hormone replacement therapy in terms of reducing cardiovascular risk. However, the benefits of hormone replacement therapy in cardiovascular disease remain unclear. We investigated the effects of estrogen on the contractile responses of the renal artery of ovariectomized Wistar rats (OVX) compared to both ovariectomized 17 β -estradiol-treated rats (OVXE) and sham-operated (control) rats. Isometric contraction of renal artery was recorded with a strain gauge transducer. The maximum contractile response of the renal artery smooth muscle to KCl (80 mM) in the OVXE group was significantly higher than that in both the control and OVX groups. The phenylephrine (PE) concentration-response curves in all three groups indicated a greater sensitivity at lower concentrations of PE following treatment with 100 μ M L-arginine methyl ester (L-NAME). The EC₅₀ values for PE in the three groups were 2 times lower in the presence of L-NAME than those lacking exposure to L-NAME. The EC₅₀ value for PE in the OVX group was ~3 times lower in the presence of L-NAME than in those lacking exposure to L-NAME and 100 nM BMY 7378, an α_{1D} -adrenoceptor antagonist. The rate of relaxation of the PE-induced contraction (T_{1/2}) was significantly reduced in the OVX group relative to both the control and OVXE groups. T_{1/2} values after treatment with 100 μ M L-NAME were slower than those lacking exposure to L-NAME in all groups. Further, the T_{1/2} value of the OVX group was 2 times greater than that of the control; this change was reversed in the OVXE group. In conclusion, our results suggest that estrogen regulates contraction and relaxation in the renal artery via NO synthase activity and alteration of the Ca²⁺ transport systems.

Key words: renal artery, ovariectomy, Ca²⁺ regulation, NO synthase

Introduction

The incidence of cardiovascular disease is markedly lower in cycling, pre-menopausal women and post-menopausal women receiving estrogen than in men or untreated post-menopausal women. Clinical studies support a protective role of estrogen in hormone replacement therapy in terms of reducing cardiovascular risk. Further, recent studies have demonstrated the benefits of hormone replacement therapy in cardiovascular disease (REF). However, an advisory from the American Heart Association did not recommend initiation of hormone replacement therapy for the sole purpose of preventing cardiovascular disease (Hulley *et al.*, 1998; Tolbert *et al.*, 2001).

It has been reported that nitric oxide (NO) release and NO synthase activity are enhanced by estrogen in the uterine arteries but not in the renal arteries of non-pregnant sheep (Vielle *et al.*, 1996). In contrast, 17β -estradiol antagonizes increases in vascular tone in hypertensive females by enhancing NO-dependent relaxation and by suppressing endothelium-derived contractile factor (EDCF)-mediated mechanisms in an NO-independent manner (Zhang *et al.*, 2002). Estrogen pretreatment of male dogs decreased both maximum contractile responses and EC_{50} value to U46619 (a TXA_2 mimetic) or PE in the coronary artery. These results suggested that smooth muscle reactivity of the dog coronary artery to both U46619 and PE might be susceptible to regulation by both androgens and estrogens (Karanian *et al.*, 1996). Changes in the vasomotor reactivity of renal arteries may similarly be altered by changes in the hormonal milieu and lead to defective autoregulation of renal hemodynamics. However, the exact contractile activity in renal arteries isolated from ovariectomized rats (OVX) remains unclear. This study examined whether estrogen altered phenylephrine (PE) contraction and the involvement of NO and Ca^{2+} regulation in the renal arteries isolated from OVX.

Materials and methods

All protocols involving animals were approved by the Animal Care and Use Committee of Showa University, Japan. Six week-old female Wistar rats were purchased from Japan Laboratory Animals, Inc., (Tokyo) and housed under a 12:12-h light-dark cycle; food and water were available *ad libitum*. At eight weeks, rats were randomly divided into three groups. Two groups were ovariectomized and the third group underwent a sham operation. After four weeks post-surgery, one of the ovariectomized groups (OVXE) was implanted subcutaneously with a silicone capsule (Osmotic pump MODEL 2ML2, Alzet, USA) containing 17β -estradiol (17 μ g). The animals in the remaining group of ovariectomized (OVX) rats and the “sham” operated rats (controls) were all implanted with a placebo pellet. Two weeks post-implantation, animals were anesthetized with diethyl ether and euthanized by decapitation and their kidneys with intact renal arteries were quickly removed for dissection of the arteries. The weight of the body, of the abdominal fat and of the wet uterine were measured to verify estrogen treatment.

Contractile studies of isolated renal arteries

Freshly isolated kidneys with their attached renal artery were immediately placed in Krebs-

Henseleit Buffer solution (KHB) containing 118 mM NaCl, 4.5 mM KCl, 2.5 mM CaCl, 1.2 mM KH_2PO_4 , 1.5 mM MgSO_4 , 25 mM NaHCO_3 and 11 mM glucose and bubbled with 95% O_2 –5% CO_2 . Ring preparations (length, 2.5–3.0 mm) of the main renal arteries were isolated from both kidneys under the dissecting microscope. Once the preparation was isolated, adherent fat tissue was removed from the vessels. The cleaned specimens were placed in a chamber (UFER, Medical Kishimoto, Tokyo, Japan) containing KHB at $37 \pm 0.5^\circ\text{C}$ and gassed with 95% O_2 –5% CO_2 . Two fine tungsten wires (100 μm diameter) were passed through the lumen of the artery. One end of each wire was connected to an isometric transducer (T7-8-240, Orientec, Tokyo, Japan), whereas the opposite end was attached to the holder; subsequently, isometric tension was recorded on a polygraph (RECTIGRAPH-8K, San-Ei, Tokyo, Japan). Experiments were monitored via computer-based analysis using Mac-Lab and Chart 3.6 software.

The arterial ring preparations were loaded to a tension of 1 g and allowed to equilibrate for 60 min in KHB maintained at $37 \pm 0.5^\circ\text{C}$, pH 7.4; subsequently, specimens were bubbled with 95% O_2 –5% CO_2 . During the equilibration, the ring preparations were washed with KHB every 15–20 min. After equilibration, preparations were contracted with 100 mM KCl, followed by washing with KHB three times. After 30 min, vessels were again contracted with 60 mM KCl in order to establish the control level. The 60 mM KCl-induced contraction served as a standard contractile response for each agonist concentration.

Fatty acid composition of uterus and aorta

The uterus and aorta from each animal were stored at -80°C by flash-freezing in liquid N_2 until use or immediately homogenized in ice-cold 0.32 mol/L sucrose buffer (pH 7.4) containing 2 mmol/L EDTA, 0.5 mg/L leupeptin, 0.5 mg/L pepstatin, 0.5 mg/L aprotinin, and 0.2 mmol/L phenylmethylsulfonyl fluoride using a Polytron homogenizer (PCU 2–110, Kinematica, Lucerne, Switzerland). The homogenates were also stored at -80°C in liquid N_2 until use.

Fatty acid composition was determined to estimate the 17β -estradiol effect on the uterus and the aorta using the one-step chromatographic analysis of Lepage and Roy (1986) described previously (Hashimoto *et al.*, 1999). The protein concentration was estimated using the method of Lowry *et al.* (1951).

Drugs

L-phenylephrine hydrochloride, N^G -nitro L-arginine methyl ester (L-NAME), leupeptin, pepstatin, aprotinin, and phenylmethylsulfonyl fluoride were purchased from Sigma-Aldrich (St. Louis, MO, USA). BMY7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride) was acquired from Funakoshi (Tokyo, Japan). All other chemicals and materials were used at the highest grade commercially available.

Statistical analyses

Analysis of data was conducted with SPSS statistical analysis software (Version 12.0; SPSS Inc, Tokyo, Japan). Results were expressed as the mean $\pm$ SEM. Baseline comparisons between the 3 groups were performed using one-way ANOVA, and post hoc analyses were performed with the Turkey test for two independent samples. Group means were compared

Table 1. Average weights of body, uterus and abdominal fat in the three groups

Groups weight (g)	Control (n=11)	OVX (n=12)	OVXE (n=12)
Body	232 ± 10	285 ± 17*	275 ± 12*
Uterus	0.5 ± 0.08	0.1 ± 0.01*	0.1 ± 0.01*
Abdominal fat	8.1 ± 0.7	10.3 ± 0.9	9.1 ± 0.6

Control: sham operated rats; OVX: ovariectomized rats; OVXE: ovariectomized rats with 17 β -estradiol treatment for 2 weeks from 4 weeks after ovariectomy. Values are mean $\pm$ SEM. *, Significantly different from control ($P < 0.05$).

Table 2. Fatty acid composition of the uterus (mol%)

FA Groups	PA	SA	OA	LA	LNA	AA
Control	28.3 $\pm$ 0.9	16.4 $\pm$ 3.6	18.0 $\pm$ 3.0	19.0 $\pm$ 5.2	0.7 $\pm$ 0.3	11.6 $\pm$ 3.0
OVX	27.6 $\pm$ 0.7	6.3 $\pm$ 0.6*	25.4 $\pm$ 0.6*	32.1 $\pm$ 0.8*	1.5 $\pm$ 0.1*	3.8 $\pm$ 0.5*
OVXE	27.9 $\pm$ 1.0	10.6 $\pm$ 4.3	22.4 $\pm$ 3.5	26.5 $\pm$ 6.0	1.2 $\pm$ 0.4	7.2 $\pm$ 3.5
Groups	EPA	DPA	DHA	n6/n3	DHA/AA	
Control	0.3 $\pm$ 0.01	1.3 $\pm$ 0.2	4.2 $\pm$ 0.8	5.1 $\pm$ 1.0	0.4 $\pm$ 0.06	
OVX	0.3 $\pm$ 0.02	0.9 $\pm$ 0.1	2.0 $\pm$ 0.2*	7.7 $\pm$ 0.4*	0.5 $\pm$ 0.03	
OVXE	0.3 $\pm$ 0.01	1.0 $\pm$ 0.2	3.0 $\pm$ 1.0	6.8 $\pm$ 1.2	0.6 $\pm$ 0.1	

Values are mean $\pm$ SEM from 6 rats in each group. *, Significantly different from control ($P < 0.05$). AA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; LA, linoleic acid; LNA: linolenic acid; OA, oleic acid; PA, palmitic acid; SA, stearic acid; DHA/AA, molar ratio of arachidonic acid and docosahexaenoic acid.

using Student's *t*-test. Unless otherwise noted, a nominal two-sided *P* value less than 0.05 was utilized to assess significance.

Results

Characteristics in body, uterus and abdominal fat weights among the three groups

Compared to that of the control group, the OVX group had a significantly increased body weight, while the OVXE group had also increased in body weight (Table 1). The weight of uterus was substantially decreased in both the OVX and OVXE groups compared to that of the control group. The weight of abdominal fat of the OVX group tended to increase compared to that of the control group, but this tendency was prevented by treatment with 17 β -estradiol. While we could detect significant differences in body and uterine weights in the OVX, the 17 β -estradiol treatment made no difference to the body weight and uterus weight in the OVXE group.

Therefore, we further investigated the fatty acid composition in both the uterus and aorta to explore the effect of 17 β -estradiol on these target organs- the uterus compared to the aorta. The detailed fatty acid composition of both the uterus and the aorta are shown in Table 2 and Table 3 respectively. The major fatty acid composition in the uteri isolated from the OVX animals was

Table 3. Fatty acid composition of the aorta (mol%)

FA Groups	PA	SA	OA	LA	LNA	AA
Control	28.7 ± 0.9	15.5 ± 2.0	20.2 ± 1.8	22.0 ± 2.7	0.6 ± 0.1	8.9 ± 1.6
OVX	31.5 ± 1.0	15.5 ± 1.0	19.3 ± 1.2	21.5 ± 1.1	0.6 ± 0.1	8.0 ± 1.0
OVXE	30.3 ± 1.0	16.3 ± 2.0	19.0 ± 1.3	21.0 ± 2.3	0.6 ± 0.1	9.1 ± 1.4
Groups	EPA	DPA	DHA	n6/n3	DHA/AA	
Control	0.2 ± 0.02	1.3 ± 0.2	2.6 ± 0.2	6.8 ± 0.7	0.3 ± 0.03	
OVX	0.2 ± 0.01	1.0 ± 0.1	2.3 ± 0.3	7.3 ± 0.6	0.3 ± 0.01	
OVXE	0.3 ± 0.01	1.1 ± 0.1	2.4 ± 0.2	7.0 ± 0.6	0.3 ± 0.01	

Values are mean ± SEM from 6 rats in each group. AA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; LA, linoleic acid; LNA: linolenic acid; OA, oleic acid; PA, palmitic acid; SA, stearic acid; DHA/AA, molar ratio of arachidonic acid and docosahexaenoic acid.

substantially different from that of the control animals. Concentrations of stearic acid (SA) and arachidonic acid (AA) in the OVX group were ~3 times less than those in the control (6.3 *vs.* 16.4 mol%, $P < 0.05$ and 3.8 *vs.* 11.6 mol%, $P < 0.05$, respectively). These differences in fatty acid in the OVX animals might be attenuated by treatment with 17 β -estradiol as shown in the OVXE animals. However, there were no differences in the major fatty acid composition in the aorta among the three groups. We also confirmed the effect of treatment with 17 β -estradiol in the OVXE group.

Contractile responses to KCl

The concentration-response curves for renal artery preparations from both OVX and OVXE rats exhibited a significantly greater increase in the maximum response to KCl. Compared to the control group, the contractile responses of the renal artery preparations to KCl in the OVXE group was significantly higher at both 80 mM and 100 mM KCl concentrations, and were 99.1 ± 1.6% *vs.* 107.7 ± 1.9% ($P < 0.05$), respectively at 80 mM KCl (Fig. 1). The response to 60 mM KCl showed no significant difference among the three groups. Therefore, the response to 60 mM KCl in each group was considered to be the 100% response when we compared the PE response.

Effects of L-NAME and BMY 7378 on PE concentration-responses

The PE concentration-response curve of the OVX group was shifted to the left compared to that of the control group (Fig. 2A). In addition, the EC₅₀ values to PE in the OVX were significantly lower compared to the control and OVXE groups (Table 4). The sensitivity of these PE concentration-response curves in the three groups was enhanced at low concentrations by treatment with 100 μ M L-NAME, a nitric oxide synthase inhibitor (Fig. 2B). The EC₅₀ value in the OVX group was significantly less than those in the OVXE and control groups. The PE concentration-response curves following treatment with L-NAME were not shifted with the introduction of 100 nM BMY 7378, an α_{1D} -adrenoceptor antagonist. The contractile response of the arterial smooth muscle to 1 μ M PE following L-NAME and BMY7378 treatment was significantly higher in the OVX group (141.6 ± 14.4 %) compared with both the control (65.9 ± 21.1 %) and OVXE (32.8 ± 19.5 %) groups (Fig. 2C). No significant difference was detected in the

Fig. 1. Contractile response curves for KCl in renal artery preparations isolated from the three groups. Control: sham operated rats (○); OVX: ovariectomized rats (●); OVXE: ovariectomized rats treated with 17β -estradiol for 2 weeks (□). *, Significant difference from Control ($P < 0.05$).

EC₅₀ values of the contractile responses after the treatment with BMY7378 between the three groups.

Rate of relaxation following PE-induced contraction

The rate of relaxation following a contraction was measured as the time required to reach 50% of the maximum tension ($T_{1/2}$) after the PE was washed out of the bath. This value was significantly higher in the OVX group (85 ± 13 sec) compared with the control group (47 ± 4 sec) as shown in Fig. 3A. The $T_{1/2}$ value of the relaxation in the OVXE group (47 ± 17 sec) was similar to the control group. Following treatment with $100 \mu\text{M}$ L-NAME, $T_{1/2}$ values were prolonged in all groups. The $T_{1/2}$ value in the OVX group (137 ± 19 sec) was significantly higher than that of the control group (66 ± 10 sec) (Fig. 3B). In contrast, the $T_{1/2}$ value of the relaxation in the OVXE group (84 ± 20 sec) was similar to that of the control group. To ensure the validity of $T_{1/2}$ as a measure of the relaxation rate, the exponential components of these processes were examined. The slopes were almost the same: -3.4 in controls, -3.7 in OVX and -3.6 in OVXE groups, respectively. While this indicates that the three groups have the same relaxation component, the $T_{1/2}$ value in the OVX group was significantly different to that in both the control and OVXE groups.

Fig. 2. A: Phenylephrine (PE) contractile-response curves in renal artery preparations isolated from the three groups. B: Phenylephrine (PE) contractile-response curves in renal artery preparations isolated from the three groups after treatment with 100 μ M L-NAME. *, Significant difference from control ($P<0.05$). #, Significant difference from OVX ($P<0.05$). C: Phenylephrine (PE) contractile-response curves in renal arteries isolated from the three groups after treatment with 100 μ M L-NAME and 100 nM BMY7378. Control: sham operated rats ($\circ$); OVX: ovariectomized rats ($\bullet$); OVXE: ovariectomized rats treated with 17 β -estradiol for 2 weeks ($\square$). *, Significant difference from control ($P<0.05$). #, Significant difference from OVX ($P<0.05$).

Table 4. Effects of L-NAME and BMY7378 on the EC_{50} (μ M) values to phenylephrine (PE) in renal artery preparations isolated from control, OVX and OVXE animals

Groups	Control (n=6)	OVX (n=6)	OVXE (n=6)
PE	10.8 ± 5.5	$4.1 \pm 1.2^*$	$16.2 \pm 5.6^{\#}$
PE with L-NAME	6.2 ± 3.9	$1.4 \pm 0.3^*$	$6.3 \pm 1.4^{\#}$
PE with L-NAME and BMY7378	5.7 ± 2.9	$1.5 \pm 0.3^*$	$6.5 \pm 1.7^{\#}$

Values are mean $\pm$ SEM. *, Significantly different from control ($P<0.05$). #, Significantly different from OVX ($P<0.05$).

Discussion

In the current study we have investigated the effect of estrogen on both the contractile and relaxation activity in the renal artery isolated from OVX rats. Our results revealed that the PE concentration responses curve in OVX rat preparations slightly but significantly shifted to the left compared to the response in control preparations, and that the response was prevented by continuous administration of 17 β -estradiol for 2 weeks. After treatment with 100 μ M L-NAME, the EC_{50} values of the PE concentration response curves decreased in the three groups, and it was still less in the OVX group than in the control and in OVXE groups. These results have demonstrated that ovariectomy affected PE-induced contraction of renal artery smooth muscle

Fig. 3. Time profiles of relaxation after washout PE of renal artery preparations isolated from the three groups in the absence (A) or presence of 100 μ M L-NAME (B). Control: sham operated rats ($\circ$); OVX: ovariectomized rats ($\bullet$); OVXE: ovariectomized rats treated with 17 β -estradiol for 2 weeks ($\square$). *, Significant difference from control ($P < 0.05$). #, Significant difference from OVX ($P < 0.05$).

slightly but significantly, and that the administration of 17 β -estradiol partly prevented this affect in the OVX group. Furthermore, 17 β -estradiol treatment significantly prevented the alterations of the relaxation as shown in Fig. 3. In order to identify whether the relaxation occurred via the endothelium, acetylcholine (ACh) was applied to the preparations. There was no significant difference in the ACh-induced relaxation between the three groups (data not shown), suggesting that renal arteries isolated from the three groups were endothelium intact preparations. In addition, we found that differences between the artery relaxation rates were not completely eliminated by L-NAME in all groups in this study. Therefore, our results suggest that 17 β -estradiol regulates renal artery contractile responses via alteration of Ca^{2+} transport systems, which also may be independent of the NO relaxation factor. After wash out of PE, a remarkably slower rate of relaxation was measured in preparations derived from the OVX preparations relative to those from both control and OVXE preparations. This reduced rate of relaxation could either be the result of lower intracellular Ca^{2+} extrusion or the result of an increase in the inward Ca^{2+} leak. An alternative explanation for the diminished relaxation rate following PE-induced contractions is that the rate of PE removal after washing is lower in the OVX group consequent to decreased dissociation or degradation of the PE. It is possible that Ca^{2+} pump activity in the smooth muscle of the renal arteries from the OVX rats was reduced to a larger extent than that in the control rats.

It has been reported that activation of α -adrenoceptors increases the concentration of

intracellular Ca^{2+} , which in turn produces contraction of smooth muscle cells (Leech and Faber, 1996). We have already demonstrated that prazosin, an α_1 adrenoceptor blocker, inhibited norepinephrine-induced contraction in renal arteries, and that nifedipine, a Ca^{2+} antagonist, also suppressed utilization of extracellular Ca^{2+} (Inazu *et al.*, 1991). Three subtypes of α_1 -adrenoceptors have been classified on the basis of pharmacological and molecular biology criteria (MacDougall *et al.*, 2006), and are referred to as α_{1A} , α_{1B} and α_{1D} receptors. BMY7378 is a highly selective antagonist of the α_{1D} -adrenoceptor subtype (Goetz *et al.*, 1995) and can be used to distinguish α_1 -adrenoceptor subtypes present in different tissues. Studies measuring mRNA have demonstrated that the rat renal artery expresses the α_{1D} -adrenoceptor subtype (Piascik *et al.*, 1994) at a level comparable to that in the aorta (Suzuki *et al.*, 1997). The α_{1D} -adrenoceptor significantly mediated rat renal arterial diameter (Villalobos-Molina *et al.*, 1997). Our previous report (Tajima *et al.*, 2002) showed that the α_{1D} -adrenoceptor was related to PE-induced hyperreactive contraction in diabetes. There was a slight inhibition by 100 nM BMY7378 at low PE concentration in the control group. However, 100 nM BMY7378 did not affect the PE concentration response curves in the three groups after treatment with 100 μM L-NAME.

Studies have shown that estrogen may regulate vascular tone in the cerebral artery by shifting cyclooxygenase-1 (COX-1)-dependent vasodilatation or vasoconstriction (Ospina *et al.*, 2003; Jun *et al.*, 1998). Indomethacine significantly, albeit slightly, affected contractile responses to PE in the current investigation; however, no differences were detected among the three groups (data not shown). This effect may be attributable to the inhibition of the COX-dependent release of PGI_2 . Studies have reported that estrogen increases the contraction caused by α_2 -adrenoceptor activation in the presence of clonidine, an α_2 -adrenoceptor agonist (Tejera *et al.*, 1999). Moreover, Mihmanli *et al.* (2002) reported that in the human renal artery, vascular tone is dependent on estrogen levels. We hypothesize that the effects of 17β -estradiol on renal arterial tone may regulate contraction through Ca^{2+} pump activity as shown in Fig. 3. While 17β -estradiol enhanced sensitivity to norepinephrine in the rabbit aorta with an intact endothelium (Miller *et al.*, 1990), there is also a report that in contrast 17β -estradiol induced inhibition of the PE-contractile response of vessels in female dogs (Karanian *et al.*, 1996). Our results with respect to the OVXE group are related to the latter report. However, other studies noted that the effects of 17β -estradiol on vessels display 'regional heterogeneity effects' (Tatchum-Talom *et al.*, 2002). Explanations regarding the protective effects of 17β -estradiol have been offered by several authors on the basis of the NO or NOS-dependent pathway (Beyer *et al.*, 2001; Geary *et al.*, 2000; McNeill *et al.*, 1999) and Ca^{2+} transport mechanisms (Crews *et al.*, 1999; Murphy *et al.*, 2000; Tep-areenan *et al.*, 2003). Consequently, we hypothesized that the effects of 17β -estradiol may influence several pathways of the contractile system in vascular smooth muscle. The current investigation revealed a significantly lower relaxation rate following washout of PE in the OVX group in comparison with the control group before and after treatment with L-NAME. This finding indicated that the relaxation effect of estrogen is not completely related to the NO or NOS dependent mechanism. Therefore, the relaxation effect of estrogen may be associated with the Ca^{2+} regulation system as well as with NO release in the OVX rats. These effects could be partially recovered upon 17β -estradiol treatment in the OVXE

(Fig. 3).

Several investigators reported that the relaxation effects of 17β -estradiol in coronary arteries of pigs (Murphy *et al.*, 1999), vascular smooth muscle cells (Murphy *et al.*, 2000) and in the aorta of rats (Tep-areenan *et al.*, 2003) occurred via inhibition of Ca^{2+} influx. Moreover, several studies noted that the effects of 17β -estradiol involved the modulation of Ca^{2+} -activated K^+ channels (Node *et al.*, 1997; Rosenfeld *et al.*, 2000). Tetraethylammonium (TEA), a K^+ channel inhibitor, did not affect our results (data not shown). The present investigation demonstrated the hyperreactivity of contractile responses to KCl in the OVXE rats. The contractile response to KCl is caused by an increased Ca^{2+} influx in the smooth muscle of OVXE rats relative to that in the control and OVX rats as shown in Figs 1 and 2.

Body weight exhibited meaningful albeit slight recovery; in contrast, uterine weight was not restored by administration of 17β -estradiol. The 17β -estradiol treatment, which was initiated one month post-ovariectomy, was continued at $17\text{ }\mu\text{g}$ for 2 weeks, which may, in part, account for our findings. Several investigations described implementation of 17β -estradiol treatment concomitant with ovariectomy; furthermore, this regime led to normalization of body and uterine weights of ovariectomized rats (Davidge *et al.*, 1998; Ospina *et al.*, 2003; Teede *et al.*, 2001). Alteration of fatty acid composition in the uterus of OVX rats was significantly different from that in control rats and the changes were slightly reduced by treatment with 17β -estradiol (Table 2). This phenomenon might be correlated with the dosage and treatment with 17β -estradiol in this study. Results from the current study suggested that estrogen regulates Ca^{2+} transport systems as well as NO release.

Acknowledgements

Dr. Budbazar Enkhjargal was a visiting research fellow from Ulaanbaatar Medical University in Mongolia at Faculty of Health Sciences, Showa University, Yokohama, Japan. This work was partly supported by the Smoking Research Foundation.

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