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Differential regulation of K^+ and Ca^{2+} channel gene expression by chronic treatment with estrogen and tamoxifen in rat aorta

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Abstract

The beneficial effect of estrogen on the vascular system is partly associated with its ability to reduce vascular contractility. Estrogen acutely activates large-conductance Ca^{2+}-activated K^+ channel (BK_{Ca}) and inhibits L-type voltage-gated Ca^{2+} channel (VGCC) in vascular smooth muscle cells. However, a long-term influence of estrogen, estrogen deficiency, or selective estrogen receptor modulators on gene expression of these ion channels is unclear. This study was therefore aimed to determine the relative mRNA expression levels of α- and β-subunits of BK_{Ca}, $K_V1.5$ subtype of delayed rectifier K^+ channel (K_V), and α_{1C} subunit of L-type VGCC in endothelium-denuded aortas from female rats by a semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis. Rats were divided into four experimental groups: (i) sham-operated control, (ii) ovariectomized, (iii) ovariectomized with 17β-estradiol treatment and (iv) ovariectomized with tamoxifen treatment. The results showed that ovariectomy decreased the mRNA expression of $K_V1.5$ while it increased the mRNA expression of α_{1C} subunit of L-type VGCC. Ovariectomy-induced modulation of gene expression of these ion channels was completely prevented in ovariectomized rats receiving chronic treatment with estrogen or tamoxifen. In contrast, the expression levels of genes encoding both α- and β-subunits of BK_{Ca} remained the same in the four animal groups. The present study has provided the first line of evidence suggesting the long-term beneficial effects of estrogen and tamoxifen therapy on vascular ion channel expressions, which may be an important mechanism by which the favorable modulation of vessel tone by estrogen or selective estrogen receptor modulators is mediated.

Keywords: Estrogen; Tamoxifen; Ovariectomy; Gene expression; K^+ channel; Ca^{2+} channel; (Rat)

1. Introduction

During the premenopausal years, women have a lower risk of getting cardiovascular disease than men of the same age, but this protection disappears after menopause (Barrett-Connor and Bush, 1991). Previous observational studies indicate a reduction in cardiovascular risk in postmenopausal women on estrogen replacement therapy (Barrett-Connor and Bush, 1991) although the benefit of estrogen may be outbalanced by its side effects (Writing Group for the Women's Health Initiative Investigators, 2002). The carcinogenic effect of estrogen has led to the development of selective estrogen receptor modulators, such as tamoxifen and raloxifene, as alternative hormonal therapies.

The estrogen receptor mRNA and protein are both expressed in vascular smooth muscle cells (Karas et al., 1994; Andersson et al., 2001). Estrogen induces endothelium-dependent and -independent vasorelaxation (Chan et al., 2001). Chronic treatment with estrogen or raloxifene up-regulates the expression of endothelial nitric oxide synthase and increases the bioavailability of nitric oxide (Gonzales et al., 2001; Rahimian et al., 2002). On the other hand, estrogen at pharmacological concentrations reduces vasoconstrictive responses to agonists that also activate L-type voltage-gated Ca^{2+} channels (VGCCs) (Shan et al., 1994; Andersen et al., 1999). Estrogen regulates vessel tone partly by inhibiting L-type VGCCs (Nakajima et al., 1995; Ogata et al., 1996; Kitazawa et al., 1997).

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Vessel tone development is associated with membrane potential, which is principally determined by the permeability of K^+ ions in vascular smooth muscle. Although the expression pattern of K^+ channels varies in different types and sizes of vessels (Archer et al., 1996; Michelaskis et al., 1997), BK_{Ca} and K_V channels are present in all vascular myocytes regulating the vasoconstriction (Nelson and Quayle, 1995).

Estrogen activates BK_{Ca} channels in myocytes from mammalian artery (Wellman et al., 1996; Rosenfeld et al., 2000; White et al., 2002), while putative BK_{Ca} blockers attenuate estrogen-induced vasorelaxation (Rosenfeld et al., 2000; Tsang et al., 2003). Vascular myocytes isolated from estrogen receptor β-deficient mice display a reduction in large voltage-gated outward current and the loss of outward current may be due to loss of either BK_{Ca} channels or K_V channels (Zhu et al., 2002). These findings indicate that estrogen regulation of vessel tone could involve vascular BK_{Ca} and K_V channels.

Tamoxifen exerts an estrogenic action by relaxing blood vessels (Figtree et al., 2000; Hutchison et al., 2001). Tamoxifen reduces L-type Ca^{2+} currents in arterial myocytes (Song et al., 1996). However, it remains to be determined whether tamoxifen could exert a similar stimulatory effect on vascular K^+ channels as to estrogen.

Both K^+ channels (especially the BK_{Ca} and K_V) and VGCCs are among the key regulators of vessel tone and they are likely effector molecules mediating endothelium-independent vasorelaxation to estrogen. In contrast, ATP-sensitive or inwardly rectifier K^+ channels play little role in estrogen-induced vascular effects (Nevala et al., 2001; Rosenfeld et al., 2002). We hypothesize that reduced vascular contractility following chronic treatment with estrogen or tamoxifen might involve increased expression of BK_{Ca} or K_V channels and decreased expression of L-type Ca^{2+} channels. This study was aimed to examine (i) the effect of estrogen deficiency on the mRNA levels of α- and β-subunits of BK_{Ca} (rSlo and rBKß), $K_V1.5$ subtype of K_V ($rK_V1.5$), and α_{1C} subunit of L-type VGCC (rαLCA) from rat aortic smooth muscle in ovariectomized rats, and (ii) the effects of chronic therapy with estrogen or tamoxifen so as to determine whether tamoxifen could mimic or antagonize the effect of estrogen.

2. Methods

2.1. Animals and hormonal treatment

A total of 30 female Sprague–Dawley rats of 7-week-old (195 ± 2.6 g) supplied by Laboratory Animal Service Center, Chinese University of Hong Kong were used in the present study. Rats were anesthetized with sodium pentabarbital (40 mg/kg, i.p.). Ovariectomy was performed via the mid-abdominal route. Sham-operated ovariectomized rats were used as controls. Two weeks after ovariectomy, all rats were randomly assigned to one of the three experimental groups. One group received subcutaneous implantation of slow timed-release 17β-estradiol pellet (0.5 mg/pellet, Innovative Research of America) and the other received tamoxifen (5 mg/pellet, Innovative Research of America). The animals were treated for 5 weeks. All rats were kept under conditions of controlled humidity (47–48%), temperature (24 °C) and light cycle (12 h of light, 12 h of dark). Animals were fed on normal laboratory chow and had free access to drinking water. Rat serum was obtained at the time of sacrifice by collection of aortic trunk blood followed by centrifugation. Serum levels of 17β-estradiol were determined by radioimmunoassay using ^{125}I-labeled estradiol (Diagnostics System Laboratories, Webster, TX, USA). The systolic blood pressure was measured using a tail cuff method.

2.2. Aortic artery preparation

Rats were sacrificed by cervical dislocation and aorta was excised and placed on a dissecting dish filled with autoclaved, ice-cold D-PBS (Gibco, USA) with the following composition (in g/l) NaCl 8, KCl 0.2, $NaH_2PO_4 \cdot 7H_2O$ 2.16, KH_2PO_4 0.2. After removing surrounding connective tissues and the endothelium, the thoracic segment of aorta was snap-frozen in liquid nitrogen and stored at −80 °C until RNA extraction. Removal of the endothelium in some segments was confirmed by lack of a relaxant response to 1 μM acetylcholine and histological examination. Immunoblotting also failed to detect the protein expression of endothelial nitric oxide synthase in endothelium-denuded aortic rings (data not shown).

2.3. RNA extraction and semiquantitative reverse transcription-polymerase chain reaction

Total RNA was extracted from the homogenized frozen tissues by TRIzol reagent (Invitrogen). Extracted RNA was dissolved in 40 μl diethylpyrocarbonate-treated water and quantified by measuring its absorbance at 260 nm. DNase I (1.5 μg; amplification grade)-treated RNA samples were reverse transcribed to first strand cDNA using a SuperScript preamplification system (Invitrogen) for each reverse transcription-polymerase chain reaction (RT-PCR). After reverse transcription, polymerase chain reaction (PCR) was performed in a thermal cycler (PTC-200, MJ Research). All reactions were terminated at 4 °C. The sequences of oligonucleotide primers used in this study and the conditions of PCR are listed in Table 1. Control reactions for RT-PCR were performed by replacing RNA sample for RT with diethylpyrocarbonate-treated water (no RNA control), using RNA sample that was not reverse transcribed to cDNA (no RT control) and replacing RT product with diethylpyrocarbonate-treated water (no cDNA control). The sequences of DNA being amplified by PCR were confirmed by direct DNA sequencing using

Table 1
Sequences of oligonucleotide primers and conditions of PCR

Primer	Sequences	PCR conditions	GenBank accession number
Rat α-subunit of BK_{Ca} (rSlo)		94 °C for 1 min, 55 °C for 1 min,	AF135265
5′ -Primer	5′ -CAA GAT GGA TGC GCT CAT CA-3′	72 °C for 1 min for 30 cycles,	
3′ -Primer	5′ -TAG AAA TTC TGG CAG GAT TC-3′ (spanning nucleotides 541-978)	72 °C for 10 min	
Rat β-subunit of BK_{Ca} (rBKβ)		94 °C for 1 min, 55 °C for 1 min,	U54495
5′ -Primer	5′ -AAG CTG GTG ATG GCC CAG AA-3′	72 °C for 1 min for 34 cycles,	
3′ -Primer	5′ -TGG TTT TGA TCC CGA GTG TC-3′ (spanning nucleotides 61–353)	72 °C for 10 min	
Rat α1C of VGCC (rαLCA)		94 °C for 1 min, 52 °C for 1 min,	L04684
5′ -Primer	5′ -CCT TCC AGG CCC TGC CGT AT-3′	72 °C for 1 min for 33 cycles,	
3′ -Primer	5′ -GTT GTC CAT GAT GAC AGC CA-3′ (spanning nucleotides 1649–2010)	72 °C for 10 min	
Rat $K_V1.5$ ($rK_V1.5$)		94 °C for 1 min, 53 °C for 1 min,	M27158
5′ -Primer	5′ -GCC ATC CTC AGG GTG AT-3′	72 °C for 1 min for 32 cycles,	
3′ -Primer	5′ -GCC CAC CAG AAG GCA TC-3′ (spanning nucleotides 1916–2148)	72 °C for 10 min	
Rat GAPDH		Same conditions as to rSlo, rBKß, rαLCA and $rK_V1.5$	M17701
5′ -Primer	5′ -ACC ACA GTC CAT GCC ATC AC-3′		
3′ -Primer	5′ -TCC ACC ACC CTG TTG CTG TA-3′ (spanning nucleotides 550–1001)		

a DNA analyzer (ABI Prism 310 Genetic Analyzer, Applied Biosystems).

Semiquantitative RT-PCR analysis of α- and β-subunits of BK_{Ca} (rSlo and rBKβ), α_{1C} subunit of VGCC (rαLCA), and $rK_V1.5$ was performed. Aortic RNA (8 μl; 1.5 μg) was treated with DNase I and reverse transcribed, and 2 μl of the resulting cDNA samples were subsequently used in PCRs for rSlo, rBKβ, rαLCA, $rK_V1.5$, and GAPDH. The optimal PCR conditions were determined so that the amplifications were within the exponential phase (linear range) of PCR with respect to the amount of input RNA. Separate reactions were also performed in parallel using primers for rat GAPDH. After PCR, the products were resolved on a 1.5% agarose gel with ethidium bromide. The fluorescent images were captured under UV transillumination with a CCD camera (Flurochem Alpha Innotech Imaging System, San Leandro, CA) and recorded on black and white photographs. The intensities of the bands were converted into digitalized signals with an image scanner and quantified by a gel documentation program (Flurochem, Alpha Innotech). Signal intensities of the rat rSlo, rBKβ, rαLCA, and $rK_V1.5$ products were normalized to those of rat GAPDH products as ratios to produce arbitrary units of relative abundance.

2.4. Statistical analysis

Results are expressed as mean ± S.E.M. of *n* aortic rings prepared from separate rats. Comparisons between groups of data were made by one-way analysis of variance (ANOVA) followed by Newman–Keuls comparison test. A level of probability (*P*) of less than 0.05 was considered significant.

3. Results

3.1. Animal treatment

17β-Estradiol but not tamoxifen therapy increased the serum levels of 17β-estradiol and uterine weight in ovariectomized rats (Table 2). The body weight of estrogen- or tamoxifen-treated ovariectomized rats was significantly less than untreated ovariectomized rats. The

Table 2
Effects of treatment with estrogen or tamoxifen of ovariectomized rats on serum 17β-estradiol levels, uterine weight, body weight, and blood pressure

Parameter	Control	Ovx	Ovx + E_2	Ovx + Tam
Serum 17β-estradiol (pg/ml)	21.7 ± 2.5	5.7 ± 0.7[a]	63.6 ± 7.1[a,b]	6.8 ± 1.5[a]
Uterine weight (mg)	477 ± 45	81 ± 3[a]	379 ± 37[a,b]	135 ± 15[a]
Body weight (g)	265 ± 6.5	342 ± 4.9[a]	260 ± 4.1[b]	262 ± 4.4[b]
Blood pressure (mm Hg)	115.1 ± 9.5	119.7 ± 6.3	111.5 ± 6.1	105.4 ± 4.9

Values of mean ± S.E.M. of six to eight animals. Statistical significance ($P < 0.05$) is indicated by [a] between control and ovariectomized (Ovx) groups and [b] between ovariectomized and groups treated with 17β-estradiol (Ovx + E_2) or with tamoxifen (Ovx + Tam).

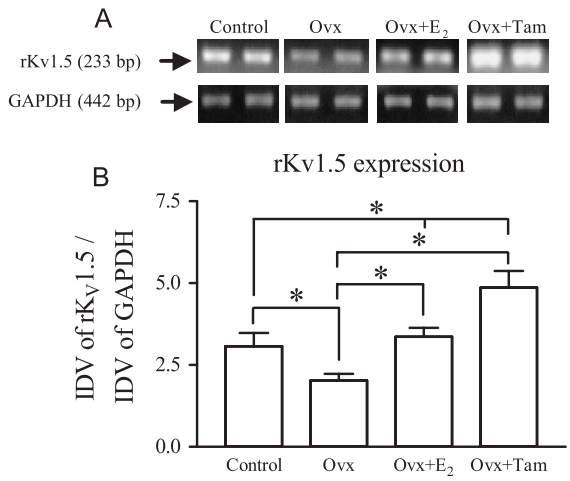

Fig. 1. Semiquantitative RT-PCR analysis demonstrating the expression of aortic $K_V1.5$ mRNA transcripts in different experimental groups (control, Ovx, Ovx + 17β-estradiol, and Ovx + tamoxifen) using gene-specific primer for $K_V1.5$. (A) RT-PCR result of $K_V1.5$ (233 bp) and GAPDH (442 bp). (B) Relative expression of aortic $K_V1.5$ mRNA normalized with the internal marker GAPDH. Results are means ± S.E.M. of six to eight rats. Significant difference is indicated (*$P < 0.05$).

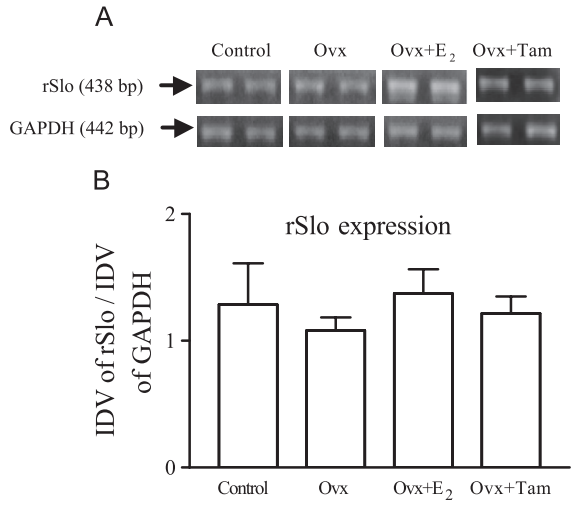

Fig. 2. Semiquantitative RT-PCR analysis demonstrating the expression of aortic α-subunit of BK_{Ca} channel (rSlo) mRNA transcripts in different experimental groups (control, Ovx, Ovx + 17β-estradiol, and Ovx + tamoxifen) using gene-specific primer for rSlo. (A) RT-PCR result of rSlo (438 bp) and GAPDH (442 bp). (B) Relative expression of aortic rSlo mRNA normalized with the internal marker GAPDH. Results are means ± S.E.M. of six to eight rats.

systolic blood pressure was similar in all animal groups (Table 2).

3.2. mRNA expression of $K_V1.5$ channels

The amount of $K_V1.5$ mRNA in aortas from ovariectomized rats was lower as compared to that from sham-operated controls (Fig. 1). The analysis showed that the aortas had a 34% decrease in $K_V1.5$ mRNA transcript level after ovariectomy ($P < 0.05$, compared with control) and this decrease was prevented in 17β-estradiol-replaced ovariectomized rats ($P < 0.05$). Chronic treatment with tamoxifen exerted an estrogenic action, fully reversing ovariectomy-induced reduction in the level of K_V mRNA transcript (Fig. 1B). Tamoxifen was more effective than 17β-estradiol ($P < 0.05$) in increasing the mRNA expression. Both tamoxifen and 17β-estradiol up-regulated the aortic $K_V1.5$ mRNA expression in ovariectomized rats by ~ 40% and ~ 66%, respectively. Tamoxifen-treated aortas had a significantly higher level of $K_V1.5$ mRNA transcript as compared with the control aortas ($P < 0.05$).

3.3. mRNA expression of α- and β-subunit of BK_{Ca} channels (rSlo and rBKβ)

Either ovariectomy or chronic therapy with estrogen or tamoxifen exhibited no significant effects on the mRNA levels of α-subunit (rSlo) of BK_{Ca} channels, as compared to their effects on $K_V1.5$ mRNA expression. The relative levels of mRNA transcript for rSlo in aortas were similar in all groups (Fig. 2). Similarly, the mRNA expression of β-subunit (rBKβ) was unaltered by either estrogen withdrawal or chronic treatment with estrogen or tamoxifen (Fig. 3).

3.4. mRNA expression of α_{1c} subunit of VGCC (rαLCA)

The mRNA level of α_{1C} subunit of VGCC (rαLCA) was significantly up-regulated in aortas from ovariectomized rats (Fig. 4). Ovariectomy induced an approximately 80% increase in the levels of rαLCA mRNA transcript as compared with sham-operated controls ($P < 0.05$). This increase trend was reversed by chronic 17β-estradiol treatment. Tamoxifen

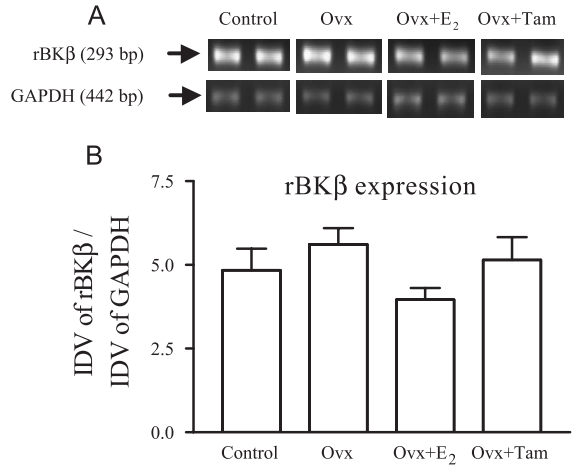

Fig. 3. Semiquantitative RT-PCR analysis demonstrating the expression of aortic β-subunit of BK_{Ca} channel (rBKβ) mRNA transcripts in different experimental groups (control, Ovx, Ovx + 17β-estradiol, and Ovx + tamoxifen) using gene-specific primer for rBKβ. (A) RT-PCR result of rBKß (293 bp) and GAPDH (442 bp). (B) Relative expression of aortic rSlo mRNA normalized with the internal marker GAPDH. Results are means ± S.E.M. of six to eight rats.

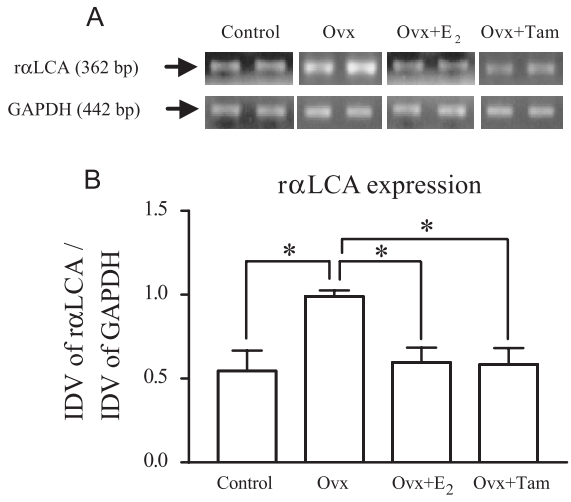

Fig. 4. Semiquantitative RT-PCR analysis demonstrating the expression of aortic α_{1C} subunit of VGCC (rαLCA) mRNA transcripts in different experimental groups (control, Ovx, Ovx + 17β-estradiol, and Ovx + tamoxifen) using gene-specific primer for rαLCA. (A) RT-PCR result of rαLCA (362 bp) and GAPDH (442 bp). (B) Relative expression of aortic $K_V1.5$ mRNA normalized with the internal marker GAPDH. Results are means ± S.E.M. of six to eight rats. Significant difference is indicated (*$P < 0.05$).

mimicked the effect of 17β-estradiol in up-regulating the α_{1C} mRNA expression. Both 17β-estradiol and tamoxifen were equally effective in inhibiting the effect of ovariectomy with approximately 40% reduction in the rαLCA mRNA expression ($P < 0.05$, Fig. 4).

4. Discussion

A direct muscle relaxant effect of estrogen may involve multiple mechanisms, such as attenuation of agonist-induced vascular smooth muscle contraction, activation of K^+ channels, and inhibition of Ca^{2+} channels in vascular smooth muscle cells. The present study is, however, the first report to demonstrate the modulatory effect of chronic treatment with estrogen or tamoxifen on the gene expression of vascular ion channels. The results are summarized as follows. (i) Estrogen treatment up-regulated the mRNA expression of vascular $K_V1.5$ channels. (ii) Estrogen treatment down-regulated the mRNA expression of α_{1C} subunit of vascular VGCCs. (iii) Tamoxifen exerted an estrogen-like effect on gene expression of $K_V1.5$ channels and α_{1C} subunit of VGCCs. (iv) Treatment with estrogen or tamoxifen did not affect the mRNA expression of both α- and β-subunits of vascular BK_{Ca} channels.

K_V α-subunit proteins ($K_V1.5$) are expressed in mammalian vascular smooth muscle cells at both mRNA (Clement-Chomienne et al., 1999; Cox et al., 2001) and protein levels (Cheong et al., 2001; Coppock and Tamkun, 2001). The K_V channel exists as hetero- or homotetramers (Korovkina and England, 2002) and several studies have shown that $K_V1.5$ is the dominant species present in vascular smooth muscle cells (Cheong et al., 2001; Cox et al., 2001). Down-regulation of $rK_V1.5$ mRNA expression following ovariectomy and reversal of this effect by estrogen therapy indicates that the circulating estrogen contributes to the regulation of gene expression of $K_V1.5$ channels in rat aortas. It remains to be determined whether estrogen exerts a physiological or pharmacological effect since the serum levels of 17β-estradiol in estrogen-treated ovariectomized rats were three folds higher than the sham-operated controls. No changes in uterine vessel estrogen receptor α and β expression are observed during the estrous cycle (Andersson et al., 2001). However, it is at present unknown whether the $K_V1.5$ mRNA expression is influenced by the stage of the estrous cycle. Although $K_V1.5$ channels have been identified in vascular myocytes (Berger et al., 1998; Belevych et al., 2002), their physiological role is still not fully understood. Activation of K_V channels by membrane depolarization may serve as a negative feedback mechanism which limit further membrane depolarization and decreases Ca^{2+} influx through L-type VGCCs. Estrogen-induced vasorelaxation is inhibited by 4-aminopyridine, a potent K_V channel inhibitor (Tsang et al., 2003), while the chronic effect of estrogen on the vascular function of K_V channels is unknown. If the effect of estrogen on K_V channel gene expression in aortas could be extended to other vascular beds and also that changes in the mRNA levels could be extrapolated to similar changes in the channel protein levels, our results would suggest that reduced vessel contractility after chronic estrogen therapy might in part result from an increased expression and function of vascular K_V channels.

In contrast, the mRNA levels of both α- and β-subunits of vascular BK_{Ca} channels were unaffected by ovariectomy or in ovariectomized rats receiving estrogen therapy. Estrogen relaxes the arteries partly via opening BK_{Ca} channels (White et al., 2002), probably through direct binding of estrogen to the β-subunit (Valverde et al., 1999). It appears that estrogen could activate vascular BK_{Ca} channels through a non-genomic mechanism probably without having a chronic impact on the gene expression. A tissue-selective influence of estrogen on the K^+ channel mRNA or protein expression may exist. For example, estrogen can up-regulate BK_{Ca} channel β_1 subunit transcript expression in the myometrium from both control and ovariectomized mice (Benkusky et al., 2002).

L-type VGCC pore-forming α-subunit, α_{1C} (Striessnig, 1999), is one of the main pathways of Ca^{2+} entry in vascular smooth muscle cells (Welling et al., 1997; Ohya et al., 2001). Any change in the gene expression of α_{1C} gene (rαLCA) and its protein level would affect the contractile state of vascular myocytes. Estrogen inhibits L-type VGCC current in both cultured and freshly isolated vascular myocytes (Shan et al., 1994; Nakajima et al.,

1995; Ogata et al., 1996) without modulating vasorelaxation induced by VGCC blockers (Tsang et al., 2002). The present study demonstrates that the rαLCA mRNA was up-regulated following ovariectomy while its expression returned to the control level in ovariectomized rats receiving estrogen treatment. This indicates chronic influence of the circulating estrogen on the gene expression of the pore-forming unit of vascular L-type VGCCs. Estrogen regulates the VGCC expression but in nonvascular myocytes. Cardiac L-type VGCC protein level is increased in estrogen-deficient mice (Johnson et al., 1997). Ovariectomy increases and estrogen decreases the L-type VGCC density in rabbit myocardium (Patterson et al., 1998). Collins et al. (1993) were the first to propose that some of the cardiovascular benefits of estrogen therapy is likely due to a long-term calcium antagonistic effect of estrogen. Therefore, our results may have clinical implications. L-type VGCC is a critical element in the vascular responses to vasoconstrictors. Chronic estrogen treatment inhibits vasoconstriction induced by various agonists (Sudhir et al., 1997; Andersen et al., 1999; Bowyer et al., 2001) and these constrictors contract the arteries largely through activation of L-type VGCCs. The present results show that estrogen reduced the mRNA expression of α_{1C} of VGCCs in aortas. This effect may explain some of the cardioprotective effects of estrogen.

Tamoxifen, the first synthetic selective estrogen receptor modulator, is a mixed agonist/antagonist of estrogen receptor. Limited studies have shown that tamoxifen could be an estrogenic agent in blood vessels. For example, tamoxifen reduces vasoconstrictions (Figtree et al., 2000; Hutchison et al., 2001), while it inhibits L-type VGCC currents in arterial myocytes (Song et al., 1996). As demonstrated in this study, tamoxifen exerts a similar effect as to estrogen on the mRNA expression of aortic ion channels. Like estrogen, tamoxifen treatment increased mRNA expression of $K_V1.5$ channels and decreased expression of α-subunit of L-type of VGCC without affecting expression of both α- and β-subunits of BK_{Ca} channels. The relative level of $K_V1.5$ mRNA from aortas was higher in tamoxifen-treated than 17β-estradiol-treated ovariectomized rats; whereas, the uterine weight was only slightly increased by tamoxifen. These findings indicate that tamoxifen may act as a full agonist for the estrogen receptors that mediate its effect on aortic $K_V1.5$ transcript expression. Despite the acute and non-genomic relaxation induced by tamoxifen, the benefit of chronic use of selective estrogen receptor modulators on the vascular reactivity is still unclear. This study indicates that suppression of L-type VGCC gene expression could be involved in the inhibitory effect of tamoxifen treatment on vasoconstriction.

The K_V current is enhanced while the L-type Ca^{2+} current is suppressed in vascular myocytes from normotensive than hypertensive rats (Cox and Lozinskaya, 1995; Martens and Gelband, 1996). Although it is unclear whether such changes are the direct cause or consequence of a hypertensive condition, our data may explain that reduced vasoconstriction by estrogen therapy could be partly caused by the enhanced function and expression of vascular K_V channels and reduced function and expression of vascular L-type VGCC. This may represent an important mechanism underlying a long-term benefit of estrogen therapy on blood pressure after menopause (Cagnacci et al., 1999; Cacciatore et al., 2001).

In summary, we have provided new evidence showing that chronic estrogen treatment increases the mRNA expression of $rK_V1.5$ channels but decreases the mRNA expression of rαLCA channels in aortas from ovariectomized rats. Such changes in gene expression may be associated with the reduced vasoconstriction in estrogen-replaced rats. In contrast, the mRNA expression of both α- and β-subunits of vascular BK_{Ca} channels is unaffected by ovariectomy or estrogen therapy in ovariectomized rats. Another novel finding of this study is the estrogen-like effect of tamoxifen on the gene expression of $rK_V1.5$ and rαLCA channels, thus indicating a benefit of using this agent in the vascular system. Further work will be undertaken (i) to examine chronic impacts of therapy with the selective estrogen receptor modulators on the functional expression of these ion channels in resistance arteries since these vessels contribute primarily to peripheral flow resistance and to the maintenance of blood pressure, and (ii) to determine whether the estrogenic effects are mediated through estrogen receptors by using aromatase inhibitors or selective estrogen receptor antagonists.

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References

Andersen, H.L., Jweis, J.U., Fjalland, B., Korsgaard, N., 1999. Effect of acute and long-term treatment with 17β-estradiol on the vasomotor responses in the rat aorta. Br. J. Pharmacol. 126, 159–168.

Andersson, C., Lydrup, M.L., Ferno, M., Idvall, I., Gustafsson, J., Nilsson, B.O., 2001. Immunocytochemical demonstration of oestrogen receptor β in blood vessels of the female rat. J. Endocrinol. 169, 241–247.

Archer, S.L., Huang, J.M.C., Reeve, H.L., Hampl, V., Tolarova, S., Michelakis, E.D., Weir, G.K., 1996. Differential distribution of electrophysiologically distinct myocytes in conduit and resistance arteries determines their response to nitric oxide and hypoxia. Circ. Res. 78, 431–442.

Barrett-Connor, E., Bush, T.L., 1991. Estrogen and coronary heart disease in women. JAMA 265, 1861–1867.

Belevych, A.E., Beck, R., Tammaro, P., Poston, L., Smirnov, S.V., 2002. Developmental changes in the functional characteristics and expression

of voltage-gated K^+ channel currents in rat aortic myocytes. Cardiovasc. Res. 54, 152–161.

Benkusky, N.A., Korovkina, V.P., Brainard, A.M., England, S.K., 2002. Myometrial maxi-K channel β1 subunit modulation during pregnancy and after 17β-estradiol stimulation. FEBS Lett. 524, 97–102.

Berger, M.G., Vandier, C., Bonnet, P., Jackson, W.F., Rusch, N.J., 1998. Intracellular acidosis differentially regulates K_V channels in coronary and pulmonary vascular muscle. Am. J. Physiol. 275, H1351–H1359.

Bowyer, L., Brown, M.A., Jones, M., 2001. Vascular reactivity in men and women of reproductive age. Am. J. Obstet. Gynecol. 185, 88–96.

Cacciatore, B., Paakkari, I., Hasselblatt, R., Nieminen, M.S., Toivonen, J., Tikkanen, M.I., Ylikorkala, O., 2001. Randomized comparison between orally and transdermally administered hormone replacement therapy regimens of long-term effects on 24-hour ambulatory blood pressure in postmenopausal women. Am. J. Obstet. Gynecol. 184, 904–909.

Cagnacci, A., Rovati, L., Zanni, A., Malmusi, S., Facchinetti, F., Volpe, A., 1999. Physiological doses of estradiol decrease nocturnal blood pressure in normotensive postmenopausal women. Am. J. Physiol. 276, H1355–H1360.

Chan, H.Y., Yao, X., Tsang, S.Y., Chan, F.L., Lau, C.W., Huang, Y., 2001. Different role of endothelium/nitric oxide in 17β-estradiol- and progesterone-induced relaxation in rat arteries. Life Sci. 69, 1609–1617.

Cheong, A., Dedman, A.M., Beech, D.J., 2001. Expression and function of native potassium channel Kv α1 subunits in terminal arterioles of rabbit. J. Physiol. 534, 691–700.

Clement-Chomienne, O., Ishii, K., Walsh, M.P., Cole, W.C., 1999. Identification, cloning and expression of rabbit vascular smooth muscle Kv1.5 and comparison with native delayed rectifier K^+ current. J. Physiol. 515, 653–667.

Collins, P., Rosano, G.M., Jiang, C., Lindsay, D., Sarrel, P.M., Poole-Wilson, P.A., 1993. Cardiovascular protection by oestrogen—a calcium antagonist effect? Lancet 341, 1264–1265.

Coppock, E.A., Tamkun, M.M., 2001. Differential expression of K_V channel α- and β-subunits in the bovine pulmonary arterial circulation. Am. J. Physiol., Lung Cell. Mol. Physiol. 281, L1350–L1360.

Cox, R.H., Lozinskaya, I.M., 1995. Augmented calcium currents in mesenteric artery branches of the spontaneously hypertensive rat. Hypertension 26, 1060–1064.

Cox, R.H., Folander, K., Swanson, R., 2001. Differential expression of voltage-gated K^+ channel genes in arteries from spontaneously hypertensive and Wistar Kyoto rats. Hypertension 37, 1315–1322.

Figtree, G.A., Webb, C.M., Collins, P., 2000. Tamoxifen acutely relaxes coronary arteries by an endothelium-, nitric oxide-, and estrogen receptor-dependent mechanism. J. Pharmacol. Exp. Ther. 295, 519–523.

Gonzales, R.J., Walker, B.R., Kanagy, N.L., 2001. 17β-Estradiol increases nitric oxide-dependent dilation in rat pulmonary arteries and thoracic aorta. Am. J. Physiol., Lung Cell. Mol. Physiol. 280, L555–L564.

Hutchison, S.J., Chou, T.M., Chatterjee, K., Sudhir, K., 2001. Tamoxifen is an acute, estrogen-like, coronary vasodilator of porcine coronary arteries in vitro. J. Cardiovasc. Pharmacol. 38, 657–665.

Johnson, B.D., Zheng, W., Korach, K.S., Scheuer, T., 1997. Increased expression of the cardiac L-type calcium channel in estrogen receptor-deficient mice. J. Gen. Physiol. 110, 135–140.

Karas, R.H., Patterson, B.L., Mendelsohn, M.E., 1994. Human vascular smooth muscle cells contain functional estrogen receptor. Circulation 89, 1943–1950.

Kitazawa, T., Hamada, E., Kitazawa, K., Gaznabi, A.K., 1997. Non-genomic mechanism of 17 β-oestradiol-induced inhibition of contraction in mammalian vascular smooth muscle. J. Physiol. 499, 497–511.

Korovkina, V.P., England, S.K., 2002. Molecular diversity of vascular potassium channel isoforms. Clin. Exp. Pharmacol. Physiol. 29, 317–323.

Martens, J.R., Gelband, C.H., 1996. Alterations in rat interlobar artery membrane potential and K^+ channels in genetic and nongenetic hypertension. Circ. Res. 79, 295–301.

Michelaskis, E.D., Reeve, H.L., Huang, J.M., Tolarova, S., Nelson, D.P., Weir, E.K., Archer, S.L., 1997. Potassium channel diversity in vascular smooth muscle cells. Can. J. Physiol. Pharm. 75, 889–897.

Nakajima, T., Kitazawa, T., Hamada, E., Hazama, H., Omata, M., Kurachi, Y., 1995. 17β-Estradiol inhibits the voltage-dependent L-type Ca^{2+} currents in aortic smooth muscle cells. Eur. J. Pharmacol. 294, 625–635.

Nelson, M.T., Quayle, J.M., 1995. Physiological roles and properties of potassium channels in arterial smooth muscle. Am. J. Physiol., Cell Physiol. 268, C799–C822.

Nevala, R., Paukku, K., Korpela, R., Vapaatalo, H., 2001. Calcium-sensitive potassium channel inhibitors antagonize genistein- and daidzein-induced arterial relaxation in vitro. Life Sci. 69, 1407–1417.

Ogata, R., Inoue, Y., Nakano, H., Ito, Y., Kitamura, K., 1996. Oestradiol-induced relaxation of rabbit basilar artery by inhibition of voltage-dependent Ca channels through GTP-binding protein. Br. J. Pharmacol. 117, 351–359.

Ohya, S., Yamamura, H., Muraki, K., Watanabe, M., Imaizumi, Y., 2001. Comparative study of the molecular and functional expression of L-type Ca^{2+} channels and large-conductance, Ca^{2+}-activated K^+ channels in rabbit aorta and vas deferens smooth muscle. Pflügers Arch. 441, 611–620.

Patterson, E., Ma, L., Szabo, B., Robinson, C.P., Thadani, U., 1998. Ovariectomy and estrogen-induced alterations in myocardial contractility in female rabbits: role of the L-type calcium channel. J. Pharmacol. Exp. Ther. 284, 586–591.

Rahimian, R., Dube, G.P., Toma, W., Dos Santos, N., McManus, B.M., van Breemen, C., 2002. Raloxifene enhances nitric oxide release in rat aorta via increasing endothelial nitric oxide mRNA expression. Eur. J. Pharmacol. 434, 141–149.

Rosenfeld, C.R., White, R.E., Roy, T., Cox, B.E., 2000. Calcium-activated potassium channels and nitric oxide coregulate estrogen-induced vasodilation. Am. J. Physiol.: Heart Circ. Physiol. 279, H319–H328.

Rosenfeld, C.R., Roy, T., Cox, B.E., 2002. Mechanisms modulating estrogen-induced uterine vasodilation. Vasc. Pharmacol. 38, 115–125.

Shan, J., Resnick, L.M., Liu, Q.Y., Wu, X.C., Barbagallo, M., Pang, P.K.T., 1994. Vascular effects of 17β-estradiol in male Sprague–Dawley rats. Am. J. Physiol.: Heart Circ. Physiol. 266, H967–H973.

Song, J., Standley, P.R., Zhang, F., Joshi, D., Gappy, S., Sowers, J.R., Ram, J.L., 1996. Tamoxifen (estrogen antagonist) inhibits voltage-gated calcium current and contractility in vascular smooth muscle from rats. J. Pharmacol. Exp. Ther. 277, 1444–1453.

Striessnig, J., 1999. Pharmacology, structure and function of cardiac L-type Ca^{2+} channels. Cell. Physiol. Biochem. 9, 242–269.

Sudhir, K., Ko, E., Zellner, C., Wong, H.E., Hutchison, S.J., Chou, T.M., Chatterjee, K., 1997. Physiological concentrations of estradiol attenuate endothelin 1-induced coronary vasoconstriction in vivo. Circulation 96, 3626–3632.

Tsang, S.Y., Yao, X., Chan, H.Y., Chen, Z.Y., Ming, W.C., Huang, Y., 2002. Effect of 17β-estradiol exposure on vasorelaxation induced by K^+ channel openers and Ca^{2+} channel blockers. Pharmacology 65, 26–31.

Tsang, S.Y., Yao, X., Chan, H.Y., Wong, C.M., Chen, Z.Y., Au, C.L., Huang, Y., 2003. Contribution of K^+ channels to relaxation induced by 17β-estradiol but not by progesterone in isolated rat mesenteric artery rings. J. Cardiovasc. Pharmacol. 41, 4–13.

Valverde, M.A., Rojas, P., Amigo, J., Cosmelli, D., Orio, P., Bahamonde, M.I., Mann, G.E., Vergara, C., Latorre, R., 1999. Acute activation of Maxi-K channels (hSlo) by estradiol binding to the β subunit. Science 285, 1929–1931.

Welling, A., Ludwig, A., Zimmer, S., Klugbauer, N., Flockerzi, V., Hofmann, F., 1997. Alternatively spliced IS6 segments of the α1C gene determine the tissue-specific dihydropyridine sensitivity of cardiac and vascular smooth muscle L-type Ca^{2+} channels. Circ. Res. 81, 526–532.

Wellman, G.C., Bonev, A.D., Nelson, M.T., Brayden, J.E., 1996. Gender differences in coronary artery diameter involve estrogen, nitric oxide, and Ca^{2+}-activated K^+ channels. Circ. Res. 79, 1024–1030.

White, R.E., Han, G., Maunz, M., Dimitropoulou, C., El-Mowafy, A.M.,

Mendelsohn, M.E., Catravas, J.D., Snead, C., Carrier, G.O., Zhu, S., Yu, X., 2002. Endothelium-independent effect of estrogen on Ca^{2+}-activated K^{+} channels in human coronary artery smooth muscle cells. Cardiovasc. Res. 53, 650–661.

Writing Group for the Women's Health Initiative Investigators, 2002. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA 288, 321–333.

Zhu, Y., Bian, Z., Lu, P., Karas, R.H., Bao, L., Cox, D., Hodgin, J., Shaul, P.W., Thoren, P., Smithies, O., Gustafsson, J.A., Mendelsohn, M.E., 2002. Abnormal vascular function and hypertension in mice deficient in estrogen receptor β. Science 295, 505–508.