Preferential Reduction of Na^+/K^+ ATPase $\alpha 3$ by 17β-Estradiol Influences Contraction Frequency in Rat Uteri

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Abstract

One $\beta 1$ and two α ($\alpha 1$ and $\alpha 3$) isoforms of Na^+/K^+-ATPase exist in rat uteri. Previous immunocytochemistry studies have suggested that the $\alpha 3$ isoform may be involved in calcium regulation indirectly. Estrogens are known to both modulate Na^+/K^+-ATPase activities in non-uterine tissues and suppress spontaneous uterine contractions in rats. Thus the purpose of this study was to examine the correlation between estrogens-modulated uterine contraction and the expression of Na^+/K^+-ATPase $\alpha 3$ isoform in rats. After 1-, 2-, and 4- day treatments with 17β-estradiol (E_2, 5 μg/ml/kg, s.c., daily), the diameter of uterine horn was measured. The contraction force of uterine strips was measured by standard muscle bath apparatus. The protein abundance and enzyme activity of Na^+/K^+-ATPase in rat uteri were measured by Western blot analysis and ATPase assay, respectively. One day of E_2 decreased both contraction frequency and $\alpha 3$-protein expression without the change in uterine diameter, enzyme activity or other isoforms. Two days of E_2 reduced contraction frequency, the enzyme activity, as well as $\alpha 3$- and $\beta 1$- protein abundance but increased $\alpha 1$-protein and uterine diameter. Four days of E_2 elicited similar effects as two days of E_2, but did not affect $\alpha 1$-protein abundance. In conclusion, E_2 elicits differential effects on isoform expression. After 1-day treatment with 17β-estradiol, the decrease in the expression of $\alpha 3$ and $\beta 1$ without a change in Na^+/K^+-ATPase activity suggests that some isoform other than $\beta 1$ exist in rat uteri. The positive correlation between the reduction of $\alpha 3$-and the decrease of contraction frequency suggests the involvement of $\alpha 3$ isoform in uterine oscillation.

Key Words: 17β-estradiol, uterus, Na^+/K^+-ATPase, contraction, oscillations

Introduction

Na^+/K^+-ATPase, a heterodimeric transmembrane protein complex, is composed of a catalytic α and glycosylated β subunits (17, 28). The former contains the binding sites for Na^+, K^+, ATP, Pi and ouabain (a specific inhibitor for Na^+/K^+-ATPase (6). The latter may help to assemble the α subunit and transport it to the plasma membrane (32). Activation of Na^+/K^+-ATPase triggers the exchange of two K^+ into and three Na^+ out of the cells (7). The imbalance of sodium homeostasis may in turn alter various cellular functions (16, 22).

Three isoforms ($\alpha 1$, $\alpha 2$, and $\alpha 3$) of α-subunit, encoded by a multigene family, exhibit various properties. The affinity of $\alpha 1$ to ouabain, a Na^+/K^+-ATPase antagonist, is much less than that of $\alpha 2$ and $\alpha 3$. In terms of localization, $\alpha 1$ appears in most tissues, $\alpha 2$ exists in fetal hearts and brains, and $\alpha 3$ is present in muscles and fetal brains. In the heart, $\alpha 1$ is ubiquitously expressed over the surfaces of these cells, whereas $\alpha 2$ are confined to a reticular distribution within plasma membrane that paralleled the underlying endoplasmic or sarcoplasmic reticulum where calcium is stored. Colocalization of Na^+/K^+-ATPase $\alpha 2$ with Na^+/Ca^{2+} exchanger on plasma membrane suggests that $\alpha 1$ may regulate bulk cytosolic Na^+, whereas $\alpha 2$ regulate Na^+ and, indirectly, Ca^{2+} (12, 27). Because intracellular calcium is known to influence muscle contraction, the unique

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Received September 30, 1999; Revised December 17, 1999; Accepted December 20, 1999.

localization implies the possible involvement of Na^+/K^+-ATPase α2 isoform in cardiac muscle contraction. However, it is not clear if the Na^+/K^+-ATPase α2 isoform is associated with smooth muscle contractions.

Estrogens are known to modify smooth muscle contraction. In uteri prolonged treatment with 17β-estradiol facilitates oxytocin-induced contractions due to the induction of oxytocin receptors (9), but suppresses spontaneous uterine contractions in rats (4, 21). However, the mechanism of estrogens' functions on spontaneous contraction in the uteri is not well understood. It has been reported that the induction of Na^+/K^+-ATPase α2 is associated with thyroxin-induced cardiac contractions (11). Thus, the purpose of this study was to examine the relationship between Na^+/K^+-ATPase α isoforms and muscle contraction after 17β-estradiol treatments in rat uteri.

The effects of 17β-estradiol on Na^+/K^+-ATPase activity in various non-uterine tissues has been reported. In vivo treatment with 17β-estradiol increases Na^+/K^+-ATPase activity in cardiac tissue (5) but inhibits the activity in the ileum (26). In vitro treatment with 17β-estradiol inhibits Na^+/K^+-ATPase activity in rat astrocytes (8). In brains 17β-estradiol increases the activity in the medial preoptic-suprachiasma region, but decreases the activity in the mediobasal hypothalamus region (24).

Even though the effect of estrogens on uterine Na^+/K^+-ATPase activity has not been well characterized in uteri, the mRNA expression of Na^+/K^+-ATPase isoforms α1 and α3 has been identified (30, 12). Compared to ovariectomized rats, pregnant rats have been reported to express greater mRNA expression of Na^+/K^+-ATPase isoforms α1 and α3 (30). Therefore, the present study was designed to examine whether 1-, 2, and 4- day treatment of 17β-estradiol affected the protein abundance of α3 isoform- and enzyme activity. The time-dependent changes in α1-, α3-, β1- protein, enzyme activity, and uterine contractions allow us to examine the relationship between α3 isoform and spontaneous contractions.

Materials and Methods

Animals and Treatments

Female Sprague-Dawley rats weighing 200-300 g housed in the colony at The Animal Center of National Cheng Kung University Medical College at 24±1 °C under a 14-h light (0500-1900) schedule were used in these experiments. Two weeks after ovariectomy, 24 rats were divided equally into three groups: the 1-day, 2-day, and 4-day group. Each group was then divided into two subgroup and each subgroup of four was then treated with 17β-estradiol (5 μg/ml/kg, s.c.), or solvent (as control) daily between 0900 h and 1000 h. The treated rats were sacrificed 24 hours after the last dose. The removed uteri were divided into two parts: one for measuring contraction force and the other for preparing tissue homogenates.

Preparation of Tissue Homogenates

Tissue homogenates were prepared based on a modification of the method of Turi *et al.* (31). Uterine horns were kept in homogenizing solution of the following composition: 150 mM-sucrose, 30 mM histidine, 1 mM EGTA, 1% deoxycholate, and 0.1 M PMSF. After adipose tissue was trimmed, the minced uteri were grounded with Tissue Tearor (Cole-Parmer, IL) and then homogenized with a Pestle/Tube homogenizer (Cole-Parmer, IL). After centrifugation at 10,000 g for 20 min, the pellet was discarded. The supernatant was further centrifuged at 100,000 g for 60 min. After the supernatant was removed, the pellet was resuspended in 50 mM Tris-HCl (pH 7.2). The tissue homogenate was further divided into two parts: one for measuring the enzyme activity and the other for analyzing protein abundance. Rat brain homogenates prepared in the same manner were used as a positive control because all the Na^+/K^+-ATPase isoforms tested in this study are expressed in rat brains.

Na^+/K^+-ATPase Assays

The Na^+/K^+-ATPase activity was determined according to the method of Lee *et al.* (15). In brief, the homogenate was transferred to an incubation medium of the following composition: 10 mM-$MgCl_2$, 3.3 mM-EDTA, 100 mM Tris (pH 7.8) and divided into two tubes. One-half of the homogenate was mixed with the preheated assay mixture with 10 mM ouabain and the other half was mixed with the same mixture without ouabain. The assay mixture contained 1.132 M NaCl, 0.2 M KCl, 0.05 M NaN_3 and ATP. Fifteen minutes later, the reaction was stopped by adding 200 μl of 30% tricholoacetic acid. The ouabain-insensitive and sensitive phosphate liberation was measured by spectrophotometer at a wavelength of 660 nm. The difference between ouabain-insensitive and sensitive phosphate can be attributed to the Na^+/K^+-ATPase enzyme activity. Protein was measured by the method of Lowry *et al.* (19). Bovine albumin was used as the standard.

Immunoblot Analysis of Na^+/K^+-ATPase Subunit Abundance

The method for immunoblot analysis is as

described previously (15). In brief, 70 μg protein/ lane of uterine tissue homogenates or 5 μg protein/ lane of brain tissue homogenates was subjected to polyacrylamide gel electrophoresis on 12.5% polyacrylamide gels in the presence of 0.1 % sodium dodecyl sulfate and then transferred to polyvinyldifluoride membranes by electroblotting. Before being probed with antibodies against the α1-, α2-, α3-, β1-, or β2- subunits of Na^+/K^+-ATPase, the blots were incubated with blocking buffer for 1.5 hr at room temperature to lower nonspecific binding. The blocking buffer contained phosphate-buffered saline (136.9 mM-NaCl, 2.7 mM-KCl, 10 mM- Na_2HPO_4, 1.8 mM-KH_2PO_4), 5% non-fat milk (Carnation) and 0.5% (vol/vol) Tween 20. After incubation with primary antibodies, the blots were then treated with secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) labeled with peroxidase (dilution 1: 5000) for 50 min. In accordance with the manufacturer's protocol (ECL, Amersham), the blots were treated with chemiluminescence reagent and then exposed to x-ray film to visualize protein bands. Each protein band was determined by comparing with prestained protein marker (Amersham) run simultaneously in the experimental lanes. As an internal control, the protein abundance of β-actin was assessed. Rat brain expressing all Na^+/K^+-ATPase subunits served as a positive control. A background control was obtained without adding the primary antibodies.

Antibodies

The following antibodies (Upstate, NY) were used: mouse anti-rabbit Na^+/K^+-ATPase α1 monoclonal antibody (1:2000), rabbit anti-rat $Na^+/^+$-ATPase α2 polyclonal antibody (1:1000), mouse anti-sheep Na^+/K^+-ATPase α3 monoclonal antibody (1:250), rabbits anti-rat Na^+/K^+-ATPase β1 polyclonal antibody (1:500) or rabbit anti-rat Na^+/K^+-ATPase β2 polyclonal antibody (1:500). All antibodies used are capable of recognizing the subunits of $Na^+/^+$-ATPase in rats.

Quantitation

Multiple exposures of autoradiograms were made to ensure that the signals were within the linear range of the film. A computerized image analyzer (Quantity One, PDI., NY) quantified bands on immunoblots. After scanning, the OD value of each Na^+/K^+-ATPase subunit was normalized by its own β-actin value. The value of the vehicle control was defined as 100%. The proportion between 17β-estradiol-treated and its own vehicle control group was expressed as % of the vehicle control.

Measurement of Uterine Contractions

A longitudinal uterine strip 1 mm wide by 15 mm long was placed in physiological salt solution (PSS). The composition of the PSS was as follows: 116 mM NaCl, 4.6 mM KCl, 1.16 mM $NaH_2PO_4 \cdot H_2O$, 1.16 mM $MgSO_4 \cdot 7H_2O$, 21.9 mM $NaHCO_3$, 1.8 mM $CaCl_2 \cdot 2H_2O$, 11.6 mM dextrose, and 0.03 mM $CaNa_2EDTA$.

The uterine strips were mounted in an organ bath containing PSS for isometric force measurement as described previously (29). The PSS was maintained at 37 °C and was aerated with a mixture of 95% O_2 and 5% CO_2. All preparations were allowed to equilibrate for at least 40 min under a constant passive force of 1. 0 g. This level of passive force was determined to be optimal for maximum force development for 60 mM potassium chloride (KCl). After a 40-min equilibration period, all strips were challenged with 60 mM KCl for determining viability and maximum contractile force. The strips which did not respond to KCl were discarded. A cycle was operationally defined as the generation of force of at least two-thirds of the KCl (60 mM)-induced contraction that initiated at and returned to the baseline.

Data Analysis and Statistical Evaluation

All data are expressed as means±SEM (standard errors of the means). The data were analyzed by one-way analyses of variance (ANOVA). If the mean values were found to be statistically different, Least Standard Division test of the Means model of SYSTAT was used for multiple comparisons. In all cases, a p value less than 0.05 was considered statistically significant.

Results

Effect of 17β-Estradiol on Uterine Na^+/K^+-ATPase Activity

To examine whether 17β-estradiol affected uterine Na^+/K^+-ATPase activity, the ovariectomized rats were treated with 17β-estradiol for 1, 2, and 4 days. A corresponding vehicle control group was established for each of the 1-, 2-, and 4- day E_2 groups. The uterine Na^+/K^+-ATPase activity in the 1-, 2-, and 4- day vehicle control groups were 4.75±0.34 (n=4), 4.27±0.40 (n=4), and 4.65±0.34 (n=4), respectively (Fig. 1, *upper panel*). No significant difference among the groups was found. Relative to the vehicle control of the same exposure period, 17β-estradiol lowered the Na^+/K^+-ATPase activity to 86.3±6.7 % in 1 day (n=4), 62.7±9.8 % in 2 days (n=4), 70.2±9.8 % in 4 days (n=4) (Fig. 1, lower

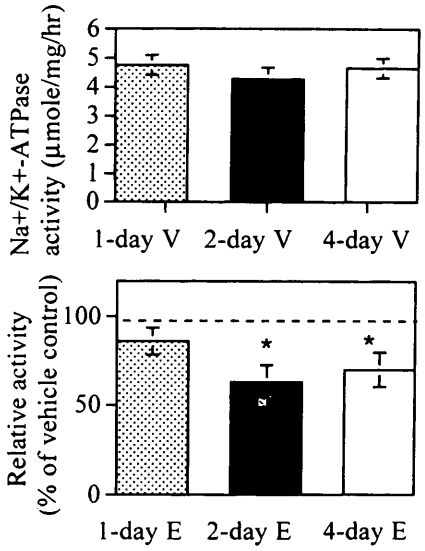

Fig. 1. Effect of 17β-estradiol on uterine Na^+/K^+-ATPase activity of ovariectomized rats. *Upper panel*: the Na^+/K^+-ATPase activity of ovariectomized rats in a corresponding vehicle control group (V): the 1-day V, 2-day V, and 4-day V groups. *Lower panel*: the relative changes in Na^+/K^+-ATPase activity after treatment with 17β-estradiol (5 μg/ml/kg, daily, s.c.) for 1 (1-day E), 2 (2-day E), or 4 days (4-day E). The Na^+/K^+-ATPase activity in each 17β-estradiol group was normalized to the value in a corresponding vehicle control and expressed as % of vehicle control. Dashed line indicates the level of enzyme activity in vehicle controls, defined as 100%. The data are presented as means±SEM of 4 rats for each group. Data were analyzed by a one-way ANOVA. * indicates $p < 0.05$ vs. vehicle controls at the same time point.

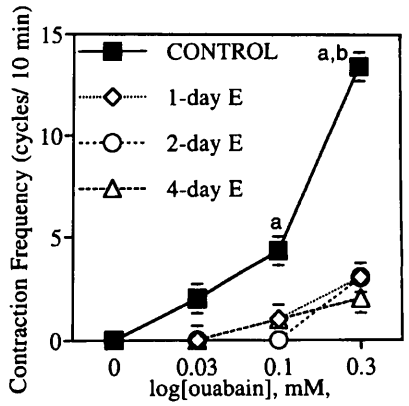

Fig. 2. Effect of 17β-estradiol on the protein abundance of Na^+/K^+-ATPase α1 subunits in rat uteri. A: representative autoradiograms of immunoblots probed for α1-protein abundance. After 1 (1D), 2 (2D), or 4 days (4D), uterine α1-protein abundance in vehicle controls (C) and 17β-estradiol (5 μg/ml/kg, daily, s.c.)-treated (E) groups was measured. The rat brain (B) was used as our positive control. B: quantitation of Western blots. Each point is presented as means±SEM of 4 rats in each group. Relative protein abundance was defined as the ratio of α1-protein abundance in estrogens-treated groups relative to that in vehicle controls after 1-(1-day E), 2-(2-day E), and 4-(4-day E) day treatment. A dashed line indicates the level of protein abundance in vehicle controls, defined as 100%. The data are presented as means±SEM of 4 rats for each group. Data were analyzed by a one-way ANOVA when examining the time-dependent changes in level of protein abundance. * indicates $p < 0.05$ vs. vehicle controls at the same time point. Positions of 113 and 45-kDa molecular protein standard are shown.

panel). Both 2 and 4 days of 17β-estradiol treatment significantly lowered uterine Na^+/K^+-ATPase activity.

Effect of 17β-Estradiol on the Protein Abundance of Na^+/K^+-ATPase Isoforms

The effects of various exposure on the protein abundance of Na^+/K^+-ATPase α1-subunits are shown in Figure 2. A representative autoradiogram of immunoblot results appears in Figure 2A. and quantitations of the Western blots are summarized in Figure 2B. When compared to brain tissues, ovariectomized uteri contained lower level of α1 subunit. Two days of 17β-estradiol increased the α1 protein more than 4-fold ($p < 0.05$), whereas 1- and 4- day estrogens treatments doubled the α1 protein abundance.

Figure 3 summarizes the effect of estrogens on the protein abundance of Na^+/K^+-ATPase α3 subunit. A representative autoradiogram of immunoblot results appears in Figure 3A and quantitations of the Western blots are summarized in Figure 3B. The protein abundance of α3 subunit decreased significantly with increasing 17β-estradiol exposure: 35.0±6.5 % in 1 day, 19.0±6.5 % in 2 days, 10.0±6.5 % in 4 days.

Figure 4 shows the effect of 17β-estradiol on

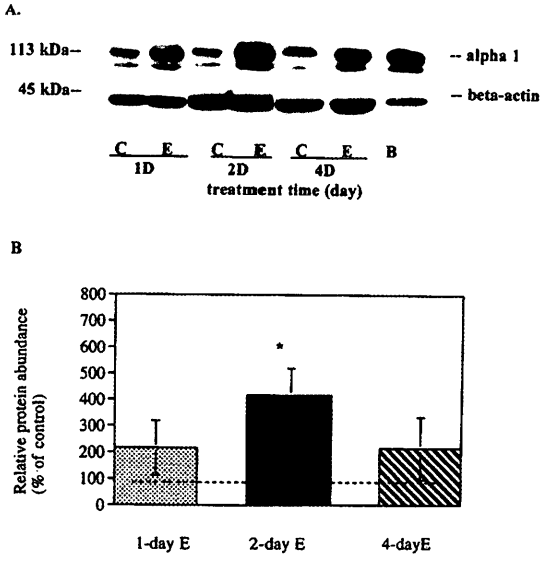

Fig. 3. Time-dependent effect of 17β-estradiol on the protein abundance of Na^+/K^+-ATPase α3- subunits in rat myometrium. A: representative autoradiograms of immunoblots probed for α3-protein abundance. B: densitometer-scanned results. Each point is presented as means±SEM of 4 rats in each group. Labels and analysis as in Figure 2. * indicates $p < 0.05$ vs. vehicle controls at the same time point. Positions of 113 and 45-kDa molecular protein standard are shown.

Table 1. Effect of 17β-Estradiol (5 μg/ml/kg) on Uterine Functions of Ovariectomized Rats

(mm)	Uterine diameter		Spontaneous contraction frequency (cycles/10 min)	
	Vehicle	17β-Estradiol	Vehicle	17β-Estradiol
n	4	4	4	4
Treatment period				
1-day	1.6±0.35[a]	3.2±0.35	8.3±1.0	0.0±0.0*
2-day	1.7±0.35	5.5±0.35*	8.9±1.0	0.0±0.0*
4-day	1.5±0.35	6.4±0.35*	9.0±1.0	1.0±0.0*

[a] The data were presented as mean±SEM. Values significantly different from the vehicle control value. Data were compared by a two-way ANOVA.

*$p < 0.05$ when compared with vehicle control of the same exposure period.

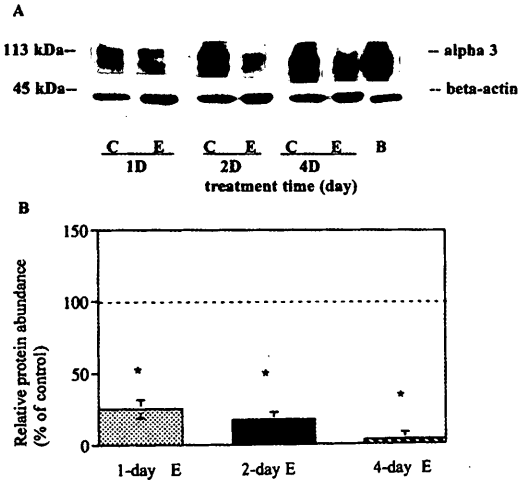

Fig. 4. Time-dependent effect of 17β-estradiol on the protein abundance of Na^+/K^+-ATPase β1 subunits in rat myometrium. A: representative autoradiograms of immunoblots probed for β1-protein abundance. B: densitometer-scanned results. Each point is presented as means±SEM of 4 rats in each group. Labels and analysis as in Figure 2. * indicates $p<0.05$ vs. vehicle controls at the same time point. Positions of 48.9 and 45-kDa molecular protein standard are shown.

the protein abundance of Na^+/K^+-ATPase β1-subunit. A representative autoradiogram of immunoblot results appears in Figure 4A and quantitations of the Western blots are summaried in Figure 4B. The protein abundance of β1 subunit was decreased significantly with increasing 17β-estradiol exposure: 26.8±10.5 % in 1 day, 38.5±10.5 % in 2 days, and 42.3±10.5 % in 4 days. When compared with brain tissues, neither α2 nor β2 subunit was expressed in rat uteri (data not shown). This result is in agreement with a previous report (30).

Effect of Estrogens on Uterine Contraction and Diameter

Since our data showed that 17β-estradiol has different effects on the expression of α1- and α3-isoforms for different treatment periods, we proceeded to determine the correlation between the expression of isoforms and uterine functions. Table 1 summarizes the effect of 17β-estradiol on uterine contraction and diameter. One-day-17β-estradiol treatment significantly suppressed spontaneous oscillatory contraction, but did not affect the uterine diameter. Two and four days of 17β-estradiol treatment significantly increased uterine diameter. This result suggests that spontaneous uterine oscillation was more susceptible to 17β-estradiol than uterine diameter.

In addition, the effect of 17β-estradiol on ouabain-induced uterine contraction was also examined. Contraction frequency was increased significantly with increasing concentrations of ouabain (0.03, 0.1, and 0.3 mM) in the vehicle control group but not in the estrogens-treated groups (Fig. 5). The contraction response to ouabain among the 1-day, 2-day, and 4-day vehicle control groups were very similar. Therefore, a representative tracing in the 4-day vehicle control was shown in Figure 5. However, the contraction force in the vehicle control was not significantly altered by ouabain (data not shown). Taken together, a reduction in Na^+/K^+-ATPase α3 by 17β-estradiol may be associated with the decreased contraction frequency.

Discussion

This is the first report to demonstrate that 17β-estradiol elicits differential effects on the protein expression of Na^+/K^+-ATPase α1 and α3 subunits. The time-dependent effect of 17β-estradiol on the expression of α3- and β1- subunit and oscillatory contraction suggests a positive correlation between Na^+/K^+-ATPase α3 subunits and frequency of spontaneous contraction. The reduction of ouabain-induced uterine oscillation by 1-, 2-, 4-day treatment

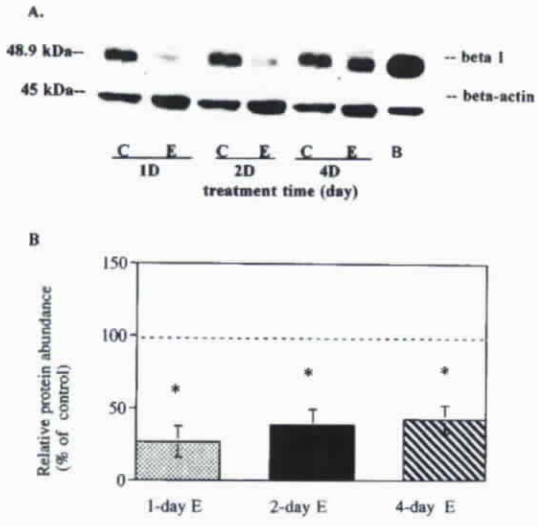

Fig. 5. Effect of 17β-estradiol on ouabain-induced uterine contraction of ovariectomized rats. Uteri were from ovariectomized rats treated with 17β-estradiol (5 μg/ml/kg, daily, s.c.) for 1 (1-day E), 2 (2-day E), 4 (4-day E) days or vehicle (control) for 4 days. Ouabain (0.03, 0.1, and 0.3 mM) was used to induce uterine oscillatory contraction. [a] indicates $p<0.05$ vs. 0 mM at the same group. [b] indicates $p<0.05$ vs. control group.

with 17β-estradiol further supports that the presence of Na^+/K^+-ATPase α3-subunit is associated with the development of spontaneous uterine contraction.

Na^+/K^+-ATPase plays an important role in muscle contractions. In skeletal muscles, training increases the concentration of Na^+/K^+-ATPase, which is associated with the faster recovery of plasma potassium induced by electrical stimulation and greater contractile performance (3). In cardiac muscles thyroid hormone increases predominantly the expression of Na^+/K^+-ATPase α2 (11) in conjunction with well established functions: including the increase in heart rate, contraction velocity, and diastolic relaxation. Our study shows that estrogens lowered both α3 expression and contraction frequency. In smooth muscles, a high concentration of ouabain (1 mM) suppressed the phasic component of oxytocin-induced contraction in uteri (2), histamine-induced oscillation in canine trachea (14), and norepinephrine-induced oscillation in small mesenteric arteries (10). All of these pharmacological data support that our observation that the reduction of Na^+/K^+-ATPase may be associated with the reduction of oscillatory contraction in smooth muscle.

Estrogens are known to suppress the frequency of spontaneous contraction in rats within 24 hours (4, 21). This result is consistent with our finding. Because Na^+/K^+-ATPase is thought to be involved in oscillatory contraction, we speculate that 17β-estradiol may lower the activity of Na^+/K^+-ATPase. Our data shows that both the enzyme activity and Na^+/K^+-ATPase α3-subunit are significantly inhibited by 1 to 4 days of treatment with 17β-estradiol. The proportional change in Na^+/K^+-ATPase α3-subunit coincides with that in contraction frequency in response to 1 to 4 days of treatment with 17β-estradiol. Our data also suggests that the abundance of Na^+/K^+-ATPase α3 subunit, rather than the overall change in enzyme activity, is the major contributor to oscillatory contraction.

The uterotrophic actions of estrogens, including the increased fluid accumulation, uterine diameter, and the organ weight, have been well confirmed. In terms of time course, a significant increase of Na^+/K^+-ATPase α1-subunit by 2 days of 17β-estradiol has been associated with an increased uterine diameter in conjunction with dramatic increased fluid accumulation (unpublished observation). Therefore, we speculate that the increase of Na^+/K^+-ATPase α1-subunit by estrogens may contribute to estrogens-induced uterotrophic actions. Katagiri et al. showed that, in response to estrogens, the Na^+/K^+ ratio was decreased from 6.0:1 to 1.3:1 in uterine luminal flushes in conjunction with an increase in fluid accumulation. (13). Na^+/K^+-ATPase is thought to be involved in regulating electrolyte environment and fluid accumulation. In addition, the delayed change in uterine diameter corresponds to the increase in the expression of the α1 isoform. Our data agree with the finding that 17β-estradiol increases Na^+/K^+-ATPase density in guinea pig uteri (18). Taken together, Na^+/K^+-ATPase α1-subunit may play a role in estrogens-induced uterotrophic actions.

We offer two possible reasons for the differential effects of estrogens on Na^+/K^+-ATPase α1 and α3 subunit: the increase of intracellular sodium and the differential regulation of both isoforms. For the former, our explanation is that 1-day treatment with 17β-estradiol significantly decreases in the protein level of α3- and β1- subunits, which causes a significant decrease in Na^+/K^+-ATPase activity. The alteration in sodium/potassium balance causes an increased intracellular sodium, which enhances the expression of α1 gene (34) and Na^+/K^+-ATPase activity (25). For the latter, our explanation is that Na^+/K^+-ATPase subunits are differentially regulated by estrogens. Recent studies have showed that the 5'-flanking region for α1-gene contains a TATA-like box, Sp1 binding site, active transcription factor consensus site (ATF-1), CREB, and two glucocorticoid-responsive element half consensus sequences (33). The upstream region for α3-gene contains ATAT sequence preceeding the transcription initiation sites, binding sites for Sp1, AP-1, AP-2, and AP-4, as well as glucocorticoid and thyroid hormone receptors (23). Although it is not clear whether estrogens response element (ERE) exists in the 5'-flanking region for genes of Na^+/K^+-ATPase isoforms,

it is still possible that estrogens induces various transcription factors to modulate the expression of α1 and α3 differently.

After estrogens treatment, the level of uterine Na^+/K^+-ATPase activity is close to that reported by Turi et al. (31). In our study, however, 1 day of 17β-estradiol did not affect Na^+/K^+-ATPase activity but did significantly suppressed the expression of α3- and β1- protein. Four days of 17β-estradiol treatment leads to a 25% decrease in Na^+/K^+-ATPase activity and more than 60% decrease in β1- and α3- protein. The decrease in protein abundance of Na^+/K^+-ATPase α3 and β1 was greater than that in activity. As our data suggests, Na^+/K^+-ATPase activity cannot be used as an indicator for Na^+/K^+-ATPase isoforms. It is known that each α subunit combines with the β1 protein in a 1:1 stoichiometry. In this study the expression of β1 protein was decreased by 1-4 days of estrogens treatments. The 60% decrease of β1 protein by 1-day treatment of estrogens did not significantly affect activity. These data indicate that some isoform other than β1 exists in rat uteri. Arystarkova *et al.* demonstrated that a new β isoform, β3, exists in the lung, liver, skeletal muscle, and testis (1). The mRNA of human β3 isoform gene in uteri elicits greater expression than that in liver or skeletal muscle (20). The presence of the new isoform, β3, may account for the decrease in β1-protein without significant decrease in activity.

In conclusion, 17β-estradiol elicited differential effects on the expression of Na^+/K^+-ATPase subunits in rat uteri. Multiple effects of 17β-estradiol on uterine functions provide a good model for examining the physiological correlation between cellular functions and the expression of Na^+/K^+-ATPase isoforms. It is likely that more than one β-isoform exists in rat uteri.

Acknowledgments

The authors wish to acknowledge Dr. Yu-Chong Lin (University of Hawaii) for his assistance in the manuscript and S.-C. Wang and B.-Y. Huang for their assistance in conducting the experiments. The project described was a partial fulfillment of C.-L. Lee's master thesis and was supported by NSC grant 87-2314-B006-099 to M.-L. Tsai.

References

1. Arystarkhova, E. and Sweadner, K.J. Tissue-specific expression of the Na^+,K^+-ATPase β3 subunit. *J. Biol. Chem.* 272: 22405-22408, 1997.
2. Ausina, P., Savineau, J.P., Hernandez, J.S., D'Ocon, M.P., Martin, J.D. and Candenas, M.L. Effect of inhibition of the electrogenic Na^+/K^+ pump on the mechanical activity in the rat uterus. *Fund. Clin. Pharmacol.* 10: 38-46, 1996.
3. Clausen, T. and Nielsen, O.B. The Na^+/K^+ pump and muscle contractility. *Acta Physiol. Scand.* 152: 365-373, 1994.
4. Downing, S.J. and Porter, D.G. Evidence that inhibition of myometrial activity by estradiol in the rat is mediated by an RNA synthetic pathway. *J. Endocrinol.* 78: 119-124, 1993.
5. Dzurba, A., Ziegelboffer, A., Vrbjar, N., Styk, J. and Slezak, J. Estradiol modulates the sodium pump in heart sarcolemma. *Mol. Cell Biochem.* 176: 113-118, 1997.
6. Eakle, K.A., Kim, K.S., Kabalin, M.A. and Farley, R.A. High-affnity ouabain binding by yeast cells expressing Na^+, K^+-ATPase α subunits and the gastric H^+, K^+-ATPase β subunit. *Proc. Natl. Acad. Sci. USA* 89: 2834-2838, 1992.
7. Ewart, H.S. and Klip, A. Hormonal regulation of the Na^+-K^+-ATPase: mechanisms underlying rapid and sustained changes in pump activity. *Am. J. Physiol.* 269: C295-C311, 1995.
8. Fraser, C.L. and Swanson, R.A. Female sex hormones inhibit volume regulation in rat brain astrocyte culture. *Am. J. Physiol.* 267: C909-C914, 1994.
9. Fuchs, A.R. Hormonal control of myometrial function during pregnancy and parturition. *Acta Endocrinol. Suppl.* 221: 1-70, 1978.
10. Gustafsson, H. and Nilsson, H. Rhythmic contractions in isolated small arteries of rat: role of K^+ channels and the Na^+,K^+-pump. Acta *Physiol. Scand.* 150: 161-170, 1994.
11. Hensley, C.B., Azuma, K.K., Tang, M.J. and McDonough, A. A. Thyroid hormones induction of rat myocardial Na^+/K^+-ATPase: α1, α2, and β1-mRNA and -protein levels at steady state. *Am. J. Physiol.* 262: C484-492, 1992.
12. Herrera, V.L., Emanuel, J.R., Ruiz-Opazo, N., Levenson, R. and Nadal-Ginard, B. Three differentially expressed Na, K-ATPase alpha subunits: structural and functional implications. *J. Cell Biol.* 105: 1855-64, 1987.
13. Katagiri, S., Ma, S., Yuen, B.H. and Moon, Y.S. Role for insulin-like growth factor I in the regulation of electrolyte composition of uterine luminal fluid. *J. Reprod. Fertil.* 109, 115-120, 1997.
14. Kong, S.K. and Stephens, N.L. Induction of rhythmic contraction in canine tracheal smooth muscle. *Can. J. Physiol. Pharmacol.* 68: 131-1316, 1990.
15. Lee, Y.C., Lin, H.H. and Tang, M.J. Glucocorticoid upregulates Na-K-ATPase alpha-and beta-mRNA via an indirect mechanism in proximal tubule cell primary cultures. *Am. J. Physiol.* 268: F862-F867, 1995.
16. Lemieux, D.R., Roberge, F.A. and Savard, P. A model study of the contribution of active Na-K transport to membrane repolarization in cardiac cells. *J. Theor. Biol.* 142: 1-34, 1990.
17. Lingrel, J.B., Orlowski, J., Shull, M.M. and Price, E. M. Molecular genetics of Na,K-ATPase. *Nucleic Acid Res. Mol. Biol.* 38: 37-89, 1990.
18. Lipton, A., Leah, J. and Parkington, H.C. Effect of ovarian steroids and prolactin on the contractility and sodium pump site density of guinea-pig myometrium. *J. Endocrinol.* 77: 43-48, 1978.
19. Lowry, D.H., Rosebrough, N. J., Farr, A.I. and Randall, R.J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265-275, 1951.
20. Malik, N., Canfield, V., Sanchez-Watts, G., Scherer, S., Beatty, B. G., Gros, P. and Levenson, R. Structural organization and chromosomal localization of the human Na,K-ATPase β3 subunit gene and pseudogene. *Mammal. Genome* 9: 136-143, 1998.
21. Massmann, G.A., Figueroa, J.P. and Nathanielsz, P.W. Further characterization of the electromyographic activity of the myometrium and mesometrium in nonpregnant sheep under estrogens supplementation. *Biol. Reprod.* 45: 605-610, 1991.
22. Navran, S.S., Allain, G., Garcia, H.F. and Allen, J.C. Sodium pump stimulation by activation of two alpha adrenergic receptor subtypes in canine blood vessels. *J. Pharmacol. Exp. Ther.* 245: 608-613, 1988.
23. Pathak, B.G., Pugh, D.G. and Lingrel, J.B. Characterization of the

5'-flanking region of the human and rat Na^+-K^+ ATPase alpha 3 gene. *Genomics* 8: 641-647, 1990.

24. Rodriguez del Castillo, A., Battaner, E., Guerra, M., Alonso, T. and Mas, M. Regional changes of brain Na^+,K^+-transporting adenosin triphosphatase related to ovarian function. *Brain Res.* 416: 113-118, 1987.
25. Rayson, B.M. and Gupta, R.K. Steroids intracellular sodium levels, and Na^+/K^+-ATPase regulation. *J. Biol. Chem.* 260: 12740-12743, 1985.
26. Schwarz, S.M., Bostwick, H.E. and Medow, M.S. Estrogens modulates ileal basolateral membrane lipid dynamics and Na^+/K^+-ATPase activity. *Am. J. Physiol.* 254: G687-G694, 1988.
27. Slezak, J., Schulze, W., Okruhlicova, L., Tribulova, N. and Singal, P.K. Cytochemical and immunocytochemical localization of Na,K-ATPase alpha subunit isoenzymes in the rat heart. *Mol. Cell Biochem.* 176: 107-112, 1997.
28. Sweadner, K.J. Isozymes of the Na^+/K^+-ATPase. *Biochim. Biophys. Acta* 988: 185-220, 1989.
29. Tsai, M.L., Webb, R.C. and Loch-Caruso, R. Congener-specific effects of PCBs on contractions of pregnant rat uteri. *Reprod. Tox.* 10: 21-28, 1996.
30. Turi, A., Marcsek, Z., Mullner, N., Kucsera, M. and Bori, Z. The activity of Na^+/K^+ ATPase and abundance of its mRNA are regulated in rat myometrium during pregnancy. *Biochem. Biophys. Res. Comm.* 188: 1191-1197, 1992.
31. Turi, A. and Torok, K. Myometrial Na^+/K^+-activated ATPase and its CA^{2+} sensitivity. *Biochim. Biophy. Acta* 940: 77-84, 1985.
32. Ueno, S., Takeda, K., Noguchi, S. and Kawamura, M. Significance of the beta- subunit in the biogenesis of Na^+/K^+-ATPase. *Biosci. Reports* 17: 173-88, 1997.
33. Yagawa, Y., Kawakami, K. and Nagano, K. Cloning and analysis of the 5'-flanking region of rat Na^+/K^+-ATPase alpha 1 subunit gene. *Biochem. Biophy. Acta* 1049: 286-292, 1990.
34. Yamamoto, K., Ikeda, U., Okada, K., Saito, T., Kawakami, K. and Shimada, K. Sodium ion mediated regulation of Na^+/K^+-ATPase gene expression in vascular smooth muscle cells. *Cardiovas. Res.* 28: 957-962, 1994.