The Differential Effects of Tamoxifen and ICI 182,780 on the Reduction of Na^+/K^+ ATPase Activity and Spontaneous Oscillations by 17β-Estradiol

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Abstract

A prolonged treatment with 17β-estradiol reduces the frequency of spontaneous oscillations and the Na^+/K^+ ATPase activity in rat uteri. Acute inhibition of Na^+/K^+ ATPase activity by a Na^+/K^+ ATPase inhibitor, ouabain, decreases the frequency of oxytocin-induced oscillations in uteri. Therefore, the purpose of this study was to examine whether the prolonged inhibition of Na^+/K^+ ATPase activity by 17β-estradiol was estrogen receptor (ER)-dependent. The uterine explants from ovariectomized rats were cultured in vitro as our experimental model to compare the effect of two antiestrogenic compounds (ICI 182,780 and tamoxifen) on the Na^+/K^+ ATPase activity and the frequency of spontaneous oscillations. ATPase assay and a standard muscle bath apparatus were to measure the activity and the contraction. When compared with the control, a 2-day treatment with 17β-estradiol in vivo or in vitro decreased the activity and the frequency. ICI 182,780 lowered the activity but tamoxifen did not. ICI 182,780 did not decrease the frequency but tamoxifen did. Even the reversal effects of these antiestrogenic compounds on the reduced activity and the frequency by 17β-estradiol were different. Tamoxifen elicited a greater reversal effect on the reduced activity but ICI 182,780 did not. In contrast, ICI 182, 780 elicited a greater reversal effect on the reduced frequency but tamoxifen did not. Prolonged inhibition of Na^+/K^+ ATPase activity by K^+-free solution suppressed the frequency with the elevation of basal tension. Addition of KCl at lower concentrations (0.3-1.2 mM) induced oscillatory contraction after reducing the basal tension. As our data suggest, the prolonged effect of 17β-estradiol may decrease uterine the activity through ER dependent and independent pathways. The reduction of uterine Na^+/K^+ ATPase activity by estrogens may increase the basal tension after each oscillatory cycle, which, in part, contributes to the reduced frequency of spontaneous oscillations.

Key Words: 17β-estradiol, ouabain, Na^+/K^+ ATPase, spontaneous uterine contractions, myometrium, rats

Introduction

Long-term elevation of 17β-estrogen is well known to influence oxytocin-induced and spontaneous uterine contractions in a different manner. In pregnant rats before term, uteri elicit a greater contraction response to oxytocin. As data indicate, only long-term exposure to 17β-estradiol increases the frequency of oxytocin-induced oscillations in uteri by increasing the expression of oxytocin receptors.

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The increased response to oxytocin by 17β-estradiol is due to the activation of estrogen receptors (ER) (7, 22). In contrast, prolonged exposure to estrogens lowers the frequency of spontaneous oscillations. In the 4-day cycle rats, the uterus exhibits cyclic changes in the frequency of spontaneous oscillations. The lowest frequency with the greatest intrauterine pressure occurs in the proestrous phase when the plasma level of estrogens reaches the highest level (10). Even though it is thought that the acute effect to 17β-estradiol on the frequency of spontaneous oscillations is ER-independent (6, 16, 17) but chronic exposure might be ER-dependent, the mechanism responsible for the reduction of spontaneous oscillations by estrogens is still under investigation.

Na^+/K^+ ATPase is essential for maintaining Na^+ and K^+ concentration gradients across the cell membrane. Immediate inhibition of Na^+/K^+-ATPase by ouabain induces colonic muscle contraction (3). Pretreatment with ouabain lowers the frequency of agonist-induced oscillations in trachea without further increasing the contraction force (12). Gustafsson (1994) proposed that pretreatment with ouabain decreases contraction frequency by decelerating the relaxation (8). Jassen et al. (1997) also showed that the inhibition of Na^+/K^+ ATPase activities by ouabain disrupts the progression of muscle relaxation, prolongs the duration of each oscillatory cycle, and reduces the rhythm in tracheal smooth muscles (11). All current data show that the acute inhibition of Na^+/K^+ ATPase activity induce contractions. In the presence of oscillatory contractions, the inhibition of Na^+/K^+ ATPase activity decreases the frequency of oscillatory contractions by blocking muscle relaxation.

Isoforms and enzyme activity of Na^+/K^+ ATPase has been reported in rat uteri (25). Pretreatment with ouabain decreases oxytocin-induced oscillatory frequency (2). It has been reported that a 2-4 day treatment with 17β-estradiol reduces Na^+/K^+ ATPase activity in rat uteri with the decrease in the protein abundance of Na^+/K^+ ATPase (24). Their data indicate that prolonged inhibition of Na^+/K^+ ATPase activity is associated with the decrease of the frequency. Thus, the purpose of our study was to examine whether the reduced Na^+/K^+ ATPase activity by a prolonged treatment with 17β-estradiol was due to the activation of estrogen receptors. Our first objective was to examine whether the prolonged effect of 17β-estradiol on uterine Na^+/K^+ ATPase activity and the frequency of spontaneous oscillations could be affected by two estrogen receptor antagonists (tamoxifen and ICI 182,780). Secondly, we would like to examine how the decreased Na^+/K^+ ATPase activity influenced uterine oscillations.

Materials and Methods

Chemicals

Ouabain, tetraethylammonium (TEA), gentamycin, 17β-estradiol, tamoxifen, and sesame oil (Sigma, MO), RPMI medium (Gibco, NY), and other chemicals (Nacalai Tesque, Japan) were used. ICI 182,780 was a gift from Zeneca Pharmaceuticals. ICI 182,780, tamoxifen, and 17β-estradiol were dissolved in 100 % ethanol and the other chemicals in double distilled water. To perform in vivo experiments, 17β-estradiol in ethanol was further diluted in sesame oil. The final concentration of 17β-estradiol was 5 μg/ml.

Use and Care of Animals

All animal studies were performed according to the protocols and procedures approved by local Animal Care and Use Committee and were in accordance with NIH standards established by the Guidelines for the Care and Use of Experimental Animals and by the American Veterinary Medical Association. Female Sprague-Dawley rats weighing 180 to 220 g were housed in a colony at National Cheng Kung University at 24±1°C under the schedule of a 14-h light (0500-1900 h). Normal virgin rats were ovariectomized following anesthesia with ether. Two weeks later, the ovariectomized rats were divided into two groups: one was injected subcutaneously with 17β-estradiol (5 μg/ml/kg) and the other was injected with seame oil (the vehicle control) on a daily basis. Exactly 24 hours after two daily injections, the uteri were removed and 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 $CaNa_2EDTA$. Longitudinal uterine strips (1 mm wide by 15 mm long) were divided into two parts: one for measuring contraction force and the other for preparing tissue homogenates.

Uterine Explant Culture

To examine whether the reduction of oscillations and Na^+/K^+ ATPase activity by 17β-estradiol was ER-dependent, explants from ovariectomized rats were pretreated with 17β-estradiol and estrogen receptor antagonists (tamoxifen and ICI 182,780) for 2 days in vitro. For pretreating explants, the uterine explants were cultured in vitro described previously (23). In brief, uterine explants (1 mm wide by 15 mm long) from ovariectomized rats were placed in a culture dish containing 5 ml RPMI medium and gentamycin

(50 mM/ml) at 37° C in a 5% CO_2 incubator for 2 days. Test compounds from stock solutions were added to the medium. The pretreated uterine strips were divided into two groups: one for measuring contraction force and the other for preparing tissue homogenates.

Measurement of Oscillatory Contractions

The uterine strips were mounted in an organ bath containing PSS for isometric force measurement as described previously (24). The PSS was maintained at 37°C and aerated with a mixture of 95% O_2 and 5% CO_2. The level of passive force had been optimized for maximum force development with 60 mM KCl. Those strips not responding to KCl were discarded. The frequency of contractions was defined as the number of oscillatory cycles over a period of 10 min. In each oscillatory cycle, the contraction force measured at the relaxation phase was defined as basal tension and that at the contraction phase was defined as peak tension. In either spontaneous or agonist-induced oscillations, the amplitude of each cycle may not be the same. In particular, agonist-induced profiles always start with tonic contractions followed by phasic contractions. To exclude tonic contractions, the cycle of oscillatory contractions was included when a force between basal to peak tension was at least two-thirds of the KCl (60 mM)-induced oscillatory contraction.

Preparation of Tissue Homogenates

Tissue homogenates were prepared as described previously (24). Uterine horns were kept in the homogenizing solution of the following composition: 150 mM-sucrose, 30-mM histidine, 1 mM EGTA, 1-% deoxycholate, and 0.1 M PMSF. After trimming the adipose tissue, the minced uteri were ground with a 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) as tissue homogenates for measuring the enzyme activity.

Na^+/K^+ ATPase Assay

Na^+/K^+ ATPase activity was determined according to the method of Tsai et al (24). In brief, tissue homogenates were transferred to an incubation medium of the following composition: 10 mM-$MgCl_2$, 3.3 mM-EDTA, 100 mM Tris (pH 7.8) and then separated into two tubes. One tube of the homogenate was mixed with the preheated assay mixture with 10 mM ouabain and the other tube was similarly mixed but without ouabain. The assay mixture contained 1.132 M NaCl, 0.2 M KCl, 0.05 M NaN_3 and ATP. Fifteen minutes later, 200 µl of 30% tricholoacetic acid was added to stop the reaction. The ouabain-insensitive and ouabain-sensitive phosphate liberations were measured by a spectrophotometer at a wavelength of 660 nm. The difference in the liberation amount between ouabain-insensitive and sensitive phosphate represented the level of Na^+/K^+ ATPase activity. Protein was measured by the method of Lowry et al. using bovine albumin as the standard.

Data Analysis and Statistical Evaluation

All data are presented as means±SEM (standard errors of the means), and analyzed by one-way analyses of variance (ANOVA). If the mean values were found to be statistically different, the LSD test of the Means model was used for multiple comparisons. In all cases, the value of *P* less than 0.05 was considered statistically significant.

Results

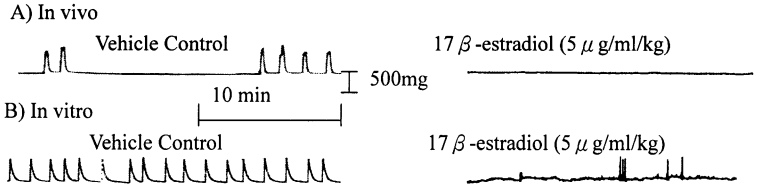
Effect of 17β-Estradiol on Uterine Na^+/K^+ ATPase Activity

Uterine Na^+/K^+ ATPase activity was measured after a 2-day treatment with 17β-estradiol in vivo or in vitro. As shown in Table 1, the 2-day treatment with 17β-estradiol in vivo significantly lowered uterine Na^+/K^+ ATPase activity from 7.5±0.5 to 4.6±0.4 µmole/mg/hr ($P<0.05$) and that in vitro lowered the activity from 3.5±0.3 to 2.1±0.4 µmole/mg/hr ($P<0.05$).

To determine whether the reduced Na^+/K^+ ATPase activity by 17β-estradiol was ER-dependent, two antiestrogenic compounds (ICI 182,780 and tamoxifen) were used in the uterine explant culture. Four treatment groups were included: vehicle (as a control), 17β-estradiol (10^{-8} M), 17β-estradiol (10^{-8} M) plus an antiestrogenic compound (3×10^{-7} M), and an antiestrogenic compound (3×10^{-7} M) alone. The first set was to examine the reversal effect of ICI 182, 780 on the decreased activity by 17β-estradiol. The activity in the each group was measured and expressed as percentage of the control. Relative to the control (as 100 %), either 17β-estradiol or ICI 182, 780 alone significantly decreased the activity to 64±8% and 50±7% of the control ($P<0.05$). The combination of 17β-estradiol and ICI 182, 780 did not significantly alter the activity ($P>0.05$) (Fig. 1A).

The second set was to examine the reversal effect of tamoxifen on the decreased activity by 17β-estradiol. Relative to the control, 17β-estradiol significantly decreased the Na^+/K^+ ATPas activity (*P*

Table 1. The influence of a 2-day exposure to 17β-estradiol in vivo and in vitro on the frequency of spontaneous oscillations and the activity of Na^+/K^+ ATPase in rat uteri. Representative tracings of uterine oscillations after treatments in vivo (A) and in vitro (B).



	In vivo treatment		In vitro treatment	
	Vehicle	17β-Estradiol (5 mg/ml/kg)	Vehicle	17β-Estradiol (10^{-8} M)
Frequency (cycles/10 min)	5.3 ±1.0	1.0±0.4*	7.3 ±1.1	3.1 ±0.7*
Na^+/K^+ ATPase (mmole/mg/hr)	7.5±0.5[a]	4.6±0.4*	3.5±0.3	2.1±0.4*

[#] These data are presented as the means ± SEM.

* Significant difference between 17β-estradiol (5 μg/ml/kg)- and vehicle- treated uteri under the same condition of the treatment (P<0.05).

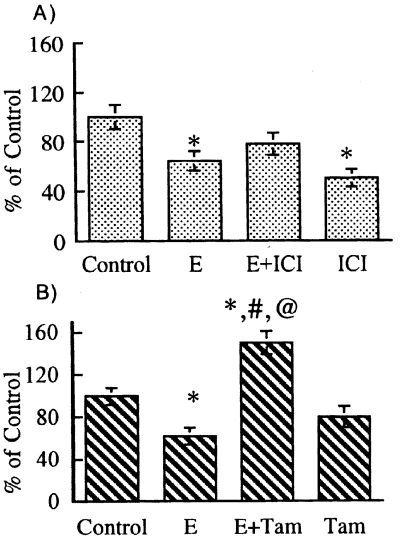


Fig. 1. The effect of ICI 182,780 (A) and tamoxifen (B) on the reduced Na^+/K^+ ATPase activity by 17β-estradiol. (A) Uterine strips were treated with vehicle (Control), 17β-estradiol (10^{-8} M) (E), 17β-estradiol (10^{-8} M) plus ICI 182,780 (3×10^{-7} M) (E+ICI), and ICI 182,780 (3×10^{-7} M), respectively, for 2 days. (B) Uterine strips were treated with vehicle (Control), 17β-estradiol (10^{-8} M) (E), 17β-estradiol (10^{-8} M) plus tamoxifen (3×10^{-7} M) (E+Tam), and Tamoxifen (3×10^{-7} M) (Tam), respectively, for 2 days. Na^+/K^+ ATPase activity measured from each group was normalized with that from the control and expressed as % of Control. The value expressed by each bar represents the mean±SEM (n=5). *Different from the control, P<0.05. [@]Different from the group of ICI or Tam, P<0.05. [#]Different from the E group, P<0.05.

<0.05) but tamoxifen did not. When compared with the control, the combination of 17β-estradiol and tamoxifen significantly increased the activity (P< 0.05). The activity in the 17β-estradiol plus tamoxifen group was significantly higher than that in the 17β-estradiol or the tamoxifen group (P<0.05) (Fig. 1B).

Taken together, ICI 182,780 alone lowers the activity but does not significantly reverse the decreased activity. Tamoxifen alone does not affect the activity but significantly reverses the decreased activity.

Effect of 17β-Estradiol on the Frequency of Spontaneous Oscillations

To confirm that the prolonged treatment with 17β-estradiol could decrease the frequency of spontaneous contractions, the contraction frequency was measured after a 2-day pretreatment with 17β-estradiol in vivo. Irregular and spontaneous oscillation occurs in rat uteri. Usually, spontaneous oscillations were followed by certain period of quiescence. Therefore, the frequency could be measured only during oscillations and before next oscillations occurred. As shown in Table 1, either in vivo or in vitro treatment with 17β-estradiol decreased the frequency of spontaneous oscillations (P<0.05).

To further examine whether the decreased frequency by 17β-estradiol was ER-dependent, both ICI 182,780 and tamoxifen were used in the uterine explant culture. First of all, we examined the reversal effect of ICI 182,780 on the decreased frequency by 17β-estradiol. Uterine explants were treated with one of the followings in vitro: vehicle (as a control), 17β-estradiol, 17β-estradiol plus ICI 182,780, and ICI 182,780 alone. After a 2-day treatment in vitro, the frequency of spontaneous oscillations in the each group was measured. Relative to the control (8.3±

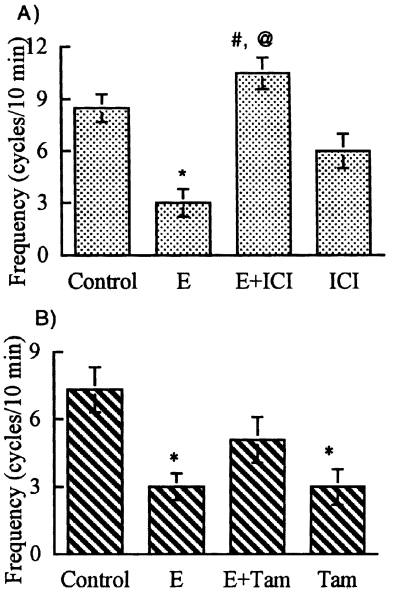


Fig. 2. The effect of ICI 182,780 (A) and tamoxifen (B) on the reduced frequency of spontaneous oscillations by 17β-estradiol. (A) Uterine strips were treated with vehicle (as a Control), 17β-estradiol (10^{-8} M) (E), 17β-estradiol (10^{-8} M) plus ICI 182,780 (3×10^{-7} M) (E+ICI), and ICI 182,780 ($3x10^{-7}$ M), respectively, for 2 days. (B) Uterine strips were treated with vehicle (as a Control), 17β-estradiol (10^{-8} M) (E), 17β-estradiol (10^{-8} M) plus tamoxifen (3×10^{-7} M) (E+Tam), and tamoxifen (3×10^{-7} M) (Tam), respectively, for 2 days. The frequency (cycles/10 min) expressed by each bar represents the mean±SEM (n=5). *Different from the control, $P<0.05$. @Different from the group of ICI or Tam, $P<0.05$. #Different from the E group, $P<0.05$.

0.6 cycles/10 min), 17β-estradiol significantly decreased the frequency to 3.1±0.6 but ICI 182,780 did not. In the presence of 17β-estradiol, ICI 182,780 significantly reversed the frequency to 10.5±0.9. The increased frequency in the combination group was higher than that in the 17β-estradiol or the ICI 182, 780 group ($P<0.05$) (Fig. 2A). Secondly, uterine strips were treated with one of the followings in vitro: vehicle (as a control), 17β-estradiol, 17β-estradiol plus tamoxifen, and tamoxifen alone. Relative to the control, 17β-estradiol or tamoxifen alone significantly decreased the frequency ($P<0.05$) but the combination of 17β-estradiol and tamoxifen did not ($P>0.05$) (Fig. 2B).

Taken together, ICI 182,780 alone does not alter the frequency but significantly reverses the decreased frequency. Tamoxifen alone decrease the frequency but does not significantly reverse the decreased frequency.

Influence of the Decreased Na^+/K^+ ATPase on the Frequency of Uterine Contractions

Even though immediate inhibition of Na^+/K^+ ATPase by ouabain can induce muscle contractions,

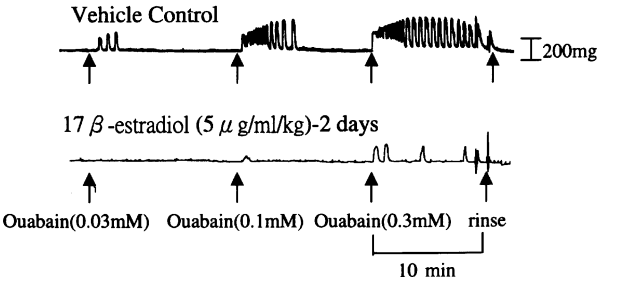


Fig. 3. A representative polygraph tracing of uterine oscillations in response to ouabain from 0.03 to 0.3 mM. After a 2-day pretreatment with 17β-estradiol (5 μg/ml/kg) or vehicle (Vehicle Control) in vivo, the contraction profiles of the vehicle-control uteri (*Top tracing)* and 17β-estradiol -treated uteri (*Bottom tracing*) were monitored. Each arrowbar indicates the addition of each treatment at the interval of 10 min. Similar results were obtained in 4 additional experiments.

the 17β-estradiol-treated uteri containing lower levels of Na^+/K^+ ATPase activity is associated with the reduction of frequency (Table 1). If the activation of Na^+/K^+ ATPase is involved in uterine oscillations, the uterine tissues containing lower levels of Na^+/K^+ ATPase activity were less responsive to ouabain-induced contractions. Therefore, the following experiment was to compare the contraction response to ouabain between 17β-estradiol- and vehicle- treated uteri. The addition of uabain from 0.03 to 0.3 mM at intervals of about 10-min could increase the frequency in a concentration-dependent manner (Fig. 3). Similar profiles were found in spontaneous oscillations. Irregular oscillations were followed by a certain period of uterine quiescence. In comparison, the frequency induced by ouabain (0.1 and 0.3 mM) was lower in the 17β-estradiol than that in the vehicle control. The data support that 17β-estradiol may lower the frequency of ouabain-induced oscillations through a Na^+/K^+ ATPase-related pathway. In addition, the reduction of ouabain-induced oscillations by 17β-estradiol was fully reversed by ICI 182,780 but not by tamoxifen (data not shown).

Even though ouabain induces muscle contractions immediately, our data show that the prolonged reduction of Na^+/K^+ ATPase activity by 17β-estradiol is associated with the decreased frequency. To further explore why the prolonged inhibition of Na^+/K^+ ATPase activity decreased the frequency of uterine oscillations, the next experiment was to examine the time-dependent effect of K^+-free solution (known to inhibit Na^+/K^+ ATPase activity) on the spontaneous oscillations. Within 5 min after replacing the PBS with K^+-free solution, the maximal force (defined initial tension) was induced without the appearance of oscillatory contractions (Fig. 4A). Only when the basal tension (measured in the relaxation phase of each oscillatory cycle) reached to

Table 2. The influence of 17β-estradiol (5 μg/ml/kg, in vivo) on the contraction response of rat uteri to K^+-free solution and KCl at 0.3 mM

	Vehicle	17β-Estradiol
Maxiaml basal tension in response to K^+ -free solution (% of the initial tension)[1]	60±8[a]	95±10[*]
Maximal frequency in response to K^+ -free solution (cycles/10 min)	21.2±3.2	10.2±2.2[*]
Duration of uterine oscillations in response to K^+ -free solution (min)	50±10	25±9[*]
Basal tension in response to 0.3 mM KCl (% of the initial tension)	30±8	90±11[*]

[1] The contraction force, which was induced in 5 min after exposure to K^+-free solution, is defined as initial tension. In each oscillatory cycle, the contraction force measured in the relaxation phase was considered as basal tension and that in the contraction phase was considered as peak tension.
Each oscillatory cycle was operationally defined when a force between basal to peak tension was at least two-thirds of the KCl (60 mM)-induced oscillatory contraction.
[a] These data are presented as the means±SEM.
[*] Significant difference between 17β-estradiol (5 μg/ml/kg)- and vehicle- treated uteri (P<0.05).

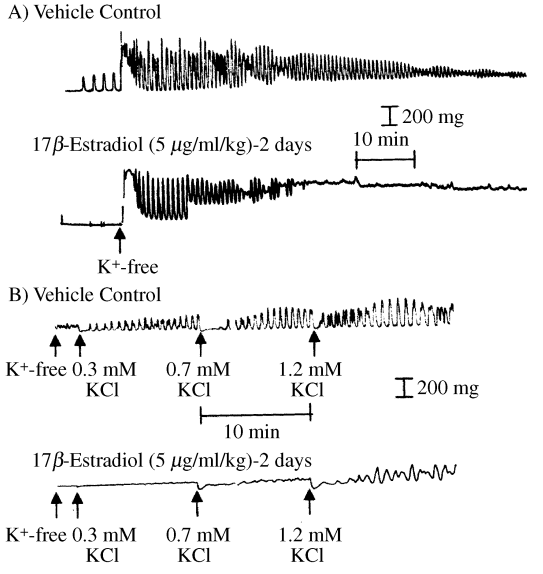


Fig. 4. A representative polygraph tracing of uterine oscillations. (A) The time-dependent effect of K^+-free solution on uterine contractions. (B) The influence of KCl (0.3 to 1.2 mM) on uterine oscillations 50 min after exposure to K^+-free solution. After a 2-day treatment, the contraction profiles of the vehicle- (*Top tracing)* and 17β-estradiol (*Bottom tracing*) -treated uteri were monitored. Arrowbar indicates the addition of each treatment. Similar results were obtained in 4 additional experiments.

the baseline, uterine oscillations started to develop oscillations and reached to the greatest frequency (21.2±3.2 cycles/10 min). The oscillations remained for about 50 min. More than 50-min exposure to K^+-free solution shifted the oscillations to tonic contractions with continuous elevation of basal tension (about 60% of the initial). In comparison, 17β-estradiol shortened the duration and lowered the maximal frequency during oscillations (P<0.05). The basal tension after a 50-min exposure to K^+-free solution was increased to about 95% of the initial tension by 17β-estradiol (P<0.05) (Table 2).

Right after 1-hr exposure to the K^+-free solution, Na^+/K^+ ATPase activity was reactivated by adding KCl from 0.3 to 1.2 mM at intervals of about 10 min. Prior to the induction of oscillatory contraction, KCl at 0.3 mM lowered the basal tension (Fig. 4B). Only the concentrations of KCl lower than physiological levels (about 5 mM) were able to induce oscillations with inducing muscle relaxation. In comparison, the 17β-estradiol-treated uteri were less responsive to KCl (0.3 to 1.2 mM) (Fig. 4B). The data suggest that the 17β-estradiol-treated uteri may have greater basal tension after inhibition of Na^+/K^+ ATPase activity.

Discussion

Our in vitro findings are consistent with in vivo observation that a 2-day treatment with 17β-estradiol decreased the frequency of spontaneous and ouabain-induced oscillations in conjunction with the decrease of Na^+/K^+ ATPase activity. Our data in Fig. 2 show that ICI 182,780 alone decreased the activity without significantly altering the frequency. In the presence of 17β-estradiol, ICI 182,780 completely reversed the decreased frequency of spontaneous oscillations to the control without fully bringing the decreased activity to the control. The data suggest that the decreased activity by 17β-estradiol may play a minor role in the decreased frequency.

It has been thought that the increase of spontaneous oscillations requires the increase in intercellular communication through gap junctions

and pacemaker-like activity. In pregnant model, chronic exposure of pregnant uteri to 17β-estradiol facilitates intercellular communication by increasing the expression of gap junction protein and enhances the regularity of synchronized contractions at term. It is thought that lack of gap junction proteins may contribute to irregular contractions in non-pregnant uteri (1, 15). This may explain why the vehicle-treated uteri elicited relatively irregular oscillations in response to ouabain (Fig. 3). Even though the increase of gap junctions by 17β-estradiol may contribute to the regularity of oscillatory contractions, it still cannot further increase the frequency. The data imply that gap junction is the unlikely target molecule for the reduction of spontaneous oscillations by estrogens. The remaining candidate responsible for the decrease of spontaneous oscillations by estrogens may be pacemaker-like activity. Kuriyama and coworkers demonstrated that pretreatment with 17β-estradiol in vivo hyperpolarizes the membrane and lowers the discharge rate of pacemaker-likely activity (13). The binding of 17β-estradiol to the beta subunit of maxi-K channels in acute exposure increases the opening of the potassium channels and hyperpolarizes the membrane (20, 26). If the increase of membrane potential contributes to the decreased frequency to the greater extent than the decreased Na^+/K^+ ATPase activity does, we postulated, ICI 182,780 might elicit a greater effect on the decreased frequency than the decreased activity. Our data in Fig. 1 and 2 support our postulation. The decreased activity by 17β-estradiol may play a minor role in the decreased frequency.

It is known that the immediate inhibition of Na^+/K^+ ATPase activity is thought to induce contraction. However, our data show that the prolonged decreased Na^+/K^+ ATPase activity by 17β-estradiol is associated with the reduction of frequency. To explore how the decreased Na^+/K^+ ATPase activity causes the reduction of contraction frequency by 17β-estradiol, we first examined the time-dependent effect of uteri to K^+-free solution. As shown in Fig. 4A, right after replacing the PBS with K^+-free solution, a transient induction of tonic contraction was followed by oscillatory contractions. The oscillatory contractions lasted for about 50 min. At the end of exposure, the oscillatory contraction became tonic with the elevation of basal tension. In the presence of K^+-free solution, re-addition of KCl from 0.3 to 1.2 mM increased the frequency of oscillatory contraction with lowering the basal tension. These data clearly illustrate that the reduction of Na^+/K^+ ATPase may decrease the frequency with the elevation of basal tension. Our data agree with previous reports that pretreatment with Na^+/K^+ ATPase inhibitors may block the appearance of relaxation phase, shifted the profile from phasic to tonic contractions, and eliminated the frequency of oscillatory contractions (8, 9, 11, 14).

As the results of Fig. 4, prolonged inhibiting Na^+/K^+ ATPase may block the development of contraction frequency by reducing the muscle relaxation during the relaxation phase. The induction of muscle relaxation by KCl (0.3-1.2 mM) is associated with the appearance of oscillatory contractions. It is known that cyclic changes in intracellular calcium are required for inducing contraction/relaxation cycles of muscle oscillations. It has been reported that ouabain increases contraction force immediately through an indirect increase in calcium retention (4, 19). Therefore, we postulate that the prolonged inhibition of Na^+/K^+ ATPase activity may increase calcium retention, which then lowers the frequency of spontaneous oscillations. However, the postulation requires further investigation.

This is the first report to compare that the effect of two antiestrogenic compounds on uterine oscillations. ICI 182,780 alone did not alter the frequency but tamoxifen did. In the presence of 17β-estradiol, the reversal effect of ICI 182,780 was greater than that of tamoxifen (Fig. 3 and 4). Since tamoxifen alone lowers the frequency, it may be plausible that tamoxifen does not effectively reverse the decreased frequency. Tamoxifen may decrease the frequency through other pathways. In fact, these two antiestrogenic compounds elicit their actions of antiestrogenicity differently. In the presence of 17β-estradiol, both are antiestrogenic. However, in the absence of 17β-estradiol, ICI 182,780 alone facilitates the degradation of estrogen receptors but tamoxifen acts as a partial agonist (18). This may explain why tamoxifen alone lowered the frequency but ICI 182,780 did not. Since tamoxifen alone lowered the frequency, it may explain why tamoxifen may not significantly reverse the decreased frerquency. The data clear demonstrate that estrogens reduce the frequency of spontaneous oscillations through an estrogen receptor-mediated pathway.

In terms of Na^+/K^+ ATPase activity, ICI 182,780 decreased the activity but tamoxifen did not. However, the reversal effect of tamoxifen on Na^+/K^+ ATPase activity was greater than that of ICI 182,780. Since either ICI 182,780 or 17β-estradiol decreased the activity, we postulate that either down-regulation of estrogen receptors by ICI 182,780 or the activation of other pathways irrelevant to estrogen receptors may decrease the activity. Since the activation of estrogen receptors by estrogens decreases the activity, we postulate that ICI 182,780 decreases the activity by activating a pathway other than estrogen receptors. As shown in Fig. 2, tamoxifen completely reversed the reduced activity to the level greater than the control ($P<0.05$). Because tamoxifen may increase Na^+/K^+ ATPase activity through a PKC-dependent

pathway without activating ER (21), the data suggest that the irreversal effect of tamoxifen on Na^+/K^+ ATPase activity may act through both ER- dependent and independent pathways. Taken together, estrogens may alter the activity through both estrogen receptors and non- estrogen receptors.

Even though spontaneous oscillation is an important function for uteri, this is the first report to compare the effects of two antiestrogenic compounds on the frequency and the activity. Since ICI 182, 780 elicited a greater reversal effect on the reduced frequency than that on the reduced Na^+/K^+ ATPase, the data further suggest that the activation of Na^+/K^+ ATPase only partially is involved in the development of spontaneous oscillatory contractions. It is likely that the activation of Na^+/K^+ ATPase may be partially involved in oscillatory contractions by lowering muscle tension in the relaxation phase. Consequently, the reduction of Na^+/K^+ ATPase activity by a 2-day treatment with 17β-estradiol disrupts progression of muscle relaxation during each oscillatory cycle and then decreases contraction frequency.

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