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Role of 4-Hydroxylated Estradiol in Reducing Ca^{2+} Uptake of Uterine Arterial Smooth Muscle Cells through Potential-Sensitive Channels[1]

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ABSTRACT

Entry of ionic Ca^{2+} into the vascular smooth muscle cell for contraction is thought to be mediated by two major membrane channels. The first are designated as potential-sensitive channels (PSCs), which are opened by membrane depolarization, and the second, as receptor-operated channels (ROCs), which are activated by α1-receptor-ligand interactions. This study was designed to determine the presence of these 2 distinct populations of Ca^{2+} entry channels in smooth muscle cells of the uterine arteries in pigs. This was studied by measuring the baseline tone and contractile properties of uterine arteries in in vitro perfusion studies, as well as their specific Ca^{2+} uptakes. These parameters showed markedly different sensitivities towards two smooth muscle inhibitors used in this study: D-600 and amrinone. D-600 specifically inhibits uptake of extracellular Ca^{2+} through PSCs, while amrinone specifically inhibits Ca^{2+} uptake through ROCs. By choosing an appropriate concentration of D-600 or amrinone, Ca^{2+} uptake and contractions of uterine arterial segments induced by high-K^+ (PSC activator) and phenylephrine (ROC activator) could be selectively inhibited. Furthermore, it was demonstrated that the blockade of Ca^{2+} uptake by D-600 and amrinone was additive, excluding the interpretation of a common Ca^{2+} pathway with two separate mechanisms for opening it. It was also determined that 4-hydoxylated estradiol (4OH-E_2), a compound known to increase uterine blood flow in pigs, decreased Ca^{2+} uptake through the PSCs and exhibited no effect on ROCs. The presence of separate Ca^{2+} pathways that can be activated independently by agonists may indicate a refined system for controlling uterine blood flow.

INTRODUCTION

The administration of estrogen results in an increase in uterine blood flow (UBF) in the pig, as has been observed in other mammalian species (Dickson et al., 1969). This increase in UBF begins after a 20- 30-min lag time, regardless of site of administration, and is inhibited by cycloheximide (Killam et al., 1973). These data suggest that estrogen exerts its vascular effects through local production of a mediator. Recent evidence from our laboratory and others suggests that catechol estrogens (2- and 4-hydroxylated estrogens) may serve as this mediator. These metabolites of estrogen are found in the circulation when estrogens are high (Fishman and Dixon, 1967; Gelbke et al., 1975) and are synthesized by the porcine conceptus (Mondschein et al., 1985), uterine artery (Van Orden et al., 1983a), and endometrium (S. P. Ford, Iowa State University, and D. E. Van Orden, University of Iowa, unpublished observations). In addition, 4-hydroxylated estradiol (4OH-E_2) is equipotent with estradiol-17α (E_2) in increasing UBF when injected into the uterine artery of gilts (Van Orden et al., 1983b), and unlike E_2, its uterine hyperemia is unaffected by simultaneous administration of cycloheximide (Ford et al., 1986). It has also been reported that there are specific membrane-bound receptors for catechol estrogen on estrogen target cells (Schaeffer et al., 1980). Catechol estrogens

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may reach the uterine artery via a lymphatic route. The pig uterus possesses a system of lymphatics that is capable of carrying estrogens from the uterine lumen to the adventitial surface of the uterine artery (Magness and Ford, 1982). Delivery of catechol estrogens to the uterine artery via a lymphatic pathway rather than a vascular route would avoid the rapid methoxylation of those compounds by catechol-O-methyltransferase, which is found in red blood cells (Ball and Knuppen, 1980).

Several lines of evidence suggest that hydroxylated estrogens act through blockage of Ca^{2+} channels. Sandahl et al. (1978) observed that nifedipine, a potential-sensitive Ca^{2+} channel blocker, caused a marked increase in UBF in the rat at doses that had no effects on the general circulation. Further, these researchers observed that rats whose uteri were under estrogen hyperemia exhibited no further increase in UBF in response to nifedipine. Since Walters and Redman (1984) observed a 20-min time lag between the administration of nifedipine and a reduction in blood pressure of pregnant women, we hypothesized a role of hydroxylated estrogens in reducing Ca^{2+} uptake by smooth muscle cells of the uterine arteries. It is known that uptake of extracellular Ca^{2+} is required for vascular smooth muscle contractions, and that vascular relaxation occurs after exposure to drugs that block these channels (Bolton, 1979). Further, membrane Ca^{2+} channel blockade requires an exposure time of several min to block Ca^{2+}-induced contractions (Meisheri et al., 1981). Thus, the 20- to 30-min time lag associated with estrogen-induced vasodilation may result from its hydroxylation and subsequent blockade of Ca^{2+} channels.

Membrane channels mediating Ca^{2+} entry have been designated as two major types. Potential-sensitive channels (PSCs) are activated by depolarization of the cell membrane after an elevation of extracellular K^+ concentration, and Ca^{2+} influx through these channels is selectively inhibited by D-600 (Bolton, 1979; Meisheri et al., 1981). Receptor-operated channels (ROCs) are opened by norepinephrine- or phenylephrine-induced activation of α1-adrenergic receptors, and Ca^{2+} influx through these channels is selectively blocked by amrinone (Meisheri et al., 1981).

This study was therefore conducted to verify the presence of each type of calcium channel (ROC and PSC) in uterine arterial smooth muscle and their possible interaction with 4OH-E_2

MATERIALS AND METHODS

General

Yorkshire gilts of similar age and weight (10–12 mo; 140–160 kg) that were exhibiting consecutive estrous cycles of normal duration (18–22 days) were assigned to be killed during the luteal phase (LP; Day 13) of the estrous cycle. The first day of behavioral estrus was designated as Day 0.

Contraction Studies

At death, a 3.5-cm segment of the middle uterine artery supplying one uterine horn of each gilt was excised immediately in front of its first bifurcation in the mesometrium. Each arterial segment was placed immediately into a container of oxygenated (95% oxygen, 5% carbon dioxide) Krebs-Ringer solution (22°C; composition in millimoles per liter = sodium chloride, 118.1; sodium bicarbonate, 25.0; potassium chloride, 4.7; calcium chloride·6 H_2O, 2.5; potassium dihydrophosphate, 1.2; magnesium sulfate·7 H_2O, 1.2; glucose, 11.1). At the laboratory, both arterial segments were prepared for simultaneous perfusion as described previously for ovine and bovine uterine arteries (Ford et al., 1976). Briefly, arterial segments were cannulated at each end with polyethylene tubing and mounted in duplicate perfusion chambers within 60 min after death. Arterial segments were perfused intraluminally and extraluminally with continuously oxygenated Krebs-Ringer solution (37°C). An extraluminal and intraluminal perfusion rate of 10 ml/min was maintained throughout the perfusion of each artery. Uterine arterial segments were allowed a 30-min equilibration period, by which time all arteries had established a constant baseline perfusion pressure (BPP) against the intra-luminal flow and were ready for the initiation of drug perfusions. Drug dosages are reported as the final concentrations in the intra-luminal perfusion flow. Changes in perfusion pressure arising from changes in resistance to flow through the arterial segments were measured with Statham pressure transducers and recorded in millimeters of mercury (mmHg) by a Hewlett Packard 7700 chart recorder.

Ca^{2+} Uptake Studies

Calcium influx was measured by using a technique previously reported by Meisheri et al. (1981). Mea-

surement of unidirectional fluxes were carried out to study phenylephrine and high-K^+-induced Ca^{2+} uptake (influx). For this, the arterial segments were exposed to ^{45}Ca-containing solutions for a short time period (10 min). The amount of ^{45}Ca entering the tissue during such short periods can be assumed to be due primarily to Ca^{2+} influx. This experimental approach allowed us to selectively determine the influence of phenylephrine or high-K^+ on the uptake of Ca^{2+} from the extracellular space. Briefly, the segment of uterine artery immediately adjacent to the section used for contraction studies was utilized for Ca^{2+} influx determinations. Segments were placed into a container of oxygenated (95% O_2, 5% CO_2) physiological salt solution (PSS) at 22°C, composition in millimoles per liter = sodium chloride, 140; potassium chloride, 4.5; D-glucose, 10; HEPES, [N - 2 - hydroxyethylpiperazine - N' - 2 - ethanesulfonic acid], 5; calcium chloride, 1.5; magnesium chloride, 1.0). At the laboratory, the excess connective tissue was trimmed away, and each artery was cut into 0.5-cm segments for Ca^{2+} influx determinations.

Two 20-cm lengths of 3-0 silk were passed through the lumen of each segment. Both ends of one length of silk were attached to a 2.4-gram weight, while the remaining length was used to lower each segment into a 12 × 75 culture tube containing 4 ml of aerated nonradioactive PSS. A polyethylene stopper was used to clamp this length of silk against the side of the tube, thus suspending the arterial segment and weight in the buffer solution. The 2.4-gram weight was used to apply a constant tension on the arterial smooth muscle, and resulted in a more uniform uptake of ^{45}Ca by triplicate 0.5-cm segments from each animal. These tubes were kept aerated and at 37°C throughout the experiment. After a 60-min equilibration period, the rings were exposed to control or experimental solutions containing E_2, 4OH-E_2, D-600, or amrinone for periods of 30 min, followed by a 10-min exposure to high-K^+, phenylephrine, and 0.5 μCi/ml of ^{45}Ca plus the inhibitor and/or steroid. This experimental protocol was designed to test the criteria of selective inhibition by E_2, 4OH-E_2, D-600, or amrinone of Ca^{2+} uptake induced by the additions of high-K^+ and phenylephrine.

At the end of the 10-min incubation period, tissues were transferred to tubes containing 10 ml of a Ca^{2+}-free buffer (PSS with $CaCl_2$ omitted) containing 10^{-3} M lanthanum chloride ($LaCl_3$) for 60 min maintained at 4°C. At this concentration, $LaCl_3$ has been shown to inhibit both efflux and uptake of Ca^{2+} from vascular smooth muscle cells (Deth and Lynch, 1981). Each segment was then blotted dry, weighed, and placed in a separate scintillation vial containing 0.5 ml of Protosol (NEN Research Products, Boston, MA) and heated at 60°C for 2 h to facilitate the breakdown of tissue. Ten milliliters of a Toluene Triton Scintillation cocktail (7.0 g PPO and 0.3 g POPOP, Fisher Scientific Pittsburgh, PA, and 250 ml Triton X-100, Research Products International Corporation, Mt. Prospect, IL, were dissolved in each liter of Toluene, Scintanalyzed, Fisher Scientific) was added to each vial and counted for radioactivity. Additionally, for each uterine artery, one 0.5-cm arterial segment was used to determine nonspecific binding of ^{45}Ca to the tissue. These segments were maintained in 4 ml of aerated Ca^{2+}-free buffer containing 10^{-3} M $LaCl_3$ for 30 min before and during the 10-min exposure to 0.5 μCi/ml of ^{45}Ca in the presence of phenylephrine and high-K^+. At the end of the incubation period, tissues were again placed in 10 ml of a Ca^{2+}-free buffer containing 10^{-3} M $LaCl_3$ at 4°C for 60 min, and ^{45}Ca uptake was determined as previously described.

The intraassay coefficient of variation, calculated by running multiple segments (n = 6) of a luteal phase uterine artery in a single assay, averaged 6.9%, with an uptake of 237.6 ± 5.7 μmoles ^{45}Ca/kg tissue (wet weight). Nonspecific binding of ^{45}Ca, which was relatively consistent for all arterial segments in this study, averaged 38.8 ± 2.6 μmoles/kg tissue and may have resulted from binding to nonmuscular components of the arterial wall. Nonspecific binding, which averaged 18.1 ± 1.2% of specific uptake, was subtracted from total ^{45}Ca influx for each artery to obtain specific influx.

Drugs and Chemicals

Phenylephrine hydrochloride (Sigma, St. Louis, MO), D-600 (A. G. Knoll Pharmaceutical Laboratories, Ludwigshafen, W. Germany), amrinone (Sterling Winthrop, Rensselaer, NY), and ^{45}Ca (12.0 mCi/mg; New England Nuclear, Boston, MA) were used in this study. The 4OH-E_2 used in the study was obtained from E_2 by direct introduction of molecular oxygen into the phenolic precursor by *Aspergillus alliaceus* and purification on high performance liquid chromatography as previously described (Williamson et al., 1985).

Statistical Analysis

For in vitro perfusion data, treatment differences in uterine arterial response were statistically evaluated by split-plot analysis for a completely randomized design with perfusion periods as the subplot; differences in ^{45}Ca uptake of arteries from gilts were analyzed by factorial design (Kirk, 1968). Simple regression analyses were used to determine relationships between inhibition of ^{45}Ca uptake of uterine arterial segments subjected to different treatments.

RESULTS

In Vitro Contractility Studies

Experiment 1a. The first study was conducted to evaluate the role of 4OH-E_2 in altering uterine arterial responsiveness in vitro. Uterine arterial segments from 5 LP gilts were subjected to in vitro perfusions. On each experimental day, both uterine arteries from each gilt were subjected to simultaneous perfusions, as previously described. After a 30-min equilibration period, BPPs were recorded and both arteries received a bolus infusion of KCl into the intraluminal flow, which elevated concentrations to 200 mM for 5 sec; the resultant increase in intraluminal pressure was recorded. Ten min later, after pressures had returned to baseline, a bolus of phenylephrine was administered into the intraluminal flow, which elevated concentrations to 7.5×10^{-5} M for 5 sec. After both arteries had again returned to their previously established BPP, one of the arteries (randomly selected) was continuously infused with 4OH-E_2 (3.5×10^{-5} M; treated vessel), and the other was infused with vehicle for 30 min (control vessel). After 15 min of 4OH-E_2 infusion, during which time arteries had again established constant BPPs, bolus injections of KCl and phenylephrine were sequentially administered as previously described, and the increases in pressure were again recorded. The increase in perfusion pressure above BPP in response to KCl and phenylephrine was used to estimate relative activity of PSCs and ROCs on the vascular smooth muscle membrane. The concentration of 4OH-E_2 used was determined from preliminary studies to decrease maximally the BPP of uterine arterial segments from LP gilts. The doses of KCl and phenylephrine used were determined from preliminary studies to be in the middle of their respective dose-response curves for increasing contraction of uterine arteries.

Uterine arterial BPP and responsiveness to KCl and phenylephrine remained constant throughout the perfusion sequence in control vessels. After perfusion of treated vessels for 15 min with 4OH-E_2, the BPP had decreased markedly ($p<0.05$; Fig. 1) to reach a new BPP, which remained constant throughout the remaining 15 min of perfusion. Corresponding to this decrease in BPP, there was, in treated vessels, approximately a 50% increase in vessel diameter (average of 3 measurements taken along the arterial segment by using a metal ruler placed against the top of the perfusion chamber), and the response of treated vessels to a depolarizing dose of KCl was significantly reduced ($p<0.05$) when compared to responses before 4OH-E_2 perfusion. The response to phenylephrine, however, was not affected by 4OH-E_2 perfusion. A perfusion of E_2, at similar concentrations, failed to alter arterial responsiveness to KCl or phenylephrine and had no effect on BPP (data not shown).

Experiment 1b. In a subsequent study, both uterine arteries from each of three LP gilts were perfused simultaneously and subjected to increasing concentrations of $CaCl_2$ in the perfusion fluid (Fig.

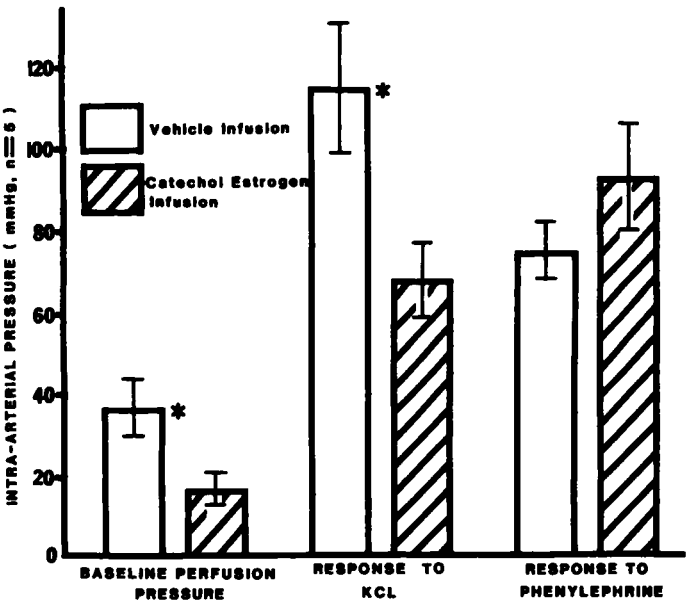

FIG. 1. Effect of 4OH-E_2 (3.5×10^{-5} M) on the baseline perfusion pressure, and response to KCl (0.20 M) and phenylephrine (7.5×10^{-5} M) of uterine arteries from Day 13 nonpregnant gilts. Within a measurement, means with and without *asterisks* differ significantly ($p<0.05$). Means ± SEM (n = 5).

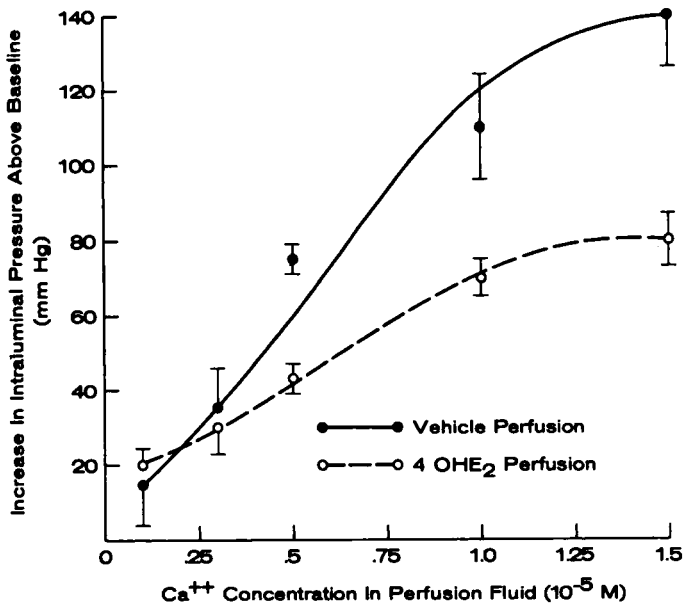

FIG. 2. The effect of 4OH-E_2 (3.5×10^{-5} M) on reducing Ca^{2+}-induced contraction of uterine arteries. The regression equations and R^2 values were y=(9,191,770.2)x + 18.1 and 0.965 for the vessels perfused with 4OH-E_2. Means ± SEM (n = 3).

2). Beginning 15 min before and continuing throughout the $CaCl_2$ perfusions, 1 of each pair of arteries was perfused continuously with vehicle and the other with 4OH-E_2 (3.5×10^{-5} M). Each dose of $CaCl_2$ was perfused for a 5-min period, followed by 5 min of vehicle perfusion during which time each artery returned to its original BPP. As can be seen in Figure 2, uterine arteries perfused with 4OH-E_2 exhibited a significantly ($p<0.05$) reduced contractile response to each extracellular calcium concentration added.

Experiment 1c. A third experiment was conducted to evaluate the effects of calcium channel blockers specific for PSCs (D-600) and ROCs (amrinone) on the responsiveness of uterine arteries in vitro. Three LP gilts were used for these studies. After simultaneous perfusion of a segment of both uterine arteries from each gilt for 30 min (control perfusion) to establish constant BPPs, both arteries were subjected to bolus infusions of KCl and phenylephrine, as outlined in Experiment 1a. One artery, randomly selected, was perfused with D-600 (2×10^{-6} M) and the other with amrinone (5.3×10^{-7} M) for 30 min. Both arteries were allowed 15 min to reestablish constant BPPs after the initiation of drug perfusions, and again subjected to bolus infusions of the KCl and phenylephrine. After perfusion of D-600, BPPs, and the response of arteries to KCl were markedly reduced to 57 ± 10% and 45 ± 14% of preinfusion levels, whereas the response to phenylephrine was slightly elevated, averaging 125 ± 18% of preinfusion levels. Amrinone perfusion had no effect on BPP or response to KCl (100 ± 2 and 125 ± 29% of control perfusion) but reduced the contractile response to phenylephrine (43 ± 12%). As stated previously, vehicle-treated vessels exhibit a constant BPP and a repeatable response to KCl and phenylephrine during multiple perfusion sequences.

$^{45}Ca^{2+}$ Uptake Studies

Experiment 2a. Parallel experiments were carried out to study the effects of E_2 (3.5×10^{-5} M) and 4OH-E_2 (3.5×10^{-5} M) on specific uptake of ^{45}Ca by uterine arteries from LP gilts (n = 6). To be consistent with the perfusion studies, tissues were pretreated with each inhibitory agent for 30 min before exposing them to 200 mM KCl or 7.5×10^{-5} M phenylephrine in the presence of 0.5 μCi/ml ^{45}Ca, also containing the steroid, for 10 min. The doses of KCl and phenylephrine used were determined from preliminary studies to consistently stimulate in vitro contractility of uterine arteries. Exposure to 4OH-E_2 markedly reduced ($p<0.01$) ^{45}Ca uptake by uterine arterial segments from LP gilts from 214 ± 14 to 142 ± 12 μmoles ^{45}Ca/kg arterial tissue during KCl

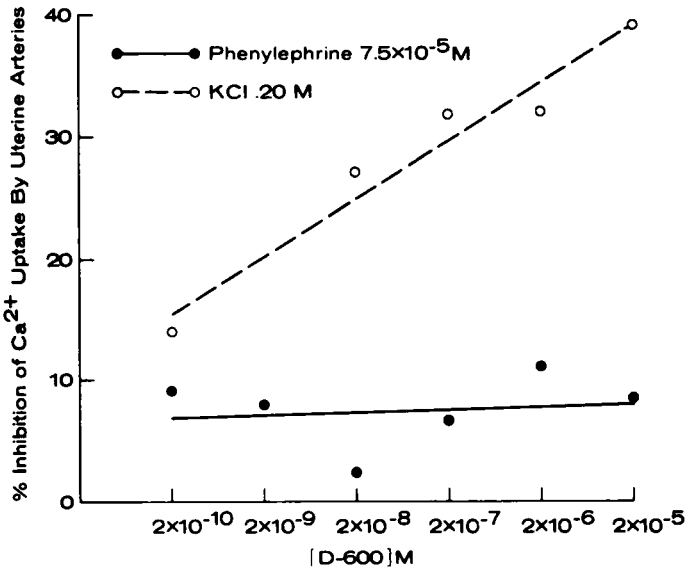

FIG. 3. The effect of increasing concentrations of D-600 on inhibiting ^{45}Ca uptake of uterine arteries stimulated with phenylephrine (7.5×10^{-5} M; regression equation: y=[78,436.1]x + 7.3; R^2 = 0.045) or KCl (0.20M; regression equation: y=(757,067.8)x + 2.5; R^2=.670). (n = 3.)

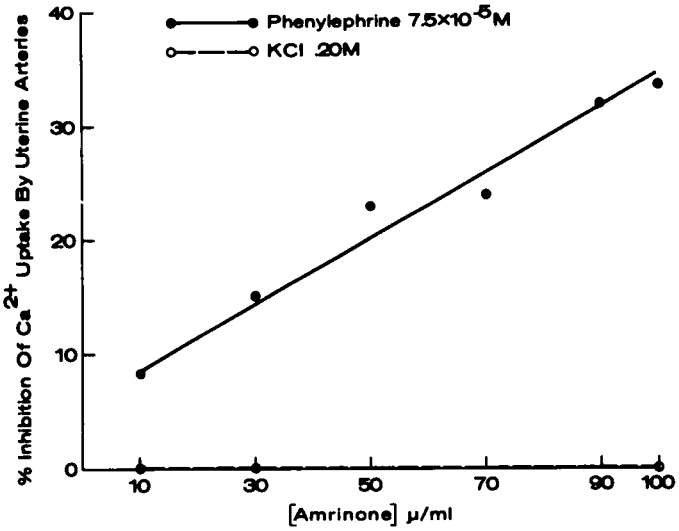

FIG. 4. The effect of increasing concentrations of amrinone on inhibiting ^{45}Ca uptake of uterine arteries stimulated with phenylephrine (7.5 ± 10^{-5} M; regression equation: $y=[0.27]x + 6.93$; $R^2=0.975$) or KCl (0.20 M; no inhibition).

exposure, but had no effect on ^{45}Ca uptake during exposure to phenylephrine. When uterine arteries were incubated with E_2 + KCl or phenylephrine, however, the uptake of ^{45}Ca was not reduced over that seen in arterial segments incubated in the absence of E_2.

Experiment 2b. The specificity of D-600 and amrinone in preventing ^{45}Ca uptake through PSCs and ROCs was investigated by inducing ^{45}Ca uptake with KCl (200 mM) or phenylephrine (7.5×10^{-5} M) in the presence of one of these antagonists. As in Experiment 2a, arterial rings were preincubated in nonradioactive PSS with D-600 or amrinone for 30 min, followed by a 10-min incubation with KCl or phenylephrine plus 0.5 µCi/ml ^{45}Ca. Increasing concentrations of D-600 in the incubation medium progressively reduced KCl-induced uptake of ^{45}Ca but had little effect (< 10%) on inhibiting phenylephrine-induced ^{45}Ca uptake (Fig. 3). Incubation of arterial segments with increasing concentrations of amrinone, however, progressively decreased phenylephrine-induced ^{45}Ca uptake, but had no effect on KCl-induced uptake of ^{45}Ca (Fig. 4). When arterial segments were incubated in medium containing high concentrations of both D-600 (2×10^{-5}) and amrinone (100 µg/ml), their effects were additive, reducing ^{45}Ca uptake induced by KCl and phenylephrine nearly 90% (Fig. 5). When 4OH-E_2 and D-600 were coincubated, however, the uptake of ^{45}Ca was not reduced over that of 4OH-E_2 or D-600 alone. Coincubation of 4OH-E_2 and amrinone resulted in additivity of their individual effects on inhibiting ^{45}Ca uptake.

DISCUSSION

As previously reported by Meisheri et al. (1981) for the rabbit aorta, D-600 greatly inhibited the KCl-induced contraction of uterine arterial segments and exhibited little effect on norepinephrine-induced contractions. At the same time, amrinone predominantly inhibited the contractile response to phenylephrine. In our studies, however, amrinone was much more selective for α_1-receptor-induced contractions than was reported by Meisheri et al. (1981). This difference may result from our use of phenylephrine, a selective α_1-agonist, whereas Meisheri et al. (1981) used norepinephrine, which will bind to α_1- and α_2-adrenergic receptors. Recent studies have indicated that activation of the α_2-receptor prompts depolarization of the smooth muscle cell and the opening of PSCs in the surface membrane (Cavero et al., 1983). Consistent with their effects on contraction, D-600 and amrinone also selectively inhibited uterine arterial uptake of extracellular ^{45}Ca in response to KCl and phenylephrine, respectively, at concentrations equivalent to those used in the in vitro perfusion studies. Thus, by choosing an appropriate concentration of D-600 or amrinone, we could selectively inhibit ^{45}Ca uptake and contraction induced by either high-K^+ or phenylephrine.

These studies are the first to demonstrate an in vitro effect of an estrogen or an estrogen metabolite

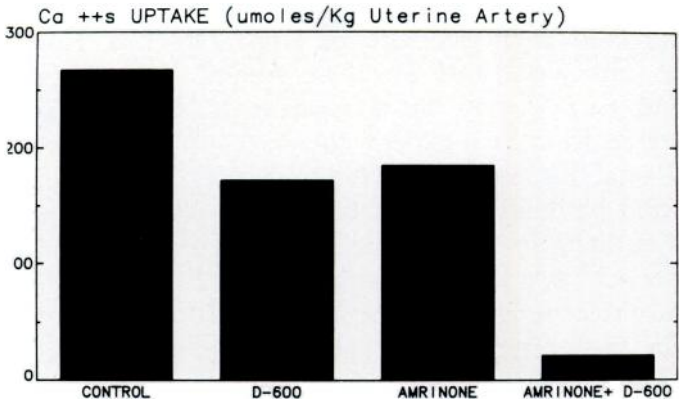

FIG. 5. Uptake of ^{45}Ca by uterine arteries from Day 13 nonpregnant gilts (n = 3) coincubated with phenylephrine (7.5×10^{-5} M) and KCl (0.20 M), before and after additions of D-600 and amrinone, or a combination of both.

on altering the responsiveness of vascular smooth muscle. Hydroxylated estrogen markedly reduced the BPP of uterine arterial segments, while approximately doubling vessel diameter in a manner similar to that elicited by perfusion of D-600. This decline in BPP in response to 4OH-E_2 or D-600 occurred progressively over a 10- to 15-min period, which suggests the necessity of a time lag between their interaction with PSCs and a reduction in ^{45}Ca uptake. Further evidence that 4OH-E_2 is interfering with the uptake of extracellular Ca^{2+} is the reduced ability of arteries to constrict to depolarizing doses of KCl or to increased extracellular Ca^{2+} after perfusion of hydroxylated estrogen. A time lag of 10–20 min between the administration of substances known to antagonize uptake of extracellular Ca^{2+} through PSCs and a reduction in arterial pressure in vivo (Walters and Redman, 1984) or reduced arterial contractility in vitro (Meisheri et al., 1981) has been reported previously. In addition, 4OH-E_2 blocked uterine arterial uptake of extracellular ^{45}Ca in our studies at the same concentrations required to inhibit in vitro responsiveness. The lack of additivity of 4OH-E_2 and D-600 in inhibiting ^{45}Ca uptake when compared to each compound's effect alone again suggests an effect of 4OH-E_2 on inhibiting ^{45}Ca uptake through PSCs.

The failure of 4OH-E_2 to block the contractile response of uterine arteries to phenylephrine and its additivity with amrinone in preventing ^{45}Ca uptake suggests that 4OH-E_2 did not block uptake of extracellular Ca^{2+} through ROCs. These data suggest an effect of 4OH-E_2 on increasing uterine arterial diameter (distensibility), independent of the contractile response of the vessel to α_1-agonist(s).

Previous research has suggested that progesterone, not estrogen, is responsible for altering in vitro contractility of uterine arteries to α_1-agonists and the numbers of α_1-receptors on uterine arterial smooth muscle cells of pigs during the estrous cycle (Ford et al., 1984). Gilts castrated during the luteal phase of the estrous cycle show a decline in uterine arterial contractility and α_1-adrenergic receptor density to levels characteristic of those observed at estrus (Van Orden et al., 1983c). In addition, luteal type contractility to α_1-agonists can be restored in uterine arteries from long-term castrates by in vivo administration of progesterone for several days prior to in vitro perfusion (Ford et al., 1977). Baseline perfusion pressure of these arteries, however, was not modified by progesterone treatment, suggesting a lack of effect on vessel diameter.

Our experiments clearly indicate the presence of 2 separate Ca^{2+} pathways in the plasma membrane of uterine arterial smooth muscle cells. This is verified by the observation that the effects of D-600 and amrinone on inhibiting extracellular Ca^{2+} uptake were additive when the 2 specific Ca^{2+} channel antagonists were applied simultaneously. The presence of 2 separate Ca^{2+} pathways for 2 different stimuli, and the fact that 4OH-E_2 was specific for PSCs, indicate a refined system for activation of smooth muscle where one mechanism of activation can exist independent of the other.

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