Antagonistic Effects of 17β-Estradiol, Progesterone, and Testosterone on Ca\textsuperscript{2+} Entry Mechanisms of Coronary Vasoconstriction

Janice K. Crews, Raouf A. Khalil

Abstract—The clinical observation that coronary artery disease is more common in men and postmenopausal women than in premenopausal women has suggested cardioprotective effects of female sex hormones including hormone-mediated coronary vasodilation. The purpose of this study was to investigate whether the sex hormone-induced coronary relaxation is caused by inhibition of Ca\textsuperscript{2+} mobilization into coronary smooth muscle. The effects of 17β-estradiol, progesterone, and testosterone on vascular reactivity and Ca\textsuperscript{2+} influx were tested in deendothelialized coronary artery strips isolated from castrated male pigs. Prostaglandin F\textsubscript{2α} (PGF\textsubscript{2α}) (10\textsuperscript{-5} mol/L) caused significant, maintained contraction of coronary artery strips. Caffeine (25 mmol/L), an activator of Ca\textsuperscript{2+} release from intracellular stores, caused transient contraction in Ca\textsuperscript{2+}-free solution whereas membrane depolarization by 96 mmol/L KCl, an activator of Ca\textsuperscript{2+} entry, caused maintained contraction in the presence of external Ca\textsuperscript{2+}. The 3 sex hormones caused significant and concentration-dependent relaxation of PGF\textsubscript{2α} and 96 mmol/L KCl-induced contractions with 17β-estradiol being the most effective. The sex hormones did not significantly affect the transient caffeine contraction in Ca\textsuperscript{2+}-free solution. In contrast, the sex hormones significantly inhibited the PGF\textsubscript{2α} and KCl-induced Ca\textsuperscript{2+} influx. 17β-Estradiol caused similar inhibition of PGF\textsubscript{2α} and KCl-induced contractions, suggesting inhibition of the same Ca\textsuperscript{2+} entry mechanism. However, progesterone and testosterone caused greater relaxation of PGF\textsubscript{2α}-induced contraction than of KCl-induced contraction. We conclude that in coronary arteries of castrated male pigs, sex hormones inhibit Ca\textsuperscript{2+} entry from extracellular space but not Ca\textsuperscript{2+} release from intracellular stores. 17β-Estradiol mainly inhibits Ca\textsuperscript{2+} entry, whereas progesterone and testosterone cause coronary relaxation by inhibiting other mechanisms in addition to Ca\textsuperscript{2+} entry. (Arterioscler Thromb Vasc Biol. 1999;19:1034-1040.)

Key Words: sex hormones ■ calcium ■ coronary ■ contraction

Coronary artery disease is one of the most common and costly diseases. Coronary vasospasm and subsequent occlusion are frequently associated with increased cardiovascular risk and may lead to myocardial infarction and death.\textsuperscript{1} Clinical data suggest that the incidence of coronary heart disease is greater in men and postmenopausal women compared with premenopausal women. This is believed to be because of putative cardioprotective effects of the female sex hormone estrogen. The cardioprotective effects of estrogen have been explained by several mechanisms, including modification of lipid and carbohydrate metabolism,\textsuperscript{2} modification of the composition of circulating lipoproteins,\textsuperscript{3-5} and changes in blood coagulation,\textsuperscript{6} as well as direct cardiovascular protective effects on hemodynamics.\textsuperscript{7} Estrogens are vasodilators. For example, it has been reported that subcutaneous administration of β-estradiol in female guinea pigs reversibly and significantly lowers both the resting systolic blood pressure and the peak systolic blood pressure induced by a pressor challenge of norepinephrine.\textsuperscript{8} Also intravenous infusion of estrogen in ovariectomized nonpregnant sheep has been shown to cause a significant increase in cardiac output and a decrease in systemic vascular resistance whereas local application of estrogen to the uterine artery only causes uterine vasodilation and increased uterine blood flow.\textsuperscript{9} The vasodilator effects of estrogen have also been observed in normal coronary arteries.\textsuperscript{10}

The vascular endothelium has been suggested to play a role in mediating the estrogen-induced vasodilation. However, indomethacin does not affect the 17β-estradiol-induced relaxation in endothelium-intact coronary arteries,\textsuperscript{10} indicating that the release of vasodilator prostanoids is not involved in the 17β-estradiol-induced coronary relaxation in vitro. Furthermore, estrogen causes vasodilation in deendothelialized rabbit coronary artery precontracted by endothelin-1, prostaglandin F\textsubscript{3α} (PGF\textsubscript{3α}), and high-potassium depolarizing solution\textsuperscript{10} suggesting that the estrogen-induced inhibition of vascular tone has an endothelium-independent component that involves direct action on vascular smooth muscle.\textsuperscript{5,11,12}
Although several studies have addressed the putative cardiovascular benefits of estrogen, there is little and rather inconsistent information on the vascular effects of other sex hormones. For example, the effects of the other female sex hormone, progesterone, and the male sex hormone, testosterone, on the reactivity of vascular smooth muscle, in general, and coronary smooth muscle, in particular, to various vasoconstrictor agonists have been less clear and have ranged from no effect or increased vascular reactivity to potent vascular relaxation.

Also, vasoactive eicosanoids are metabolites of arachidonic acid synthesized by and released from platelets, white blood cells, and vascular smooth muscle cells in response to tissue injury. Among these eicosanoids, the cyclooxygenase metabolite PGF₂₅₅ has a potent vasoconstrictive effect in various vascular beds, including coronary vessels. Although vasoactive eicosanoids have been implicated in the pathogenesis of coronary vasospasm, little is known about the modulation of their vasoconstrictive action by sex hormones.

Furthermore, the cellular mechanisms of the sex hormone-induced changes in the contractility of vascular smooth muscle, in general, and coronary smooth muscle, in particular, have not been clearly elucidated. Specifically, the effects of sex hormones on the Ca²⁺ mobilization mechanisms in coronary smooth muscle, namely Ca²⁺ release from the intracellular stores and Ca²⁺ entry from the extracellular space, remain unclear.

The purpose of the present study was the following: (1) To determine whether sex hormones other than estrogen cause coronary vascular relaxation and the magnitude of this relaxation, if any, compared with that induced by estrogen. Therefore, the effects of estrogen and the other major female sex hormone, progesterone, as well as the male sex hormone, testosterone, on the contraction of coronary artery strips induced by the vasoactive eicosanoid PGF₂₅₅ were compared.

(2) To determine whether sex hormones inhibit Ca²⁺ release from the intracellular stores. Therefore, the effects of sex hormones on caffeine-induced contraction were investigated.

(3) To determine whether sex hormones inhibit Ca²⁺ entry from the extracellular space and to investigate the possible Ca²⁺ entry pathways involved. Therefore, the effects of sex hormones on PGF₂₅₅-depolarization-induced Ca²⁺ influx were investigated.

Methods

Tissue Preparation

Castrated male Yorkshire pigs (20 to 30 kg) were anesthetized by inhalation of isoflurane (Ohio Medical Products). This animal model was used to minimize the contribution of endogenous sex hormones. The abdominal cavity was opened and the animal was bled by severing the renal artery. The chest cavity was opened and the heart was removed rapidly and immersed in normal Krebs solution. All procedures were performed in accordance with the guidelines of the Animal Care and Use Committee at the University of Mississippi Medical Center and the American Physiological Society. The left anterior descending coronary artery was dissected under a dissection microscope, cleaned of connective tissue, and cut transversely into 3-mm-wide rings. The endothelium was removed by gently rubbing the vessel interior with forceps.

Isometric Tension

Coronary artery rings were cut open into strips. One end of the strip was attached to a glass hook using a thread loop and the other end was connected to a Grass force transducer (FT03, Astro-Med). Strips were stretched to 2 g of tension and allowed to equilibrate for 1 hour in a water-jacketed, temperature-controlled organ bath filled with 50 mL Krebs solution continuously bubbled with 95% O₂/5% CO₂ at 37°C. Preliminary experiments with coronary artery strips equilibrated to 0.5, 1, 1.5, and 2 g of resting tension showed that maximal 96 mmol/L KCl-induced contraction was obtained in tissues adjusted to 2 g of resting tension. This resting tension was equivalent to stretching the tissue to approximately 1.5 times its original length. Further increases in resting tension did not significantly change the maximal contraction to 96 mmol/L KCl. The changes in isometric tension were recorded on a Grass polygraph (model 7D, Astro-Med). Removal of the endothelium was routinely verified by the absence of acetylcholine (10⁻⁶ mol/L)-induced vasorelaxation in coronary strips precontracted with PGF₂₅₅ (3×10⁻⁷ mol/L).

Three different protocols were followed in this study. In the first protocol, PGF₂₅₅ (10⁻⁵ mol/L) was added to the Krebs solution. When the contraction reached a plateau, the tissue was rinsed with Krebs solution 3 times for a duration of 10 minutes each. The whole procedure of contraction and washing was repeated 2 times. PGF₂₅₅ (10⁻⁵ mol/L) was then added to the Krebs solution to induce a maximal control contraction. The sex hormones 17β-estradiol, progesterone, and testosterone were added individually, 1 hormone per chamber. One chamber was used as a control in which the tissue was treated with the vehicle. The hormones were added cumulatively at concentrations ranging from 10⁻⁷ to 10⁻⁴ mol/L, and changes in isometric tension were recorded and presented as percentage of the maximal PGF₂₅₅-induced contraction. For each hormone concentration, the effect of the hormone on contraction reached a plateau before the next hormone concentration was added.

In the second protocol, the bathing solution was changed to 96 mmol/L KCl to induce contraction. The whole procedure of contraction and washing was repeated 2 times. The bathing solution was changed to 96 mmol/L KCl to induce a maximal control contraction. The sex hormones were added individually, 1 hormone per chamber. The hormones were added cumulatively at concentrations ranging from 10⁻⁷ to 10⁻⁴ mol/L, and changes in isometric tension were recorded and presented as percentage of the maximal 96 mmol/L KCl contraction.

In the third protocol, the tissues were stimulated with 96 mmol/L KCl to induce contraction. Once the KCl contraction reached a plateau, the tissue was rinsed with Krebs solution 3 times for a duration of 10 minutes each. The whole procedure of contraction and washing was repeated 2 times. The tissues were incubated in Ca²⁺-free (2 mmol/L EGTA) Krebs for 5 minutes, and then stimulated with caffeine (25 mmol/L) for a duration of 2 minutes or until the transient contraction returned to the baseline. The bathing solution was then changed to normal Krebs (2.5 mmol/L Ca²⁺) solution, and the tissue was allowed to equilibrate for 1 hour to replenish the intracellular Ca²⁺ stores. After the basal tone reached a steady-state the sex hormones (10⁻⁵ mol/L) were added, 1 hormone per chamber, for a duration of 1 hour. One chamber was used as a control in which the tissue was treated with the vehicle. After 1 hour the Krebs solution was replaced with Ca²⁺-free (2 mmol/L EGTA) Krebs in the continuous presence of the hormone (10⁻⁵ mol/L) or the vehicle. The tissues were incubated in the Ca²⁺-free (2 mmol/L EGTA) Krebs for 5 minutes, and then stimulated with caffeine (25 mmol/L) to elicit a second caffeine contraction.

⁴⁵Ca²⁺ Influx

Coronary artery strips were incubated in normal Krebs solution for 1 hour in preparation for measurement of Ca²⁺ influx as previously described. The strips were stimulated with 10⁻⁵ mol/L PGE₂ or 96 mmol/L KCl for 30 minutes, and then transferred to PGE₂ or 96 mmol/L KCl solution supplemented with 10⁻⁵ mol/L or 6×10⁻⁵ mol/L 17β-estradiol, progesterone, or testosterone, or the vehicle, for 1 hour. The incubation times were determined by the time the sex hormone effect on contraction reached a steady-state. The tissues were then transferred to the respective radioactive ⁴⁵Ca²⁺-labeled solution (specific activity, 2 μCi/mL; ICN Radiochemical) for 90 seconds. Preliminary experiments showed that the relationship between ⁴⁵Ca²⁺ uptake versus time is linear during 15-, 30-, 60-, and 90-second exposures to ⁴⁵Ca²⁺-labeled solution. The tissues were
transferred to ice-cold Ca\(^{2+}\)-free (2 mmol/L EGTA) Krebs for 45 minutes to quench extracellular \(^4\text{Ca}^{2+}\) label. The tissues were weighed and placed in 2 mL hypotonic (5 mmol/L) EDTA for 24 hours at 4°C to disrupt the cell membranes and release the intracellular content of \(^4\text{Ca}^{2+}\). The next day, 4 mL of Ecolite scintillation cocktail was added, and the samples were counted in a scintillation counter (LS 6500, Beckman Instruments).

**Solutions**

Normal Krebs solution contained, in mmol/L: NaCl, 120; KCl, 5.9; NaHCO\(_3\), 25; NaH\(_2\)PO\(_4\), 1.2; dextrose, 11.5; MgCl\(_2\), 1.2; CaCl\(_2\), 2.5. The solution was bubbled with 95% O\(_2\)/5% CO\(_2\) to adjust the pH to 7.4. For the Ca\(^{2+}\)-free Krebs solution CaCl\(_2\) was omitted and 2 mmol/L EGTA was added. The high-KCl depolarizing solution was prepared as Krebs solution but with equimolar substitution of NaCl with KCl.

**Drugs and Chemicals**

Stock solution of PGF\(_{2\alpha}\) was prepared as \(10^{-2}\) mol/L in distilled water. Caffeine was prepared as 25 mmol/L in Ca\(^{2+}\)-free (2 mmol/L EGTA) Krebs solution. Stock solution of 17β-estradiol (2,3,5[10]-estratriene-3,17β-diol; Sigma Chemical Co) was prepared as 5\(\times\)10\(^{-3}\) mol/L in 100% ethyl alcohol. Stock solutions of progesterone (4-pregnene-3,20-dione; Sigma) and testosterone (4-androsten-17β-ol-3-one; Sigma) were prepared as 10\(^{-5}\) mol/L in 100% ethyl alcohol. Diluted 17β-estradiol, progesterone, and testosterone solutions were also made in 100% ethyl alcohol. All other chemicals were of reagent grade or better.

**Statistical Analysis**

The developed force in the presence of the hormone was presented as the percentage of maximal PGF\(_{2\alpha}\)-, caffeine-, or KCl-induced force in the absence of the hormone. Data were analyzed and expressed as mean±SEM. Data were compared using 1-way ANOVA with Scheffé test and unpaired Student’s t test. Differences \(p<0.05\) were considered statistically significant.

**Results**

In normal Krebs (2.5 mmol/L Ca\(^{2+}\)) solution PGF\(_{2\alpha}\) (10\(^{-5}\) mol/L) caused a significant contractile response of coronary artery strips that was 48.87±9.21% (n=7) of control 96 mmol/L KCl contraction and was maintained for at least 4 hours. The 3 sex hormones 17β-estradiol, progesterone, and testosterone caused significant relaxation of the PGF\(_{2\alpha}\)-induced contraction (Figure 1). The sex hormone-induced relaxation of PGF\(_{2\alpha}\)-induced contraction was concentration-dependent (Figure 2). 17β-Estradiol (IC\(_{50}\)=8\(\times\)10\(^{-6}\) mol/L) was more effective at inducing coronary relaxation than progesterone (IC\(_{50}\)=2.7\(\times\)10\(^{-5}\) mol/L) or testosterone (IC\(_{50}\)=2.3\(\times\)10\(^{-7}\) mol/L). Similar concentrations of the nonsex steroid hormones 11-deoxycorticosterone and deoxycorticosterone acetate did not cause any significant inhibition of PGF\(_{2\alpha}\)-induced contraction.

Agonist-induced contraction of vascular smooth muscle may involve Ca\(^{2+}\) release from the intracellular stores or Ca\(^{2+}\)-free entry from the extracellular space.\(^{22}\) Agonist-induced contraction in Ca\(^{2+}\)-free solution has been used to measure the Ca\(^{2+}\) release component of smooth muscle contraction and has been shown to be rapid and transient.\(^ {23}\) In Ca\(^{2+}\)-free (2 mmol/L EGTA) Krebs, PGF\(_{2\alpha}\) caused a small but maintained contraction that reached 5.52±1.07% (n=7) of control 96 mmol/L KCl contraction. The observation that the PGF\(_{2\alpha}\)-induced contraction in Ca\(^{2+}\)-free Krebs was maintained suggested that it might not solely reflect Ca\(^{2+}\) release from the intracellular stores. In contrast, caffeine is known to stimulate Ca\(^{2+}\) release from the intracellular stores and to cause transient contraction in vascular smooth muscle.\(^ {23}\) In the control experiments, caffeine (25 mmol/L) caused a small transient contraction in Ca\(^{2+}\)-free (2 mmol/L EGTA) solution that was 7.38±0.70% (n=22) of the control 96 mmol/L KCl contraction. The tissues were washed 3 times in normal Krebs (2.5 mmol/L Ca\(^{2+}\)) solution to replenish the intracellular Ca\(^{2+}\) stores. Under these conditions a second caffeine contraction in Ca\(^{2+}\)-free (2 mmol/L EGTA) was 97.4±7.32% (n=4) of the first caffeine contraction (Figure 3). The caffeine response in the tissues pretreated with the sex hormones was not
significantly different from that in the control tissues treated with the vehicle (Figure 3).

To test whether the sex hormones inhibit coronary artery contraction by inhibiting Ca\textsuperscript{2+} entry into the coronary smooth muscle, we tested the effect of the 3 sex hormones on PGF\textsubscript{2α}-induced 45 Ca\textsuperscript{2+} influx. In unstimulated coronary artery the basal 45 Ca\textsuperscript{2+} influx was 11.76±0.57 µmol/kg/min (n=10). PGF\textsubscript{2α} (10\textsuperscript{-5} mol/L) caused a significant (P=0.049) increase in Ca\textsuperscript{2+} influx to 28.27±6.64 µmol·kg\textsuperscript{-1}·min\textsuperscript{-1} (n=14). The 3 sex hormones significantly reduced the PGF\textsubscript{2α}-induced Ca\textsuperscript{2+} entry (Figure 4).

We investigated the possible Ca\textsuperscript{2+}-entry pathways that might be modulated during sex hormone-induced inhibition of coronary artery contraction. Membrane depolarization by high KCl solution is known to stimulate Ca\textsuperscript{2+} entry through voltage-gated Ca\textsuperscript{2+} channels. Figure 5 shows that the 3 sex hormones caused significant relaxation of 96 mmol/L KCl-induced Ca\textsuperscript{2+} entry with 17β-estradiol being more effective than progesterone or testosterone.

To further investigate the effects of sex hormones on Ca\textsuperscript{2+} entry through voltage-gated Ca\textsuperscript{2+} channels, we measured the effect of the sex hormones on the 45Ca\textsuperscript{2+} influx induced by 96 mmol/L KCl depolarizing solution (Figure 6). The 96 mmol/L KCl-induced 45Ca\textsuperscript{2+} influx in tissues treated with the vehicle was 32.93±4.78 µmol·kg\textsuperscript{-1}·min\textsuperscript{-1} (n=15). In tissues treated with 17β-estradiol (10\textsuperscript{-5} mol/L) the 96 mmol/L KCl-induced 45Ca\textsuperscript{2+} influx was significantly (P=0.033) reduced to 19.03±2.04 µmol·kg\textsuperscript{-1}·min\textsuperscript{-1} (n=10). In contrast, the 96 mmol/L KCl-induced Ca\textsuperscript{2+} influx in the presence of 10\textsuperscript{-5} mol/L progesterone and testosterone was slightly but insignificantly reduced (Figure 6A). However, when the tissues were treated with a higher concentra-
Sex Hormones Inhibit Ca$^{2+}$ Influx in Coronary Artery

The present study demonstrates that (1) both female and male sex hormones cause relaxation of PGF$\alpha_{2}$- and depolarization-induced contractions of coronary artery with 17β-estradiol being the most effective, (2) sex hormones do not inhibit caffeine-induced transient contraction of coronary artery, (3) sex hormones inhibit both PGF$\alpha_{2}$- and depolarization-induced Ca$^{2+}$ entry, and (4) 17β-estradiol causes similar inhibition of PGF$\alpha_{2}$- and depolarization-induced coronary artery contractions, whereas progesterone and testosterone cause greater inhibition of the PGF$\alpha_{2}$-induced contraction than the depolarization-induced contraction.

The present study showed that 17β-estradiol caused significant relaxation of PGF$\alpha_{2}$-induced contraction of coronary artery smooth muscle. These results are consistent with the clinical data that suggested cardiovascular protective effects of estrogen.24–26 The results are also in agreement with experimental data that have shown that estrogen causes vascular relaxation in constricted blood vessels such as rabbit coronary artery.10,12 and human coronary artery.27

We have also found that both progesterone and testosterone cause significant relaxation of the coronary artery, although the inhibitory effect of these hormones was less potent than that of 17β-estradiol. Our results with progesterone are different from other reports, which have shown that progesterone induces a negative or opposing effect on blood flow and vasodilation and a minimal effect on canine coronary artery relaxation.13 Other studies, however, have shown that progesterone induces endothelium-independent relaxation of rabbit coronary artery.28 The cause of the difference between the results is not clear but may be related to differences in experimental animal species. There have also been inconsistent reports on the effects of testosterone on vascular reactivity. Several studies have reported that testosterone enhances the pressor response to norepinephrine in spinal cat14 and increases the vascular reactivity to norepinephrine in the perfused hindlimb of dogs.15 Other studies have shown that testosterone has a potent vasorelaxant effect in the rabbit coronary artery and aorta.16 The present results provide evidence that testosterone has a potent vasorelaxant effect in porcine coronary artery.

One purpose of the present study was to investigate the effects of sex hormone on Ca$^{2+}$-mobilization mechanisms in coronary smooth muscle, specifically Ca$^{2+}$ release from the intracellular stores and Ca$^{2+}$ entry from the extracellular space. The present results showed that 17β-estradiol, progesterone, and testosterone did not cause any significant inhibition of the transient caffeine-induced contraction. These results suggest that the vasorelaxant mechanism used by the sex hormones may not involve inhibition of releasable intracellular Ca$^{2+}$ stores. However, the results should be interpreted with caution because the conclusion was based on the experiments with caffeine.

We found that the sex hormones caused significant inhibition of PGF$\alpha_{2}$-induced Ca$^{2+}$ entry into coronary arterial smooth muscle. Agonist-stimulated Ca$^{2+}$ influx could be through voltage-gated and receptor-operated Ca$^{2+}$ channels.29,30 The present results showed that each of the 3 sex hormones caused significant inhibition of the depolarization-induced contraction of the coronary artery. We also found

![Figure 7](image-url)

**Figure 7.** Effect of individual sex hormones on the initial maximal steady-state contraction induced by either 10$^{-5}$ mol/L PGF$\alpha_{2}$ (open symbols) or 96 mmol/L KCl (closed symbols) in coronary artery strips. Inhibition was measured at increasing concentrations of 17β-estradiol (A), progesterone (B), or testosterone (C), and presented as percentage of the initial maximal contraction to either PGF$\alpha_{2}$ or 96 mmol/L KCl. Data represent mean±SEM of measurements in individual coronary artery strips from 6 to 8 pigs. *Indicates inhibition of 96 mmol/L KCl-induced contraction by a specific concentration of sex hormone is significantly different (P<0.05) from inhibition of PGF$\alpha_{2}$-induced contraction by the same concentration of the same sex hormone.
that the sex hormones caused significant inhibition of depolarization-induced Ca\(^{2+}\) influx. These results provide the first evidence that sex hormones inhibit coronary artery contraction by inhibiting Ca\(^{2+}\) entry through voltage-gated Ca\(^{2+}\) channels in coronary smooth muscle. To our knowledge, 1 study has shown that estrogen blocks voltage-gated Ca\(^{2+}\) channels in cultured A7r5 cells.31 Although the Ca\(^{2+}\) permeability through voltage-gated channels may be different in cultured cells, our present results in coronary smooth muscle are still consistent with the findings of this report and should represent an important area for future electrophysiology investigations.

Finally, we investigated whether the sex hormones inhibit PGF\(_{2\alpha}\) and depolarization-induced coronary smooth muscle contractions by inhibiting the same Ca\(^{2+}\)-entry pathway. We did not find a significant difference between the 17\(\beta\)-estradiol-induced relaxation of PGF\(_{2\alpha}\)- and KCl-induced contractions, suggesting that, regardless of the type of stimulant, estrogen probably inhibits the same Ca\(^{2+}\)-entry mechanism, namely Ca\(^{2+}\) entry through voltage-gated Ca\(^{2+}\) channels. However, these findings should be interpreted with caution because estrogens may be equally potent in inhibiting Ca\(^{2+}\) entry through voltage-gated Ca\(^{2+}\) channels as well as other types of excitable Ca\(^{2+}\) channels. Interestingly, the progesterone- and testosterone-induced relaxation of PGF\(_{2\alpha}\)-induced contraction was significantly greater than the relaxation of KCl-induced contraction. This suggests that progesterone and testosterone not only inhibit Ca\(^{2+}\) entry through voltage-gated channels but may also inhibit other mechanisms activated by PGF\(_{2\alpha}\). These possible mechanisms may include the following. (1) PGF\(_{2\alpha}\) may stimulate Ca\(^{2+}\) entry through other types of Ca\(^{2+}\) channels, for example the receptor-operated Ca\(^{2+}\) channels.29,30 If this is the case, then progesterone and testosterone are probably more effective at inhibiting Ca\(^{2+}\) entry through receptor-operated Ca\(^{2+}\) channels than through voltage-gated Ca\(^{2+}\) channels. This is supported by the present observation that relatively lower concentrations (10\(^{-7}\) mol/L) of progesterone or testosterone were required to significantly inhibit the PGF\(_{2\alpha}\)-induced Ca\(^{2+}\) influx compared with the relatively greater hormone concentrations required to significantly inhibit the depolarization-induced Ca\(^{2+}\) influx. (2) PGF\(_{2\alpha}\) may stimulate other mechanisms in addition to stimulation of Ca\(^{2+}\) entry from extracellular space. For example, PGF\(_{2\alpha}\) may activate the enzyme protein kinase C by increasing the formation of diacylglycerol.32 If this is the case, then progesterone and testosterone may be acting by inhibiting these additional contractile mechanisms.

It is important to note that the present experiments were conducted on coronary arteries from castrated male pigs. Therefore, we cannot generalize that the observed vascular relaxation by sex hormones is the general effect of the hormones on coronary arteries from either males with intact gonads or females independent of their hormonal status because expression of estrogen, progesterone, or testosterone receptors in coronary arteries may vary depending on sex and the status of the gonads. Comparison of the vascular effects of sex hormones on coronary arteries from male and female pigs with and without intact gonads should, therefore, represent an interesting area for future investigation. Also, in the present study, micromolar concentrations of sex hormones caused significant vascular relaxation and decreased Ca\(^{2+}\) entry in isolated coronary artery strips from castrated male pigs. It remains to be investigated whether similar vascular effects also occur under the more physiological in vivo conditions in which levels of the sex hormones and expression of the sex hormone receptors may vary depending on sex and the presence or absence of functioning gonads.

In conclusion, both the female sex hormones, estrogen and progesterone, and the male sex hormone, testosterone, cause significant relaxation of PGF\(_{2\alpha}\)- and depolarization-induced contractions of coronary arteries of castrated male pigs, with estrogen being the most effective. Sex hormones inhibit Ca\(^{2+}\) entry into coronary smooth muscle, but not Ca\(^{2+}\) release from intracellular stores. The results suggest that estrogen mainly inhibits Ca\(^{2+}\) entry through voltage-gated Ca\(^{2+}\) channels, whereas progesterone and testosterone may inhibit Ca\(^{2+}\) entry through other types of Ca\(^{2+}\) channels or suppress other contractile mechanisms in addition to Ca\(^{2+}\) entry. Further studies are needed to investigate the effects of sex hormones on these possible additional contractile mechanisms.

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References

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