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Progesterone modulates estradiol actions: acute effects at physiological concentrations

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Abstract

The progestin element of hormone replacement therapy may reduce the cardioprotective actions of the estrogen component. Only high concentrations (μM) of progesterone directly relaxed U46619 (9,11-dideoxy-9α, 11α-methanoepoxy prostaglandin F2α)-pre-contracted porcine coronary artery rings. A low concentration of progesterone (1 nM), with no effects of its own, shifted the relaxation curves of bradykinin and calcium ionophore A23187 to the right while not affecting those of sodium nitroprusside and levcromakalim. The negative influence that 1 nM progesterone exerted on bradykinin- and A23187-mediated relaxation was diminished when 1 nM 17β-estradiol was concomitantly added to the bathing medium. Conversely, the potentiating actions of 1 nM 17β-estradiol on relaxations elicited by sodium nitroprusside and levcromakalim were reduced following simultaneous treatment with the same concentrations of progesterone. These findings represent the first evidence for an acute in vitro vascular effect of progesterone at a physiologically relevant concentration and concur with previous in vivo reports demonstrating that progesterone may diminish the beneficial effects of estrogens.

Keywords: Progesterone; 17β-Estradiol; Vasorelaxation; Coronary artery; (Porcine)

1. Introduction

There is compelling evidence for the cardioprotective actions of estrogen. Pre-menopausal women are less susceptible to coronary heart disease than age-matched men (Barrett-Connor, 1995) and postmenopausal women (Saaman and Crawford, 1995). Epidemiological and clinical studies carried out with postmenopausal women suggest that hormonal replacement therapy not only improves vasodilatation (Gilligan et al., 1994; Lieberman et al., 1994; Reis et al., 1994) but also favourably alters lipid profiles (Ettinger et al., 1996). Furthermore, data derived from animal and human models have indicated that such regimens can diminish coronary artery atherosclerosis (Clarkson et al., 1996) and reduce coronary heart disease mortality (Ettinger et al., 1996).

In clinical practice, an estrogen–progesterone combination is commonly recommended to postmenopausal women (American College of Physicians, 1992) as cumulative unopposed estrogen usage is believed to increase the risk of thrombosis as well as uterine and breast malignancies (Belchetz, 1994; Colditz et al., 1995). Some findings have however suggested that concurrent progesterone therapy may oppose the beneficial effects of estrogen. Estrogen-enhancement of endothelium-dependent vasodilatation was reduced in isolated canine coronary artery rings after progesterone was co-administered with estrogen to the dogs for 14–21 days (Miller and Vanhoutte, 1991). Similarly, medroxyprogesterone acetate appeared to decrease the positive effects estrogen had on coronary vasoreactivity in ovariectomised monkeys that had been given hormonal therapy for at least a month (Williams et al., 1994; Miyagawa et al., 1997). Furthermore, progesterone appeared to also antagonise the inhibitory influence estrogen had on intimal plaque formation in the aortic tissues of hypercholesterolemic female rabbits (Hanke et al., 1996a,b). Taken together, these studies have provided preliminary evidence for the antagonistic actions of progesterone on the positive vascular effects of estrogen.

To the best of our knowledge, most, if not all, of the work concerned with the interactive relationship of progesterone and estrogen has been carried out with in vivo

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animal models. Due to limited investigations in in vitro settings, the mechanisms behind the reported negative regulatory activity of progesterone on the effects produced by estrogen remain unresolved. Previously, we demonstrated that endothelium-independent relaxation could be enhanced in porcine coronary artery rings following short-term (20 min) exposure to a physiologically relevant concentration (1 nM) of 17β-estradiol (Teoh et al., 1999). The current experiments were therefore designed to determine whether or not acute treatment with the same physiological level (1 nM) of progesterone could itself alter endothelium-dependent and endothelium-independent relaxations and/or modulate the potentiating effects of 1 nM 17β-estradiol in the same model.

2. Materials and methods

2.1. Tissue preparation

Hearts from pigs of either sex were collected from the local abattoir and rinsed in cold, oxygenated (95%O_2: 5%CO_2) Krebs–Henseleit solution (KHS; composition in mM: 120 NaCl, 4.76 KCl, 25 $NaHCO_3$, 1.18 $NaH_2PO_4 \cdot H_2O$, 1.25 $CaCl_2$, 1.18 $MgSO_4 \cdot H_2O$, 5.5 glucose) before the left anterior descending and right coronary arteries were isolated. After removal of connective tissue, intact coronary artery rings (3 mm wide) were suspended in organ baths filled with oxygenated KHS maintained at 37°C. Artery segments were placed under a 2 g tension for at least 100 min before commencement of the experiment. Except for the last 30 min, bath KHS was changed every 20 min during the entire equilibration period. Isometric tension was determined with force transducers coupled to an amplifier and a personal computer for data collection.

2.2. Experimental protocol

The viability of each porcine coronary artery ring was determined by eliciting contraction with 30 nM U46619 in the presence of 10 μM indomethacin and relaxation with 1 μM bradykinin. Only rings that produced ≥ 4 g contraction and demonstrated $\geq 80\%$ relaxation were used for further studies. After repeated washing to remove these drugs, rings were again exposed to 10 μM indomethacin for 20 min. Where required, vehicle or progesterone and/or 17β-estradiol (at a final concentration of 1 nM) were added to the bathing medium for 20 min before contraction was elicited with 30 nM U46619. The hormones, when added, remained present throughout the experiment. After a stable contraction was achieved, cumulative concentration–response curves were recorded for bradykinin, calcium ionophore A23187, sodium nitroprusside or levcromakalim. In all cases, only one relaxing agent was applied to each ring segment.

2.3. Drugs

U46619 (9,11-dideoxy-9α, 11α-methanoepoxy prostaglandin F2α) was obtained from Biomol, PA, USA. Levcromakalim was a gift from SmithKline Beecham, UK. All other chemicals were from Sigma, St. Louis, MO, USA. Stocks of 17β-estradiol, levcromakalim and U46619 were made up in ethanol. The final concentration of ethanol in each bath was always $\leq 0.2\%$. A23187 was dissolved in dimethyl sulphoxide (final concentration 0.1%) and indomethacin in a buffered 1 mM sodium carbonate solution. Ethanol and dimethyl sulphoxide at the highest bath concentrations had no effect on the responses measured. Working solutions were obtained by dilution with KHS.

2.4. Data and statistical analysis

All data denote means ± S.E.M. with N indicating the number of porcine hearts. Relaxations elicited by the vasodilators are expressed as percentages of the U46619-induced contraction. Log(EC_{50}) values were determined using a curve-fitting program (SigmaPlot, Jandel Scientific Software, CA, USA). Analysis of variance (ANOVA) and Bonferroni's test were applied where appropriate to determine individual differences between multiple groups of data, using a computer statistical package (SigmaStat, Jandel Scientific Software). Significance was set at $P < 0.05$.

3. Results

The artery rings used ($N = 30$) contracted 6.81 ± 0.21 g in response to 30 nM U46619 and relaxed 92.68 ± 0.94% in response to 1 μM bradykinin. Progesterone (10–100 μM) directly relaxed U46619-pre-contracted rings resulting in a mean maximum response of 86.45 ± 4.20% (Fig. 1). Parallel experiments indicated that the vehicle (final

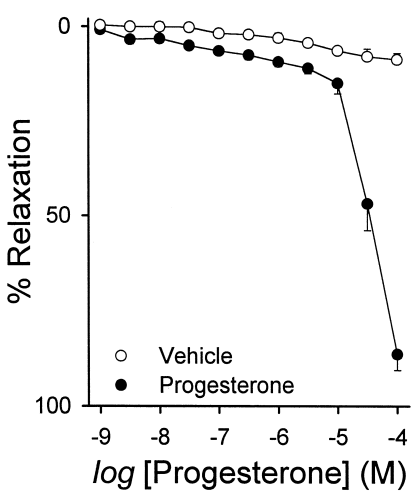

Fig. 1. Direct relaxing effect of progesterone in isolated porcine coronary artery rings. Tissue segments were contracted with 30 nM U46619 and relaxed with cumulative addition of ethanol vehicle or progesterone ($N = 6$). Data represent means ± S.E.M.

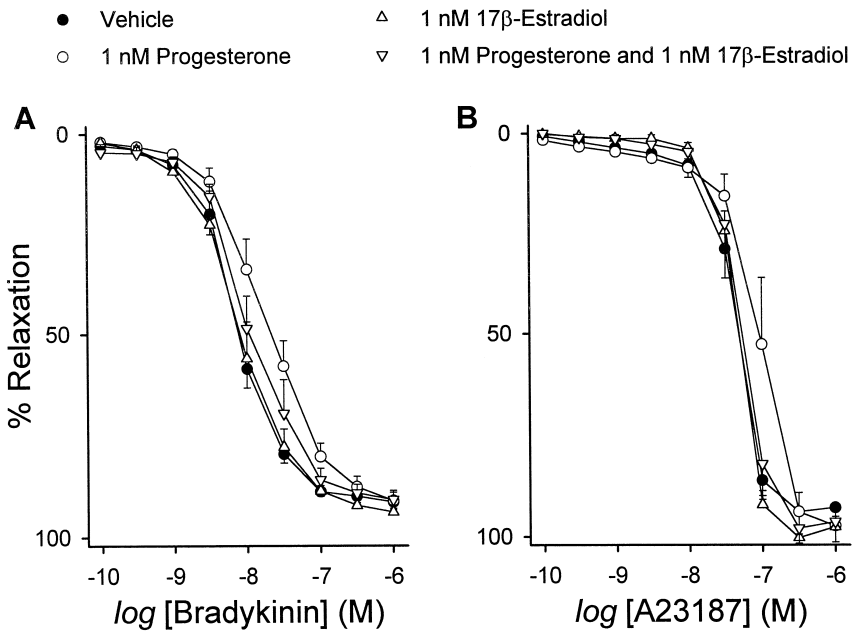

Fig. 2. Effect of progesterone and/or 17β-estradiol on endothelium-dependent relaxation in isolated porcine coronary artery rings. Ring segments were exposed to vehicle, 1 nM progesterone and/or 1 nM 17β-estradiol for 20 min before being contracted with 30 nM U46619. Cumulative relaxation–response curves for (A) bradykinin ($N = 6$) or (B) calcium ionophore A23187 ($N = 5$) were then recorded. Data represent means ± S.E.M.

bath concentration = 0.2%) did not relax U46619-contracted rings appreciably (Fig. 1).

We reported in our previous work that short-term exposure (20 min) to 1 nM 17-estradiol enhanced endothelium-independent relaxation in the same preparation (Teoh et al., 1999). Hence, our subsequent studies involved investigation of the acute vasorelaxant effects of the same concentration of progesterone since this level of progesterone also falls within the physiological range (Case records of the Massachusetts General Hospital, 1986). As illustrated in Figs. 2 and 3, U46619-pre-contracted rings relaxed in response to bradykinin (0.1 nM–1 μM), calcium ionophore

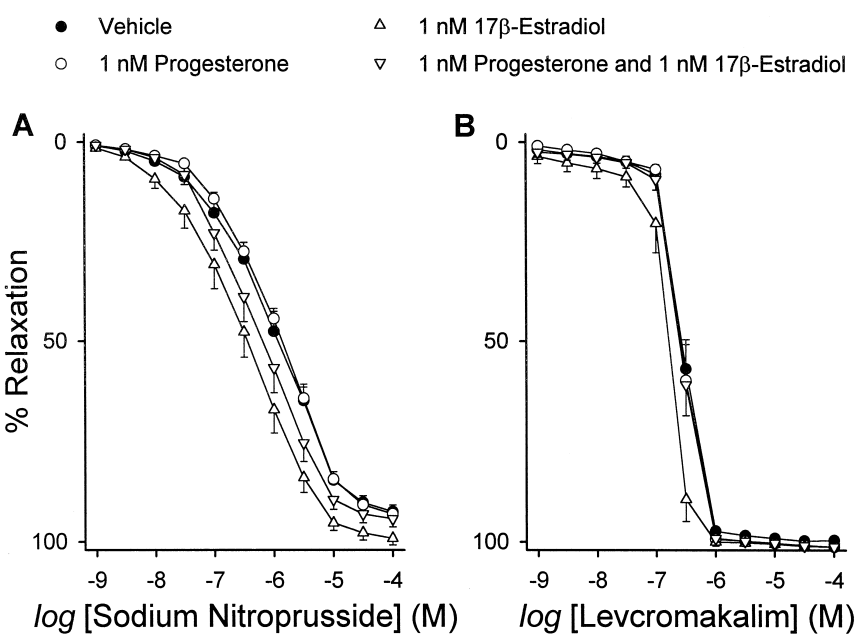

Fig. 3. Effect of progesterone and/or 17β-estradiol on endothelium-independent relaxation in isolated porcine coronary artery rings. Ring segments were exposed to vehicle, 1 nM progesterone and/or 1 nM 17β-estradiol for 20 min before being contracted with 30 nM U46619. Cumulative relaxation–response curves for (A) sodium nitroprusside ($N = 6$) or (B) levcromakalim ($N = 6$) were then recorded. Data represent means ± S.E.M.

Table 1
Log(EC_{50}) values for bradykinin, A23187, sodium nitroprusside and levcromakalim after acute treatment with 1 nM progesterone and/or 1 nM 17β-estradiol
Data represent mean ± S.E.M. with $N = 5–6$. Values in square brackets indicate mean EC_{50} values in nM.

	Log(EC_{50}) M			
	Vehicle	1 nM progesterone	1 nM 17β-estradiol	1 nM progesterone + 1 nM 17β-estradiol
Bradykinin	−8.13 ± 0.02 [7.35]	−7.73 ± 0.02 [18.71][a]	−8.11 ± 0.02 [7.85][b]	−7.97 ± 0.04 [10.72][a,b]
A23187	−7.36 ± 0.02 [43.85]	−7.02 ± 0.03 [96.61][a]	−7.34 ± 0.01 [45.39][b]	−7.28 ± 0.01 [52.00][b]
Sodium nitroprusside	−5.96 ± 0.05 [1086]	−5.91 ± 0.04 [1227]	−6.43 ± 0.03 [372.0][a,b]	−6.24 ± 0.04 [573.0][a,b]
Levcromakalim	−6.54 ± 0.00 [291.1]	−6.55 ± 0.00 [282.5]	−6.77 ± 0.01 [169.8][a,b]	−6.56 ± 0.00 [276.7][c]

[a] $P < 0.05$ compared with corresponding control (vehicle) value.
[b] $P < 0.05$ compared with corresponding progesterone value.
[c] $P < 0.05$ compared with corresponding 17β-estradiol value. (ANOVA followed by Bonferroni's test).

A23187 (0.1 nM–1 μM), sodium nitroprusside (1 nM–100 μM) and levcromakalim (1 nM–100 μM) in a concentration-dependent manner. The contractions elicited by 30 nM U46619 were unaffected by a 20-min pre-incubation with 1 nM progesterone. In contrast, the cumulative relaxation curves of bradykinin and A23187 were shifted to the right considerably (Fig. 2), resulting in significant increases in log(EC_{50}) values (Table 1, $P < 0.05$). Sodium nitroprusside- and levcromakalim-mediated relaxations were unaltered following progesterone pre-treatment (Fig. 3). In accord with our previous report (Teoh et al., 1999), pre-incubation with 1 nM 17β-estradiol significantly enhanced the sensitivity of the ring segments to sodium nitroprusside and levcromakalim but not to bradykinin and A23187 (Figs. 2 and 3; Table 1).

The inhibitory effect that 1 nM progesterone had on endothelium-dependent relaxation decreased when 1 nM 17β-estradiol was concomitantly added to the baths (Fig. 2). Log(EC_{50}) values for bradykinin and A23187 after co-incubation with the two hormones were lower than those calculated following exposure to 1 nM progesterone alone (Table 1). The leftward shift in the endothelium-independent relaxation curves produced by 1 nM 17β-estradiol also diminished after simultaneous incubation with 1 nM progesterone (Fig. 3; Table 1).

4. Discussion

The results from this study indicate that acute treatment of porcine coronary artery rings with 1 nM progesterone reduces bradykinin- and calcium ionophore A23187-mediated relaxations and that this effect is limited when 1 nM 17β-estradiol is concomitantly administered. Our data further suggest that the enhanced sensitivity to sodium nitroprusside and levcromakalim observed following short-term (20 min) exposure to 1 nM 17β-estradiol (Teoh et al., 1999; present study) is diminished when progesterone (1 nM) is simultaneously applied. In agreement with Glusa et al. (1997) and Jiang et al. (1992a), we also show that progesterone at supraphysiological concentrations (micromolar range), and thus physiologically irrelevant levels, can directly relax U46619-contracted porcine coronary artery rings.

Due to the widespread belief that estrogens are cardioprotective (Barrett-Connor, 1995; Saaman and Crawford, 1995), much attention has been focused on the influence estradiol has on the vasculature. To date, evidence from both clinical and scientific studies indicates that acute and chronic exposure to 17β-estradiol not only promotes relaxation but also reduces contraction (e.g., Jiang et al., 1992b; Gilligan et al., 1994; Teoh et al., 1999). Recently, however, there has been a burgeoning interest in the effects of testosterone on blood vessel function. In vivo trials have since provided preliminary documentation for the possibility that testosterone can be detrimental to the vascular network (Adams et al., 1995; Herman et al., 1997) and that this could in part account for the higher prevalence of coronary heart disease in men (Barrett-Connor, 1995).

Relative to the estrogens and testosterone, little attention has been paid to the actions of the progestins in the circulatory system and in the process of atherosclerosis. In fact, the limited data available are contradictory, with positive, negative and no effects having been reported. Prolonged treatment with progesterone enhanced adenosine diphosphate-mediated relaxation in canine coronary arteries (Miller and Vanhoutte, 1991) but had no effect on either vasoconstricting or vasorelaxing reponses in rat aortic rings (Vedernikov et al., 1997). Interestingly, while long-term exposure to progesterone did not affect plaque development in female rabbits, it did cause a considerable increase in intimal plaque size in the male animals (Hanke et al., 1996b).

To date, only two other groups have managed to demonstrate that progesterone can modulate vasorelaxation following acute application (Jiang et al., 1992a; Glusa et al., 1997). However, as in the present model (see Fig. 1), high concentrations of progesterone (≥ 1 μM) were required to record a significant direct response. Since the level of progesterone in the normal ovulating woman is in the range of 0.6–102 nM (Case records of the Massachusetts General Hospital, 1986), it would seem that this

property of progesterone is irrelevant to its physiological effects in vivo and may only be of pharmacological interest. In contrast, the negative influence we observed with short-term administration of progesterone (1 nM) might be of physiological importance since this concentration of the hormone falls within the range of concentrations measured during the menstrual cycle. Hence, to the best of our knowledge, this is the first account documenting that a physiologically relevant concentration of progesterone acutely (20 min) modulates agonist-stimulated relaxation of coronary arteries in vitro.

Progesterone receptors have been detected in the endothelial and smooth muscle cells of baboon (Lin et al., 1986) and rabbit (Hegele-Hartung et al., 1997) aortae, monkey (Minshall et al., 1998) and human (Ingegno et al., 1988) coronary arteries as well as human saphenous veins (Perrot-Applanat et al., 1995). The rapid onset observed upon acute application of progesterone in the current work is, however, inconsistent with the response being mediated by the intrinsically slow gene induction process of classical steroid receptors. As to whether this effect is due to activation of progesterone receptors that act independently of the genomic pathway remains to be determined.

Our findings demonstrating that progesterone can oppose the actions of 17β-estradiol concur with previous work (Miller and Vanhoutte, 1991; Williams et al., 1994; Hanke et al., 1996a,b; Miyagawa et al., 1997). This antagonistic relationship between the two female sex hormones might also be of physiological relevance since the concentration of 17β-estradiol used (i.e., 1 nM) in this study falls within the physiological range of the normal ovulating woman (0.84–1.33 nM; Case records of the Massachusetts General Hospital, 1986). However, the levels of the individual sex hormones in a pre-menopausal woman fluctuate according to the stage of the menstrual cycle, and the opposing actions we have discovered in our preparation may not occur at all the permutations of progesterone versus estrogen concentrations.

Perhaps more interesting is the observation that although 1 nM progesterone and 1 nM 17β-estradiol, each on their own, did not affect the endothelium-independent and endothelium-dependent concentration-relaxation curves, respectively, they were able to blunt the modulatory actions of the other female sex hormone. Inasmuch as the actions of progesterone were endothelium-dependent and those of 17β-estradiol were mediated at the level of the smooth muscle, it is plausible that these two hormones enforce their actions via different mechanisms. Further work will have to be carried out to investigate this possibility.

Extrapolating data from in vitro animal tissue experiments to the human situation is often a problem. In the current project, the rationale for using the porcine coronary artery model was the close similarity between the hearts of pigs and humans. As the porcine hearts were from a local abattoir, we were unable to determine or control the sex distribution of the hearts obtained. While we recognise this as a limitation of our study, the data we present were reproducible in every batch of hearts investigated.

In conclusion, we report that a low concentration (1 nM) of progesterone can acutely impair bradykinin- and A23187-produced relaxation. Our finding that short-term (< 30 min) exposure to a low concentration of progesterone can reduce the enhancing effects of 17β-estradiol on endothelium-independent relaxation in vitro supports speculations that the progestin element of hormone replacement therapy may partially oppose the cardioprotective effect of the estrogen component. These results further suggest that besides antagonising the effects of estrogens on lipid metabolism and cell proliferation, progestins can also diminish the vascular influence of estrogen.

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