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Investigation of the negative inotropic effects of 17β-oestradiol in human isolated myocardial tissues

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1 The aim of the present study was to evaluate the effects of 17β-oestradiol in human myocardium. The effects of 17β-oestradiol, progesterone and testosterone on force of contraction were investigated in electrically driven isolated atrial trabeculae and ventricular papillary muscles from human hearts in the presence and absence of Bay K 8644, a calcium channel agonist. In addition, the effects of 17β-oestradiol, progesterone and testosterone on binding of [^{3}H]-PN 200 110 were assessed in membranes prepared from human ventricular myocardium.

2 17β-Oestradiol elicited a negative inotropic effect in atrial (IC_{50}: 7.1 μmol l^{-1}, confidence interval 3.8 to 13.4, $n=3$) and ventricular preparations (IC_{50}: 4.6 μmol l^{-1}, confidence interval 2.2 to 9.4, $n=3$) as compared with solvent controls. There was no significant difference ($P>0.05$) of IC_{50} values in the absence and presence of isoprenaline (0.01 μmol l^{-1}) in atrial (IC_{50}: 10.8 μmol l^{-1}, confidence interval 9.1 to 12.9, $n=6$) and ventricular preparations (IC_{50}: 9.4 μmol l^{-1}, confidence interval 7.3 to 11.9, $n=8$).

3 17β-Oestradiol at 30 μmol l^{-1} induced a significant rightward shift of the concentration-response curves for the positive inotropic effect of Bay K 8644 in atrial preparations (EC_{50}: 0.13 μmol l^{-1}, confidence interval 0.08 to 0.19, $n=6$; EC_{50} with 17β-oestradiol: 0.58 μmol l^{-1}, confidence interval 0.33 to 0.83, $n=6$, $P<0.05$) and ventricular preparations (EC_{50}: 0.07 μmol l^{-1}, confidence interval 0.04 to 0.11, $n=8$; EC_{50} with 17β-oestradiol: 0.3 μmol l^{-1}, confidence interval 0.18 to 0.49, $n=8$, $P<0.05$). Testosterone, progesterone at 30 μmol l^{-1} and the solvent control had no significant effect on the concentration-response curves to Bay K 8644.

4 In membranes prepared from human ventricular myocardium the effect of 17β-oestradiol on binding of [^{3}H]-PN 200 110, an antagonist at the 1,4 dihydropyridine binding site, was not different from that observed with progesterone, testosterone or solvent controls.

5 In myocardial membranes no specific oestrogen receptors were demonstrated by [^{3}H]-oestradiol binding studies.

6 Thus, the calcium antagonistic property of 17β-oestradiol cannot be attributed to a direct interaction with 1, 4 dihydropyridine binding sites.

Keywords: 17β-Oestradiol; calcium antagonism; cardiac muscle; contractility

Introduction

In postmenopausal women on oestrogen replacement therapy, the rate of cardiovascular mortality is reduced by 30 to 50% compared to nontreated individuals (Stampfer *et al.*, 1991). This has been attributed to effects on cholesterol metabolism and deposition (Kushawaha, 1992), effects on carbohydrate metabolism (Gaspard *et al.*, 1995), beneficial effects on platelet aggregation, reductions of arterial blood pressure (Nabulski *et al.*, 1993) and increases in coronary blood flow (Williams *et al.*, 1992). Recently, a calcium antagonistic effect of oestrogen has been reported in uterine vascular smooth muscle (Stice *et al.*, 1987), and in preparations from rat aortae and rabbit coronary arteries (Jiang *et al.*, 1991). A nifedipine-like effect of diethylstilboestrol on the contractile response of human and rat isolated detrusor muscles has been demonstrated (Elliot *et al.*, 1992). Furthermore, in guinea-pig isolated cardiac myocytes a negative inotropic effect of oestrogen due to inhibition of the calcium inward current I_{Ca} and a decrease of systolic free Ca^{2+} was observed (Jiang *et al.*, 1992a). This corresponds well to the demonstration of a negative inotropic effect of 17β-oestradiol in rabbit perfused hearts (Raddino *et al.*, 1986). These observations seem to be inconsistent with reports of an increase in cardiac output in the presence of oestradiol in male transsexuals (Slater *et al.*, 1986), in non-pregnant ovariectomized ewes (Magness & Rosenfeld, 1989), in pregnant women (Longo, 1989) and in postmenopausal women treated with oestrogen (Luotola, 1983). However, it has not yet been established whether there is a direct effect of 17β-oestradiol on myocardial force of contraction in human hearts. *In vivo*, this effect might be obscured by counteracting effects like coronary and peripheral vasodilatation (Volterrani *et al.*, 1995). Therefore, we characterized the effect of 17β-oestradiol, progesterone and testosterone on the positive inotropic response to the Ca^{2+}-channel agonist, Bay K 8644, in isolated atrial and ventricular muscle strips from human hearts. In order to provide more information on the underlying mechanisms, we studied the effect of 17β-oestradiol on the binding of [^{3}H]-PN 200-110 to 1, 4 dihydropyridine binding sites of the Ca^{2+}-channel in human myocardial membrane preparations.

Methods

Myocardial tissue

Experiments were performed on human isolated, electrically stimulated, ventricular papillary muscle strips and right auricular trabeculae or on membrane preparations from human left ventricular and right atrial myocardium. Tissue was obtained during aortocoronary bypass operations from patients without heart failure ($n=16$, 4 female, 12 male; age: mean 61.4 years, range 41-71) or during cardiac transplantation from patients with dilated cardiomyopathy ($n=10$, 4 female, 6 male; age: mean 45.3 years, range 31-64). All patients gave written informed consent before surgery. Medical therapy consisted of

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diuretics, nitrates, angiotensin-converting enzyme inhibitors and cardiac glycosides. Patients receiving catecholamines, β-adrenoceptor or Ca^{2+}-channel antagonists were not eligible for the study. Cardiac surgery was performed with cardioplegic arrest during hypothermia. The cardioplegic solution was a modified Bretschneider solution containing (mmol l^{-1}) NaCl 15, KCl 10, $MgCl_2$ 4, histidine 180, tryptophan 2, mannitol 30 and potassium dihydrogen oxoglutarate 1.

Contraction experiments

Immediately after excision, the papillary muscles and atrial trabeculae were placed in ice-cold preaerated modified Tyrode solution (composition see below). The experiments were performed on isolated electrically driven (1 Hz) muscle preparations. Muscle strips were dissected under microscopic control in aerated bathing solution at room temperature. Connective tissue, if visibly present, was trimmed away carefully. The preparations were attached to a bipolar platinum stimulating electrode and suspended individually in 75 ml glass tissue chambers for recording of isometric contractions. The bathing solution used was a modified Tyrode solution containing in mmol l^{-1}: NaCl 119.8, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 22.6, Na_2EDTA 0.05, ascorbic acid 0.28, glucose 5.0. It was continuously gassed with 95% O_2 and 5% CO_2 and maintained at 37°C; its pH was 7.4 Isometric force of contraction was measured with an inductive force transducer (W. Fleck, Mainz, Germany) attached to a Gould recorder (Brush 2400, Gould Inc, Cleveland, Ohio, U.S.A.). Each muscle was stretched to the length at which force was maximal. The preparations were paced electrically at 1 Hz with rectangular pulses of 5 ms duration (Grass stimulator SD 9), the voltage was 20% above threshold. All preparations were allowed to equilibrate at least 90 min in a drug-free bathing solution until complete mechanical stabilization. After 45 min, the solution was changed. Concentration-dependent mechanical effects were obtained. Control strips kept in Tyrode solution, identical in composition to the original experiments, revealed maximally 10% reduction of baseline isometric tension over the period necessary to complete pharmacological testing. Agents were applied cumulatively to the organ bath. Each muscle was used only once to record a concentration-response curve.

Human myocardial membrane preparation

Left ventricular myocardium was chilled in 30 ml ice-cold homogenization buffer (20 mmol l^{-1} Tris/HCl, 1 mmol l^{-1} EDTA, 1 mmol l^{-1} dithiothreitol, pH 8.0). Connective tissue was trimmed away and myocardial tissue was minced with scissors, disrupted with an Ultraturrax (Janke and Kunkel, Staufenbreisgau, Germany) and homogenized with a motor-driven glass Teflon potter for 1 min. The homogenate was spun at 480 *g* for 10 min (JA 20, Beckman, Palo Alto, U.S.A.). The supernatant was retained and the pellet was discarded. This homogenate was diluted with an equal volume of ice-cold 1 mol l^{-1} KCl and stored on ice for 10 min. The supernatant was centrifuged at 100,000 *g* for 45 min. The pellet was resuspended in 50 volumes of homogenization buffer and recentrifuged at 100,000 *g* for 45 min. The final pellet was resuspended in incubation buffer (see below).

Radioligand binding studies

Radioligand studies were done as described elsewhere (Schmidt *et al.*, 1993). In brief, the final pellet was resuspended in incubation buffer (10 mmol l^{-1} HEPES, 2 mmol l^{-1} $MgSO_4$, 1 mmol l^{-1} EGTA) and homogenized by hand for 1 min. After centrifugation of the homogenate (100,000 *g* for 30 min) the pellet was resuspended in a total volume of 500 μl. Incubation was carried out at 30°C for 60 min to allow complete equilibration of ligand and receptor. The reaction was stopped by rapid vacuum filtration through Whatman GF/C filters (Whatman, Clifton, New Jersey, U.S.A.); the filters were washed immediately three times with 10 ml ice-cold incubation buffer. All experiments were performed in triplicate. Filters were dried at 90°C and placed in 10 ml scintillation fluid (Quickszint 501, Zinsser analytics, Frankfurt, Germany) and radioactivity was determined in a liquid scintillation counter. Dihydropyridine binding sites were determined by using [^{3}H]-PN 200 110 (specific activity 86.2 Ci $mmol^{-1}$) as radioligand. Non-specific binding was assessed in presence of nifedipine, 10 μmol l^{-1}. Saturation experiments with ^{3}H-17β-oestradiol were performed in presence of 1 μmol l^{-1} non-radioactive 17β-oestradiol. The maximal density (B_{max}) and apparent affinity (K_D) of binding sites were obtained in individual experiments from Scatchard plots determined by linear regression analysis (Scatchard, 1949). Competition experiments with 17β-oestradiol, testosterone, progesterone and DMSO were performed in presence of 0.5 nmol l^{-1} [^{3}H]-PN 200 110. All tubes were protected from light and all experiments were done in red light.

Materials

Radioligands, [^{3}H]-isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-methoxycarbonylpyridine-3-carboxylate ([^{3}H]-PN 200 110) and [^{3}H-17β]-oestradiol, were purchased from New England Nuclear, Dreieich, Germany. 17β-Oestradiol, progesterone and testosterone were generous gifts from Schering AG, Berlin, Germany. Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) was from Bayer AG, Leverkusen, Germany. All other compounds used were of analytical or best grade commercially available. Only deionized and twice distilled water was used throughout. Dimethylsulphoxide (DMSO) was used as solvent.

Statistics

All data are shown as means ± s.e.mean. IC_{50}- and EC_{50}-values are given as means with 95% confidence intervals in parentheses. Statistical significance was estimated using Student's *t* test for paired and unpaired observations. A *P* value of less than 0.05 was considered significant.

Miscellaneous

Protein concentrations were determined according to Lowry *et al.* (1951) with bovine serum albumin used as standard.

Results

Effects of 17β-oestradiol on force of contraction in human atrial and ventricular myocardium

Original recordings of the impact of 17β-oestradiol on the force of contraction in isolated electrically driven preparations from human atrial and ventricular myocardium are shown in Figure 1. In tissues from both regions in the presence and absence of isoprenaline (10 nmol l^{-1}), 17β-oestradiol (30 μmol l^{-1}) produced a negative inotropic effect which reached its maximum within less than 5 min. Bolus application of the steroid hormones, progesterone and testosterone, had no significant effect on force of contraction in either atrial or ventricular myocardium (data not shown) suggesting a specificity of the effect of 17β-oestradiol. This suggestion is further supported by concentration-response curves of the negative inotropic effect of 17β-oestradiol alone and in presence of isoprenaline in human atrial and ventricular preparations (Figure 2a and b). The potency and efficacy of 17β-oestradiol did not differ significantly when applied in the absence or presence of isoprenaline (atria: IC_{50} alone 7.1 μmol l^{-1} [3.8 to 13.4], in the presence of isoprenaline 10.8 μmol l^{-1} [9.1 to 12.9]; ventricles: IC_{50} alone 4.6 μmol l^{-1} [2.2 to 9.4] in pre-

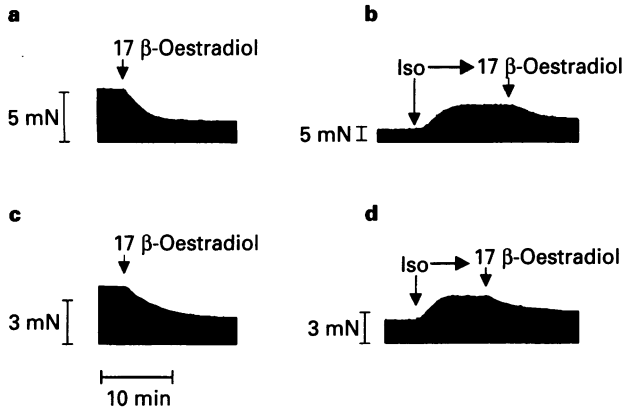

Figure 1 Original recordings of isometric force of contraction in isolated, electrically driven human atrial trabeculae (a;b) and human papillary muscle strips (c;d) after application of 30 μmol l^{-1} 17β-oestradiol alone (a;c) or in the presence of 0.01 μmol l^{-1} isoprenaline (Iso)(b;d). 17β-oestradiol exerted negative inotropic effects in both tissues, alone and in the presence of isoprenaline.

sence of isoprenaline 9.4 μmol l^{-1} [7.3 to 11.9]). Though a concentration-dependent negative inotropic effect of DMSO was demonstrated in atria and ventricles, curves obtained in the presence of 17β-oestradiol were significantly different from those observed in solvent alone.

Functional characterization of the L-type Ca^{2+}-channel antagonistic properties of 17β-oestradiol in human atrial and ventricular myocardium

Since there is experimental evidence of an interaction of 17β-oestradiol with the L-type Ca^{2+}-channel (Jiang *et al.*, 1992a), the positive inotropic effect of the L-type Ca^{2+}-channel agonist Bay K 8644 on human myocardium was investigated in the presence of 17β-oestradiol (30 μmol), progesterone (30 μmol l^{-1}) and testosterone (30 μmol l^{-1}) in left ventricular and in the presence of 17β-oestradiol (30 μmol l^{-1}) in right atrial preparations. In presence of 17β-oestradiol there was a significant ($P<0.05$) rightward parallel shift of the concentration-response curve to Bay K 8644 in atrial and ventricular preparations (Figure 2c and d). In contrast, progesterone and testosterone had no significant effect on the potency of Bay K 8644 in human ventricle (Figure 2d). Thus, these experiments provided further evidence of a calcium-channel antagonistic activity of 17β-oestradiol in human atrial and ventricular myocardium. Absolute values of basal force of contraction, maximal inotropic effects and EC_{50}-values of these experiments are listed in Table 1.

Radioligand binding studies

In order to examine the underlying mechanisms of the negative inotropic effects of 17β-oestradiol binding studies were performed. Saturation experiments with [^{3}H]-PN 200 110 revealed the presence of 1,4 dihydropyridine binding sites of the L-type Ca^{2+}-channel in myocardial membrane preparations. Scatchard transformations were linear, indicating the presence of one binding site (data not shown). The density of binding sites (B_{max}) was 75.6±3.4 fmol mg^{-1} protein, the K_D-value amounted to 0.17±0.04 nmol l^{-1} ($n=4$). Specific binding of [^{3}H]- 17β-oestradiol was not shown (data not shown). Therefore, in order to characterize the interaction of steroids with the 1,4 dihydropyridine binding site, competition experiments in presence of 0.5 nmol l^{-1} [^{3}H]-PN-200 110 were done. Non-specific binding at this concentration was less than 10% of total binding. The binding of the radioligand was diminished only at very high doses ($IC_{50}>100$ μmol l^{-1}) by progesterone, testosterone and 17β-oestradiol (data not shown). Despite a tendency of the binding data of 17β-oestradiol to be shifted leftwards to those obtained with the other sex steroids, no significant difference from those assessed in the presence of the solvent alone could be established. Therefore, the diminuation of [^{3}H]-PN-200 110- binding is not specific for 17β-oestradiol and seems to be due to a non-specific solvent effect.

Thus, 17β-oestradiol exerts a negative inotropic effect in atrial and ventricular isolated muscle which cannot be attributed to a direct effect at 1,4 dihydropyridine binding sites.

Discussion

The present study showed, for the first time, a negative inotropic effect of 17β-oestradiol, the primary physiological oestrogen in man, in isolated atrial and ventricular strips from human myocardium. This is in accordance with a decrease of contractility observed in presence of 17β-oestradiol in rabbit perfused hearts (Raddino *et al.*, 1986). As progesterone and testosterone had no effect on myocardial contractility, this effect cannot be assumed to be a common property of steroid hormones. These data are in good accordance with the observation of relaxation of precontracted rabbit coronary arteries in the presence of 17β-oestradiol but not in the presence of testosterone (Jiang *et al.*, 1991). The rightward shift of the dose response curve for Bay K 8644-induced positive inotropism in atrial and ventricular isolated muscle observed with 17β-oestradiol corresponds well to the marked reduction of Bay K 8644-induced contraction reported in rabbit coronary arteries (Jiang *et al.*, 1992b) and to the reduction of coronary perfusion pressure induced by Bay K 8644 in isolated, perfused heart preparations in the presence of oestradiol (Raddino *et al.*, 1986). It suggests a calcium antagonistic effect and could well be due to an interaction with the 1,4 dihydropyridine binding site of L-type calcium channels (Brown *et al.*, 1984).

Indeed it has been suggested that 17β-oestradiol may behave as a Ca^{2+}-channel antagonist (Collins *et al.*, 1993). This suggestion is supported by several experiments giving evidence of a nifedipine-like effect of 17β-oestradiol in uterine vascular smooth muscle cells (Stice *et al.*, 1987), in human detrusor muscles (Elliot *et al.*, 1992), in rat aortae and in rabbit coronary arteries (Jiang *et al.*, 1991). Moreover, Sheldon & Argentieri (1995) reported recently a direct concentration-dependent inhibition of a dihydropyridine-sensitive inward current in guinea-pig isolated detrusor myocytes. However, very few studies are available in human isolated tissues of cardiac origin. Mück *et al.* (1994) reported effects of oestradiol on calcium influx in human umbilical cord endothelial cells. Jiang *et al.* (1992a) demonstrated a concentration- and voltage-dependent inhibition of the slow calcium inward current induced by 17β-oestradiol in isolated ventricular myocytes from guinea-pig hearts. Additionally, these investigators observed diminished systolic intracellular levels of free calcium ions in the presence of 17β-oestradiol. Therefore, there is good evidence that calcium release from the sarcoplasmic reticulum is under the control of calcium ion influx through calcium channels (Fabiato, 1989). Nevertheless, the involvement of other mechanisms mediating intracellular calcium reduction (e.g. direct affection of the Na^{2+}/Ca^{2+}-exchanger) cannot be ruled out.

To our knowledge, the interactions of 17β-oestradiol and calcium channel binding sites have never been studied before. The rightward shift of the Bay K 8644 concentration-effect curve induced by 30 μmol l^{-1} 17β-oestradiol without effect on the calcium channel agonist's efficacy indicates competitive antagonism at 1,4 dihydropyridine binding sites. This suggestion is endorsed by the demonstration of a reversible and concentration-dependent inhibition of voltage-operated inward Ca^{2+} currents by 10 to 30 μmol l^{-1} 17β-oestradiol in isolated cardiac myocytes from guinea-pigs (Jiang *et al.*, 1992a) and in rat aortic smooth muscle cells (Nakajima *et al.*, 1995) as well as by 4OH-oestradiol in uterine smooth muscle cells (Stice

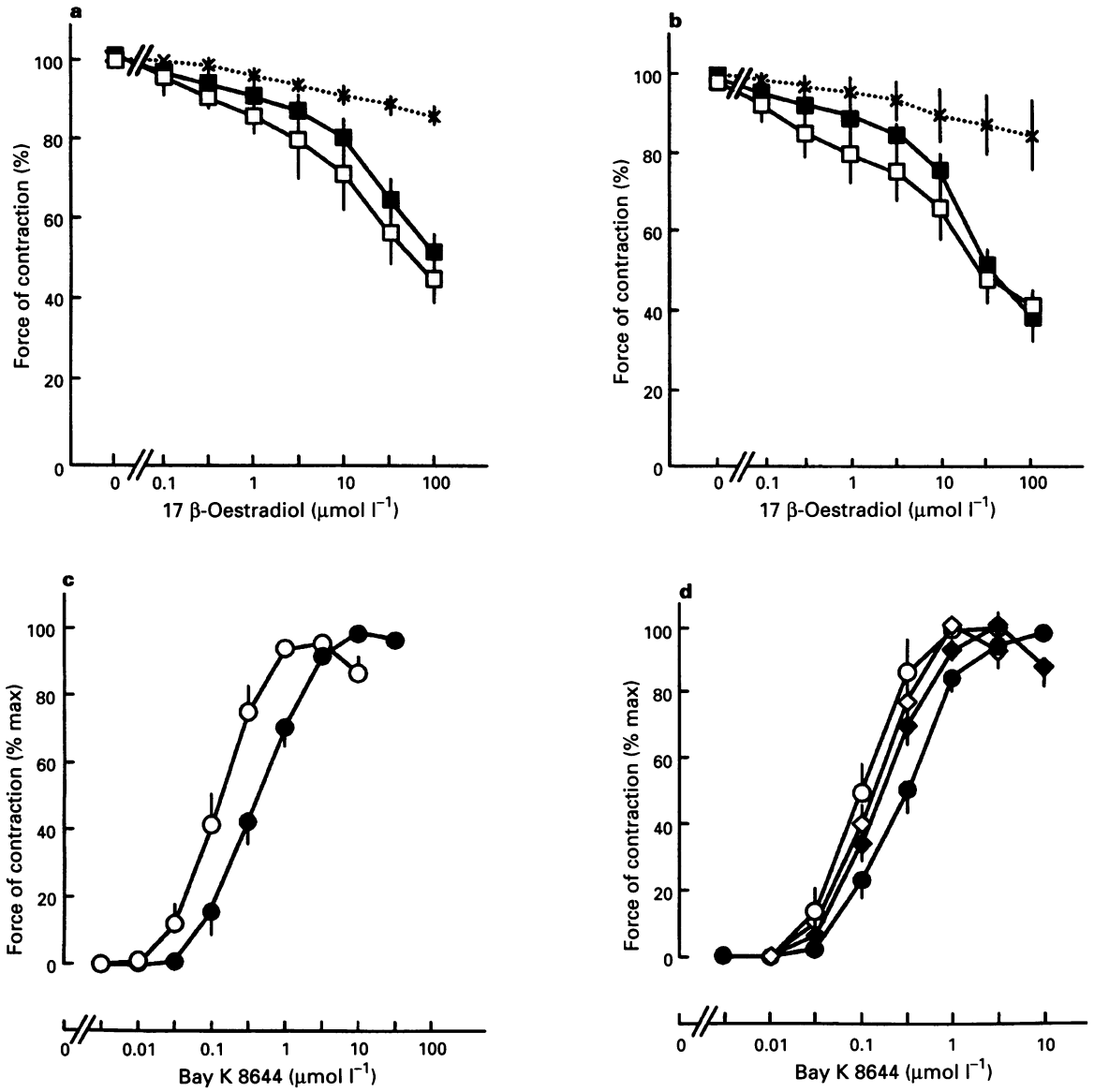

Figure 2 Cumulative concentration-response curves of the negative inotropic effect of 17β-oestradiol alone (□) and in the presence of 0.01 μmol l^{-1} isoprenaline (■) in human atrial (a) and human ventricular (b) myocardium compared to the solvent medium DMSO (×). The ordinate scales represent the force of contraction as a percentage of the value before the addition of 17β-oestradiol or solvent control. In the atrial myocardium (a) the basal force of contraction was 3.8±0.7 mN. It was increased to 320.4±35.1% by isoprenaline ($n=10$). At 100 μmol l^{-1}, 17β-oestradiol reduced force of contraction by 56.5±8.3% (alone, $n=3$) and by 52.9±4.8% (in the presence of isoprenaline, $n=6$). In the ventricular myocardium (b) the basal force of contraction was 1.9±0.3 mN. It was increased to 202.8±29.6% by isoprenaline ($n=10$). At 100 μmol l^{-1}, 17β-oestradiol reduced force of contraction by 59.6±5.3% (alone, $n=3$) and by 61.4±7.3% (in presence of isoprenaline, $n=8$). There were no significant differences between the negative inotropic effects of 17β-oestradiol in the absence and presence of isoprenaline in either preparation. Panels (c) and (d) illustrate cumulative concentration-response curves of the effect of Bay K 8644 (0.03 to 100 μmol l^{-1}) alone (○; $n=6$) and in the presence of 17β-oestradiol (30 μmol l^{-1})(●, $n=6$) on force of contraction in human isolated, electrically driven trabeculae from the right atrium (c) and in isolated, electrically driven papillary muscle strips from the left ventricle (d) in the presence of 17β-oestradiol (30 μmol l^{-1})(●, $n=8$), progesterone (30 μmol l^{-1})(◆, $n=6$) and testosterone (30 μmol l^{-1})(◇, $n=8$).

et al., 1987). This effect was enhanced in depolarizing conditions. In contrast, in our hands, 17β-oestradiol had no specific effect on the binding of [^{3}H]-PN 200 110, an antagonist at the 1,4 dihydropyridine binding site of the L-type calcium channel in (depolarized) myocardial membranes. However, it has been suggested that Bay K 8644, being a partial agonist 1,4 dihydropyridine analogue, interacts at two functionally different binding sites in cardiac tissue with agonist and antagonist properties (Schwartz *et al.*, 1984, Dube *et al.*, 1985). In contrast, 1,4 dihydropyridines with antagonistic properties are assumed to bind to one high-affinity binding site only (Janis *et al.*, 1984). Furthermore, it has been suggested that non-dihydropyridine calcium channel blockers in cardiac cells act at binding sites linked allosterically to the 1,4-dihydropyridine binding site (Janis *et al.*, 1984). Thus, it might be speculated that our binding studies reflect an interaction of 17β-oestradiol with the L-type calcium channel in a manner which modulates the effects of Bay K 8644 but not of antagonists at 1,4 dihydropyridine binding sites.

The fact that we could not detect any specific binding of [^{3}H]-17β-oestradiol in myocardial ventricular preparations corresponds well to the finding that the effects of 17β-oestradiol are mediated by intracytoplasmic and intranuclear receptors leading to gene induction (Karas *et al.*, 1994). However, recently evidence has accumulated that steroid hormones have rapid membrane effects not involving 'classical'

Table 1 Force of contraction in human atrial trabeculae and papillary muscle strips

	n	*Basal* (mN)	*Max. PIE* (mN)	EC_{50} (μmol l^{-1})
Human atrial myocardium				
Bay K 8644	6	3.9±0.4	+4.7±0.2	0.13 (0.08–0.19)
Bay K 8644 in the presence of 17β-oestradiol (30 μmol l^{-1})	6	2.3±0.5	+6.4±0.7	0.58 (0.33–0.83)*
Human ventricular myocardium				
Bay K 8644	8	1.4±0.5	+1.7±0.3	0.07 (0.04–0.11)
Bay K 8644 in the presence of 17β-oestradiol (30 μmol l^{-1})	8	1.6±0.3	+1.5±0.4	0.3 (0.18–0.49)*
Bay K 8644 in the presence of progesterone (30 μmol l^{-1})	6	2.2±0.5	+2.0±0.6	0.17 (0.08–0.36)
Bay K 8644 in the presence of testosterone (30 μmol l^{-1})	8	2.1±0.8	+1.8±0.6	0.13 (0.084–0.23)

*$P < 0.05$ vs. predrug value.
EC_{50} values are quoted as geometric means (with 95% confidence limits). Abbreviation: PIE: positive inotropic effect.

receptors (Schumacher, 1990). In support of this the maximal negative inotropic effect of 17β-oestradiol in our studies as well as effects reported in guinea-pig isolated cardiomyocytes (Jiang *et al.*, 1992a) were observed within minutes. Though the effects observed in our study were seen at very high concentrations of 17β-oestradiol, much higher than those observed in plasma samples from premenopausal (midcycle 785 to 1,840 pmol l^{-1}) and from pregnant women (6,000 to 8,000 pmol l^{-1}) (Volterrani *et al.*, 1995), they agree well in magnitude with concentrations reported to be effective in inducing relaxation of coronary arteries and inhibiting calcium currents in myocytes (Jiang *et al.*, 1991; 1992a, b) amounting to 10 to 30 μmol l^{-1}. Due to their lipophilicity, steroids may accumulate in cells and their membranes to achieve effective concentrations and, therefore, act on cell membrane receptors yet to be identified in a way unrelated to genomic physiological effects.

As there is some experimental evidence that oestrogen can increase intracellular levels of cyclic AMP and cyclic GMP (Szego & Davis, 1967; Mügge *et al.*, 1993) perhaps due to Ca^{2+}-calmodulin interaction (Farhat *et al.*, 1992), it might be speculated that these effects may finally lead to alterations of Ca^{2+} available for binding to the contractile apparatus (Fischmeister & Hartzell, 1990; Lohmann *et al.*, 1995).

In conclusion, a negative inotropic effect of 17β-oestradiol has been demonstrated in human isolated heart preparations. Though, 17β-oestradiol seems to interfere with the availability of Ca^{2+} for the contraction machinery by antagonizing calcium- and Bay K 8644-induced contractions. The underlying mechanisms remain obscure especially in the myocardium because a direct interaction with dihydropyridine binding sites did not occur in our studies.

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