Oestrogen as a calcium channel blocker

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This paper reviews the evidence for calcium-antagonist properties of oestrogen which may offer long-term protective effects on the cardiovascular system in post-menopausal women. Oestrogen has already been shown to have a beneficial effect on cholesterol metabolism and deposition, thereby inhibiting the formation of atherosclerotic plaque and coronary atheroma. Calcium antagonism, resulting in both acute and chronic modulation of coronary and peripheral vasomotion, is another mechanism by which oestrogens may provide cardiovascular benefits to women, including those with existing cardiovascular disease.

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Introduction

Oestrogen replacement therapy in post-menopausal women reduces the risk of cardiovascular and cerebrovascular disease[1]. Large-scale epidemiological studies indicate that post-menopausal women who receive oestrogens appear to have one-third to one-half the cardiovascular mortality rate, and one-half the cerebrovascular mortality rate, when compared to untreated women[2-5]. These findings are supported by the evidence that oestrogen has a beneficial effect on cholesterol metabolism and deposition, contributing to the inhibition of atherosclerotic plaque formation in arterial walls[6]. Angiographic studies also show a protective effect of post-menopausal oestrogens on coronary atheroma development[7,8]. The favourable changes in lipid profiles in post-menopausal women taking oestrogens, however, do not fully account for the cardiovascular protective effects[9], and probably account for only 30% of the protective effect[10]. It is important, therefore, to try to identify other mechanisms by which oestrogens may provide cardiovascular benefits to women. The mechanisms that contribute to the acute and chronic modulation of coronary[11-13] and peripheral[14,15] vasomotion may play an important role in this cardiovascular protection. There is now evidence that oestrogen has calcium antagonistic properties which may be involved in the long-term protective effect of oestrogen on the cardiovascular system.

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suggest that 17β-oestradiol may be a calcium-antagonist in these arterial preparations.

Experiments in isolated guinea pig ventricular myocytes confirmed a calcium antagonistic property of oestrogen[20]. The effect of 17β-oestradiol on cardiac cell contraction, inward calcium current, and intracellular free calcium was investigated using a photodiode array, voltage-clamp, and Fura-2 fluorescence techniques, respectively. 17β-oestradiol was shown to have a negative inotropic effect by inhibiting inward calcium current and so reducing intracellular free calcium. This calcium antagonistic property could be one of the mechanisms of 17β-oestradiol-induced endothelium-independent relaxation in isolated rabbit and human coronary arterial preparations[21].

**Calcium antagonism in vascular smooth muscle**

Recent work has confirmed that oestrogen can relax epicardial coronary arteries by inhibiting calcium influx without changing calcium sensitivity of contractile elements[22]. Fura-2-loaded porcine coronary arterial strips were contracted by the thromboxane A2 analogue U-46619 and high potassium depolarization. Cytosolic calcium concentration and contraction were measured simultaneously in the presence and absence of 17β-oestradiol. 17β-oestradiol (30 nmol. 1−1 to 30 μmol. 1−1) inhibited the sustained contraction induced by U46619 and the sustained elevation of intracellular calcium. High concentrations of 17β-oestradiol only partially inhibited the U46619-induced contraction, despite complete inhibition of the increase in intracellular calcium. 17β-oestradiol inhibited the increase in calcium-induced contraction, and the contraction induced by the cumulative addition of calcium in strips pretreated with potassium. However, 17β-oestradiol did not change the slope of the intracellular calcium tension curve and had no effect on levels of cyclic AMP or cyclic GMP. It therefore appears that 17β-oestradiol inhibits the contraction of coronary vascular smooth muscle by inhibiting calcium influx without changing the calcium sensitivity of the contractile elements.

Further evidence that 17β-oestradiol has effects on calcium channels in vascular smooth muscle cells is demonstrated in work on an isolated vascular smooth muscle cell line (A7r5)[23]. The effects of 17β-oestradiol on voltage-dependent calcium channels in these cells were studied using whole patch-clamp techniques. Supraphysiological concentrations of 17β-oestradiol significantly reduced L-type barium currents and T-type calcium currents within 1–2 min. 17a-oestradiol, however, caused significantly less reduction in both types of current. Most of the acute effects in vascular smooth muscle cells occurred at concentrations three to four orders of magnitude higher than achieved in the normal female circulatory system. However, it is possible that tissues accumulate steroids, and local concentrations may indeed reach this level.

As well as effects on calcium channels, oestrogen has been shown to affect large-conductance chloride channels by a direct membrane effect[24]. Large-conductance chloride channels in cultured fibroblasts were shown to be inhibited by exposure to extracellular, but not intracellular, anti-oestrogen, and this effect could be prevented by extracellular 17β-oestradiol but not intracellular 17β-oestradiol or extracellular 17α-oestradiol. This work demonstrates another regulatory role for ovarian steroids, affecting plasma ion channels via membrane binding sites, distinct from the classical oestrogen receptor and subsequent activation of intracellular second-messenger pathway(s).

**Long-term calcium antagonistic effects on the cardiovascular system in vivo**

It has been demonstrated that calcium channel blockers such as nifedipine can alter the progression of atherosclerosis in animals fed a cholesterol-rich diet[25], and clinical studies suggest a beneficial effect of nifedipine[26] and nicardipine[27] on the development of early atheromatous lesions seen in coronary angiography. Similar results have been reported in cynomolgus monkeys[28] where oestrogens inhibit the progression of atherosclerosis in the coronary arteries. If 17β-oestradiol does have a calcium antagonistic effect in the human, then its protective effect on atherosclerotic disease may also involve this mechanism[21].

**Endothelium-derived relaxing factor (nitric oxide)—an 'indirect' calcium antagonist**

Nitric oxide (NO) causes vasorelaxation in endothelium-intact coronary arteries and is a product of the conversion of L-arginine by NO synthesis (NOS) to NO and citrulline. Oestrogen can stimulate calcium-dependent NOS causing synthesis of NO[29], NO stimulates guanylate cyclase, thus increasing intracellular levels of cyclic GMP and relaxing vascular smooth muscle. Cyclic GMP induces vascular smooth muscle relaxation by reducing calcium influx and calcium release from the sarcoplasmic reticulum[30], and by decreasing the phosphorylation of contractile proteins[31]. This indirect calcium antagonistic effect, via cyclic GMP, may contribute to both the short-term vascular effects of oestrogen on blood flow and long-term protective effects of oestrogen on atherosclerosis.

Oestrogen-induced increases in blood flow in the uterine artery can be antagonized by NOS inhibition[32], and oestrogen can stimulate constitutive NOS in cultured bovine endothelial cells[33]. Increased basal release of NO in endothelium-intact aortic rings from female rabbits, compared with those from males, has been reported[34]. Recent experiments show oestrogen-induced stimulation of basal NOS six-fold in human
umbilical vein endothelial cultured monolayers within 30 min. This may be due to an acute effect on the constitutive enzyme or on the inducible enzyme. An in vivo study examining the effects of oestrogen on NOS activity in heart, kidney, and skeletal muscle showed increased NO in female guinea pigs after 5 days' treatment; however, this occurred in males only after 10 days. It was suggested that the number or availability of oestrogen receptors in male tissues is initially too low, requiring a period of oestrogen priming. Variations in expired NO production with cyclical hormone changes in pre-menopausal women has recently been reported, with NO levels peaking at the middle of the menstrual cycle. This suggests a possible influence of gonadal hormones on production of NO in humans.

NO has been observed to slow the development of atheroma by inhibiting smooth cell proliferation while stimulating proliferation of endothelial cells. Oestrogen is a potent antioxidant of lipids, and oxidized lipids inhibit NO. Oestrogen may, therefore, be cardioprotective via enhanced NO production, or reversal of its inhibition, which in turn has antiatherogenic properties; however, the time course for this effect may be relevant only with long-term oestrogen treatment.

**In vivo effects of oestrogen on endothelium-dependent relaxation**

Recent results suggest that the sex hormone status of the animal may be important in determining whether endothelium-derived relaxing factor (EDRF), thought to be NO, plays a role in oestrogen-induced coronary relaxation. Oestrogen-treated and then acutely oestrogen-withdrawn female rabbits showed increased sensitivity to the relaxing effect of 17β-oestradiol. The relaxation was endothelium-dependent. Acetylcholine (ACh) induces endothelium-dependent vascular relaxation mediated by the release of EDRF. Treatment with oestradiol modulates responses to ACh in coronary arteries in cynomolgus monkeys and humans, suggesting an endothelium-dependent mechanism in vivo. Infusion of ACh into atherosclerotic coronary arteries of ovariectomized, hypercholesterolaemic cynomolgus monkeys caused contraction, while a comparison group of animals treated with long-term (2 years) and acute (20 min) 17β-oestradiol showed a relaxation response to ACh. In humans, oestrogen has little direct effect, but ACh-induced vasoconstriction is attenuated, or abolished, after acute administration of oestradiol in post-menopausal women with atherosclerotic coronary arteries. These studies indicate that oestrogen is affecting vascular tone by an EDRF-dependent mechanism in vivo. The response of coronary arteries to ACh after exposure to 17β-oestradiol has recently been shown to be gender dependent. Acute intra-coronary administration of oestrogen reversed the constrictor response to ACh in females but not in males.

**Acute beneficial effects on myocardial ischaemia in women**

A reduction in myocardial ischaemia has been demonstrated in post-menopausal women with proven coronary artery disease given acute sublingual 17β-oestradiol, suggesting that oestrogen may be of therapeutic benefit to women with established coronary heart disease. In a double-blind crossover study, each patient was randomized to treatment with sublingual 17β-oestradiol or placebo before undergoing exercise ECG testing. Oestradiol administration significantly increased the time to 1 mm ST segment depression and total exercise time, indicating an improvement in exercise-induced myocardial ischaemia by oestrogen. The mechanism of action of oestrogen is unknown, but may be the result of a beneficial effect on coronary blood flow, or a reduction in peripheral vascular resistance, or a combination of these mechanisms.

**Conclusions**

17β-oestradiol induces relaxation of animal and human coronary arteries independent of the endothelium in vitro. This effect may play an important role in the regulation of coronary tone in vivo, and may partially explain the reduction in risk of coronary heart disease in post-menopausal women taking hormone therapy. 17β-oestradiol has effects on vascular smooth muscle contraction evoked by activation of both receptor- and potential-operated calcium channels and on calcium currents in isolated myocytes and vascular smooth muscle, and may possibly have a calcium antagonistic property in vivo in humans. Many studies have demonstrated the effect of calcium antagonists on animal experimental atheroma, causing regression. It has been shown that calcium antagonists have similar effects on human coronary atherosclerosis, but only with long-term therapy. If oestrogen does have a calcium antagonistic effect in humans, then its protective effect on atherosclerotic disease may also involve this mechanism.

Oestrogen also has effects on endothelium-derived NO production in vessels from animals. The stimulation of NO by oestrogen and other factors may be involved in the blood flow responses in a number of vascular beds, including the human coronary circulation. This effect of oestrogen on NO could be involved in the mechanism of acute blood flow response and chronic long-term protection from vascular atherosclerotic disease.

There is increasing evidence that oestrogen has beneficial effects on both coronary and peripheral blood flow in humans. These effects may directly relate to both the short- and long-term beneficial effects of oestrogen replacement therapy on the cardiovascular system of post-menopausal women.
References


