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# Oestradiol inhibits smooth muscle cell proliferation of pig coronary artery

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**1** The effect of oestradiol 17β on vascular smooth muscle proliferation was examined in segments of the pig left anterior descending coronary artery (LAD). It was established by cytochemical techniques that out-growth from the segments was composed of vascular smooth muscle cells.

**2** [$^3$H]-thymidine uptake by pig LAD segments was used as an index of vascular smooth muscle cell proliferation. Nitroprusside and forskolin significantly inhibited [$^3$H]-thymidine uptake and were used as positive controls.

**3** Oestradiol 17β (180–360 nM) inhibited thymidine uptake by pig LAD segments ($P<0.05$). The inhibition was observed only in the absence of phenol red, which is a weak oestrogen receptor agonist. The anti-oestrogens tamoxifen and its more potent metabolite 4-hydroxytamoxifen, both of which are partial oestrogen receptor agonists, also significantly inhibited thymidine uptake. However, pretreatment with either tamoxifen or 4-hydroxytamoxifen did not signficantly block oestradiol 17β-induced inhibition of thymidine uptake.

**4** The LAD segments bound [$^3$H]-oestradiol 17β in a time-dependent manner and about 20 to 30% was displaced by an excess of unlabelled oestradiol 17β. Autoradiography showed [$^3$H]-oestradiol 17β was evenly distributed in the cytosol and nuclei of cells in the three layers of the vessel wall.

**5** The data suggest that oestradiol 17β inhibits smooth muscle cell proliferation in porcine LAD segments, possibly through an oestrogen receptor mechanism. This *in vitro* effect suggests an *in vivo* role for oestradiol 17β in directly protecting coronary arteries against myointimal proliferation in premenopausal women.

**Keywords:** Proliferation; oestradiol 17β; tamoxifen; coronary artery

## Introduction

The physiological and therapeutic role of oestrogen in coronary heart disease (CHD) is of considerable interest to the large number of premenopausal women taking oral contraceptives and postmenopausal women taking oestrogen.

Atherosclerosis as well as other vascular responses to injury have a proliferative component (Ross & Glomset, 1973) which includes smooth muscle cell migration and proliferation (Clowes *et al.*, 1983; Walker *et al.*, 1986). We have shown oestradiol 17β to prevent myointimal proliferation in rabbit cardiac and aortic allografts (Foegh *et al.*, 1987; Cheng *et al.*, 1991). Oestrogen has been reported to suppress surgically induced vascular intimal hyperplasia in rabbits (Rhee *et al.*, 1977).

Oestradiol also inhibits diet-induced atherosclerosis in rat and rabbit (Moskowitz *et al.*, 1956; Constantinides *et al.*, 1962; Renaud, 1970). Ethinyl oestradiol has been reported to reduce plaque area in the aorta of cholesterol-fed female *Macaca fascicularis* (Clarkson *et al.*, 1987; 1990). This effect of ethinyl oestradiol was attributed to the increased HDL-cholesterol induced by the drug. However, ethinyl oestradiol diminished the plaque area in the aorta even when its anti-atherogenic effect on the plasma pattern of lipoproteins was counteracted with progestins. The lack of correlation between the pattern of plasma lipoproteins and the effect on the degree of atherosclerosis suggests a direct effect of ethinyl oestradiol on cellular events occurring in the vessel wall. In this context it is noteworthy that Fischer-Dzoga *et al.* (1983) found oestradiol 17β to inhibit the proliferation induced by hyperlipemic serum in cell cultures of rabbit aorta smooth muscle cells.

The obvious question arises as to the presence and localization of oestrogen receptors in vascular tissue. Oestrogen receptors have been identified in endothelium and vascular smooth muscle cells of several mammalian species (Stumpf *et al.*, 1977; Lin *et al.*, 1982; Lin & Shain, 1986). Oestradiol treatment of oophorectomized baboons (*Papio* sp.) caused the redistribution of oestrogen receptors from cytoplasmic to the nuclear fraction in vascular smooth muscle cells (Lin *et al.*, 1986). These findings imply a role for oestrogen in the regulation of vascular cell function but whether or not oestrogen receptors are implicated in the control of vascular smooth muscle cell proliferation is not established.

At this time there is no evidence that oestradiol 17β inhibits smooth muscle proliferation in the coronary vasculature except in rabbit cardiac allografts (Foegh *et al.*, 1987). The purpose of this study was to evaluate the effect of oestradiol 17β on coronary artery smooth muscle cell proliferation *in vitro* using the uptake of radioactive thymidine into isolated segments of the left anterior descending branch of porcine coronary artery tree. The porcine coronary artery was chosen because of its similarity to that of man. A preliminary account of this work was presented at the 63rd Scientific Session of the American Heart Association in Dallas, Texas, November 12–15, 1990 (Vargas *et al.*, 1990).

## Methods

### *Vessel preparation*

Hearts were obtained from pigs slaughtered at Horst Co. (Hagerstown, MD, U.S.A.). The hearts (10) were harvested and transported to our laboratory in ice-cold Ringer-lactate. Hearts were obtained from male pigs castrated before sexual maturity and from sexually mature female pigs. Segments were prepared from the left anterior descending coronary artery 5 to 10 mm from the origin. Adherent tissue and fat

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were removed from the vessels. Segments of 3 to 5 mm were prepared (5 to 7 for each heart) under sterile conditions and placed in ice-cold minimum essential medium, Eagle's Dulbecco's modification (DMEM) containing gentamicin (50 $\mu g\ ml^{-1}$).

*Time course of [$^3$H]-thymidine uptake*

LAD segments were incubated overnight in DMEM culture medium containing gentamicin (25 $\mu g\ ml^{-1}$) and supplemented with L-glutamine (2 mM) in a humidified 5% $CO_2$ atmosphere at 37°C. After this incubation period, the test drugs and [$^3$H]-thymidine (sp. activity 2.6–3.1 TBq $mmol^{-1}$) were added to the medium and the segments were incubated for an additional 24 to 96 h, depending on the experimental protocol. The experiments were performed in serum-free DMEM without phenol red. The experiments in which the effect of serum was evaluated were performed in the same way as described above, but different concentrations of foetal calf serum (1, 5 and 10%) were added to the culture medium. The tissues were incubated with [$^3$H]-thymidine for 24 h. All concentrations of serum were tested with each experiment. Medium without serum was used as a control. The exposure to [$^3$H]-thymidine was terminated by washing the tissue, replacing the medium with ice-cold phosphate buffer saline (PBS, pH 7.2 without $Ca^{2+}$ and $Mg^{2+}$) and further incubating the segments with PBS containing unlabelled thymidine (1 mM) for 20 to 30 min at 4°C. The tissues were then digested in NaOH (0.5 N) for 18–24 h at 60°C. Aliquots were taken for measurement of radioactivity (Beckman, LS-3150 T scintillation counter). Protein content was determined by the method of Lowry *et al.* (1951).

*Immunofluorescent staining for α-actin and desmin of smooth muscle cells*

LAD explants were placed upright and incubated in 300 µl of serum-free DMEM for up to one week. The segments were removed after three days incubation or when cell out-growth was observed under the inverted microscope. Fresh medium was added every day. The cells growing from the explants were allowed to attach to cover slips. The cover slips were washed with PBS and the cell permeability was increased with lysophosphatidyl choline for 15 min at 37°C. The permeabilized cells were treated for 10 to 20 min with rhodamine labelled phalloidin and washed with PBS. The cover slips were mounted on slides for fluorescence microscopy to observe actin staining.

Cells were also stained for desmin. The cover slips were incubated with 1:30 dilution of a polyclonal antibody for desmin (Sigma Chemicals Co., St. Louis, MO, U.S.A.) for 15 min at room temperature. After washing with PBS, the cells were incubated with a 1:100 dilution of fluorescein isocyanate-labelled goat anti-rabbit IgG (Sigma Chemicals Co., St. Louis, MO, U.S.A.) for 30 min. After additional washing with PBS, the slides were mounted for fluorescence microscopy.

*Uptake of [$^3$H]-oestradiol 17β into vascular segments*

Segments from porcine LAD were isolated as described above. They were exposed to [$^3$H]-oestradiol 17β (sp. activity 5.2 to 6.3 TBq $mmol^{-1}$) for varying time periods (0.5 to 60 min) in Krebs-Ringers bicarbonate buffer at 37°C. For each time point a control with unlabelled oestradiol 17β (100 µM) was used. After the incubation period the rings were frozen in liquid nitrogen and later digested in NaOH (0.5 N) overnight at 60°C. Aliquots were taken for scintillation counting and for determination of protein content.

*Autoradiography*

In another series of experiments, vascular rings were incubated with [$^3$H]-oestradiol 17β for periods ranging from 10 to 20 min. The rings were fixed overnight in 10% formalin and then placed in fresh formalin for two additional days. Progressive dehydration of the tissues was accomplished with increasing concentrations of ethanol. The tissues were then embedded in paraffin and cross sections of 4 to 6 µm were prepared. The sections were stained with Mayer's haematoxylin. Stained sections were rehydrated overnight and coated with photographic emulsion $NTB_2$ (the emulsion was warmed at 40°C for 12 h prior to application to the slides). After a two weeks exposure the sections were developed with Dektol for 45 s and fixed with GBX for 5 min.

*Drugs and materials*

Gentamicin, L-glutamine, PBS and DMEM were purchased from Biofluids (Rockville, MD, U.S.A.). [$^3$H]-thymidine and [$^3$H]-oestradiol 17β were obtained from Amersham (Arlington Heights, IL, U.S.A.). Thymidine, oestradiol 17β, tamoxifen, phalloidin, lysophosphatidyl choline and anti-desmin polyclonal antibody were obtained from Sigma Chemicals Co. (St. Louis, MO, U.S.A.). Rhodamine labelled phalloidin was obtained from Molecular Probes Inc. (Eugene, OR, U.S.A.). Phosphate buffered formalin 10% was purchased from Fischer Scientific (Fair Lawn, NJ, U.S.A.). $NTB_2$, Dektol and GBX were obtained from Eastman Kodak Co. (Rochester, NY, U.S.A.). 4-Hydroxytamoxifen was kindly donated by Dr Robert Clarke. [$^3$H]-thymidine was prediluted with sterile PBS (500 µCi $ml^{-1}$) and was kept refrigerated. Oestradiol, tamoxifen and 4-hydroxytamoxifen were prepared in ethanol in a stock solution (1 mg $ml^{-1}$) and further diluted in the same solvent to keep the volume of administration constant.

*Statistics*

The data calculated as absolute values in c.p.m. $mg^{-1}$ of protein. The [$^3$H]-oestradiol 17β uptake experiments are expressed as individual points. Each point is the mean of duplicated measurements. The [$^3$H]-thymidine uptake experiments are presented as mean ± s.e.mean of the percentage of uptake in the control group. The means are compared by analysis of variance (ANOVA), using a Newman-Keuls test for post-hoc comparisons. $P<0.05$ is accepted as statistical significance.

## Results

The cells migrating from porcine LAD explants after 1 to 2 weeks incubation in medium without serum, stained pos-

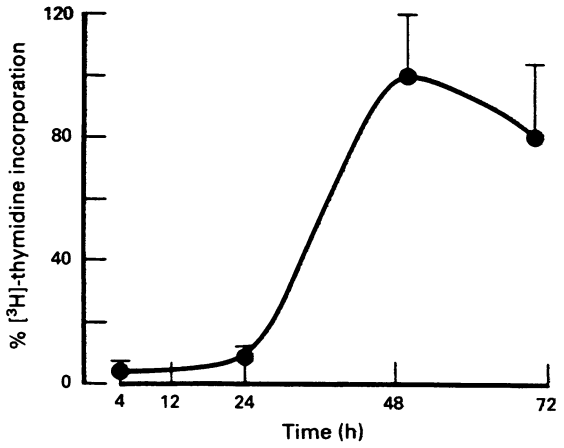


**Figure 1** Time course of [$^3$H]-thymidine uptake into segments of porcine left anterior descending coronary artery (LAD). Each point is a mean ± s.e.mean ($n = 4$ or 6 segments) of the total uptake at each time point, expressed as a percentage of the maximum uptake at 48 h (195,234 ± 70,779 c.p.m. $mg^{-1}$ of protein).

**Table 1** Effect of forskolin and sodium nitroprusside (SNP) on the uptake of [$^3$H]-thymidine into rings of pig LAD

| *Agent* | μM | *[$^3$H]-thymidine uptake* (c.p.m. mg$^{-1}$ protein) | % |
|---|---|---|---|
| Control | | 234,771 ± 40,018 | 100 |
| Forskolin | 100 | 147,641 ± 25,373[a] | 63 |
| Control | | 151,035 ± 25,103 | 100 |
| SNP | 10 | 137,232 ± 18,123 | 91 |
| SNP | 50 | 78,389 ± 14,782[b] | 52 |
| SNP | 75 | 45,932 ± 6,172[b] | 30 |
| SNP | 100 | 3,498 ± 419[b] | 2 |

[a]$P<0.05$; [b]$P<0.01$.
The segments were incubated overnight in DMEM with glutamine and gentamicin. The drugs were added at the indicated final concentration together with [$^3$H]-thymidine (2.5 μCi ml$^{-1}$) and incubated for 24 h. The incubation was ended by washing the segments with ice-cold buffer. The results are mean ± s.e.mean of the absolute values (c.p.m. mg$^{-1}$ protein). The experiment was performed three times. Six segments were used for each concentration.

itively for α-actin and desmin. The stain identified >90% of the cells as vascular smooth muscle.

### *[$^3$H]-thymidine uptake*

Vascular segments of pig LAD incorporated radioactive thymidine in a time-dependent manner. Significant uptake of [$^3$H]-thymidine occurred after 24 h exposure and reached a plateau at 48 h (Figure 1).

In Table 1 the uptake of thymidine into LAD segments is shown to be inhibited by approximately 40% by forskolin (100 μM). Sodium nitroprusside (10 to 1000 μM) nearly abolished [$^3$H]-thymidine uptake with an approximate $IC_{50}$ of 50 μM.

Uptake of [$^3$H]-thymidine was also affected by experimental conditions other than time and antiproliferative drugs. For example, when the concentration of foetal bovine serum in the medium was 5 or 10% the [$^3$H]-thymidine uptake was significantly reduced to about 50% of that incorporated into LAD segments when serum was absent (Figure 2).

### *[$^3$H]-oestradiol 17β uptake and autoradiographic studies*

[$^3$H]-oestradiol 17β was taken up by pig LAD segments in a time-dependent manner (Figure 3a). Equilibrium was achieved between 30 to 45 min of incubation. The total uptake of [$^3$H]-oestradiol 17β was higher in segments from sexually mature female pigs than in tissues from castrated males. [$^3$H]-oestradiol was partially displaced (30%) by an excess of unlabelled oestradiol 17β (Figure 3b).

The [$^3$H]-oestradiol 17β taken up by the tissues was localized by autoradiography. The silver grains were distributed evenly between the different cells within the vessel wall, and as expected, between both cytoplasmic and nuclear compartments (Figure 4).

### *Effect of oestradiol 17β on [$^3$H]-thymidine uptake*

Oestradiol 17β inhibited [$^3$H]-thymidine uptake into pig LAD segments. Oestradiol 17β, at concentrations of 180, 360 and 720 nM, significantly reduced the uptake to 45 ± 16, 48 ± 12 and 25 ± 10% of the controls (Figure 5). This inhibitory effect of oestradiol 17β was concentration-dependent and was significantly expressed only when the weak oestrogen receptor agonist, phenol red, was absent from the culture medium.

The oestrogen receptor partial agonists, tamoxifen and 4-hydroxytamoxifen, at concentrations of 0.1 and 1 μM, significantly inhibited [$^3$H]-thymidine uptake. Equimolar con-

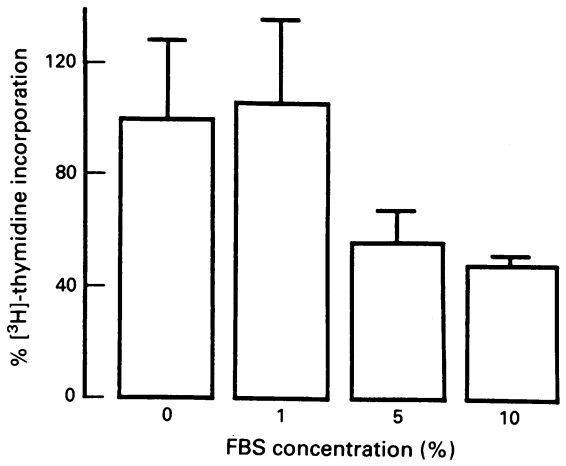


**Figure 2** Effect of three different concentrations of foetal bovine serum (FBS) on [$^3$H]-thymidine uptake (24 h incubation) into segments of porcine LAD. Each column represents the mean ± s.e.mean ($n = 5$ or 6 segments) of the uptake expressed as a percentage of the total uptake into segments when foetal bovine serum was absent from medium (3,824 ± 890 c.p.m. mg$^{-1}$ of protein).

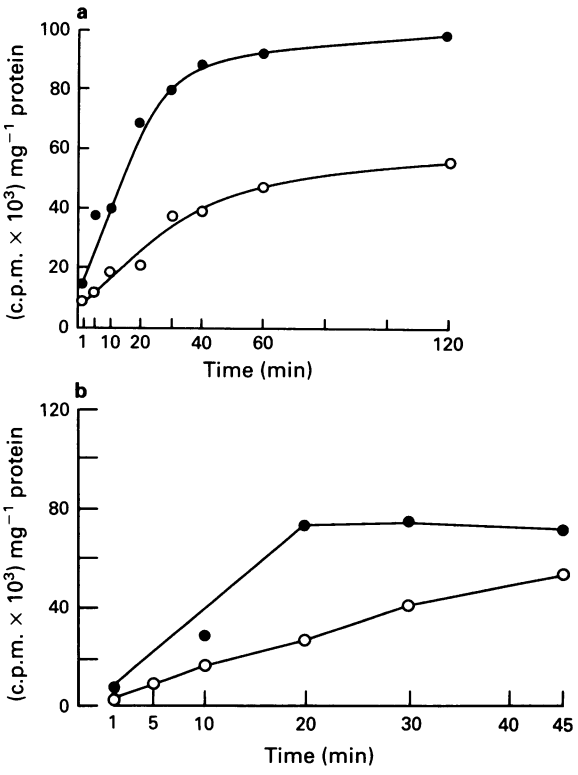


**Figure 3** (a) Time course of [$^3$H]-oestradiol 17β uptake by segments of porcine LAD from castrated male (○) and intact female (●) pigs. Each point is a duplicate measurement of the total uptake expressed as c.p.m. mg$^{-1}$ protein. The experiment was replicated three times. In all three experiments the total uptake of [$^3$H]-oestradiol 17β by vessels from intact females was higher than in vessels from castrated males. The total uptake, at 60 min, in vessels from males expressed as a percentage of the uptake in those from females was: 51, 62 and 64% in each experiment. The final concentration of [$^3$H]-oestradiol 17β in the incubation medium was 400 ng ml$^{-1}$. The maximum oestradiol uptake at 120 min in each experiment was: females, 96,223, 266,585 and 186,000 c.p.m. mg$^{-1}$ protein; males, 54,067, 97,247 and 120,000 c.p.m. mg$^{-1}$ protein. (b) Time course of [$^3$H]-oestradiol 17β uptake by segments of LAD from male (○) and female pigs (●). The two lines represent the total uptake in the presence of an excess of unlabelled oestradiol 17β (100 μM) in the incubation medium.

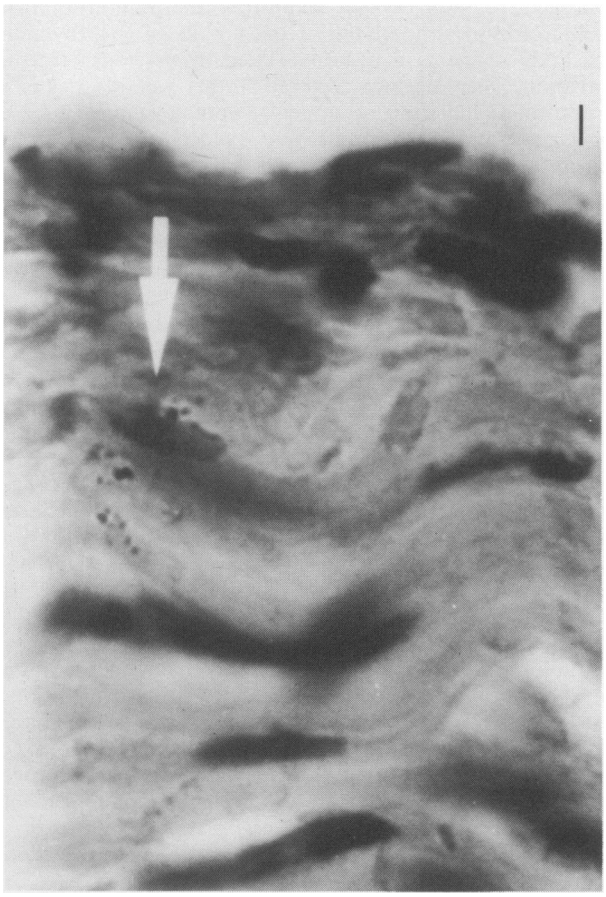

**Figure 4** Autoradiograph of an LAD explant incubated with [$^3$H]-oestradiol 17$\beta$ for 60 min. The photograph shows the silver grains over the nucleus and in the cytoplasm of the smooth muscle cells. $NTB_2$ emulsion. Staining with Mayer's haemotoxylin. The bar represents 10 $\mu$m.

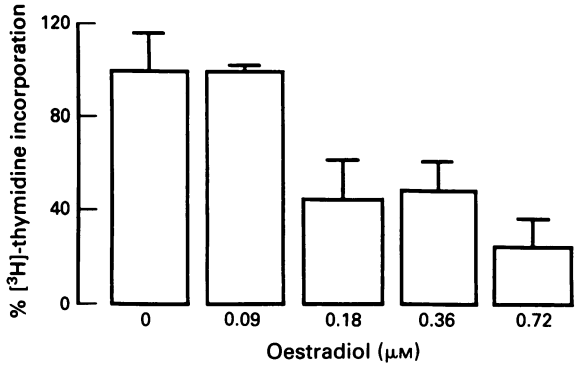


**Figure 5** Oestradiol 17$\beta$ inhibition of [$^3$H]-thymidine uptake into segments of porcine LAD. Vascular segments were exposed to oestradiol 17$\beta$ and radioactive thymidine for 48 h. The results are mean ± s.e.mean ($n = 4$) of [$^3$H]-thymidine uptake into the control tissues treated with the vehicle (152,876 ± 31,694 c.p.m. mg$^{-1}$ protein).

centrations (1 $\mu$M) of tamoxifen and its active metabolite 4-hydroxytamoxifen had oestrogen-like effects in reducing [$^3$H]-thymidine uptake to 28 and 16%, of the control respectively (Figure 6). In the same experiment oestradiol 17$\beta$ (360 nM) was more active and reduced thymidine uptake to 41 ± 5% of the control.

However, pretreatment of the segments with either of the two partial agonists, tamoxifen or 4-hydroxytamoxifen, failed to antagonize the inhibitory effect of oestradiol 17$\beta$.

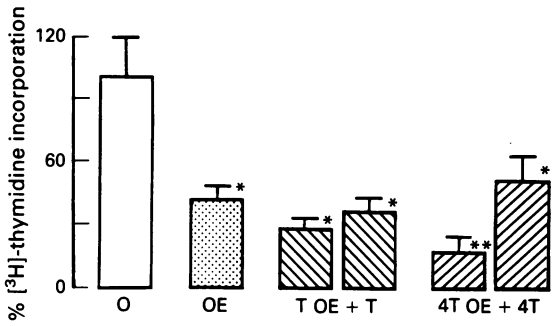


**Figure 6** Effects of 1 $\mu$M of either tamoxifen (T) or 4-hydroxytamoxifen (4T) on oestradiol 17$\beta$ (OE) inhibition of [$^3$H]-thymidine uptake into segments of porcine LAD (48 h incubation). The antioestrogens were added to the medium 3 h before oestradiol 17$\beta$ (360 nM). The columns represent mean ± s.e.mean ($n = 4$) of the percentage of the total uptake into segments treated only with the vehicle (301,999 ± 60,236 c.p.m. mg$^{-1}$ protein).

## Discussion

The purpose of these studies was to determine the effect of nanomolar concentrations of oestradiol 17$\beta$ on the proliferation of coronary artery smooth muscle cell *in vitro*. Oestradiol 17$\beta$ (180–720 nM) significantly inhibited [$^3$H]-thymidine uptake into porcine LAD segments. This suggests that in our experimental conditions, oestradiol 17$\beta$ inhibits smooth muscle cell proliferation in the pig (LAD) coronary artery.

The LAD coronary artery preparation used in this study was evaluated with several compounds known to affect cellular proliferation. Firstly, treatment with either forskolin or sodium nitroprusside is reported to inhibit cellular replication, probably by increasing basal levels of cyclic AMP and cyclic GMP, respectively, as described by Friedman (1976); Kempski *et al.* (1987) and by Garg & Hassid (1989). In our studies, both forskolin (100 $\mu$M) and sodium nitroprusside (50, 75 and 100 $\mu$M) significantly inhibited [$^3$H]-thymidine uptake in a concentration-dependent manner.

Serum is expected to increase cell proliferation due to the presence of growth factors. However, serum can also inhibit thymidine uptake into cells in culture medium (Berthois *et al.*, 1986) depending on concentration, pretreatment with activated charcoal, and cell types. In the LAD segments, a concentration of 1% had no effect, but a significant decrease of [$^3$H]-thymidine uptake was observed after incubation with 5 and 10 fold higher concentrations of foetal bovine serum. Serum is reported to increase prostacyclin production which acts in an autocrine fashion (McIntyre *et al.*, 1978) to inhibit cell proliferation indirectly by increasing basal levels of cyclic AMP. In addition, serum is also required for endothelial growth and preservation (Diglio *et al.*, 1988). Thus, the inhibition of [$^3$H]-thymidine uptake by 5 to 10% which we observed may relate to preservation of the endothelium resulting in inhibition of SMC growth.

The cells (>90%) growing out from the LAD segments were identified by specific immunofluorescence staining of the cytoskeleton proteins $\alpha$-actin and desmin (Skaill *et al.*, 1986; Kocher & Gabbiani, 1986) as smooth muscle cells. It remains to be determined to what extent [$^3$H]-thymidine was incorporated into the proliferating smooth muscle cells.

Oestradiol 17$\beta$ is reported to inhibit proliferation in primary cultures of smooth muscle cells from rabbit aorta (Fischer-Dzoga *et al.*, 1983). This effect of oestradiol 17$\beta$ on vascular SMC proliferation may be mediated directly by activation of oestrogen receptors and/or indirectly by releasing other hormones and growth factors (Sutherland *et al.*, 1983; Davidson & Lippman, 1989). Support for a direct effect of oestrogens on the vessel wall stems from the finding of high affinity oestrogen binding sites in extracts prepared from rabbit aorta ($K_d$ $2.8 \times 10^{-10}$ M) by Colburn & Buon-

assisi (1978), from rat aorta smooth muscle cell ($K_d$ $5.0 \times 10^{-8}$ M) by Nakao *et al.* (1981) and from dog coronary artery ($K_d$ $3.6 \times 10^{-9}$ M) by Harder & Coulson (1979).

Oestrogen appears to activate vascular oestrogen receptors as suggested by its ability to increase progesterone binding sites in baboon (*Papio* sp.) aortic SMC (Lin *et al.*, 1986) which is similar to its effects in uterus, ovary and breast where the response is widely believed to be receptor-mediated. Other effects of oestradiol 17β on SMC which may also involve oestrogen receptor activation include: augmented prostacyclin production by smooth muscle cells obtained from rat aorta (Chang *et al.*, 1980) as well as inhibition of collagen synthesis and hydroxy proline incorporation into rabbit aorta (Fischer & Swain, 1985).

We found radiolabelled oestradiol 17β to be incorporated into the porcine LAD segments in a time-dependent manner. The autoradiographs demonstrated that the tracer was present in the cytoplasm as well as in the nucleus of the different cells. Previous studies by Stumpf *et al.* (1977) have shown that nuclear localization of radioactive oestradiol 17β occurs in vascular tissues as well as in other target tissues for the hormone (Stumpf *et al.*, 1977; Haras *et al.*, 1989). Our finding of nuclear localization of oestradiol as well as in the cytoplasm indicates that the porcine LAD may indeed be a target for oestradiol 17β. Thus, the inhibition by oestradiol 17β of [$^3$H]-thymidine uptake into pig LAD segments may relate to oestrogen receptors. The distribution of [$^3$H]-oestradiol 17β in the segments further suggests that oestradiol may act directly on the smooth muscle cells and/or indirectly by affecting paracrine mechanisms involving other cells within the vessel wall.

The presence of phenol red, a weak oestrogen agonist, completely prevented the inhibitory effect of oestradiol 17β on [$^3$H]-thymidine uptake into LAD segments. In this context it is relevant to recall that oestrogen increases proliferation of MCF 7 cells and that this effect is also prevented by the presence of phenol red in the medium (Berthois *et al.*, 1986; Welshons *et al.*, 1988); this effect of phenol red was attributed to its weak oestrogen activity and its occupation of oestrogen receptor, which reinforces the notion that the inhibition by oestradiol 17β of the uptake of [$^3$H]-thymidine into LAD segments may be mediated by oestrogen receptor activation. Therefore, we explored further whether this effect of oestradiol 17β is an oestrogen-receptor-mediated response by using classical anti-oestrogens, which are used in the treatment of human breast cancer.

Tamoxifen and its active metabolite, 4-hydroxytamoxifen, are both non-steroidal anti-oestrogens but they are also partial agonists (Jordan, 1984). The side effects of tamoxifen treatment in women are typical of those resulting from administration of oestrogen. We found these two anti-oestrogens, in the absence of oestradiol 17β, significantly to inhibit [$^3$H]-thymidine uptake into the pig LAD. This inhibition may be an oestrogen-like effect of these partial agonists. These findings suggest that there may be at least two different oestrogen receptors. One is the classical uterine oestrogen receptor antagonized by tamoxifen. Conversely, tamoxifen is an oestrogen agonist for the other oestrogen receptor at least at the μM concentration used. These concentrations are those normally used of tamoxifen known to antagonize oestrogen in breast cancer cell lines. Our data cannot exclude completely the possibility of non-receptor-mediated actions of oestradiol participating in the described inhibitory effect. However, they reinforce the notion that oestradiol-induced inhibition of [$^3$H]-thymidine incorporation may be mediated through oestrogen receptors.

In summary, we describe for the first time an inhibitory effect of oestradiol 17β on thymidine uptake into segments of pig LAD. This effect seems to be mediated through oestrogen receptors. We suggest that oestradiol inhibition of smooth muscle cell proliferation may be one of the factors involved in the decreased incidence of coronary artery disease in young women.

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