6

Estrogens and the arterial wall

The protective effect of estradiol-17β (E2) in women is well recognized, but the underlying mechanisms are not fully understood. In addition to its beneficial effect on plasma lipoproteins, estradiol exerts a direct effect on the cells of the vessel wall itself. Based on the literature published during 1990-1997, mechanisms of estrogen action in the vascular wall include hormone-induced :

- maintenance of vasodilatory capacity ;
- protection against LDL oxidative modification ;
- action on the metabolism of lipoproteins after they entered the arterial wall ;
- vasorelaxation ;
- inhibition of smooth muscle cell proliferation ;
- stimulation of endothelial cell proliferation, both *in vivo* and *in vitro*.

Most of these effects are probably mediated via functional estrogen receptors present in the cells of the vascular wall.

Vasomotricity and cell-mediated metabolism of steroids and lipids

The studies published on « estrogen and the arterial wall » can be presented under two headings :

- definition of the target site and molecular mechanisms of action by *in vitro* and *in vivo* studies ;
- pharmacological studies on molecules which mimic the action of natural estrogens.

Experimental studies strongly support the atheroprotective effect of estrogens. They have been carried out in monkey, rabbit and swine (Hough and Zilversmit, 1986 ; Adams et al., 1990 ; Hayashi et al., 1992) and more recently in apolipoprotein E-deficient (apoE KO) mice generated by gene targeting (Plump et al., 1992 ; Zhang et al., 1992 ; Van Ree et al., 1994 ; Bourassa et al., 1996). These animals develop pronounced hypercholesterolemia on a normal chow diet, with chylomicron and very low lipoprotein remnant

accumulation in plasma probably resulting from abnormal receptor-mediated lipoprotein removal (Ishibashi et al., 1994 ; Mortimer et al., 1995), an increase in atherogenic lipoprotein retention by matrix components, as well as slowing down of reverse cholesterol transport from the arterial wall (Saxena et al., 1993 ; Hayek et al., 1994). Under these conditions, the mice develop lesions similar to those seen in humans, with foam cell-rich fatty and fibrous lesions including both proliferating smooth muscle cells and calcium deposits (Bourassa et al., 1996).

Definition of the target site using experimental models

None of the studies available explain the mechanisms involved. Although a firm answer cannot be given, favourable changes in blood lipids and lipoproteins do not appear to be the major target (table 6.Ia).

Vasodilation

Studies (table 6.Ib) suggest that the vasodilatory capacity can result from a direct action of estrogens on smooth muscle cells (SMC), by a non genomic effect involving ion channels. The molarities used in these experiments, in the range of µM, cannot be reached with HRT. They may be reached by local administration, as during coronary angiography. Other studies (table 6.Ic) suggest the possibility of an increased bioavailability of endothelium-derived relaxing factors, such as nitric oxide (NO) or a related nitrosocompound, either by increasing NO synthase expression or by decreasing superoxide anion production, preventing the degradation of NO. In both cases, increased NO would promote vasodilation but also inhibit proliferation of the adjacent vascular smooth muscle, reduce platelet aggregation and inhibit monocyte adhesion to the endothelium and the inflammatory reaction induced by cytokines, all of which are key contributors to the development of atherosclerosis (Förstermann et al., 1994 ; Cooke, 1994 ; Kubes et al., 1991 ; Bath et al., 1991 ; De Caterina et al., 1995 ; Khan et al., 1996). However, the fact that medroxyprogesterone acetate (MPA) interferes with this effect (Miyagawa et al., 1997) but not with the protective effect of HRT (Grostein et al., 1996), suggests that this mechanism does not play a major role in the atheroprotective effect.

Protection of LDL against oxidation

Few studies (table 6.Id) have considered the protection of LDL against oxidative modification. Most *in vitro* studies have used estrogen concentrations that, again, are in the µM range and cannot be achieved with HRT. They probably reflect the intrinsic antioxidant structure of the estrogen molecule. In contrast, *in vivo* studies or *ex vivo* studies (cell cultures) suggest that an antioxidant effect could be mediated by estrogen receptors, at physiological molarities. Whether this effect results in protection of LDL against oxidative modification or reduces NF-KB activation (Flohé et al., 1997) is uncertain.

Table 6.Ia : Definition of the target site using animal experimental models : favourable changes in blood lipids and lipoproteins do not appear to constitute the major target

Authors	Experimental model*	Technique/Protocol	Observations/Conclusions
Srivastava et al. (1993)	Rat and mouse	Lipid metabolism. mRNA measurments by Northern blot.	Estrogen up regulates LDL-R in rat but not in mice.
Haarbo et al. (1991)	Rabbit	Cholesterol content in aortic tissue	Estrogen attenuates atherogenesis in cholesterol-fed ovariectomized animals. Progestins (Levonorgestrel and norethisterone acetate) do not counteract the effects.
Williams et al. (1995)	Monkey	Histomorphometry	Lipid lowering induces artery and lumen enlargment and improves reactivity of large epicardial coronary arteries. E2 do not add to these improvements except for dilator capacity.

Table 6.Ib : Definition of the target site using animal experimental models : vasodilator capacity (I)

Authors	Experimental model*	Technique/Protocol	Observations/Conclusions
Salas et al. (1994)	Pig-coronary artery	Arterial tension	17 α-estradiol (2μM) causes relaxation of K+-contracted coronary artery.
White et al. (1995)	Pig	Arterial tension studies patch-clamp	Estradiol (5μM) relaxes coronary arteries by opening Ca++ and voltage-activated K+ channels by a direct action at the level of SMC (inducible NOS).
Ravi et al. (1994)	Rat aorta	Arterial tension studies	Estradiol (μM) attenuates vasoconstrictor effect of phenylephrine and angiotensin II.
Rodriguez et al. (1996)	Rat aorta	Arterial tension studies	17β-estradiol and DES have relaxant effect. 17β is inactive
Jiang et al. (1992)	Rabbit coronary arteries	Arterial tension studies	Estradiol (μM) attenuates coronary artery contraction induced by endothelin-1 via an endothelium-independant mechanism.

Table 6.Ic : Definition of the target site using animal experimental models : vasodilator capacity (II)

Authors	Experimental model*	Technique/Protocol	Observations/Conclusions
Mugge et al. (1993)	Human coronary arteries	Arterial tension studies	Estradiol produces an endothelium-independent relaxation.
Mikkola et al. (1995)	Human umbilical vein endothelial cells.	6-keto-PGF1a and ET-1 RIA.	Estrogens induce stimulation of PGI2 but has no effect on ET-1 production. TX is inhibitor.
Lamping and Nuno (1996)	Dog coronary microvessels	Videomicroscopy	Estradiol (0.1-1 μM) attenuates constriction of coronary microvessels to endothelin-1.
Wellman et al. (1996a)	Rat	Intra vascular pressure. Membrane potential measurements. Patch-Clamp studies.	E2 increases basal NO release from endothelial cells. Part of the effect is through activation of Ca++-dependent K+ channels. TX is antagonist.
Keaney et al. (1994)	Pig-coronary artery	Arterial tension Oxydative modification of LDL.	Estradiol preserves endothelial function in cholesterol-fed swine in association with protection of LDL against oxydative modification.
Brosnihan et al. (1994)	Rat	Transgenic hypertensive rat (mouse Ren-2 gene)	Estrogen countermodulates the renin-mediated hypertension by enhancing the activity of endothelial-derived relaxing factors.
Hayashi et al. (1994)	Rabbit	Conversion arginine/citrulline	Estradiol (10-7 M) enhances activity of neuronal NOS in tissue homogenates by a possible effect on Ca++-calmodulin.
Hayashi et al. (1995)	Human umbilical vein endothelial cells	Biochemistry	Estrogen increases endothelial nitric oxide by a receptor-mediated pathway.
Hishikawa et al. (1995)	Human aortic endothelial cells.	Cell biology and biochemistry	Estradiol increases endothelial cNOS.
Arnal et al. (1996)	Aortic bovine endothelial cells	Cell biology	Estrogens induce a receptor mediated antioxydant effect

Table 6.Id : Definition of the target site using animal experimental models : protection of LDL against oxidative modification

Authors	Experimental model*	Technique/Protocol	Observations/Conclusions
Mazière et al. (1991)	Human LDL	TBARS assay*	Estrogens (μM) inhibit copper and cell-mediated modification of LDL.
Nègre-Salvayre et al. (1993)	Human LDL Aortic bovine endothelial cells	TBARS assay Cell culture	Estradiol (μM) inhibits LDL oxydation and increases cellular resistance against the cytotoxic effect of oxidized LDL
Ayres et al. (1996)	Human LDL	TBARS assay Diene conjugation method oxysterol measurement	Estradiol (μM) is as or more effective an antioxydant as α-tocopherol.
Tang et al. (1996)	Human LDL	TBARS assay and oxysterol measurement	Different estrogens (μM) might act preferentially on distinct lipid substrates in exhibiting antioxidant effects.
Miller et al. (1996)	Rabbit LDL	TBARS assay	Antioxydant efficacy can be separated from estrogenicity
Keaney et al. (1994)	Pig-coronary artery	Arterial tension Oxydative modification of LDL.	Estradiol preserves endothelial function in cholesterol-fed swine in association with protection of LDL against oxydative modification.
Arnal et al. (1996)	Aortic bovine endothelial cells	Cell biology	Estrogens induce a receptor mediated antioxydant effect.

* measure of lipid peroxidation

Table 6.Ie : Definition of the target site using animal experimental models : metabolism of lipoproteins in the arterial wall

Authors	Experimental model*	Technique/Protocol	Observations/Conclusions
Wagner et al. (1991)	Monkey	Catabolic rate and arterial accumulation of labeled tyramine cellobiose LDL.	Direct effect of estrogens at the level of the arterial wall: suppression of uptake and/or degradation of LDL.
Wagner et al. (1992)	Monkey	Catabolic rate and arterial accumulation of labeled tyramine cellobiose LDL.	Regional differences among arterial sites : coronary arteries > carotid bifurcation.
Haarbo et al. (1994)	Rabbit	Cholesterol content in plasma aortic tissue and use of labeled LDL.	The atheroprotective effect of estradiol is not mediated through an effect on aortic permeability to LDL but rather to their metabolism in the arterial wall.
Haarbo and Christiansen (1996)	Rabbit	Cholesterol content in plasma and aortic tissue.	Estradiol inhibits progression of atherosclerosis and may be important in secondary prevention.
Colvin (1996)	Rabbit	Turnover studies of labeled native and chemically modified LDL (methyl-LDL)	In addition to any effect on LDL receptor, estrogen promotes the activity of LDL receptor-independent pathways.
Schwartz et al. (1996)	Rat chondrocyte	Cell biology and biochemistry	Estradiol (10-10 M) has a rapid sex-dependent (F) action on chondrocyte membrane lipid metabolism.

Table 6.II : Definition of the target site using in vivo studies in human

Authors	Experimental model*	Technique/Protocol	Observations/Conclusions
Akkad et al. (1996)	Human carotid plaque	Duplex ultrasound	Estrogen replacement therapy is associated with significant plaque regression.
Taddei et al. (1996)	Forearm blood flow	Strain-gauge plethysmography	Estrogens exert a protective effect on endothelial function.
Cicinelli et al. (1997)	Human	Nitric oxide plasma levels	Transdermal estrogen increases plasma levels of NO.
Wilcox et al. (1997)	Human	Plasma endothelin levels by RIA	Baseline concentrations are positively correlated to plasma cholesterol and decrease with estrogen treatment.
Kishikawa et al. (1993)	Human aorta	Immunohistochemistry	Cytokine-mediated cellular interaction plays a pivotal role in the atherosclerotic process.

Table 6.III : Pharmacological studies

Authors	Molecule/species	Protocol	Observations/Conclusions
Clarkson et al. (1990)	Ethinylestradiol, Norgestrel Ethynodiol diacetate/Monkey	Histomorphometry	Coronary artery atherosclerosis is lessened by the estroprogestative associations despite a decrease in HDL.
Subbiah et al. (1993)	Equine estrogens (equilin)/Human	TBARS assay	Equilin exhibits higher antioxidant potency than E1 and E2
Sulistiyani et al. (1995)	17 α-dihydroequilin sulfate Ethynylestradiol/Rabbit	Lipid metabolism/ Atherosclerotic plaque measurement/Cholesterol tissue content	Estrogen exerts its beneficial effect at the level of the arterial wall.
Taniguchi et al. (1994)	Catecholestrogens/Human	TBARS assay	Catecholestrogens (μM) may be more important antioxydants that estrogens.
Honore et al. (1997)	Phytoestrogens/Monkey	Quantitative coronary angiography	Soy isoflavones, like estrogen, enhance the dilator response to acetylcholine of atheroscleortic arteries.
Grainger et al. (1995)	Tamoxifen/Mouse	Histomorphometry TGF-β assay	Tamoxifen suppresses diet-induced fatty streaks and increases TGF-β concentration in serum and vascular wall
Klinge et al. (1996)	Calf estrogen receptor	Gel mobility shift assay	Differences in receptor conformation when liganded with 4-hydroxytamoxifen vs estradiol.
Chailleux et al. (1994)	Rat liver	Biochemistry	Partial purification of the antiestrogen binding site I.
Poirot et al. (1994)	Rat liver	Biochemistry	Partial purification of the antiestrogen binding site II

Table 6.IV : Presence of ER in the vascular wall

Authors	Experimental model*	Technique/Protocol	Observations/Conclusions
Campisi et al. (1993)	Human-aorta-whole tissue	binding assay	64 kDa ER, *in situ*
Knauthe et al. (1996)	Rat-aorta- veins	RT-PCR	sex differences and E2 induction in venous vessels, not in aorta
Bayard et al. (1995)	Rat- aorta- SMC	RT-PCR, ICC; luciferase assay	functional ER
Bei et al. (1996)	Rat- coronary- SMC	RT-PCR, binding assay	binding properties
Karas et al. (1994) and Baysal and Losordo (1996) review-	Human SMC - saphenous vein-mammary artery	RNAse protection assay, ICC, WB, luciferase assay, binding assay, gel mobility assay	functional ER
Perrot-Applanat et al. (1988)	Human and rabbit uterus	ICC	presence *in situ* in SMC
Perrot-Applanat et al. (1995) and Perrot-Applanat (1996) review	Human saphenous vein Cardiovascular system	RT-PCR, EIA, ICC	most biopsies are ER–/PR+, SMC *in situ*
Kim-Schulze et al. (1996)	Endothelial cells - Human coronary artery-HUVEC-	ICC, RNAse protection assay, binding assay, gel mobility shift assay	nuclear and functional ER
Venkov et al. (1996)	Endothelial cells- Human aorta-HUVEC- bovine aorta	gel mobility shift assay, WB, ICC	ER (67 kDa)
Isoforms			
Karas et al. (1995)	Human- saphenous vein-mammary artery- SMC	RT-PCR	ER Δ4 (cytoplasmic, 55kD) ; no ER-dependent transactivation
Inoue et al. (1996)	Rat-aorta- SMC	RT-PCR ; CAT assay	3 ER Δ4, Δ 4/5 (dominant negative) and Δ 3/4 isoforms + WT ; no ER-dependent transactivation
Lehrer et al. (1993)	hypertensive women	RT-PCR	higher incidence (48 %) of ER variant in hypertensive women / 10 % in normal

Table 6.Va : Modulation of vasomotricity by estradiol : *in vivo* studies

Authors	Experimental model	Technique/Protocol	Observations/Conclusions
Barber et al. (1996)	Pig- coronary artery	Receptor binding assay for endothelin measurement of isometric force (rings)	Endothelin receptors are modified in association with endogenous fluctuation in estrogens (endothelium independant) ; greater contractions to ET1 in females versus males
Cheng et al. (1994)	Rat aorta - prepubertal	measurement of isometric force (rings)	enhance endothelium-dependent vasorelaxation induced by acetycholine
Collins et al. (1994)	Rabbit - coronary artery (castrated, estrogen treated or acutely estrogen withdrawn)	measurement of isometric force (rings)	induce relaxation in artery precontracted with PGF2α (20%, 42% and 75% at 10^{-9}, 10^{-8} and 10^{-6}, respectively) ; endothelium and NO - dependent
Thompson and Weiner (1997)	ovariectomized - guinea pig-coronary artery-long term estradiol replacement	measurement of isometric force (rings)	E2, at low doses, decrease contractility of arteries to thromboxan A2 (endothelium - derived NO dependent)
Collins et al. (1995)	Human- coronary artery	measurement of coronary artery diameter (quantitative angiography) and coronary blood flow (intracoronary doppler)	attenuates acetylcholine-induced constriction, in women but not in men, with coronary heart disease
Gilligan et al. (1994)	Post menopausal women - brachial artery	measurement of blood flow by intra-arterial catheter and resistance after E2 infusion (plasma level 350pg/ml	potentiates endothelium-dependent vasodilator response to ACh in healthy women ;
	- coronary artery		potentiates endothelium-dependent and endothelium-independent in women with risk factor for atherosclerosis and impaired vascular function high estradiol level?
Gilligan et al. (1995)	idem	after E2 replacement therapy (Estraderm Ciba, 0.1mg/day, 3 weeks of transdermal administration)	no effect with chronic estradiol administration (3 times lower E2 level : 120 pg/ml, as compared to acute administration (340 pg/ml). Dose dependent effect ?

Table 6.Vb : Modulation of vasomotricity by estradiol : in vitro studies

Authors	Experimental model*	Technique/Protocol	Observations/Conclusions
Bell et al. (1995a)	pig coronary artery (castrated)	measurement of isometric force (rings) - overnight E2 (1nM)	Enhances endothelium-dependent NO-mediated vasorelaxation ; blocked by tamoxifen
Bell et al. (1995b)	idem	acute E2 (1nM)	Enhances acute noradrenergic (but not isoproterenol) vasorelaxation ; endothelium independent
Chester et al. (1995)	Human- coronary artery	measurement of isometric force (rings)	Induces relaxation (7-15 min) in artery precontracted with thromboxan A2 (endothelium independent) ; sex-dependent (greater in females)
Thomas et al. (1995)	Rat-aorta	measurement of isometric force (rings)	Induces relaxation in aorta precontracted with adrenaline and potassium chloride (max 120 min) ; endothelium independent

Table 6.VI : Vascular (SMC) antiproliferative effects of estradiol : *in vivo* and *in vitro* studies

Authors	Experimental model	Technique/Protocol	Estradiol concentration	Observations/Conclusions
Clarkson (1994)	Monkey coronary, cholesterol diet	angiography	norgestrel	E2 diminish atherosclerosis, independently of plasma lipoprotein concentration
Williams et al. (1994)	idem		1 mg E2/kg/week	
Hanke et al. (1996)	Rabbit aorta, cholesterol diet	intimal thickening	2.5-25 mg P capronate/kg/week	E2 (and E2+ « reduced » P) diminish intimal plaque size ; No effect of P « high doses » alone or E2+P « high dose » independent of plasma cholesterol concentration
Kolodgie et al. (1996)	Rat aorta, SMC	Microwell-Boyden chamber	0.5-10 ng/ml	E2 (and DES) diminish growth factor (PDGF) - induced migration of rat aorta SMC
Moraghan et al. (1996)	Pig coronary, SMC	3H thymidine incorporation	10^{-11}-10^{-7}	E2 (physiol doses) diminish 2 % FCS-induced proliferation of pig coronary SMC, in female only Inhibition by P (10^{-9}M)
Suzuki et al. (1996)	Human aorta, SMC	idem	E2 10^{-9}-10^{-6} or E2 10^{-7}+P (10^{-9}-10^{-6})	E2 diminish growth factor (EGF, PDGF, bFGF)-induced proliferation and PDGF-induced migration of human aorta SMC ; dose dependent no effect of P (10^{-9} to 10^{-6}), or E2 10^{-7}+P (10^{-9}-10^{-6})
Espinoza et al. (1996)	Rat SMC	idem		E2 diminish 1% FCS-induced proliferation only after E2 preincubation
Dai-Do et al. (1996)	Post menopausal women and men saphenous vein	idem	10^{-8} - 10^{-6}	E2 diminish growth factor (PDGF) induced proliferation and migration, regardless of gender.
Vargas et al. (1993)	Pig coronary segment, SMC	idem	0.2-0.4 10^{-6}	diminution of proliferation in segments and organ culture
Vargas et al. (1996)	Rat coronary, organ culture	idem	idem	

Table 6.VII : Proliferative effects of estradiol on endothelial cells : *in vitro* and *in vivo* studies

Authors	Experimental model	Technique/Protocol	Estradiol concentration	Observations/Conclusions
Morales et al. (1995)	HUVEC	3H thymidine incorporation + Boyden chamber migration + Matrigel test	1.5 ng/ml	proliferation (max 4 days); migration
Kim-Schulze et al. (1996)	Human coronary artery + HUVEC		3.5 10^{-9} (1ng/ml)	proliferation (max 24-30 hours) ; prevented by ICI 182780
Schnaper review (1996)		ICC, RNAse protection assay ligand binding 5.10^4 R/cell - gel shift assay		Presence of functional ER
Morales et al. (1995)	*in vivo* murine model	Matrigel phys coinjected with bFGF	17β E2 pellet (21 days) - 750 pg/ml	increase of angiogenesis to the level non ovariectomized mice

ICI 182 780 : specific ER antagonist (Zeneca Pharm.)

Lipoproteins metabolism

Some studies (table 6.Ie) have considered the metabolism of lipoproteins in the arterial wall ; an effect on aortic permeability to LDL has not been confirmed, although it should be stressed that the focal nature of plaque formation is closely related to high permeability at certain arterial sites, induced by hemodynamic forces which stimulate the rate of transcytosis (Nilsen, 1996 ; Davies et al., 1984) and/or endothelial cell turnover and death (Davies et al., 1986 ; Lin et al., 1990 ; Chang and Harley, 1995). E2 could then be active on lipoprotein transfer by stimulating the rate of cell renewal, an effect which could also prevent SMC proliferation (Krasinski et al., 1997 ; Foegh et al., 1994 ; Chen et al., 1997 ; Iafrati et al., 1997). Other studies suggest that the plasma lipid-independent antiatherogenic effect of estradiol could be related to the metabolism of lipoproteins after they have entered the arterial wall. Such a mechanism is not clear and may be related to the captation of modified LDL by macrophages, which represent an opportunistic, but constant, component of atherosclerotic plaque. Indeed, estrogens also inhibited monocyte adhesion to the endothelium, although the molarities used in the study appear unphysiological and the data conflict with another paper (Cid et al., 1994). The relationship between monocytes and endothelium is one aspect of the local immune response in atherosclerosis (Libby and Hansson, 1991). Caulin-Glaser et al. (1996) showed that estradiol inhibits IL-1-mediated membrane E-selectin, vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expression.

For the definition of the target site in human studies, five studies are reported in table 6.II. These studies, using different physical or biochemical techniques, confirm the above experimental studies. They again support the atheroprotective effect (Akkad et al., 1996). They also confirm the probable role of estrogens in increasing NO bioavailability in the vascular wall (Taddei et al., 1996 ; Cicinelli et al., 1997) and a possible action on vasoconstrictors such as endothelin (Wilcox et al., 1997). Attention should also be drawn to a possible immunological mechanism.

Pharmacological approach

A series of studies have been reported (table 6.III), using either estrogen analogs or drugs displaying a so-called « antiestrogen effect ». Synthetic or natural phytoestrogens have been dealt with in the first category. These estrogen agonists do not in general have appreciable beneficial effects compared to estradiol (Clarkson et al., 1990 ; Taniguchi et al., 1994 ; Honore et al., 1997). Some studies have also analyzed the activities of equine estrogens without showing any significant advantages either (Subbiah et al., 1993 ; Sulistiyani et al., 1995).

Probably of greater interest are the reported activities of « antiestrogen » compounds. Triphenylethylene derivatives exert beneficial effects in animal

studies. The most appealing of the different molecules is probably Tamoxifen (Grainger et al., 1995) and possibly some of its analogues such as Raloxifen® (LY139481 Hcl). The mechanism of their action on the cardiovascular system is not known. It is not clear whether this atheroprotective effect results from a partial agonistic effect that these molecules have in tissues (Klinge et al., 1996) like the liver, or from a different nonestrogenic pathway, involving specific binding sites called « Antiestrogen Binding Sites » (Chailleux et al., 1994 ; Poirot et al., 1994). Further studies are necessary in this field, particularly in the comparison of molecules with partial and complete antiestrogen activities.

Cell proliferation, relaxation and endothelial cell adhesion

Different publications have been selected : original and relevant papers published in journals with a high impact factor in the vascular field (*Circulation, Circulation Research, Cardiovascular Research*) or the endocrinologic field (*Endocrinology, Journal of Clinical Endocrinology and Metabolism, American Journal of Obstetrics and Gynaecology*), as well as significative reviews ; papers from the same groups were analyzed together. *In vivo* and *in vitro* studies were analyzed separately ; the methodology is given in the tables. Studies using pharmacological doses of E2 were not selected. The mechanism of action of estradiol on the vascular wall (direct effects on the cells of the arterial wall, or effects via paracrine mechanism(s)) is mentioned when available.

Results will be presented as follows : analysis of estradiol effects on vascular tone and arterial structure (proliferation), angiogenesis, and cell adhesion. A few data related to the role of progesterone (P) have been included.

Specific estrogen receptors and mechanism of action

Estrogen has many effects on the vasculature. Some of these are rapid, membrane effects of the hormone on ion channels that do not require transcriptional activation of genes. The major positive effects of estrogen on vascular tissue involve estrogen receptors (ER).

Numerous studies concerning the presence of estrogen receptors in the vascular wall have been published over the 10 last years, linked to the production and widespread utilisation of monoclonal antibodies against the receptor proteins and the use of reverse transcriptase polymerase chain reaction (RT-PCR) and RNase protection assays. These more recent studies are presented in table 6.IV.

The expression of ER and PR (progesterone receptors) has now been demonstrated *in situ* in normal and pathological samples from the vascular system and most of these studies agree that the receptor content is low. Significant

differences are observed in arteries of the female genital tract between different physiological and experimental situations, but the expression of ER has not been clearly linked to sex differences in most vascular tissues (Perrot-Applanat et al., 1988 ; Perrot-Applanat, 1996 - review ; Knauthe et al., 1996). Nuclear ER and PR are present *in situ* in myocytes (tunica media) of arteries, with occasionally labeled endothelial cells ; in a recent autopsy study, ER was also identified in vascular smooth muscle cells (SMC) from coronary arteries of premenopausal women (Losordo et al., 1994) ; the expression was much lower in atherosclerotic vessels than in normal arteries, suggesting a role of ER or its deficiency in atherosclerosis. Two papers report the absence (or low level) of expression of ER in biopsies from saphenous veins, while PR is expressed in the SMC of the intima and tunica media (Perrot-Applanat et al., 1995).

Since the pioneering work of Colburn and Buonassi (1978) demonstrating the presence of ER in bovine endothelial cell cultures several papers have reported the presence of ER in endothelial cells and smooth muscle cells prepared from human and animal arteries (aorta, coronary artery) and veins (table 6.IV).

Classically, ER, activated through estrogen binding, interacts with DNA (estrogen-responsive element, ERE) and transactivating proteins which regulate transcriptional activity. Based on the capability of E2-induced transcriptional transactivation in transient transfection assays, a number of *in vitro* studies have revealed that these receptors are functional (Karas et al., 1994 ; Bayard et al., 1995 ; Kim-Schulze et al., 1996 ; Baysal and Losordo, 1996 - review).

Recently, Iafrati et al. (1997) reported that one of the well-documented vascular effects of estrogen (inhibition of the response to vascular injury in the carotid artery) remains unchanged in ER-KO mice ; the recent discovery of ERb (Iafrati et al., 1997) should also help to explain the effects of estrogen on the vasculature.

Effects of estradiol on vascular tone

The effect of estradiol on coronary artery reactivity has been studied for several years. In the early 1980's it was reported that super-physiological doses of estrogen improved endothelium-mediated dilation of rabbit arteries. In the early 1990's it was reported that physiological doses of estrogen increased dilatory responses of atherosclerotic coronary arteries.

The *in vivo* studies are presented in table 6.Va. E2 increases basal coronary flow and epicardial coronary artery diameter in post-menopausal women (Reis et al., 1994). The impact of estrogen deficiency on the endothelium has been evaluated by observing endothelial function following E2 infusion and replacement of estrogen (table 6.Va). Acute administration of estrogen enhances endothelium-dependent vasodilation to acetylcholine in rat aorta

(Cheng et al., 1994) and in both the peripheral (forearm) and coronary circulation of healthy postmenopausal women (Gilligan et al., 1994a,1995) ; in one of these studies, this effect was not apparently maintained with a 3-week cycle of systemic estradiol administration. Women with risk factors for vascular dysfunction had significantly reduced vasodilatory responses to acetylcholine as compared with healthy subjects (Gilligan et al., 1994b). Two *in vivo* studies demonstrated that estrogen-induced, endothelium-dependent vasorelaxation of coronary arteries may depend on sex hormone status : it is observed in women, but not in men with coronary heart disease (Collins et al., 1995). In the female rabbit (Collins et al., 1994), relaxation is greater in acutely estrogen-withdrawn animals (versus castrated or estrogen treated). The possibility of the involvement of NO *in vivo* has recently been strenghtened by evidence that estrogen-induced increases can be antagonized by NO synthase inhibitors (Thompson and Weiner, 1997). Long-term E2 replacement therapy has also been shown to decrease the contractility of coronary arteries to thromboxane A2 in the guinea-pig (Thompson and Weiner, 1997).

Independently of NO synthesis, endogenous fluctuations of estrogen modulate endothelin receptors in coronary arterial smooth muscle cells of female pigs (Barber et al., 1996)

The *in vitro* studies are presented in table 6.Vb. It is generally agreed that the tone of underlying smooth muscle cells is regulated by a variety of agents such as EDRF (or NO) (Furchgott and Zawadski, 1980), prostacyclin (Moncada et al., 1976), endothelin (Yanagisawa et al., 1988), angiotensin (Ryan, 1989), free radicals and adrenaline. Several *in vitro* experiments have studied the incubation of arterial rings and measured isometric forces during E2 exposure.

E2 induces rapid relaxation of rat aorta and human coronary arteries precontracted with adrenaline (Thomas et al., 1995) and thromboxane A2 (Chester et al., 1995), respectively, through endothelium-independent mechanism(s). The relaxation by adrenergic stimulation is enhanced by acute, direct exposure to E2 (Bell et al., 1995a). In the model of the rat mesenteric artery, male gender and ovarian hormone deficiency reduce the vasodilatory effects of endothelium-independent relaxing agents (Nevala et al., 1996). A direct smooth muscle relaxing effect of estradiol may be mediated by calcium channel blockade (for review, see Collins et al., 1994) when used for a short time at relatively high concentrations. The inhibitory effect of estradiol may be mediated via non genomic effects ; a 5-min incubation of cultured rat pulmonary vascular SMC with 17b-estradiol significantly increases the basal intracellular cAMP level in a concentration-dependent manner (Farhat et al., 1996).

Acetylcholine (Ache) induces endothelium-dependent vasorelaxation *in vitro* by the release of NO. Disruption of the endothelium or inhibition of NO synthesis by selective NO inhibitors contribute to coronary vasoconstriction ; the increase in acetylcholine-induced relaxation by E2 is mediated through endothelium-dependant mechanism(s). Estrogen, at low doses, acts indirectly

through the release of endothelium-derived NO (Collins et al., 1994) and involves ER (Cheng et al., 1994) blockage by Tamoxifen. Also, the ability of estrogen to act as an antioxidant has been postulated to account for the rapid restoration of endothelium-dependent vasodilation. In the other hand, endothelium-dependent, NO-mediated relaxation (by A23187) of porcine coronary arteries is enhanced by overnight, but not acute, exposure to physiological doses of estrogen (Bell et al., 1995b).

Effects of estradiol on vascular cell proliferation

Vascular smooth muscle cell (SMC) proliferation and migration are believed to be critical for SMC accumulation in the intima, and this may be important for luminal narrowing in atherosclerosis, restenosis and venous bypass graft disease. Although SMC are in a contractile phenotype, they may change their properties following a disease state and start to proliferate, a property which is also shown for SMC in culture. Estradiol inhibits smooth muscle cell proliferation both *in vivo* and *in vitro*.

The *in vivo* studies are presented in table 6.VI. An anti-atherosclerotic effect of estrogen has been found in a variety of animal models, such as pig, primates (Clarkson, 1994), rabbits (Hanke et al., 1996a) and mouse. It is partially mediated by the decrease in LDL uptake and/or degradation, but also by an improvement in coronary artery function. The effect is sex-dependent in the rabbit (Hanke et al., 1996a). Estrogen also reduces myointimal proliferation after ballon injury of rat carotid artery (Chen et al., 1996).

The *in vitro* studies are presented in table 6.VI. Three studies demonstrate that 17b-estradiol inhibits the proliferation and migration of SMC from human or rat coronary arteries (Kolodgie et al., 1996 ; Suzuki et al., 1996) and human saphenous veins (Dai-Do et al., 1996) stimulated by mitogens such as PDGF and bFGF ; this antiproliferative effect of estradiol was also obtained with SMC from coronary artery (pig) or aorta (rat) cultured in 1-2 % FCS (Espinosa et al., 1996 ; Moraghan et al., 1996), or in carotid segments (Vargas 1996). However, conflicting results are reported concerning the sex-dependency of the anti-proliferative effect of physiological doses of E2. Two studies reported a differential response in coronary arteries from pig (Moraghan et al., 1996) and rat (Vargas et al., 1996) ; however, a recent study on SMC from human saphenous veins provides evidence that the antiproliferative and antimigratory effects occurred in post-menopausal women as well as in age-matched men, indicating that SMC can respond to 17β estradiol regardless of the gender (Dai-Do et al., 1996). These different observations may be due to the different cell types and/or species used.

The mechanisms of the inhibition of cell proliferation by E2 remain to be clarified. Estradiol could inhibit SMC proliferation directly or indirectly by inhibiting the synthesis of growth factors ; other actions can be considered, such as endothelial-derived nitric oxide, in the inhibition of SMC prolifera-

tion, or the rapid (5 min) increase in cAMP (an important intracellular signaling molecule in the response of SMC to vasoactive and mitogenic factors) during E2 incubation (Farhat et al., 1996), features compatible with non genomic actions.

Effect of estradiol on endothelial cell functions : proliferation, migration and adhesion

Three articles from Schnaper's group (Morales et al., 1995 ; Kim-Schulze et al., 1996 ; Schnaper et al., 1996) have evaluated the effect of estrogen on the angiogenic behavior of endothelial cells (table 6.VII). Under *in vitro* conditions (20 % serum), estrogen treatment enhances several endothelial cell functions, including migration, proliferation, attachment and formation of capillary-like tubes, steps that are critical to angiogenesis. This estrogen enhancement of human endothelial cell (HUVEC and coronary artery) proliferation is reduced by the ER antagonist ICI 182780. Furthermore, estrogen increases the angiogenic effect of bFGF *in vivo* in mice (Morales et al., 1995 ; table 6.VII).

A group of molecules called adhesion receptors is involved in cell-cell and cell-matrix recognition. One class, integrins, are the primary receptors for the extracellular matrix (ECM). In addition, endothelial cell adherence, considered as the initial step in leukocyte recruitment in autoimmune diseases, is mediated by adhesion molecules such as ICAM-1, VCAM-1 and E-selectin. Two important papers report a modulation of adhesion molecule expression by estradiol in endothelial cells (HUVEC) (Cid et al., 1994 ; Aziz and Wakefield, 1996) : increases in E-selectin, ICAM and VCAM under TNF-a (but not under IL1a) stimulation. The effect of estradiol (versus progesterone which leads to a decrease in E-selectin after IL1 stimulation) may be important in females in maintaining a regulatory control of E-selectin expression under immunophysiological conditions, but perhaps also in inflammatory situations. *In vivo*, E2 lowers cP-selectin expression (Jilma et al., 1996), a plasma form of P-selectin (produced by endothelial cells and platelets) lacking a hydrophobic transmembrane sequence and derived from an alternatively spliced form. The mechanisms of E2-modulated selectin expression are not clear.

There are no recent reports concerning either modifications of the extracellular matrix composition, or the activity of proteases involved in vascular remodeling (metalloproteases...) and affecting its contribution to vessel wall stability.

Impact of progesterone

Most studies have used estrogen alone. In current practice, estrogen is combined with a progestogen (administered either continuously or cyclically) to avoid the risk of abnormal endometrial proliferation and endometrial cancer.

The impact of progesterone on vasoreactivity and experimental atherosclerosis has not been clearly analyzed and conflictory results have been reported. Hormone replacement therapy effect on vasoreactivity of atherosclerotic

coronary artery in *Cynomolgus* monkey (Williams et al., 1994 ; Miyagawa et al., 1997) showed a diminution of the protective effect of estradiol following the addition of medroxyprogesterone acetate (MPA), but not of progesterone ; MPA also attenuates the estrogen-mediated inhibition of neointima formation after ballon injury in the rat carotid artery (Levine et al., 1996) ; progesterone inhibits SMC proliferation in pig coronary artery (Moraghan et al., 1996), but not (either alone or in combination with E2) in human aorta (Suzuki et al., 1996) ; another study suggests that progesterone is dose-dependently able to inhibit completely the beneficial effect of estrogen in experimental (rabbit) atherosclerosis (Hanke et al., 1996a). The beneficial effect of estradiol and progesterone was confirmed by an epidemiological study of post-menopausal women (Grodstein et al., 1996).

To conclude, experimental studies suggest that the vascular wall is the target of the antiatherogenic effect of estrogens, meaning that the estradiol effect on lipid concentrations and the lipoprotein profile is not the main mechanism. This protective effect could in part be explained by at least three mechanisms : maintenance of vasodilatory capacity, protection of LDL against oxidation, and modification of lipoprotein metabolism in the arterial wall, resulting in less accumulation of atherogenic lipoproteins in the intimal space. Preliminary clinical studies are in accordance with experimental results. Further experimental studies, using animal models, will be necessary to determine the mechanism(s) of the atheroprotective effect of estrogens.

Animal and human studies indicate that physiological doses of estradiol protect against coronary arteriosclerosis by inhibition of smooth muscle cell proliferation. Another effect of estrogen is enhanced relaxation in response to acetylcholine of arterial rings from estrogen-treated ovariectomized animals as compared with untreated animals. Women presenting a risk factor for vascular dysfunction had significantly reduced vasodilatory responses to acetylcholine compared with healthy subjects. However, there is no good animal model for arterosclerosis and results are sometimes conflictory, depending of the species, the type of artery or the dose of E2. Angiogenesis is also enhanced by 17β-estadiol *in vitro* and *in vivo* (mice) but this result needs confirmation. The applicability of these findings to patients with coronary disease needs to be investigated.

Although the presence of ER has been demonstrated in vascular tissues, together with a link between their stimulation and physiological effects, the cellular mechanism(s) whereby the presence of estrogen is translated into an effect on the biology of the arterial wall remains to be identified.

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