Biochemical Pharmacology, Vol. 51, pp. 571–576, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
SSDI 0006-2952(95)02159-0

COMMENTARY

Non-Genomic Effects of Estrogen and the Vessel Wall

Michel Y. Farhat, Sylvie Abi-Younes and Peter W. Ramwell*
DEPARTMENT OF PHYSIOLOGY AND BIOPHYSICS, GEORGETOWN UNIVERSITY MEDICAL CENTER, WASHINGTON, DC 20007, U.S.A.

ABSTRACT. Estrogen, like other steroids, is now believed to possess rapid membrane effects independent of the classical gene activation pathway of steroid action. The presence of membrane estrogen receptors has been demonstrated in different cell types, but not yet in vascular tissue. *In vivo*, estrogen administration rapidly promotes acetylcholine-induced vasodilation of the coronary and peripheral vascular beds of postmenopausal women. Estrogen also causes relaxation of precontracted isolated arterial segments and perfused organ preparations, within minutes of administration of the hormone. These rapid vasomotor effects of estrogen may be related to blockade of the cell membrane voltage-dependent calcium channels, resulting in inhibition of extracellular Ca^{2+} mobilization and flux. Recently, estradiol has been shown to rapidly affect cyclic nucleotide turnover in vascular segments, smooth muscle, and epithelial cell cultures, suggesting the possibility of a "cross-talk" between membrane-mediated events and nuclear receptor activation. BIOCHEM PHARMACOL 51;5:571–576, 1996.

KEY WORDS. estrogen; steroids; non-genomic; vascular smooth muscle; calcium influx; cyclic AMP; vascular reactivity

Several members of the steroid superfamily, namely progesterone, aldosterone, testosterone, and vitamin D, clearly act through non-genomic, as well as genomic mechanisms. The classical model of steroid action involves rapid diffusion of the hormone into the target cell and combination with a high affinity cytosolic/nuclear receptor. The hormone–receptor complex then binds to specific DNA sequences, the hormone response elements, resulting in altered transcription of specific mRNA and subsequent protein synthesis [1, 2]. Most effects of steroids are mediated by this genomic pathway, and can be detected within an hour. However, many cellular responses, which are clearly non-genomic, are also observed within seconds of hormone administration. Thus, pregnane steroids, particularly 3α-hydroxylated metabolites of progesterone, are known to have rapid and profound effects on brain excitability mediated by the γ-aminobutyric $acid_A$–benzodiazepene receptor–chloride ionophore complex [3]. In human sperm, progesterone, within seconds, elevates intracellular Ca^{2+}, and elicits the acrosome reaction [4]. Similarly, aldosterone increases Na^+ influx and stimulates intracellular inositol 1,4,5–triphosphate levels in vascular smooth muscle cells within 30 sec. The effect of aldosterone on the cell membrane Na^+/H^+ antiport is observed in human mononuclear leukocytes with an acute onset of 1–2 min [5]. Also, 1,25-dihydroxycalciferol rapidly affects calcium transport in several cell systems. In osteogenic sarcoma ROS cells, vitamin D specifically activates both phospholipase C and dihydropyridine-sensitive Ca^{2+} channels [6]. Similarly, in male rat osteoblasts, testosterone increased intracellular Ca^{2+} flux and increased inositol 1,4,5-triphosphate and diacylglycerol within seconds [7]. The rapid nature and the pharmacological characteristics of these effects are incompatible with the classical genomic pathway of steroid action, involving protein synthesis.

In the present review, we focus on the non-genomic effects of estrogen particularly in vascular tissue. Evidence for a direct or non-genomic effect of estrogen comes from several sources. In the central nervous system, estradiol influences neural activity and induces alterations in the electrical properties of neurons within seconds or minutes of application of the hormone [8, 9]. Estrogen also alters Ca^{2+} influx in uterine smooth muscle cells [10, 11], and increases intracellular cyclic adenosine monophosphate cAMP† in human breast cancer cells [12]. These effects include rapid changes in membrane polarization and electrical activity, membrane permeability, and alteration in intracellular signalling pathways. We also discuss the physiological relevance of these membrane effects of estrogen with respect to vascular function and their possible role in genomic activation.

In addition to its effect on hepatic lipoprotein metabolism, estrogen can act directly on the vessel wall to modify vasomotion [13–15] and to inhibit vascular smooth muscle cell proliferation in response to injury [16–18]. The mechanism(s) involved in the vascular antiproliferative effect of estrogen has not been identified. However, the nature of the response and

* Corresponding author. Tel. (202) 687-1050; FAX (202) 687-7400.

† *Abbreviations:* cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; ER, estrogen receptor; and LAD, left anterior descending coronary artery.

its dependence on protein synthesis suggest a nuclear event involving genomic activation. On the other hand, the vasomotor effect of estrogen is observed within minutes of application of the hormone and does not appear to involve gene transcription.

ESTROGEN AND VASOMOTOR TONE

In Vivo *Studies*

The relationship between pregnancy and the degree of hyperemia in uterine and umbilical arteries, as well as changes in peripheral vascular resistance [19], led investigators to evaluate the role of estrogen in regulating vascular tone. In the uterus, 17β-estradiol (10 μg/kg) rapidly increases uterine blood flow in oophorectomized ewes [20] and rabbits [21] via a mechanism not affected by simultaneous administration of actinomycin D. Similarly, catecholestrogens markedly induced uterine hyperemia in pigs, which was not inhibited by cyclohexamide.*

Williams *et al.* [22] studied the short-term effects of ethinyl estradiol administration on coronary vascular tone in ovariectomized monkeys on an atherogenic diet. Acetylcholine induced a vasoconstrictor response that changed to vasodilation 20 min following estrogen infusion. In postmenopausal women, Gilligan *et al.* [23] showed that intracoronary infusion of physiological levels of 17β-estradiol prevented epicardial coronary artery constriction induced by acetylcholine, and resulted in increased coronary flow and decreased coronary resistance. Similarly, intravenous administration of ethinyl estradiol also attenuated the abnormal coronary vasomotor responses to acetylcholine in postmenopausal women 15 min after administration of the hormone [24]. Analogous rapid effects of estradiol were demonstrated in the peripheral circulation. Intra-arterial infusion of physiological concentrations of 17β-estradiol potentiated the forearm vascular response to both acetylcholine and sodium nitroprusside in post-menopausal women with atherosclerosis risk factors [25]. Further, Rosano *et al.* [26] reported a beneficial effect of acute estrogen administration on myocardial ischemia in women with coronary artery disease subjected to treadmill testing. This effect was observed 40 minutes, after administration of sublingual 17β-estradiol (1 mg), and may be mediated by a direct coronary relaxing effect, peripheral vasodilation, or a combination of both.

In Vitro *Studies*

Data from isolated organ perfusion and arterial segment preparations further support a non-genomic effect of estrogen on vasomotor tone. Catecholestrogens markedly reduce, within 20 min, the responses to depolarizing concentrations of KCl in uterine arterial segments of gilts [27]. Similarly, micomolar concentrations of estradiol relaxed isolated vascular segments of human umbilical artery within 5 min of addition of the hormone [28]. In these experiments, however, it is not clear whether the observed relaxation is mediated by a specific estrogenic effect since a similar response was observed with weak estrogens, such as estrone and estriol.

Raddino *et al.* [29] studied the effect of 17β-estradiol on coronary perfusion pressure in the isolated rabbit heart. In this preparation, estradiol (10^{-7} M) elicited immediate vasodilation during vasopressin-induced coronary vasospasm. This effect was independent of gender and may be mediated by an effect on smooth muscle cell calcium transport. Similarly, Harder and Coulson [30] described a change in membrane electrical properties of canine coronary artery smooth muscle cells in response to estrogen. A hyperpolarizing response was observed in the vascular smooth muscle cells within 15 min of administration of the synthetic estrogen, diethylstilbestrol (10^{-6} M). Moreover, micromolar concentrations of 17β-estradiol were shown to inhibit the contractile response of LAD segments from rabbits [31] and pigs [32] to various pressor agonists, namely endothelin-1, calcium, and the voltage-dependent channel agonist BAY K 8644 and prostaglandin $F_{2\alpha}$. 17β-Estradiol also rapidly relaxed pre-contracted arterial rings from human coronary artery [33] and rabbit basilar artery [34] by an endothelium-independent mechanism.

In all these experiments, the acute nature of the vascular responses to estrogen indicates the involvement of membrane-mediated mechanisms of action. However, the fact that pharmacological concentrations of estrogen (micromolar) are required to produce these responses would suggest a non-specific effect of the hormone. In addition, the lack of proper controls, such as other estrogens, ER antagonists, and other steroids in these studies, makes the interpretation of the data more questionable. Moreover, while we have shown that 17β-estradiol is more potent than its 17α-isomer, a weaker estrogen, in attenuating the contractile response of porcine LAD segments to prostaglandin $F_{2\alpha}$ [32], Salas *et al.* [35] have reported recently that both isomers are equally potent in eliciting an acute endothelium-independent vasorelaxation in pig coronary artery segments. Both studies suggest that the estrogenic effect is mediated by blockade of voltage-dependent Ca^{2+} channels.

The effect of estrogen on vascular reactivity may depend on the nature of the vascular bed. In contrast to the vasodilatory effect in coronary vessels, estrogen administration potentiates vasopressor responses of isolated mesenteric and pulmonary vascular beds. We showed low nanomolar concentrations of 17β-estradiol potentiate significantly vasoconstrictor responses of the rat isolated mesenteric preparation elicited by norepinephrine, K^+, and the prostaglandin endoperoxide U46619 [36]. This rapid response (<4 min) was also observed with 17β-estradiol conjugated to bovine serum albumin, suggesting a membrane effect. Potentiation of the pressor response with estrogen was also observed in the isolated perfused rat lung preparation. A 5-min perfusion with 10 nM 17β-estradiol diethylstilbestrol enhanced the pressor response to U46619, a thromboxane A_2 analog, and angiotensin II. Infusion of 17α-estradiol and testosterone had no significant effect on pulmonary perfusion pressure, suggesting stereospecificity of the estrogenic response [37].

* Ford S, Van Orden D and Farley D, Effect of cycloheximide on (catechol) estrogen uterine hyperhemia. *Proc Soc Gynecol Invest*, 378, 1986.

TABLE 1. Effect of estrogen on cyclic nucleotides in vascular and other tissues

Tissue	Estrogen (concentration)	Incubation time (min)	Effect	Ref.
		Vascular tissue		
Rabbit aorta	$E_{2\alpha}$ (20 nM)	5.0	cAMP	43
Pig coronary	$E_{2\beta}$ (μM)	5.0	cGMP	32
Rat pulmonary VSMC	$E_{2\beta}$ (μM)	5.0	cAMP	*
Human coronary	E_2 (μM)	30.0	cAMP, cGMP	33
		Non-vascular tissue		
Rat uterus	$E_{2\beta}$ (0.5 μg/100 g, i.v.)	0.5	cAMP	42
	DES (5 μg/100 g, i.v.)	5.0	cAMP	44
	$E_{2\beta}$ (0.02–0.1 μg/100 g, i.v.)	8.0	cAMP, cGMP	45
	$E_{2\beta}$ (1–100 nM)	15.0	cGMP	46
	$E_{2\alpha}$ (20 nM)	5.0	cAMP	43
	$E_{2\beta}$, DES, 2-OHE (pM)	60.0	cAMP	12
Rat hypothalamus	$E_{2\beta}$, DES (20 μM)	40.0	cAMP	47
Human breast cancer cells	$E_{2\beta}$, DES, 2-OHE (pM)	60.0	cAMP	12
Rat pituitary GH3 cells	$E_{2\beta}$ (10 nM)	15.0	cGMP	48

Abbreviations: $E_{2\alpha}$, 17α-estradiol; $E_{2\beta}$, 12β-estradiol; DES, diethylstilbestrol; and VSMC, vascular smooth muscle cells.

* Farhat MY, Abi Younes S, Dingaan B, Vargas R and Ramwell PW, Estradiol stimulates cyclic AMP production in rat pulmonary smooth muscle cells by a non-genomic mechanism. *Proceedings of the 75th Annual Meeting of the Endocrine Society*, Las Vegas, NV, 1233B, 1993.

ESTROGEN AND INTRACELLULAR SIGNALLING

The rapid effects of estrogen may be mediated by regulation of intracellular signalling mechanisms. Two intracellular second messenger systems have been suggested in this respect, namely Ca^{2+} and the cyclic nucleotides.

Intracellular Calcium

Estrogen may inhibit vascular smooth muscle cell contractility by blocking Ca^{2+} mobilization and flux. Raddino *et al.* [29] showed that Ca^{2+} reversed the inhibitory effect of 17β-estradiol on the contractile response of the rabbit coronary artery. Similarly, in isolated rabbit coronary artery segments, a 20-min incubation with 17β-estradiol (1–10 μM) shifted the concentration-response curve to Ca^{2+} to the right [27]. Moreover, the increased uterine blood flow accompanying either ovarian (estrus) or conceptus (day 13) production of estrogen is related to the blockade of voltage-sensitive Ca^{2+} channels of vascular smooth muscle [31, 38].

Using whole cell patch clamping, Zhang *et al.* [39] attenuated voltage-dependent Ca^{2+} currents in the A7r5 vascular smooth muscle cell line with estrogen. They showed that 17β-estradiol (10 μM) significantly reduced peak L-type Ba^{2+} and T-type Ca^{2+} current within 1–2 min. 17α-Estradiol was less potent than its β-isomer. 17β-Estradiol inhibited angiotensin II as well as α-adrenergic-induced contractions. These agonists increase intracellular Ca^{2+} by activating receptor-mediated Ca^{2+} channels or releasing Ca^{2+} from intracellular stores. Estradiol is reported to regulate Ca^{2+} influx and mobilization from intracellular stores in cardiac myocytes [27, 40] and chicken granulosa cells [41]. 17β-Estradiol did not affect intracellular mobilization of Ca^{2+} in A7r5 vascular smooth muscle cells when measured with fura 2 [39]. On the other hand, the effect of estradiol on receptor-operated Ca^{2+} channels has not been investigated.

Cyclic Nucleotides

A number of studies show estrogen to induce rapid changes in both cAMP and cGMP in various estrogen target tissues (Table 1). The first study in this regard was by Szego and Davis [42], who showed that intravenous administration of 17β-estradiol (0.5 μg/100 g) to ovariectomized rats evoked within 30 sec, a 2- to 3-fold increase in uterine cAMP. Recently, Aronica *et al.* [12] reported significant increases in cAMP in rat uterus and in MCF-7 human breast cancer cells evoked by very low concentrations of physiologically active estrogens and anti-estrogens. This increase in cAMP resulted from activation of the membrane adenylate cyclase enzyme and was not blocked by inhibitors of RNA and protein synthesis.

Estrogen may also have a direct effect on the turnover of cyclic nucleotides in vascular tissue. We find in rat pulmonary vascular smooth muscle cell cultures that 35-min incubation with 17β-estradiol increases intracellular cAMP in a concentration-dependent manner. This effect was specific since it was observed with diethylstilbestrol but not with testosterone or the α-isomer of estradiol.* These findings were later confirmed by Mügge *et al.* [33], who found that a 30-min incubation of human coronary arteries *in vitro* with 3 μM 17β-estradiol significantly increased both cAMP and cGMP by 88 and 182%, respectively.

In contrast, other studies have demonstrated an inhibitory effect of estrogen on both cAMP and cGMP production in a number of vascular preparations. Thus, Kishi and Numano [43] reported that short-term (5 min) incubation with 17α-estradiol (20 nM) decreases both basal and epinephrine-stimulated cAMP levels in aortic segments from oophorectomized rabbits.

* Farhat MY, Abi Younes S, Dingaan B, Vargas R and Ramwell PW, Estradiol stimulates cyclic AMP production in rat pulmonary smooth muscle cells by a non-genomic mechanism. *Proceedings of the 75th Annual Meeting of the Endocrine Society*, Las Vegas, NV, 1233B, 1993.

In contrast, uterine tissue from the same animals showed increased cAMP levels under the same conditions. Micromolar concentrations of 17β-estradiol were also shown to inhibit rapidly (5 min) both basal and stimulated levels of cGMP in isolated arterial segments from porcine LAD [32]. These observations suggest that the difference in the effect of estrogen on cyclic nucleotide turnover may relate to the nature of the vascular bed [43–48].

PUTATIVE ESTROGEN MEMBRANE BINDING SITES

The cytoplasmic membrane is a potential site of estrogen action [49–56] (Table 2). Although there have been no direct attempts at identifying membrane estrogen receptors in vascular tissue, the presence of putative membrane receptor sites for estrogen has been claimed in other tissues known to be targets for estrogen action. Using ligand–receptor binding assays and derivatized estrogen conjugates, Pietras and Szego [57–60] provided evidence that ligand-specific, temperature-dependent binding sites for 17β-estradiol are present on plasma membrane of isolated cells and hepatocytes of ovariectomized rats. Other studies characterized specific binding sites for estradiol in male rat synaptic plasma membrane [61], female rat pituitary [62–64] and human breast cancer cells [65, 66]. Pappas *et al.* [67] also demonstrated the presence of a membrane ER on the surface of GH3/B6 rat pituitary tumor cells, using confocal laser scanning microscopy and immunolabelling. Moreover, recent western blot studies have suggested the presence of three major estrogen binding proteins with M_r values from 23 to 33 kDa in synaptosomal membrane fractions isolated from rat brain.* One of these proteins was identified as the oligomycin-sensitivity conferring protein. The nature of these binding components in smooth muscle cell membrane, and their role in mediating the various short-term and transcription-independent responses to estrogen in target cells remain to be determined. However, the possibility that estrogen binds different membrane proteins may explain the qualitative difference in the estrogenic response between different vascular beds. The presence of subtypes of the membrane ER, like those observed for the nuclear ER in mammalian uterus [68], may be an intriguing possibility.

TABLE 2. Steroid membrane binding sites

Steroid	Tissue	Technique	Refs.
Progesterone	Rat brain	Ligand binding	49,50
Aldosterone	Human lymphocytes	Photoaffinity labeling	51
	Rat kidney	Ligand binding	52,53
Glucocorticoids	Rat liver	Ligand binding	54
	Frog brain	Ligand binding	55
Vitamin D	Bone	Ligand binding	56
Estradiol	Rat endometrium	Ligand binding	57,58
		Derivatized estrogen conjugates	59
	Rat liver	Ligand binding	60
		Derivatized estrogen conjugates	59
	Brain	Ligand binding	61
		Western blot	56
	Breast cancer cells	Fluorescence	62
			63
	Pituitary cells	Ligand binding	64,65
		ER antibodies	66
		Confocal laser microscopy	67

PHYSIOLOGICAL RELEVANCE

The effect of estrogen on calcium transport and cyclic nucleotides may explain some of the rapid effects of estrogen on vascular reactivity, but their contribution to the known physiological effects of the hormone is yet to be determined. Ca^{2+} and cyclic nucleotides are important intracellular second messengers involved in mediating many smooth muscle cell functions, such as contractility [69, 70] and proliferation [71, 72]. Estrogen may act through the cAMP pathway to regulate cAMP-mediated gene expression, or enhance transcription of estrogen-regulated genes [12]. Drugs that increase intracellular cAMP concentration inhibit transmission of growth stimulatory signals in the cell. cAMP blocks one of the major signal conduction pathways (the "Ras pathway") by which growth factors exert their genomic effects [73, 74]. cAMP may also interact with the ER and so modify its transcriptional activity [75, 76]. Similarly, Power *et al.* [77] showed that a dopamine membrane receptor-mediated phosphorylation cascade involving intracellular cAMP can activate progesterone receptors in transfected monkey kidney (CV_1) cells. Progesterone receptor-negative CV_1 cells transfected with a chicken progesterone receptor A (PR_A) form expression vector responded equally to progesterone and dopamine treatment by increasing PR_A-mediated transcription. *In vitro*, dopamine mimicked the effect of progesterone resulting in translocation of chicken progesterone receptor from cytoplasm to nucleus. Studies with other receptors also indicate that the human estrogen receptor, the human vitamin D receptor, and the human thyroid hormone receptor also activate transcription from target genes in response to dopamine.

Wehling [78] recently proposed a two-step model of aldosterone action in many cell types, which may be applicable to vascular smooth muscle cells. The primary response involves steroid binding to membrane receptors initiating rapid changes in electrolyte balance, while a later genomic activation results in protein synthesis and requires 1–2 hr to develop. This model may apply to the mechanisms of action of other steroid hormones, which are known to mediate some of their *in vivo* effects via non-genomic pathways. The anesthetic action of progesterone and its analogs and its effect on oocyte maturation and the spermatozoa acrosome reaction are examples of such mechanisms.

* Zheng J and Ramirez VD, Neural membrane estrogen binding proteins: Western (ligand) blot studies. *Proceedings of the 77th Annual Meeting of the Endocrine Society*, Washington, DC, P1 422, 1995.

CONCLUSIONS

Estrogen joins progesterone, aldosterone, and vitamin D in possessing non-genomic properties. There is overwhelming evidence to suggest that beside its role as a transcriptional regulator, estrogen may induce rapid cellular events through interaction with specific membrane binding sites. In vascular tissue, estrogen may mediate its non-genomic effects by altering membrane ionic permeability, regulation of cyclic nucleotide turnover, and membrane bound enzyme activity. The nature of the membrane estrogen receptor and the significance of the second messengers are yet to be identified. Moreover, the possibility of "cross-talk" between membrane receptor-mediated events and activation of nuclear steroid receptors as reported for dopamine, progesterone, and aldosterone is noteworthy.

References

1. Katzenellenbogen B, Dynamics of steroid hormone receptor action. *Annu Rev Physiol* **42:** 17–35, 1980.
2. Evans R, The steroid and thyroid hormone receptor superfamily. *Science* **240:** 889–895, 1988.
3. Blackmore PF, Beebe SJ, Danforth DR and Alexander NA, Progesterone and 17α-hydroxyprogesterone: Novel stimulators of calcium influx in human sperm. *J Biol Chem* **265:** 1376–1380, 1990.
4. Lan NC, Chen JS, Belelli D, Pritchett DB, Seeburg PH and Gee KW, A steroid recognition site is functionally coupled to an expressed GABA benzodiazepene receptor. *Eur J Pharmacol* **188:** 403–406, 1990.
5. Wehling M, Christ M and Theisen K, Membrane receptors for aldosterone: A novel pathway for mineralocorticoid action. *Am J Physiol* **263:** E974–E979, 1992.
6. Civitelli R, Kim YS, Gunsten SL, Fujimori A, Huskey M, Avioli LV and Hruska KA, Nongenomic activation of the calcium message system by vitamin D metabolites in osteoblast-like cells. *Endocrinology* **127:** 2253–2262, 1990.
7. Lieberherr M and Grosse B, Androgens increase intracellular calcium concentration and inositol 1,4,5-triphosphate and diacylglycerol formation via a pertussis toxin-sensitive G-protein. *J Biol Chem* **269:** 7217–7223, 1994.
8. McEwen B, Non-genomic and genomic effects of steroids on neural activity. *Trends Pharmacol Sci* **12:** 141–147, 1991.
9. Nabekura J, Oomura Y, Minami T, Mizuno Y and Fukuda A, Mechanism of the rapid effect of 17β-estradiol on medial amygdala neurons. *Science* **233:** 226–228, 1986.
10. Pietras RJ and Szego CM, Endometrial cell calcium and oestrogen action. *Nature* **253:** 357–359, 1975.
11. Batra S, Effect of estrogen and progesterone treatment on calcium uptake by the myometrium and smooth muscle of the lower urinary tract. *Eur J Pharmacol* **127:** 37–42, 1986.
12. Aronica SM, Kraus WL and Katzenellenbogen BS, Estrogen action via the cAMP signaling pathway: Stimulation of adenylate cyclase and cAMP-regulated gene transcription. *Proc Natl Acad Sci USA* **91:** 8517–8521, 1994.
13. Gisclard V, Miller VM and Vanhoutte PM, Effect of 17β-estradiol on endothelium-dependent responses in the rabbit. *J Pharmacol Exp Ther* **244:** 19–22. 1988.
14. Williams JK, Adams MR and Klopfenstein HS, Estrogen modulates responses of atherosclerotic coronary arteries. *Circulation* **81:** 1680–1687, 1990.
15. Jiang C, Sarrel PM, Poole-Wilson and PA and Collins P, Acute effect of 17β-estradiol on rabbit coronary artery contractile responses to endothelin-1. *Am J Physiol* **163:** H271–H275, 1992.
16. Rhee CY, Spaet TH, Stemerman MB, Lajam F and Shiang HH, Estrogen suppression of surgically induced vascular intimal hyperplasia in rabbits. *J Lab Clin Med* **90:** 77–84, 1977.
17. Foegh ML, Khirabadi BS, Nakanishi T, Vargas R and Ramwell PW, Estradiol protects against experimental cardiac transplant atherosclerosis. *Transplant Proc* **19:** 90–95, 1987.
18. Foegh ML, Asotra S, Howell M and Ramwell PW, Estradiol inhibition of neointimal hyperplasia after balloon injury. *J Vasc Surg* **19:** 722–726, 1994.
19. Ueland K and Parer JT, Effects of estrogens on the cardiovascular system of the ewe. *Am J Obstet Gynecol* **96:** 400–406, 1966.
20. Resnik R, Battaglia F, Makowski E and Meschia G, The effect of actinomycin D on estrogen induced uterine blood flow. *Am J Obstet Gynecol* **122:** 273–277, 1975.
21. Penney L, Frederick R and Parker G, 17β-Estradiol stimulation of uterine blood flow in oophorectomized rabbits with complete inhibition of uterine ribonucleic acid synthesis. *Endocrinology* **109:** 1672–1676, 1981.
22. Williams JK, Adams MR, Herrington DM and Clarkson TB, Short-term administration of estrogen and vascular responses of atherosclerotic coronary arteries. *J Am Coll Cardiol* **20:** 452–457, 1992.
23. Gilligan DM, Quyyumi AA and Cannon RO III, Effects of physiological levels of estrogen on coronary vasomotor function in postmenopausal women. *Circulation* **89:** 2545–2551, 1994.
24. Reis SE, Gloth ST, Blumenthal RS, Resar JR and Zacur HA, Ethinyl estradiol acutely attenuates abnormal coronary vasomotor responses to acetylcholine in postmenopausal women. *Circulation* **89:** 52–60, 1994.
25. Gilligan DM, Badar DM, Panza JA, Quyyumi AA and Cannon RO III, Acute vascular effects of estrogen in postmenopausal women. *Circulation* **90:** 786–791, 1994.
26. Rosano GMC, Sarrel PM, Poole-Wilson PA and Collins P, Beneficial effect of oestrogen on exercise-induced myocardial ischaemia in women with coronary artery disease. *Lancet* **342:** 133–136, 1993.
27. Stice SL, Ford SP, Rosazza JP and Van Orden DE, Interaction of 4-hydroxylated estradiol and potential-sensitive Ca^{2+} channels in altering uterine blood flow during the estrous cycle and early pregnancy in gilts. *Biol Reprod* **36,** 369–375, 1987.
28. De Sa M and Meirelles R, Vasodilating effect of estrogen on the human umbilical artery. *Gynecol Invest* **8:** 307–313, 1977.
29. Raddino R, Manca C, Poli E, Bolognesi R and Visioli O, Effects of 17β-estradiol on the isolated rabbit heart: *Arch Int Pharmacodyn* **281:** 57–65, 1986.
30. Harder D and Coulson P, Estrogen receptors and effects of estrogen on membrane electrical properties of coronary vascular smooth muscle. *J Cell Physiol* **100:** 375–382, 1979.
31. Jiang C, Poole-Wilson PA, Sarrel PM, Mochizuki S, Collins P and MacLeod KT, Effect of 17β-oestradiol on contraction, Ca^{2+} current and intracellular free Ca^{2+} in guinea-pig isolated cardiac myocytes. *Br J Pharmacol* **106:** 739–745, 1992.
32. Vargas R, Thomas G, Wroblewska B and Ramwell PW, Differential effects of 17α and 17β estradiol on $PGF_{2\alpha}$ mediated contraction of the porcine coronary artery. *Adv Prostaglandin Thromboxane Leukot Res* **19:** 277–280, 1989.
33. Mügge A, Riedel M, Barton M, Kuhn M and Lichtlen PR, Endothelium independent relaxation of human coronary arteries by 17β-oestradiol *in vitro*. *Cardiovasc Res* **27:** 1939–1942, 1993.
34. Futo J, Shay J, Block S, Holt J, Beach M and Moss J, Estrogen and progesterone withdrawal increases cerebral vasoreactivity to serotonin in rabbit basilar artery. *Life Sci* **50:** 1165–1172, 1992.
35. Salas E, López MG, Villarroya M, Sáanchez-García P, De Pascual R, Dixon WR and García AG, Endothelium-independent relaxation by 17-α-estradiol of pig coronary arteries. *Eur J Pharmacol* **258:** 47–55, 1994.
36. Vargas R, Delaney M, Farhat MY, Wolfe R, Rego A and Ramwell PW, Effect of estradiol 17β on pressor responses of rat mesenteric

bed to norepinephrine, K^+, and U-46619. *J Cardiovasc Pharmacol* **25:** 200–206, 1995.
37. Farhat MY and Ramwell PW, Estradiol potentiates the vasopressor response of the isolated perfused rat lung to the thromboxane mimic U46619. *J Pharmacol Exp Ther* **261:** 686–691, 1992.
38. Ford S and Stice S, Effects of the ovary and conceptus on uterine blood flow in the pig. *J Reprod Fertil* **33:** 83–90, 1985.
39. Zhang F, Ram JL, Standley PR and Sowers JR, 17β-Estradiol attenuates voltage-dependent Ca^{2+} currents in A7r5 vascular smooth muscle cell line. *Am J Physiol* **266:** C975–C980, 1994.
40. De Beer EL and Keizer HA, Direct action of estradiol-17β on the atrial action potential. *Steroids* **40:** 223–231, 1982.
41. Morley P, Whitfield JF, Vanderhyden BC, Tsang BK and Schwartz JL, A new nongenomic estrogen action: The rapid release of intracellular calcium. *Endocrinology* **131:** 1305–1312, 1992.
42. Szego C and Davis J, Inhibition of estrogen-induced cyclic AMP elevation in rat uterus by glucocorticoids. *Life Sci* **8:** 1109–1116, 1969.
43. Kishi Y and Numano F, A study of the mechanism of estrogen as an antiatherosclerotic: The inhibitory effect of estrogen on A23187-induced contraction of the aortic wall. *Mech Ageing Dev* **18:** 115–123, 1982.
44. Rosenfeld M and O'Malley B, Steroid hormones: Effects on adenyl cyclase activity and adenosine 3′,5′-monophosphate in target tissues. *Science* **168:** 253–255, 1970.
45. Flandory L and Galand P, Changes in cGMP and cAMP content in the estrogen-stimulated rat uterus: Temporal relationship with other parameters of hormonal stimulation. *J Cyclic Nucleotide Res* **4:** 145–158, 1978.
46. Kuehl FA Jr, Ham EA, Zanetti ME, Sanford CH, Nicol SE and Golberg ND, Estrogen-related increases in uterine guanosine 3′: 5′ cyclic monophosphate levels. *Proc Natl Acad Sci USA* **71:** 1866–1870, 1974.
47. Weissman B, Daly J and Skolnick P, Diethylstilbestrol-elicited accumulation of cyclic AMP in incubated rat hypothalamus. *Endocrinology* **97:** 1559–1566, 1975.
48. Kuehl F Jr, Zanetti ME, Cirillo VJ and Ham EA, Estrogen-induced alterations in cyclic nucleotide and prostaglandin levels in target tissue. *J Steroid Biochem* **6:** 1099–1105, 1975.
49. Ke F-C and Ramirez VD, Binding of progesterone to nerve cell membranes of rat brain using progesterone conjugated to ^{125}I-bovine serum albumin as a ligand. *J Neurochem* **54:** 467–472, 1990.
50. Majewska MD, Demirgoren S and London ED, Binding of pregnenolone sulfate to rat brain membranes suggests multiple sites of steroid action of the $GABA_A$ receptor. *Eur J Pharmacol* **189:** 307–315, 1990.
51. Wehling M, Eisen C, Aktas J, Christ M and Theisen K, Photoaffinity labeling of plasma membrane receptors for aldosterone from human mononuclear leukocytes. *Biochem Biophys Res Commun* **189:** 1424–1428, 1992.
52. Christ M, Sippel K, Eisen C and Wehling M, Non-classical receptors for aldosterone in plasma membranes from pig kidneys. *Mol Cell Endocrinol* **99:** R31–R34, 1994.
53. Ožegovíc B, Dobrović-Jenik D and Milković S, Solubilization of rat kidney plasma membrane proteins associated with ^{3}H-aldosterone. *Exp Clin Endocrinol* **92:** 194–198, 1988.
54. Suyemitsu T and Terayama H, Specific binding sites for natural glucocorticoids in plasma membranes of rat liver. *Endocrinology* **96:** 1499–1508, 1975.
55. Orchinik M, Murray TF and Moore FL, A corticosteroid receptor in neuronal membranes. *Science* **252:** 1848–1851, 1991.
56. Baran DT, Ray R, Sorensen AM, Honeyman T and Holick MF, Binding characteristics of a membrane receptor that recognizes 1α,25-dihydroxyvitamin D_3 and its epimer, 1β,25-dihydroxyvitamin D_3. *J Cell Biochem* **56:** 510–517, 1994.
57. Pietras RJ and Szego CM, Specific binding sites for oestrogen at the outer surface of isolated endometrial cells. *Nature* **265:** 69–72, 1977.
58. Pietras R and Szego C, Estrogen receptors in uterine plasma membrane. *J Steroid Biochem* **11:** 1471–1483, 1979.
59. Pietras RJ and Szego CM, Metabolic and proliferative responses to estrogen by hepatocytes selected for plasma membrane binding-sites specific for estradiol-17β. *J Cell Physiol* **98:** 145–160, 1979.
60. Pietras RJ and Zsego CM, Partial purification and characterization of estrogen receptors in subfractions of hepatocyte plasma membranes. *Biochem J* **191:** 743–760, 1980.
61. Towle A and Sze P, Steroid binding to synaptic plasma membrane: Differential binding of glucocorticoids and gonadal steroids. *J Steroid Biochem* **18:** 135–143, 1983.
62. Bression D, Michard M, Le Dafniet M, Pagesy P and Peillon F, Evidence for a specific estradiol binding site on rat pituitary membranes. *Endocrinology* **119:** 1048–1051, 1986.
63. Schaeffer J, Stevens S, Smith R and Hsueh A, Binding of 2-hydroxyestradiol to rat anterior pituitary cell membranes. *J Biol Chem* **255:** 9838–9843, 1980.
64. Pappas TC, Gametchu B, Yannariello-Brown J, Collins TJ and Watson CS, Membrane estrogen receptors in GH3/B6 cells are associated with rapid estrogen-induced release of prolactin. *Endocrine* **2:** 813–822, 1994.
65. Nenci I, Marchetti E and Marzola A, Affinity cytochemistry visualizes specific estrogen binding sites on the plasma membrane of breast cancer cells. *J Steroid Biochem* **14:** 1139–1146, 1981.
66. Berthois Y, Pourreau-Schneider N, Gandilhon P, Mittre H, Tubiana N and Martin PM, Estradiol membrane binding sites on human breast cancer cell lines. Use of a fluorescent estradiol conjugate to demonstrate plasma membrane binding systems. *J Steroid Biochem* **25:** 963–972, 1986.
67. Pappas TC, Gametchu B and Watson CS, Membrane estrogen receptors identified by multiple antibody labeling and impeded-ligand binding. *FASEB J* **9:** 404–410, 1995.
68. Thampan TNRV and Clark JH, An estrogen receptor activator protein in rat uterine cytosol. *Nature* **290:** 152–154, 1981.
69. Somlyo AP and Somlyo AV, Signal transduction and regulation in smooth muscle. *Nature* **372:** 231–236, 1994.
70. Parfenova H, Shibata M, Zuckerman S and Leffler CW, CO_2 and cerebral circulation in newborn pigs: Cyclic nucleotides and prostanoids in vascular regulation. *Am J Physiol* **266:** H1494–1501, 1994.
71. Metcalfe JC, Moore JP, Smith GA and Hesketh TR, Calcium and cell proliferation. *Br Med Bull* **42:** 405–412, 1986.
72. Assender JW, Southgate KM, Hallett MB and Newby AC, Inhibition of proliferation, but not of Ca^{2+} mobilization, by cyclic AMP and GMP in rabbit aortic smooth-muscle cells. *Biochem J* **288:** 527–532, 1992.
73. Cook SJ and McCormick F, Inhibition by cAMP of Ras-dependent activation of Raf. *Science* **262:** 1069–1072, 1993.
74. Wu J, Dent P, Jelinek T, Wolfman A, Weber MJ and Sturgill TW, Inhibition of the EGF activated MAP kinase signalling pathway by adenosine 3′,5′ monophosphate. *Science* **262:** 1065–1069, 1993.
75. Cho H and Katzenellenbogen BS, Synergistic activation of estrogen receptor-mediated transcription by estradiol on protein kinase activators. *Mol Endocrinol* **7:** 441–452, 1993.
76. Fujimoto N and Katzenellenbogen BS, Alteration in the agonist/antagonist balance of antiestrogens by activation of protein kinase A signaling pathways in breast cancer cells: Antiestrogen selectivity and promotor dependence. *Mol Endocrinol* **8:** 296–304, 1994.
77. Power RF, Mani SK, Codina J, Conneely OM and O'Malley BW, Dopaminergic and ligand-independent activation of steroid hormone receptors. *Science* **254:** 1636–1639, 1991.
78. Wehling M, Nongenomic actions of steroid hormones. *Trends Endocrinol Metab* **5:** 347–353, 1994.