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THE ROLE OF EOSINOPHIL RECEPTORS IN THE NON-GENOMIC RESPONSE TO OESTROGENS IN THE UTERUS

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SUMMARY

Three independent groups of parameters of oestrogen stimulation in the rat uterus are mediated by three independent mechanisms of oestrogen action.

The genomic response to oestrogens (increase in RNA and protein content) is mediated by the cytosol-nuclear receptor system. This response is suppressed by Actinomycin D. The cytosol-nuclear receptors have a higher affinity for oestradiol than for oestriol, therefore oestradiol is the more potent oestrogen for the genomic response to oestrogens. Any condition interfering with oestrogen binding by this system, or with cytosol oestrogen-receptor complex transfer to the nucleus, interferes with the genomic response to oestrogens.

Oestrogen-induced uterine oedema, increase in vascular permeability, release of histamine, uterine eosinophilia and some other parameters of oestrogen stimulation are mediated by the eosinophil oestrogen receptor system. This response is not blocked by Actinomycin D. Therefore it is a non-genomic response. The eosinophil receptors have a higher affinity for oestriol than for oestradiol. Therefore oestriol is the stronger oestrogen for the response mediated by eosinophils. Any agent or condition interfering with migration of eosinophils to the uterus, *e.g.* cortisol, colchicine, blood eosinopenia of young animals, selectively blocks eosinophil-mediated response to oestrogens, but does not interfere with other oestrogenic responses. We propose a mechanism for eosinophil migration to the uterus and for the role of eosinophils in oestrogen action.

Cyclic AMP is involved in a third mechanism of oestrogen action upon a separate group of parameters.

INTRODUCTION

My interest in uterine eosinophils began in 1965, with the finding that uterine eosinophils bind tritiated oestradiol [1]. Since then, an in depth study of the characteristics of the oestrogen receptors in the uterine eosinophils has been pursued in my laboratory. The experiments we have done have demonstrated that the eosinophil oestrogen receptor system is a newly established system, independent of Jensen's cytosol-nuclear receptor system. The experiments proved that uterine eosinophils are involved in the mechanisms of oestrogen action in the uterus and that they are responsible for some early non-genomic parameters of oestrogen stimulation. The cytosol-nuclear receptor system on the other hand, is responsible for the genomic response to oestrogens. We also found that the response to oestrogens mediated by one of the receptor systems could be selectively stimulated, inhibited or blocked without interfering with parameters of oestrogen stimulation mediated by another receptor system.

After a brief description of the cytosol-nuclear receptor system, the evidence for the involvement of eosinophils in oestrogen action will be reviewed in depth and the mechanisms of action of this system will be discussed and compared with other independent mechanisms.

THE CYTOSOL-NUCLEAR RECEPTOR SYSTEM AND THE GENOMIC RESPONSE TO OESTROGENS

The cytosol-nuclear receptor system for oestrogens has been described and studied in detail by the groups of Jensen, Baulieu and others (see [2] for a review). It consists of a cytosol receptor and a nuclear receptor [2]. The receptors are present in cells of the luminal and glandular epithelium, the smooth muscle and the stroma of the uterus [3–6]. The interaction of oestrogens with the cytosol-nuclear receptor system involves a two-step mechanism [2]. The hormone is first bound to the cytosol receptor. The oestrogen-cytosol receptor complex is then translocated to the nucleus and undergoes some structural changes. After entering the nucleus, the oestrogen-nuclear receptor complex interacts with a specific acceptor site, possibly in the chromatin [2]. This interaction induces genomic activation or derepression, resulting in an increased transcription of specific messenger RNA, which in turn codes for the synthesis of specific proteins. This results in increased uterine RNA and protein synthesis, "true uterine growth" measured as increased uterine dry weight, increased content of some specific uterine enzymes and biochemical, morphological and functional differentiation of target cells [2, 7]. All effects mediated by the cytosol-nuclear receptor system may be regarded as the genomic response to oestrogens [7].

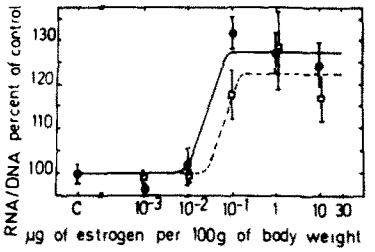

Fig. 1. Dose response of oestrogen-induced increase in uterine RNA content. Oestradiol-17β (●) or oestriol (□) was given intravenously 6 h before the animals were killed (from ref. 7).

Genomic activation can be blocked at the level of transcription by Actinomycin D. Indeed, Actinomycin D was found to selectively block the genomic response to oestrogens in the uterus [8, 9].

There should be a direct relationship between the genomic response to oestrogens and the amount of oestrogen carried to the nucleus by the cytosol-nuclear receptor system. The following three studies support this relationship.

Firstly, the cytosol-nuclear oestrogen receptor system has higher affinity for oestradiol-17β than for oestriol [10, 11]. Accordingly, Figs 1 and 2 show that oestradiol is the more potent oestrogen for increased uterine RNA and protein content [7, 12].

Secondly, progesterone selectively inhibits oestrogen binding by the nuclei of the luminal epithelial cells but not by the other uterine cells [4–6]. Since the direct competition of progesterone and oestradiol for the receptor sites is unlikely, it was proposed that progesterone has a specific inhibitory effect on either the synthesis of oestrogen receptors or the transfer of the oestrogen-cytosol receptor complex from the cytoplasm to the nucleus in the luminal epithelial cells [5]. This inhibition in oestrogen binding restricted to one cell type parallels the inhibition by progesterone of clearly genomic responses, namely mitotic activity, uridine uptake and cell hypertrophy in these same cells [5].

Thirdly, it has been found that oestrogen-induced RNA and protein synthesis first appears in 10-day old rats [13]. The cytosol receptors however are already present in the neonatal and very young rats. Nuclear receptors are present only in very low

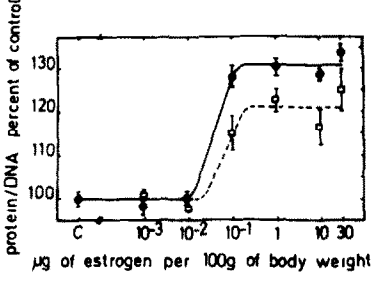

Fig. 2. Dose response of oestrogen-induced increase in uterine protein content. Oestradiol-17β (●) or oestriol (□) was given intravenously 6 h before the animals were killed (from ref 7).

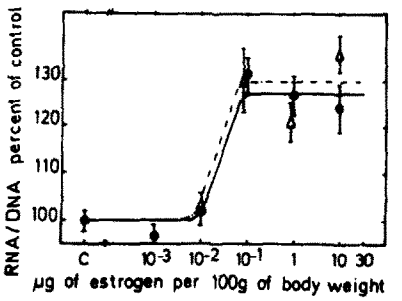

Fig. 3. Effect of cortisol on the oestrogen-induced increase in uterine RNA content 6 h after oestrogen administration. Animals without cortisol (●) and animals with cortisol injected intraperitoneally (△) are compared. The effect of the intravenous injection of cortisol is not shown, as the results are identical to those obtained with the intraperitoneal injection (from ref. 7).

amounts in animals younger than 10 days of age [14]. It can be concluded that an impairment of the transfer of oestrogen-cytosol receptor complex to the nucleus in very young animals may explain their lack of genomic response [13].

Cortisol and DL-propranolol are antioestrogenic agents which counteract some non-genomic parameters of oestrogen stimulation, but do not interact with the genomic response to oestrogens.

Cortisol is a lysosome membrane stabilizing agent and a blood eosinopenia-inducing hormone, known to limit the number of eosinophils entering the uterus after oestrogen administration [7, 15]. It blocks those parameters of oestrogen stimulation which are mediated by the eosinophil oestrogen receptor system [7, 15]. Figs 3 and 4 show that cortisol does not modify the oestrogen-induced increase in uterine RNA and protein content [7, 15]. Therefore, it can be assumed that neither lysosome stability nor uterine eosinophils play any role in the genomic response to oestrogens.

DL-propranolol is known to block the oestrogen-induced increase in uterine cyclic AMP content (see 16 for a review). Table 1 shows that propranolol does not modify the oestrogen-induced increases in uterine RNA and protein content [16]. Therefore, it can be

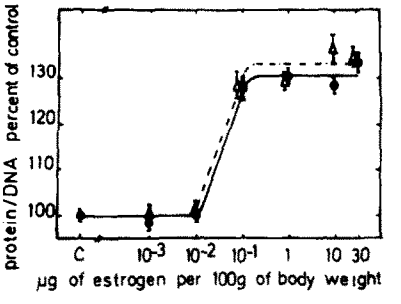

Fig. 4. Effect of cortisol on the oestrogen-induced increase in uterine protein content 6 h after oestrogen administration. Animals without cortisol (●) and animals with cortisol injected intraperitoneally (△) are compared. The effect of the intravenous injection of cortisol is not shown, as the results are identical to those obtained with the intraperitoneal injection (from ref. 7).

Table 1. Effect of propranolol on the oestrogen-induced uterine eosinophilia and other parameters of oestrogen stimulation, 6 h after the administration of 30 μg oestradiol per 100 g b. wt [16]

Parameter of oestrogen stimulation	Experimental condition			
	Control	Propranolol	Oestrogen	Propranolol + oestrogen
Total number of uterine eosinophils	30 ± 11	127 ± 64	26333 ± 1825	31423 ± 3298
Uterine wet weight in % of controls	100 ± 9.3	101.6 ± 11.2	203.9 ± 22.9	209.3 ± 14.1
Uterine protein/DNA in % of controls	100 ± 2	101 ± 2	128 ± 7	121 ± 5
Uterine RNA/DNA in % of controls	100 ± 5	103 ± 7	127 ± 9	127 ± 7
Uterine glycogen/DNA in % of controls	100 ± 16	95 ± 15	134 ± 32*	102 ± 17*

* Not significant, as compared to controls.

assumed that the genomic response to oestrogens is independent from the oestrogen-induced increase in uterine cyclic AMP content.

THE EOSINOPHIL RECEPTOR SYSTEM AND THE NON-GENOMIC RESPONSE TO OESTROGENS

Oestrogen receptors in oesinophil leukocytes

Uterine eosinophils bind oestradiol *in vitro* [1, 11, 17–19] and *in vivo* [3–6]. The *in vivo* studies included a dry radioautographic technique for diffusible compounds. Oestrogen binding was localized in the cytoplasm [1, 3], probably in the peroxidasosomes [20, 21] and along cellular membranes [1, 3]. Endogenously produced oestrogens compete with [^{3}H]-oestradiol for binding sites [17, 22]. Oestrogen binding sites in uterine eosinophils have high affinity, limited binding capacity and great specificity for oestrogens but not for other steroids [1, 11, 17, 19, 22].

The eosinophil oestrogen receptors have been localized in the 24,000 *g* fractions from rat uterus and from eosinophil-rich human blood leukocyte preparations, but not in the 24,000 *g* fractions from human blood leukocyte preparations deficient in eosinophils [20, 21]. The total number of binding sites per human blood eosinophil leukocyte was found to be 7400 sites per cell, and the K_D for oestradiol-17β was 5.6×10^{-10} M, in both human blood eosinophil and rat uterine eosinophil receptor preparations [20, 21].

The eosinophil receptor system for oestrogens has been demonstrated in the mature rat [1, 3–6, 17, 19–24] and mature Syrian hamster [25] uterus, in the human endometrium [18, 26] and in the human eosinophil leukocytes of the blood [20, 21].

Eosinophil migration to the uterus

Without oestrogen there are no eosinophils in the rat uterus (see [27] for a review). Eosinophils are attracted to the uterus of immature rats a few minutes after the intravenous injection of oestrogens [27, 28] (Fig. 5). This requires that migrating eosinophils recognize uterine blood vessels in the presence of oestrogen [27, 28]. The recognition could be based upon the simultaneous presence of oestrogen receptors on the eosinophil surface and in the wall of small uterine blood vessels. Oestrogen receptors were found in or near the cytoplasmic membrane of the eosinophils [1, 3] and in the wall of small uterine blood vessels, but not in the blood vessels from other organs [27, 28]. It is possible that free oestrogen receptors from the capillary wall have affinity for oestrogen-receptor complexes located in the surface of eosinophils, or that free oestrogen receptors from the surface of eosinophils, have affinity for oestrogen-receptor complexes located on uterine blood vessel wall. This would cause the coupling of oestrogen simultaneously to both the eosinophil and the uterine blood vessel receptors in an "oestrogen-bireceptor complex", and produce the attachment of eosinophil leukocytes to uterine blood vessels by oestrogen-receptor bridges. This attachment is the initial step for eosinophil penetration into uterine stroma.

Other explanations can be proposed to account for the eostrogen-induced uterine eosinophilia. Oestrogens might change uterine levels of histamine, serotonin, bradikinin, cyclic AMP, cyclic GMP or prostaglandin E_1, substances commonly considered to in-

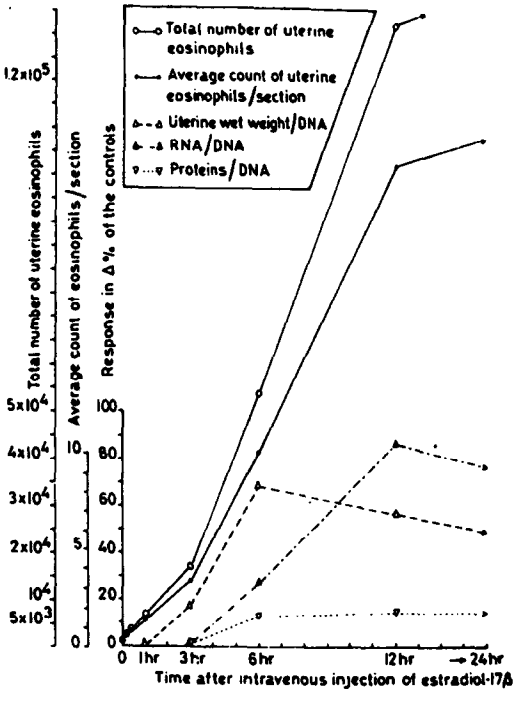

Fig. 5. Kinetics of oestrogen-induced uterine eosinophilia and other parameters of oestrogen stimulation (from ref. 28).

teract with eosinophil migration or to be themselves eosinotactic. Their role in eosinophil migration to the uterus may be ruled out, based on the following data: Eosinophils are not attracted by serotonin or bradikinin [29]. Histamine is eosinotactic in horses, but not in other species, including guinea pigs, dogs, mice, rats and man [see 29 for a review]. Cyclic AMP does not play a role in eosinophil migration to the uterus since this migration is not prevented by the uterine cyclic AMP blocker propranolol [16]. Colchicine, which inhibits the release of enzymes mediating the inflammatory response by increasing cyclic GMP levels and inhibits prostaglandin E_1 synthesis, release and/or effects, does not block oestrogen-induced recognition of uterine blood vessels by eosinophils [30]. Therefore, neither cyclic GMP nor prostaglandin E_1 are involved in eosinophil migration to the uterus.

The mechanisms of eosinophil-mediated oestrogen effects

Some of the early non-genomic parameters of oestrogen stimulation in the uterus, such as oedema (water inhibition), increase in vascular permeability, release of histamine [7, 12, 15, 19, 27, 31–33] and possibly, anti-immune protection for the blastocyst [33] have been ascribed to presence of eosinophils.

When eosinophils enter uterine stroma, they undergo several changes which can be observed at the ultrastructural level. Uterine eosinophils are found closely apposed (100–200 Å) to the plasma membranes of other uterine stromal cells [32, 34, 35]. This is accentuated under hyperoestrogenic conditions [34, 35]. After a prolonged hyperoestrogenic treatment a close relationship among the connective tissue cells surrounding eosinophils produces a configuration that resembles nuclei of decidualization [35, 36]. Further, the eosinophils release their peroxidasosomes (specific granules) and their dense granules into uterine ground substance [32].

The release of peroxidasosomes and dense granules from uterine eosinophils to uterine stroma suggests that their content may act on uterine ground substance and/or neighboring cells. Eosinophil peroxidasosomes contain a basic protein [37] and several enzymes, *e.g.* a hemoprotein with peroxidase activity, phospholipase D, beta glucuronidase, cathepsin, arylsulfatase, histaminase (see [29] and [32] for a review) and collagenase [38]. In addition, eosinophils were shown to have both kinin-producing and kininase activities and to release prostaglandins E_1 and/or E_2 [29].

The release of eosinophil collagenase, beta glucuronidase, arylsulfatase and cathepsin to uterine stroma may be responsible for the oestrogen-induced depolymerization of ground substance mucopolysaccharides [39] and collagen [36]. This depolymerization would osmotically increase uterine extracellular water content (*i.e.* oestrogen-induced oedema) [7, 32, 35, 40].

Released eosinophil collagenase also causes depolymerization and disaggregation of basement membrane collagen fibrils in small uterine blood vessels, noted following oestrogen treatment [35, 40]. Perhaps this contributes to the increase in vascular permeability. The release of prostaglandins E_1 and/or E_2 from eosinophils may also play a role in oestrogen-induced increase in vascular permeability.

Oestrogen-induced mast cell histamine release into the uterus may be mediated by eosinophils [7, 19, 27] via the release of the basic protein contained in eosinophil peroxidasosomes. It is well-established that basic proteins stimulate mast cells to degranulate and release histamine (see [41] for a review). One can also speculate that histamine release could result from prostaglandin E_1 or E_2 release from eosinophils [42]. The activity of the histamine released from mast cells is limited by degradation by eosinophil histaminase.

Eosinophil peroxidase may participate in the activity of oestrogen as an intermediate hydrogen and electron carrier [32]. Hydrogen peroxide was proposed to act as a terminal hydrogen acceptor in a hypothetical redox cycle [23].

Uterine eosinophils were proposed to play a role in sperm capacjtation [43]. Eosinophils were also found to migrate to male ductus deferens under the effect of oestrogens [44], where they could also play a role in sperm capacitation.

Eosinophils may interfere with blood coagulation at the site of blastocyst implantation by producing hydrogen peroxide, which inhibits platelet aggregation. Eosinophils, with their known fibrinolytic activity, are attracted to sites of fibrin deposition, explaining in part uterine fibrinolytic properties (see [29] for a review).

Eosinophils are usually considered as promoting immune reactions. This conception arose primarily because of the cortisol-induced blood eosinopenia, which accompanies cortisol-induced suppression of immunity and cortisol-induced involution of lymphoid organs (see [45] for a review). However, it must be noted that circulating eosinophils are low after cortisol precisely because they migrate to lymphoid tissue [45], the very location where the immune reaction is being suppressed. Therefore, it is not unreasonable that oesinophils in the uterus protect blastocyst from its rejection as a homograft [33]. Indeed, intrauterine skin homograft rejection can be prevented by oestrogen administration [46]. Sequellae of certain immune reactions have also been shown to be suppressed by eosinophils [29]. Eosinophil phospholipase D inactivates the platelet-activating factor and eosinophil arylsulfatase inactivates the slow reacting substance of anaphylaxis (see [29] for a review). We propose that eosinophils may play a role in decidualization and/or implantation by interfering with blastocyst immune rejection.

Oestrogen binding—oestrogen stimulation relationship

There is a direct relationship between oestrogen binding by uterine eosinophils and eosinophil-

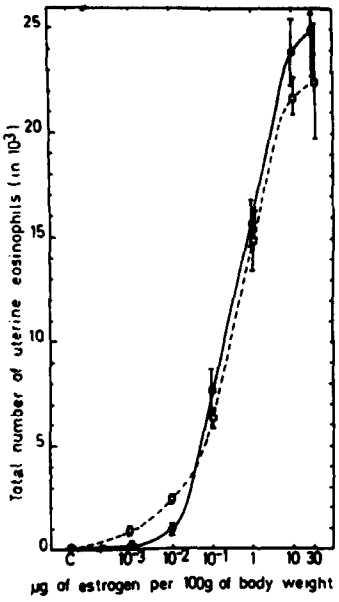

Fig. 6. Dose response of oestrogen-induced uterine eosinophilia. Oestradiol-17β (●) or oestriol (□) was given intravenously 6 h before the animals were killed (from ref. 7).

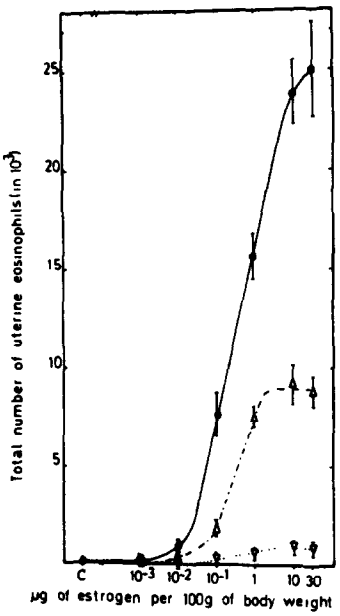

Fig. 8. Effect of cortisol on the oestrogen-induced uterine eosinophilia. Oestradiol was given *in vivo* 6 h before the animals were killed. Three groups of animals are compared: 1 animals without cortisol (●), 2 animals injected intravenously with 2 mg of cortisol acetate/100 g b. wt, simultaneously with the oestrogen injection (▽), and 3 animals injected intraperitoneally with 2 mg of cortisol/100 g b. wt 12 h before the oestrogen injection (△) (from ref. 7).

mediated parameters of oestrogen stimulation. The eosinophil oestrogen receptors have a higher affinity for oestriol than for oestradiol [19], and correspondingly oestriol is the stronger oestrogen for the response mediated by eosinophils (Figs 6 and 7) [7, 12]. Aminophylline increases the *in vitro* binding of oestrogens by uterine eosinophils [24]. Accordingly, it was found in the immature rat that aminophylline increases oestrogen-induced uterine oedema at physiological doses of oestradiol [47].

Selective inhibition of eosinophil-mediated parameters of oestrogen stimulation

Eosinophils are attracted to the uterus by oestrogens, and their number is proportional to the dose of oestrogens administered [7, 12]. Any condition interfering with the migration of eosinophils to the uterus should block the oestrogenic response mediated by eosinophils.

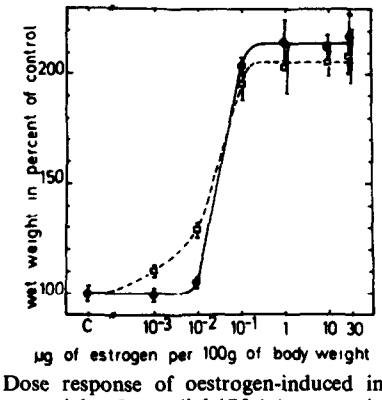

Fig. 7. Dose response of oestrogen-induced increase in uterine wet weight. Oestradiol-17β (●) or oestriol (□) was given intravenously 6 h before the animals were killed (from ref. 7).

Cortisol is known to drastically decrease the number of circulating eosinophils and therefore limit their availability for migration to the uterus under oestrogenic conditions [7, 15]. This results in a suppression of the oestrogen-induced uterine eosinophilia and the uterine wet weight response to oestrogens (Figs 8 and 9) [7, 15].

The small increase in uterine wet weight in cortisol-treated animals 6 h after oestrogen administration (Fig. 9) [7, 15] possibly reflects the oestrogen-induced increase in protein content (a genomic response) and is probably not a true uterine oedema.

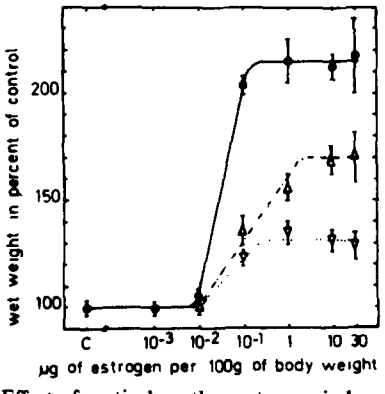

Fig. 9. Effect of cortisol on the oestrogen-induced increase in uterine wet weight 6 h after oestrogen administration. Animals without cortisol (●), animals with cortisol injected intravenously (▽) and animals with cortisol injected intraperitoneally (△) are compared (from ref. 7).

The lysosome membrane-stabilizing properties of cortisol were proposed to account for the antioestrogenic effects of cortisol. We had ruled out this possibility since propranolol, a drug with lysosome membrane-stabilizing properties similar to cortisol, failed to inhibit both uterine eosinophilia and the water imbibition oestrogen effects [16].

Colchicine was also found to interfere with oestrogen-induced uterine eosinophilia and therefore, to block the oestrogen-induced uterine oedema [30]. Colchicine does not interfere with the recognition by eosinophils of uterine blood vessels in the presence of oestrogens, as discussed previously, but interferes instead with eosinophil migration to uterine stroma by a disassembly of microtubular system [30].

In 10-day old rats, in which the number of circulating eosinophil leukocytes is physiologically very low, there is no measurable eosinophil-mediated parameters of oestrogen stimulation [13]. There is, however, already a mature cytosol-nuclear oestrogen receptor system and a genomic response to oestrogens [13].

Agents not inhibiting eosinophil-mediated parameters of oestrogen stimulation

The following data provides evidence for independence of eosinophil-mediated parameters of oestrogen stimulation from either genome activation, cyclic AMP, cyclic GMP, prostaglandins, serotonin, bradikinin or histamine.

Actinomycin D does not block oestrogen-induced uterine eosinophilia [48] and oestrogen-induced uterine oedema [48, 49]. Therefore, those parameters of oestrogen stimulation are responses independent from genome activation or derepression.

Propranolol, an agent suppressing oestrogen-induced increase in uterine cyclic AMP levels does not block oestrogen-induced uterine eosinophilia and oedema (Table 1) [16]. Therefore, these responses are independent from cyclic AMP.

Experiments with colchicine show that at least oestrogen-induced uterine eosinophilia is independent from the action of cyclic GMP or prostaglandin E_1 [30]. Serotonin and bradikinin [29] as well as histamine [29] were also shown to be non-eosinotactic substances (see section on eosinophil migration).

Experiments with indomethacin and D-2-bromolysergic acid diethylamide indicate that neither prostaglandins (derived from *de novo* synthesis) nor serotonin are involved in the mediation of oestrogen-induced uterine oedema [50].

CYCLIC AMP-MEDIATED PARAMETERS OF OESTROGEN STIMULATION

There is evidence that some parameters of oestrogen stimulation are mediated via cyclic AMP. Exogenously administered cyclic AMP produces oestradiol-like induction of several uterine glycogenolytic enzymes and an increase in the production of a specific, oestradiol-sensitive cervicovaginal antigen (see [16] for a review). Propranolol was found to inhibit this specific cervicovaginal antigen, but failed to inhibit the eosinophil-mediated and the genomic parameters of oestrogen stimulation (Table 1) [16]. This suggests that cyclic AMP is involved in some but not all oestrogenic responses, probably as a separate mechanism of oestrogen action.

We have already proposed that the glycogen response to oestrogens is independent from both the cytosol-nuclear [12] and the eosinophil [15] oestrogen receptor systems. This provides further support to the possibility that the glycogen response to oestrogens is mediated by a third mechanism of oestrogen action.

INDEPENDENCE OF OESTROGEN ACTIONS

Evidence shows that two or possibly three independent mechanisms of oestrogen action exist in the uterus, each one mediating a separate group of parameters of oestrogen stimulation. This fact strongly suggests that any study of oestrogen action should include several parameters of oestrogen stimulation. In the early days of oestrogen research, the parameter measured was uterine wet weight 6 h after oestrogen administration. Therefore, oestriol (or "theelol", as it was first called), was considered to be the strongest oestrogen. Later on, sophistication of biochemical techniques changed the method of study, and the measurement of RNA or proteins became the new fashion. Then oestriol fell into disrepute, and oestradiol became the strongest oestrogen. Why do scientists, in their wish to *believe* they understand everything, draw broad general conclusions from single, restricted observations?

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