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Effect of Oestradiol on Progesterone Receptors in Normal Mammary Glands and its Relationship with Lactation

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Mammary glands of lactating mice, either intact or ovariectomized, do not contain detectable amounts of progesterone receptors and this lack of receptors persists also in tissues of animals treated with oestradiol. In contrast with lactators, mammary glands of virgin mice contain progesterone receptors whose amounts are augmented by oestradiol administration.

During lactation mammary glands do not contain detectable amounts of cytoplasmic progesterone receptors (Shyamala & McBlain, 1979). From studies on rats, it is known that lactation is accompanied by a pseudopregnant state characterized by an inhibition of gonadotropin secretion, an increase in prolactin secretion and maintenance of corpus-luteum function (McCann *et al.*, 1967; Ford & Melampy, 1973; Hammons *et al.*, 1973): this pseudopregnant state is associated with very low serum concentrations of oestradiol during early lactation and a high serum concentration of progesterone (Smith & Neill, 1977). Studies on progesterone receptors from several laboratories indicate that oestradiol can cause an increase in cytoplasmic progesterone receptors in target tissues such as uterus, vagina and certain experimentally induced mammary tumours (Toft & O'Malley, 1972; Rao *et al.*, 1973; Milgrom *et al.*, 1973; Leavitt *et al.*, 1974; Horowitz & McGuire, 1977; Koenders *et al.*, 1977) and progesterone can inhibit the progesterone-receptor synthesis either by antagonizing the action of oestrogen or by deactivating the progesterone receptors directly (Milgrom *et al.*, 1973; Hseuh *et al.*, 1975). Thus a lack of progesterone receptors in mammary glands during lactation can result from either an inhibitory effect of progesterone on progesterone-receptor synthesis and/or its deactivation or from a lack of an oestrogenic environment necessary for the synthesis of progesterone receptors. The present studies were therefore undertaken to elucidate the physiological basis for the lack of progesterone receptors in lactating mammary glands. A preliminary report of this work has been published (McBlain *et al.*, 1978).

Experimental

Female Balb/c mice from our own colony were used. The day of parturition was counted as day 0 of lactation and where indicated animals were ovariectomized on day 2 of lactation. Virgin mice were ovariectomized between 2 and 4 months of age and were used between 2 and 3 weeks after castration. Oestradiol-17β was given subcutaneously as a 10μg/ml solution of 0.9% NaCl containing 1% ethanol.

For measurements of progesterone receptors compound R5020 (17,21-dimethyl-19-norpregna-4,9-diene-3,20-dione), a synthetic progestin that has been used successfully to characterize progesterone receptors in several tissues (Philibert *et al.*, 1977), was used as the ligand in the present studies. Both ^{3}H-labelled compound R5020 (sp. radioactivity 86.0Ci/mmol) and unlabelled compound R5020 were purchased from New England Nuclear Corp., Boston, MA, U.S.A. Tissue homogenates were prepared in a phosphate/glycerol buffer (5mM-sodium phosphate, 10mM-thioglycerol, 10% glycerol, pH7.4) and centrifuged at 12350*g* for 1h. Unless otherwise specified, the supernatants designated as cytoplasmic extracts were incubated with either 20nM- (mammary glands) or 50nM- (uterus) ^{3}H-labelled compound R5020 alone or in the presence of a 100-fold excess of unlabelled compound R5020. Since we have previously reported that under certain experimental conditions in mammary tissues ^{3}H-labelled compound R5020 can bind to sites that resemble glucocorticoid-binding sites (Shyamala & McBlain, 1979) in parallel sets, cytoplasmic extracts were also incubated with ^{3}H-labelled compound R5020 in the presence of a 100-fold excess of unlabelled dexamethasone. The specific binding data reported in the present studies represent the binding of ^{3}H-labelled compound R5020 observed in incubations containing unlabelled dexamethasone minus that observed in incubations containing unlabelled compound R5020 and thus represent binding to high-affinity sites only. Unless otherwise specified all other experimental details, including the dextran-coated-charcoal assay

for measurements of progesterone receptors, were as described previously (Shyamala & McBlain, 1979). DNA content of tissues was determined by the method of Cerriotti (1952) and protein concentration was assayed by the method of Lowry *et al.* (1951). In all studies, the uteri of the same animals served as control tissues.

Results

Effect of oestradiol on progesterone-receptors of mammary glands and uteri of lactating mice

As shown in Fig. 1, in both control and oestradiol-treated lactators, mammary tissue did not have detectable amounts of progesterone receptors, whereas the uteri of the same animals had progesterone receptors; furthermore the amount of uterine progesterone receptors was significantly stimulated by oestradiol administration. In experiments where lactating mice received two doses of oestradiol spaced 24h apart, there were still no detectable amounts of progesterone receptors in mammary tissues (results not shown). The results of similar experiments performed with castrated lactators are shown in Fig. 2. It can be seen that ovariectomy did not result in the appearance of progesterone receptors in mammary tissue of lactators and also did not significantly affect the amount of progesterone receptors in the uterus compared with intact lactators. However, in oestradiol-treated castrated animals, the amount of uterine progesterone receptors was significantly higher than control values and, in contrast with the uteri, oestradiol once again failed to stimulate progesterone-receptor synthesis in mammary glands. Measurement of progesterone concentrations in the plasma revealed a significant decrease in that present in castrated lactators compared with intact lactators (B. E. P. Murphy & G. Shyamala, unpublished work). From the results presented above, it was concluded that neither a lack of an oestrogenic stimulus nor an inhibitory influence of progesterone led to the apparent lack of progesterone receptors in mammary tissue during lactation.

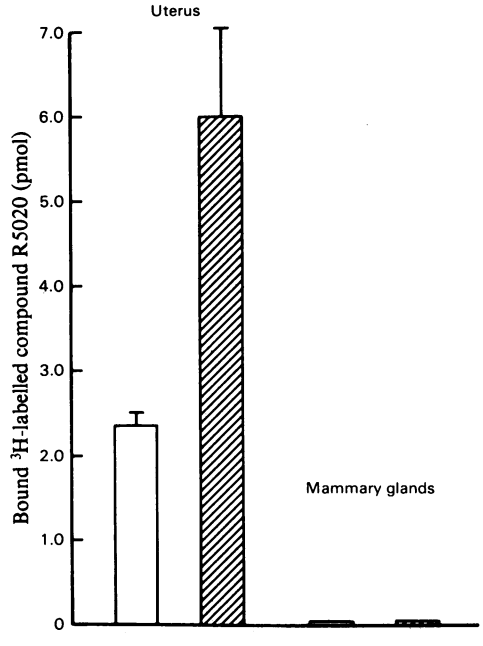

Fig. 1. *Effect of oestradiol on the amounts of cytoplasmic progesterone receptor in the uterus and mammary glands of lactating mice*
Two groups of lactating mice between day 7 and day 10 of lactation were given a single injection of either 0.9% NaCl □ or 3 μg of oestradiol (■) 24h before killing as described in the text. The results are expressed as means ± S.E.M. for four experiments and represent the binding per g of mammary tissue or per uterus.

Effect of oestradiol on progesterone receptors in mammary glands and uteri of virgin mice

As mentioned above, it is known that oestradiol can stimulate the synthesis of progesterone receptors in certain target tissues for oestrogen, including certain mammary tumours. However, it remained to be established whether normal mammary tissues behave

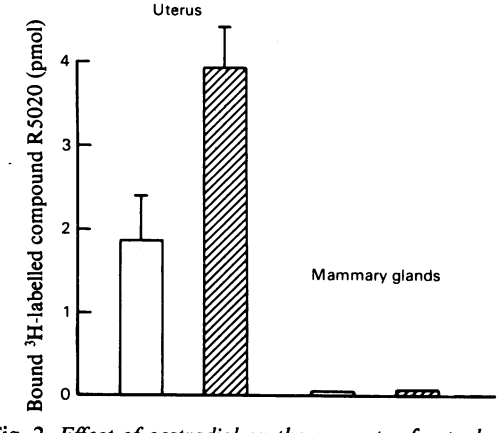

Fig. 2. *Effect of oestradiol on the amounts of cytoplasmic progesterone receptor in the uterus and mammary glands of castrated lactating mice*
Mice were castrated at day 2 of lactation, a single injection of 0.9% NaCl (□) or 3 μg of oestradiol (■) were given on day 9 and tissues were assayed at day 10. The results are expressed as means ± S.E.M. for five experiments and represent binding per g of mammary tissue or per uterus.

similarly. To this end, the effect of oestradiol on progesterone receptors in mammary glands and uteri of castrated virgin mice was examined. The specific binding data from a typical experiment as analysed by Scatchard plots are shown in Fig. 3. It can be seen that progesterone receptors are present in mammary glands of castrated virgin mice and the amounts are significantly higher in the tissues from oestradiol-treated animals. The K_d of the progesterone–receptor complex of mammary tissues of oestradiol-treated animals (0.97 nM) was similar to that of the 0.9% NaCl-treated animals (1.5 nM) and both values are similar to the reported K_d values for progesterone–receptor complexes in various target tissues (Philibert *et al.*, 1977). The increase in progesterone receptors due to oestradiol was also apparent when the results were expressed either on the basis of the DNA content of the tissues or on the basis of protein concentration of the cytoplasmic extracts (Table 1). Thus it is clear that, similar to other target tissues for oestradiol, progesterone receptors in normal mammary glands can be increased by oestradiol and such increases are in all probability due to a cellular increase in progesterone receptors.

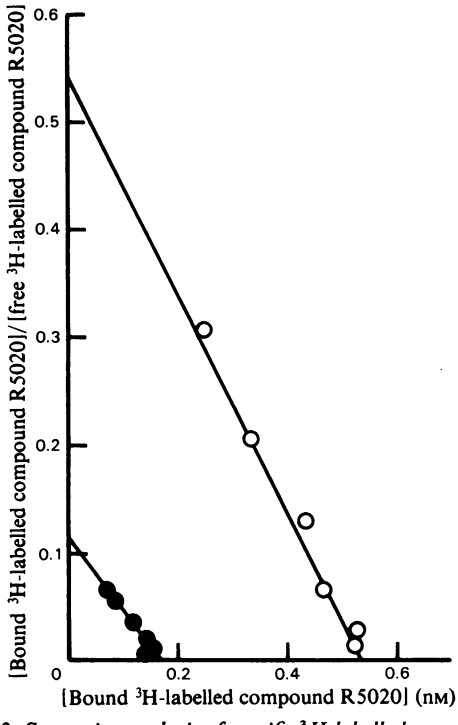

Fig. 3. *Saturation analysis of specific ^{3}H-labelled compound R5020 binding in cytoplasmic extracts of mammary glands of castrated virgin mice*

Portions of cytoplasmic extracts from 0.9% NaCl-(●) or oestradiol-treated (○) mice were incubated for 4h at 4°C with various concentrations of ^{3}H-labelled compound R5020 either alone or in the presence of a 500-fold excess of unlabelled compound R5020. Bound ^{3}H-labelled compound R5020 was determined on duplicate portions. The results represent specific binding per g of mammary tissue.

Discussion

During lactation there is a decrease in uterine progesterone receptors and it was suggested that the hormonal milieu of lactation might have been non-conducive to the synthesis of progesterone receptors (Gomez *et al.*, 1977). The results reported in the present paper indicate that the uterine tissue of lactators can be stimulated to synthesize progesterone receptors under the influence of oestradiol. Thus lack of an adequate oestrogenic environment may be one aspect of the hormonal milieu of lactation that contributes to decreases in progesterone receptors in the uterus. However, in the case of mammary glands, the lack of detectable amounts of progesterone receptors during lactation and the inability of these tissues to synthesize progesterone receptors under the influence of oestradiol in both intact and castrated states led us to believe that the hormonal milieu of lactation alone cannot be responsible for the absence of progesterone receptors in

Table 1. *Effect of oestradiol on the amounts of cytoplasmic progesterone receptor in the uterus and mammary glands of castrated virgin mice*

Two groups of ovariectomized virgin mice were given a single injection of either 0.9% NaCl or 3 μg of oestradiol 24h before being killed. The results are expressed as means ± S.E.M. for five experiments and represent specific binding to high-affinity sites.

	Receptor concentration			
	Mammary glands		Uterus	
Group	(fmol/mg of DNA)	(fmol/mg of protein)	(fmol/mg of DNA)	(fmol/mg of protein)
0.9% NaCl	487 ± 54	12.4 ± 1.4	4066 ± 458	414 ± 47
Oestradiol-treated	993 ± 53	23.5 ± 1.4	15548 ± 976	1278 ± 80

mammary glands. This, taken together with the observation that mammary tissues of virgin mice do contain progesterone receptors and respond to oestradiol with increased amounts of progesterone receptors, leads us to conclude that tissue-specific factors related to lactation may be responsible for the lack of progesterone receptors in mammary tissues. In rodents, a principal index of oestrogen action in mammary tissue is enhanced epithelial-cell proliferation (Lyons *et al.*, 1958; Nandi, 1958; Bresciani, 1965) and during established lactation there is no significant increase in the mammary gland's DNA content (Munford, 1963; Banerjee & Walker, 1967; Hseuh *et al.*, 1973). On the basis of this information, and the results on progesterone receptors presented above, it would appear that the lactating mammary tissue might be 'refractory' to the effects of oestradiol. One of the causes for the lack of, or decreased, ability of a target tissue to respond to oestradiol may be an absence or decreased amounts of cytoplasmic oestrogen receptors; this is clearly not the situation with mammary glands during lactation since oestrogen receptors are present in the lactating mammary tissue (Shyamala & Nandi, 1972; Gardner & Wittliff, 1973) and, in fact, the amounts of cytoplasmic oestrogen receptors expressed on the basis of DNA or protein increase in mammary tissue during lactation (Hseuh *et al.*, 1973; Leung *et al.*, 1976; Hunt & Muldoon, 1977). Previous reports have also demonstrated that the cytoplasmic oestrogen receptors of lactating mammary glands can be translocated to the nucleus after administration of oestradiol *in vivo* (Shyamala & Nandi, 1972; Hseuh *et al.*, 1973; Gardner & Wittliff, 1973). It has been proposed that in the chain of events leading to the synthesis of progesterone receptors under an oestrogenic stimulus, in addition to binding of oestradiol to cytoplasmic oestrogen receptors and its translocation to the nucleus, the oestrogen receptors need to undergo processing in the nucleus (Horowitz & McGuire, 1978). It could be that in the lactating mammary glands, the nuclear processing of oestrogen receptors is altered such that there is no synthesis of progesterone receptors; it might also be that the altered nuclear processing of oestrogen receptors leads to a state that is 'refractory' to an oestrogenic stimulus. A vast number of studies have shown that the concentration of oestrogen receptors in human breast tumours has prognostic value for hormonal therapy (McGuire *et al.*, 1975). More recently it has been proposed that the presence of progesterone receptors may be an additional sensitive marker for oestrogen responsiveness, since they are synthesized under the influence of oestrogen (Terenius, 1973; Horowitz *et al.*, 1975). The significance of the present observations lies in the fact that we have identified a particular developmental state of the normal mammary gland that appears to be insensitive to an oestrogenic stimulus even in the presence of an adequate concentration of oestrogen receptors. Since during the normal course of lactational involution the oestrogenic response of mammary tissue to synthesize progesterone receptors is restored, an understanding of the molecular basis of 'refractoriness' to oestrogen during lactation may have far-reaching consequences in understanding the hormonal regulation of mammary neoplasia.

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