dependent. At a concentration of 5 µg/ml, only 28.6% of the embryos showed an open cranial neural tube. At 25 µg/ml of BUdR, however, all the embryos examined had an open cranial neural tube with a significantly lower amount of protein as compared with the controls ($p < 0.001$, table). A concentration of 100 µg/ml of BUdR was highly toxic and none of the embryos had the visceral yolk sac circulation. At concentrations above 10^{-7}–10^{-6} M the analog has previously been reported to be lethal to preimplantation mouse embryos[9]. 10-day embryos generally survived free from effects for 24 h in the presence of 100–150 µg/ml of the analog[10]. Therefore, the mouse embryos of the early somitic stage seem to be more sensitive than the 10-day embryos.

The effects of 3 M excess thymidine and deoxycytidine on the inhibition of development by 25 µg/ml of BUdR were investigated. As shown in the table, thymidine almost completely reversed the morphological effects of the analog. A similar result has been reported on the inhibition of development of cleavage stages of mouse embryos[11]. In contrast to thymidine, deoxycytidine produced only a marginal effect in reversing the inhibition of the cranial neural tube closure. The result obtained by cotreatment with thymidine suggests that the cranial neural tube defects probably result from BUdR incorporation into DNA in place of thymidine. On the other hand, the effect of deoxycytidine can better be explained in terms of the nucleotide pool[12].

BUdR has been reported to inhibit differentiation in a number of different in vitro culture systems[13,14], whereas in vivo experiments have shown that the analog can cause many different types of abnormalities[1,2]. The mode of action of BUdR has not yet been satisfactorily explained. Nevertheless, the analog appears to be a valuable tool for investigating the mechanism of teratogenesis.

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0014-4754/83/020202-02$1.50 + 0.20/0

Peroxidase activity (PA) and progesterone receptors (PgR) in normal rat mammary glands

R. Seshadri, A.P. Banerji and P.N. Shah[1,2]

Division of Endocrinology, Cancer Research Institute, Parel, Bombay-400012 (India), December 15, 1981

Summary. PA and PgR appear to be reliable markers of estradiol action in normal female rat mammary glands, as seen by simultaneous expression of both proteins between the ages of 61 and 131 days. However, expression of PA at certain periods, when PgR was undetectable suggests, that the extent of availability of both estradiol and progesterone at target sites may act as controlling factors in synthesis of one protein over another.

As far back as 1955, Lucas et al.[3] observed that estradiol could stimulate peroxidase activity (PA) in the uteri of immature rats. However, it was only after 1972 that various groups of investigators[4-7] established that PA was a reliable indicator of estrogen stimulation of uterine growth, both by biochemical and histochemical methods. Further, this enzyme could only be induced by estradiol in target glands such as uterus, vagina and cervix of immature rats[5-7]. Our laboratory was the first to report that physiological doses of estradiol could induce PA in yet another target tissue, the mammary gland of rats, the highest inducibility of PA by estradiol being noted between 40 and 90 days of age[8].

Furthermore, as estradiol was the only hormone that could induce PA in a dose-dependent way in the mammary tissue, it appeared to be a reliable biochemical end-product of estrogenic action. Similar results have been recently reported in mammary glands of virgin mice[9].

Progesterone receptor (PgR) is another protein that is considered to be a marker of estradiol action in target tissues[10-12]. Very recently, Haslam and Shyamala[13] showed that in mammary glands of virgin mice, PgR is augmented by estradiol administration in a manner similar to that observed in the uterus. In this investigation we have simultaneously determined in the mammary glands of young, pubertal and adult intact female Holtzman rats PA and PgR formed as a result of endogenous estrogen stimulation, to see if there exists any correlation between these 2 parameters.

Materials and methods. Virgin female Holtzman rats aged 21–171 days with free access to food and water were used independently of their estrous status. For each value presented, mammary tissues from 3 to 6 rats were individually analyzed. The rats were sacrificed by an overdose of ether.

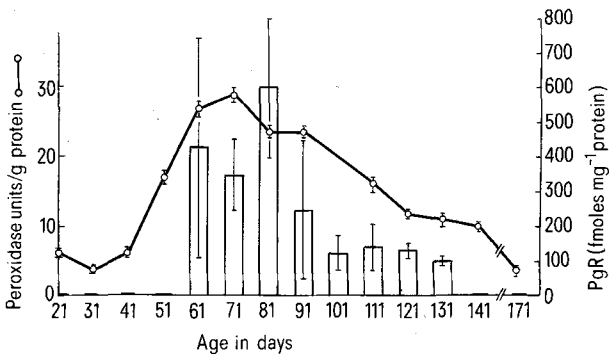
For the assessment of PA, the method of Lyttle and DeSombre[14], was essentially followed, except that crude enzyme extract (5000 g supernatant) of the mammary tissue was used. An enzyme unit was defined as the amount of enzyme required to produce an increase of 1 absorbance unit per min, and activity was expressed as units per g of protein.

The entire procedure for PgR assay was carried out at 0–4 °C. Freshly excised mammary tissue, or tissues stored in liquid nitrogen, were homogenized in 2 vol. of chilled 10 mM Tris-HCl buffer, pH 7.4 containing 1 mM Na_2EDTA and 2 mM mercaptoethanol and centrifuged for 1 h at 2 °C in a Beckman ultracentrifuge at 150,000 × g to obtain the clear cytosol. Concentrations of the specific ligand, R5020 (17,21-dimethyl-19nor-4,9 pregnadiene-3,20-dione [6,7^{3}H], 51.4 Ci/mmole) varying from 0.5 to 20 pmoles/ml, in triplicates, were added to glass tubes and to each tube,

200 µl of cytosol was added and vortexed. Parallel incubations with excess nonradioactive R5020 and blanks with buffer were also processed. All the tubes were incubated at 4 °C for 16 h. To determine specific steroid binding[15], 0.5 ml of dextran coated charcoal suspension (0.25% Norit A-0.0025% dextran-70 in 0.01 M Tris-HCl buffer, pH 8.0) was added to each tube, shaken thoroughly for 30 min and spun at 3200 rpm in a Sorvall cold centrifuge for 10 min. Aliquots (200 µl) were added to 10.0 ml scintillation fluid containing 10% Triton X-100 and counted in an LS 100 Beckman LS Counter with an efficiency of 53%. Quench corrections were done by using the external standard ratio. The K_d-values and specific PgR binding sites were determined by Scatchard analysis[16]. Protein was measured by the method of Lowry et al.[17].

Results. PA was present in the mammary tissues of virgin female rats from 21 to 171 days of age, the levels ranging from 3 to 28 units g^{-1} protein (fig.). PgR, on the other hand, was detectable only from 61 to 131 days of age, and ranged from 100 to 600 femtomoles mg^{-1} cytosol protein. The K_d-value for PgR as calculated by employing Scatchard analysis averaged around 4.6×10^{-9} M. It was interesting to observe that both PA and PgR were expressed in maximal levels between the age of 61 to 91 days. A differential expression of these 2 marker proteins was especially noticeable at the age of 51 days when PA was as high as 15 units g^{-1} protein and PgR was undetectable.

Discussion. It is common knowledge that when a hormone and its receptor interact in target tissues, specific proteins are produced. In the case of estradiol, 2 specific proteins viz. the peroxidase enzyme and PgR are known to be produced in the mammary glands of mice[9,13] and rats[8]. The present study showed that both PA and PgR were measurable in appreciable amounts from 61 to 131 days of age (fig.) though there was a gradual decline in both from day 91. This correlates well with our previous observation[18] that the mammary gland of the Holtzman rat attained maximum growth and differentiation by day 91, and subsequently no significant change could be noted. Similarly[8], estradiol-induced PA in rat mammary glands declined after day 91 and became negligible after 131 days. Though the PA levels never showed wide variability, the mammary tissue PgR concentration exhibited such a phenomenon at certain days (fig.). This could be the consequence of using intact animals in cycle, (irrespective of their estrous status) in which varying levels of circulating estradiol influenced PgR synthesis and/or endogenous progesterone levels interfered with the PgR estimation. An additional contributory factor could be the experimental variations inherent in the PgR assay.



The endogenous peroxidase activity (PA) and progesterone receptor (PgR) concentrations of the mammary tissues of intact female virgin Holtzman rats (no exogenous estradiol administered) were determined on the days shown by the methods described in the text. Each point is an average of 3 or more determinations.

Lack of correlation in the expression of these 2 proteins in the mammary tissue was observed mainly at the age of 51 days, when PA was 15 units g^{-1} protein and PgR was undetectable. Surprisingly, the level of PgR in the mammary tissue of 51-day-old rats was found to be 150 femtomoles mg^{-1} protein when they were hysterectomized a week earlier (unpublished data). This may be due to greater availability of endogenous estradiol to the mammary gland in the absence of the uterus, the preferred target organ of that steroid. This preference could also be inferred from our earlier reports on higher levels of PA in the uterus than in the mammary glands of 51-day-old intact Holtzman rats[8]. Another possible explanation for the lack of correlation between these parameters at certain ages could be based on the observed antagonistic action of progesterone on estradiol-induced synthesis of PA in rat mammary glands[8] and mammary neoplasms[19]. Furthermore, induction of PA and not PgR by estradiol in the mammary glands of rats during pregnancy and lactation was interpreted as the negative effect of progesterone on the estrogen-mediated increase in PgR[19]. Therefore it is not illogical to conclude that the extent of availability of both estradiol and progesterone to the mammary gland may be decisive factors controlling the suppression or synthesis of one estrogen-induced protein over the other.

Since PA, and not PgR was measureable in the rat mammary tissue at all ages studied, (fig.) there may be preferential expression of the peroxidase enzyme due to its possible participation in estrogen action at target sites. Nevertheless, under favorable conditions existing in rats between the ages of 61 and 131 days it appears that estrogen can simultaneously induce both its marker proteins in the mammary gland.

1 Acknowledgments. The authors are deeply indebted to Miss Shampa Dutta and Mr G.K. Kapse for technical assistance.
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