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SHORT COMMUNICATION

11β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN THE MAMMARY GLAND

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Summary—Levels of 11β-hydroxysteroid dehydrogenase activity in mammary gland homogenates from pregnant and lactating Sprague–Dawley rats were determined by incubation with [^{3}H]corticosterone under standard conditions, followed by thin-layer chromatography of incubated media. Enzyme activity was high in virgin and pregnant rats, but fell soon after parturition, suggesting a possible role for this enzyme in the co-ordinate regulation of glucocorticoid effects on milk protein synthesis.

INTRODUCTION

A variety of hormones—adrenal and ovarian steroids, and peptides such as insulin and prolactin—are important regulatory factors in mammogenesis and lactogenesis during the physiological cycle of pregnancy and lactation [1–3]. In rat and mouse mammary gland explants taken from animals at mid-pregnancy, glucocorticoids increase the synthesis of caseins [4] and α-lactalbumin, major milk proteins [4, 5]; in contrast, progestins inhibit their synthesis [6, 7]. Further *in vitro* studies on α-lactalbumin production by ourselves and others have shown that the response of rodent mammary gland explants to glucocorticoids and progestins diminishes several days before parturition in both rat and mouse [7, 8], and that explants from post-partum animals are unresponsive to glucocorticoids and progestins post-partum [8]. Thus the response of the mammary gland to hormones can change according to the physiological status of the animal.

Steroid hormones act by binding to intracellular receptors capable of interacting with regulatory sequences in specific genes, thereby altering their transcription rate [9]. Progestin receptors are absent from the lactating mammary gland [10–13], explaining the lack of observed effect of progestins after parturition [14]. In contrast, glucocorticoid receptor levels either remain unaltered in rat mammary gland [15], or may even increase post-partum [16].

Metabolism of steroids, or sequestration by binding proteins such as transcortin [16, 17], may alter tissue levels of active hormone. The fall in blood progesterone levels which precedes parturition and leads to lactogenesis partly reflects metabolism of progesterone in the ovary to 20α-hydroxypregn-4-ene-3-one, which has much lower progestational activity and thus is unable to block lactogenesis [18]. The enzyme 11β-OH steroid dehydrogenase catalyzes the conversion of corticosterone (B) to 11-dehydrocorticosterone (A), and of cortisol (F) to cortisone (E) [19, 20]; it appears particularly active in tissues where glucocorticoids may play a developmental role [19]. During pregnancy 11β-hydroxysteroid dehydrogenase modulates maternal glucocorticoid access to the uterus and placenta as well as to the foetus, by controlling the tissue concentration of the active steroids (F and B), since E and A are inactive [20].

Recently, a further role for this enzyme has been postulated, that of conferring aldosterone selectivity on Type I receptors in mineralocorticoid target tissues such as kidney, in which these receptors would otherwise be occupied by physiological glucocorticoids which have affinity equal to that of aldosterone [21]. In the mammary gland, a potential physiological role for 11β-hydroxysteroid dehydrogenase has not been explored. The aim of the present study was thus to examine the activity of 11β-hydroxysteroid dehydrogenase activity in the rat mammary gland through pregnancy and lactation.

EXPERIMENTAL

Materials

[^{3}H]1,2,6,7-corticosterone (specific activity ~90 Ci/mmol) was obtained from Amersham, North Ryde, N.S.W., Australia. Corticosterone (B) and 11-dehydrocorticosterone (A) were from Sigma, St Louis, Mo. Carbenoxolone sodium was the generous gift of Biorex, London, England. Dulbecco modified Eagle's medium (DMEM) and HEPES were supplied by Flow Laboratories (North Ryde, N.S.W., Australia). All organic solvents were analytical grade. Thin-layer chromatography (TLC) Merck TLC plates, silica gel 60 F_{254} precoated (cat. No. 5715), were obtained from Crown Scientific, Burwood, Victoria, Australia. Scintillant was Emulsifier Safe from Canberra Packard, Mt Waverley, Victoria, Australia. Plug-mated rats were from Central Animal House, Monash University and were housed in the Prince Henry's Hospital Animal house on a 12 h light-dark regime with rat chow and water *ad libitum*.

Methods

Abdominal mammary glands were removed and placed in DMEM–HEPES pH 7.4 at room temperature during transport from the animal house to the laboratory. The tissue was removed onto a petri dish and minced with a razor blade. For measurement of 11β-hydroxysteroid dehydrogenase activity minced tissue (0.2 g) was added to 2 ml DMEM–HEPES with ~0.4 μCi of [^{3}H]corticosterone in a 20 ml glass scintillation vial. Incubation was at 37°C for 2 h in a rotary shaking water bath; at the end of incubation, the tissue and medium were frozen in the vial on solid CO_2, and kept at −20°C until extracted. Zero time incubation samples were taken, and blank samples without tissue were

taken both at zero time and after incubation at 37°C. Carbenoxolone, an inhibitor of 11β-hydroxysteroid dehydrogenase [21], was used at a final concentration of 24.5 μM or 245 μM.

For extraction, the vials were thawed, 5 ml ethyl acetate added and the mixture vigorously shaken for 1 min after thorough vortexing. The organic phase was removed into a test tube after the aqueous and organic phases cleared. The aqueous phase (containing the tissue) was extracted with a further 5 ml ethyl acetate, and the organic phase removed as before. The organic phases were pooled and evaporated to dryness either under air or under partial vacuum. The lipid residue was stored at −20°C until analysis.

For analysis by TLC, 400 μl of acetate was added to the sample residue and 40 μl removed for recovery estimation. Samples (20 μl) were streaked (1 cm) onto a Merck TLC plate, together with standard corticosterone and 11-dehydrocorticosterone, and the plate developed in chloroform:EtOH (95% v/v) 92:8 (v/v). Steroids were visualized by u.v. light, and the areas corresponding to B and A scraped into scintillation vials. The remainder of the plate was divided into 8 or 9 sections and similarly scraped into scintillation vials to enable recovery from the plate to be estimated. Scintillant fluid (4 ml) was added to each minivial (Pony vials, Packard) and the radioactivity measured in a Packard scintillation counter with automatic quench correction. Results were expressed as % conversion of B to A and correction was made for any non-tissue conversion of B to A during incubation.

To confirm the identity of the radioactive metabolite as A, a second solvent system $CHCl_3$:MeOH, 90:10 was used in TLC analysis. After development, the plate was divided into 0.5 cm strips, scraped, and counted as before. Authentic B and A were included on the same plate as the tissue extract, and no significant radioactivity was detected except where authentic B and A were found. Finally, an extracted sample from an incubation performed on day 14 of pregnancy was analyzed by HPLC, as previously described [21], except that the acetonitrile concentration during elution was 19%. Significant radioactivity eluted only at positions corresponding to authentic B or A.

RESULTS

When mammary gland tissue homogenates were incubated with [^{3}H]B, conversion of B occurred to a compound with identical chromatographic characteristics as A, in TLC in two solvent systems, and on HPLC analysis. Recovery from the plates was >85% and no significant spots of radioactivity other than B or A were detected. In all experiments, at least 18,000 dpm of the radioactivity were spotted onto the plates; individual spots corresponding to [^{3}H]B and [^{3}H]A contained at least 1800 dpm except in the studies with carbenoxolone (see below).

As shown in Fig. 1, high levels of enzyme activity, as measured by percent conversion of B to A, were seen in mammary gland from virgin rats and in pregnant rats up to day 20 of pregnancy, with 80% conversion under standard conditions. At parturition, conversion was ~60%. Levels on day 1 of lactation were similar (~70%); by day 4 of lactation, however, levels had fallen to 33% and continued to decrease to ~20% at day 21 of lactation. This represents a conservative reckoning of the extent of change; the standard conditions that we used almost certainly underestimate the differences between the various stages of pregnancy and lactation.

This activity was very markedly inhibited by carbenoxolone at 24.5 μM in explants of day 13 of gestation; conversion of B to A was 7.6% ($n = 2$) compared with 80% in the absence of carbenoxolone. With 245 μM carbenoxolone, conversion was 1.5%.

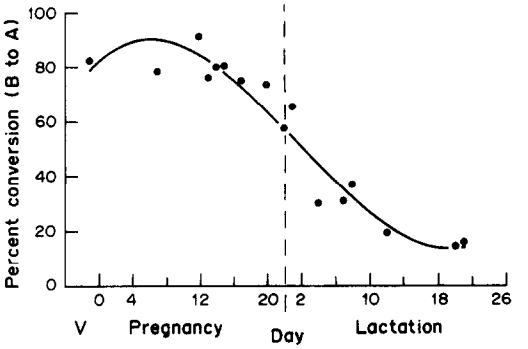

Fig. 1. Percent conversion of corticosterone (B) to 11-dehydrocorticosterone A by rat mammary gland homogenates. Shown are mean values of 2–3 animals for each time point, except for virgin (V), day 12 of pregnancy (P12), P14, and L7 which were single determinations. Each determination was done in duplicate. Mammary gland homogenate was incubated with [^{3}H]corticosterone as described in Materials and Methods. Incubate was extracted with ethyl acetate, and analyzed by TLC. Results are expressed as percent conversion of B to A and were calculated by summing the radioactivity of the areas identified as A and B and expressing the radioactivity in A as a percentage thereof. Correction was made for conversion of B to A in non-tissue blanks (less than 3%).

DISCUSSION

In these experiments, we have shown that mammary tissue from virgin, pregnant and lactating rats can convert the active glucocorticoid B to A, which has about 300-fold lesser affinity for the glucocorticoid receptor [21]. The conversion of B to A was almost completely inhibited by carbenoxolone, a known inhibitor of 11β-hydroxysteroid dehydrogenase. Previously, a major active metabolite of [^{3}H]corticosterone in rat mammary acini has been postulated to be a 21-acyl derivative [22, 23]; in contrast, other workers using cortisol in an explant system claimed that no equivalent metabolism occurred [24]. In our systems the radioactive metabolite formed co-chromatographed with A in two TLC systems and on HPLC, with no evidence of any other major metabolite.

Conversion of B to A decreased markedly after day 1 lactation, and continued to decline less rapidly until day 20 of lactation, when percent conversion was only 20% compared with 80% during pregnancy. One question arising from the observations that we had made on the conversion of B to A is whether the high level of enzyme activity during pregnancy is enough to decrease levels of B available to the glucocorticoid receptor, and thus prevent premature milk production. Early work *in vivo* by Talwalker *et al.*[25] showed that massive doses of B (12–16 mg/day) were necessary to initiate milk production, but no estimates of conversion of B to A were carried out; similarly, Davis *et al.*[26] showed that 6 mg/day of cortisol acetate was ineffective.

The time-course of the changes in enzyme activity differs from the changes in glucocorticoid response of explants in terms of α-lactalbumin and whey acidic protein synthesis [8, 27]. For both of these major milk proteins, the effects of glucocorticoids in increasing protein production are lessened by day 19 and day 20 of pregnancy, almost completely lost by day 1 of lactation, and totally absent by day 5 of lactation; in contrast, the major fall in enzyme activity that we have observed in the present study occurs after day 1 of lactation.

The reason for the fall in 11β-hydroxysteroid dehydrogenase activity after parturition may be related to cellular location. Since enzyme activity was high in virgin rats and

decreased after parturition, it is possible that adipose cells may be the main source of this enzyme. Preliminary studies in this laboratory (Smith and Funder, unpublished) have shown varying levels of 11β-hydroxysteroid dehydrogenase activity in adipose tissue from other sites. Separation of cell types and determination of the levels of the enzyme activity in both adipocytes and epithelial cells of mammary glands is at present being undertaken to address the questions of tissue localization in the mammary gland, and the possibility of cell-cell interactions in such pre-receptor mechanisms of steroid specificity.

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