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RETENTION OF GLUCOCORTICOID BY ISOLATED MAMMARY TISSUE MAY COMPLICATE INTERPRETATION OF RESULTS FROM IN VITRO EXPERIMENTS

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SUMMARY: When mammary gland explants from mid-pregnant rats were incubated with insulin (5 μg/ml) and [^{3}H]cortisol (5 μg/ml) for one day, the tissue accumulated 1.69 μg cortisol/g wet tissue. During a second incubation with insulin and prolactin (5 μg/ml), only 20% of the steroid was lost per day. Such retention of glucocorticoid had an important biological consequence: the tissue exposed for one day to insulin and cortisol showed a transient stimulation of casein synthesis during a subsequent, five-day incubation with insulin and prolactin. No casein synthesis was detected, if the first culture medium contained only insulin. In conclusion, mammary gland explants from mid-pregnant rats require a glucocorticoid for casein synthesis, but this requirement may be obscured if the explants are initially incubated in medium containing cortisol, since they are capable of accumulating and retaining this steroid. Similar interpretative difficulties may arise in studies on other steroid-tissue relationships.

INTRODUCTION

The interaction of a hormone with its target tissue can trigger a series of events which becomes transiently independent of the continued presence of that hormone in the external milieu. Insulin activity was demonstrated in the diaphragm (1), mammary gland (2) and adipose tissue (3) several hours after initial exposure to the hormone. Mammary gland explants exhibited enhanced RNA synthesis 4 hours after initial exposure to prolactin (4). In contrast to the short-lived effects of protein hormones, both the effects and the intracellular presence of estradiol can be detected for several days after it has been removed from the culture medium (5).

Evidence which suggests that either glucocorticoid or its effects persist in mammary tissue has also been reported. The synthesis of

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casein by mouse mammary explants requires insulin, glucocorticoid and prolactin (6); yet, a short-lived stimulation of casein synthesis can occur in mammary explants cultured with only insulin and prolactin after previous exposure to cortisol (F)[1] in vitro (7). Even freshly isolated tissue contains enough endogenous glucocorticoid to stimulate casein synthesis transiently, when the tissue is incubated without F (8).

This study further investigates the question of whether or not mammary tissue, which has been exposed to glucocorticoid in vitro, can retain the steroid and whether such residual hormone might exert biological activity. Answers to these questions would be relevant to the interpretation of earlier studies.

MATERIALS AND METHODS

Ovine prolactin (NIH-P-S-13) was kindly provided by the Hormone Distribution Program, NIAMDD, crystalline porcine insulin (lot 615-08E-220) was a gift from Eli Lilly Company and cortisol was purchased from Calbiochem. Casein (Hammerstein) and rennin were obtained from ICN Pharmaceuticals, Inc., Medium 199 was from Grand Island Biological Company and thin-layer chromatography plates (Sil G-50 UV_{254}, 0.5 mm thick) were purchased from Brinkman Instruments, Inc. [1,2,6,7,-^{3}H(N)]-cortisol (91 Ci/mmol), [4-^{14}C]cortisol (55 mCi/mmol) and [^{33}P]orthophosphoric acid, carrier-free, were obtained from New England Nuclear.

Mammary gland explants were prepared from 10-12 day pregnant rats (Sprague-Dawley) and incubated in Medium 199 as described previously (6); media and lens paper supports were changed daily. All experiments involved a double incubation: initially, explants were cultured with either I (5 µg/ ml) or I and F (5 µg/ml). After one day, the media were changed to ones containing either I, IP or IFP; incubation was continued an additional five days (days 2-6).

Casein was measured by calcium-rennin precipitation (6); the explants were pulsed with [^{33}P]orthosphosphoric acid (10 µCi/ml) for 4 h. Using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (9), 88% of the radioactivity in the precipitates from stimulated explants was identified as rat casein.

Cortisol uptake and retention were measured by incubating the explants in Medium 199 containing I (5 µg/ml), F (5 µg/ml) and [^{3}H]F (12.4 µCi/ml); after one day, the medium was changed to IP. Then at daily intervals, one set of explants was washed on filter paper with 5 x 2 ml of 0.9% saline (w/v). [^{3}H]F was extracted from the homogenates and purified as described by Carson et al. (10), except that the Sephadex LH-20 chromatography step was omitted.

1/ Abbreviations: I, insulin; F, cortisol; P, prolactin.

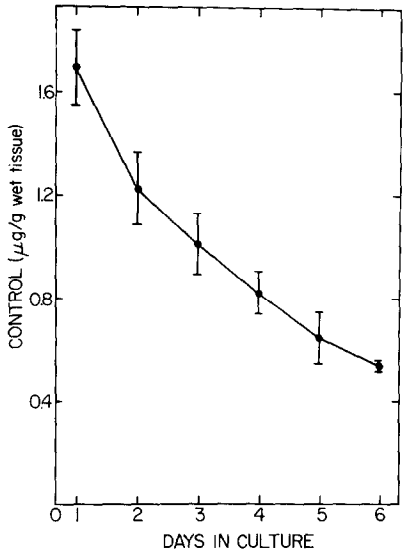
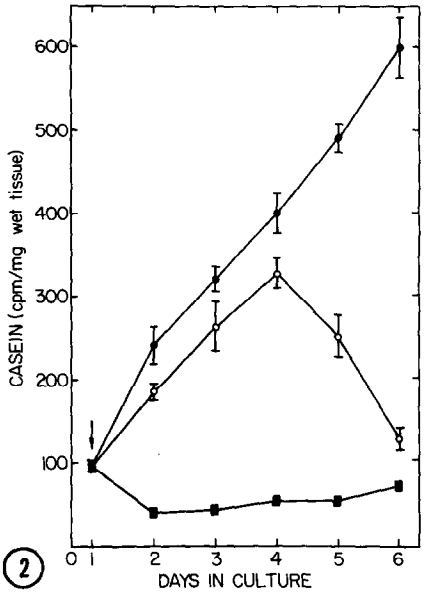


Figure 1: The accumulation and retention of F in mammary gland explants from mid-pregnant rats. Explants were cultured in Medium 199 containing I (5 μg/ml), F (5 μg/ml) and [^{3}H]F (12.5 μCi/ml) for one day, followed by five days with I and P (5 μg/ml). [^{3}H]F was extracted from homogenates of the washed explants and purified as described in Materials and Methods. Each point represents the mean from a single experiment performed in triplicate; the brackets represent one S.E.M.

RESULTS AND DISCUSSION

When mammary gland explants from mid-pregnant rats were incubated with I and [^{3}H]F for one day, the F content of the explants was 1.69 μg/g wet tissue, and the level declined approximately 20% each day during the subsequent incubation with IP (Fig. 1). It is noteworthy that 99.0 ± 0.2% of all the radioactivity remained as [^{3}H]F. This lack of metabolism may be due to the fact that corticosterone, rather than F, is the natural glucocorticoid in rats. However, bovine mammary gland slices do not metabolize F either (11), even though this steroid comprises 50% of the serum glucocorticoid activity in the cow (12).

In order to investigate the biological effects of the retained steroid, casein synthesis was measured during the second incubation. When explants are initially incubated with IF for one day and then cultured with IP, a stimulation of casein synthesis can be detected by day 2 (i.e., one day following exposure to IP) and synthesis rises



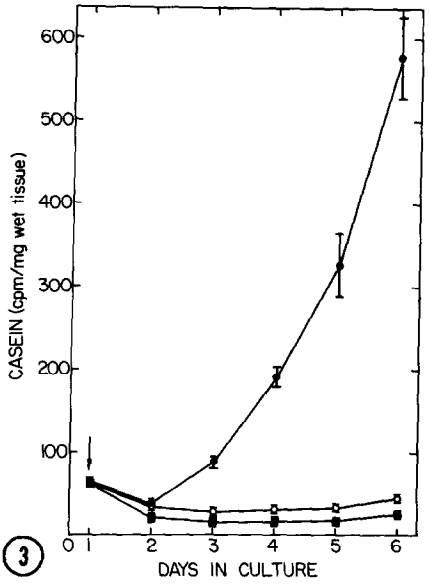


Figure 2: Time course for casein synthesis under different culture conditions. Mammary gland explants from mid-pregnant rats were cultured in Medium 199 containing I (5 µg/ml) and F (5 µg/ml) for one day; then the medium was changed (arrow) to one containing I (■), IP (o) or IFP (●). P was present at a concentration of 5 µg/ml; casein was determined by calcium-rennin precipitation (6). Each point represents the mean of three experiments, each performed in triplicate; the brackets represent one S.E.M.

Figure 3: Time course for casein synthesis under different culture conditions. Mammary gland explants from mid-pregnant rats were cultured in Medium 199 containing I (5 µg/ml) for one day; then the medium was changed (arrow) to one containing I (■), IP (o) or IFP (●). F and P were present at concentrations of 5 µg/ml, each; casein was determined by calcium-rennin precipitation (6). Each point represents the mean of three experiments, each performed in triplicate; the brackets represent one S.E.M.

progressively through day 4 (Fig. 2). During this period, casein synthesis in the IP system almost parallels that in the IFP system, although it averages 20-25% less in the former system. However, after day 4, casein synthesis declines markedly in the IP system, while it continues to rise in the IFP system. When the concentration of F in the first incubation medium is reduced to 1 µg/ml, the stimulation of casein synthesis is similar to that observed with 5 µg/ml but is more transient, becoming maximal on day 2 or 3 (data not shown). If the explants are initially cultured with I alone, instead of IF, there is never any stimulation of casein synthesis detectable in the IP system, although

casein synthesis in the IFP system increases normally after a short delay (Fig. 3).

Mammary gland accumulation of glucocorticoid is not unique to the rat. Using isolated perfusion techniques, the guinea pig lactating gland takes up 0.9 μg F/g/h, when F is present in the perfusate at a concentration of 1.3-3 μg/ml (13); and the goat lactating gland accumulates 60 ng F/g/h, when the concentration of F is only 92 ng/ml perfusate (14). In vitro, acini from rat lactating gland can take up corticosterone, although only 31% of the accumulated steroid is corticosterone, since the remainder becomes metabolized (15). In all three species, the uptake of glucocorticoid is proportional to the steroid concentration in the perfusate or medium.

Recently, similar step-down experiments have been reported by Matusik and Rosen (16). Mammary gland explants from pregnant rats were incubated for two days with IF; after a second two-day incubation with IP, casein mRNA was induced. It was concluded that F is not necessary for the formation of casein mRNA, because the second incubation medium did not contain F. However, the present study demonstrates that F is retained for several days during the second incubation, and that the residual F is necessary for the observed stimulation of casein synthesis. The conclusion that formation of casein mRNA is essentially independent of glucocorticoid may be correct, if the hormonal requirements for transcription and translation differ; however, the question needs to be re-examined using tissue which does not contain detectable levels of the hormone. More generally, caution should be exercised in the interpretation of tissue responses to multi-hormone systems, since an observed effect may not be exclusively attributable to the hormone added last.

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