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Is EGF a Physiological Inhibitor of Mouse Mammary Casein Synthesis? Unphysiological Responses to Pharmacological Levels of Hormones

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Summary. It has been observed that EGF inhibits the induction of casein synthesis by mouse mammary tissue in vitro in addition to acting as a promoter of mammary epithelial proliferation. However, since the circulating level of EGF increases during lactation, and since functional EGF receptors are retained by the lactating cells, it seemed unlikely that EGF is an inhibitor of mammary differentiation in vivo. The current studies demonstrate, in fact, that EGF inhibits the induction of casein synthesis in vitro only when insulin is present in the culture medium at unphysiologically high concentrations. Other artifactual responses to high levels of hormones are described.

Epidermal growth factor has been implicated in the growth of mouse mammary epithelium (1,2). The factor also has been reported (3-5) to inhibit casein and α-lactalbumin syntheses by the epithelial cells from pregnant and lactating mice in vitro, in the presence of insulin, glucocorticoid and prolactin. Accordingly, it has been postulated (3) that EGF has a dual function in mouse mammary development. However, the increased synthesis of these milk proteins that occurs in the transition from pregnancy to lactation and the concomitant increase in the plasma level of EGF (6), appear inconsistent with the postulate that EGF is an inhibitor of mammary differentiation in the intact animal. These considerations prompted a re-investigation of this problem in relation to

Abbreviations: I, insulin; F, hydrocortisone; P, prolactin; EGF, epidermal growth factor.

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casein synthesis. α-Lactalbumin represents a more complex situation, and its relationship to EGF will be presented in another report.

Materials and Methods

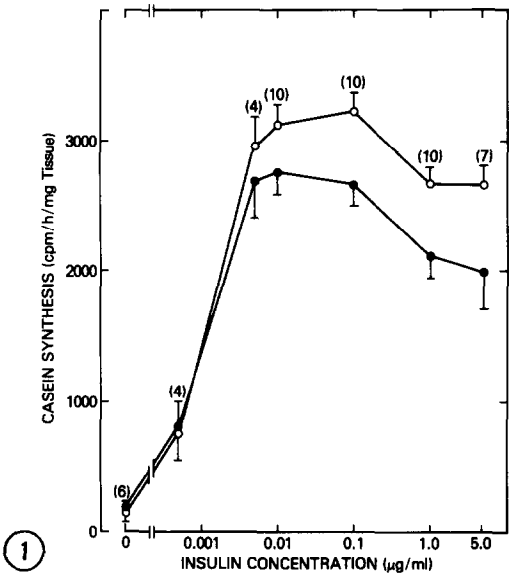
C_3H/HeN, mmtv+ strain of mice, 16-17 days in their first pregnancy, obtained from the NIH animal care facility, were used in these studies. Ovine prolactin (NIH PRL-17) was a gift from the Hormone Distribution Program, NIDDK, NIH, Bethesda, MD. Crystalline porcine insulin was a gift from Eli Lilly and Co., Indianapolis, IN. Epidermal growth factor, receptor grade, and rabbit anti-mouse EGF serum were obtained from Collaborative Research, Inc., Bedford, MA. Hydrocortisone, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), bovine serum albumin, penicillin G and Triton X-100 were obtained from Sigma Chemical Company, St. Louis, MO. Medium 199 with Hanks' salts was purchased from Grand Island Biological Co., Grand Island, NY. Gentamicin was obtained from Microbiological Associates, Walkersville, MD. Culture dishes were obtained from Costar, Cambridge, MA. L-[^{3}H]-Aminoacid mixture was purchased from ICN Pharmaceuticals, Irvine, CA. [^{125}I]-EGF (160 uCi/ug) and Protosol were obtained from Dupont, Boston, MA. Rabbit antisheep IgG was obtained from Pelfreeze Biologicals, Rogers, AR. Production of anti-mouse casein serum in sheep and its characterization have been described previously (7). This anti-serum is directed against all the major mouse caseins.

Culture: Abdominal mammary glands were removed under sterile conditions and explants were cultured as described (8). Media were changed daily.

Casein synthesis: The explants were pulsed with 25 uCi/ml L-[^{3}H]-aminoacid mixture for the last 4 hours of the culture period, and the rate of casein synthesis was determined in explant homogenate supernatants as described previously (7). The culture medium contained less than 5 percent of the total synthesized casein.

Results and Discussion

Figure 1 represents an insulin titration curve for the induction of casein synthesis by mammary explants from 17-day pregnant mice, cultured in the presence of glucocorticoid and prolactin, in the absence or presence of EGF. The antiserum used for the assay of casein synthesis is directed at all the major mouse caseins (see Materials and Methods). It can be seen that casein synthesis is dependent on insulin as shown previously, and that the shape of the titration curve is similar to the one reported earlier (8). Of particular interest is the observation that at the lowest insulin concentrations, 20 ng/ml of EGF exerts very little inhibitory effect on the induction of casein synthesis, but that at unphysiologically high concentrations of insulin, i.e., 100 ng/ml and higher, EGF does inhibit the induction by 20 - 25%. Figure 2, derived from Fig. 1, shows the



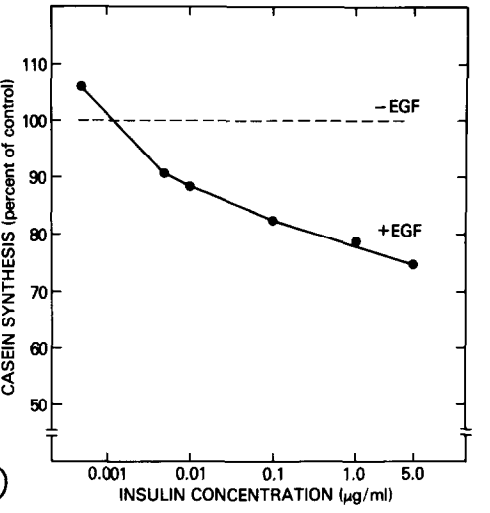


Fig. 1. Insulin titration curve for the induction of casein synthesis in the absence (○) and presence (●) of epidermal growth factor. Mouse mammary explants were cultured for 44h in media containing F (0.1 ug/ml), P (1.0 ug/ml) and I (concentrations as indicated) without or with EGF (0.02 ug/ml), and then pulsed for 4h with [^{3}H]-L-aminoacid mixture (25 uCi/ml). Casein synthesis was determined by immunoprecipitation. Results are mean ± SEM for the number of experiments shown in parentheses.

Fig. 2. Effect of EGF on the induction of casein synthesis as a function of the insulin concentration. The calculated values are derived from the experimental results shown in Fig. 1.

progressively increasing inhibition by EGF as the insulin level is elevated. Greater inhibition by this amount of EGF in the presence of high levels of insulin was observed previously (4). The point to be emphasized is that, with insulin at levels approaching the physiological, EGF is not an effective inhibitor; at unphysiologically high levels of insulin, EGF becomes increasingly inhibitory for casein synthesis.

Even more persuasive arguments which oppose the notion that the mitogenic agent, EGF, is also an inhibitor of lactogenesis derive from considerations of the intact animal. Progesterone is a case in point. It is recognized that this steroid hormone exerts a dual influence on mammary development during pregnancy. Its serum level is high at this time during which it promotes mammary epithelial proliferation and inhibits differentiation. However, about the time of parturition, the serum progesterone level falls precipitously, and lactogenesis ensues shortly

thereafter. It has been established in a number of ways that a major impetus for lactogenesis is, in fact, the decline in the level of progesterone (9). In addition, lactating cells appear to be deficient in progesterone receptors (10).

The EGF pattern contrasts markedly with that of progesterone. The level of EGF in the circulation actually increases as the mouse enters the lactating state (6); this has been confirmed in the present study (data not shown). Moreover, the lactating cell retains functional EGF receptors (4).

We conclude that EGF is not a physiological inhibitor of the induction of mouse casein synthesis. The inhibition which has been observed *in vitro* results from the use of unphysiologically high levels of insulin in the culture medium. Furthermore, the increasing levels of plasma EGF, and the retention of functional EGF receptors as the gland in the intact animal progresses to its most highly differentiated state, the lactating state, are inconsistent with the postulate (3,5) that EGF is an inhibitor of this differentiation.

Artifactual, i.e., unphysiological, effects of pharmacological concentrations of insulin *in vitro* have been reported previously (11,12). Insulin is known to be an anti-lipolytic hormone *in vivo* and also *in vitro* when present at approximately physiological levels. However, it promotes lipolysis at high concentrations (11,12). Unphysiologically high concentrations of prolactin can also produce artifactual effects *in vitro*. Thus, when prolactin is used at 5 ug/ml, hydrocortisone at $2.8X10^{-7}M$ produces a differential effect on the induction of casein and α-lactalbumin syntheses by mouse mammary explants (13). Under these conditions, casein synthesis is increased, while α-lactalbumin synthesis is inhibited. However, when prolactin is present at a more physiological level (0.5 ug/ml), a level which is more than enough to induce casein synthesis maximally *in vitro* (14), this differential effect is markedly reduced (15).

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