ACTIVATION OF CASEIN SYNTHESIS BY PROSTAGLANDINS PLUS SPERMIDINE IN MAMMARY GLAND EXPLANTS OF MICE*

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SUMMARY: Prostaglandins B_2, E_2 or $F_{2\alpha}$ in combination with 0.5 mM spermidine stimulated casein synthesis in mouse mammary gland explants in a prolactin-like manner. Also, methyl GAG, an inhibitor of polyamine synthesis, abolished the stimulation of casein synthesis by prolactin but did not abolish the effect of spermidine plus a prostaglandin.

INTRODUCTION

Prostaglandins (PG)B_2, E_2 and $F_{2\alpha}$ stimulate RNA biosynthesis in a prolactin-like manner in mouse mammary gland explants that have been preincubated with insulin plus hydrocortisone for 2 days (1, 2). These same prostaglandins, however, have no effect on the rate of casein biosynthesis under identical experimental conditions. But they do attenuate the time of onset of the prolactin stimulation of casein synthesis (2). Indomethacin, an inhibitor of prostaglandin biosynthesis, abolishes the effects of prolactin on both RNA and casein biosynthesis (1, 2). The participation of one or more of the prostaglandins in the regulation of the production of milk substances thus seems possible.

Recent studies have also suggested that the polyamines may participate in the regulation of lactational processes. Concentrations of spermidine and spermine increase several fold in the mammary glands of rats during pregnancy and lactation (3). Also, prolactin in concert with insulin plus hydrocortisone increases spermidine levels more than three fold in mouse mammary gland explants incubated for 2 days with these hormones (4). It has further been shown that

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spermidine can be substituted for hydrocortisone but not for prolactin in the triple hormone combination (insulin, a glucocorticoid and prolactin) needed to stimulate the production of milk proteins in mouse mammary gland explants (4, 5). Interestingly, methylglyoxal bis-guanylhydrazone (methyl GAG), an inhibitor of polyamine synthesis, abolishes the stimulation of casein synthesis by prolactin when insulin and hydrocortisone are also present in the incubation medium (4). Methyl GAG, however, does not suppress the stimulation of casein synthesis by this triple hormone combination when spermidine is also added to the incubation medium. It thus seems possible that, in part, the stimulation of milk protein synthesis by prolactin in the mammary gland may be mediated by elevated levels of spermidine. The alternative is that elevated levels of spermidine may merely be required for prolactin to express its actions on milk protein synthesis. In any event, it seems clear that the polyamines are not the sole mediators of prolactin's effects on milk protein synthesis since spermidine will not substitute for prolactin in the triple hormone combination needed to activate casein and lactalbumin synthesis (5). The present studies were carried out to determine if both the prostaglandins and polyamines may also be involved in the activation of milk protein synthesis by prolactin.

MATERIALS AND METHODS

Swiss-Webster female mice were purchased from Spartan Research Animals, Inc., Haslett, Michigan. Mammary gland explants were prepared from these animals when they were 12-14 days pregnant. Methods used to prepare the explants and details of incubation procedures used were described earlier (6). Substances donated for use in these experiments were from the following sources: Ovine prolactin (NIH-P-S-10) from the National Institute of Arthritis and Metabolic Diseases; Porcine insulin from the Eli Lilly Company; Prostaglandins from the Upjohn Company. Methyl GAG, arachidonic acid and spermidine were purchased from the Sigma Chemical Company.

The extent of [^{3}H]-leucine incroporation into casein in the mammary gland explants was estimated by methods described earlier (6).

RESULTS AND DISCUSSION

Table 1 shows the effects of combinations of prostaglandins, arachidonic acid, spermidine and prolactin on the rate of [^{3}H]-leucine incorporation into

Table 1. Effect of various combinations of prolactin, prostaglandins, arachidonic acid and spermidine on casein synthesis in mammary gland explants.

Agent Added	^{3}H-Leucine incorporation into casein (d.p.m./mg wet wt.)				
	Control	Spermidine	Prolactin	Spermidine + Agent	Prolactin + Spermidine + Agent
Arachidonic Acid	293 ± 7	---	442 ± 27	379 ± 14	458 ± 29
PGB_2	387 ± 10	---	624 ± 36	595 ± 37	623 ± 44
PGE_2	284 ± 22	297 ± 11	397 ± 13	390 ± 23	384 ± 15
$PGF_{2\alpha}$	342 ± 4	344 ± 21	457 ± 37	460 ± 36	477 ± 31
$PGF_{1\alpha}$	209 ± 7	---	316 ± 28	215 ± 6	---

Mammary gland explants from midpregnant Swiss-Webster mice were preincubated for 2 d in medium containing insulin (2.5 µg/ml) and hydrocortisone (2.5 µg/ml) by methods described earlier (6). A PG (50 µg/ml), arachidonic acid (50 µg/ml), prolactin (2.5 µg/ml) and (or) 0.5 mM spermidine was then added to the medium and incubation was continued for 16 h. ^{3}H-Leucine (0.5 µCi/ml, 50 Ci/mmole) was added to the incubation vessels 2 h prior to termination of the incubations. Numbers are means ± standard errors of explants from 7 flasks.

casein in mammary gland explants that had been preincubated for 2 days with insulin plus hydrocortisone. Neither spermidine, by itself, nor the prostaglandins, by themselves (2), affected the rate of [^{3}H]-leucine incorporation into casein. The prostaglandins B_2, E_2 and $F_{2\alpha}$, however, when combined with 0.5 mM spermidine acted to enhance the rate of casein synthesis. Further, the effects of the prostaglandins plus spermidine were non-additive to a maximally stimulatory concentration of prolactin; this observation is compatible with the idea that the actions of these agents is via a mechanism similar to that of prolactin. $PGF_{1\alpha}$ when combined with spermidine had no effect on the rate of [^{3}H]-leucine incorporation into casein. $PGF_{1\alpha}$ also has no effect on the rate of RNA synthesis in mammary gland explants; this is in contrast to the PGs B_2, E_2 and $F_{2\alpha}$ which stimulate the rate of RNA synthesis(2). Arachidonic acid, a precursor for the synthesis of the prostaglandins of the 2 series, also acted in concert with spermidine to stimulate casein synthesis. Interestingly, arachidonic acid (like PGs B_2, E_2 and $F_{2\alpha}$) stimulates the rate of RNA synthesis in mammary gland explants in a prolactin-like manner (7).

Table 2. Effect of methyl-GAG on prolactin and $PGF_{2\alpha}$ plus spermidine stimulation of casein synthesis in mammary gland explants

Agent(s)	Added	^{3}H-Leucine incorporation into casein (d.p.m./mg wet wt.)
Experiment 1	Control	283 ± 15
	Prolactin	526 ± 42
	Methyl GAG	232 ± 17
	Methyl GAG + Prolactin	210 ± 15
Experiment 2	Control	259 ± 17
	Spermidine + $PGF_{2\alpha}$	373 ± 30
	Methyl GAG	173 ± 12
	Spermidine + $PGF_{2\alpha}$ + Methyl GAG	247 ± 17

Incubation conditions were the same as for Table 1 except that prolactin (2.5 μg/ml), 20 μM Methylglyoxal bis-guanylhydrazone (Methyl-GAG), 50 μg/ml $PGF_{2\alpha}$ and(or) 0.5 mM spermidine were present in the medium during the final 16 h incubation period.

Table 2 shows that methyl GAG, an inhibitor of polyamine synthesis, suppresses the early (14-16 hr.) effect of prolactin on casein synthesis. This drug did not, however, abolish the stimulatory effect of spermidine plus $PGF_{2\alpha}$ on casein synthesis. A concentration of methyl GAG 10 fold higher than that needed to abolish the later (2 day) effect of prolactin on casein synthesis (3) was needed to abolish the earlier (14-16 hr.) effect.

The data presently available therefore seems to indicate that the stimulation of casein synthesis by prolactin may involve both the prostaglandins and polyamines. This idea is supported by two types of evidence. First, inhibitors of polyamine and prostaglandin synthesis (2) abolish the stimulation of casein synthesis by prolactin. Second, the action of prolactin on casein synthesis can be mimicked by incubation with the prostaglandins B_2, E_2 or $F_{2\alpha}$ plus spermidine. The acceleration of the rate of spermidine synthesis in the mammary gland may

involve all three of the hormones (insulin, a glucocorticoid and prolactin) required to stimulate the rate of milk protein synthesis since each of these hormones has been shown to activate enzymes within the biosynthetic pathway of the polyamines (3,4,8,9).

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