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Phorbol Myristate Acetate Stimulates RNA and Casein Synthesis in Cultured Mouse Mammary Gland Tissues[1] (42302)

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Abstract. Prolactin and phorbol myristate acetate (TPA) stimulate the rate of [^{3}H]uridine incorporation in cultured mouse mammary gland explants in a similar fashion. Both the time-courses and magnitude of responses were the same; in addition, maximum stimulatory concentrations of TPA and prolactin elicited a nonadditive response when tested together. Nordihydroguaiaretic acid (NDGA), a lipoxygenase inhibitor, abolished both the TPA and prolactin effects on [^{3}H]uridine incorporation. TPA also effected an enhanced rate of [^{3}H]leucine incorporation into a casein-rich phosphoprotein fraction, but only if the explants were also cultured with spermidine.

We have recently reported that phorbol myristate acetate (TPA), a protein kinase C activator, stimulates ornithine decarboxylase (ODC) activity in cultured mouse mammary gland tissues (1). This effect of TPA is expressed in a fashion similar to that elicited by prolactin (PRL). The onset of the TPA and PRL effects on ODC activity occur within 1 hr after exposing cultured mammary tissue to these agents (1, 2). The onset of the effects of prolactin on RNA synthesis and the formation of several milk products occur several hours after exposing mammary gland explants to prolactin (1). The purpose of the present studies was to determine if TPA will behave in a prolactin-like fashion in stimulating macromolecular processes which are activated subsequent to the stimulation of ODC activity in cultured mouse mammary tissues.

Materials and Methods. Midpregnant (10–14 days of pregnancy) Swiss–Webster mice, used in all experiments, were purchased from Harlan Laboratories, Inc. (Indianapolis, Ind.). Ovine PRL (NIH-P-S-14) was a gift from NIAMDD. Other substances were from the following sources: cortisol from Charles Pfizer (New York, N.Y.); medium 199 (M-199) Earle's salts from K. C. Biol., Inc. (Lexexa, Kans.); [5,6-^{3}H]uridine (41.3 Ci/mmole) and [4,5-^{3}H]leucine (59.2 Ci/mmole) from New England Nuclear Corporation (Boston, Mass.); porcine insulin, penicillin, and streptomycin, from Eli Lilly Company (Indianapolis, Ind.); and 4,4′-(2,3-dimethyl-1,4-butanediyl)bis[1,2-benzenediol] (NDGA) and 4-phorbol 12-myristate 13-acetate (TPA) from Sigma, Inc. (St. Louis, Mo.).

Methods used to culture tissues were described earlier (3). Briefly, mice were killed by cervical dislocation and the caudal pair of mammary glands were removed aseptically and placed in M-199 Earle's salts. The glands were cut into pieces weighing 3–5 mg, and 24 explant pieces, four from each of six animals, were placed on siliconized lens paper floating on 5 ml M-199 Earle's salts in sterile tissue culture dishes. All incubations were carried out in a 37°C water bath in an atmosphere of 95% air–5% CO_2. Explants were incubated in media containing insulin (1 μg/ml) plus cortisol (10^{-7} *M*) for 36 hr. PRL and/or other agents were then added and incubations were continued for 6 hr (RNA experiments), or 16 hr (casein experiments). For the RNA experiment [^{3}H]uridine was added to the culture medium for the final hour of incubation and the specific activity of the ^{3}H in the RNA was subsequently assessed by the methods of Munro and Fleck (5) with minor modifications (3). For the casein experiments [^{3}H]leucine was added to the culture medium for the final 2 hr of incubation and the extent of [^{3}H]leucine incorporated into a casein-enriched fraction containing phosphoproteins which are isoelectrically precipitable at pH 4.6 was determined (6). Statistical

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TABLE I. EFFECT OF TPA CONCENTRATION ON [^{3}H]URIDINE INCORPORATION INTO RNA[a]

[TPA] (μg/ml)	[^{3}H]Uridine incorporation into RNA (dpm/μg RNA)	P
0	136 ± 5*	—
0.1	147 ± 6	N.S.
1.0	160 ± 7	<0.05
5.0	187 ± 9	<0.05
10.0	179 ± 6	<0.05
20.0	183 ± 9	<0.05

[a] Explants were cultured for 36 hr with 10^{-7} *M* cortisol plus 1 μg/ml insulin. TPA, at the concentrations indicated above, was then added to certain flasks and incubations continued for 6 hr. [^{3}H]Uridine (0.5 μCi/ml) was present for the final 1 hr of incubation.

* Numbers represent the means ± SE of six pools of explants derived from six animals.

TABLE III. TIME-COURSE OF TPA AND PROLACTIN STIMULATION OF [^{3}H]URIDINE INCORPORATION INTO RNA[a]

Incubation time (hr)	[^{3}H]Uridine incorporation into RNA (dpm/μg RNA)		
	Control	Prolactin	TPA
2	106 ± 5*	101 ± 6*	107 ± 9*
3.5	115 ± 8	115 ± 5	131 ± 7
6	121 ± 7	166 ± 4**	163 ± 6**
8	118 ± 3	177 ± 8**	174 ± 7**

[a] Explants were cultured for 36 hr with 10^{-7} *M* cortisol plus 1 μg/ml insulin. TPA (10 μg/ml) or prolactin (1 μg/ml) was then added to certain flasks and incubations continued for the times indicated. [^{3}H]Uridine (0.5 μCi/ml) was present for the final 1 hr of incubation.

* Numbers represent the means ± SE of six pools of explants derived from six animals.

** Significantly greater than control with $P < 0.05$.

comparisons were made with Student's *t* test or analysis of variance.

Results. Table I shows the effect of TPA at various concentrations on the rate of [^{3}H]uridine incorporation into RNA in mouse mammary gland explants. TPA at 1 μg/ml was the lowest concentration that elicited a response and all TPA concentrations up to 20 μg/ml elicited stimulatory effects. Table II shows the nonadditivity of the prolactin and TPA responses when these agents were tested together at maximum stimulatory concentrations. The time-courses for the prolactin and TPA actions on [^{3}H]uridine incorporation into RNA are shown in Table III. Both TPA and prolactin effected significant responses at 6 and 8 hr but not at earlier times.

In recent studies it has been shown that NDGA, an inhibitor of lipoxygenase activity, abolishes several of the actions of prolactin in cultured mammary tissues (7). To provide further evidence that TPA and prolactin are functioning via similar mechanisms on stimulating RNA synthesis, the effect of NDGA on the TPA response was tested. Table IV

TABLE II. NONADDITIVITY OF PROLACTIN AND TPA ACTIONS ON THE RATE OF [^{3}H]URIDINE INCORPORATION INTO RNA

Addition (μg/ml)	[^{3}H]Uridine incorporation[a] into RNA (dpm/μg RNA)	P
—	109 ± 4*	—
10, TPA	182 ± 9	<0.05
1, Prolactin	181 ± 9	<0.05
10, TPA plus 1, Prolactin	218 ± 16	<0.05

[a] Explants were cultured for 36 hr with 10^{-7} *M* cortisol plus 1 μg/ml insulin. TPA (10 μg/ml) and/or prolactin (1 μg/ml) was then added to certain flasks and incubation continued for 6 hr. [^{3}H]Uridine (0.5 μCi/ml) was present for the final 1 hr of incubation.

* Numbers represent the means ± SE of six pools of explants derived from six animals.

TABLE IV. EFFECT OF NDGA ON TPA AND PROLACTIN STIMULATION OF THE RATE OF [^{3}H]URIDINE INCORPORATION INTO RNA[a]

Addition	[^{3}H]Uridine incorporation into RNA (dpm/μg RNA)		
	Control	TPA (10 μg/ml)	Prolactin (1 μg/ml)
—	182 ± 11*	331 ± 19	278 ± 9[c]
50 μ*M* NDGA	190 ± 6	217 ± 8[b]	218 ± 9[b]

[a] Explants were cultured for 36 hr with 10^{-7} *M* cortisol plus 1 μg/ml insulin. TPA, prolactin, and/or NDGA were then added to certain flasks and incubation continued for 6 hr. [^{3}H]Uridine (0.5 μCi/ml) was present during the final hour of incubation.

[b] Less than TPA or prolactin stimulated increments.

[c] Greater than control with $P < 0.05$.

* Numbers represent the means ± SE of six pools of explants derived from six animals.

shows that 50 μM NDGA significantly reduced the magnitude of the TPA and prolactin actions on the rate of [^{3}H]uridine incorporation into RNA in the mammary gland explants.

Table V shows the results of an experiment in which the effect of TPA on the rate of [^{3}H]leucine incorporation into casein was assessed. By itself, TPA had no effect on casein synthesis. In the presence of 0.5 or 1.0 mM spermidine, however, TPA did elicit a response which was nonadditive to that caused by prolactin.

Discussion. These studies clearly show that TPA has prolactin-like actions on RNA and casein synthesis in cultured mouse mammary gland explants. The characteristics of the TPA response on RNA synthesis were not different from those effected by PRL in that both the time-course and maximum responses were the same. In addition, maximum stimulatory concentrations of TPA and PRL exhibited a nonadditive response when these agents were tested in concert. All these observations are compatible with the conclusion that the effects of TPA and PRL on RNA synthesis are carried out via a common mechanism. Similar observations were reported earlier (1) regarding the actions of PRL and TPA on ornithine decarboxylase activity (ODC) in cultured mouse mammary tissues. The effect of TPA on casein synthesis was apparent only if spermidine was present in the culture medium. In addition, the magnitude of the TPA stimulation of casein synthesis was never as great as that evoked by prolactin. In studies not presented, TPA was not found to have a prolactin-like effect on the rate of [^{14}C]acetate incorporation into triglycerides; this was the case both in the presence and absence of spermidine in the culture medium. The effects of prolactin on milk product formation, i.e., casein and triglyceride synthesis, appear to be more complex than the earlier expressed effects on ODC activity and RNA synthesis.

The fact that TPA effects prolactin-like changes in the mammary gland is compatible with earlier studies in which phospholipase C (PLC) was shown to elicit prolactin-like effects on ODC activity (8), and RNA synthesis (9). PLC also was shown to effect an increased rate of casein synthesis, but only when spermidine was also present in the culture medium (9). PLC cleaves phosphodiester bonds of phospholipids to yield a diglyceride plus a phosphorylated moiety characteristic of the phospholipid serving as its substrate. The diglycerides are known to activate an enzyme, protein kinase C, which catalyzes the phosphorylation of certain tyrosyl residues on specific proteins. Since the only known specific action of TPA on cells is its stimulation of protein kinase C activity (10, 11), it would seem that the actions of TPA, PLC, and perhaps PRL may all be channeled through a similar mechanism; i.e., they may all involve the activation of protein kinase C. Since protein kinase C is a calcium dependent enzyme, the above conclusion is supported by the observation that all of prolactin's actions in mammary tissues are abolished when calcium is unavailable to the tissues (12) or available at extracellular concentrations below 10 μM (13).

TABLE V. EFFECT OF TPA, PROLACTIN, AND SPERMIDINE ON THE RATE OF [^{3}H]LEUCINE INCORPORATION INTO CASEIN[a]

Spermidine concentration (mM)	[^{3}H]Leucine incorporation into casein (dpm/mg wet tissue weight) Control	+10 μg/ml TPA	+1 μg/ml prolactin
0	276 ± 12*	309 ± 30	468 ± 36[b]
0.25	345 ± 39	303 ± 27	585 ± 36[b]
0.5	312 ± 18	426 ± 30[b]	519 ± 48[b]
1.0	363 ± 6	450 ± 33[b]	684 ± 54[b]

[a] Explants were cultured for 36 hr with 10^{-7} M cortisol plus 1 μg/ml insulin. Prolactin, TPA, and/or spermidine were then added to certain flasks and incubations were continued for 16 hr. [^{3}H]Leucine (1 μCi/ml) was in the culture medium for the final 2 hr of incubation.

[b] Greater than with control with $P < 0.05$.

* Numbers represent the means ± SE of six pools of explants derived from six animals.

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