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PROLACTIN STIMULATION OF ORNITHINE DECARBOXYLASE ACTIVITY IN THE MAMMARY GLAND MAY INVOLVE AN ACTIVATION OF PROTEIN KINASE C[1]

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Phorbol myristate acetate (TPA), a protein kinase C activator, stimulates ornithine decarboxylase (ODC) activity in mammary gland explants derived from 12-14 day pregnant mice. The calcium ionophore A23187 similarly stimulates ODC activity. Maximally stimulatory concentrations of TPA and A-23187 produce additive responses. The prolactin (PRL) stimulation of ODC activity is nonadditive to that caused by TPA, A23187 or TPA plus A23187. These observations are compatible with the thesis that the stimulation of ODC activity by PRL may occur via an activation of protein kinase C.

We have recently observed that exogeneously added phospholipase C (PLC) will stimulate ornithine decarboxylase (ODC) activity in a manner similar to prolactin (PRL) in cultured mouse mammary gland explants (1). PLC cleaves phosphodiester bonds of phospholipids to yield a diglyceride plus a phosphorylated moiety characteristic of the phospholipid serving as the substrate.

Protein kinase C is a recently characterized plasma membrane constituent which is a calcium dependent enzyme that is activated by diacyl glycerides (2,3). We reasoned that the effect of PRL and PLC on stimulating ODC activity in the mammary gland may occur via a release of diglycerides and a consequential activation of protein kinase C in the plasma membrane. Accordingly, phorbol myristate acetate (TPA), a substance known to stimulate protein kinase C (2,3), was tested for its ability to stimulate ODC activity in cultured mouse mammary gland explants. Since protein kinase C is known to be a calcium dependent enzyme, the TPA effect on ODC activity was also tested in the presence of A23187, a calcium ionophore.

MATERIALS AND METHODS

Midpregnant (10-14 days of pregnancy) Swiss-Webster mice, used in all experiments, were purchased from Harlan Laboratories, Inc. (Indianapolis, IN); ovine prolactin (NIH-P-S-14, 31.0 I.U./mg) was a gift from NIAMD. Other substances were from the following sources: Cortisol from Charles Pfizer (New York, NY); Medium 199 Earle's Salts from K.C. Biol. Inc. (Lenexa, KA); [1-^{14}C] ornithine (49.1 mCi/mmol) and hyamine hydroxide from New England Nuclear Corp. (Boston, MA); Bovine insulin, penicillin and streptomycin from Eli Lilly Co. (Indianapolis, IN); 4 -Phorbol 12-myristate 13-acetate (TPA) and A23187 from Sigma Chemical Co. (St. Louis, MO).

Mice were killed by cervical dislocation, and the caudal pair of mammary glands were removed and placed

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Table I. Effect of TPA on ODC activity*

TPA concentration (μg/ml)	ODC activity (pmols/30 min/10 mg tissue)
0	0.56 ± 0.30
0.1	0.92 ± 0.25
1.0	1.78 ± 0.36**
10.0	1.86 ± 0.32**

*Explants were incubated for 24 hours with 1 μg/ml insulin plus 10^{-7}M cortisol. TPA was then added to certain flasks and incubations continued for 6 hours. Numbers in the Table represent the mean ± SE of 3 observations with tissue derived from 6 animals.
**Significantly greater than control with $p < .05$.

in medium 199 Earle's salts. Explants (about 3 mg each were then prepared as described earlier (4). After preparation, the explants were placed on siliconized lens paper floating on 2 ml medium 199 Earle's salts containing 1 μg/ml insulin plus 10^{-7}M cortisol. All incubations were carried out in polypropylene vials maintained at 37C in an atmosphere of 95% O_2 - 5% CO_2. After a one day culture, PRL, TPA and/or A23187 were added to certain vials and incubations were continued for the times specified in the Tables. Concentrated stock solutions of TPA were contained in ethanol; A23187 was contained in dimethyl sulfoxide (DMSO). Ethanol and DMSO concentrations in the culture medium never exceeded 0.125%; neither ethanol nor DMSO at concentrations of 0.125% or less affected basal ODC activities or the magnitude of PRL stimulation of ODC activity. After culture, the tissues were homogenized in 50 mM Tris buffer (pH 7.4), and ODC activity was determined using a modification (5) of the methods described by Janne and Williams-Ashman (6). ODC activity is expressed as picomoles of CO_2 produced per 30 min per 10 mg wet tissue weight. Statistical comparisons were made with Student's t-test or an analysis of variance where appropriate.

RESULTS

Tables I and II show the effects of TPA and A23187 respectively on ODC activity. TPA at concentrations of 1 and 10 μg/ml were equally efficacious in stimulating ODC activity. A23187 was maximally stimulatory when concentrations between 10 and 50 μM were employed.

Table III shows the time-course for the effects of PRL, TPA and TPA

Table II. Effect of A23187 on ODC activity*

A23187 concentration (μM/ml)	ODC activity (pmols/30 min/10 mg tissue)
0	0.53 ± 0.08
1	0.94 ± 0.19
10	2.06 ± 0.17**
25	2.44 ± 0.19**
50	3.08 ± 0.72**

*Explants were incubated for 24 hours with 1 μg/ml insulin plus 10^{-7}M cortisol. A23187 was then added to certain flasks and incubations continued for 6 hours. Numbers in the Table represent the mean ± SE of 3 observations with tissue derived from 6 animals.
**Significantly greater than control with $p < .05$.

Table III. Time-course for PRL, TPA, and TPA + A23187 stimulation of ODC activity*

Time (hours)	ODC activity (pmol/30 min/10 mg tissue) control	PRL	TPA	TPA +A23187
1	3.14±0.77	8.61±0.19**	2.06±0.53	1.36±0.25
2	6.39±1.97	23.7 ±0.61**	8.94±1.80	9.22±2.02
4	3.19±0.02	40.0 ±6.7**	7.06±0.94**	16.5 ±2.0**+
6	0.44±0.11	9.81±1.75**	2.61±0.14**	4.97±0.64**+
8	0.44±0.08	6.94±1.39**	1.61±0.08**	4.75±0.36**+

*Explants were incubated for 24 hr with 1 μg/ml insulin plus 10^{-7}M cortisol. PRL (1 μg/ml), 5 μg/ml TPA or 5 μg/ml TPA + 25 μM A23187 were then added to certain flasks and incubations continued for the times listed above. Numbers in the Table represent the mean ± SE of 3 observations with tissue derived from 6 or more animals.
**Significantly greater than control with $p < .05$.
+Significantly greater than TPA by itself with $p < .05$.

plus A23187 on ODC activity in cultured mouse mammary tissues. As was reported earlier (1), ODC activity increases transiently merely because of the initial change of culture medium. Increased ODC activities in response to PRL are apparent at all times between 1 and 8 hours; maximum ODC activity was noted 4 hours after PRL exposure. Significant effects of TPA on ODC activity were observed at 4 hours and subsequent times, but not at 1 or 2 hours.

TPA plus A23187 also effected a stimulation of ODC activity with a time-course similar to that elicited with TPA by itself. The magnitude of response however was about twice as great when A23187 was added with TPA thus demonstrating additivity of the TPA and A23187 responses. For the time-course of the TPA plus A23187 response, maximal stimulatory concentrations of TPA and A23187 were employed. These concentrations were determined by the titration experiments shown in Tables IV and V. TPA concentrations above 2.5 μg/ml were maximally stimulatory (Table IV) and A23187 concentrations above 10 μM maximally potentiated the TPA response (Table V). Also apparent from Table IV is the fact that the TPA and PRL effects on ODC activity were nonadditive, i.e. when TPA was added with PRL, ODC activity was not greater than that which occurred when PRL was added, by itself, to the cultures.

The data in Table VI shows that the TPA plus A23187 effect on ODC activity is also nonadditive to a maximally stimulatory concentration of PRL. This was true when 10, 25 or 50 μM A23187 was present in the culture medium.

Table IV. Effect of TPA concentration on the PRL or TPA plus A23187 actions on ODC activity*

TPA concentration (μg/ml)	ODC activity (pmol/30min/10 mg tissue) Control	Plus PRL	Plus A23187
0	0.61±0.06	9.36±0.78**	
0.1		9.69±1.81**	1.42±0.22**
1.0		8.86±1.11**	2.14±0.14**
2.5		8.56±1.02**	2.97±0.33**+
5.0		10.7 ±1.4**	3.25±0.08**+
10.0	1.98±0.14**	7.36±0.67**	3.81±0.27**+

*Explants were incubated for 24 hours with 1 μg/ml insulin plus 10^{-7}M cortisol. PRL (1 μg/ml), 25 μM A23187 and/or TPA were then added to certain flasks and incubations continued for 7 hours. Numbers in the Table represent the mean ± SE of 3-6 observations with tissue derived from 6 or more animals.
**Greater than control with $p < .05$.
+Greater than the 10 μg/ml TPA value with $p < .05$.

Table V. Effect of A23187 concentration on the PRL or TPA plus A23187 actions on ODC activity*

A23187 concentration (μM)	ODC activity (pmols/30 min/10 mg tissue) Control	Plus PRL	Plus TPA
0	1.00±0.22	11.8 ±2.1**	
5		11.4 ±3.6**	1.44±0.28
10		9.17±2.10**	3.33±0.55**+
25		9.00±1.83**	3.33±0.50**+
50		6.39±0.67**	3.97±0.28**+

*Explants were incubated for 24 hours with 1 μg/ml insulin plus 10^{-7}M cortisol. PRL (1 μg/ml), 10 μg/ml TPA and/or A23187 were then added to certain flasks and incubation continued for 7 hours. Numbers in the Table represent the mean ± SE of 3-6 observations with tissue derived from 6 or more animals.
**Greater than control with $p < .01$.

DISCUSSION

These studies show that the phorbol ester TPA or the combination of TPA plus A23187 stimulates ODC activity in mammary gland explants in a manner similar to that of prolactin. The TPA and TPA plus A23187 responses were nonadditive to that of PRL, thus indicating that these agents are likely functioning via a similar mechanism. Although the time of onset of the TPA and TPA plus A23187 responses were extended (4 hr) from that occuring with PRL (1 hr), maximum ODC activities were observed at the same time (4 hr) after administration of each of these stimulatory agents. The delayed time of onset of the TPA and TPA plus A23187 responses could be due to the diffusion times required for these substances to accumulate at an effective concentration in the PRL-responsive cells.

In earlier studies we observed that the PRL stimulation of ODC activity can be reproduced by administering phospholipase C (PLC) to cultured mammary tissues (1). Since the only currently known specific action of TPA is to stimulate protein kinase C in plasma membranes, and since diacyl glycerides, products of phospholipase C action, are also known to stimulate protein kinase C (2,3), we would conclude that these substances may be having their actions on ODC activation via a stimulation of protein kinase C activity. Because of the similar actions of TPA, PLC and PRL on ODC activation, it would also seem that the effect of PRL on ODC activity may be carried out via an activation of protein kinase C.

Table VI. Nonadditivity of PRL and TPA plus A23187 effects on ODC activity*

Treatment	ODC activity (pmols/30 min/10mg tissue) Experiment	1 Experiment	2 Experiment 3
Control	1.05±0.38	0.61±0.06	0.94±0.19
PRL	14.1±3.3	9.36±0.78	9.47±1.80
TPA	1.53±0.22	1.08±0.14	1.63±0.19
PRL+A23187	10.8±3.6	9.08±0.83	6.39±0.66
TPA+A23187	2.72±0.55	3.81±0.27	3.97±0.27
TPA+A23187+PRL	9.83±2.83**	8.69±0.89**	5.67±0.44**

*Explants were incubated for 24 hours with 1 μg/ml insulin plus 10^{-7}M cortisol. PRL (1 μg/ml), 10 μg/ml TPA and/or A23187 at 10 μM (Experiment 1), 25 μM (Experiment 2), or 50 μM (Experiment 3) were then added to certain flasks and incubations continued for 7 hours. Numbers in the Table represent the mean ± SE of 3 observations with tissue derived from at least 6 animals.
**Nonadditive response.

Further supporting the idea that the TPA activation of ODC activity in the mammary gland may occur via an activation of protein kinase C is the observation that the calcium ionophore A23187 potentiated the magnitude of the TPA stimulation of ODC activity. Protein kinase C is known to be highly sensitive to calcium (2,3), and it is reasonable that the provision of calcium to target cells by the administration of a calcium ionophore, would potentiate the effect of an activator on a calcium dependent enzyme. Regarding the PRL action on ODC activity, it was observed that A23187 did not potentiate the magnitude of the PRL-induced response. This makes sense if PRL is having its primary effect on mammary cells by activating PLC; this mechanism is known to be the way by which many regulatory agents cause perturbations in target cells (2,3). The diacyl glycerides formed from the PLC actions are known to activate protein kinase C; the action of PLC on polyphosphoinositides also releases inositol triphosphate which is known to increase intracellular calcium ion concentrations. Maximal activation of protein kinase C can thus be achieved by raising intracellular calcium and providing diacyl glycerides. It is also of relevance that we have earlier observed that many of the actions of PRL in the mammary gland are calcium dependent (7). It is of further interest that maximal responses in other biological systems have also been found to require the synergistic action of protein kinase C activators (diacylglycerides or TPA) and calcium ionophores (8-11).

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