

## Effects of Mezerein and Diglycerides on Ornithine Decarboxylase Activity in Mouse Mammary Gland Explants<sup>1</sup> (42684)

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*Abstract.* One of the most rapid actions of prolactin in mouse mammary gland explants is the stimulation of ornithine decarboxylase (ODC) activity. Several protein kinase C activators including mezerein, dicaprin, diolein, and 1-oleoyl-2-acetyl-*rac*-glycerol were found to stimulate ODC activity as does prolactin. Both mezerein and the diglycerides produced nonadditive responses when tested with maximum stimulatory concentrations of prolactin. The results of these studies therefore provide further evidence that the prolactin stimulation of ODC activation in the mammary gland may involve an activation of protein kinase C. © 1988 Society for Experimental Biology and Medicine.

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In earlier studies from this laboratory we reported that phorbol myristate acetate (TPA), a protein kinase C activator (1, 2), stimulates ornithine decarboxylase (ODC) activity in mammary gland explants derived from 12- to 14-day pregnant mice (3). Since maximum stimulatory concentrations of TPA and prolactin, when tested together, caused a nonadditive stimulation of ODC activity, we concluded that prolactin may be stimulating ODC activity in the mammary gland via an activation of protein kinase C.

Mezerein, a non-phorbol ester diterpene that is a second stage tumor promoter (4, 5) like TPA, has also been shown to be an activator of protein kinase C (6). Accordingly, mezerein was employed in these studies to determine if it would activate ODC in cultured mouse mammary gland explants. The possible activation of ODC by three diglycerides is also reported in these studies.

**Materials and Methods.** Midpregnant (12-14 days of pregnancy) Swiss-Webster mice, used in all experiments, were purchased from Harlan Laboratories, Inc. (Indianapolis, IN); ovine prolactin (NIH-P-S-16, 30.5 IU/mg) was a gift from NIAID. Other substances were from the following sources: Cortisol from Chas. Pfizer (New York, NY); medium 199 Earl's salts from GIBCO Labo-

ratories (Grand Island, NY); [1-<sup>14</sup>C]-ornithine (57.6 mCi/mmol) and hyamine hydroxide from New England Nuclear Corp. (Boston, MA); bovine insulin, penicillin, and streptomycin from Eli Lilly Co. (Indianapolis, IN); mezerein and 1-oleoyl-2-acetyl-*rac*-glycerol (OAG) from Sigma Chemical Co. (St. Louis, MO); 1,2-diolein and *D*-1,2-dicaprin from Serdary Research Laboratories (London, Ont., Canada).

Methods used in these studies were as follows. Mice were killed by cervical dislocation, and the caudal pair of mammary glands were removed and placed in Hanks' balanced salt solution (HBSS). Explants (about 3 mg each) were then prepared as described earlier (7). The explants were next placed on siliconized lens paper (Dow Corning Corp., Greensboro, NC) floating on 2 ml medium 199 Earl's salts containing 1 µg/ml insulin and 10<sup>-7</sup> M cortisol. All incubations were carried out in sterilized polypropylene vials maintained at 37°C in a humidified atmosphere of 95% O<sub>2</sub>-5% CO<sub>2</sub>. After a 24-h culture, prolactin, mezerein, and/or one of the diglycerides were added to certain vials and incubations were continued for the times specified in the tables and figure. Concentrated stock solutions of mezerein and the diglycerides were contained in dimethyl sulfoxide (DMSO). DMSO concentrations were maintained below 0.2%; at concentrations below 0.2%, DMSO neither affected basal ODC activity, nor did it affect the magnitude of the prolactin stimulation of ODC activity.

After culture, the explants were homoge-

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nized (ground glass homogenizers) in ice-cold 50 mM Tris buffer (pH 7.4). ODC activity was then determined using a modification (8) of the methods described by Williams-Ashman (9). ODC activity is expressed as picomoles produced per 30 min per 10 mg wet tissue weight. Statistical comparisons were made with the Student *t* test or with an analysis of variance followed by Duncan's test.

**Results.** Table I shows the effects of various concentrations of mezerein on ODC activity in mouse mammary gland explants. The lowest mezerein concentration that effected a stimulation of ODC activity was 5  $\mu\text{g/ml}$ . At concentrations of 10  $\mu\text{g/ml}$  and above maximum stimulatory effects of mezerein were noted: the magnitude of these responses was equivalent to that elicited with prolactin. Also, when tested together at maximum stimulatory concentrations, prolactin and mezerein evoked nonadditive responses (Table I).

The time-course for the mezerein stimulation of ODC activity is shown in Fig. 1. A significant stimulation was initially observed 4 hr after exposing the explants to mezerein. Maximum stimulatory effects were observed between 5 and 6 hr and by 8 hr there no longer was a significant response.

The data in Table II show the effects of three diglycerides on ODC activity. We chose to test these particular diglycerides because of their relative water solubility and because they have been shown to be useful as protein kinase C activators in other biological systems.

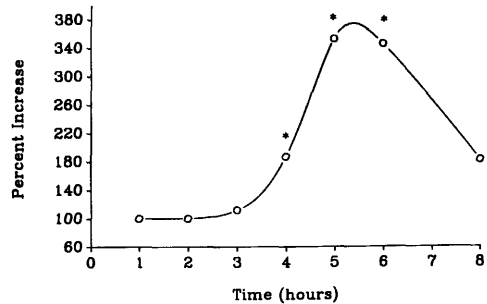


FIG. 1. Time-course for the effect of mezerein on ODC activity in mammary gland explants. Explants were incubated for 24 hr with 1  $\mu\text{g/ml}$  insulin plus  $10^{-7}$  M cortisol. Mezerein (10  $\mu\text{g/ml}$ ) was then added to certain explants and incubation was continued for the times indicated in the figure. ODC activity was then determined. \*Significantly greater than control with  $P < 0.05$ .

cal systems. At a concentration of 50  $\mu\text{g/ml}$ , dicaprin, diolein and OAG approximately doubled ODC activity. At a concentration of 10  $\mu\text{g/ml}$ , only OAG effected a stimulatory response. Each of the diglycerides at 50  $\mu\text{g/ml}$  elicited a nonadditive response with a maximum stimulatory concentration of prolactin.

**Discussion.** The results of these studies clearly show that mezerein as well as three diglycerides stimulate ODC activity in mouse mammary gland explants as does prolactin. The maximum mezerein response was not different from that elicited with prolactin. In addition, prolactin and mezerein evoked nonadditive responses when these agents were tested together. We therefore conclude that prolactin and mezerein are likely functioning via a common metabolic pathway in stimulating ODC activity in the mammary gland.

The time-course for the mezerein stimulation of ODC activity is not identical to that elicited by prolactin. In earlier studies (8) we reported that the onset of the prolactin stimulation of ODC activity is 30–60 min after adding prolactin to the culture medium of mouse mammary explants; this is in contrast to the earliest detectable mezerein response which occurred at 4 hr. Also different is the time of maximum response. A maximum prolactin stimulation of ODC activity is apparent after 2–4 hr, whereas a maximum mezerein response is observed at 5–6 hr. The reason for the time delay in the mezerein

TABLE I. EFFECT OF MEZEREIN AND/OR PROLACTIN ON ODC ACTIVITY<sup>a</sup>

Mezerein concentration ( $\mu\text{g/ml}$ )	ODC activity (pmole/30 min/10 mg tissue)		
	Control	Prolactin	<i>P</i>
0	0.173 $\pm$ 0.023	0.708 $\pm$ 0.094	<0.05
1	0.228 $\pm$ 0.055	0.693 $\pm$ 0.079	<0.05
5	0.543 $\pm$ 0.094*	0.780 $\pm$ 0.055	—
10	0.748 $\pm$ 0.118*	0.772 $\pm$ 0.063	—
20	0.708 $\pm$ 0.055*	0.866 $\pm$ 0.063	—

<sup>a</sup> Explants were incubated for 24 hr with 1  $\mu\text{g/ml}$  insulin plus  $10^{-7}$  M cortisol. Prolactin (1  $\mu\text{g/ml}$ ) and/or mezerein was then added to certain explants and incubation continued for 5 hr. Numbers in the table represent the means  $\pm$  SE of six observations with tissues pooled from six animals.

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

TABLE II. EFFECT OF DIGLYCERIDES AND/OR PROLACTIN ON ODC ACTIVITY<sup>a</sup>

Diglyceride	Diglyceride concentration ( $\mu\text{g/ml}$ )	ODC activity (pmole/30 min/10 mg tissue)		
		Control	+1 $\mu\text{g/ml}$ PRL	<i>P</i>
—	—	0.142 $\pm$ 0.024	0.758 $\pm$ 0.016	<0.05
Diolen	10	0.121 $\pm$ 0.032	—	—
Diolen	50	0.290 $\pm$ 0.032*	0.903 $\pm$ 0.040	<0.05
Dicaprin	10	0.226 $\pm$ 0.032	—	—
Dicaprin	50	0.371 $\pm$ 0.032*	0.742 $\pm$ 0.032	<0.05
OAG	10	0.234 $\pm$ 0.024*	—	—
OAG	50	0.315 $\pm$ 0.016*	0.661 $\pm$ 0.064	<0.05

<sup>a</sup> Explants were incubated for 24 hr with 1  $\mu\text{g/ml}$  insulin plus  $10^{-7}$  M cortisol. Prolactin (1  $\mu\text{g/ml}$ ) and/or the diglycerides were then added to certain explants and incubation continued for 5 hr. Numbers in the table represent the means  $\pm$  SE of at least six observations with tissues pooled from six animals.

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

response may be explained by the time required for mezerein to accumulate at an effective concentration at its site of activation of protein kinase C.

The fact that three diglycerides also activate ODC activity in the mammary gland and the effect of these agents is nonadditive to a maximum stimulatory concentration of prolactin further supports the thesis that prolactin may be stimulating ODC activity via an activation of protein kinase C. Supporting this conclusion are studies in which prolactin-like effects on ODC activity in the mammary gland have been elicited by a number of other agents including mezerein, TPA (3), and phospholipase C (10). Since phospholipase C cleaves phosphodiesterase bonds of phospholipids to yield diglycerides, it is entirely possible that the activation of ODC activity in mammary gland explants by phospholipase C may occur via the generation of diglycerides and their consequent activation of protein kinase C. The results in Table II clearly show that three diglycerides will stimulate ODC activity as does prolactin and the protein kinase C activators mezerein and TPA.

The results of these and earlier studies therefore support the conclusion that the prolactin stimulation of ODC activity in the mammary gland likely involves an activation of protein kinase C.

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