Effects of Mezerein and Diglycerides on the PRL Stimulation of Cell Replication in Nb_2 Node Lymphoma Cells (42959)

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Abstract. **These studies provide further support for the thesis that the activation of protein kinase C is likely involved in the prolactin (PRL) stimulation of mitogenesis in the Nb_2 node lymphoma cell line. The diterpene mezerein is shown to potentiate the mitogenic effect of PRL at a hormone concentration which elicits a less than maximum response. A similar response was observed with two diglycerides, diolein and dicaprin. Neither mezerein nor the diglycerides affected the magnitude of response to a maximum stimulatory concentration of PRL.** [P.S.E.B.M. 1989, Vol 192]

In recent studies it has been reported that 12-*O*-tetradecanoylphorbol 13-acetate (TPA), an activator of protein kinase C (PKC), will potentiate the mitogenic effect of prolactin (PRL) on Nb_2 node lymphoma cells when PRL is employed at a less than maximum stimulatory concentration (1). TPA, like PRL, also stimulates the rate of [^{3}H]thymidine incorporation into DNA in the Nb_2 node lymphoma cells (2, 3). In additional studies inhibitors of PKC were shown to inhibit the PRL stimulation of cell division (4), as well as the PRL stimulation of [^{3}H]thymidine incorporation into DNA in the Nb_2 node cells (5). Taken together, the above observations suggest that the PRL stimulation of cell division in the Nb_2 node lymphoma cells may involve an activation of PKC.

To provide further support for this conclusion, additional activators of PKC were tested on the Nb_2 node lymphoma cells. Substances tested included the diterpene mezerein (6) as well as diglycerides; the diglycerides represent one class of physiological activators of PKC (7–9).

Materials and Methods

The Nb_2 node lymphoma cells were provided by Dr. C. T. Beer of the Cancer Control Agency of British Columbia (Vancouver, British Columbia, Canada). Ovine prolactin (NIH-P-S-17) was a gift from the NIAMDD. Other materials used in these studies were from the following sources: fetal calf serum (FCS), horse serum (HS), and Fisher's medium from Gibco Laboratories (Grand Island, NY); 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, Ontario, Canada); penicillin and streptomycin from Eli Lilly Co. (Indianapolis, IN).

The Nb_2 cells were maintained as suspension culture in 25-cm^2 culture flasks containing "growth medium" (Fisher's medium supplemented with 10% FCS, 10% HS, 1×10^{-4} *M* 2-mercaptoethanol, 50,000 IU/liter penicillin, and 50,000 μg/liter streptomycin). The cells were incubated at 37°C in the presence of a 95% air/5% CO_2 mixture. Fresh medium was added every 72 hr. Twenty-four hours before beginning an assay, the cells were collected by centrifugation at 300*g*. The cells were then resuspended and cultured for 24 hr in "stationary medium" (same components as growth medium except for the deletion of FCS and the reduction of HS from 10 to 4%). The cells were next collected by centrifugation at 300*g*, resuspended in stationary medium, and used for experimentation. When the effects of PRL and/or mezerein or the diglycerides on cell number were to be determined, 1-ml aliquots ($1–1.5 \times 10^5$ cells/ml) were transferred to the wells of sterile multiwell tissue culture plates (24 plates/well). The cells were then cultured for an additional 24–72 hr in the presence of the appropriate concentration of PRL, mezerein, and/or diglycerides. Concentrated stock solutions of mezerein and the diglycerides were contained in

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dimethyl sulfoxide (DMSO). DMSO concentrations were maintained below 0.1%; at concentrations below 0.1%, DMSO affected neither cell number by itself after a 72-h culture nor the magnitude of the PRL stimulation of cell number. Following this incubation the cells were transferred to vials containing 9 ml of Hematall isotonic fluid, and cell number was determined on a Coulter model 2 M counter. Statistical comparisons were made using an analysis of variance and Tukey's HSD procedure or a paired t test where appropriate.

Results

Table I shows the complex effects of various concentrations of mezerein and/or PRL on cell number of Nb_2 node lymphoma cells. By itself, mezerein elicited a concentration-response decrease in cell number after the 72-hr culture period. In the presence of PRL, at a concentration (55 pg/ml) that causes a less than maximum response, mezerein at concentrations between 0.005 and 5 ng/ml potentiated the mitogenic effect of PRL. Finally, in the presence of a maximum stimulatory concentration of PRL (20 ng/ml), mezerein at concentrations of 0.5–5 ng/ml suppressed the magnitude of the PRL response.

The time course for the mezerein effect on potentiating the mitogenic action of an intermediate concentration of PRL is shown in Table II. The potentiation of the PRL response is clearly apparent at 48 and 72 hr, but not at 24 hr, even though a significant PRL response is already apparent by 24 hr.

The effects of two diglycerides, diolein and dicaprin, on the mitogenic effect of PRL are shown in Tables III and IV, respectively. Neither of these diglycerides, by themselves or in the presence of a maximum stimulatory concentration of PRL, had an effect on cell number. In the presence of an intermediate concentration of PRL, however, both diglycerides potentiated the PRL response at diglyceride concentrations between 0.1 and 100 ng/ml.

Table I. Effect of Mezerein on Mitogenesis in Nb_2 Cells[a]

Mezerein concentration (ng/ml)	Number of Cells ($\times 10^{-3}$) after 72 hr		
	0 PRL	55 pg/ml PRL	20 ng/ml PRL
0	155 ± 6[b]	631 ± 8	803 ± 12
0.005	128 ± 4[c]	668 ± 13[d]	777 ± 23
0.05	134 ± 4[c]	749 ± 8[d]	796 ± 20
0.5	91 ± 1[c]	727 ± 18[d]	752 ± 6[c]
1	85 ± 2[c]	720 ± 8[d]	740 ± 4[c]
5	81 ± 2[c]	680 ± 15[d]	712 ± 4[c]

[a] Cells were cultured for 72 hr in the presence of mezerein and/or PRL at the concentrations indicated. Cell number was then determined.
[b] Mean ± SE of determinations from six wells.
[c] Significantly less ($P < 0.05$) than in the absence of mezerein.
[d] Significantly greater ($P < 0.05$) than in the absence of mezerein.

Table II. Time Course of Mezerein Effect on Cell Division[a]

Incubation time (hr)	Numbers of cells ($\times 10^{-3}$)		
	75 pg/ml PRL	75 pg/ml PRL ± 0.1 ng/ml Mezerein	P
0	100 ± 3[b]	99 ± 4	NS
24	125 ± 2	116 ± 3	NS
48	233 ± 6	271 ± 3	<0.05
72	509 ± 15	606 ± 10	<0.01

[a] Cells were cultured for 0–72 hr in the presence of 75 pg/ml PRL ± 0.1 ng/ml mezerein. Cell numbers were then determined.
[b] Mean ± SE of determinations from six wells.

Table III. Effect of Diolein on Mitogenesis in Nb_2 Cells[a]

Diolein concentration (ng/ml)	Numbers of cells ($\times 10^{-3}$) after 72 hr		
	0 PRL	100 pg/ml PRL	20 ng/ml PRL
0	180 ± 5[b]	820 ± 15	993 ± 10
0.1	186 ± 2	851 ± 19	988 ± 19
1	184 ± 2	870 ± 6[c]	1007 ± 11
10	188 ± 5	861 ± 13[c]	1011 ± 30
100	179 ± 4	891 ± 8[c]	979 ± 36
1000	182 ± 4	840 ± 28	936 ± 12[d]

[a] Cells were cultured for 72 hr in the presence of diolein and/or PRL at the concentrations indicated. Cell number was then determined.
[b] Mean ± SE of determinations from four wells.
[c] Significantly greater than in the absence of diolein.
[d] Significantly less than in the absence of diolein.

Table IV. Effect of Dicaprin on Mitogenesis in Nb_2 Cells[a]

Dicaprin concentration (ng/ml)	Number of cells ($\times 10^{-3}$) after 72 hr		
	0 PRL	100 pg/ml PRL	20 ng/ml PRL
0	253 ± 6[b]	648 ± 3	741 ± 10
0.1	244 ± 3	697 ± 3[c]	769 ± 8
1	243 ± 6	679 ± 6[c]	739 ± 7
10	250 ± 5	681 ± 2[c]	766 ± 5
1000	259 ± 3	677 ± 17	758 ± 5

[a] Cells were cultured for 72 hr in the presence of dicaprin and/or PRL at the concentrations indicated. Cell number was then determined.
[b] Mean ± SE of determinations from four wells.
[c] Significantly greater than in the absence of dicaprin.

In several experiments, the commonly used synthetic diglyceride OAG was also tested; at concentrations between 0.1 and 1000 ng/ml, OAG had no effects, either by itself or in the presence of an intermediate or maximum stimulatory concentration of PRL (data not included). Although OAG is an activator of PKC in many cell types, OAG may be an ineffective activator of PKC in the Nb_2 node lymphoma cells under the experimental conditions employed in these studies.

Discussion

These studies show that low concentrations of the diterpene mezerein, as well as two diglycerides, potentiate the mitogenic action of PRL at PRL concentrations which have a less than maximum effect on cell division in Nb_2 node lymphoma cells. The phorbol ester TPA was shown to have a similar effect ((1); Rillema, Waters, and Tarrant, unpublished studies). Since all of these substances are known to be activators of PKC, it seems likely that the potentiation of the mitogenic effect of PRL occurs via an elevation of PKC activity. We reported earlier that when phospholipase C is added exogenously to cultured Nb_2 cells, it also potentiates the mitogenic effect of a less than maximally effective concentration of PRL in the Nb_2 cells (10). Presumably, this potentiation is also carried out via the activation of PKC consequent to the generation of diglycerides.

At high concentrations mezerein inhibited cell number by itself, as well as in the presence of a maximum stimulatory concentration of PRL. High concentrations of TPA were shown earlier to attenuate the magnitude of the PRL stimulation of cell division in the Nb_2 cells (1). In unpublished studies, we (Rillema, Waters and Tarrant) have observed that high concentrations of TPA down-regulate PKC activity in Nb_2 node cells. Assuming that high concentrations of mezerein have a similar effect, the suppression of the PRL response in Nb_2 cells treated with high concentrations of TPA or mezerein is likely caused by the down-regulation of PKC activity.

Taken together, the above observations, along with the fact that a variety of PKC inhibitors have been shown to suppress or abolish the PRL stimulation of mitogenesis in the Nb_2 cells (4, 5), suggest that PKC is involved in the PRL regulation of cell division in the Nb_2 cells.

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1. Gertler A, Walker A, Friesen HG. Enhancement of human growth hormone-stimulated mitogenesis of Nb_2 node lymphoma cells by 12-*O*-tetradecanoylphorbol-13-acetate. Endocrinology **116**:1636–1644, 1985.
2. Buckley AR, Montgomery DW, Kibler R, Putnam CW, Zukoski CR, Gout PW, Beer CT, Russell DH. Prolactin stimulation of ornithine decarboxylase and mitogenesis in Nb_2 node lymphoma cells: The role of protein kinase C and calcium mobilization. Immunopharmacology **12**:37–51, 1987.
3. Emoto N, Tsushima T, Shizume K, Tanaka T, Saji M, Ohba Y, Wakai K, Arai M, Ohmura E. Biological activities of human growth hormone and its derivatives estimated by measuring DNA synthesis in Nb_2 node rat lymphoma cells. Acta Endocrinol **114**:283–291, 1987.
4. Too C-KL, Walker A, Murphy PR, Crague CR, Jacobs HK, Friesen HG. Identification of amiloride-sensitive Na^+/K^+ exchange in rat Nb_2 node lymphoma cells. Stimulation by 12-*O*-tetradecanoyl-phorbol-13-acetate. Endocrinology **121**:1503–1511, 1987.
5. Russell DH, Buckley AR, Montgomery DW, Larson NA, Gout PW, Beer CT, Putnam CW, Zukoski CF, Kibler R. Prolactin-dependent mitogenesis in Nb_2 node lymphoma cells: Effects of immunosuppressive cyclopeptides. J Immunol **138**:276–284, 1987.
6. Miyake R, Tanaka Y, Tsuda T, Kaibucki K, Kikkawa U, Nishizuki Y. Activation of protein kinase C by non-phorbol tumor promoter, mezerein. Biochem Biophys Res Commun **121**:649–656, 1984.
7. Berridge MJ. Inositol lipids and cell proliferation. Biochim Biophys Acta **907**:33–45, 1987.
8. Hishizuka Y. Perspectives on the role of protein kinase C in stimulus-response coupling. JNCI **76**:363–370, 1986.
9. Alkon DL, Rasmussen H. A spatial-temporal model of cell activation. Science **239**:998–1005, 1988.
10. Ofenstein JP, Rillema JA. Possible involvement of the phospholipases in the mitogenic actions of prolactin on Nb_2 node lymphoma cells. Proc Soc Exp Biol Med **185**:147–152, 1987.