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Prolactin Stimulation of Nb 2 Node Lymphoma Cell Division Is Inhibited by Polyamine Biosynthesis Inhibitors[1] (42170)

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Abstract. The mitogenic action of prolactin in Nb 2 node lymphoma cells was inhibited by two drugs which interfere with polyamine biosynthesis. At concentrations of 0.5 m*M* and above α-difluoromethyl ornithine (DFMO), which inhibits ornithine decarboxylase and the conversion of ornithine to putrescine, significantly attenuated the mitogenic effect of prolactin. This inhibition was prevented by the addition of putrescine, spermidine, or spermine to the culture medium. At concentrations of 1 μ*M* and above methylglyoxal bis(guanylhydrazone) (MGBG), which inhibits *S*-adenosylmethionine decarboxylase and hence the conversion of putrescine to spermidine and spermine, abolished the mitogenic action of prolactin. This inhibition was prevented by the addition of spermidine or spermine, but not putrescine, to the culture medium. These studies show that ongoing polyamine biosynthesis is essential for prolactin to express its mitogenic effect in this lymphoma cell line.

Cell division in the Nb 2 node lymphoma cell line is stimulated specifically by prolactin or other hormones containing lactogenic activities (1–4). This cell line can therefore be employed as a model system for studying the mechanism by which prolactin has its mitogenic actions on cells.

The regulation of the replication of many plant and animal cells involves perturbations in the metabolism of the polyamines (5, 6). In the Nb 2 node lymphoma cells, the prolactin stimulation of DNA synthesis and cell division occurs in concert with an enhanced activity of two polyamine biosynthetic enzymes, ornithine decarboxylase and *S*-adenosylmethionine decarboxylase, in these cells (7). The polyamines are also known to be involved in the actions of prolactin on protein synthesis and other differentiation functions in the mammary gland and other cell types (8–14).

To determine whether ongoing polyamine biosynthesis is required for prolactin to express its mitogenic action on the Nb 2 node lymphoma cells, two inhibitors of polyamine biosynthesis were employed. These included (a) α-difluoromethyl ornithine (DFMO) which is an irreversible inhibitor of the enzyme ornithine decarboxylase (15), and (b) methylglyoxal bis(guanylhydrazone) (MGBC) which inhibits the enzyme *S*-adenosylmethionine decarboxylase (16).

Materials and Methods. The Nb 2 node lymphoma cells employed in these studies were a gift from Dr. C. T. Beer of the Cancer Control Agency of British Columbia (Vancouver, British Columbia, Canada). The α-difluoromethyl ornithine was a gift from Merrell Dow Pharmaceuticals Inc. (Cincinnati, Ohio). Ovine prolactin (NIH-P-S-12) was provided by the NIAMDD. Other materials used in these studies were purchased from the following sources: Fisher's medium, L-glutamine and fetal calf serum (FCS) from KC Biologicals Inc. (Lenexa, Kans.); horse serum from Hyclone (Logan, Utah); methylglyoxal bis-(guanylhydrazone) from Aldrich Chemical Company Inc. (Milwaukee, Wis.); spermidine, putrescine, and spermine from Sigma Chemical Company (St. Louis, Mo.); penicillin and streptomycin from Eli Lilly Inc. (Indianapolis, Ind.).

The Nb 2 node lymphoma cells were maintained as suspension cultures in 25-cm^2 culture flasks containing Fisher's medium supplemented with 10% FCS, 10% horse serum, 1×10^{-4} *M* 2-mercaptoethanol, 50 U/ml penicillin, 50 μg/ml streptomycin, and 204 ng/ml L-glutamine. They were maintained at 37°C in an atmosphere consisting of 5% CO_2 and 95% air. For 24 hr before treatments were to

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begin, the cells were cultured with the medium described above with the exceptions that it contained 20% horse serum and no FCS. In the absence of FCS, the Nb 2 lymphoma cells only divide if a lactogenic hormone is added to the culture medium.

After the 24-hr culture in the absence of FCS, 1 ml of the cells was aliquoted into wells (Corning, No. 25820) with about 1×10^5 cells per well. The cells were then cultured for an additional 72 hr (unless otherwise specified) in the presence of various combinations of drugs or hormones including MGBG, DFMO, prolactin, putrescine, spermidine, and spermine. After the 72-hr culture periods, the cells were added to 10 ml of Hematall isotonic diluting fluid and counted on a Model ZM Coulter counter. The data in the tables and figures represent the means of at least three replicates, and statistical comparisons were made using an analysis of variance.

Results. Table I shows the effect of DFMO, an irreversible inhibitor of ODC activity on the PRL stimulation of cell division in the Nb 2 node lymphoma cells. At concentrations of 0.5 to 5 m*M* DFMO elicited a concentration-dependent inhibition of the PRL stimulation of cell proliferation. At concentrations between 5 and 40 m*M* (data not presented), DFMO abolished the PRL stimulation of cell division; DFMO at these high concentrations, however, also significantly reduced the number of cells in the cell cultures that were incubated for 72 hr in the absence of prolactin.

TABLE I. THE EFFECTS OF DFMO ON THE PROLACTIN STIMULATION OF PROLIFERATION OF Nb 2 NODE LYMPHOMA CELLS[a]

DFMO concentration (m*M*)	Number of cells/ml ($\times 10^{-3}$)		Fold increase over control
	No PRL	PRL (20 ng/ml)	
0	282 ± 3.5	1520 ± 65.9	5.39
0.01	259 ± 2.8	1448 ± 49.7	5.59
0.1	276 ± 8.4	1456 ± 19.7	5.28
0.5	255 ± 4.8	1039 ± 106	4.07*
1.0	264 ± 2.0	858 ± 36.2	3.25*
2.0	285 ± 2.9	563 ± 11.0	1.98*
5.0	300 ± 5.0	519 ± 14.8	1.73*

[a] PRL (20 ng/ml) and/or DFMO at the concentrations indicated in the table were added to stationary lymphoma cells. Cell number was then determined after a 72-hr incubation period.

* Significantly less than in the absence of DFMO ($P < 0.05$).

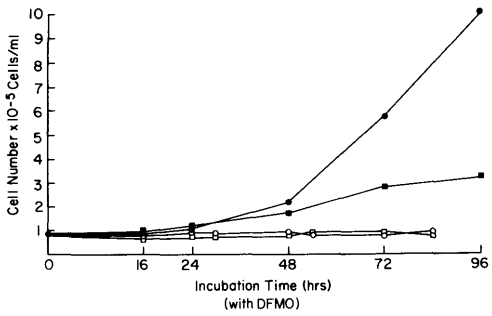

FIG. 1. Time course of DFMO inhibition of PRL stimulation of cell division. Cells were cultured for 16–96 hr with no addition (○), 20 ng/ml PRL (●), 1 m*M* DFMO (□), or PRL plus DFMO (■). Values in the figure are the means of triplicate determinations; in all cases the standard error of the mean was less than 3% of the mean.

The time course for the effect of DFMO on the proliferation of the Nb 2 node lymphoma cells is shown in Fig. 1. DFMO at a concentration of 1 m*M* had no effect by itself on cell number during a 96-hr culture period. At all times during this culture period, however, this drug significantly attenuated the prolactin stimulation of cell proliferation.

The specificity of the DFMO inhibition of cell division was established by the studies shown in Fig. 2. Putrescine, spermidine, and spermine, which are formed downstream from ODC in the polyamine biosynthetic pathway, were each able to reverse the DFMO inhibition of the PRL responses. None of the polyamines at the concentrations employed had an effect, by themselves, on the proliferation of the Nb 2 node lymphoma cells. The polyamine concentrations employed were selected from preliminary titration studies which are not presented in this text.

MGBG, a drug that inhibits the conversion of putrescine to spermidine and spermine, was also found to alter the responsivity of the Nb 2 lymphoma cells to PRL. Table II shows that, at a concentration of 1 μM, MGBG significantly attenuated the PRL stimulation of cell division; MGBG at concentrations between 10 and 40 μM abolished the PRL response. It must be noted, however, that MGBG at concentrations of 1 μM and above significantly

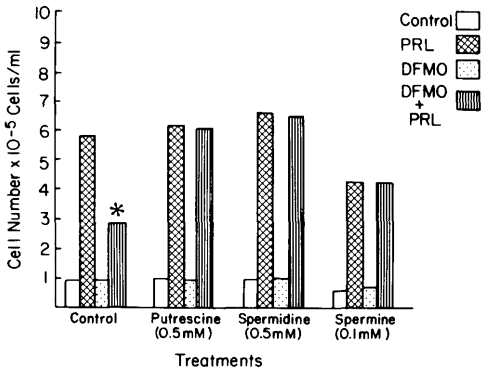

FIG. 2. Effect of polyamines on the DFMO inhibitor of the PRL stimulation of cell division. Cells were cultured for 72 hr in the presence of the combinations of agents indicated in the figure. Numbers represent the means of triplicate determinations; the standard error of the mean in all cases was less than 3% of the mean. *Significantly less ($P < 0.01$) than the PRL-stimulated cultures.

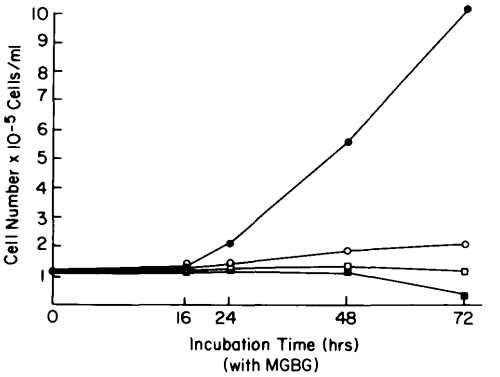

FIG. 3. Time course of MGBG inhibition of PRL stimulation of cell division. Cells were cultured for 16–72 hr with no additions (○), 20 ng/ml PRL (●), 20 μM MGBG (□), or MGBG plus PRL (■). Numbers are the means of triplicate determinations; the standard error of the mean in all cases was less than 3% of the mean.

reduced the number of cells in cell preparations that were cultured in the absence of PRL.

The time course for the MGBG inhibition of cell division in the Nb 2 node lymphoma cells is shown in Fig. 3. At all times during a 72-hr culture period 20 μM MGBG abolished PRL's mitogenic effect. The decrease in cell number due to 20 μM MGBG, by itself, was only noted after 48 hrs of culture.

Finally, the specificity of the MGBG inhibition of cell division in the Nb 2 node lymphoma cells was established by the results of the experiment shown in Fig. 4. The abolition of the PRL stimulation of mitogenesis by MGBG was ameliorated by adding spermidine or spermine, but not putrescine, to the culture medium. It is also of interest that all of these polyamines, including putrescine, prevented the decrease of cell number which occurred with exposure times to MGBG of longer than 48 hr.

TABLE II. THE EFFECTS OF MGBG ON THE PROLACTIN STIMULATION OF PROLIFERATION OF Nb 2 NODE LYMPHOMA CELLS[a]

MGBG concentration (μM)	Number of cells/ml ($\times 10^{-3}$)		Fold increase over control
	No PRL	PRL (20 ng/ml)	
0	171 ± 0.2	851 ± 3.4	4.98
0.01	167 ± 6.0	988 ± 1.0	5.92
0.1	164 ± 9.4	969 ± 6.8	5.91
1.0	118 ± 7.6	228 ± 4.4	1.93*
10.0	79.8 ± 3.6	90.4 ± 2.0	1.13*
20.0	67.2 ± 2.8	77.8 ± 2.6	1.16*
40.0	61.6 ± 3.0	69.6 ± 1.0	1.13*

[a] PRL (20 ng/ml) and/or MGBG at the concentrations indicated in the table were added to stationary lymphoma cells. Cell number was then determined after a 72-hr culture period.

* Significantly less than in the absence of MGBG ($P < 0.05$).

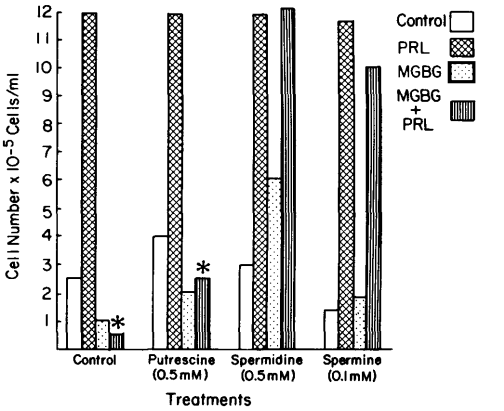

FIG. 4. Effect of polyamines on the MGBG inhibition of the PRL stimulation of cell division. Cells were cultured for 72 hr in the presence of the combinations of agents indicated in the figure. Numbers represent the means of triplicate determinations; standard errors of the mean in all cases are less than 3% of the mean. *Significantly less ($P < 0.01$) than the PRL-stimulated cultures.

Discussion. These studies clearly show that ongoing polyamine biosynthesis is essential for prolactin to express its action on mitogenesis on the Nb 2 node lymphoma cells. Both DFMO, an inhibitor of ornithine decarboxylase activity, and MGBG, an inhibitor of *S*-adenosylmethionine decarboxylase activity, were found to interfere with the PRL stimulation of cell division in this cell line. The specificity of action of these drugs was established by showing that the inhibition of PRL responses were reversed by culturing the cells with polyamines that in the polyamine biosynthetic pathway are produced downstream from the site of inhibition of the drugs.

In accord with our work were earlier studies carried out by Richards *et al.* (7). They observed that PRL has a profound effect on ODC and SAMD activities in the Nb 2 node lymphoma cells. In addition, the effect on these enzymes correlated with the prolactin stimulation of [^{3}H]thymidine incorporation into DNA and cell division. Both the studies on the polyamine enzymes and enzyme inhibitors therefore support the conclusion that the PRL stimulation of polyamine biosynthesis is at least a part of the mechanism by which PRL expresses its mitogenic action in the Nb 2 node lymphoma cells. The mechanism of action of PRL on cell division would, therefore, not be unlike that on stimulating lactogenic processes in that the PRL stimulation of milk product synthesis is also known to involve the polyamines (8).

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