# Cyclic AMP Impairs the PRL Stimulation of Iodide Uptake into Mouse Mammary Tissues (44313)

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*Abstract.* **In earlier studies the uptake of iodide into mammary cells was found to occur by a mechanism similar to that in thyroid cells (i.e., the uptake occurs *via* a sodium-iodide symporter that is inhibited by perchlorate and thiocyanate). Although cyclic AMP stimulates the iodide transport mechanism in thyroid cells, the present studies show that cyclic AMP, as well as pharmacological agents that elevate cyclic AMP, impair iodide uptake into mammary cells. In addition, elevated cyclic AMP levels interfere with the PRL stimulation of iodide uptake as well as iodide incorporation into proteins in mammary tissues. Clearly, cyclic AMP has opposite effects on regulating iodide uptake processes in mammary versus thyroid cells.** [P.S.E.B.M. 1998, Vol 219]

In the alveolar epithelial cells of the mammary gland, prolactin (PRL) stimulates the uptake of an array of substances (1–4) including iodide (5). The uptake of iodide into mammary cells appears to occur *via* a transport mechanism similar to that present in thyroid cells (i.e., iodide uptake occurs *via* a sodium-iodide symporter that is inhibited by perchlorate and thiocynate (6–8). Many of the effects of TSH on thyroid cells, including the stimulation of iodide uptake, are mediated by a cyclic AMP dependent mechanism. Accordingly, DBcAMP and agents that elevate cyclic AMP in isolated thyroid cells stimulate the iodide concentrating mechanism. Since the characteristics of the sodium-iodide symporter are similar in thyroid and mammary cells, we carried out studies to determine the possible role of cyclic AMP in regulating iodide uptake in cultured mammary tissues. Of further interest was the possible effect of cyclic AMP on the PRL stimulation of iodide transport, since cyclic AMP has been reported earlier to interfere with the PRL regulation of a plethora of other lactational processes in the mammary gland (1).

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## Material and Methods

Midpregnant (10–14 days of pregnancy) Swiss-Webster mice were used in all experiments; they were purchased from Harlan Laboratories (Indianapolis, IN). Ovine prolactin (PRL; National Institutes of Health PS-19) was a gift from the National Institutes of Health. Other substances were purchased from the following sources: cortisol from Charles Pfizer (New York, NY); Hank's balanced salt solution (HBSS), and medium 199-Earle's salts from GIBCO Laboratories (Grand Island, NY); $^{3}$HOH and [carboxy-$^{14}$C] inulin (405.8 mCi/g) from New England Nuclear (Boston, MA); $^{125}$I from Amersham Life Sci., Inc. (Arlington Heights, IL); porcine insulin, penicillin, and streptomycin from Eli Lilly (Indianapolis, IN); and $N^6$, $O^{21}$-dibutyryl-adenosine $3^1$, $5^1$-cyclic monophosphate (DBcAMP), adenosine $3^1$, $5^1$-cyclic-monophosphoric acid (cyclic AMP), guanosine $3^1$, $5^1$-cyclic monophosphate (cyclic GMP), 3-isobutyl-1-methyl-xanthine (IBMX), caffeine, theophylline, and cholertoxin (CT) from Sigma Chemical Co. (St. Louis, MO).

Explants of mouse mammary tissues were prepared and cultured as described earlier (10). The explants were cultured on siliconized lens paper floating on 6-ml medium 199-Earle's salts containing 1 μg/ml insulin plus $10^{-7}$ *M* cortisol; all incubations were carried out in 60 × 15-mm petri dishes maintained at 37°C in an atmosphere of 95% $O_2$-5% $CO_2$. In experiments where the effects of PRL on iodide transport were to be determined, the tissues were initially cultured for 24–36 hr in the absence of PRL or other substances; PRL or various inhibitors were then

added, and incubations continued for 24 hr. For the final 2 hr of culture, the tissues were transferred to vessels containing $^{125}I$ (0.25 μCi/ml; 0.3 ng/ml iodide) in 4 ml media; incubations were carried out in a rotary water bath at 37°C (120 cycles/min). The tissues were then weighed and homogenized in 2 ml 10% trichloroacetic acid (TCA). After a determination of total radioactivity, the samples were centrifuged at 2000 *g* for 210 min. After washing the pellet with an additional 5 ml 10% TCA, radioactivity in the TCA insoluble fraction was determined. The intracellular accumulation of radiolabeled iodide was calculated by subtracting the amount of radiolabel in the extracellular space from the total radioactivity in the tissue homogenates (3, 4, 10). For these calculations the total water content (51.0%) and extracellular space (24.6%) were determined by the volume of distribution of $^{3}HOH$ and [$^{14}C$]inulin (1 m*M*), respectively. In time course studies, equilibration was achieved with $^{3}HOH$ and [$^{14}C$]inulin by 15 min after their addition. PRL had no effect on the volumes of distribution of these substances under the conditions employed in these experiments. Results of the iodide uptake studies are expressed as a distribution ratio that represents the ratio of the intracellular specific activity divided by the extracellular specific activity of the radiolabeled iodide. The results of the incorporation studies are expressed as DPM/mg wet weight of tissues.

Statistical comparisons were made with Student's *t* test when two means were compared with an analysis of variance followed by Dunnet's test for multiple comparisons.

## Results

Figure 1 shows the effects of a 24-hr treatment with DBcAMP on iodide uptake during a subsequent 1-hr culture period. DBcAMP, an analog of cyclic cAMP that is more permeable to cells, elicited a concentration-dependent (0.1–0.5 m*M*) inhibition of iodide uptake. In addition, the magnitude of PRL stimulation of iodide uptake was also significantly attenuated in tissues that were cultured with both PRL and DBcAMP for a 24-hr period. Similar results were obtained when tissues were treated with cyclic AMP (Fig. 2), although much higher concentrations of cyclic AMP were needed (5 and 10 m*M*). Cyclic GMP (5 m*M*) had no effect on iodide uptake, nor did it affect the magnitude of the PRL response. Cyclic GMP, therefore, serves as a nonspecific control for the results generated with cyclic AMP and DBcAMP. In addition, in earlier studies (11) as well as in unpublished studies, we have shown that neither butyrate nor adenosine at concentrations up to 10 m*M* affect the PRL regulation of RNA, casein, lactose, or lipid synthesis in cultured mouse mammary tissues.

Figure 3 shows the effects of several drugs, known to elevate cyclic AMP levels in cells, on iodide uptake into cultured mammary cells. Three phosphodiesterase inhibitors, 1 m*M* IBMX, 10 m*M* theophylline, and 2 m*M* caffeine attenuated the magnitude of the PRL stimulation of iodide uptake, whereas 5 m*M* caffeine abolished this response.

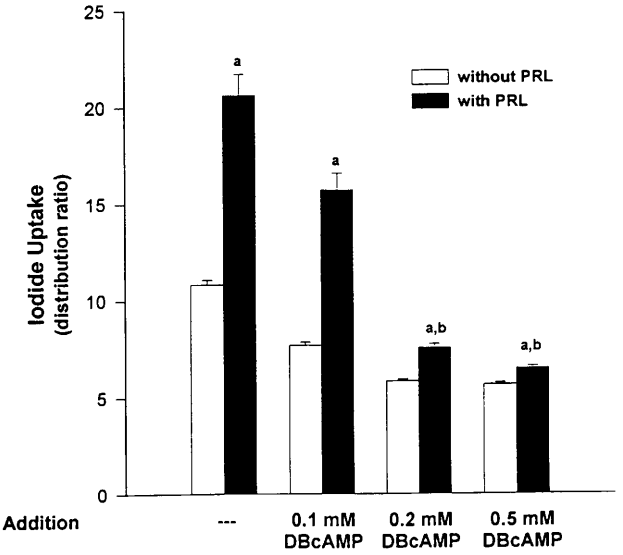


**Figure 1.** Effect of DBcAMP on PRL stimulation of iodide uptake. Tissues were cultured for 24 hr with the substances listed in the Figure. $^{125}I$ (0.25 μCi/ml, 0.3 ng/ml) was present for the final 2 hr of culture. Values in the figure represent the mean ± SEM of six observations. (a) Significantly greater than control with $P < 05$. (b) Significantly attenuated PRL response with $P < 05$.

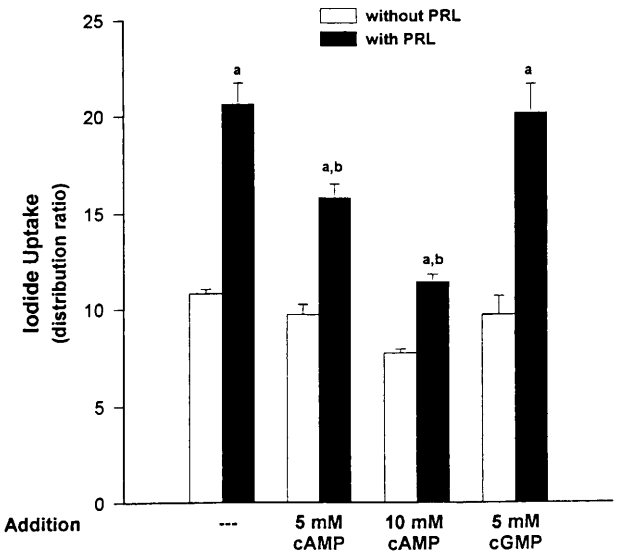


**Figure 2.** Effect of cyclic nucleotides on PRL stimulation of iodide uptake. Tissues were cultured for 24 hr with the substances listed in the figure. $^{125}I$ (0.25 μCi/ml, 0.3 ng/ml) was present for the final 2 hr of culture. Values in the figure represent the mean ± SEM of six observations. (a) Significantly greater than control with $P < 05$. (b) Significantly attenuated PRL response with $P < 05$.

Theophylline (10 m*M*) and 5 m*M* caffeine also reduced the basal level of iodide uptake. Choleratoxin, which elevates tissue cyclic AMP levels by activating the Gs protein and hence stimulating adenylate cyclase, also attenuated the magnitude of the PRL stimulation.

A significant fraction of iodide taken up into mammary cells was incorporated into tyrosyl residues of proteins, primarily caseins. PRL is also known to stimulate this process. Figures 4–6 show the effects of the cyclic nucleotides, as well as the agents that elevate tissue cyclic AMP levels

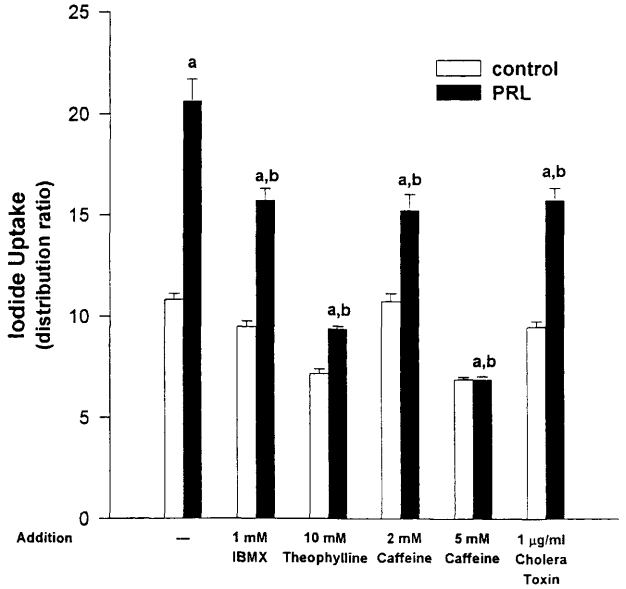


**Figure 3.** Effect of phosphodiesterase inhibitors and choleratoxin on PRL stimulation of iodide uptake. Tissues were cultured for 24 hr with the substances listed in the figure. $^{125}I$ (0.25 µCi/ml, 0.3 ng/ml) was present for the final two hr of culture. Values in the figure represent the mean ± SEM of six observations. (a) Significantly greater than control with $P < 05$. (b) Significantly attenuated PRL response with $P < 05$.

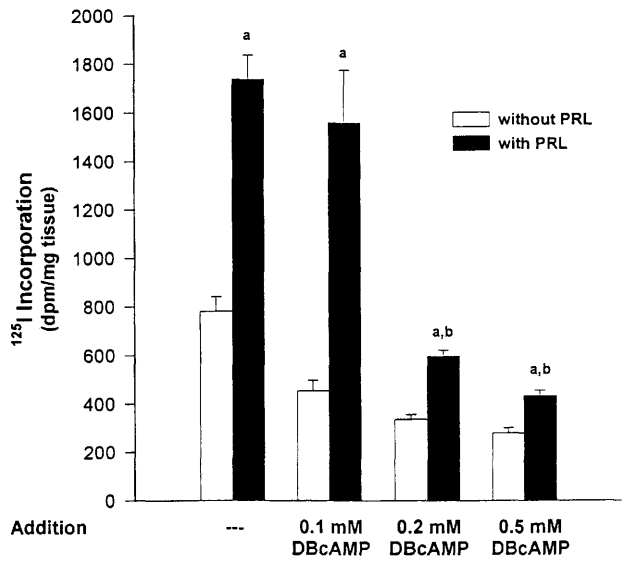


**Figure 4.** Effect of DBcAMP on PRL stimulation of $^{125}I$ incorporation. Tissues were cultured for 24 hr with the substances listed in the figure. $^{125}I$ (0.25 µCi/ml, 0.3 ng/ml) was present for the final 2 hr of culture. Values in the figure represent the mean ± SEM of six observations. (a) Significantly greater than control with $P < 05$. (b) Significantly attenuated PRL response with $P < 05$.

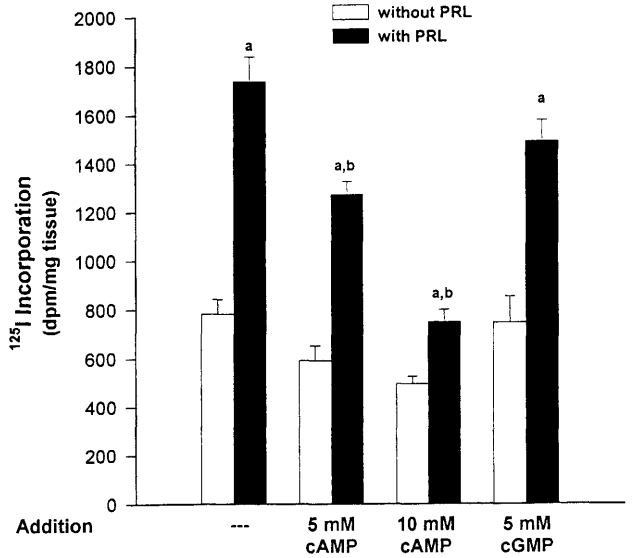


**Figure 5.** Effect of cyclic nucleotides on PRL stimulation of $^{125}I$ incorporation. Tissues were cultured for 24 hr with the substances listed in the figure. $^{125}I$ (0.25 µCi/ml, 0.3 ng/ml) was present for the final 2 hr of culture. Values in the figure represent the mean ± SEM of six observations. (a) Significantly greater than control with $P < 05$. (b) Significantly attenuated PRL response with $P < 05$.

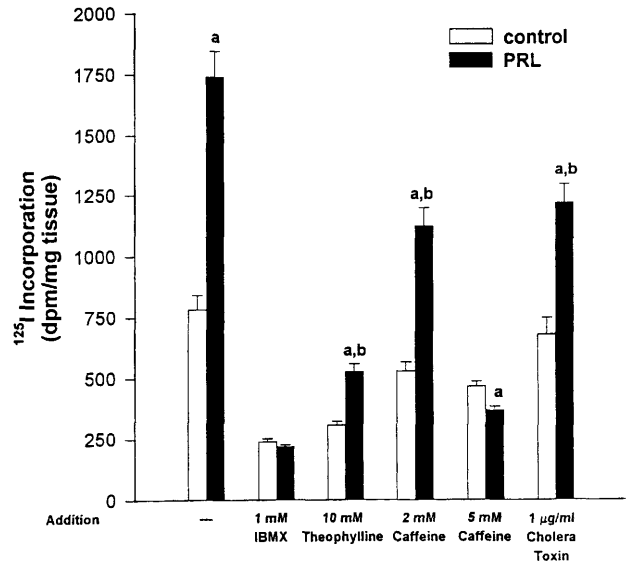


**Figure 6.** Effect of phosphodiesterase inhibitors and choleratoxin on PRL stimulation of $^{125}I$ incorporation. Tissues were cultured for 24 hr with the substances listed in the figure. $^{125}I$ (0.25 µCi/ml, 0.3 ng/ml) was present for the final 2 hr of culture. Values in the figure represent the mean ± SEM of six observations. (a) Significantly greater than control with $P < 05$. (b) Significantly attenuated PRL response with $P < 05$.

on iodide incorporation in cultured mammary tissues. DBcAMP (Fig. 4) and cyclic AMP (Fig. 5) caused a concentration-dependent inhibition of iodide incorporation, whereas cyclic GMP was without effect. DBcAMP and cyclic AMP also caused a concentration-dependent inhibition of the PRL stimulation of iodide incorporation. All of the phosphodieterase inhibitors significantly reduced the extent of iodide incorporation into TCA-precipitable proteins (Fig. 6). In addition all the phosphodiesterase inhibitors as well as choleratoxin abolished or attenuated the magnitude of the PRL response.

## Discussion

Although the characteristics of the iodide-symporter are the same in mammary and thyroid cells, cyclic AMP stimulates the iodide transport processes in thyroid cells but in-

hibits it in mammary cells. In addition, cyclic AMP suppresses the PRL stimulation of iodide uptake into mammary cells, consistent with the cyclic AMP inhibition of the PRL regulation of a number of other lactogenic processes (1). In thyroid cells the cyclic AMP stimulation of iodide uptake is consistent with the fact that many of the effects of TSH in thyroid cells, including the stimulation of iodide transport, occur *via* a cyclic AMP signaling mechanism. It is well documented that TSH elevates cyclic AMP levels in thyroid cells by stimulating adenylate cyclase; prolactin in contrast, has no effect on adenylate cyclase activity on cyclic AMP levels in mammary cells. Since inhibitors of protein and RNA synthesis abolish the TSH and PRL regulation of iodide uptake in thyroid and mammary cells respectively (6, 9), both hormone effects appear to occur, at least in part, by an RNA-DNA-dependent mechanism. Accordingly, it will be of interest to determine the molecular basis for the cyclic AMP stimulation of transcription from the iodide transporter gene in thyroid cells and the inhibition of this gene in mammary cells. It is possible that the two cell types have two different tissue-specific isoforms that are regulated differently. In thyroid cells, recent studies by Koyai *et al.* (12) reported that cyclic AMP elevates the mRNA and protein levels of the sodium-iodide symporter.

The possible physiological role of cyclic AMP in regulating lactational processes is suggested from the fact that there is a precipitous decrease in cyclic AMP levels in the mammary glands of several species immediately following parturition (1). Accordingly, cyclic AMP may suppress lactational processes, including iodide transport, during pregnancy. And subsequent to parturition, the decreased cyclic AMP levels may function in concert with other signaling cascades to enhance milk product synthesis and secretion.

In earlier studies, PRL was also shown to stimulate the rate of iodide incorporation into milk proteins (5). It was concluded from those studies that PRL also likely stimulates the activity of the peroxidase enzyme, which catalyzes the incorporation of iodide into proteins. Cyclic AMP suppressed the rate of iodide uptake as well as the PRL regulation of this process in cultured mammary cells. Since cyclic AMP clearly interfered with the uptake of iodide into mammary cells, the limited intracellular availability of iodide in cyclic AMP-treated cells could explain, at least in part, the incorporation data. On the other hand, cyclic AMP may also interfere with peroxidase activity and perhaps the PRL regulation of this enzyme, and this may also contribute to the experimental results. Further experiments will be required to resolve this issue.

In summary, these studies show that cyclic AMP suppresses iodide uptake as well as the PRL stimulation of this process in mammary cells. This contrasts with thyroid cells where cyclic AMP stimulates iodide transport.

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