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# THE ACTIVATION OF cAMP-DEPENDENT PROTEIN KINASE IS DIRECTLY LINKED TO THE REGULATION OF OSTEOBLAST PROLIFERATION (UMR-106) BY PARATHYROID HORMONE

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In order to characterize the direct involvement of cAMP in the change of osteoblast proliferation by parathyroid hormone(PTH), we employed the diastereoisomers of adenosine 3',5'-cyclic phosphorothioate, Sp-cAMPS and Rp-cAMPS, which have been recently shown to act directly as agonist and antagonist, respectively in the activation of cAMP-dependent protein kinase(PKA). Dibutyryl cAMP (dbcAMP) and cholera toxin as well as human(h)PTH-(1-34) significantly inhibited [$^3$H]thymidine incorporation(TdR) in osteoblastic osteosarcoma cells, UMR-106. Sp-cAMPS ($10^{-6}$-$10^{-4}$M)inhibited TdR in a dose-dependent manner. Although Rp-cAMPS ($10^{-6}$-$10^{-4}$M) itself did not affect TdR, it significantly blocked dbcAMP-, cholera toxin- and Sp-cAMPS-induced suppression of TdR. Moreover, Rp-cAMPS ($10^{-6}$-$10^{-4}$M) dose-dependently antagonized hPTH-induced suppression of TdR. Present studies first indicated that the activation of PKA is directly linked to the change of osteoblast proliferation by PTH. 

Several lines of evidence indicate that cAMP may regulate osteoblast proliferation by parathyroid hormone (PTH) (1-3) , but no reports have been available now that the activation of cAMP-dependent protein kinase(PKA) is directly coupled to the change in osteoblast proliferation by PTH. Recently, the diastereoisomers of the phosphorothioate analogue of cAMP, Sp-cAMPS and Rp-cAMPS which directly stimulated and inhibited PKA, respectively, were developed and employed to examine hormone-stimulated cellular responses and to distinguish cAMP-dependent from cAMP-independent events in various kinds of cells including hepatocytes, kidney, thyroid and Leydig tumor cells (4-7), but these cAMP analogues have not been examined in bone cells. In osteoblastic osteosarcoma cells, UMR-106, PTH has been reported to inhibit cell proliferation (1,3) and activate PKA as well as adenylate cyclase (8,9), therefore, these cells are suitable to determine the role of PKA in the control of osteoblast proliferation by PTH. In the present studies, Sp-cAMPS and Rp-cAMPS were employed to determine whether or

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Abbreviations: cAMPS, adenosine 3',5'-monophosphorothioate; EDTA, ethylene diaminetetraacetic acid.

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not the activation of PKA would be directly linked to the suppression of the cell proliferation by PTH in UMR-106 cells.

## Materials and Methods

### Materials

UMR-106 cells were the generous gift from Dr. T.J. Martin (Melbourne, Australia). Human(h)-PTH-(1-34) was obtained from Peptide Institute Inc. (Osaka, Japan). Sp-cAMPS and Rp-cAMPS from Biolog Life Science Institute (Bremen, W. Germany), $N^6$, $O^{2'}$-dibutyryl adenosine 3',5'-cyclic monophosphate (dbcAMP) from Sigma Co. (St. Louis, MO), cholera toxin from List Biological Lab (Campbell, CA) and methyl-[$^3$H]-thymidine from Amersham Japan (Tokyo, Japan). All other chemicals were of analytical grade.

### Cell culture

UMR-106 cells were maintained in Dulbecco's Modified Eagle Medium containing 10% fetal calf serum in a 5% $CO_2$-95% air atmosphere at 37$^0$C, as previously described (10). Cells were weekly passed using 0.05% trypsin-0.02% EDTA solution and plated in appropriate clusters at 1.0-1.5 x $10^5$ cells/cm$^2$.

### [$^3$H]thymidine incorporation

Twenty-two hrs after treatment with indicated concentration of substances, the cells were pulsed with [$^3$H]thymidine (0.2uCi/ml). Two hrs later, the incubation was terminated by removal of the medium and the addition of 5% trichloroacetic acid (TCA). After removal of TCA, the precipitated layer was washed with ethanol and the wells were desicated at room temperature. The residuum was dissolved in 20mM NaOH and 1% sodium dodecyl sulfates and scintilation cocktail was added. Samples were counted in a liquid scintilation counter. Data are expressed as the mean ± SEM. Statistical analysis was performed, using student's t test or Duncan's multiple range test.

## Results and Discussion

The effect of Sp-cAMPS or Rp-cAMPS on [$^3$H]thymidine incorporation (TdR) was examined, using UMR-106 cells which possessed the osteoblastic characteristics and compared to that of dbcAMP or cholera toxin, a compound which activated endogenous cAMP production. As shown in Figure 1, cholera toxin (1ug/ml) as well as dbcAMP ($10^{-4}$-$10^{-3}$M) significantly inhibited TdR. Sp-cAMPS ($10^{-6}$-$10^{-4}$M) dose-dependently inhibited TdR with a minimal effective dose at $10^{-5}$M, indicating that the activation of PKA would have been coupled to the suppression of cell proliferation in UMR-106 cells. On the other hand, Rp-cAMPS ($10^{-6}$-$10^{-4}$M) by itself did not affect TdR, which was compatible with a previous report showing that the action of Sp-cAMPS on electrolyte excretion through the isolated perfused rat kidney was similar to that of PTH and that Rp-cAMPS alone unaffected the electrolyte excretion (5). These results indicated that Sp-cAMPS mimicked cAMP-induced effect and that Rp-cAMPS did not affect the cell proliferation. Next experiments were performed to examine whether or not Rp-cAMPS would be able to antagonize cAMP-induced suppression of osteoblast proliferation. As

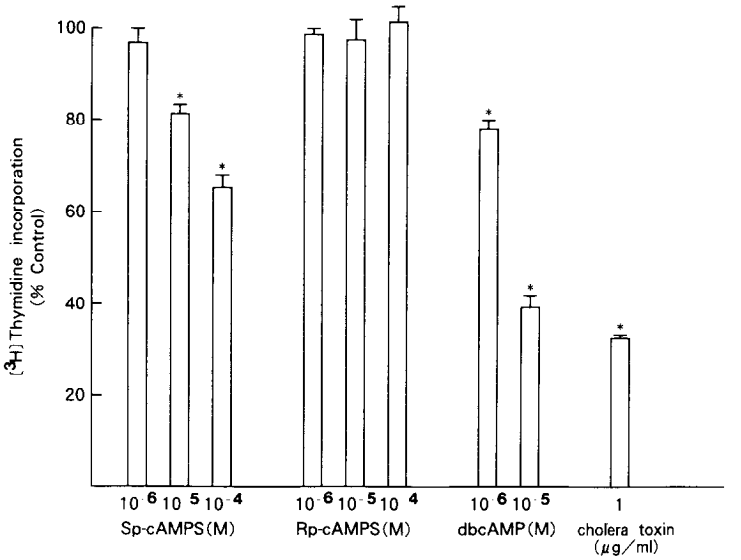


FIG. 1. Effect of Sp-cAMPS, Rp-cAMPS, dbcAMP and cholera toxin on [$^3$H]thymidine incorporation. [$^3$H]thymidine incorporation was measured as described in Materials and Methods. Data are expressed as % control. Each bar represents the mean ± SEM of quadruplicate determinations. * $P < 0.01$, compared to control.

shown in Figure 2, Rp-cAMPS ($10^{-4}$M) significantly antagonized the effect of both dbcAMP and cholera toxin. Moreover, Rp-cAMPS also antagonized the effect of Sp-cAMPS on the cell proliferation, indicating that Rp-cAMPS acted as an inhibitor of PKA in UMR-106 cells. Next, we studied to clarify a role of PKA in PTH-induced change of

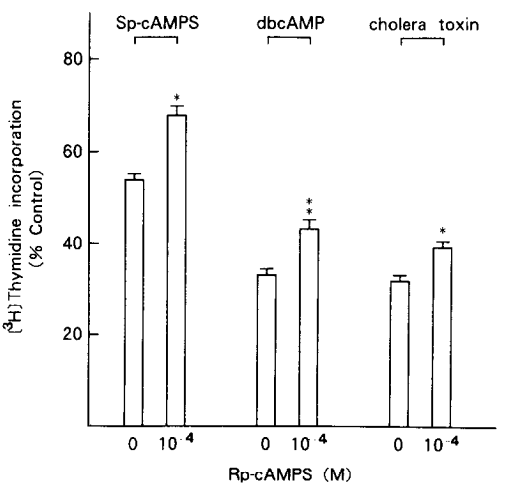


FIG. 2. Effect of Rp-cAMPS on Sp-cAMPS-, dbcAMP- and cholera toxin-induced suppression of [$^3$H]thymidine incorporation. Cells were treated with Sp-cAMPS($10^{-4}$M), dbcAMP($10^{-3}$M) or cholera toxin(1ug/ml) 30 min after the addition of Rp-cAMPS($10^{-4}$M). Each bar represents the mean ± SEM of quadruplicate determinations. * $P < 0.01$, compared to Rp-cAMPS-untreated cells, ** $P < 0.05$, compared to Rp-cAMPS-untreated cells.

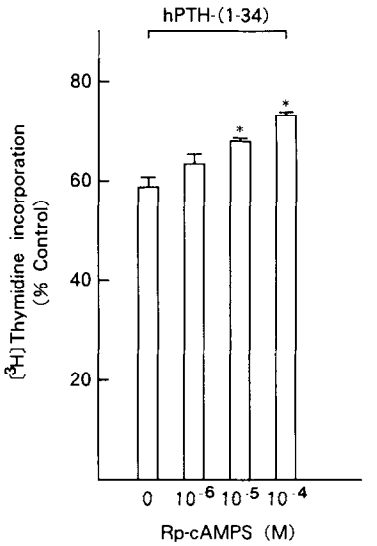


FIG. 3. Effect of Rp-cAMPS on hPTH-(1-34)-induced suppression of [$^3$H]thymidine incorporation. Cells were treated with hPTH-(1-34) ($10^{-7}$M) 30 min after the addition of various concentration of Rp-cAMPS ($10^{-6}$-$10^{-4}$M). Each bar represents the mean ± SEM of quadruplicate determinations. * P <0.01, compared to Rp-cAMPS-untreated cells.

osteoblast proliferation. Human-PTH-(1-34) at $10^{-7}$M caused significant suppression of TdR in UMR-106 cells, which was compatible with recent reports (1,3). As shown in Figure 3, Rp-cAMPS ($10^{-6}$-$10^{-4}$M) dose-dependently antagonized PTH-induced suppression of cellular proliferation with a minimal effective dose at $10^{-5}$M. Since Rp-cAMPS antagonized the effect of PTH as well as that of dbcAMP, cholera toxin and Sp-cAMPS, present data indicate that the activation of PKA is directly linked to PTH-induced suppression of cellular proliferation in UMR-106.

In the present studies, however, Rp-cAMPS did not completely antagonize the PTH-induced suppression of osteoblast proliferation. To explain these results, following possibilities must be considered. First, it seems probable that Rp-cAMPS even at high concentration of $10^{-4}$M does not have the ability completely to block PTH-stimulated PKA activity, since Rp-cAMPS failed to inhibit Sp-cAMPS-induced effect completely. Second, it might also be presumed that PKA is not the only second messenger involved in the suppression of osteoblast proliferation by PTH. Because, in UMR-106 cells, PTH has recently been reported to act on phosphoinositide metabolism, another pathway of intracellular messenger system, resulting in an activation of protein kinase C and an increase in cytosolic calcium (11, 12). Third, Rp-cAMPS as well as Sp-cAMPS have been reported to inhibit phosphodiesterase activity in cultured Leydig tumor cells (7). Therefore, it is possible that a partial inhibition of phosphodiesterase activity in turn might allow endogenous cAMP to accumulate, resulting in partial compensation of PKA inhibition. Further studies are necessary to clarify these possibilities and these studies are in progress in our laboratories. An important conclusion derived from the present studies is that the activation of PKA is an obligatory initiator of the suppressin of cell

proliferation by PTH in UMR-106. Rp-cAMPS can be used to identify the PKA-dependent mechanism in osteoblasts.

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