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

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The present study was performed to compare the effect of parathyroid hormone-related protein(PTHrP) on the proliferation of osteoblastic osteosarcoma cells (UMR-106) with that of PTH and characterize the direct involvement of cAMP in the change of osteoblast proliferation by PTHrP. Human(h)PTHrP-(1-34) (10^{-11}-10^{-7}M) dose-dependently inhibited [^{3}H]thymidine incorporation (TdR) in the same manner as hPTH-(1-34).The simultaneous addition of PTHrP and PTH at a maximal effective dose of 10^{-7}M did not cause additive suppressive effect on cell proliferation. Rp-cAMPS,which has been recently shown to act directly as antagonist in the activation of cAMP-dependent protein kinase (PKA), dose-dependently (10^{-6}-10^{-4}M) antagonized PTHrP-induced suppression of TdR in the same manner as PTH. Present study indicated that PTHrP has the same effect on osteoblast proliferation as PTH and that the activation of PKA is directly linked to the change of osteoblast proliferation by PTHrP.

Parathyroid hormone-related protein (PTHrP), a causative peptide associated with humoral hypercalcemia of malignancy, has been purified from the conditioned medium derived from a human lung cancer cell line and its amino acid sequencing revealed high similarity with PTH at the amino-terminus (1,2). PTHrP and PTH have been to have similar effect in the target cells of PTH (3,4), although there have also been reports about contradictory results especially on bone resorption and anabolic effect (5,6). As for osteoblast proliferation, PTH has been reported to inhibit the proliferation of osteoblastic osteosarcoma cells (UMR-106) (7,8). Moreover, our recent studies (9) demonstrated that the activation of cAMP-dependent protein kinase (PKA) was directly linked to the inhibitory effect of PTH on cell proliferation in UMR-106 cells, using the direct PKA antagonist, Rp-cAMPS, and also revealed that this cAMP analogue was a useful probe to distinguish the PKA-dependent mechanism from others. In the present study, therefore, we examined the effect of PTHrP on the proliferation of UMR-106 cells and compared

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

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this with that of PTH and clarified the participation of the activation of PKA in PTHrP-induced change of osteoblast proliferation.

Materials and Methods

Materials

UMR-106 cells were the generous gift from Dr. T. J. Martin (Melbourne, Australia). Human(h)PTHrP-(1-34) and hPTH-(1-34) were obtained from Peptide Institute Inc. (Osaka, Japan). Rp-cAMPS from Biolog Life Science Institute (Bremen, W. Germany) 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°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 $\times$ 10^5 cells/cm^2.

[^{3}H]thymidine incorporation

Twenty-two hrs after treatment with the indicated concentration of substances, the cells were pulsed with [^{3}H]thymidine (0.2μCi/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 $\pm$ SEM. Statistical analysis was performed, using student's t test or Duncan's multiple range test.

Results and Discussion

First experiments were performed to examine the effect of PTHrP on [^{3}H]thymidine incorporation (TdR) and to compare its effect to that of PTH. As shown in figure 1, hPTH-(1-34) (10^{-11}-10^{-7}M) caused the dose-dependent suppression of TdR with minimal effective dose at 10^{-10}M. Human PTHrP-(1-34) also inhibited TdR in the same dose-response pattern. Next experiments were performed to examine whether or not simultaneous treatment with PTHrP and PTH at a maximal effective dose caused additive inhibitory effect on TdR. As shown in figure 2, the degree of inhibitory effect by the simultaneous treatment was almost equal to that by PTHrP alone or PTH alone, suggesting that PTHrP inhibited osteoblast proliferation presumably through PTH receptor and/or the same mechanism as PTH. Recently, Rp-cAMPS, the diastereoisomer of the phosphorothioate analogue of cAMP, which directly inhibited PKA, was developed and employed to examine hormone-stimulated cellular response and to distinguish cAMP-dependent from cAMP-independent events in various kinds of cells (11-14). Our recent study (9) demonstrated that Rp-cAMPS, which did not affect osteoblast proliferation by itself, significantly antagonized PTH-induced inhibition of TdR, indicating that the activation of PKA is directly linked to the change of osteoblast proliferation by PTH. In the present study, we examined whether or not the activation of PKA was directly coupled to PTHrP-induced inhibition of the proliferation in UMR-106 cells. Rp-cAMPS (10^{-6}-10^{-4}M) dose-dependently antagonized PTHrP-induced inhibition of TdR with a

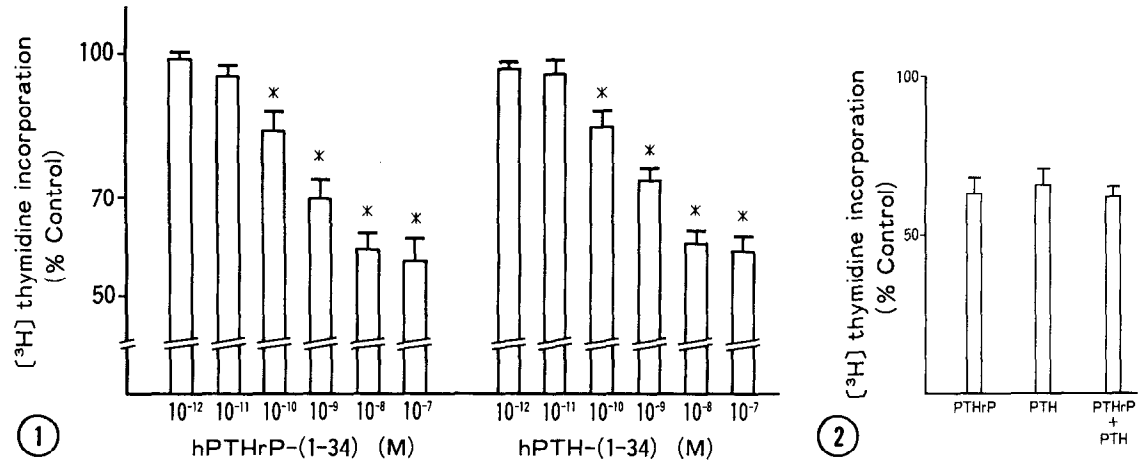

Fig. 1. Dose-response effect of hPTHrP-(1-34) and hPTH-(1-34) on [^{3}H]thymidine incorporation. [^{3}H]thymidine incorporation was measured as described in Materials and Methods. Data are expressed as % control. Each bar represents the mean ± SEM of quadruplicate determinations.
* $P<0.01$, compared to control.

Fig. 2. Combined effects of hPTHrP-(1-34) and hPTH-(1-34) on [^{3}H]thymidine incorporation. After treatment with 10^{-7}M hPTHrP-(1-34) and/or 10^{-7}M hPTH-(1-34), [^{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.

minimal effective dose at 10^{-5}M in the same manner as PTH (Table 1). Present results strongly indicate that PTHrP inhibited the proliferation of UMR-106 cells presumably through the same mechanism, namely the activation of PKA.

Table 1. Effect of Rp-cAMPS on PTHrP- or PTH-induced suppression of [^{3}H]thymidine incorporation

	[^{3}H]thymidine incorporation (% Control)	
Rp-cAMPS (M)	hPTHrP-(1-34)	hPTH-(1-34)
0	56 ± 2	59 ± 2
10^{-6}	61 ± 1	64 ± 2
10^{-5}	72 ± 2 [a]	68 ± 1 [a]
10^{-4}	74 ± 3 [a]	73 ± 2 [a]

Cells were treated with 10^{-7}M hPTHrP-(1-34) or hPTH-(1-34) 30 min after the addition of various concentrations of Rp-cAMPS (10^{-6}-10^{-4} M). Data are expressed as %control. Each bar represents the mean ± SEM of quadruplicate determinations.
[a] $p<0.01$, compared to Rp-cAMPS-untreated group.

In the present study, however, Rp-cAMPS did not completely antagonize the PTHrP-induced suppression of TdR as well as PTH-induced one. As discussed in our recent report (9), three possibilities must be considered concerning these results. First, it might be possible that Rp-cAMPS even at high concentration of 10^{-4}M does not have the ability completely to block PTHrP-stimulated PKA activity. Second, Rp-cAMPS has been reported to inhibit phosphodiesterase activity in cultured Leydig tumor cells (14). 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. Third, it might also be presumed that PKA is not the only second messenger involved in the suppression of osteoblast proliferation by PTHrP. There has been recent evidence that dual second messenger systems (cAMP and calcium/protein kinase C) exist in manifestation of hormonal activity by PTH in UMR-106 cells (15-17). It is, therefore, possible that PTHrP might also possess such dual second messenger signaling in these cells. Indeed, Civitelli et al. recently reported that PTHrP as well as PTH caused a transient increase in cytosolic calcium in UMR-106 cells (3) and our preliminary study also reconfirmed this phenomenon (data not shown). Therefore, in addition to the activation of PKA, calcium/protein kinase C system might be also involved in PTHrP-induced inhibition of cell proliferation. Further studies are necessary to clarify these possibilities and these studies are in progress in our laboratories. An important conclusion derived from the present study is that the activation of PKA is an obligatory initiator of the suppression of cell proliferation by PTHrP in UMR-106 cells.

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