THE EFFECT OF PARATHYROID HORMONE (PTH) AND 1,25 DIHYDROXY VITAMIN D₃ (1,25(OH)₂D₃) ON RNA AND HEME SYNTHESIS BY ERYTHROID PRECURSORS

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Summary

PTH was shown to stimulate RNA and heme synthesis in 12–13 days embryonic mouse liver erythroid cells, thus expressing an erythropoietin-like effect. Incubation of erythroid precursors with 1,25(OH)₂D₃ had no effect on heme synthesis, and increased the RNA synthesis only slightly, thus showing no enhancement effect of 1,25(OH)₂D₃ on the PTH stimulating effect on erythropoiesis.

Introduction

Anaemia is almost invariable in patients with chronic renal failure (CRF) and frequently complicates the course of a wide spectrum of other chronic disease. Reticulocytopenia, bone marrow histology, and ferrokinetic data suggest that the anaemia in chronic renal failure is due primarily to underproduction of red cells.

The presence of a serum factor toxic to erythropoiesis, has been suggested in patients with CRF. It has been shown that erythroblast maturation and DNA synthesis in vitro was inhibited by serum from uraemic patients. In addition the decreased response of uraemic animals to erythropoietin and the ability of CRF patients to increase their haematocrit following haemodialysis support this theory [1].

High levels of PTH have been suggested as one of the causes for the development of anaemia in patients with adenoma of the parathyroid as well as in uraemic patients. Recent data have suggested an improvement in the anaemia of haemodialysed patients following subtotal parathyroidectomy, and after the removal of the adenoma in primary hyperparathyroid states [2,3].

The aim of the present study was to examine the in vitro effect of PTH and 1,25(OH)₂D₂ on RNA and heme synthesis by erythroid cells using the embryonic mouse liver erythroid precursors as a model system.
Material and methods

Tissue culture

Erythroid precursors were obtained from embryonic livers of C₅₇Bl/6J mice.

RNA synthesis Twelve-day embryonic liver erythroblasts were incubated with different concentrations of PTH and 1,25(OH)₂D₃. ³H-uridine was added and the incorporation of the ³H-uridine into RNA was detected as described previously [4].

Heme synthesis Thirteen-day embryonic liver cells were incubated with PTH and 1,25(OH)₂D₃. At the end of incubation ⁵⁹FeCl₃ was added and its incorporation into heme was determined using the method of Teale [5].

Results

Effect of 1,25(OH)₂D₃ on RNA and heme synthesis

A dose dependent effect of 1,25(OH)₂D₃ on ³H-uridine incorporation into erythrocyte cells is shown in Figure 1; 0.5ng/ml of 1,25(OH)₂D₃ produced maximal

![Figure 1](image)

Figure 1. The effect of 1,25(OH)₂D₃ on RNA synthesis by 12th-day embryonic mouse liver erythroblasts

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increase of $17 \pm 5.4\%$ (mean $\pm$ SE, $p < 0.02$) whereas with higher concentrations a decrease was observed. Four ng/ml caused a statistically significant inhibitory effect of $20 \pm 5\%$ ($p < 0.01$).

Preincubation of the cells with the same concentrations of $1,25(OH)_2 D_3$ for

![Graph](image)

**Figure 2.** Effect of PTH and $1,25(OH)_2 D_3$ on heme synthesis by 13th-day embryonic mouse liver erythroblasts

4 and 24 hours did not have any additional effect on RNA synthesis. $1,25(OH)_2 D_3$ had no effect on heme synthesis (Figure 2).

**Effect of $1,25(OH)_2 D_3$ and PTH on RNA synthesis**

As seen in Figure 3 the addition of $1,25(OH)_2 D_3$ to erythroid precursors incubated with PTH in a concentration known to stimulate RNA synthesis did not further increase $^3$H-uridine incorporation. The addition of $1,25(OH)_2 D_3$ in its stimulatory concentration did not show any effect on the inhibition observed using high concentrations of PTH (8u/ml) (Figure 3).
Figure 3. Effect of PTH and 1,25(OH)$_2$D$_3$ on RNA synthesis

Figure 4. Effect of preincubation periods with PTH on RNA synthesis of 12th-day embryonic mouse erythroblasts
Effect of PTH on RNA synthesis

The stimulatory effect of PTH (1u/ml) on RNA synthesis persisted even after the washing of the PTH from the erythroid cells. The effect was time dependent during the first 30 min, whereas longer incubation time produced no further response (Figure 4). Abolition of the inhibitory effect on higher concentrations of PTH (8u/ml) was achieved upon removal of the PTH from the incubation media.

Conclusion

It was shown that PTH stimulates RNA and heme synthesis in 12–13 day embryonic mouse liver erythroid cells, thus demonstrating an erythropoietin-like effect [6]. The stimulatory effect of PTH on RNA synthesis was persistent following the removal of the PTH from the erythroid cells. This observation suggests a possible signal given by the hormone which activates cell proliferation via elevation of intracellular cAMP levels. Abolition of the inhibitory effect of high concentration of PTH was achieved upon removal of the hormone from the incubation media. This observation correlates well with the in vivo studies in patients suffering from hyperparathyroidism with anaemia, showing improvement in erythropoiesis following decreased PTH levels post surgery.

1,25(OH)₂D₃ had no effect on heme synthesis and the slightly increased RNA synthesis seems to be non specific.

These results suggest no enhancement effect of 1,25(OH)₂D₃ on the PTH stimulating effect on erythropoiesis.

References

4 Agam G, Bessler H, Djaldetti M. Biochim biophys Acta 1976; 425: 41
5 Teale FWJ. Biochim biophys Acta 1959; 35: 543

Open Discussion

DRÜEKE (Paris) Did you use the entire parathyroid hormone or hormone fragments for your studies?

LEVI Well, we used the pure commercial entire PTH. We did not perform any segmental studies: it’s not available for us.

DRÜEKE What was the calcium concentration of your incubation medium and did the effect of PTH depend on the medium’s calcium concentration?

LEVI We are carrying out right now some experiments with verapamil to study the role of calcium.