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# 1,25-DIHYDROXYVITAMIN $D_3$ INCREASES SERUM LEVELS OF THE VITAMIN K-DEPENDENT BONE PROTEIN*

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## SUMMARY

1,25-dihydroxyvitamin $D_3$ increases serum levels of bone Gla protein (BGP). The maximal increase occurs 12 h after injection and is given by 350 ng $1,25(OH)_2D_3$ per 180 g body weight. In both 2 and 11 month-old male rats, the maximal increase is about 3 times the normal level, while in 2 month old female rats, the maximal increase is 2 times the normal level. These effects of $1,25(OH)_2D_3$ in rats parallel the previously described effects of the vitamin on BGP secretion by rat osteosarcoma cells in culture.

BGP is the first bone-specific protein whose synthesis in animals is dramatically increased by $1,25(OH)_2D_3$. The possible functions of BGP in the biological actions of $1,25(OH)_2D_3$ on bone are discussed.

## INTRODUCTION

The vitamin K-dependent bone protein is a 49-residue protein of known structure (1-3) which is found in the extracellular matrix of bone (4,5) and in blood plasma (6,7). This protein contains three residues of the vitamin K-dependent amino acid, γ-carboxyglutamic acid (Gla) and has been termed bone Gla protein or BGP.[1] The best insight into probable functions for BGP has come from the analysis of bones from animals

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1. Gla, γ-carboxyglutamic acid; bone Gla protein (BGP), γ-carboxyglutamic acid-containing protein of bone.

2. $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin $D_3$.

3. CaBP, duodenal vitamin D-dependent calcium-binding protein.

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maintained chronically on Warfarin, a vitamin K antagonist which inhibits the formation of γ-carboxyglutamic acid. These bones have only 5% of normal BGP levels, yet have normal mineral and protein contents, normal morphology, and normal strength (8). The absence of structural abnormalities in these bones has led to the suggestion that BGP has an informational rather than a structural role in bone.

The presence of BGP in plasma has raised the possibility that the plasma protein plays a messenger role (8). Clinical studies support this possibility since plasma BGP levels are dramatically elevated in patients with metabolic bone disease characterized by increased bone turnover (9). These increased plasma BGP levels could reflect a role for BGP in the regulation of calcium or skeletal homeostasis.

The recent discovery that 1,25-dihydroxyvitamin $D_3$[2] regulates the synthesis of bone Gla protein (BGP) by rat osteosarcoma cells has raised the possibility that BGP may mediate some action of vitamin D on bone (10). To understand the nature of this action, it is clearly necessary to determine the circumstances under which 1,25-dihydroxyvitamin $D_3$ regulates BGP synthesis in animals. In the present experiments, we have accordingly determined the plasma BGP response to intravenously administered $1,25(OH)_2D_3$ in male and female rats.

## EXPERIMENTAL PROCEDURES

Materials. Sprague-Dawley derived rats were purchased from Simonsen Laboratories. Normal rodent chow was obtained from Ralston Purina. $1,25(OH)_2D_3$ was a generous gift from Hoffman-LaRoche.

Methods. Rats were anaesthetized with ether and then injected intrajugularly with 25 μl of the desired amount of $1,25(OH)_2D_3$ in 95% ethanol. After the injections, rats were given food and water ad libitum. Animals were exsanguinated at the indicated times by cardiac puncture and the blood samples were allowed to clot overnight at 4°. Serum samples were stored frozen prior to assay.

Triplicate aliquots of serum from each rat were assayed for BGP by radioimmunoassay using procedures described previously (7). All data points given here are the average serum BGP levels for 4 rats exsanguinated at the indicated time after $1,25(OH)_2D_3$ administration.

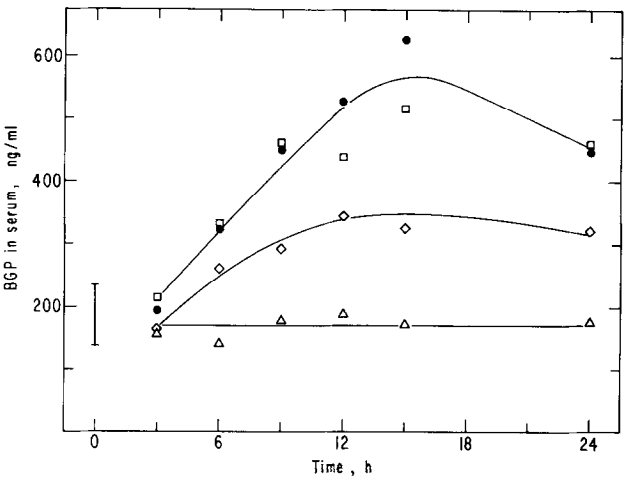


Fig. 1. Dose dependence of serum BGP on the level of $1,25(OH)_2D_3$. Forty-seven day old male rats were intravenously injected with 80 (◇), 350 (●), and 1500 (□) ng $1,25(OH)_2D_3$ per 180 g body weight. Control rats (△) were injected with EtOH alone. Each point represents the average serum BGP level of four rats sacrificed at the indicated times.

## RESULTS

The response of serum BGP levels to intravenously administered $1,25(OH)_2D_3$ was first determined in young male rats. As can be seen in Figure 1, serum BGP levels begin to rise 6 hours after the administration of 350 ng $1,25(OH)_2D_3$ and reach levels which are three times control values by 15 h. Increasing the amount of $1,25(OH)_2D_3$ administered from 350 to 1500 ng does not increase the serum BGP response while reducing the level of $1,25(OH)_2D_3$ injected to 80 ng decreases the serum BGP response to approximately half the maximum level. Figure 2 shows that serum BGP levels return to control values 48 hours after the administration of 350 ng $1,25(OH)_2D_3$.

The serum BGP response of $1,25(OH)_2D_3$ in 11 month-old adult male rats is compared with that of the younger animals in Figure 3. The time course of the serum BGP response to $1,25(OH)_2D_3$ is identical in the young and the adult rat. In addition, both animals give the same maximal response of three times the control values. It should be noted

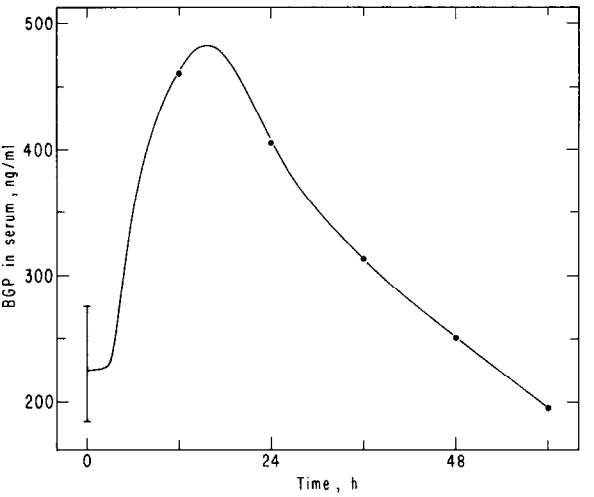


Fig. 2. Time course of the maximal serum BGP response to $1,25(OH)_2D_3$. Fifty-three day old male rats were injected intravenously with 350 ng $1,25(OH)_2D_3$ per 180 g body weight. Each point represents the average serum BGP level of four rats sacrificed at the indicated times.

that serum BGP levels do decrease significantly with age, a result noted also in previous studies (6), and that the scales used for young and adult rats have been adjusted to better facilitate a comparison of the relative response to $1,25(OH)_2D_3$.

The serum BGP response in young female rats is compared with that of age-matched young male rats in Figure 4. The time course of the serum BGP response to 350 ng $1,25(OH)_2D_3$ is identical in male and female rats while the magnitude of the response is significantly lower for the female animals.

## DISCUSSION

The present experiments clearly demonstrate that $1,25(OH)_2D_3$ administration elevates serum BGP levels in the rat. The probable explanation for this effect is that $1,25(OH)_2D_3$ administration increases the synthesis of BGP and that this increased BGP synthesis rate results in elevated serum BGP levels. The best evidence for this interpretation

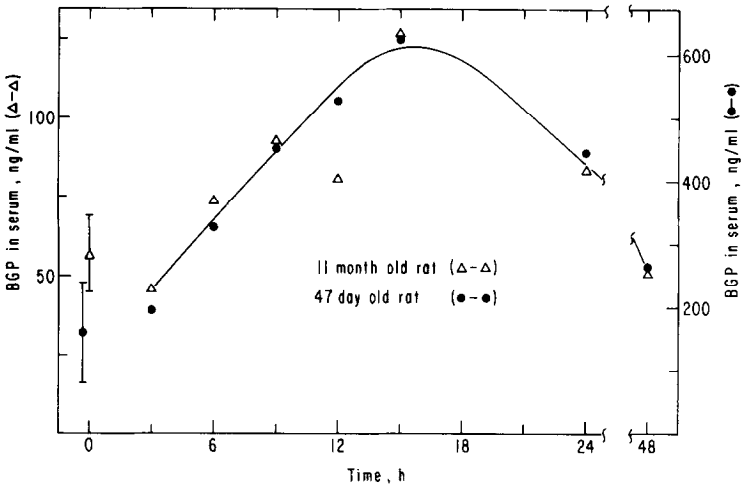


Fig. 3. Effect of age on the serum BGP response to $1,25(OH)_2D_3$. The serum BGP response to an intravenous injection of 350 ng $1,25(OH)_2D_3$ per 180 g body weight is compared in forty-seven day (●) and eleven month (△) male rats. Each point represents the average serum BGP level of four rats sacrificed at the indicated times.

is the similarity in the $1,25(OH)_2D_3$ response of serum BGP levels in rats to the previously determined response of BGP secretion by rat osteosarcoma cells. In both systems there is a 3 to 6 h delay before BGP levels are first increased above control levels. In addition, the maximum response is reached by 12 to 15 h in both systems. Finally, the fact that BGP levels in serum increase 2 to 3 fold while BGP secretion rates by osteosarcoma cells increase 6-fold is consistent with the fact that half of the cellular response is achieved by levels of $1,25(OH)_2D_3$ normally found in plasma.

An alternative explanation for the serum BGP response to $1,25(OH)_2D_3$ is that $1,25(OH)_2D_3$ increases the rate of bone resorption which in turn releases BGP from the bone matrix into serum. One observation in conflict with this interpretation is the fact that the administration of $1,25(OH)_2D_3$ to rats on a normal diet does not increase the rate of bone resorption (11). In addition, developmental studies in the rat have shown that

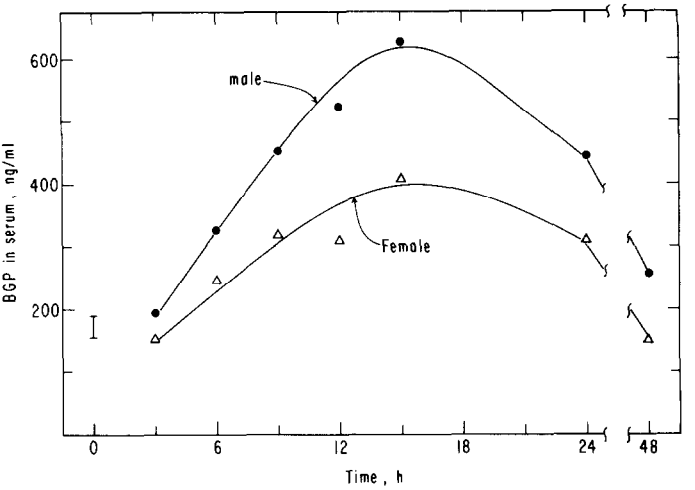


Fig. 4. Comparison of the serum BGP response of $1,25(OH)_2D_3$ in males and females. Forty-seven day male (●) and female (△) rats were injected intravenously with 350 ng $1,25(OH)_2D_3$ per 180 g body weight. Each point represents the average serum BGP level of four rats sacrificed at the indicated times.

serum BGP comes from new synthesis rather than from the release of BGP from the extracellular bone matrix during bone resorption (7).

The effect of $1,25(OH)_2D_3$ on serum BGP levels is almost identical to the effect seen in earlier studies on the intestinal calcium binding protein (CaBP).[3] CaBP levels are approximately doubled by $1,25(OH)_2D_3$ administration to D replete rats comparable in weight to those used in the present study (12). Half of the maximal CaBP response is reached at 125 ng of $1,25(OH)_2D_3$ and the maximal response at 500 ng (12). For comparison, $1,25(OH)_2D_3$ approximately triples serum BGP levels and the dosages needed for half maximal and maximal responses are 80 and 350 ng. The BGP and CaBP responses have similar duration, both returning to basal levels in about 48 h. The major difference in response is that CaBP levels are maximal 1 to 2 h after $1,25(OH)_2D_3$ administration (12). while BGP levels reach maximal values only after 12 to 15 h. Although the difference in the kinetics of the BGP and CaBP responses to $1,25(OH)_2D_3$ remain to be explained, the striking parallel in the amplitude, duration, and dose dependence of these responses strongly indicates that the

elevation of serum BGP levels is, like the elevation in CaBP, part of the normal physiological response to vitamin D.

While the actions of vitamin D on bone are incompletely understood, it is clear that vitamin D can stimulate the release of calcium from bone (13). Presumably, $1,25(OH)_2D_3$ elicits this response by acting on bone cells to increase the synthesis of proteins such as BGP. We can therefore propose either a direct or an indirect role for BGP in carrying out this action of vitamin D on bone (10). BGP may be a direct component of the mechanism which pumps calcium from bone, either reducing the rate of mineral deposition by inhibiting mineral growth (5) or by increasing the rate of mineral dissolution. Alternatively, BGP may be an informational protein which acts to regulate the cellular activities involved in bone turnover (8). For example, BGP could recruit osteoclats to loci of bone remodeling or increase the activity of such cells at the remodeling sites. Experiments are in progress to test these and other models for the role of BGP as mediator of the action of vitamin D on bone.

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