Vitamin D Metabolites Affect Serum Calcium and Phosphate in Freshwater Catfish, Heteropneustes fossilis

Authors: Srivastav, Ajai K., Srivastav, Sunil K., Sasayama, Yuichi, Suzuki, Nobuo, and Norman, Anthony W.

Source: Zoological Science, 14(5) : 743-746

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.14.743
Vitamin D Metabolites Affect Serum Calcium and Phosphate in Freshwater Catfish, *Heteropneustes fossilis*

Ajai K. Srivastav¹*, Sunil K. Srivastav¹, Yuichi Sasayama², Nobuo Suzuki² and Anthony W. Norman³

¹Department of Zoology, University of Gorakhpur, Gorakhpur 273 009, India  
²Noto Marine Laboratory, Faculty of Science, Kanazawa University, Ogi-Uchiura, Ishikawa 927-05, Japan  
³Department of Biochemistry, University of California, Riverside, CA 92521, USA

ABSTRACT—The effects of vitamin D₃, 24,25(OH)₂ vitamin D₃, 25(OH) vitamin D₃ and 1,25(OH)₂ vitamin D₃ were investigated on the serum calcium and phosphate levels of freshwater catfish, *Heteropneustes fossilis*. The fish were injected daily intraperitoneally with these secosteroids for 10 days. Blood samples were collected at day 1, 3, 5 and 10. Serum calcium and inorganic phosphate levels were elevated by all of the treatments except for 24,25(OH)₂ vitamin D₃.

INTRODUCTION

Bony fishes, particularly those inhabiting seawater, contain large hepatic stores of vitamin D (Copping, 1934; Urist, 1976; Takeuchi et al., 1984). Vitamin D₃, which itself apparently lacks direct biological activity, produces a number of metabolites after sequential hydroxylation in liver and kidney (Norman et al., 1982). Teleosts inhabiting freshwater and seawater are able to convert vitamin D₃ and 25(OH)D₃ to more polar metabolites (Hayes et al., 1986; Takeuchi et al., 1991). Moreover, fish contains circulating levels of vitamin D₃, 25(OH)D₃, 1,25(OH)₂D₃ and 24,25(OH)₂D₃ (Hay and Watson, 1976; Nahm et al., 1979; Avioli et al., 1981; Takeuchi et al., 1991; Sundell et al., 1992; Rao and Raghuaramulu, 1995). Furthermore, specific binding proteins for 1,25(OH)₂D₃ have been demonstrated in tissues from European eel and Atlantic cod (Marcocci et al., 1982; Sundell et al., 1992). These studies suggest a physiological role for vitamin D₃ system in fishes.

The effects of vitamin D₃ and its metabolites on calcium homeostasis have been studied in a few freshwater teleosts (*Amphipnous cuchia*; Srivastav, 1983; *Anguilla rostrata*; Fenwick et al., 1984; *Clarias batrachus*; Swarup and Srivastav, 1982; Swarup et al., 1984; Srivastav and Srivastav, 1988; *Cyprinus carpio*; Swarup et al., 1991; Srivastav et al., 1993: *Carrassius auratus*; Fenwick, 1984: *Heteropneustes fossilis*; Srivastav and Singh, 1992) and a few marine species (*Gadus morhua*; Sundell et al., 1993; *Pagophenia bernachii*; Fenwick et al., 1994). Nevertheless there is still considerable controversy regarding the physiological role of this vitamin and its metabolites in teleosts as many of the previous reports are contradictory. Administration of vitamin D or its metabolites has been reported to cause either (i) no significant change (Urist, 1962; MacIntyre et al., 1976; Lopez et al., 1977), (ii) increase (Srivastav, 1983; Fenwick, 1984; Swarup et al., 1984, 1991; Fenwick et al., 1984, 1994; Srivastav and Srivastav, 1988; Srivastav and Singh, 1992), or (iii) decrease (Sundell et al., 1993) in the blood calcium content. Moreover, the effect of 24,25(OH)₂D₃ has been investigated only in *Sarotherodon mossambicus* (a freshwater species, Wendelaar Bonga et al., 1983) and *Gadus morhua* (a marine species, Sundell et al., 1993).

The present study was undertaken to investigate the effects of vitamin D and some of its major metabolites on serum calcium and phosphate of a freshwater catfish, *Heteropneustes fossilis*.

MATERIALS AND METHODS

Freshwater catfish, *H. fossilis* of both sexes were procured and acclimated to laboratory conditions at 27 ± 2°C for one week prior to the experiment. The fish weighed between 45-64 g and were not fed following their capture. Blood samples from six fish was taken prior to the start of the experiment (zero hour). The remaining fish were randomly divided into five groups of 24 fish each. These groups received daily intraperitoneal injections of either vehicle (95% ethanol; group A), vitamin D₃ (5 µg; group B), 24,25(OH)₂D₃ (2 µg; group C), 25(OH)D₃ (1 µg; group D), or 1,25(OH)₂D₃ (0.5 µg; group E). The doses indicated are per 100 g body wt of fish/0.5 ml. The doses of various vitamin D metabolites used in the present study correspond more or less to the doses used in other teleosts by previous investigators (Wendelaar Bonga et al., 1983; Sundell et al., 1993).

Six fish from each group were anesthetized with MS222 and blood samples were collected 4 hr after the last injection (by a syringe from the caudal vessels) after 1, 3, 5 and 10 days of treatment. Sera were separated by centrifugation and total calcium and phosphate were measured according to Trinder (1960) and Fiske and Subbarow (1925).
methods, respectively. Calcium from serum was precipitated as an insoluble orange-red complex by an alkaline solution of naphthol-hydroxamic acid. After centrifugation the precipitate was dissolved in alkaline disodium ethylenediamine tetraacetate, then treated with ferric nitrate and the resultant amber colour was measured colorimetrically. For phosphate, the serum was deproteinized by adding trichloroacetic acid. To the filtrate, ammonium molybdate was added followed by 1,2,4-aminonaphthalene sulfonic acid. The resultant blue colour was measured colorimetrically.

Student's t test was used to determine statistical significance. In all cases, the experimental group was compared with the vehicle-injected group sampled at the same time. The data were also subjected to two-way ANOVA.

RESULTS

Serum calcium levels of fish treated with various vitamin D analogs are shown in Fig. 1. Both vitamin D3 and 25(OH)D3 increased the serum calcium levels at day 3 and day 5. No changes were observed in calcium concentrations following 24,25(OH)2D3 treatment. The serum calcium level of 1,25-(OH)2D3 treated fish increased more rapidly and showed a significant increase on day 1 which progressively increased till day 5. All groups were normocalcemic by day 10.

Serum phosphate levels were unaffected through day 3 except for the 1,25(OH)2D3 treated fish which were hyperphosphatemic. By day 5, all the treated groups were hyperphosphatemic with the exception of the 24,25(OH)2D3 treated group (Fig. 2). Unlike the situation with calcium which return to normal values by day 10, the hyperphosphatemic effect, when stimulated, remained so up to day 10.

Comparing (two-way ANOVA) serum calcium and phosphate levels of *H. fossilis* treated with various vitamin D metabolites, it has been observed that these electrolytes differed significantly between the exposure period (for calcium F=4.581 and P<0.01; for phosphate F=3.465 and P<0.04), whereas between the various treatments used in this study, only phosphate levels differed significantly (for calcium F=2.002 and P<0.16, not significant; for phosphate F=4.323 and P<0.02).

DISCUSSION

The data shows that vitamin D3, 25(OH)D3 and 1, 25(OH)2D3 affect calcium homeostasis in *H. fossilis*. These observations are in accord with the results of other investigations in which administration of vitamin D2 and these metabolites elevated the serum/plasma calcium (total) concentrations in other fishes (Swarup and Srivastav, 1982; Srivastav, 1983; Fenwick, 1984; Swarup *et al.*, 1984, 1991; Srivastav and Swarup, 1988; Srivastav and Singh, 1992; Fenwick *et al.*, 1984, 1994). Administration of 1,25(OH)2D3 to marine fishes has been reported either to increase (*Gadus morhua*; Sundell *et al.*, 1993) or decrease (*Pagotenia bernachi*; Fenwick *et al.*, 1994) the ionized calcium concentration without altering the total plasma calcium levels. In contrast to the present study, daily injections (for seven days) of 25(OH)D3 to Atlantic cod lowered the total calcium levels (Sundell *et al.*, 1993). 25(OH)D3 treatment produced no significant effect on either ionized or total calcium concentration of *Pagotenia bernachii* (Fenwick *et al.*, 1994).

24,25(OH)2D3 injections to *H. fossilis* did not affect serum calcium levels and this agrees with the studies of Sundell *et al*.
Vitamin D Metabolites in Fish

more active form, probably 1,25(OH)_2D_3 as these secosteroids produced an effect only on day 3 whereas 1,25(OH)_2D_3 produced an effect in one day. The present results together with those of previous report (Sundell and Björnsson, 1990) suggest that there exists different functional aspects in the actions of vitamin D_3 and its metabolites in freshwater teleosts (freshwater environment is hypotonic in relation to the blood — where vitamin D_3 analogs affect calcium homeostasis) and marine teleosts (sea water is hypertonic in relation to the blood — where vitamin D_2 and 1,25(OH)_2D_2 produced contradictory effects; Sundell et al., 1993; Fenwick et al., 1994).

REFERENCES


al. (1993) who have also noticed no change in calcium contents of 24,25(OH)_2D_3 treated Atlantic cod.

In fishes vitamin D_3 and 1,25(OH)_2D_3 increased calcium uptake (Charlier et al., 1979; Flik et al., 1982; Fenwick, 1984; Fenwick et al., 1984). In Atlantic cod 25(OH)D_3 stimulated intestinal calcium absorption whereas vitamin D_3 and 1, 25(OH)_2D_3 did not affect the calcium influx across the intestinal mucosa (Sundell and Björnsson, 1990). The observed hypercalcemia in H. fossilis may be explained by mobilization of calcium from internal stores and/or increased renal retention of calcium. Indeed, 1,25(OH)_2D_3 was shown to increase bone mineralization in teleosts (Lopez et al., 1977; Wendelaar Bonga et al., 1983). Moreover, an increased calcium uptake by the gills from the environment after treatment with these metabolites can not be ruled out.

The hyperphosphatemia evoked by the administration of vitamin D_3, 25(OH)D_3 and 1,25(OH)_2D_3 to H. fossilis is similar to that reported previously (MacIntyre et al., 1976; Fenwick et al., 1984; Swarup et al., 1984, 1991; Srivastav and Singh, 1992). In contrast, Sundell et al. (1993) and Fenwick et al. (1994) have found no effect of these secosteroids on plasma phosphate content. It is of interest to note that in H. fossilis 24,25(OH)_2D_3 produced elevated phosphate levels although this increase was not statistically significant. The hyperphosphatemic response of vitamin D_3 and its metabolites in H. fossilis suggests that the nondietary phosphorus, possibly from the bone and/or from the soft tissues, can be mobilized. The increased renal retention of phosphate also can not be ruled out.

The different outcomes in the calcium and phosphate levels of H. fossilis at some time intervals in response to vitamin D_3 and its metabolites administration may be due to reported differences in the mechanism of actions of these metabolites— a slow genome-mediated and a rapid nongenome-mediated transcalcatic response (Larsson et al., 1995).

In the present study serum calcium levels returned to normal at day 10 whereas phosphate levels were still increased. The recovery of serum calcium may be attributed to increased release of the hypocalcemic factor stanniocalcin from the corpuscles of Stannius after continuous hypercalcemic challenge induced by vitamin D_3 and its metabolites. Stanniocalcin has been reported to inhibit branchial Ca^{2+} influx (Lafeber et al., 1988; Verboss and Fenwick, 1995). An increased activity of corpuscles of Stannius after vitamin D_3/ 1,25(OH)_2D_3 has been reported in a freshwater catfish (Clarias batrachus) (Srivastav et al., 1985; Srivastav and Srivastav, 1988). The persisting increased serum phosphate levels at day 10 could be ascribed to the possible renal retention of phosphate by enhanced secretion of stanniocalcin which has been shown to stimulate the net renal phosphate reabsorption (Renfro et al., 1996).

The present study concludes that vitamin D_3 and two of its prime metabolites, 25(OH)D_3 and 1,25(OH)_2D_3 can affect both calcium and phosphate metabolism in a freshwater teleost, H. fossilis. We also do not feel it unreasonable to speculate that vitamin D_3 and 25(OH)D_3 have to be converted to a...
82: 396–402

(Received April 23, 1997 / Accepted July 1, 1997)