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# PROLACTIN AND CALCIUM METABOLISM IN A TELEOST FISH, *Sarotherodon mossambicus*

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## PROLACTIN AND PLASMA ELECTROLYTES

Many freshwater adapted fish demonstrate an efficient hypercalcemic control of plasma calcium levels, but the identity of the endocrine factors involved is still in discussion. Parathyroid glands are lacking in fish, and, although the PAS-positive pars intermedia cells in trout (1) and the Stannius corpuscles in eel (2) have been shown to contain substances with some immunological reactivity to antibodies raised against mammalian parathyroid hormone (PTH), the presence of a hypercalcemic factor in fish equal to PTH is unlikely. The involvement of PTH in hypercalcemic control is apparently restricted to terrestrial vertebrates (3, 4).

A possible role of the pituitary gland in teleost calcium regulation was first indicated by observations that hypophysectomy of freshwater eels led to reduction of the plasma calcium levels (5,6). Since the concentrations of other plasma electrolytes were also reduced, the effects of hypophysectomy were interpreted as signs of general osmotic imbalance rather than as specific effects on plasma calcium. However, Pang *et al.* (7) observed that hypophysectomized *Fundulus heteroclitus* adapted to calcium-deficient seawater showed reduced plasma calcium levels without noticeable effects on other plasma electrolytes. They conclude, therefore, that the pituitary gland might exert a hypercalcemic influence distinct from its effects on osmoregulation. This was supported by the observation that injection of pituitary homogenates or implantation of whole pituitary glands corrected the hypocalcemia. The active compound turned out to be prolactin (8,9). Ovine prolactin also induced hypercalcemia in intact *F. heteroclitus* adapted to seawater, to calcium-deficient seawater, or to saline, i.e. fish with supposedly low endogenous prolactin levels (10). In intact sticklebacks (*Gasterosteus aculeatus*) and tilapia (*Sarotherodon mossambicus*) adapted to freshwater, administration of ovine prolactin for 6 or 9 days, respectively, induced an increase in plasma total calcium (Table I).



Table I. Effects of prolactin on blood plasma

	calcium (mmol/l)	Na <sup>+</sup> (mmol/l)	osmolarity (mosmol/l)
<i>S. mossambicus</i> (n=10)			
F.W.: prolactin	3.06 $\pm$ 0.12 <sup>a</sup>	141 $\pm$ 4	327 $\pm$ 3
controls	2.84 $\pm$ 0.08	136 $\pm$ 3	323 $\pm$ 4
S.W.: prolactin	3.27 $\pm$ 0.05 <sup>a</sup>	164 $\pm$ 5 <sup>a</sup>	358 $\pm$ 5 <sup>a</sup>
controls	3.11 $\pm$ 0.10	143 $\pm$ 4	344 $\pm$ 4
<i>G. aculeatus</i> (n=6)			
F.W.: prolactin	2.43 $\pm$ 0.09 <sup>a</sup>	128 $\pm$ 4	322 $\pm$ 6
controls	2.21 $\pm$ 0.11	127 $\pm$ 5	311 $\pm$ 7
S.W.: prolactin	3.20 $\pm$ 0.10	163 $\pm$ 5 <sup>a</sup>	363 $\pm$ 7
controls	3.14 $\pm$ 0.08	141 $\pm$ 7	349 $\pm$ 8

<sup>a</sup>) Significantly different from controls,  $P < 0.01$ .

The effect of ovine prolactin on plasma total calcium, Na<sup>+</sup> and osmolarity in mature *S. mossambicus* and immature female *G. aculeatus*. Prolactin was administered to *S. mossambicus* for 9 days at a constant rate in a dose of 0.15 I.U./g/day by Alzet osmotic minipumps implanted into the peritoneal cavity; controls received a solvent releasing pump. The sticklebacks received 6 daily injections (prolactin: 0.15 I.U./g/day; controls: solvent). Means  $\pm$  S.D.; F.W.: freshwater fish; S.W.: fish adapted to seawater for 3 weeks.

However, the rise in plasma calcium was small. This is unlikely to be related to the high endogenous prolactin secretion rate that characterizes freshwater teleosts. In seawater adapted fish, which have inactive prolactin cells, the increase in plasma calcium following prolactin treatment was similar (tilapia) or even less (sticklebacks) than in freshwater fish (Table I). In addition, the plasma sodium levels were significantly elevated in seawater adapted fish of both species (Table I). More pronounced effects of ovine prolactin on plasma sodium levels in seawater adapted *S. mossambicus* have been reported by others (11-13). Prolactin further prevents the decline in plasma osmolarity and sodium following hypophysectomy in tilapia (14,15) and following transfer of intact sticklebacks from seawater to freshwater (16,17). Effects on plasma chloride have also been reported (13,17). Thus, the effects of prolactin on plasma electrolytes in these species are certainly not restricted to calcium. Nevertheless, the results of our experiments with sticklebacks (18,19) and tilapia (20) showed an inverse relationship between prolactin cell activity and the external calcium concentration. High calcium levels in fresh-



water reduce prolactin secretion almost to the low levels characteristic for seawater fish. Although the osmolarity of the ambient water also affects prolactin secretion, this effect is only noticeable in freshwater or seawater with low calcium levels (21). Magnesium ions have similar effects on prolactin secretion as calcium ions, but only at concentrations about six times higher than those of calcium (19). These observations point to a specific involvement of prolactin in calcium metabolism. We therefore determined the effects of ovine prolactin on total calcium uptake and on the calcium concentrations of the major calcium-containing compartments: bone, scales and muscular tissue.

#### PROLACTIN AND CALCIUM UPTAKE

Whole body calcium uptake was studied by exposing animals to  $^{47}\text{Ca}$ -labeled freshwater. Male tilapia (about 18 g body weight) received 6 daily injections with ovine prolactin and  $^{47}\text{Ca}$  uptake was determined with a gamma ray spectrometer equipped with a NaI(Tl) scintillation crystal. Compared to solvent injected controls, the  $^{47}\text{Ca}$  uptake was significantly elevated ( $P < 0.01$ ; Fig. 1). The  $^{47}\text{Ca}$  content of blood plasma and bones was also significantly enhanced in the prolactin-treated fish (results not shown here).

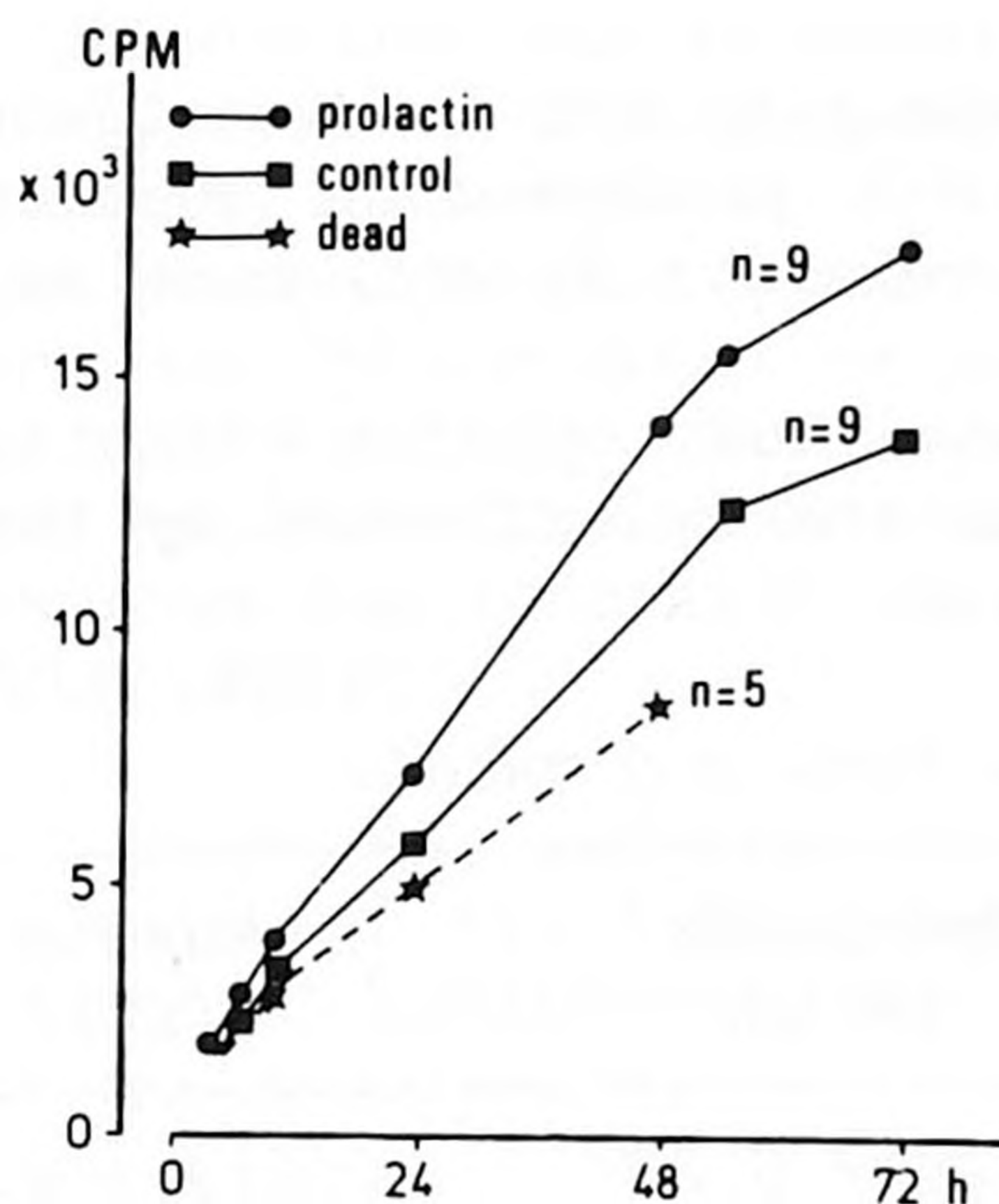


Fig. 1. The effect of 6 daily injections with ovine prolactin (0.15 I.U./g/day) on whole body  $^{47}\text{Ca}$  uptake of freshwater male *S. mossambicus*; 4 injections were given before, two during exposure to  $^{47}\text{Ca}$  (start:  $t = 0$ ); controls were injected with solvent (0.6% saline); dead: untreated fish killed at  $t = 0$ , indicating that a significant part of the  $^{47}\text{Ca}$  is bound to the body, probably by exchange of calcium at the body surface, without entering the blood circulation.



## PROLACTIN AND CALCIUM IN BONE AND MUSCLE

The effects of prolactin on the calcium content of skeletal and scalar bone and of the dorsal body musculature were examined in male *S. mossambicus* of about 30 g body weight. Technical details are described elsewhere (22). Treatment for 9 days with ovine prolactin induces a significant rise in the calcium concentration of opercular bone and fin rays (about 14%), and an even more marked increase in the calcium concentration of the scales (26%). The phosphate concentrations in bones and scales were increased to the same extent (data not shown here). The  $\text{Ca}/\text{PO}_4$  molar ratio was about 1.6 in both the prolactin treated fish and in the controls. This is close to the ratio found for hydroxyapatite. Prolactin may also exert some effect on muscle calcium but the present data are not conclusive (Table II).

If we assume that the distribution of calcium over the body compartment in *S. mossambicus* is similar to that in *Fundulus kansae* (bone: 78.06%; skin and scales: 19.25%; soft tissue: 2.69%; 23), the increase calculated for total body calcium caused by prolactin amounts to about 16%. About 11% of this increase in total calcium is accounted for by the skeletal bones, and 5% by the scales. Less than 0.1% was left in the blood. No differences could be detected in the total body sodium content (data not presented here).

## CONCLUSION

We conclude that prolactin has specific and marked effects on calcium and phosphate metabolism in *S. mossambicus*. Whether this conclusion pertains to other teleosts as well remains to be established.

The extent of the increase in total body calcium effected by prolactin in *S. mossambicus* is only weakly reflected by the changes noticeable in plasma calcium. This does not necessarily

Table II. *Effects of prolactin on bone and muscle*

	Scales (n=10)	Operculum (n=10)	Muscle (n=5)
prolactin	6.44 $\pm$ 0.34 <sup>a</sup>	6.56 $\pm$ 0.36 <sup>a</sup>	0.014 $\pm$ 0.002
control	5.01 $\pm$ 0.29	5.69 $\pm$ 0.30	0.010 $\pm$ 0.003

<sup>a</sup>) Significantly different from controls,  $P < 0.001$

*Effects of ovine prolactin (0.15 I.U./g/day), administered for 9 days by Alzet osmotic minipumps (see Table I), on calcium concentrations (mmol/g dry weight) in bone and dorsal musculature of freshwater *S. mossambicus*; means  $\pm$  S.D.*



imply that prolactin is no significant factor in the homeostatic control of plasma calcium. Although the total amount of calcium present in the blood is negligible from a quantitative point of view, even slight changes in the blood level of ionized calcium may have considerable physiological effects, for instance on the permeability for water and ions of cell membranes in general, and on those of the integumental gill epithelium in particular. In this respect it is of interest that prolactin has been implied in the control of water and ion permeability of the gills and other epithelia in fish (24-26), including *S. mossambicus* (15, 21). One of our observations indicated that the control of permeability, more than stimulation of calcium uptake, is the main function of prolactin in tilapia. Whereas prolactin cells are highly active in fish from freshwater with low calcium levels, they become inactivated when fish are transferred to a solution of NaCl in freshwater that is iso-osmotic with the body fluids, but with the same calcium concentration as in freshwater (21). Under iso-osmotic conditions there is no osmotic water flow across the integument while the passive fluxes of  $\text{Na}^+$  and  $\text{Cl}^-$  are very low, and thus there is no necessity for the fish to reduce the permeability of the integument for water and for these ions. This may explain the low prolactin cell activity and the high branchial osmotic water permeability that is found in fish from iso-osmotic saline (21).

Calcium ions are known to reduce the permeability of membranes, including the permeability of fish gills for water and ions (27, 28). We have suggested that the effects of prolactin on branchial permeability are mediated by calcium, for instance via the plasma calcium concentration (21). Such a link would explain the relationship we have found in tilapia between prolactin cell activity, the branchial osmotic water permeability, and the ambient calcium levels.

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