THE ROLE OF ENVIRONMENTAL CALCIUM AND MAGNESIUM IONS IN THE CONTROL OF PROLACTIN SECRETION IN THE TELEOST \textit{Gasterosteus aculeatus}

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INTRODUCTION

In teleosts, prolactin is essential for maintaining ionic and osmotic homeostasis in a freshwater (FW) environment. In euryhaline fish prolactin secretion is greatly reduced upon migration or transfer from FW to seawater (SW). This reduction has been ascribed to the high osmolarity or the high Na\(^+\) content of SW\(^1\),\(^2\). This effect of the environment has been supposed to be mediated by the rise in plasma osmolarity and Na\(^+\) that occurs in fish entering SW\(^1\),\(^3\). We have found evidence, however, that for sticklebacks the relatively high Ca\(^{2+}\) and Mg\(^{2+}\) concentrations of the environment are the main factors responsible for the reduced prolactin secretion in SW, whereas the high secretory activity in FW fish has been related to the low content of these ions in FW\(^3\). In this paper we first report the effects of varying concentrations of environmental Na\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\) on prolactin cell activity of sticklebacks in FW.

We have also suggested that plasma Ca\(^{2+}\), rather than osmolarity or Na\(^+\), is the relevant plasma factor for the control of prolactin secretion\(^3\). Pilot studies showed that changing the external Ca\(^{2+}\) concentration in FW resulted in parallel changes in plasma calcium, Na\(^+\) and osmolarity. Consequently, it proved hard to distinguish between the effects of these internal factors on prolactin secretion. Therefore, we investigated the effect of transferring fish directly from SW to SW with greatly reduced Ca\(^{2+}\) and Mg\(^{2+}\) concentrations. This treatment appeared to result in a rise of plasma osmolarity and Na\(^+\), and a decrease of plasma calcium.

MATERIALS AND METHODS

Techniques used and maintenance of the fish were the same as described earlier\(^3\).

**Experiment 1.** Groups of five FW fish were exposed to the following solutions:

a. artificial FW containing 0.06 mmol/l K\(^+\), 0.2 mmol/l Mg\(^{2+}\), 0.1 mmol/l Ca\(^{2+}\), and varying Na\(^+\) concentrations, namely 0.5; 4; 10; or 600 mmol/l NaCl;

b. artificial FW containing 2.1 mmol/l Na\(^+\), 0.06 mmol/l K\(^+\), 0.01 mmol/l Mg\(^{2+}\), and varying Ca\(^{2+}\) concentrations, namely 0.01; 2.0; 5.0; 10.2 or 20.0 mmol/l CaCl\(_2\cdot2\text{H}_2\text{O}\);

c. artificial FW containing 2.1 mmol/l Na\(^+\), 0.06 mmol/l K\(^+\), 0.01 mmol/l Ca\(^{2+}\), and varying Mg\(^{2+}\) concentrations, namely 0.01; 10.2; 20.0; 33.0 or 50.0 mmol/l MgCl\(_2\cdot6\text{H}_2\text{O}\).

Fish were gradually adapted to the above FW solutions in a period of six days (6 steps) to which they were then exposed for another 10 days.

**Experiment 2.** Fish maintained for at least six weeks in artificial SW\(^3\) containing
10.2 mmol/l Ca\(^{2+}\) and 56.0 mmol/l Mg\(^{2+}\) were transferred directly to artificial SW containing 1.0 mmol/l Ca\(^{2+}\) and 4.0 mmol/l Mg\(^{2+}\). In this experiment plasma calcium and osmolality were determined 12 h, 48 h and 10 days after transfer.

RESULTS

Experiment 1 (fig. 1). Increasing the Na\(^{+}\) concentration of FW affected prolactin cell size, but only the highest concentration used, namely 600 mmol/l (i.e. 1.5 x the Na\(^{+}\) concentration in SW), caused a significant reduction in the volume of the cells (p < 0.05). Contrastingly, the addition of small amounts of Ca\(^{2+}\) or Mg\(^{2+}\) led to a considerable reduction of prolactin cell size. Values as low as those normally found in SW fish occurred already after exposure to 5 mmol/l Ca\(^{2+}\) and of 33 mmol/l Mg\(^{2+}\) (i.e. at 50 and 75% respectively of the concentrations of these ions in SW).

Experiment 2 (fig. 2). Transfer of SW fish to SW with Ca\(^{2+}\) and Mg\(^{2+}\) concentrations as low as in FW led to a marked rise in plasma osmolality (p < 0.01) and a reduction of plasma Ca\(^{2+}\) (p < 0.05) within the first 12 h. Prolactin cell size remained unchanged during this period, but examination of the ultrastructure of the cells showed signs of increased hormone synthesis and release. The Golgi areas showed many presecretory granules (fig. 3) that are hardly found in normal SW fish. The number of mature granules was reduced.
to less than half of the control value, while indications of exocytosis, seldomly observed in SW fish, were occasionally seen.

After 48 h plasma osmolality had decreased, but was still above the initial level. A slightly elevated level was still found after 10 days. Plasma calcium remained below the initial values after 48 h and 10 days. Growth of the prolactin cells, evident already after 48 h, continued till day 10, but it remained well below the values typical for FW fish. It is concluded that the prolactin cells are activated by transfer to Ca\(^{2+}\) - and Mg\(^{2+}\)-low SW.

DISCUSSION

The capacity of fish to maintain ionic and osmotic homeostasis is mainly determined by the efficiency of its mechanisms to reduce the permeability of the integument for monovalent ions and water. In SW, the high external Ca\(^{2+}\) and Mg\(^{2+}\) levels are known to reduce the integumental permeability\(^5,6\). In the absence of high Ca\(^{2+}\) and Mg\(^{2+}\) levels, as is common for FW, prolactin is considered the principal hormonal factor that secures low permeability for monovalent ions\(^6,7\) and, at least as far as sticklebacks\(^7\), goldfish, trout and eel\(^8,9\) are concerned, also for water. The present study shows that in sticklebacks environmental Ca\(^{2+}\) and, to a lesser extent, Mg\(^{2+}\) are important factors in the control of prolactin secretion.

Earlier observations on sticklebacks showed that removal of the Stannius bodies, which led to a marked increase in plasma calcium while plasma osmolarity remained unaffected, was followed by considerable reduction in prolactin cell activity\(^10\). The prolactin cells of stanniectomized fish did not react to changes in environmental Ca\(^{2+}\). This finding indicated that these cells respond primarily to plasma calcium and we suggested, therefore, that in intact fish environmental Ca\(^{2+}\) controls prolactin secretion through modification of plasma calcium levels\(^10,11\). This suggestion is substantiated by the present results and may hold for Mg\(^{2+}\) as well (fig. 4).

For several other teleosts (Tilapia mossambica\(^2\), Gillichthys mirabilis\(^2\) and Anguilla anguilla\(^4\)) it has been suggested that plasma Na\(^+\) or plasma osmolarity are involved in controlling prolactin cell activity. This idea was based on observations on cultured pituitary glands. We found, however, that prolactin cells in Tilapia mossambica react essentially similar to changes in environmental Ca\(^{2+}\) and to stannietomy as prolactin cells in sticklebacks\(^12\). The question arises whether reactions of prolactin cells in \textit{vitro}, in the absence of inhibitory hypothalamic connections\(^13,14\), reflect the behaviour of these cells \textit{in situ}.

In addition to its role in maintaining ionic homeostasis, prolactin unmistakably
has hypercalcemic activities, at least in some species of teleosts. Injection of ovine prolactin \(^{10}\) and transplantation of prolactin lobes \(^{12}\) in FW sticklebacks raise plasma calcium levels. Similar results were obtained in other species \(^{15,16,17}\). The interesting possibility presents itself that in FW fish the direct action of prolactin concerns calcium metabolism and that an increase in plasma calcium levels and/or enhanced binding of Ca\(^{2+}\) to the integument, e.g. via induction of Ca-binding proteins \(^{18}\), in turn, controls plasma ionic composition and osmolarity. The results of the present study would then suggest that Ca\(^{2+}\), and possibly also Mg\(^{2+}\), exert a negative feedback control on prolactin secretion. The effects of these ions may be modulated by certain hormones and other regulatory factors \(^{19}\).

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