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ROLE OF PROLACTIN AND SOMATOLACTIN IN CALCIUM REGULATION IN FISH

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Summary

The endocrine control of calcium metabolism in fish is performed by hyper- and hypocalcaemic hormones as in terrestrial vertebrates. However, the hormones involved in calcium regulation in fish, which lack parathyroid glands, differ from those in terrestrial vertebrates. The pituitary is important in hypercalcaemic regulation in fish; prolactin exerts a hypercalcaemic action in addition to its well-established hypernatraemic effect. However, alternation of plasma calcium concentration may not be the primary factor influencing prolactin secretion; changes in osmolality or sodium levels seem to be more critical for the regulation of prolactin release. Somatolactin, a putative pituitary hormone related structurally to both growth hormone and prolactin, is another possible factor responsible for hypercalcaemic regulation in fish. Exposure of rainbow trout to high-calcium environments reduces the activity of somatolactin-producing cells located in the hypophysial pars intermedia. Conversely, an increased activity of somatolactin cells is observed in low-calcium environments. Somatolactin has also been implicated in fat metabolism from comparisons of normal rainbow trout with a blue-coloured variant, which lacks most of the somatolactin cells normally present in the pituitary. Diverse functions for somatolactin, in maturation, in the stress response, in acid–base regulation and in background adaptation, have also been proposed. More detailed studies are needed to define the function of somatolactin.

Introduction

Ionic calcium is a critical factor in a wide variety of biological processes, including neuronal excitability, muscle contraction, cell permeability, cell division, hormone release and mineralization of bone tissues. Extracellular calcium concentration is thus maintained at a relatively constant level, and endocrine systems play an important role in calcium metabolism throughout the vertebrate classes. However, the mechanisms of hormonal control of calcium homeostasis in fish are still poorly understood.

The pituitary gland has long been considered to be important in hypercalcaemic regulation in fish, which lack parathyroid glands. Prolactin (PRL) has been implicated in calcium regulation in addition to its well-established role as a freshwater-adapting hormone. Somatolactin (SL), a putative pituitary hormone produced in the pars intermedia, is another candidate for a hypercalcaemic hormone. In the present paper, we shall briefly review the pertinent literature to elucidate the involvement of PRL and SL in calcium metabolism, and also consider other possible functions of SL.

Key words: calcium, pituitary, prolactin, somatolactin, cobalt rainbow trout.

Endocrine control of calcium regulation in fish

In mammals, endocrine control of calcium regulation is performed by hyper- and hypocalcaemic hormones (Wendelaar Bonga and Pang, 1991). It is well established that parathyroid hormone (PTH) and a vitamin D_3 metabolite are hypercalcaemic hormones, whereas calcitonin exerts a hypocalcaemic action.

Fish seem to adopt the same strategy to maintain plasma calcium concentration within a narrow range between 1.3 and 1.5mmol l^{-1}. However, the hormones involved in calcium regulation in fish are apparently different from those in higher vertebrates. First, fish lack the parathyroid glands that secrete PTH. Instead, it has been suggested that the pituitary gland contains a hypercalcaemic factor(s). PRL and SL, the main subjects of this paper, are the most promising candidates as factors responsible for hypercalcaemic regulation, and the details will be discussed later. Calcitonin is secreted from the ultimobranchial body in fish, and its biochemical properties have been well characterized. Knowing the hypocalcaemic action of this hormone in mammals, investigators have attempted to determine its function in fish. The results are, however, equivocal, and a hypocalcaemic effect of calcitonin has not been established in fish. It is now accepted, however, that the corpuscles of Stannius (CS), unique to teleostean and holostean fish, are involved in hypocalcaemic regulation. Since Fontaine (1964) found that the removal of the CS induced hypercalaemia in the European eel (*Anguilla anguilla*), the CS have been shown to contain a hypocalcaemic factor. The bioactive substance produced in the CS is now known as stanniocalcin, often referred to in the past as hypocalcin or teleocalcin, and seems to be a major hypocalcaemic hormone in fish (Hirano, 1989; Wendelaar Bonga and Pang, 1991).

It is not surprising that the endocrine systems for calcium regulation differ between terrestrial and aquatic vertebrates. Fish are in constant contact with the surrounding water. In sea water, the external calcium level is much higher (approximately 10mmol l^{-1}) than the internal level, and there is a tendency for calcium to enter the body through gills and other body surfaces. In contrast, the lower calcium level of fresh water causes a loss of calcium. However, calcium is readily available as long as fish possess a way to take it up through body surfaces such as the gills against the concentration gradient. This situation is quite different from that in terrestrial vertebrates, in which no exchange of calcium takes place between the body and the surrounding medium.

The endocrine control of calcium regulation has been modified during the water-to-land transition, accompanied by changes in hormone functions and the appearance of new endocrine systems. One would expect the endocrine mechanisms of calcium regulation in fish to be different from those in terrestrial vertebrates because the target organs are very different.

Involvement of the pituitary in calcium regulation

As mentioned earlier, the pituitary has been implicated in calcium regulation in fish (Pang *et al.* 1980; Wendelaar Bonga and Pang, 1989, 1991). Removal of the pituitary leads to hypocalcaemia (Fontaine, 1956; Olivereau and Chartier-Baraduc, 1965; Chan and Chester Jones, 1968; Chan *et al.* 1968). In most cases, however, the hypocalcaemia is

accompanied by decreases in the concentration of other electrolytes. This makes it difficult to separate the specific effect of pituitary hormones on calcium regulation from their known osmoregulatory effects.

Control of calcium levels by the pituitary was first shown in the killifish, *Fundulus heteroclitus*. When killifish were adapted to artificial calcium-deficient sea water, hypophysectomy elicited a significant decrease in plasma calcium concentration, but not in the concentrations of other electrolytes (Pang *et al.* 1971). When calcium was present in the environment, hypophysectomy did not cause hypocalcaemia. Furthermore, replacement therapy or injections of pituitary homogenate were both effective in correcting the hypocalcaemia (Pang *et al.* 1973).

Prolactin

PRL is well established as an important hormone for freshwater adaptation in euryhaline teleosts (Clarke and Bern, 1980; Brown and Brown, 1989). For several species, such as salmonids, eel and tilapia, transfer from sea water to fresh water always leads to an activation of PRL cells and an increase in plasma PRL levels. There is a large body of evidence that PRL cells become activated in response to a reduction in environmental osmolality or sodium level (Nishioka *et al.* 1988; Suzuki *et al.* 1991). The hypernatraemic or sodium-retaining action of PRL has long been known in many teleost species. Pickford and Phillips (1959) demonstrated, for the first time, that hypophysectomized killifish failed to survive in fresh water, largely because of a reduction in the plasma sodium level, which was restored by treatment with PRL (Clarke and Bern, 1980; Hasegawa *et al.* 1986; Brown and Brown, 1989).

Calcium regulation

PRL is also implicated in calcium homeostasis: the reduced plasma calcium levels, caused by removal of the pituitary of killifish in calcium-deficient sea water, were restored by mammalian PRL (Pang *et al.* 1978). Injection with mammalian or teleost PRL often induced hypercalcaemia in intact freshwater fish, although negative results were also reported (Wendelaar Bonga and Pang, 1989). Flik *et al.* (1989) showed that ovine PRL caused a distinct hypercalcaemia in the American eel, *Anguilla rostrata*, and stimulated a specific Ca^{2+}-ATPase activity in the gills, which is probably the driving force for branchial calcium uptake.

An inverse relationship between calcium levels in the external environment and PRL cell activity has been reported in tilapia (Wendelaar Bonga, 1978; Wendelaar Bonga and Van der Meij, 1980; Wendelaar Bonga *et al.* 1985): a reduction in external calcium concentration stimulates PRL cell activity while increased external calcium levels reduce the activity, suggesting a hypercalcaemic action of PRL. However, there are data which do not support this hypothesis (Nicoll *et al.* 1981; Olivereau and Olivereau, 1983). An inverse relationship is also reported between extracellular calcium and PRL levels in stickleback, *Gasterosteus aculeatus* (Wendelaar Bonga and Greven, 1978), and coho salmon, *Oncorhynchus kisutch* (Fargher and McKeown, 1989). However, no such correlation has been observed in rainbow trout, *O. mykiss* (Hirano, 1986).

We have also examined the effects of changes in ambient calcium level on the secretion of PRL in the Japanese eel, *A. japonica*, both *in vivo* and *in vitro* (Arakawa *et al.* 1993). Transfer of freshwater- or seawater-adapted eel to fresh water, fresh water containing 10mmol l^{-1} calcium, sea water, calcium-free sea water or deionized water was accompanied by significant changes in plasma calcium levels (Table 1). Changes in external calcium concentrations, however, did not affect plasma PRL levels, although plasma PRL levels as well as pituitary PRL levels were always greater in fish in hypotonic environments than in those in hypertonic environments, regardless of the external calcium concentration. Moreover, hypercalcaemia induced by the removal of the CS also failed to alter plasma PRL levels (Arakawa *et al.* 1993). These results indicate that changes in ambient calcium concentration may not be the primary factor influencing PRL secretion, at least in the eel; environmental osmolality or sodium levels seem to be more critical for the regulation of PRL secretion (Suzuki *et al.* 1991).

Control of prolactin secretion

The effects of extracellular calcium on PRL release have been examined in organ-cultured pituitary. MacDonald and McKeown (1983) reported that incubation of coho salmon pituitary in a medium with a physiological level of calcium stimulated maximal PRL release. According to Johnston and Wigham (1990), both release and synthesis of PRL in rainbow trout were stimulated as increasing amounts of calcium were added to the medium up to the physiological level, suggesting that the gradient between intracellular and extracellular calcium levels is important for PRL release in salmonids. In our experiments, incubation of the eel pituitary in a medium with different concentrations of calcium up to 2.9mmol l^{-1} did not affect the basal release of PRL, except in an extremely low-calcium medium, where PRL as well as growth hormone (GH) release were inhibited (Arakawa *et al.* 1993). Similarly, changes in medium calcium concentrations did not affect PRL release from the pituitary of tilapia, *Sarotherodon mossanbicus* (Grau *et al.* 1981).

Assuming that environmental calcium is an important regulator of PRL secretion, at least two control mechanisms can be considered: one mediated by alterations in plasma calcium concentration and the other mediated by an unknown calcium sensory system or, more indirectly, mediated by changes in integumental permeability. Since fish can maintain plasma calcium at a relatively constant level, small changes in plasma calcium would affect plasma PRL levels if alteration of plasma calcium concentration is a primary regulator of PRL cell activity. This possibility, however, seems to be ruled out, at least in the eel, because there is no correlation between plasma calcium and PRL concentrations. In tilapia, Wendelaar Bonga and van der Meij (1980, 1981) suggested an indirect effect of ambient calcium on PRL cell activity, possibly mediated by changes in integumental or branchial permeability, based on the observation that the reduction in ambient calcium concentration was always accompanied by an increase in the osmotic water permeability of the gills.

Somatolactin

Somatolactin (SL) is a putative pituitary hormone, structurally related to both growth hormone and PRL. Rand-Weaver *et al.* (1991*b*) were the first to demonstrate the presence

Table 1. *Effects of changes in environmental [Ca^{2+}] on plasma Ca^{2+} and prolactin levels in Japanese eel*

	Plasma Ca^{2+} concentration (mmol l^{-1})			Plasma prolactin concentration (ng/ml^{-1})		
Transfer	Day 0	Day 2	Day 7	Day 0	Day 2	Day 7
From FW[a] to:						
FW	3.36±0.04	3.24±0.07	3.42±0.07	4.01±1.34	2.91±1.10	3.60±0.89
DW[b]	3.44±0.11	3.07±0.08*	3.14±0.10	2.19±1.26	1.57±0.65	1.62±0.96
Ca^{2+}-rich FW[c]	3.42±0.05	3.53±0.10	3.67±0.27	2.00±0.86	1.33±0.60	2.34±0.67
SW[d]	3.76±0.05	4.50±0.19**	3.88±0.08	1.84±0.74	0.32±0.20	0.08±0.02
Ca^{2+}-free SW[e]	3.45±0.10	2.98±0.13**	2.53±0.12**	2.50±0.96	0.32±0.10	0.24±0.13
From SW to:						
SW	2.97±0.05	2.90±0.05	2.87±0.04	0.28±0.12	0.28±0.09	0.26±0.11
FW	2.94±0.03	2.86±0.04	2.75±0.03***	0.33±0.13	1.93±0.63	2.13±0.64*
Ca^{2+}-free SW	3.15±0.10	2.70±0.09*	2.27±0.26*	0.84±0.40	0.35±0.07	0.26±0.03

[a]Fresh water; [b]deionized water; [c]FW containing 10 mmol l^{-1} Ca^{2+}; [d]sea water; [e]Ca^{2+}-free SW.
Values represent mean ± S.E.M. (N=4–7).
*, **, ***Significantly different from the initial value at P<0.05, P<0.01 and P<0.001, respectively.

of this novel protein in the fish pituitary. In the course of characterizing GH from the Atlantic cod (*Gadus morhua*), they found a new glycoprotein with a molecular weight of 26kDa and consisting of 209 amino acids including eight cysteine (Cys) residues. The protein has three disulphide bonds between residues Cys^{5}–Cys^{15}, Cys^{65}–Cys^{181} and Cys^{198}–Cys^{206}. The Cys residues at positions 42 and 180 are not involved in disulphide bonding. Interestingly, the positions of these disulphide bonds are homologous to those found in GH and PRL. Sequence comparison also revealed cod SL to be similarly related to both GH and PRL in fish and other vertebrates, suggesting that SL is a new member of the GH/PRL family. In view of its structural similarity to these hormones, it was named somatolactin, a hybrid between somatotropin (GH) and prolactin (Ono *et al.* 1990). There has been a remarkable proliferation of GH/PRL-like proteins, most of which are produced in the placenta (Wallis, 1992). SL appears to originate from a common ancestral gene for the GH/PRL family.

The corresponding proteins have been analyzed in several teleost species. An SL protein was isolated from the pituitary of Japanese flounder, *Paralichthys olivaceus*, and the complete structure was elucidated by the cDNA sequence (Ono *et al.* 1990). The notable difference between Atlantic cod and Japanese flounder SLs is that the flounder SL is two amino acids shorter at the C-terminal end and contains seven Cys residues instead of eight. Rand-Weaver *et al.* (1992) also purified SL from coho salmon and developed a homologous radioimmunoassay. Furthermore, clones coding for SL were isolated from cod and chum salmon (*Oncorhynchus keta*) cDNA libraries (Takayama *et al.* 1991*a*) and from a chum salmon genomic DNA library (Takayama *et al.* 1991*b*).

In spite of its structural similarity to GH and PRL, the glycosylation status of SL is rather unique. Atlantic cod SL possesses two possible glycosylation sites, but only one appears to have N-linked sugars attached (Rand-Weaver *et al.* 1991*b*). The second site may be located inside the molecule. In flounder, the SL molecule contains only one possible glycosylation site (Ono *et al.* 1990). However, glycosylation is not a common property of SL molecules. Glycosylation sites are absent from the amino acid sequence of chum salmon SL, elucidated from the cDNA sequence (Takayama *et al.* 1991*a*). This clearly indicates the non-glycosylated nature of chum salmon SL, and this has been confirmed by the amino acid sequence of purified SL and the genomic DNA analysis (Takayama *et al.* 1991*b*). Thus, the SL proteins seem to exist in a glycosylated form in some species and in a non-glycosylated form in the others, including chum and coho salmon. The glycosylation status of SLs reflects the presence or absence of periodic acid–Schiff (PAS)-positive cells of the pars intermedia (PIPAS cells) in the pituitary. The presence of glycosylated and non-glycosylated SLs may have some physiological significance.

No protein corresponding to SL has been reported in higher vertebrates. However, in view of the fact that all pituitary hormones present in mammals have been isolated from fish, it seems likely that SL will be shown to be present in the pituitaries of mammals and other higher vertebrates.

Location of somatolactin cells

The successful purification of SL was followed by the production of a specific

antiserum, which made it possible to identify SL-producing cells by immunocytochemistry (Fig. 1). Rand-Weaver *et al.* (1991*a*) demonstrated, for the first time, the cellular localization of SL in the fish pituitary, using an antiserum against Atlantic cod SL. Histological studies have revealed that the pars intermedia in most teleosts contain two distinct cell types, which are distinguishable by their staining reactions (Ball and Baker, 1969). One cell type, lying further from the neurohypophysis, stains with lead–haematoxylin (PbH) but is PAS-negative, whereas the other cell type bordering the neurohypophysial tissue is PbH-negative and usually PAS-positive; these cells are often referred to as PIPAS cells. Immunocytochemical staining, using anti-cod SL serum, revealed that SL-immunoreactive cells are located in the pars intermedia, bordering the neurohypophysial tissue. These cells correspond to PAS-positive cells, and are distinct from the melanocyte-stimulating hormone (MSH)-producing cells, which are PAS-negative. Using the same antiserum, SL cells are also detectable in the same location in other teleost species, such as flounder, rainbow trout, killifish, molly, catfish and eel (Rand-Weaver *et al.* 1991*a*). SL-immunoreactive cells are more or less PAS-positive in most fish examined, but are chromophobic in rainbow trout. Since PAS stains carbohydrate-containing materials, these findings also support the idea that the SL protein can exist in either a glycosylated or a non-glycosylated form depending on species.

The gene expression and intracellular localization of SL have also been investigated in the rainbow trout pituitary. Using an *in situ* hybridization technique with a cDNA probe encoding chum salmon SL, Kaneko *et al.* (1993*b*) demonstrated the gene expression of the SL molecule in SL-immunoreactive cells bordering the neurohypophysial tissue in the pars intermedia. Electron-microscopic immunocytochemistry using the protein A–gold technique also revealed that the SL-immunoreactivity was largely localized on the

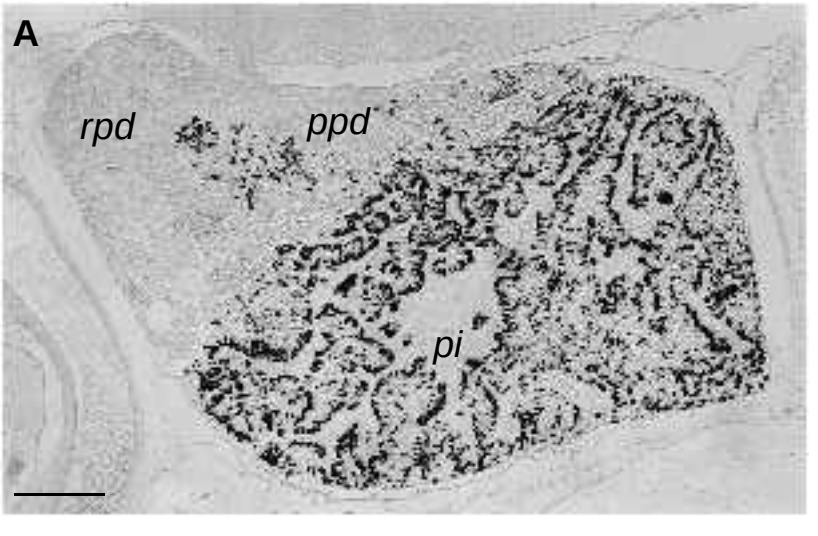

Fig. 1. Mid-sagittal section of rainbow trout pituitary, immunocytochemically stained with an antiserum raised against chum salmon somatolactin. *pi*, pars intermedia; *ppd*, proximal pars distalis; *rpd*, rostral pars distalis. Scale bar, 100 μm.

secretory granules. These results clearly indicate that the SL molecule is biosynthesized and stored in the secretory granules in these cells. These features of SL cells meet, in part, criteria for endocrine cells that produce and secrete protein hormones, although SL is not fully established as a 'hormone', since its physiological significance is still unknown. From these histological studies, SL is now known to be produced in the PIPAS cells or equivalent cells in the fish pituitary and it is reasonable to speculate that the proposed functions of the PIPAS cell are attributable to those of SL.

Proposed functions of PIPAS cells

The presence of PAS-positive cells in the pars intermedia of the fish pituitary has been described in earlier literature (Ball and Baker, 1969). These cells, which are distinct from MSH cells, are often referred to as PIPAS cells. Diverse functions of PIPAS cells have been suggested from histological studies.

Olivereau *et al.* (1980, 1981*a*) observed a striking stimulation of PIPAS cells during adaptation to deionized water in goldfish and eel. The marked stimulation was inhibited in deionized water supplemented with 2mmol l^{-1} calcium, but supplementation with sodium, potassium or magnesium failed to restore it (Olivereau *et al.* 1981*b*; Olivereau and Olivereau, 1982*a*), suggesting the involvement of PIPAS cells in calcium regulation. In view of their responsiveness to calcium, they referred to these PAS-positive cells as 'calcium-sensitive cells' instead of PIPAS cells. Activation of PIPAS cells was also observed in killifish adapted to calcium-free sea water, concomitant with hypocalcaemia (Ball *et al.* 1982). A similar result was obtained in eel transferred to calcium-free sea water (Olivereau and Olivereau, 1982*b*). These histological observations suggest that PIPAS cells produce a factor responsible for hypercalcaemic regulation. In contrast, Wendelaar-Bonga *et al.* (1986) reported that the response of PIPAS cells was dependent on water pH rather than on calcium concentration in goldfish, although the evidence for this was indirect.

PIPAS cells have also been implicated in background adaptation, since these cells became activated when fish were placed on dark backgrounds (Baker and Ball, 1970; van Eys, 1980; Ball and Batten, 1981).

Possible involvement of somatolactin in hypercalcaemic regulation

Since PIPAS cells are identical to SL cells, SL might be expected to be the predicted hypocalcaemic factor produced by PIPAS cells. Attempts to elucidate the function(s) of PIPAS cells have been made by means of morphological observations and morphometric analysis. This is a useful approach when investigating unknown functions of endocrine cells. The difficulty, however, is that one can detect morphological differences only when the change is drastic enough; the cellular activity could be altered without apparent changes in morphological appearance. Over the past few years, not only purified SL but also specific antisera and cDNAs encoding SL molecules have become available for physiological studies. Such highly specific 'probes' enable us to examine the activity of SL cells with greater accuracy, by using more advanced techniques such as immunocytochemistry, radioimmunoassay, western and northern blot analyses and *in situ* hybridization.

In view of the proposed hypercalcaemic action of PIPAS cells or calcium-sensitive cells, we have examined the effects of chronic changes in environmental calcium level on SL cell activity in rainbow trout (Kakizawa *et al.* 1993). First, rainbow trout were transferred from fresh water to calcium-rich fresh water (10mmol l^{-1}) or 80% sea water. Changes in the cellular activity were assessed by (1) sectional nuclear areas of SL cells identified immunocytochemically, (2) SL mRNA levels determined by *in situ* hybridization, and (3) plasma SL levels measured by radioimmunoassay (Fig. 2). The nuclear areas were reduced 10 and 21 days after transfer to Ca^{2+}-rich fresh water and 80% sea water. The levels of SL mRNA, expressed as the density of autoradiographic grains, were also lower 10 days after transfer to calcium-rich fresh water, suggesting that exposure to high-calcium environments reduced the SL cell activity. However, there were no significant differences in plasma SL levels. Since plasma hormone levels reflect an equilibrium between hormone secretion and consumption, it is possible that the turnover

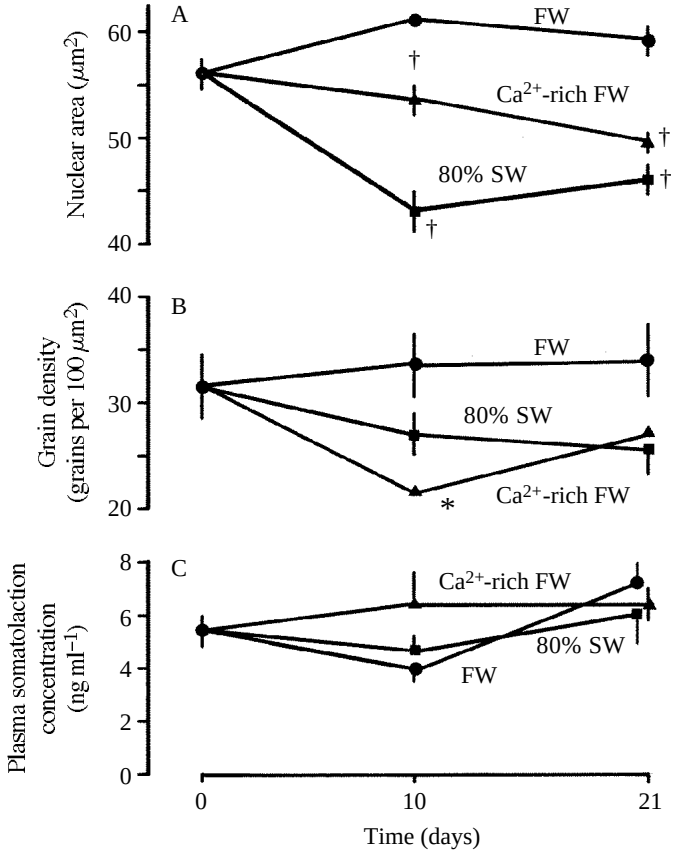

Fig. 2. Changes in (A) nuclear areas of SL cells, (B) SL mRNA levels and (C) plasma SL levels in rainbow trout transferred from fresh water (FW) to FW, to FW containing 10mmol l^{-1} Ca^{2+} (Ca^{2+}-rich FW) or to 80% sea water (80% SW). Each point represents the mean and S.E.M. (N=5). Marked values are significantly different from control (freshwater) values on the same day at *P<0.05 and †P<0.01.

rate of SL decreased after transfer to high-calcium environments without apparent changes in plasma SL levels. When the fish adapted to calcium-rich fresh water for 3 weeks were transferred back to normal fresh water, the nuclear areas of SL cells were increased and there were significant increases in SL mRNA and plasma SL levels. The increased activity of SL cells in fish in a low-calcium environment supports a hypercalcaemic action of SL. In addition, a significant reduction in PRL cell activity occurred after transfer from fresh water to 80% sea water, but not after transfer to calcium-rich fresh water. The activity of PRL cells seems to be affected by changes in osmolality rather than by ambient calcium concentration, in accordance with its well-established role as a freshwater-adapting hormone (Hirano, 1986; Brown and Brown, 1989).

Although SL cells (PIPAS cells) appear to be involved in calcium homeostasis in some way, evidence supporting the hypocalcaemic action of SL is indirect. To establish a specific function of SL, direct evidence is required.

Other proposed functions of somatolactin

SL has also been implicated in some biological events other than calcium metabolism. Rand-Weaver *et al.* (1992), who developed a specific radioimmunoassay for coho salmon SL, reported that plasma SL levels increased during the period of gonadal development and were highly correlated with oestradiol levels in females and 11-ketotestosterone levels in males. Peak SL levels were observed at the time of final maturation and spawning in both sexes. It has therefore been hypothesized that SL regulates some physiological aspects of reproduction. Furthermore, in coho salmon, SL has been reported to stimulate the production of 11-ketotestosterone and testosterone by testicular fragments and the production of oestradiol by ovarian follicles in a dose-dependent manner (Planas *et al.* 1992). However, since the observed steroidogenic activity is minimal and much less than that of gonadotrophin I (GTH I), no conclusion can be drawn as to the specific involvement of SL in reproduction.

Recently, Rand-Weaver and Sumpter (1993) claimed that plasma SL levels were markedly elevated in response to stress. Rainbow trout exposed to acute or short periods of handling and confinement stress showed a rapid increase in plasma SL levels. During acute confinement stress, a significant increase occurred within 2min, a more rapid response than that to cortisol. During short-term stress, SL levels peaked between 1 and 2 h, declined over the next 3h, and then showed an additional increase again at 24h. These results indicate that non-specific environmental stress activates SL cells, suggesting that SL is important in the adaptive response of fish to stress. Because most physiological experiments are performed under confinement stress and since blood sampling is usually accompanied by stress, plasma SL levels determined by radioimmunoassay could be overestimates. Care should be taken to avoid imposing stress on fish during experiments.

Cobalt variant of rainbow trout

In general, the participation of a hormone in physiological regulation can be demonstrated when specific deficiency symptoms occur after surgical removal of the

endocrine tissue. However, it is often difficult to remove a specific tissue without affecting other endocrine tissues. For instance, it is practically impossible surgically to remove only SL cells from the pituitary.

While characterizing a malformed pituitary from a blue-coloured variant of rainbow trout, we have recently shown that the variant lacks most of the pars intermedia in the pituitary (Kaneko *et al.* 1993*a*). Since SL cells are located in the pars intermedia, we expected this variant to be a useful model in the search for the functions of SL.

The blue-coloured variant of rainbow trout occurs occasionally at trout experimental stations and commercial trout farms in Japan (Yamazaki, 1974; Oguri, 1974). The variant is often termed 'cobalt' because of its characteristic cobalt blue body colour. In anatomical and histological studies, Yamazaki (1974) concluded that the cobalt variant lacked a pituitary. However, Oguri (1974) found a pituitary remnant, either adhering to the hypothalamus or detached from the brain, showing that the cobalt variant of rainbow trout has a malformed pituitary.

Our observations (Kaneko *et al.* 1993*a*) showed that the pituitary remnant was completely detached from the hypothalamus in all but one of the fish studied, and in this one a remnant of the pituitary was associated with the hypothalamus. Immunocytochemical staining showed that PRL and GH cells were the predominant cell types in all the pituitary remnants examined. There were far fewer SL and MSH cells than in normal fish, while corticotropin (ACTH) cells are rare in both cobalt and normal trout. In agreement with the histology of the SL cells in cobalt fish, plasma SL levels were extremely low (Fig. 3). Although plasma GH levels were significantly lower in the cobalt than in the normal fish, the levels seemed to be within the physiological range. There

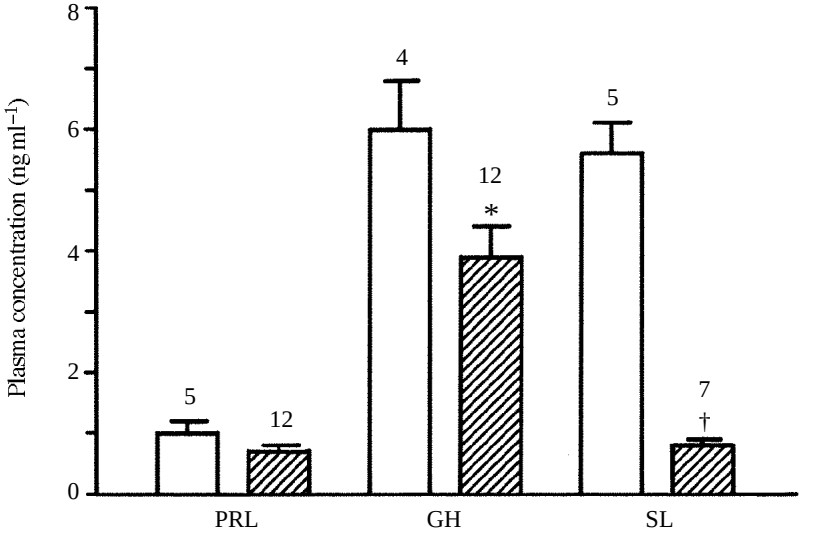

Fig. 3. Plasma concentrations of prolactin (PRL), growth hormone (GH) and somatolactin (SL) in normal (open columns) and cobalt (hatched columns) rainbow trout. Marked values are significantly different from normal values at $*P<0.05$ and $\dagger P<0.001$. Values are mean + S.E.M. Values of N are given above the column.

were no differences in PRL levels between the two groups. These findings clearly indicate that the cobalt variants lack the hypophysial pars intermedia.

The most obvious difference between cobalt and normal trout was the deposition of fat in the abdominal cavity. The ratio of fat mass in the abdominal cavity to body mass was 1.6% in normal fish, but as much as 12% in the cobalt variants. Thus, the most notable peculiarity of the cobalt fish seems to be abnormal fat metabolism, in addition to its abnormal body colour and malformed pituitary.

Although the neurohypophysial tissues are missing in the pituitary remnant of the cobalt fish, the hypothalamus–neurohypophysial neurones are present in the brain, although their fibres do not reach the pituitary. Therefore, the abnormalities of body colour and fat metabolism may be caused by the absence of the pars intermedia. The abnormal body colour may be related to the scarcity of MSH cells. Oguri (1983) reported a reduction in the numbers of dermal melanophores and renal melanin-containing cells in cobalt trout. In view of the marked deposition of fat in the cobalt variant, SL may be responsible for fat mobilization. However, further studies are required to verify this suggestion.

Conclusion

The participation of PRL and SL in calcium regulation has been considered with reference to the current literature. It is difficult at present to draw definite conclusions from the limited data available. Nevertheless, both PRL and SL appear to be involved in hypercalcaemic regulation.

It is well established that PRL exerts a hypernatraemic action, which enables fish to survive in fresh water. The difficulty lies in the fact that one cannot differentiate between the hypercalcaemic action and the hypernatraemic action of PRL. It is also possible that the hypernatraemic action of PRL is accompanied by hypercalcaemia, which could be restored rapidly to a normal level by a hypocalcaemic hormone, presumably stanniocalcin.

Although most data are in favour of the hypercalcaemic action of SL, more detailed studies are needed before we can clearly delineate its function. Several other possible roles for SL have been suggested, including roles in maturation, in the stress response, in acid–base regulation, in background adaptation and in fat metabolism. Since a definitive function of SL has not been determined, it would not be appropriate to refer to SL as a ‘hormone’. However, SL has a number of features that meet, in part, the criteria for defining a hormone: (1) SL is structurally related to GH and PRL; (2) SL-immunoreactive cells are located in the pars intermedia of the pituitary; (3) SL is present mostly in granules in the SL cells; (4) the expression of the SL gene is detectable in SL cells; and (5) SL is secreted into the blood circulation. The identification of target organs or the localization of its receptor would provide useful information on its function.

The endocrine control of calcium metabolism has been modified during the evolution of vertebrates: the changes are closely associated with the water-to-land transition. The evolution of the endocrine system includes alterations in the functions of existing hormones and the appearance and disappearance of endocrine systems. PRL is present

throughout vertebrate species, but its functions differ greatly between classes. The parathyroid glands exist in terrestrial vertebrates but are absent from aquatic vertebrates. However, some endocrine glands, such as the corpuscles of Stannius and the urophysis, are found only in fish. The evolution of the endocrine system is also accompanied by changes in target organs or receptors. Insights into the evolution of endocrine systems may shed some light on the physiological significance of SL.

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