INFLUENCE OF AMBIENT CALCIUM LEVELS ON STANNIOCALCIN SECRETION IN THE EUROPEAN EEL (*ANGUILLA ANGUILLA*)

By R. G. J. M. HANSSEN[1], N. MAYER-GOSTAN[2], G. FLIK[1]
AND S. E. WENDELAAR BONGA[1]

[1]*Department of Animal Physiology, Faculty of Science, University of Nijmegen, Toernooiveld 25, 6525 ED Nijmegen, The Netherlands* and [2]*Laboratoire de Physiologie Cellulaire et Comparée, Faculté des Sciences et des Techniques, Université de Nice, Parc Valrose, 06034 Nice Cedex, France*

Summary

The cells of the corpuscles of Stannius appeared to be more active in eels acclimated to sea water than in eels acclimated to fresh water. In acclimated eels, however, total and ionic plasma calcium concentrations and stanniocalcin titers did not differ. This suggests that levels of stanniocalcin in freshwater-acclimated eels are sufficient to maintain normocalcemia in sea water. When freshwater-acclimated eels were transferred directly to sea water, total and ionic calcium concentrations in the plasma increased significantly within 24 h but there was no apparent effect on the corpuscles of Stannius within the same time. This suggests that changes in secretory activity of the corpuscles of Stannius do not occur rapidly when they are presented with a hypercalcemic challenge. Conversely, when seawater-acclimated eels were transferred to fresh or distilled water there appeared to be a very rapid reduction in secretory activity in the corpuscles of Stannius, as indicated by the rapid accumulation of secretory granules. These data suggest that stanniocalcin turnover is more rapid in seawater-acclimated eels and that the secretory activity of the corpuscles of Stannius is rapidly reduced when a hypercalcic challenge is removed.

Introduction

Euryhaline fish, like the eel, face extensive changes in environmental calcium concentrations upon migration between fresh water and sea water. In fresh water the ionic calcium level can be as low as one-tenth of that of the blood, whereas in sea water the ionic calcium level may reach values 10 times higher than that of the blood. Since euryhaline fish maintain their blood calcium levels quite precisely in both fresh and sea water (Chan and Chester Jones, 1968), they must be able to regulate their blood calcium in accordance with the environmental calcium challenges associated with migration.

Key words: calcium, eel, fish, stanniocalcin, *Anguilla anguilla*.

Of all the fish endocrine systems studied, the corpuscles of Stannius (CS) have been most consistently implicated in the control of plasma calcium metabolism (Wendelaar Bonga and Pang, 1991). Stanniocalcin (STC), the hormone secreted by the CS, lowers plasma calcium levels by reducing gill calcium uptake (Fenwick and So, 1974; So and Fenwick, 1979; Milet *et al.* 1979; Lafeber and Perry, 1988; Lafeber *et al.* 1988*a*). Histological examination of the CS has revealed that the glands are consistently more active in seawater-adapted fish than in freshwater-adapted fish (Olivereau, 1964; Hanke *et al.* 1967; Wendelaar Bonga *et al.* 1976; Meats *et al.* 1978). In addition, CS of fish kept in calcium-deficient sea water appear to be inactive (Cohen *et al.* 1975; Wendelaar Bonga *et al.* 1980), suggesting that glandular activity is directly related to environmental calcium concentrations. Such observations have led to the suggestion that the release of STC is controlled directly by changes in plasma calcium levels (Aida *et al.* 1980; Wagner *et al.* 1989). This hypothesis was supported by observations on the effects on STC secretion of exposure of cells from the corpuscles of Stannius *in vitro* to media of various calcium concentrations. However, the rise in the extracellular calcium level required to stimulate STC release *in vitro* is outside the physiological range ($[Ca^{2+}] > 2 \, \text{mmol} \, l^{-1}$; Hanssen *et al.* 1991).

We have studied the regulation of STC release by examining CS from eels exposed to changes in the water calcium concentration. The effects of rapid transfers between fresh water and sea water on the ultrastructure of the CS and on the plasma calcium and STC concentrations were examined.

Materials and methods

Animals

Sexually immature freshwater European eels (*Anguilla anguilla* L.) weighing between 100 and 300 g were obtained from a commercial fish dealer in the Camarque, France. Fish were kept in well-aerated 1000 l tanks at approximately 12 °C. Eels were acclimated to circulating city of Nice tap water ($1.63 \, \text{mmol} \, l^{-1}$ Ca^{2+}, $0.12 \, \text{mmol} \, l^{-1} \, Na^+$, $0.09 \, \text{mmol} \, l^{-1} \, Cl^-$) or Mediterranean sea water ($12.50 \, \text{mmol} \, l^{-1} \, Ca^{2+}$, $530 \, \text{mmol} \, l^{-1} \, Na^+$, $552 \, \text{mmol} \, l^{-1} \, Cl^-$) for at least 3 weeks. The eels were not fed.

Experimental protocol

Freshwater-acclimated eels were netted and transferred directly to 200 l tanks containing circulating fresh water or sea water for 24 h. Seawater-acclimated eels were transferred for a 24 h period to seawater tanks, freshwater tanks or tanks containing running distilled water (Ca^{2+} not detectable with a commercial calcium kit, pH 7.0). At the end of the experiment fish were anesthetized in ethylaminobenzoate (MS222, $2.5 \, \text{g} \, l^{-1}$, pH 7.8) and blood samples were taken by puncture of the caudal vessels. The CS were removed as described before (Hanssen *et al.* 1989), and prepared for electron microscopy.

Plasma analysis

Plasma ionic and total calcium concentrations were measured as previously described (Hanssen *et al.* 1989). Plasma ionic calcium was determined with an ionic calcium analyzer (ICA-1, Radiometer). Total plasma calcium was measured with a commercial calcium reagent kit (Sigma).

Enzyme-linked immunosorbent assay (ELISA)

A competitive ELISA technique, developed and validated by Mayer-Gostan *et al.* (1991), was used to quantitate STC levels in plasma samples. This technique is based on competition between free STC in standard or plasma samples and STC immobilized on microtiter plates for the STC antibodies. The trout STC antiserum used (RADH-1; Kaneko *et al.* 1988) has a high degree of crossreactivity with eel STC. Purified trout STC (Lafeber *et al.* 1988*b*) served as a standard.

The wells of microtiter plates were coated with trout STC ($1.85 \, \text{nmol} \, \text{l}^{-1}$) in $200 \, \mu\text{l}$ of coating buffer ($0.05 \, \text{mol} \, \text{l}^{-1}$ bicarbonate buffer, pH 9.6), except for the wells in the first column of the microtiter plate (blanks), which received $200 \, \mu\text{l}$ of a bovine serum albumin (BSA) solution of equivalent protein content in coating buffer. Coating lasted for 1 h at 37°C; coated plates were stored at 4°C. Between incubation steps, coated plates were rinsed with washing buffer ($0.01 \, \text{mol} \, \text{l}^{-1}$ phosphate-buffered saline; PBS, pH 7.4, with 0.05 % Tween-20; Bio-Rad). For competition, antigen and RADH-1 antiserum were diluted in dilution buffer (i.e. washing buffer containing 2 % porcine serum). Equal volumes of antigen and diluted antiserum were incubated in 4 ml Minisorp tubes (Nunc) overnight at room temperature (final antiserum dilution 1:80 000). Tubes containing diluted antiserum (1:80 000) only were incubated under the same conditions. After washing, the wells of coated plates were blocked with $200 \, \mu\text{l}$ of dilution buffer (containing 2 % porcine serum) at 37°C for 1 h. The plates were washed and filled with $200 \, \mu\text{l}$ per well of the antigen/antiserum mixture except for the wells in columns 1 (blanks) and 2 (maximum absorbance) which received $200 \, \mu\text{l}$ of the diluted antibody. Plates were incubated for 2 h at 37°C. The wells were washed and incubated with $200 \, \mu\text{l}$ of 1:5000 goat anti-rabbit peroxidase immunoconjugate (Nordic) in dilution buffer for 1 h at 37°C. For quantification of the peroxidase immunoconjugate bound to the wells, an enzymatic reaction was used with *o*-phenylenediamine (OPD; Sigma) as a substrate. After washing, $200 \, \mu\text{l}$ of substrate (0.05 % OPD in citrate/phosphate buffer; $0.2 \, \text{mol} \, \text{l}^{-1}$ Na_2HPO_4, $0.1 \, \text{mol} \, \text{l}^{-1}$ citric acid, pH 5.0, with 0.025 % hydrogen peroxide) was added at room temperature. The reaction was allowed to proceed for 20–30 min in the dark and was stopped by the addition of $50 \, \mu\text{l}$ of $2 \, \text{mol} \, \text{l}^{-1}$ H_2SO_4. Absorbance was measured at 492 nm in a microplate reader (Titertek).

Electron microscopy

Freshly dissected CS were prefixed for 10 min in 3 % glutaraldehyde in $0.1 \, \text{mol} \, \text{l}^{-1}$ cacodylate buffer, pH 7.4, at room temperature. The CS were then

fixed for 1 h at 0°C in $0.1 \, mol \, l^{-1}$ cacodylate buffer containing 1% (v/v) glutaraldehyde, 0.66% (w/v) osmium tetroxide and 1.66% (w/v) potassium dichromate. Post-fixation was in 2% (w/v) uranyl acetate in water. After dehydration in graded ethanols, the tissues were embedded in Spurr's resin. Ultrathin sections were post-stained with Reynold's lead citrate and examined in a Jeol 100 CX II electron microscope.

Calculations and statistics

ELISA results were evaluated using a logit transformation (Mayer-Gostan et al. 1991). A least-squares linear regression of the transformed linear part of the standard curve was used to estimate the STC concentration of the samples. All data are presented as means±s.e.m. The Mann–Whitney U-test was used for statistical evaluation. Significance was accepted for $P<0.05$.

Results

Ultrastructurally, two types of endocrine cells may be distinguished in the CS of eels: large rounded cells with large secretory granules (type 1 cells) and small cells with long cytoplasmic extensions and small secretory granules (type 2 cells; Wendelaar Bonga and Pang, 1986). STC was immunocytochemically localized in the secretory granules of both cell types, indicating that both either sequester or produce stanniocalcin (Kaneko et al. 1989; Wendelaar Bonga et al. 1989). Because no differences were observed in the structure of the type 2 cells when eels were transferred between fresh water and sea water our present analyses focused on the type 1 CS cells.

The ultrastructure of eel CS cells before and after transfer is shown in Fig. 1. The cells of fish acclimated to fresh water appear to be quite inactive (Fig. 1A), being characterized by an abundance of large secretory granules and relatively little granular endoplasmic reticulum (GER). In contrast, the cells of fish acclimated for 3 weeks to sea water contain a well-developed and extensive GER and few secretory granules (Fig. 1B). Twenty-four hours after transfer of freshwater-adapted eels to sea water, the CS still did not exhibit the secretory features typical of the cells of seawater-acclimated fish (Fig. 1C) and the cells were similar in appearance to those of the freshwater-acclimated fish, containing many secretory granules. Examination of the cells of seawater-adapted fish transferred to fresh water for 24 h (Fig. 1D) or distilled water (Fig. 1E) does not reveal noticeable differences in the extent of the GER compared to the CS cells of the seawater-acclimated fish. However, accumulation of secretory granules is observed in the cells of both groups of fish, particularly in those transferred to distilled water.

The ionic and total calcium levels of the plasma of freshwater- and seawater-adapted eels did not differ significantly (Fig. 2). Twenty-four hours after transfer of freshwater-adapted eels to sea water, total plasma calcium level was significantly increased and the change was, for the most part, accounted for by an
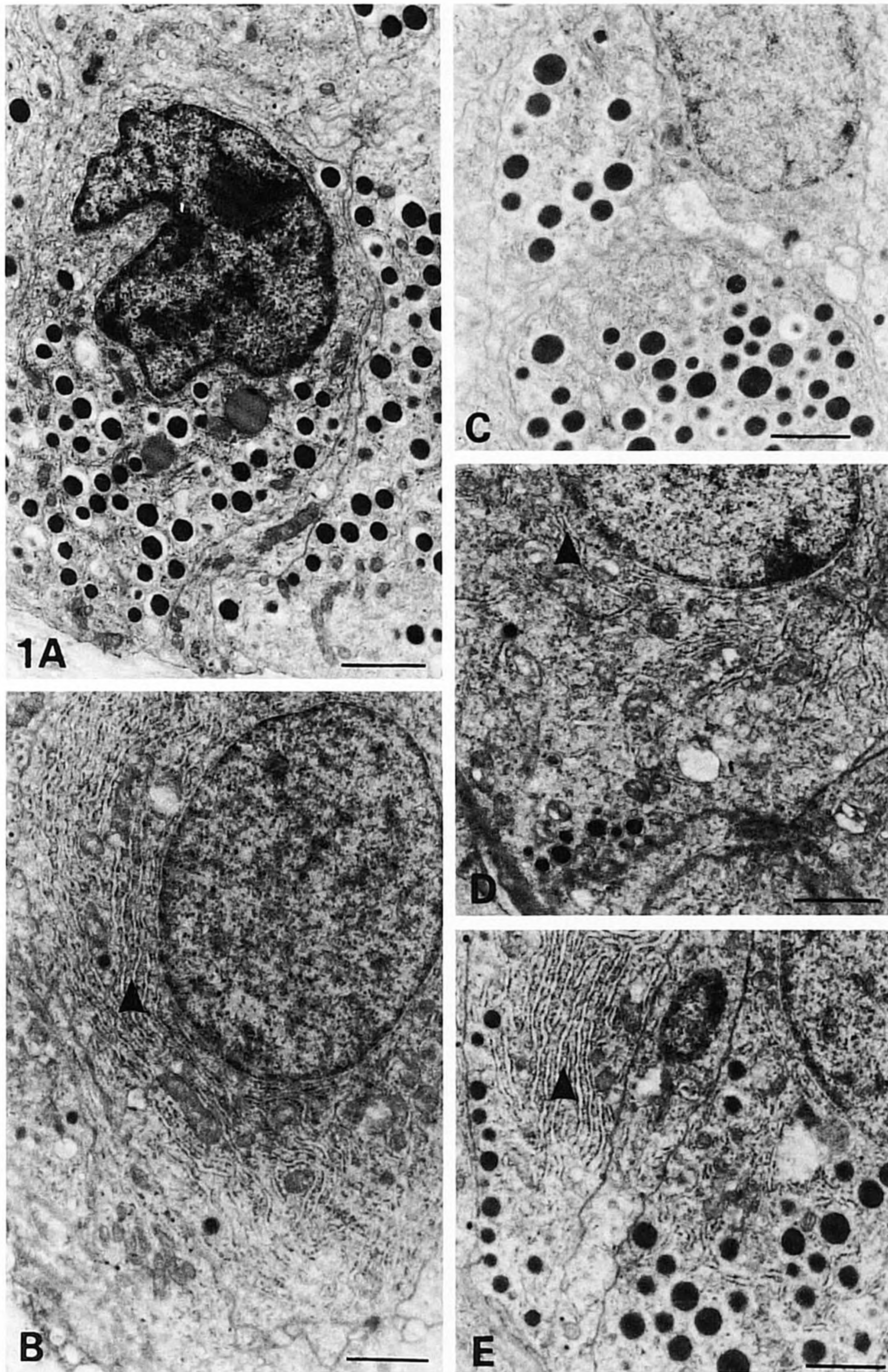
Fig. 1. Electron micrographs of the corpuscles of Stannius of eels 24 h after transfer from fresh water to fresh water (Fig. 1A) or sea water (Fig. 1C). Note the abundance of secretory granules. Electron micrographs of the corpuscles of Stannius of eels 24 h after transfer from sea water to sea water (Fig. 1B), fresh water (Fig. 1D) or distilled water (Fig. 1E). Note the extended granular endoplasmic reticulum indicated by arrowheads. Scale bars, $0.67\,\mu$m.
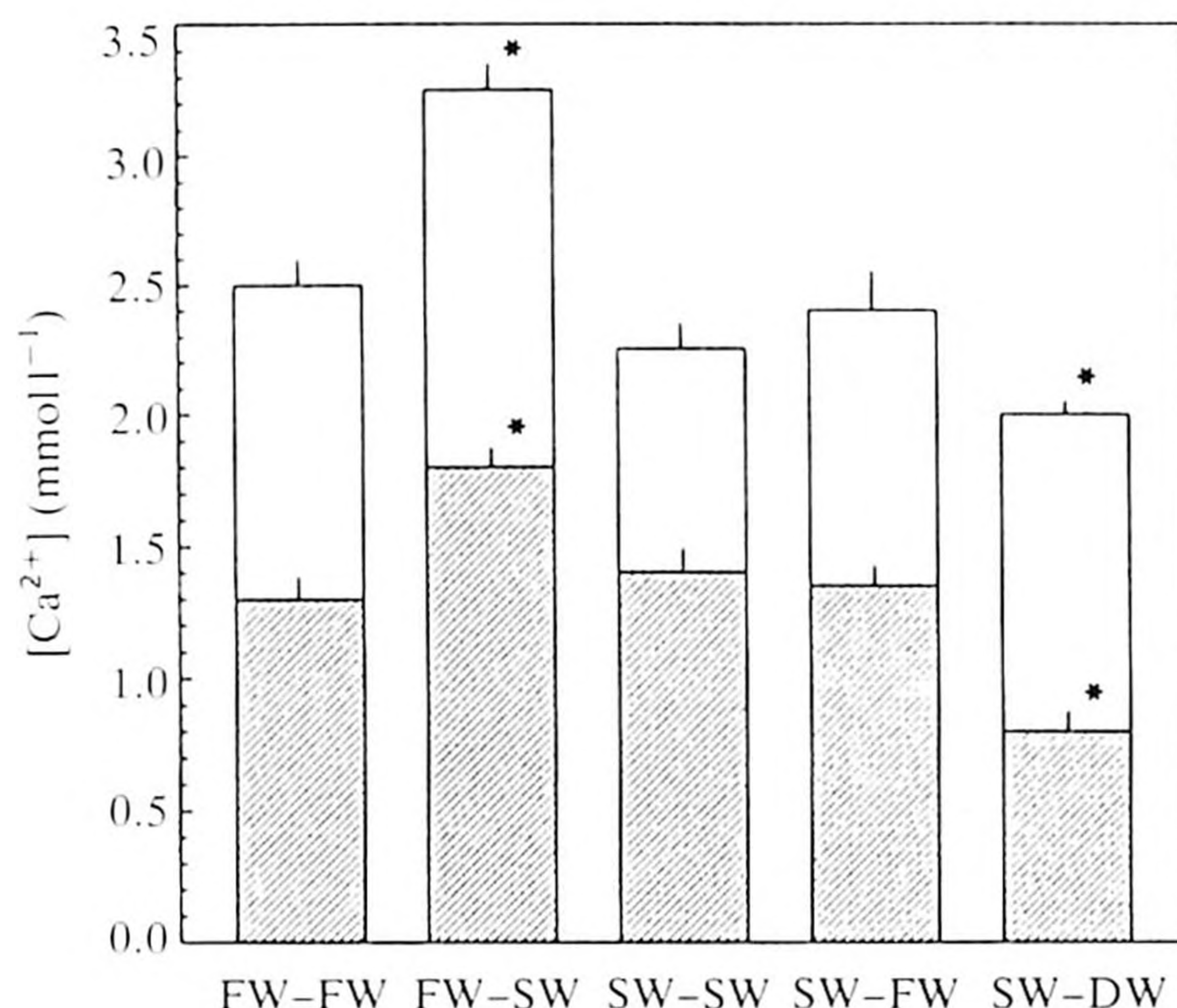
Fig. 2. Ionic (hatched columns) and total (open columns) calcium levels in plasma of eels 24 h after transfer from fresh water to fresh water (FW–FW: $N=13$) or sea water (FW–SW: $N=12$) and eels transferred from sea water to sea water (SW–SW: $N=9$), fresh water (SW–FW: $N=8$) or distilled water (SW–DW: $N=4$). * denotes a value significantly different from FW–FW levels. Values are mean+s.e.m.

increase in plasma ionic calcium. Seawater-adapted eels 24 h after transfer to fresh water had plasma ionic and total calcium concentrations that were not significantly different from those of seawater-acclimated eels. Transfer to distilled water, however, produced a significant fall in plasma ionic calcium, which was reflected in the decreased plasma total calcium level.

Plasma STC concentrations in freshwater- and seawater-adapted eels were not significantly different. Values ranged from 0.90 to 1.30 nmol l^{-1} (Fig. 3). Plasma STC levels of freshwater-adapted eels transferred to sea water were not significantly altered, but seawater-adapted eels transferred to fresh water or distilled water showed a significant drop in plasma STC levels to values around 0.50 nmol l^{-1}.

Discussion

In this study we investigated the relationship between the calcium concentration of the water and the CS activity in eels. In the short term, a transfer to hypercalcic water causes hypercalcemia but does not stimulate CS activity. This observation does not favor a direct regulatory role for plasma Ca^{2+} in STC secretion. Transfer to hypocalcic environments, however, induced a decrease in CS activity within 24 h, suggesting that a hypocalcic challenge is more adequately counteracted than a hypercalcic challenge.

A relationship between the activity of the CS and the calcium concentration of the water has been suggested previously. Urasa and Wendelaar Bonga (1987)
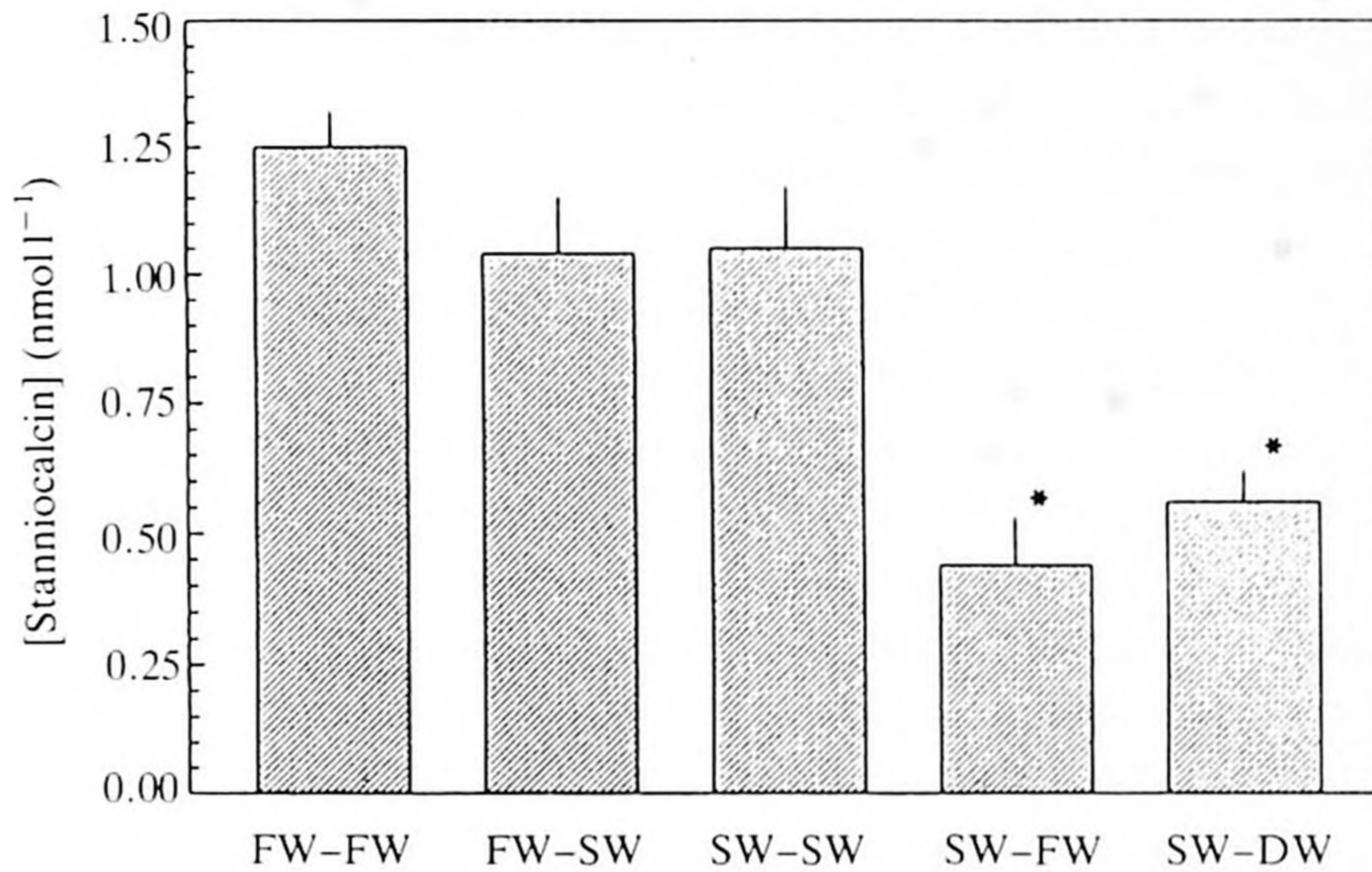
Fig. 3. Plasma stanniocalcin levels in eels transferred from fresh water to fresh water (FW–FW: $N=13$) or sea water (FW–SW: $N=5$) and eels transferred from sea water to sea water (SW–SW: $N=9$), fresh water (SW–FW: $N=8$) or distilled water (SW–DW: $N=6$). *denotes a value significantly different from FW–FW levels. Values are mean+S.E.M.

reported that in tilapia a long-term elevation of the calcium concentration of the water led to an increase in CS volume accompanied by an elevation of the plasma calcium concentration. Their results were in line with the assumption of Bailey and Fenwick (1975) that a negative feedback exists between the secretory activity of the CS and the ionic calcium concentration of the plasma. Further circumstantial evidence for such a relationship came from *in vitro* studies by Aida *et al.* (1980) and Wagner *et al.* (1989), who concluded that increased medium calcium concentrations enhanced the depletion of CS secretory granules and the release of immunoreactive STC. However, recent *in vitro* studies by our group on eel CS showed that STC release was only altered by an increase in extracellular calcium concentration well above that shown to follow most hypercalcic challenges (Hanssen *et al.* 1991).

Ultrastructural appearance

In CS cells of freshwater-adapted eels we observed a moderately developed GER and an abundance of secretory granules, indicative of a relatively inactive cell. CS cells of seawater-adapted eels were more active than those of freshwater-adapted eels judging from the absence of many secretory granules and the presence of an extensive GER. Although granule depletion has been interpreted as evidence of high secretory activity of CS cells (Cohen *et al.* 1975), this does not necessarily apply to the CS cells of all seawater-adapted euryhaline teleost fish. Granular depletion, as found in the European eel (this study), was also evident in the killifish (Cohen *et al.* 1975; Wendelaar Bonga *et al.* 1980) but not in the stickleback (Wendelaar Bonga *et al.* 1976) or trout (Meats *et al.* 1978). However,

in these two species a high incidence of exocytotic phenomena, indicating high secretory activity, was observed. Ultrastructural studies on CS cells have been carried out for a number of euryhaline teleost species. In these studies it was shown that CS cells of killifish (Cohen *et al.* 1975; Wendelaar Bonga *et al.* 1980), sticklebacks (Wendelaar Bonga *et al.* 1976), trout (Meats *et al.* 1978) and tilapia (Urasa and Wendelaar Bonga, 1987) are activated by transfer of the fish from fresh water to sea water. This change in glandular activity appears to be correlated to the calcium concentration of the water (Cohen *et al.* 1975; Wendelaar Bonga *et al.* 1980). Our transfer experiments show that transfer of an eel from fresh water to sea water for 24 h was not accompanied by activation of the CS. Transfer of an eel from sea water to fresh water or distilled water for 24 h did not affect the amount of GER in the CS cells. CS cells showed an extensive GER typical of activated cells. The CS of seawater-acclimated eels transferred to distilled water, and to a lesser extent the CS of seawater-acclimated eels transferred to fresh water, showed an accumulation of secretory granules. Accumulation of secretory granules was also found after a 24 h seawater–freshwater transfer of sticklebacks (Wendelaar Bonga *et al.* 1976). We conclude from our results that in acclimated eels the activity and the degree of granulation of CS cells are related to the calcium concentration of the water. In the short term, however, transfer between water containing high and low calcium levels does not necessarily have immediate effects on CS cell activity. It may have small effects on granulation, in particular after a hypocalcic challenge. This indicates that CS cell activity in the eel is not rapidly changed by fluctuations in water calcium concentration.

Plasma calcium levels

In freshwater-acclimated eels, ionic and total calcium concentrations of the plasma did not differ from previously reported values (1.3 mmol l^{-1} and 2.5 mmol l^{-1}, respectively). Plasma calcium concentrations in seawater-acclimated eels did not differ significantly from those measured in freshwater-acclimated eels. Similarly, in the striped mullet no differences in plasma calcium levels were observed between fish adapted to freshwater or seawater habitats (Johnson, 1972). However, plasma calcium concentrations in trout (Meats *et al.* 1978) and tilapia (Urasa and Wendelaar Bonga, 1987) differed significantly between fish adapted to high or low levels of calcium in the water. A 24 h transfer of our freshwater-acclimated eels to sea water induced a significant increase in ionic calcium in the plasma to 1.8 mmol l^{-1} and in total calcium to 3.25 mmol l^{-1}. These elevated plasma calcium levels upon transfer to sea water corroborate the report of Ogasawara and Hirano (1984) on Japanese eels, who found a transient elevation of the total plasma calcium level 24 h after transfer. We found that the ionic and total calcium concentrations in the plasma of seawater-acclimated eels after a 24 h transfer to fresh water did not change significantly. Similar observations were made by Ogasawara and Hirano (1984), who did not find significant effects on total plasma calcium in the Japanese eel after these transfers. Our seawater-acclimated eels transferred for 24 h to distilled water showed a decrease in both ionic and total

plasma calcium levels to values around 0.8 and 2.0 mmol l^{-1}, respectively. This indicates that the eels lost control over their branchial Ca^{2+} permeability and we predict an increased net Ca^{2+} efflux under these conditions. A transfer to distilled water may lead to a shift of the transepithelial potential (TEP) across the gills to more negative values (with the plasma compartment negative relative to the ambient medium). Indeed, McWilliams and Potts (1978) demonstrated a positive TEP across the gills of brown trout at ambient calcium levels of 2.0 mmol l^{-1} and higher; the TEP shifted to negative values at ambient calcium levels of 1.0 mmol l^{-1} and lower. As a presumed shift in the TEP to negative values would facilitate Ca^{2+} uptake from the water, the loss of calcium in our fish when challenged with low ambient Ca^{2+} levels indicates a severe disturbance of the integumental permeability to Ca^{2+} and, hence, a net loss of this ion. Contrary to the effects on CS cellular activity, plasma ionic and total calcium concentrations were not related to the calcium concentration of the water after long-term acclimation, and one may interpret this to indicate calcium homeostasis. Transfer of eels between high- and low-calcium waters as reported here provokes only transient changes in plasma calcium levels.

Plasma stanniocalcin concentrations

In freshwater-adapted eels the plasma immunoreactive STC concentration was 1.25 nmol l^{-1}. The plasma STC concentration in seawater-adapted eels did not differ significantly from this value. These values are lower than the 2.33 nmol l^{-1} reported recently by Mayer-Gostan *et al.* (1991) for freshwater-adapted eels. The increased calcium concentration of the plasma after a 24 h transfer of freshwater-adapted eels to sea water was not accompanied by a significant increase of the plasma STC concentration and thus plasma Ca^{2+} is not the primary trigger for STC release under these conditions. A 24 h transfer of seawater-adapted eels to fresh water or distilled water reduced the plasma STC concentration by 50%. In acclimated eels plasma STC concentrations appear to be very constant and were not related to the calcium concentration of the water. A short-term hypocalcic challenge, however, immediately affects the plasma STC concentration. This indicates that such a challenge inhibits the release of STC.

In this first report dealing with the relationship between environmental calcium, plasma calcium and plasma STC concentrations we conclude that the CS of freshwater-adapted eels exhibit a relatively low secretory activity and that an increase in the calcium concentration of the water activates the CS, but only in the long term. A short-term increase in the calcium concentration of the water does not stimulate the CS despite a 30% increase in the total calcium concentration of the plasma and a 38% increase in ionic calcium. Increased plasma calcium concentrations of this magnitude may well be within the physiological range of eel plasma. Indeed, *in vitro* experiments have shown that a 60% increase in the calcium concentration of the incubation medium did not affect STC release (Hanssen *et al.* 1991). The present *in vivo* experiments confirm our conclusion, based on the *in vitro* experiments, that plasma calcium fluctuations within the

physiological range do not regulate STC release. High secretory activity was observed in the CS of seawater-adapted eels. This activity is not reflected by an increased plasma STC level, suggesting that the metabolic clearance of STC is higher in seawater-adapted eels. When seawater-adapted eels are transferred to fresh water or distilled water, the high synthetic activity of the endocrine cells of the CS seems to be maintained for at least 24 h, but increased granulation indicates that the release of secretory material is inhibited. The drop in plasma STC levels after transfer to low-calcium water suggests that although STC clearance remains high there is a decreased release of STC.

An anti-hypercalcemic role was attributed to STC by Fenwick and Forster (1972) on the basis of the ability of CS homogenates to prevent hypercalcemia after removal of the CS. Our present finding, that a hypercalcemic state evoked by transferring eels for 24 h from fresh water to sea water is not accompanied by a stimulated release of STC, indicates that a physiologically induced hypercalcemia is not a trigger for STC release. Rapid antihypercalcemic responses of the CS cells have so far only been reported after experimentally induced high plasma calcium levels well above the physiological range. Because a direct role of calcitonin in the immediate control of plasma calcium level is very unlikely in fish (Wendelaar Bonga and Pang, 1991), we conclude that regulatory mechanisms for the minute-to-minute control of plasma calcium are absent in eels, and possibly in fish in general. Calcium levels in plasma appear to be less tightly controlled in fish than they are in terrestrial vertebrates.

References

AIDA, K., NISHIOKA, R. S. AND BERN, H. A. (1980). Degranulation of the Stannius corpuscles of coho salmon (*Oncorhynchus kisutch*) in response to ionic changes *in vitro*. *Gen. comp. Endocr.* **41**, 305–313.

BAILEY, J. R. AND FENWICK, J. C. (1975). Effect of angiotensin II and corpuscle of Stannius extract on total and ionic plasma calcium levels and blood pressure in intact eels (*Anguilla rostrata* LeSueur). *Can. J. Zool.* **53**, 630–633.

CHAN, D. K. O. AND CHESTER JONES, I. (1968). Regulation and distribution of plasma calcium and inorganic phosphate in the European eel (*Anguilla anguilla* L.). *J. Endocr.* **32**, 109–117.

COHEN, R. S., PANG, P. K. T. AND CLARK, N. B. (1975). Ultrastructure of the Stannius corpuscles of the killifish, *Fundulus heteroclitus*, and its relation to calcium regulation. *Gen. comp. Endocr.* **21**, 413–424.

FENWICK, J. C. AND FORSTER, M. E. (1972). Effects of stanniectomy and hypophysectomy on total plasma cortisol levels in the eel (*Anguilla anguilla* L.). *Gen. comp. Endocr.* **19**, 184–191.

FENWICK, J. C. AND SO, Y. P. (1974). A perfusion study of the effect of stanniectomy on the net influx of calcium 45 across an isolated eel gill. *J. exp. Zool.* **188**, 125–131.

HANKE, W., BERGERHOFF, K. AND CHAN, D. K. O. (1967). Histological observations on pituitary ACTH-cells, adrenal cortex and the corpuscles of Stannius of the European eel (*Anguilla anguilla* L.). *Gen. comp. Endocr.* **9**, 64–75.

HANSSEN, R. G. J. M., AARDEN, E. M., VAN DER VENNE, W. P. H. G., PANG, P. K. T. AND WENDELAAR BONGA, S. E. (1991). Regulation of the secretion of the teleost fish hormone stanniocalcin: effects of extracellular calcium. *Gen. comp. Endocr.* (in press).

HANSSEN, R. G. J. M., LAFEBER, F. P. J. G., FLIK, G. AND WENDELAAR BONGA, S. E. (1989). Ionic and total calcium levels in the blood of the European eel (*Anguilla anguilla*): effects of stanniectomy and hypocalcin replacement therapy. *J. exp. Biol.* **141**, 177–186.

JOHNSON, D. W. (1972). Variations in the interrenal and corpuscles of Stannius of *Mugil cephalus* from the Colorado river and its estuary. *Gen. comp. Endocr.* **19**, 7–25.

KANEKO, T., FRASER, R. A., LABEDZ, T., HARVEY, S., LAFEBER, F. P. J. G. AND PANG, P. K. T. (1988). Characterization of antisera raised against hypocalcin (teleocalcin) purified from corpuscles of Stannius of rainbow trout, *Salmo gairdneri. Gen. comp. Endocr.* **69**, 238–245.

KANEKO, T., HARVEY, S., KLINE, L. W. AND PANG, P. K. T. (1989). Localization of calcium regulatory hormones in fish. *Fish Physiol. Biochem.* **7**, 337–342.

LAFEBER, F. P. J. G., FLIK, G., WENDELAAR BONGA, S. E. AND PERRY, S. F. (1988a). Hypocalcin from Stannius corpuscles inhibits gill calcium uptake in trout. *Am. J. Physiol.* **254**, R891–R896.

LAFEBER, F. P. J. G., HANSSEN, R. G. J. M., CHOY, Y. M., FLIK, G., HERMANN-ERLEE, M. P. M., PANG, M. P. M. AND WENDELAAR BONGA, S. E. (1988b). Identification of hypocalcin (teleocalcin) isolated from trout Stannius corpuscles. *Gen. comp. Endocr.* **69**, 19–30.

LAFEBER, F. P. J. G. AND PERRY, S. F. (1988). Experimental hypercalcemia induces hypocalcin release and inhibits branchial Ca^{2+} influx in freshwater trout. *Gen. comp. Endocr.* **72**, 136–143.

MAYER-GOSTAN, N., FLIK, G. AND PANG, P. K. T. (1991). An enzyme-linked immunosorbent assay for stanniocalcin, the major hypocalcemic hormone in fish. *Gen. comp. Endocr.* (in press).

McWILLIAMS, P. G. AND POTTS, W. T. W. (1978). The effects of pH and calcium concentrations on gill potentials in the brown trout, *Salmo trutta. J. comp. Physiol.* B **126**, 277–286.

MEATS, M., INGLETON, P. M., CHESTER JONES, I., GARLAND, H. O. AND KENYON, C. J. (1978). Fine structure of the corpuscles of Stannius of the trout, *Salmo gairdneri*: structural changes in response to increased environmental salinity and calcium ions. *Gen. comp. Endocr.* **36**, 451–461.

MILET, C., PEIGNOUX-DEVILLE, J. AND MARTELLY, E. (1979). Gill calcium fluxes in the eel, *Anguilla anguilla* (L.). Effects of Stannius corpuscles and ultimobranchial body. *Comp. Biochem. Physiol.* A **63**, 63–70.

OGASAWARA, T. AND HIRANO, T. (1984). Changes in osmotic water permeability of the eel gills during seawater and freshwater adaptation. *J. comp. Physiol.* B **154**, 3–11.

OLIVEREAU, M. (1964). Corpuscles of Stannius in seawater eels. *Am. Zool.* **4**, 445 (Abstr.).

SO, Y. P. AND FENWICK, J. C. (1979). *In vivo* and *in vitro* effects of Stannius corpuscle extract on the branchial uptake of ^{45}Ca in stanniectomized North American eels (*Anguilla rostrata*). *Gen. comp. Endocr.* **37**, 143–149.

URASA, F. M. AND WENDELAAR BONGA, S. E. (1987). Effects of calcium and phosphate on the corpuscles of Stannius of the teleost fish, *Oreochromis mossambius. Cell Tissue Res.* **249**, 681–690.

WAGNER, G. F., GELLERSEN, B. AND FRIESEN, H. G. (1989). Primary culture of teleocalcin cells from rainbow trout corpuscles of Stannius: regulation of teleocalcin secretion by calcium. *Molec. cell. Endocr.* **62**, 31–39.

WENDELAAR BONGA, S. E., GREVEN, J. A. A. AND VEENHUIS, M. (1976). The relationship between the ionic composition of the environment and the secretory activity of the endocrine cell types of Stannius corpuscles in the teleost *Gasterosteus aculeatus. Cell Tissue Res.* **175**, 297–312.

WENDELAAR BONGA, S. E. AND PANG, P. K. T. (1986). Stannius corpuscles. In *Vertebrate Endocrinology, Fundamentals and Biomedical Implications* (ed. P. K. T. Pang and M. P. Schreibman), pp. 439–464. New York: Academic Press.

WENDELAAR BONGA, S. E. AND PANG, P. K. T. (1991). Control of calcium regulating hormones in the vertebrates: parathyroid hormone, calcitonin, prolactin and stanniocalcin. *Int. Rev. Cytol.* (in press).

WENDELAAR BONGA, S. E., SMITS, P. J. W. M., FLIK, G., KANEKO, T. AND PANG, P. K. T. (1989). Immunocytochemical localization of hypocalcin in the endocrine cells of the corpuscles of Stannius in three teleost species (trout, flounder and goldfish). *Cell Tissue Res.* **255**, 651–656.

WENDELAAR BONGA, S. E., VAN DER MEIJ, J. C. A. AND PANG, P. K. T. (1980). Evidence for secretory cell types in the Stannius bodies of the teleosts *Fundulus heteroclitus* and *Carassius auratus*. *Cell. Tissue Res.* **212**, 295–306.