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Effects of the Removal of Corpuscles of Stannius on the Transport of Calcium across the Intestine of Rainbow Trout

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ABSTRACT — Effects of stanniectomy (CSX) on the absorption of Ca in the intestine of rainbow trout were examined by incubating the everted gut sac in Ringer solution. The serum Ca concentration increased by CSX in fish adapted to 1/3 SW but not in those adapted to FW. Ca moved from mucosa to serosa in the anterior intestine from FW-shams and the movement was not affected by CSX. The absorptive movement of Ca was not evident in 1/3 SW-shams but became apparent following CSX. Transports of water and other electrolytes (Na, Cl, K, Mg and Pi) showed no significant changes by CSX. The results indicated that the intestine is a target organ for an active principle of the CS, which are activated in a high-Ca environment to prevent Ca entry from the ambient water.

INTRODUCTION

The corpuscles of Stannius (CS) are small endocrine bodies located on or in the kidney of holostean and teleostean fishes [1]. Since Fontaine [2] first demonstrated that the removal of the CS developed a marked hypercalcemia and injections of CS extracts restored the elevated level of serum calcium in European eels, the hypocalcemic nature of extracts or homogenates of the CS has been confirmed in many fish species [3, 4]. Recent studies [5–8] have shown that the hypercalcemia following stanniectomy in eels is a result of an increased rate of branchial calcium influx and the gills are the primary target for active CS principle, which controls uptake of calcium from ambient water.

The gut is another site of calcium entry from ingested food and swallowed water [9–11], though not considered as important as the gills in fishes [12]. It is not known whether the gut is also a target organ for the CS hormone. In this paper, we will report the effects of stanniectomy on the transepithelial transports of calcium and other electrolytes in the intestine of rainbow trout, employing the method of *in vitro* incubation of everted gut sacs [13].

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MATERIALS AND METHODS

Material fish

Cultured rainbow trout, *Salmo gairdneri* Richardson, were purchased from a fish farm and maintained in a freshwater pond feeding on a commercial diet (Nippai, No. 7P) until use. They were 21.7–26.4 cm in standard length and immature with the exception of a few males. Determinations of the transepithelial transport of calcium and other electrolytes were made by incubating in a Ringer solution an everted sac of the intestine removed from stanniectomized or sham-operated fish. The experiments were performed with fish kept in fresh water (FW) and those acclimatized to one-third seawater (1/3 SW) for two weeks. Concentrations of major ions contained in FW and 1/3 SW were; 1.16 mM Na, 0.35 mM K, 0.15 mM

Cl, 0.64 mM Ca and 0.41 mM Mg, and 177 mM Na, 4.08 mM K, 180 mM Cl, 4.34 mM Ca and 17.3 mM Mg, respectively.

Stanniectomy

The fish was anesthetized with 0.002% ethyl p-aminobenzoate, and the body wall was incised about 3 cm just anterior to the pelvic fins to open the body cavity. Several milky white bodies of the CS, 1–4 mm in diameter, were found in contact with the posterior kidney. The number of the CS varied from 2 to 7 in individuals but 3 or 4 in most fish. All the visually recognized CS were removed with fine forceps and the body wall was sewed. Sham-operated fish were treated in the same way except that the CS were not removed. After the operation, the fish were then kept in FW (through a transient transfer to a 1% NaCl solution) or in 1/3 SW for two weeks and fed the diet, but they seemed not to feed well.

Preparation and incubation of everted gut sacs

Two weeks after the operation, the fish were given a blow on the head and blood was collected by caudal section. The intestine was removed and turned mucosal side out using a glass rod. The everted gut was washed lightly with Ringer solution and the anterior and the posterior parts were cut into segments of roughly equal length. The anterior part (Segment I) was thinner than the posterior part (Segment II) which had the more developed muscular layer.

One end of the segment was ligated with a cotton thread to make a sac and weighed (W1). The sac was then filled with Ringer solution, tied at the opposite end and weighed again (W2). The initial volume of Ringer solution (Vo), that being the difference of W2–W1, was in a range of 0.3–0.6 ml. The sac was put in a 30 ml Erlenmeyer flask containing 10 ml of Ringer solution. The flask was filled with oxygen gas, sealed tightly and shook at 60 oscillations/min. The incubation time was 1 hr at 14°C for FW-fish and at 11°C for 1/3 SW-fish, each adjusted to the acclimatization temperature.

After incubation the sac was blotted and weighed (W3). The fluid in the sac (serosal fluid) and the incubation medium (mucosal fluid) were collected separately in test tubes. The serosal wall was blotted and the sac was weighed again (W4). The final volume of serosal fluid (Vs) was represented by the difference of W3–W4. Net water movement (Jw) and change in quantity of an electrolyte in the mucosal (Qm) or the serosal (Qs) side during the incubation were obtained by the following formulae:

$$Jw=(Vs-Vo)/W1\times 1000 \quad (\mu l/g \text{ tissue}\cdot hr)$$

$$Qm=(Vm\cdot Cm-10\cdot Co)/W1 \quad (\mu moles/g \text{ tissue}\cdot hr)$$

$$Qs=(Vs\cdot Cs-Vo\cdot Co)/W1 \quad (\mu moles/g \text{ tissue}\cdot hr)$$

Where Co is the initial concentration of an electrolyte in Ringer solution, and Cm and Cs are the final concentrations of the ion in the mucosal and serosal fluids, respectively, all expressed in mmoles/l. Vm is the final volume (ml) of the mucosal fluid determined by subtracting the difference of W3–W2 from the initial volume (10 ml).

The Ringer solution was prepared by dissolving 8.4738 g NaCl, 0.1864 g KCl, 0.1665 g $CaCl_2$, 0.2958 g $MgSO_4\cdot 7H_2O$, 0.3402 g KH_2PO_4, 0.8954 g $Na_2HPO_4\cdot 12H_2O$ and 2.3830 g HEPES in distilled water to make up 1 litre. NaOH was added to adjust pH to 7.4. This solution contained 159 mM Na, 5.06 mM K, 144 mM Cl, 1.46 mM Ca, 1.30 mM Mg and 5.11 mM Pi.

Collection of serum samples

Blood was collected into an unheparinized test tube and allowed to coagulate for 1 hr at 4°C. Serum was separated by centrifugation at 3,000 rpm.

Measurements of electrolyte concentrations

Na and K were determined by flame spectrometry and Ca and Mg by atomic absorption spectrometry using a Hitachi-518 atomic absorption spectrometer. Cl was determined by coulometric analysis with a Jookoo C-50 chloridometer. The colorimetric method of Goldenberg and Fernandez [14] was applied for measurements of Pi.

Statistical analyses

All values were expressed as means±S.E. Statistical comparisons were made with Student's *t*-tests for paired and unpaired measurements.

RESULTS

Between the sham-operated and the stanniectomized FW-fish, there were no significant changes in serum concentrations of any measured electrolytes, though Ca tended to increase and Mg to decrease in the stanniectomized fish (Table 1).

By adapting the fish to 1/3 SW, the serum levels of Na, K, Cl and Ca tended to rise slightly but none of these changes were statistically significant. In the 1/3 SW-fish, however, the stanniectomized fish showed a significantly higher serum Ca level compared to the shams (Table 1). Stanniectomy did not cause any significant changes in serum concentrations of other electrolytes.

In all FW- and 1/3 SW-fish, regardless of they were stanniectomized or sham, a net mucosa-to-serosa movement of water was always observed (Table 2). The flux was greater in Segment I than in Segment II. No effect of stanniectomy was observed in both FW- and 1/3 SW-fish.

Quantitative changes of electrolytes in the mucosal and serosal fluids after incubation were shown in Tables 3 (Segment I) and 4 (Segment II). There were significant losses of Na and Cl in the mucosal side and gains in the serosal side. The absorptive movements of these ions were more apparent in Segment I than in Segment II. The losses and gains were roughly balanced and did not differ between the FW- and 1/3 SW-fish. K did not show significant changes from the initial value except that a gain was observed in the mucosal fluid of Segment II from FW-shams. Mg in the serosal side consistently increased but it was highly variable in the mucosal side and not significantly changed from the initial. The gains in the serosal fluid were larger in 1/3 SW-fish than in FW-fish. Pi showed obvious and consistent increases in the serosal side. In the mucosal side, it tended to decrease in Segment I and to increase in Segment II. Effects of stanniectomy on the

TABLE 1. Serum electrolyte concentrations (mM) two weeks after the sham-operation and stanniectomy (CSX) of rainbow trout adapted to fresh water (FW) and one-third seawater (1/3 SW)

	FW		1/3 SW	
	Sham (6)	CSX (5)	Sham (5)	CSX (6)
Na	172±4	175±5	181±7	201±9
K	1.92±0.59	1.86±0.86	2.11±0.40	1.77±0.45
Cl	141±5	150±7	153±5	160±6
Ca	2.41±0.07	2.88±0.27	2.79±0.10	3.87±0.27*
Mg	1.02±0.05	0.88±1.17	1.10±0.10	1.04±0.11
Pi	3.42±0.38	3.53±0.26	3.08±0.07	2.48±0.27

Figure in parentheses denotes number of individuals examined.
* Significantly different compared to the shams ($P<0.05$).

TABLE 2. Net volume of water (μl/g tissue) moved from mucosa to serosa after 1 hr incubation of everted gut sacs of the anterior (Segment I) and the posterior (Segment II) intestines from sham-operated and stanniectomized (CSX) rainbow trout adapted to fresh water (FW) and one-third seawater (1/3 SW)

	FW		1/3 SW	
	Sham (6)	CSX (5)	Sham (5)	CSX (6)
Seg. I	210±31	125±38	224±33	175±24
Seg. II	86±20	101±17	93±23	79±22

Figure in parentheses denotes number of individuals.

TABLE 3. Changes in quantities (μmoles/g tissue) of electrolytes in the mucosal (M) and serosal (S) fluids after 1 hr incubation of everted sacs of the anterior intestine (Segment I) from sham-operated and stanniectomized (CSX) rainbow trout adapted to fresh water (FW) and one-third seawater (1/3 SW)

		FW		1/3 SW	
		Sham (6)	CSX (5)	Sham (5)	CSX (6)
Na	M	−35.7±4.9	−32.3±3.4	−46.5±4.2	−37.8±4.7
	S	37.2±6.4	29.5±5.5	37.0±1.7	32.6±4.4
K	M	0.526±0.567*	−0.477±0.970*	−0.615±0.957*	−0.097±0.767*
	S	0.121±0.346*	0.226±0.597*	0.254±0.423*	0.087±0.252*
Cl	M	−49.1±5.8	−34.6±8.8	−32.4±5.8	−39.8±7.5
	S	34.8±6.2	20.7±4.7	34.4±1.5	27.6±3.7
Ca	M	−0.140±0.304*	−0.789±0.308*	0.369±0.572*	−0.793±0.211
	S	0.358±0.049	0.211±0.056	0.340±0.015	0.441±0.020**
Mg	M	0.033±0.232*	−1.238±0.645*	−0.188±1.223*	−4.491±2.595*
	S	0.186±0.055	0.097±0.064*	0.772±0.316*	0.314±0.057
Pi	M	−1.389±0.384	−0.251±0.922*	−0.843±0.943*	−0.736±0.637*
	S	1.587±0.210	1.222±0.215	1.943±0.141	1.677±0.449

Figure in parentheses denotes number of individuals examined.
* Not significantly different from the initial value ($P \geq 0.05$).
** Significantly different compared to the shams ($P < 0.01$).

TABLE 4. Changes in quantities (μmoles/g tissue) of electrolytes in the mucosal (M) and serosal (S) fluids after 1 hr incubation of everted sacs of the posterior intestine (Segment II) from sham-operated and stanniectomized (CSX) rainbow trout adapted to fresh water (FW) and one-third seawater (1/3 SW)

		FW		1/3 SW	
		Sham (6)	CSX (5)	Sham (5)	CSX (6)
Na	M	−8.0±7.1*	−9.4±28.7*	−15.5±6.6*	−4.8±1.9*
	S	16.6±3.4	19.2±2.6	15.9±3.9	13.6±3.5
K	M	2.853±0.857	1.505±1.068*	−0.684±1.174*	1.791±0.951*
	S	0.782±0.504*	0.006±0.392*	0.273±0.476*	0.124±0.359*
Cl	M	−29.6±4.4	−20.9±8.2	−8.6±10.9*	−14.7±4.5
	S	13.3±2.9	15.1±2.4	14.4±3.7	12.2±3.1
Ca	M	0.208±0.322*	0.345±0.503*	1.455±0.523	−0.810±0.647*
	S	0.117±0.037	0.227±0.054	0.239±0.052	0.149±0.037
Mg	M	1.080±0.878*	1.257±0.941*	0.696±1.352*	−0.097±0.673*
	S	0.280±0.090	0.204±0.040	0.808±0.188	0.652±0.101
Pi	M	2.450±0.477	2.665±1.036*	3.161±1.545*	0.430±0.966*
	S	0.811±0.185	0.824±0.122	0.801±0.099	0.673±0.101

Figure in parentheses denotes number of individuals examined.
* Not significantly different from the initial value ($P \geq 0.05$).

movements of the above mentioned ions could not be observed either in Segment I or Segment II from FW- and 1/3 SW-fish.

Ca in the serosal fluid increased significantly after incubation in all the cases. On the other hand, a loss in the mucosal fluid was observed in

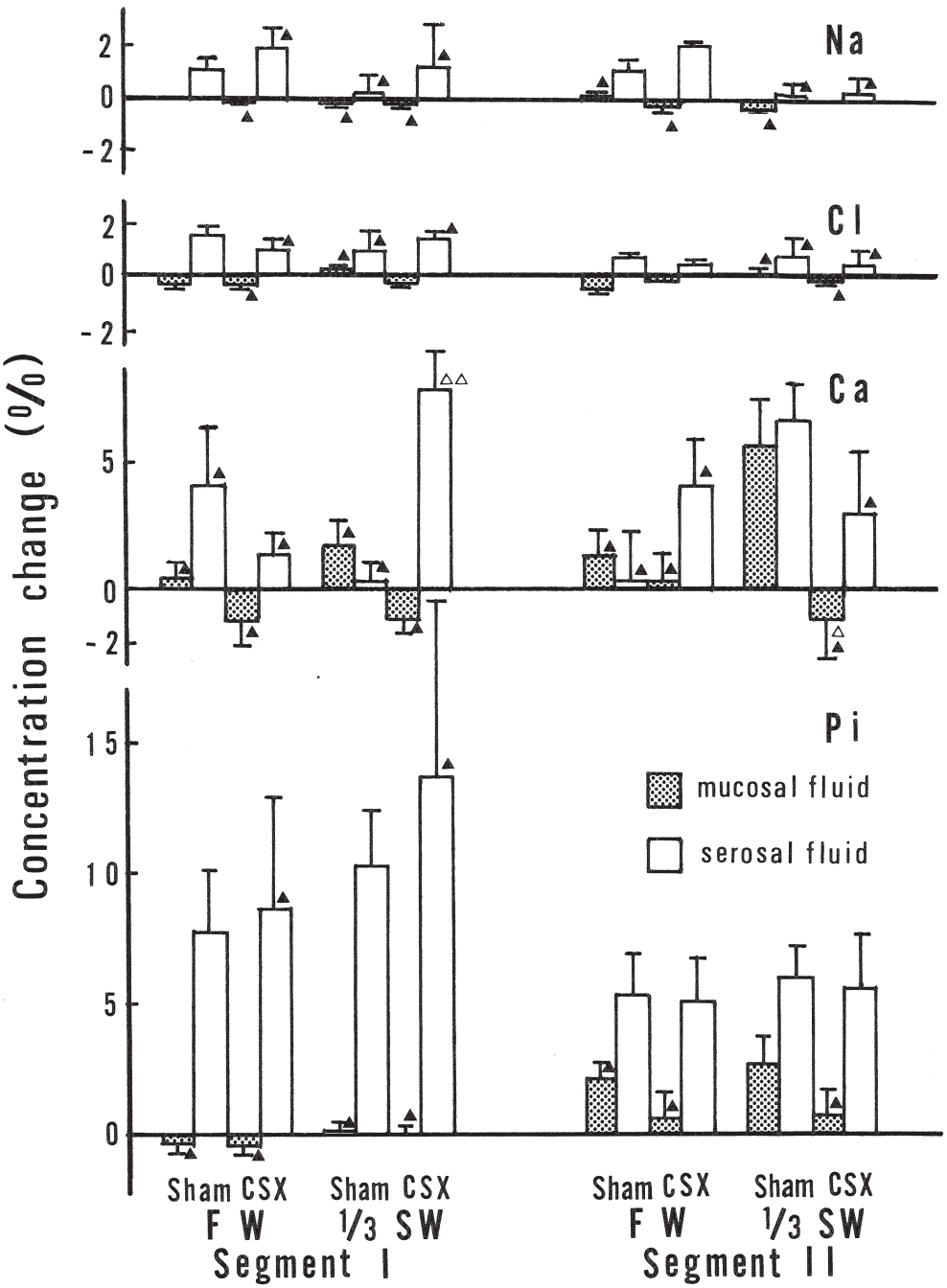

FIG. 1. Percent changes in concentrations of electrolytes in the mucosal and serosal fluids after 1 hr incubation of everted gut sacs of the anterior (Segment I) and the posterior (Segment II) intestines from sham-operated and stanniectomized (CSX) rainbow trout adapted to fresh water (FW) and one-third seawater (1/3 SW).

▲ Not significantly different from the initial level ($P \geq 0.05$).
△ Significantly different compared to the shams ($P < 0.05$).
△△ Significantly different compared to the shams ($P < 0.01$).

FW-shams (Segment I) but not in 1/3 SW-shams. Although the loss in the FW-shams was statistically insignificant, the absorptive movement of Ca was more apparent in FW-fish than in 1/3 SW-fish. Both the mucosal and serosal changes in the FW-shams were not affected by stanniectomy. An obvious effect of stanniectomy was observed in 1/3 SW-fish (Segment I); a gain in the serosal side significantly increased and a loss in the mucosal side became apparent by stanniectomy. This enhancement of Ca absorption was also suggestive in Segment II; a gain in the mucosal side in the shams was reversed to a loss, though not significant, in the stanniectomized fish.

The final mucosal and serosal concentrations of electrolytes, except K and Mg whose quantitative changes were inconsistent and judged unreliable, were illustrated as percent changes to each initial level in Figure 1. The Na and Cl levels in both sides changed little or insignificantly after incubation. In contrast, large concentration gradients of Pi were always produced as a result of marked elevations of the serosal level, though the mucosal level exhibited no appreciable falls. The gradient was larger in Segment I than in Segment II. Stanniectomy did not seem to have affected the final concentrations of Na, Cl and Pi. A clear concentration gradient of Ca could be seen only in Segment I from stanniectomized 1/3 SW-fish, where the serosal concentration distinctly increased and the mucosal concentration tended to lower compared to the initial level. The elevation of the serosal level in the stanniectomized fish was significantly larger compared to that in the shams.

DISCUSSION

Krishnamurthy and Bern [15] reported that the number of the CS in *Salmo gairdneri* varied among individuals from 4 to 6. As we have removed 2–7 visually recognized corpuscles at stanniectomy, it is possible that some of the CS had been left behind in some stanniectomized fish. However, the stanniectomy could elicit hypercalcemia in fish adapted to 1/3 SW (Ca 4.34 mM) and not in those kept in FW (Ca 0.64 mM). Pang *et al.* [16] obtained similar results in *Fundulus heteroclitus* and explained that the CS may serve in prevention of hypercalcemia in a high-calcium environment. Activation of the CS in seawater adapted *F. heteroclitus* was reported by Cohen *et al.* [17]. This seems to be the case also in the rainbow trout.

As the fish used in the present experiment did not feed well after the operation, increased Ca in the serum of 1/3 SW-adapted fish must have been derived from the ambient water through the gills and the body surface [12, 18, 19]. When a fish drinks much water to compensate an osmotic loss of water in a high osmotic environment, Ca might also be absorbed through the intestine. In the fish adapted to 1/3 SW in the present experiment, augmentation of the drinking rate could have occurred [9], but the net volume of water which moved across the gut sac was not different from that in FW-fish.

A gain of an electrolyte in one side of the gut sac was not necessarily balanced with a loss in the other side. This may have been caused by mobilization of the element initially contained in the tissue. Absorptive movements of Na and Cl known by a loss in the mucosal fluid and a gain in the serosal fluid were consistently observed in both the anterior and posterior intestine. The extent of transport of these ions paralleled with the bulk of water movement and little or no concentration gradients were produced between the mucosal and the serosal fluids. These observations substantiate the ability of the intestine to absorb water dragged by the active transport of Na and Cl. An active mucosa-to-serosa transport of Pi was also clearly recognized in the anterior intestine, whereas directional movements of K and Mg were indistinct in both parts of the intestine. The general features in behavior of the above ion species are in accord with those observed in the carp intestine by Nakamura [13]. No effects of stanniectomy were noticed regarding all of these ions.

Ca in the serosal fluid showed a consistent increase after incubation but a decrease in the mucosal fluid corresponding to the serosal increase was not evident. This inconsistency in the mucosal fluid, not only of Ca but also of other scantily presented ions, may be at least partly due to its large volume relative to the serosal fluid. The mucosa-to-serosa movement of Ca in Segment I was more apparent in FW-shams than in SW-

shams and this movement was not enhanced by stanniectomy in FW-fish. In 1/3 SW-fish, however, a mucosal gain in the shams was reversed to a significant loss in the stanniectomized fish. In addition, the serosal gain increased significantly following stanniectomy. This indicate that the absorption of Ca in the anterior intestine is enhanced by the removal of the CS, which are activated in a high-calcium environment such as 1/3 SW. The observation that an uphill concentration gradient developed in the stanniectomized 1/3 SW-fish further supports the idea that the CS are involved in prevention of Ca entry through the intestine.

A marked elevation of branchial uptake of Ca in stanniectomized eels and attenuation of the influx rate by administration of CS extracts have been reported by Fenwick and So [5], So and Fenwick [6, 7] and Milet *et al.* [8], and the gills have been considered to be the primary target for an active principle of the CS. The present results indicate that the intestine is also a target organ for a hypocalcemic factor of the CS. Further studies will be needed to confirm the effect of CS extracts using the gut sac incubation system.

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