VASCULAR AND RENAL ACTIONS OF SALMON CALCITONIN IN FRESHWATER- AND SEAWATER-ADAPTED EUROPEAN EELS (*ANGUILLA ANGUILLA*)

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SUMMARY

When freshwater eels were given either 5 or 15 i.u. calcitonin kg^{-1} body weight, there was an increase in dorsal aortic blood pressure as well as a rise in urine volume, glomerular filtration rate and electrolyte excretion rate, 1 h after injection. None of these changes was shown by seawater-adapted eels. When freshwater-adapted eels received 15 i.u. calcitonin kg^{-1} body weight there were significant changes in plasma electrolyte levels, again a response not shown by seawater-adapted fish. These changes occurred in 1 h after the injection of hormone, during which time plasma levels of sodium, chloride and calcium fell whilst plasma potassium levels increased.

At no time within the first hour were there changes in the relative handling of electrolytes. However, seawater-adapted eels showed hypercalcaemia and a reduced relative calcium clearance 3 h after receiving 15 i.u. calcitonin kg^{-1} body weight.

INTRODUCTION

Fish calcitonin was first demonstrated from the ultimobranchial (suprapericardial) body of dogfish by Copp, Cockroft & Kueh (1967), who later showed that calcitonin of salmon origin was a more potent hypocalcaemic agent in mammalian systems than were naturally produced mammalian calcitonins (Copp *et al.* 1969). However, in spite of this potency, the role(s) of fish calcitonin in teleosts is equivocal.

There are conflicting reports on both its ability to affect significantly the ionic composition of the body fluids and also the possible target tissues involved (Louw, Sutton & Kenny, 1967; Chan, Chester Jones & Smith, 1968; Pang, 1971; Copp *et al.* 1972; Lopez *et al.* 1976; Yamauchi, Matsuo, Yoshida & Orimo, 1978; Milhaud, Bolis & Benson, 1980; Hirano, Hasegawa, Yamauchi & Orimo, 1981).

In a preliminary study, Wales & Barrett (1983) have shown that in 1 h after intraperitoneally administered salmon calcitonin, albeit in higher doses of 50 or 100 i.u. kg^{-1} body weight, there was a significant depression of sodium, chloride and calcium ions in the plasma of goldfish and freshwater- and seawater-adapted eels.

The present series of experiments seeks to establish whether a similar response

Key words: Calcitonin, blood pressure, kidney, fish, electrolytes.

could be detected in eel plasma following intravenous injections of more physiologi-cally related amounts of hormone whilst extending the investigation to include poss-ible actions on blood pressure and renal handling of electrolytes.

MATERIALS AND METHODS
Animals

Freshwater-adapted eels (*Anguilla anguilla* L.) were held for 2 months at 14 °C in running Sheffield tap water containing $0.50\ \text{mmol}\,l^{-1}$ Na$^+$, $0.51\ \text{mmol}\,l^{-1}$ Cl$^-$ and $0.25\ \text{mmol}\,l^-$ Ca^{2+}, whilst seawater-adapted animals were maintained for 2 months at 12 °C in aerated recirculated artificial sea water (Natura Sea Aquariums Ltd, Jungle Laboratories Corporation, Sanford, Florida, U.S.A.) containing $450\ \text{mmol}\,l^{-1}$ Na$^+$, $530\ \text{mmol}\,l^{-1}$ Cl$^-$ and $9\ \text{mmol}\,l^{-1}$ Ca^{2+}. The eels did not feed after their arrival.

Surgical procedure

Prior to surgery, animals were anaesthetized in a solution of MS 222 (Tricaine methanesulphonate, Sigma Chemical Company, Poole, England) and weighed. A ventral incision was then made alongside the liver to expose both the pneumogastric artery and vein. Details of this method have been given before (Henderson & Wales, 1974). Implantation of polythene cannulae (Laboratoire Portex SA, Berck-sur-Mer, France) enabled arterial blood sampling at selected intervals during otherwise continu-ous recording of blood pressure in the dorsal aorta (Harvard Model 377 Pressure Trans-ducer, Harvard Apparatus Ltd, Edenbridge, Kent, England) as well as a slow venous infusion of saline ($20\,\mu l\ \text{min}^{-1}$ 0.9% w/v NaCl). The urinary bladder was also cannu-lated as a device for collecting urine samples. This cannulation was achieved with non-compressible autoanalyser tubing (Altec Laboratory Supplies & Systems Ltd, Alton, England) of 0.9 mm internal diameter. The tubing was held in place by a purse-string suture around the cloaca and by stitching the rigid orange collar to the skin surface. The operated fish were then transferred to individual black polythene tanks designed in such a way that when the tank lid was fitted the cannulae could be freely manipulated from the outside without disturbing the animals but with minimal dead space.

Experimental procedure

Some 48 h following surgery, the eels were given a priming intravenous injection of $5\,\mu\text{Ci}$ ^{3}H-inulin (Amersham International PLC, Amersham, England) followed by a sustained infusion of $2\,\mu\text{Ci}\,\text{ml}^{-1}$ ^{3}H-inulin in 0.9% w/v saline solution (Meltic Infusion Pump, Meltec Ltd, Buckingham, England) at a rate of $20\,\mu l\ \text{min}^{-1}$. This infusion was maintained throughout the experiment. The renal clearance of inulin was taken as an index of glomerular filtration rate. Urine samples were collected at intervals in tared polythene vials, whilst $150\,\mu l$ mid-point blood samples were with-drawn from the arterial cannula and immediately centrifuged. Blood remaining in the cannula was returned into the body with heparinized isotonic saline. The samples, when collected, were stored deep frozen for subsequent analysis. When stable rates of urine flow and blood pressure were evident, a single acute injection of 0, 5 or $15\,\text{i.u.}\,\text{kg}^{-1}$ of synthetic salmon calcitonin (Calsynar, Armour Pharmaceutical

Table 1. *Renal function in freshwater- and seawater-adapted eels 1 h following injection of either 5 or 15 i.u. calcitonin kg⁻¹ body weight*

5 i.u. calcitonin kg⁻¹ body weight

	Freshwater			Seawater		
	Control	Preinjection	Postinjection	Control	Preinjection	Postinjection
Urine volume (μl min⁻¹ kg⁻¹)	51·1 ± 4·2	53·2 ± 3·9	78·6 ± 7·4**	10·4 ± 2·6	10·6 ± 2·4	11·5 ± 2·3
Inulin clearance (μl min⁻¹ kg⁻¹)	60·4 ± 8·6	63·0 ± 7·3	81·3 ± 8·1*	14·8 ± 2·6	14·3 ± 3·9	17·0 ± 2·8
Relative sodium clearance	0·05 ± 0·01	0·05 ± 0·01	0·05 ± 0·01	0·15 ± 0·04	0·15 ± 0·04	0·14 ± 0·05
Relative potassium clearance	0·27 ± 0·04	0·28 ± 0·04	0·27 ± 0·06	0·37 ± 0·04	0·37 ± 0·04	0·39 ± 0·06
Relative chloride clearance	0·01 ± 0·004	0·01 ± 0·004	0·01 ± 0·003	0·60 ± 0·06	0·63 ± 0·06	0·60 ± 0·05
Relative calcium clearance	0·21 ± 0·03	0·20 ± 0·02	0·21 ± 0·03	1·71 ± 0·16	1·74 ± 0·16	1·64 ± 0·20

15 i.u. calcitonin kg⁻¹ body weight

	Freshwater			Seawater		
	Control	Preinjection	Postinjection	Control	Preinjection	Postinjection
Urine volume (μl min⁻¹ kg⁻¹)	52·2 ± 9·6	55·2 ± 10·9	90·0 ± 15·3**	12·6 ± 3·7	12·4 ± 4·1	12·7 ± 7·0
Inulin clearance (μl min⁻¹ kg⁻¹)	65·19 ± 4·5	67·7 ± 8·4	132·7 ± 19·3**	15·5 ± 5·6	16·0 ± 7·0	16·0 ± 6·7
Relative sodium clearance	0·08 ± 0·03	0·06 ± 0·02	0·07 ± 0·02	0·17 ± 0·06	0·17 ± 0·06	0·17 ± 0·09
Relative potassium clearance	0·38 ± 0·03	0·38 ± 0·03	0·39 ± 0·03	0·38 ± 0·08	0·39 ± 0·05	0·40 ± 0·07
Relative chloride clearance	0·01 ± 0·002	0·01 ± 0·002	0·01 ± 0·002	0·62 ± 0·09	0·62 ± 0·04	0·62 ± 0·05
Relative calcium clearance	0·27 ± 0·03	0·26 ± 0·03	0·30 ± 0·05	1·53 ± 0·41	1·51 ± 0·12	1·20 ± 0·37

Control values obtained after injection with acetate saline.
Values are means ± s.d.; $N = 6$ for each group.
* $P = < 0.01$
** $P = < 0.001$ (paired t-test).

Table 2. *Plasma electrolyte composition of freshwater and seawater adapted eels one hour following acute injection of 15 i.u. calcitonin kg body weight*

Plasma concentration (mmol l⁻¹)	Freshwater			Seawater		
	Control	Preinjection	Postinjection	Control	Preinjection	Postinjection
Sodium	151 ±4	152 ±6	132 ±9**	162 ±7	164 ±8	152 ±13
Potassium	3·4±0·3	3·6±0·3	4·8±0·5**▪	3·6±0·4	3·7±0·5	3·4±0·7
Chloride	96 ±4	96 ±4	84 ±8*	150 ±9	148 ±6	151 ±5
Calcium	3·40±0·3	3·45±0·3	2·61±0·5*	3·10±0·5	3·14±0·4	3·24±0·6

Control values obtained after injection with acetate saline.
Values are mean ± s.d.; $N = 6$ for each group.
** $P = <0.001$; * $P < 0.01$ (paired t-test).

Company Ltd, Eastbourne, England) was given into the venous cannula by microlitre syringe and the infusion restored. Acetate saline similar to that in which the calcitonin was supplied was also tested for vascular activity. The animals were usually monitored continuously for up to 5 h or more following injection.

After appropriate dilutions, the sodium levels were measured by flame emission using a Corning 400 spectrophotometer. Chloride levels were assayed using a Corning 925 chloride analyser. Calcium analysis was performed on a Perkin-Elmer 360 atomic absorption spectrophotometer using an acetylene/air flame. The method for calcium determination was that described by Trudeau & Freier (1967) using a lanthanum chloride/acetic acid releasing solution to remove interference from phosphate and protein, giving a value for total plasma calcium. The urinary and plasma levels of ^{3}H-inulin used to give an estimate of the glomerular filtration rates were measured by liquid scintillation (Kontron MR 300) from $20\,\mu$l of sample in 2 ml of scintillation fluid (Cocktail T, BDH Chemicals Ltd, Poole, England).

RESULTS

Freshwater-adapted eels responded to calcitonin injections with an increase in dorsal aortic blood pressure whilst seawater-adapted eels did not. No vascular responses were seen when control injections of either saline or acidified saline were given. The increase in dorsal aortic systolic pressure in response to 5 i.u. calcitonin kg^{-1} body weight was from $24 \cdot 8 \pm 0 \cdot 42$ to $27 \cdot 94 \pm 0 \cdot 46$ mmHg (mean $\pm$ s.D.; $N = 6$), whilst that to 15 i.u. calcitonin kg^{-1} body weight was from $23 \cdot 9 \pm 0 \cdot 39$ to $31 \cdot 32 \pm 0 \cdot 94$ mmHg (mean $\pm$ s.D.; $N = 6$). The duration of these responses was between 8 and 15 min respectively.

The results in Table 1 are values of renal function recorded from freshwater- and seawater-adapted eels both before and 1 h after either calcitonin or acetate saline injection.

The freshwater eels showed a significant increase in urine volume coupled to an increase in glomerular filtration rate during the 1 h collection period after calcitonin injection. At the same time there were no indications of changes in the relative handling of electrolytes. Injections of 5 i.u. calcitonin kg^{-1} body weight failed to elicit any significant changes in the plasma electrolyte composition of either freshwater- or seawater-adapted eels. However, the results summarized in Table 2 show that administration of 15 i.u. calcitonin kg^{-1} body weight did significantly affect plasma electrolyte levels in freshwater-adapted eels within 1 h of injection when there was a significant decrease in plasma sodium, chloride and calcium levels coupled with a significant increase in plasma potassium. These changes did not persist beyond this 1 h interval and did not occur at all either in seawater-adapted fish or in acetate saline injected control animals. However the seawater-adapted fish did show a response to 15 i.u. calcitonin kg^{-1} body weight some 3 h after injection, when there was a mean 40 % increase in plasma calcium levels from $3 \cdot 14 \pm 0 \cdot 4$ to $4 \cdot 38 \pm 0 \cdot 4$ mmol l^{-1} as well as a significant reduction in relative calcium clearance from $1 \cdot 51 \pm 0 \cdot 12$ to $1 \cdot 06 \pm 0 \cdot 33$ (mean $\pm$ s.D.; $N = 6$). This effect was continued for at least 5 h whilst no similar response was seen in freshwater-adapted fish.

DISCUSSION

The ability of calcitonin to induce diuresis in freshwater eels has been reported before (Hirano *et al.* 1981), although these workers did not find corresponding actions on either blood pressure or plasma electrolyte composition. This apparent discrepancy in calcitonin activity could be due to the fact that Hirano *et al.* (1981) were using 1 i.u. kg^{-1} of synthetic eel calcitonin given *via* the caudal vein whilst the results presented here were taken after giving 5 and 15 i.u. kg^{-1} of synthetic salmon calcitonin injected almost directly into the heart. The lack of any immediate vascular or renal responses by seawater-adapted eels when compared with their freshwater-adapted counterparts may point to a difference in the degree of sensitivity to calcitonin which is related to external salinity. The electrolyte changes seen in freshwater fish following injection of 15 i.u. kg^{-1} calcitonin might further indicate a branchial role of calcitonin as relative ion clearances remained unchanged at a time when plasma levels of sodium, chloride and calcium were falling and potassium levels rising. This suggestion would certainly find some support (see Milhaud *et al.* 1980), although significant falls in plasma calcium levels could give rise to altered membrane permeabilities to other electrolytes.

In an earlier paper, Wales & Barrett (1983) showed a depression of plasma sodium chloride and calcium ions in both freshwater- and seawater-adapted immature eels following intraperitoneal injections of 50 and 100 i.u. of salmon calcitonin kg^{-1} body weight. If such responses are taken as being physiological rather than pharmacological, it could be that calcitonin plays a role which alters as the eel at first increases its skeletal growth and then later faces hypoosmotic challenges prior to catadromous migration. The apparent hypercalcaemia which was seen in seawater-adapted eels was quite unexpected. The fish used in these experiments were large female eels and there is evidence that oestrogens may increase circulating plasma calcium levels (Woodhead, 1969) presumably for oocyte development. One explanation of the delayed hypercalcaemic action of calcitonin in sea water might be that it is mediated *via* a stimulatory action on gonadal steroid production.

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