THYROCALCITONIN STUDIES IN ELASMOBRANCH FISH

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INTRODUCTION

We recently reported a hypocalcaemic and hypophosphataemic action of mammalian thyrocaltitonin in the teleost fish *Ictalurus melas* (Louw, Sutton and Kenny 1967). A logical sequence to these studies would be to examine the action of the same hormone in elasmobranch fish. The absence of an osseous skeleton in the latter provides ideal material to test the hypothesis that the mode of action of thyrocalcitonin is due to a direct effect upon bone. Moreover, in contrast to the diffusely distributed thyroid follicles of the teleost fish, the thyroid of the elasmobranch fish is a discrete gland which greatly facilitates extraction procedures. We have therefore examined the effects of porcine thyrocalcitonin on the levels of serum calcium and phosphate in the lazy shark *Poroderma africanum* (Gmelin) (Smith 1949). In addition the thyroid glands from the school shark *Galeorhinus galeus* (Linnaeus) (Smith 1949), were assayed for hypocalcaemic activity.

PROCEDURE

In order to test the effects of increasingly large doses of porcine thyrocalcitonin on serum calcium the following experiment was carried out. Twenty lazy sharks were randomly assigned to four treatments consisting of an acid saline (pH 4) control and three doses (10, 30 and 100 mg/kg) of porcine thyrocalcitonin (100-200 MRC m units/mg, Wilson Laboratories TCA powder) dissolved in acid saline. The average weight of the sharks was 1·2 kg and they were maintained in very large aerated marine aquaria. The average water temperature during the course of the experiment was 17°C. All doses, including the acid saline control, were administered intraperitoneally in equal volumes of 5 ml. The sharks were bled by heart puncture 60 min after injection and the serum calcium was determined (Kessler and Wolfman 1964).

RESULTS

The results showed no effect of increasing doses of the extract. The mean serum calcium values obtained for the control, 10 mg/kg, 30 mg/kg and 100 mg/kg dosage levels were 13·2, 14·3, 14·8, and 14·2 mg/100 ml respectively. A second experiment was essentially the same as the first, the only differences being the exclusion of the 10 mg/kg body weight dosage level

and the inclusion of serum inorganic phosphorous determinations. In order to obtain accurate results it was found necessary to remove serum lipids prior to the phosphorus determination which was performed by the method described by Varley (1962).

TABLE 1

EFFECT OF PORCINE THYROCALCITONIN ON SERUM CALCIUM AND PHOSPHORUS IN THE LAZY SHARK.

		Serum calcium (mg/100 ml)			*Serum phosphorus (mg/100 ml)*		
Treatment	*Dose IP*	*No. of Sharks*	*Mean*	*± S.D.*	*No. of Sharks*	*Mean*	*± S.D.*
Acid saline (pH4) ..	5 ml	5	15·3	± 0·23	5	4·3	± 0·26
Porcine TC[1]	30 mg/kg	5	15·5	± 0·52	4	4·2	± 0·37
Porcine TC	100 mg/kg	5	16·2	± 0·22	4	4·3	± 0·30

[1] Porcine TC: Porcine Thyrocalcitonin, 100-200 MRC m units/mg, injected in total volume of 5 ml.

The results of the second experiment are summarized in Table 1 and these data again show no effect of the extract upon either serum calcium or serum inorganic phosphorus levels. The values obtained are also within the normal range for sharks reported by Urist (1961).

Table 2

EFFECT OF SCHOOL SHARK THYROID EXTRACT UPON SERUM CALCIUM IN THE RAT

	Dose		*Serum Calcium (mg/100 ml)*		
Treatment	*ml/rat*	*mg fresh tissue/rat*	*No. of rats*	*Mean*	*± S.D.*
Control O.IN HCL	1·0	—	4	9·3	± 0·18
Shark thyroid extract ..	0·5	200	4	9·1	± 0·09
Shark thyroid extract ..	1·0	400	4	9·3	± 0·20
Shark thyroid extract ..	2·0	800	4	9·2	± 0·13

Having established that the lazy shark was unresponsive to porcine thyrocalcitonin (10,000-20,000 MRC m units/kg, IP) an extract of shark thyroid tissue was assayed in the rat to find out if an endogenous source of the hormone was present in this gland. Fresh thyroid tissue was collected from 20 school sharks and a crude extract of this tissue was prepared, using a modification of the method of Stahl *et al.* (1967). The glands were homogenized in 0·1N HCL (1 g/2·5 ml acid) and the resulting homogenate, after heating to 70°C in a boiling water bath, was centrifuged at 4°C and 10,000 *g* for 90 minutes. The supernatant was then frozen and assayed at a later date for any hypocalcaemic or hypophosphataemic properties by injecting it at three dosage levels (200, 400 and 800 mg fresh thyroid/rat) into 33-day old Holtzman male rats which had been fasted for 1 day. Serum calcium (Kessler and Wolfman 1964) was determined 60 minutes after injection and compared with a similar control group which received 1 ml of 0·1N HCL. The results of this assay are contained in Table 2; no effect of the extract was apparent. As this assay is capable of detecting as little as 10 MRC m units of porcine thyrocalcitonin (Cooper *et al.* 1967), it can be concluded that the shark thyroid extract did not contain more than 0·012 MRC m units/mg fresh thyroid tissue.

DISCUSSION

The previously demonstrated sensitivity of teleost fish to porcine thyrocalcitonin (Louw *et al.* 1967) and the apparent absence of sensitivity in the elasmobranch shown in this investigation, lends considerable support to the hypothesis that the mode of action of thyrocalcitonin is due to an effect upon osseous tissue which is absent in the elasmobranch. Moreover, the apparent absence of an endogenous source of thyrocalcitonin in the thyroid tissue of both the teleost (Louw *et al.* 1967) and the elasmobranch (Table 2), and the recently demonstrated presence of a hypocalcaemic factor in the ultimobranchial bodies of both a teleost (Copp *et al.* 1967) and an elasmobranch (Copp *et al.* 1968), poses interesting problems in regard to the evolutionary aspects of this hormone.

The above remarks should, however, be considered of a tentative nature in view of the negative findings of Pang and Pickford (1967) in regard to the action of thyrocalcitonin in the teleost *Fundulus heteroclitus* and because of the possibility of interspecific differences in molecular structure of the hormone confounding the results.

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