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Responses of the Ultimobranchial Body in Eels (*Anguilla anguilla* L.) Maintained in Sea Water and Experimentally Matured, to Injections of Synthetic Salmon Calcitonin

J. Peignoux-Deville, E. Lopez, F. Lallier, E. Martelly-Bagot, and C. Milet*

Laboratoire de Physiologie générale et comparée, Muséum national d'Histoire naturelle, Laboratoire d'Endocrinologie comparée, associé au C.N.R.S., Paris, France

Summary. 1. The effect of a synthetic salmon calcitonin (SCT) treatment on ultimobranchial body (UB) activity in eels (*Anguilla anguilla* L.) maintained in sea water and submitted to experimental maturation, has been studied histologically.

2. The activity of the glands of a control group of eels maintained in sea water was taken as a reference.

3. The UB parenchyma showed a marked atrophy in the fish treated with SCT alone, and serum calcium decreased significantly in this group.

4. Immature ♀ silver eels receiving carp pituitary extract (CPE 1 mg/100 g body wt. per injection) until complete maturation presented high hypercalcemia associated with cellular hypertrophy and hyperplasia in the UB.

5. SCT treatment did not prevent the hypercalcemia provoked by CPE injections. UB activity was strongly increased in this case.

6. These data indicate that the activity of the UB in eels varies with both physiological and experimental hypercalcemia, and responds to SCT injections.

Key words: Ultimobranchial body activity – Teleosts – Calcitonin – Maturation.

Résumé. 1. L'action de la calcitonine synthétique de Saumon (SCT) sur l'activité du corps ultimobranchial (CUB) chez des Anguilles (*Anguilla anguilla* L.) maintenues en eau de mer et soumises à maturation expérimentale, a été étudiée histologiquement.

2. Les Anguilles du groupe témoin, maintenues en eau de mer, ont une glande dont l'activité modérée est considérée comme l'activité de référence.

3. L'épithélium du corps ultimobranchial est atrophié chez les animaux traités à la calcitonine et la calcémie montre une baisse légère mais significative.

Send offprint requests to: J. Peignoux-Deville, Laboratoire de Physiologie générale et comparée, Muséum national d'Histoire naturelle, 7, rue Cuvier, 75005 Paris, France.

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4. Les Anguilles recevant de l'extrait hypophysaire de Carpe jusqu'à maturation complète présentent une calcémie très élevée et l'épithélium du corps ultimobranchial montre une hyperplasie: les cellules sont hypertrophiées et on constate de nombreuses divisions cellulaires.

5. Le traitement à la calcitonine avant la maturation ne prévient pas l'hypercalcémie provoquée par les injections d'extrait hypophysaire de Carpe. L'activité du corps ultimobranchial est aussi très augmentée dans ce cas.

6. Les résultats exposés ici semblent montrer que l'activité du corps ultimobranchial chez l'Anguille, est sous la dépendance des variations tant physiologiques qu'expérimentales de la calcémie, et que cette glande répond à des injections de calcitonine.

Introduction

Calcitonin is secreted by the ultimobranchial body of several fish including dogfish (Copp *et al.*, 1967), sharks (Urist, 1967) and teleosts (Copp *et al.*, 1968). The effects of calcitonin on fish have been disputed (Urist, 1967; Pang and Pickford, 1967; Louw *et al.*, 1967; Chan *et al.*, 1968) and we wish to summarize our results on the study of the ultimobranchial body (UB) so as to marshal all our evidence for the action of calcitonin (CT), both endogenous and exogenous, on calcium metabolism in the teleost fish studied in our laboratory.

During previous experiments we were never able to detect any difference, in UB activity, between eels maintained in fresh and in sea water (Peignoux-Deville, unpublished data).

A study, made in our laboratory (Fontaine *et al.*, 1973a) on parrotfish (*Scarus* sp.) from the shore of the Gambier Island which were fed by grazing on madrepores, revealed marked functional activity of the UB and of the Stannius corpuscles. These two glands, whose common function is to lower serum calcium, appear in this case to prevent the hypercalcemia provoked by the high calcium diet of these fish (Fontaine *et al.*, 1974).

To check whether the hyperactivity of UB and Stannius corpuscles is not a characteristic of all parrotfish, we have studied another species of scaridae from the shores of Asiatic Turkey (*Sparisoma cretense*) that do not feed by grazing on coral reefs. The two glands in *Sparisoma cretense* showed less activity than the glands of the Scarus sp. (from the Gambier Islands). These findings confirmed a relationship between the activity in the glands responsible for lowering the level of serum calcium (UB and Stannius corpuscles) and the excess of calcium in the diet (Fontaine *et al.*, 1973b).

Histological and histochemical studies of the UB of the salmon (*Salmo salar* L.) showed different degrees of glandular activity at different phases of its migratory life (Deville and Lopez, 1970). In the adult salmon, when the animal begins to swim up river towards the reproduction sites, the gland appears to store an abundance of secretory material. Depletion occurred when the salmons were at the spawning grounds. In fact, in these fish, a fall of serum calcium concentration was noted (Fontaine *et al.*, 1969) and bone resorption was also observed (Tchernavin, 1938–1940). These modifications probably depend upon hyperactivity of the UB.

In previous experiments, we pointed out that the removal of the Stannius corpuscles in the eel (*Anguilla anguilla* L.) which is followed by hypercalcemia (Fontaine, 1964–1967) stimulates the UB (Lopez *et al.*, 1968). Furthermore, the UB exhibited a strong stimulation during maturation of the eel (*Anguilla anguilla* L.) induced experimentally by the injection of carp pituitary extract (CPE) (Lopez *et al.*, 1968). Moreover, naturally-matured conger eel (*Conger conger* L.) showed the same glandular hyperactivity of UB (Peignoux-Deville, unpublished results) always connected with hypercalcemia (Fontaine *et al.*, unpublished data) and an acute bone catabolism (Lopez, 1973).

Prolonged treatment with synthetic salmon calcitonin (SCT) during the maturation of the eels, did not bring about a decrease of the hypercalcemia, and the UB remained highly stimulated. On the other hand, when CT is administered after spawning (when the eels are no longer being injected with CPE) the effect of CT is important: we observed a decrease in the hypercalcemia of approximately 50%, and apparent regression of the UB, associated with a decrease of osteoclasis and of osteocytic osteolysis in the bone (Lopez and Deville, 1973).

We have thus attempted to complete the above studies, with the object of elucidating the effect on serum calcium level and on UB and osseous activity, of treatment of experimentally-matured eels with SCT. In this paper we especially want to demonstrate the strong relationship between UB activity and the serum calcium level in the eel (*Anguilla anguilla* L.).

Material and Methods

Immature ♀ silver eels (*Anguilla anguilla* L.) 400 to 600 g in weight, were obtained in Peronne (Oise). They were placed in aerated fresh water and allowed to adapt to laboratory conditions for at least one month. Then, one week before the experiment, they were progressively adapted to sea water.

A control group (first group) of 6 fish was kept in sea water during the whole experiment. They received a gelatine injection three times a week.

A second group (5 fish) was injected intraperitoneally with SCT three times a week, over a period of seven weeks (3 MRC[1] mU for every gram of body weight dissolved in gelatin solution).

A third group (4 fish) was treated similarly. Then, they were given intraperitoneal injections of carp pituitary extract[2] (CPE: 1 mg/100 g body wt. per injection) three times a week, until complete maturation, according to the method described by Fontaine *et al.* (1964).

A fourth group (7 fish) received CPE three times a week (1 mg/100 g body wt. per injection) until complete maturation. Immediately after spawning, the eels of the third and fourth groups were killed. The eels of the control and second groups were sacrificed at the end of the experiment.

$$\text{Gonosomatic ratio (GSR)} = \frac{\text{gonadal weight} \times 100}{\text{body weight}}$$

During the experiment, blood was obtained by cardiac puncture. Total serum calcium was determined with an atomic absorption spectrophotometer (Perkin Elmer).

Tissues containing the UB were removed and fixed in Bouin's fluid for 48 hours, washed in 70% alcohol dehydrated, cleared in butylic alcohol, and embedded in paraffin.

Serial sections were cut at 5 μ and stained with Hemalun-Eosin to locate the gland. When the sections containing the UB were located, Cleveland-Wolfe and Periodic-Acid Schiff stains were employed.

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[1] MRC: Medical Council Research.

[2] From the Stoller Fisheries, Spirit Lake, Iowa, U.S.A.

Results

The ultimobranchial body of the control group maintained in sea water (first group) had a parenchyma of normal appearance which was taken as a reference. It possessed a follicular structure, with a large lumen. The pseudostratified epithelium surrounded by a reticular connective tissue, contained two cell types: 1) secretory or storage cells, with a homogeneously-stained nucleus and 2) degenerating cells, with an irregular densely-stained nucleus close to the surface of the epithelium. The surrounding vascular network was not well developed (Figs. 1–2). In this group, the serum calcium level remained constant for the duration of experiment (Table 1). GSR = 2, 3.

SCT injected into immature ♀ silver eels, (second group) over a period of three weeks, brought about a slight but significant decrease of the serum calcium concentration (Table 1). The UB showed a marked atrophy in this second group, treated with SCT alone. The epithelium was reduced to one layer of storage cells with a very small amount of cytoplasm (Figs. 3–4). Glandular activity seemed to be completely arrested.

In the third group treated with SCT followed by CPE, the CPE elicited hypercalcemia, in spite of the preventive hypocalcemic effect of the SCT injections (Table 1). Though a decrease of bone catabolism was shown (Lopez *et al.*, in press) the exogenous CT did not succeed in reducing hypercalcemia and the UB was stimulated. We observed in this case, a glandular hyperplasia, in increase in the height of the epithelium and, consequently, a smaller central cavity. The cells were elongated and clear, with large lightly-stained nuclei containing prominent nucleoli (Fig. 6). Numerous degenerating cells were found at the apical surface of the epithelium near the lumen, making a "festoon-like layer" on the epithelial surface (Fig. 5). The central cavity of the gland contained large amounts of mucoïd material, pycnotic nuclei and cellular debris. The surrounding vascular network appeared to be greatly enlarged (Fig. 5). The characteristics of the UB in hypercalcemic eels have been described previously (Lopez *et al.*, 1968).

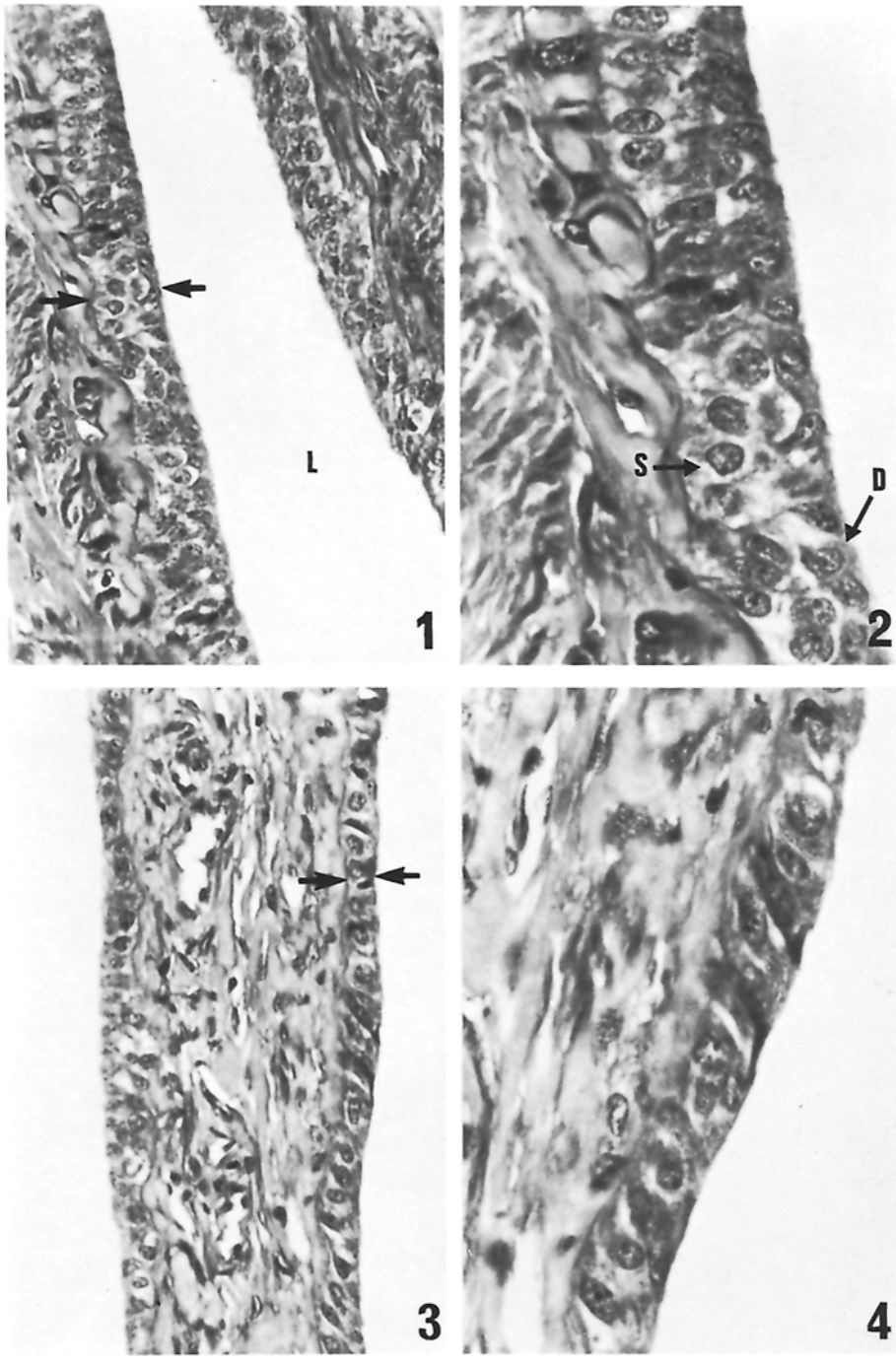
The fish treated with carp pituitary extract until complete maturation (fourth group) showed as previously reported (Fontaine *et al.*, 1964) a strong hypercalcemia (Table). [50 < GSR < 60]. The ultimobranchial parenchyma of these eels

Fig. 1. Ultimobranchial gland from control immature silver eel, maintained in sea water. Normal epithelial structure with a large central lumen (*L*). Note the small capillary network (× 360 Cleveland-Wolfe Stain)

Fig. 2. Enlargement of part of Fig. 1 showing the cells of the glandular epithelium at different functional stages: secretory cells (*S*) and degenerating cells (*D*) at the apex. (× 800 Cleveland-Wolfe Stain)

Fig. 3. Ultimobranchial gland from immature silver eel injected with synthetic salmon calcitonin, illustrating the marked atrophy of the epithelium (× 360 Cleveland-Wolfe Stain)

Fig. 4. A portion of the parenchyma of the gland shown in Fig. 3 formed by a single layer of secretory cells. (× 800 Cleveland-Wolfe Stain)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S
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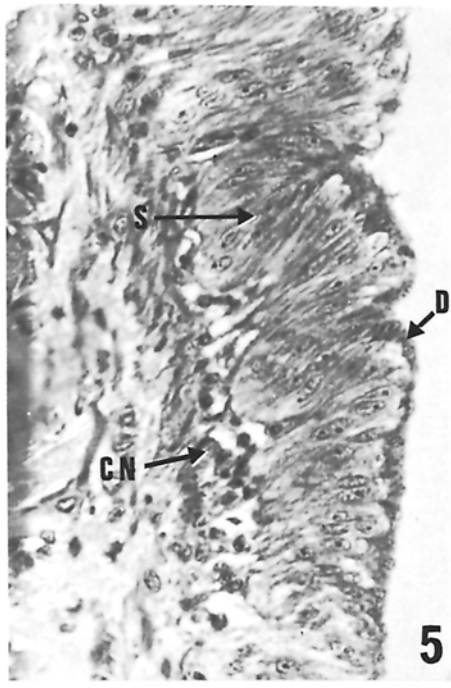
Table 1

♀ Silver eels	Serum Ca^{++} level mg/l first week before treatment		Treatment	Serum Ca^{++} level mg/l 3rd week after treatment		Stop SCT treatment 7th week	Serum Ca^{++} level mg/l 8th week		Start of carp pituitary extract treatment 8th week	Serum Ca^{++} level mg/l one week after carp pituit. extract treatment		Serum Ca^{++} level mg/l end of the experiment	
First group controls $n=6$	120 133 129 130 – –	128,0 ±2.79	Gelatine solution									129 133 120 130 104 104	120.0 ±5.36
			$0.01 < p < 0.05$										
Second group $n=5$	98 91 93 94 91	93.4 ±1.28	Synthetic salmon CT 3 MRC mu/ g/wt 21 injections	83 94 76 86 89	85.6 ±3.01		91 81 89 81 114	91.2 ±6.05				130 110 110 106 114	114.0 ±4.19
Third group $n=4$	110 110 115 –	111.6 ±1.66	,, ,,	94 – 94 102	96.6 ±2.66				carp pituitary extract 1 mg/100 g/wt	145 135 135 156	142.7 ±5.00	304 870 1280 440	723.5 ±221.26
			$p < 0.01$										
Fourth group $n=7$	115 – 125 110 110 120 125	117.5 ±2.81	carp pituitary extract 1 mg/100 g/wt			115 115 105 107 105 110 107	109.14 ±1.64			140 173 117 173 145 131 128	142.85 ±8.24	1474 419 323 331 492 628 361	575.42 ±155.15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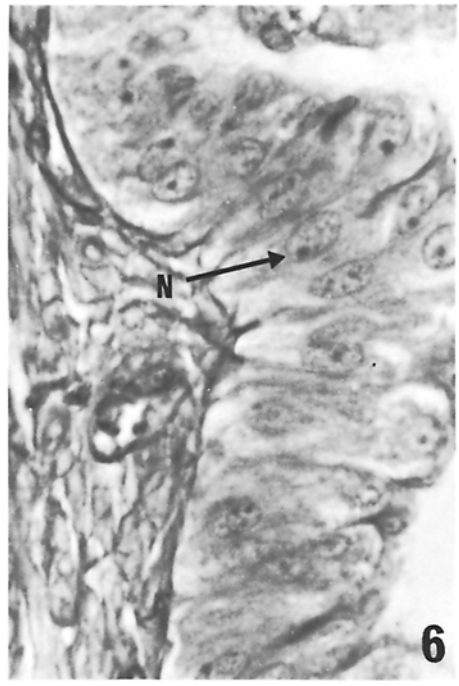
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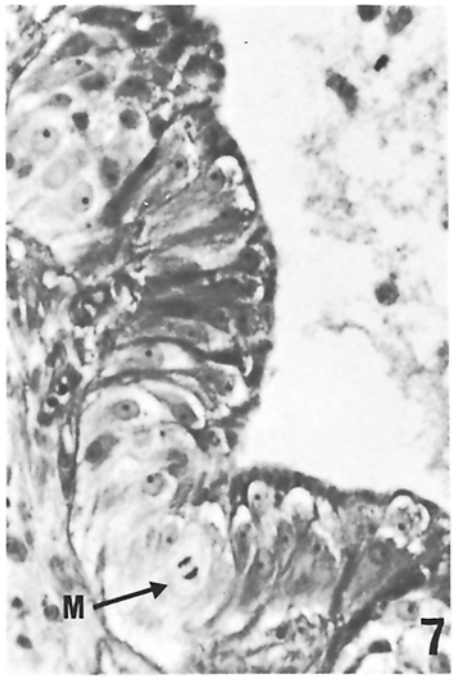
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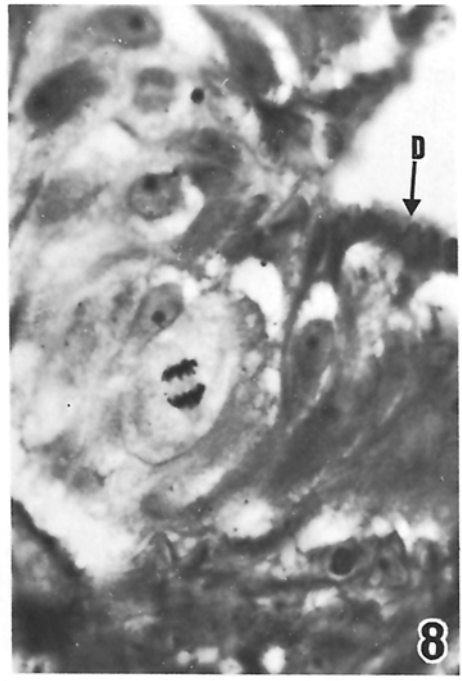
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resembled that of eels pretreated with SCT (third group): viz glandular hyperplasia, increased epithelial height, nearly-obliterated central cavity and enlarged capillaries (Fig. 7). The epithelium was formed essentially by enlarged secretory cells, whose mitotic activity was greatly increased. A stratified cell layer was formed by the degenerating cells along the luminal border (Fig. 8) as described by Robertson (1968) in *Rana pipiens*.

During previous experiments on eel maturation, in which eels were sacrified during treatment with carp pituitary extract, at different stages of the hypercalcemia, we had shown that the UB hyperactivity only began when the level of hypercalcemia was high (220 mg/l) and that this UB activity increased progressively in parallel with the serum calcium concentration. Therefore, the amount of CPE injected seemed to have no direct influence on the UB activity. Histological study of the bony tissues of these fish has indicated that the evolution of UB activity accompanied osteoclastic resorption (Lopez, 1973). Thus, a high hypercalcemia and a high rate of bone catabolism seemed to be necessary for stimulation of the UB.

Discussion

It was originally reported that mammalian calcitonin had no hypocalcemic effect on a teleost, *Fundulus heteroclitus* (Pang and Pickford, 1967). Subsequently, others have shown that calcitonin is effective in catfish, *Ictalurus melas* (Louw *et al.*, 1967) and in eels, *Anguilla japonica* (Chan *et al.*, 1968). Pang and Griffith (1971, unpublished data) have observed hypocalcemia and hypophosphatemia in fresh water-adapted *Anguilla rostrata*, injected with salmon calcitonin, but the same treatment was ineffective in sea water-adapted animals. The results reported in this paper, are inconsistent with these previously described findings, since we have shown a decrease of serum calcium level after injections of synthetic salmon calcitonin, in eels (*Anguilla anguilla* L.) maintained in sea water (Lopez *et al.*, in press) and a marked atrophy of the UB in these fish. This could be explained by a "feed-back" mechanism provoked by the injection of exogenous hormone. If we accept this hypothesis it is possible that salmon calcitonin has a non-specific biological activity in eels.

Fig. 5. Ultimobranchial gland from immature silver eel injected with synthetic salmon calcitonin, followed by carp pituitary extract until maturation. Note the increased epithelial height and the enlarged capillary network (CN). *S* secretory cells. *D* degenerating cells. (× 360 Cleveland-Wolfe Stain)

Fig. 6. Enlargement of part of Fig. 5 showing hypertrophied secretory epithelial cells containing large lightly staining nuclei (*N*). (× 800 P.A.S.)

Fig. 7. Ultimobranchial gland from immature silver eel injected with carp pituitary extract until complete maturation, illustrating the same glandular hyperplasia as in Figs. 5–6. Note the increased mitotic activity (*M*). (× 360 P.A.S.)

Fig. 8. Enlargement of part of Fig. 7. Note the numerous degenerating cells (*D*) at the apex. (× 800 P.A.S.)

In addition, we pointed out that suppression of endogenous CT in the eel by removal of the ultimobranchial body, brings about a significant increase of the serum calcium with a maximal response after two weeks. Subsequently, the serum calcium concentration returned to physiological levels. This rise of serum calcium had been shown to be due to demineralization of bone intercellular substance (Lopez *et al.*, in press) and also to a modification of the calcium flux across the gills. Preliminary results on measuring these fluxes on control and ultimobranchialectomized eels, *in vivo* by the method of Maetz (1958) and Motais (1967) and in isolated perfused gills (Shuttleworth, 1972) indicate that CT acts on influx and outflux to reduce serum calcium concentration. The regulation of the serum calcium level that occurred in the fifth week after removing the UB, was apparently due to active secretion of the Stannius corpuscles (Lopez *et al.*, in press) which are known to be hypocalcemic glands (Fontaine *et al.*, 1964 and Fontaine, 1967). Histological study of these glands indicates an active state as opposed to the results of Chan (1972) who maintained that the Stannius corpuscles of UBX eels were atrophic.

The endogenous CT also seems to be effective during the natural and experimental maturation experiments carried out in our laboratory. Histological study of the UB of eels sacrificed at different stages of maturation induced by CPE, has shown that the UB activity increased progressively with serum calcium. This hyperactivity of the glands was seen when the eels exhibited a high serum calcium level (from 220 mg/l). At this stage, the eels had already received 17 CPE injections of a total of 20 injections. These results seem to us to prove that the UB is stimulated by the serum calcium concentration and not by the pituitary hormones as Pang (1971) thought. Another experimental proof is the observed increase in UB activity (in the eel) following removal of the Stannius corpuscles which is accompanied by hypercalcemia (Lopez *et al.*, 1968).

Moreover, Robertson (1971) working on *Rana pipiens* and *Rana catesbeiana* noted that "the UB could be precociously induced to hyperactivity by a high calcium challenge" and Belanger (1971) referring to different studies on birds emphasized that "it seemed that the gland could respond to a prolonged hypercalcemic stress by hypertrophy and hyperplasia".

Furthermore, in mammals, the secretion of thyrocalcitonin was shown to be directly related to the blood calcium concentration (Care *et al.*, 1968; Cooper *et al.*, 1971). Milhaud and Moukhtar (1965) had pointed out that the adenohypophysis is not essential for the action of thyrocalcitonin in the rat.

It is evident that the effect of CT on serum calcium level in fish is difficult to study. Clark (1971) for example, testing calcitonin or ultimobranchial extracts from hogs, turtles and codfish in turtles, lizards and snakes, has obtained negative results. She thinks that "any change in blood calcium concentration induced by calcitonin might be masked by the normal calcium fluctuations". One might also suppose that since both the UB and Stannius corpuscles are hypocalcemic glands in teleosts, there may be a reciprocal interaction between them, with their different effects masking the expected decrease in serum calcium following CT injections.

However, the data summarized in this paper all seem to support the hypothesis – well established in other groups of vertebrates but disputed in fish – that

the ultimobranchial body in teleosts responds to physiological or experimental hypercalcemia, as well as to hypocalcemia induced by SCT injections.

References

Belanger, L.F.: The ultimobranchial gland of birds and the effect of nutritional variations. J. Exp. Zool. **178**, 125–138 (1971)

Care, A.D., Cooper, C.W., Duncan, T., Orimo, H.: A study of the thyrocalcitonin secretion by direct measurement of in vivo secretion rates in pigs. Endocrinology **83**, 161–169 (1968)

Chan, D.K.O.: Hormonal regulation of calcium balance in teleost fish. VIème Symposium international d'Endocrinologie comparée. Banff, Canada, Juin 1971. Gen. Comp. Endocr., Suppl. **3**, 411–420 (1972)

Chan, D.K.O., Chester-Jones, I., Smith, R.N.: The effect of mammalian calcitonin on the plasma levels of calcium and inorganic phosphate in the European eel (*Anguilla anguilla* L.). Gen. Comp. Endocrinol. **11**, 243–245 (1968)

Clark, N.B.: The ultimobranchial body of reptiles. J. Exp. Zool. **178**, 115–124 (1971)

Cooper, C.W., Deftos, L.J., Potts, Jr., J.T.: Direct measurement of in vivo secretion of pig thyrocalcitonin by radioimmunoassay. Endocrinology **88**, 747–754 (1971)

Copp, D.H., Cockroft, D.W., Kueh, Y.: Calcitonin from ultimobranchial glands of dogfish and chicken. Science **158**, 924–925 (1967)

Copp, D.H., Cockroft, D.W., Kueh, Y., Melville, M.: Calcitonin ultimobranchial hormone. In: Calcitonin (S. Taylor, ed.), pp. 306–321. New York: Springer-Verlag 1968

Deville, J., Lopez, E.: Le corps ultimobranchial du Saumon *Salmo salar* L. Etude histophysiologique à diverses étapes de son cycle vital en eau douce. Arch. Anat. Micros. **59**, 393–402 (1970)

Fontaine, M.: Corpuscles de Stannius et régulation ionique (Ca, K, Na) du milieu intérieur de l'Anguille (*Anguilla anguilla* L.). C.R. Acad. Sci. (Paris) **259**, 875–878 (1964)

Fontaine, M.: Intervention des corpuscles de Stannius dans l'équilibre phosphocalcique du milieu intérieur d'un poisson Téléostéen, l'Anguille. C.R. Acad. Sci. (Paris) **264**, 736–737 (1967)

Fontaine, M., Bertrand, E., Lopez, E., Callamand, O.: Sur la maturation des organes génitaux de l'Anguille femelle (*Anguilla anguilla* L.) et l'émission spontanée des œufs en aquarium. C.R. Acad. Sci. (Paris) **259**, 2907–2910 (1964)

Fontaine, M., Chartier-Baraduc, M.M., Deville, J., Lopez, E., Poncet, M.: Sur les variations de la calcémie observées chez *Salmo salar* L. à diverses étapes de son cycle vital. Leur déterminisme endocrinien et leur intervention éventuelle sur le comportement. C.R. Acad. Sci. (Paris) **268**, 1958–1961 (1969)

Fontaine, M., Deville, J., Lopez, E.: Ecology and regulation of calcium metabolism in Coral Grazer Parrotfish. Oceanography of the South Pacific 1972. Wellington: Comp. R. Fraser New-Zealand National Commission for UNESCO, p. 367–371, 1973a

Fontaine, M., Lopez, E., Peignoux-Deville, J.: Des relations existant entre l'activité de deux formations endocrines (corps ultimobranchial et corpuscules de Stannius), l'importance du squelette, la nature de la biocoenose et le régime alimentaire chez certains Scaridés (Pacifique et Méditerranée). C.R. Acad. Sci. (Paris) **276**, 2969–2972 (1973b)

Fontaine, M., Baud, C.A., Chartier-Baraduc, M.M., Deville, J., Lopez, E.: De quelques particularités du métabolisme du calcium chez les Scaridés des îles Gambier. Cahiers du Pacifique, 18, t. II, 579–584 (1974)

Kenny, A.D.: Introductory remarks. In: Calcium Parathyroid Hormone and the Calcitonins (R.V. Talmage and P.L. Munson, eds.), p. 9–11. Amsterdam: Excerpta Medica 1972

Lopez, E.: Etude morphologique et physiologique de l'os cellulaire des poissons Téléostéens. Thèse de Doctorat d'Etat (1972). Mémoires du Muséum national d'Histoire naturelle (nouvelle série). Série A. Zoologie, T. LXXX (1973)

Lopez, E., Deville, J., Bagot, E.: Etude histophysiologique du corps ultimobranchial d'un Téléostéen »*Anguilla anguilla*« au cours d'hypercalcémies expérimentales. C.R. Acad. Sci. (Paris) **267**, 1531–1534 (1968)

Lopez, E., Deville, J.: Effect of prolonged administration of synthetic salmon calcitonin (SCT) on vertebral bone morphology and on the ultimobranchial body (UB) activity of the mature female eel (*Anguilla anguilla* L.). Ninth European Symposium on Calcified Tissues in Baden near Vienna, October 1st to 6th (1972). Proceedings (eds. H. Czitober, J. Eschberger) 1973

Lopez, E., Peignoux-Deville, J., Lallier, F., Martelly, E., Milet, C.: Effect of calcitonin on calcium and bone metabolism in the eel *Anguilla anguilla* L. (in press)

Louw, G.N., Sutton, W.S., Kenny, A.D.: Action of thyrocalcitonin in the teleost fish *Ictalurus melas*. Nature **215**, 888–889 (1967)

Maetz, J.: Les échanges de sodium chez le poisson *Carassius auratus* L. Action d'un inhibiteur de l'anhydrase carbonique. J. Physiol. (Paris) **48**, 1085–1099 (1956)

Milhaud, G., Moukhtar, M.S.: Hypophysectomie et thyrocalcitonine. C.R. Acad. Sci. (Paris) **260**, 3179–3182 (1965)

Motais, R.: Les mécanismes d'échanges ioniques branchiaux chez les Téléostéens. Leur rôle dans l'osmorégulation. Annales de l'Institut océanographique, **45** fasc. 1, 1–84 (1967)

Pang, P.K.T.: Calcitonin and ultimobranchial glands in fishes. J. Exp. Zool. **178**, 89–100 (1971)

Pang, P.K.T., Pickford, G.E.: Failure of hog thyrocalcitonin to elicit hypocalcemia in the teleost fish *Fundulus heteroclitus*. *Comp. Biochem. Physiol.* **21**, 573–578 (1967)

Pang, P.K.T., Griffith, R.W.: Unpublished data in: Calcitonin and ultimobranchial glands in fishes. P.K.T. Pang. Symposium. J. Exp. Zool. **178**, 89–100 (1971)

Robertson, D.R.: The ultimobranchial body in *Rana pipiens*. IV. Hypercalcemia and glandular hypertrophy. Z. Zellforsch. **85**, 441–452 (1968)

Robertson, D.R.: Endocrinology of Amphibian ultimobranchial glands. J. Exp. Zool. **178**, 101–114 (1971)

Tchernavin, V.: Changes in the salmon skull. Trans. Zool. Soc., London **24**, 104–157 (1938–1940)

Urist, M.R.: Avian parathyroid physiology including a special comment on calcitonin. Amer. Zool. **7**, 883–895 (1967)

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