Ultimobranchial calcitonin of the eel,
Anguilla japonica

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Synopsis

Calcitonin activity was demonstrated in the ultimobranchial gland of *Anguilla japonica*, the Japanese eel, with a wide distribution over an area involving the esophagus and pericardium. Total calcitonin content was approximately 10–40 MRC u/kg body weight. Purification of eel calcitonin through gel filtration on Sephadex G-75 and SE Sephadex G-75 yielded a preparation with a potency of 1360 MRC u/mg. While this preparation is already more potent than porcine and other mammalian calcitonin, further purification is indicated.

Calcitonin has been extracted from the ultimobranchial body (UBB) of various species of lower vertebrates (Copp *et al.*, 1970). The recent discovery of Copp *et al.*, (1970) of salmon calcitonin with a potent biological activity has prompted us to study the calcitonin activity in the UBB of various fishes. In the course of such studies, we have incidentally found a substantial amount of calcitonin in the UBB of the Japanese eel, *Anguilla japonica*. Since the cultured eel is available in large quantities in Japan, we have attempted to purify eel calcitonin from the UBB of this species.

Materials and Methods

In *Anguilla Japonica*, UBB is usually located as an unpaired organ with a rather wide distribution between the esophagus and the pericardium, Part of the UBB is also found within the pericardium and esophagus.

In an attempt to establish the extent of the distribution of calcitonin activity, the esophagus and pericardium were cut into small pieces of 5 mm thickness

respectively and calcitonin was extracted from each piece with 0.1 N HCl. Subsequently the hypocalcemic activities of each extract in young rats were measured according to the method of Cooper *et al.* (1967). After establishing the distribution of calcitonin activity, esophagi and pericardia were collected from 6,000 eels and the purification of calcitonin was performed according to the following procedure (Table 1) (Gudmundsson *et al.*, 1968). Three hundred gram desiccated and defatted UBB was extracted with 12 litre of butanol, pyridine, acetic acid, water mixture (21:12:2:15) for 24 hr and the extract was dried under the negative pressure at 50°. The concentrate was fractionated with water and ether, freeze dried and was further fractionated by gel filtration on Sephadex G-75. 2.1 gm of crude extract, dissolved in 50 ml of 0.2 M ammonium acetate, was applied to 7.5 × 90 cm Sephadex G-75 column and eluted with the same ammonium acetate buffer. The most active fraction thus obtained was further applied to 1.2 × 20 cm SE Sephadex G-50 column, eluted with 0.1 M ammonium formate and further purified by analytical gel electrophoresis containing 15% w/v polyacrylamide.

Results

As shown in Figure 1, most of the calcitonin activities were demonstrated at the shaded area of the esophagus and pericardium. Total

Fig. 1. Distribution of calcitonin activity in the ultimobranchial body of *Anguilla Japonica*. The esophagus and pericardium were cut into small pieces of 5 mm thickness respectively and the hypocalcemic activity in rat of each piece was compared. Each bar represents the mean of 5 assay rats and the vertical bars represent the standard error.

calcitonin content in the UBB was approximately 10–20 MRC u/gland or 40 MRC u/kg b.w, suggesting the highest activity per body weight ever reported (Copp *et al.*, 1968). The elution pattern of eel calcitonin from gel filtration on Sephadex G-75 was shown in Figure 2. The most active fraction contained the biological activity of 415 MRC u/mg. This active fraction was further fractionated by SE Sephadex G-75 and the active fraction containing 1360 MRC u/mg was obtained. This active fraction was further purified by analytical gel electrophoresis. However, it is clear that the preparation is still

Table 1. Purification of eel calcitonin.

Acetone powder, 300g (800MRC mU/mg)	
extracted with BuOH, Pyridine ACOH, H ₂ O (21: 12: 2: 15 W/v)	
Filtrate	Residue
	condensed in vacuo at 50°C
Residue	extracted with H ₂ O
Extract	extracted with ether
Water layer	lyophilized
Residue, 6.3g (34MRC U/mg)	Sephadex G-75
Active fraction, 53 mg (415MRC U/mg)	SE Sephadex
Highly purified calcitonin, (1360 MRC Clmg)	

heterogenous and further purification was required.

Discussion

Calcitonin has been extracted from the thyroid gland of various mammals including porcine, bovine, ovine, rat and human and their chemical structures have subsequently been elucidated. Recently, Copp *et al.* (1970) found a potent calcitonin in the salmon ultimobranchial body and its chemical structure was determined by Niall *et al.* (1969). Of particular interest is the fact that salmon calcitonin has a more potent specific activity and more prolonged hypocalcemic action than the other calcitonins. It was also found that this prolonged hypocalcemic action of salmon calcitonin was due to its resistance to *in vivo* enzymatic inactivation (Copp *et al.*, 1970). Although eel calcitonin is not yet completely purified, the specific activity of the most active Sephadex fraction is 1360 MRC u/mg, which is apparently more potent than the other mammalian calcitonins. Since the specific activity of the purified salmon calcitonin is

Fig. 2. The elution pattern of eel calcitonin from gel filtration on Sephadex G-75. The upper insert indicates the specific biological activity per mg and the lower insert indicates the total activity per tubes. Sephadex G-75 column 7.5×90 cm, solvent 0.2 M AcNH_4 (pH 4.7).

Fig. 3. Fractionation of Sephadex G-75 fraction of eel calcitonin by 15% w/v polyacrylamide gel electrophoresis. The activity was demonstrated on the top band.

2400–2700 MRC u/mg (Keutmann *et al.*, 1971), the specific activity of eel calcitonin may be similar to or greater than that of salmon calcitonin. According to our preliminary experiment, eel calcitonin was found to be more stable to inactivation than porcine or salmon calcitonins, when incubated with human plasma.

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