Hormonal control of Ca homeostasis in lower vertebrates: Considering the evolution

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[REVIEW]

Hormonal Control of Ca Homeostasis in Lower Vertebrates: Considering the Evolution

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ABSTRACT—Early vertebrates may have had genes that would later produce hormones concerning Ca homeostasis in advanced vertebrates. These genes may possibly have expressed the respective product for the local control of Ca in some tissues, which must be a paracrine control. When vertebrates acquired the jaw, however, delicate but systemic Ca control system may have been needed to cope with fluctuations in blood Ca levels resulting from the digestion of food. Furthermore, when vertebrates were transferred from freshwater to seawater or to land, new control systems must have been required. Therefore, such vertebrates might have coped with two ways in their evolution. One is the increase of the number of local production sites of hormones, for the delicate control of Ca in various tissues, which must be the paracrine control. The other is the creation of new glands for the systemic control of Ca homeostasis by making paracrine cells independent from tissues. This style must be an endocrine control. Therefore, in modern vertebrates, in various tissues, calcitonin, stanniocalcin, parathyroid hormone-related protein, and maybe also prolactin, or at least substances immunoreactive to antibodies against these hormones, are expressed locally as paracrine tissues. At the same time, these hormones or similar molecules are also produced in special endocrine glands such as the ultimobranchial glands, Stannius corpuscles, the parathyroid glands and the pituitary gland, respectively. This supposition is summarized in Fig. 1.

Various circumstances in various animal groups

Blood Ca levels are controlled by some endocrine glands in vertebrates

Endocrine glands concerning Ca homeostasis in vertebrates are cited in Fig. 2. It is well known that in fish, the pituitary gland raises blood Ca levels (Olivereau and Olivereau, 1978). Although hagfish, the most primitive fish in modern times, is found only in the sea, some lamprey live in freshwater, while others spend at least the initial stages of their life in freshwater. These jaw-less fishes are relatives of placoderms the first vertebrates, which are now extinct. Hagfish and lampreys have the pituitary gland; thus, the placoderm must also have had the pituitary gland. Placoderms lived in freshwater such as estuary waters and lakes (Colbert and Morales, 1991). Therefore, the risk of decline of their blood Ca levels was possible. Taking these circumstances into consideration, I would like to propose the idea that the first endocrine gland concerning Ca homeostasis is the pituitary gland, for whose mechanism the hormone, prolactin is responsible.

In vertebrates other than fish, the endocrine gland responsible for raising blood Ca levels is the parathyroid gland, which secretes the parathyroid hormone. In some primitive species or in larval stages inhabited freshwater, it is the pituitary gland that is responsible, the same as in fish (Oguro *et al.*, 1978; Sasayama and Oguro, 1982). Although the active type of vitamin D functions to raise blood Ca levels in most vertebrates, it will not be mentioned in this review.

On the other hand, jawed fishes, amphibians, reptiles, birds and mammals use the ultimobranchial gland that secretes calcitonin, as the endocrine organ to lower blood Ca levels. In mammals, the ultimobranchial gland is separated from the original position and broken up during the processes of development of the embryo and then incorporated into the thyroid gland. Former ultimobranchial cells are called C-cells.

In amia, garpike and teleosts only, another endocrine gland named the Stannius corpuscle is present. This organ secretes stanniocalcin and suppresses excessive rises of blood Ca levels as well. Therefore, these fishes have dual hypocalcemic systems, since the ultimobranchial gland and the Stannius corpuscle are present.

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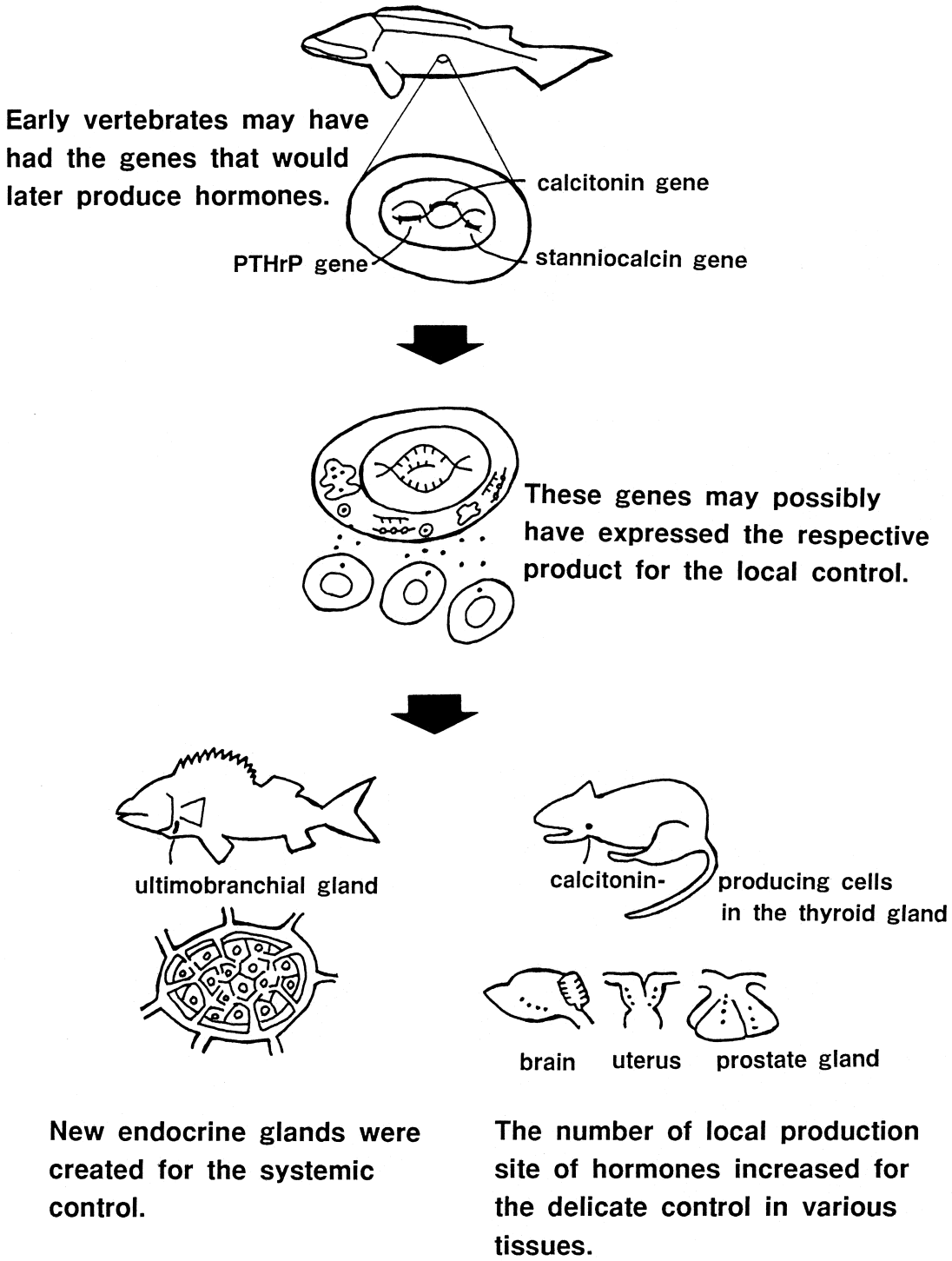

Fig. 1. A hypothesis that explains the reason why the same hormone is expressed in paracrine tissues and endocrine glands. The location of genes of those hormones does not exhibit real sites. Detailed explanation is described in the abstract of this review article.

Although jaw-less fishes have no endocrine glands except for the pituitary gland for the control of blood Ca levels, their Ca levels are kept constant

Modern hagfish only inhabit the sea. The blood Ca level of the hagfish, *Eptatretus stoutii* is 22 mg/100 ml, which is almost half of the value of seawater Ca levels (Urist, 1963). Namely, the blood Ca levels of the hagfish are controlled at lower levels than that of seawater, although the mechanisms have not been clarified yet. Jaw-less fishes do not have ultimobranchial glands, Stannius corpuscles, or parathyroid glands. When vertebrates acquired a jaw, they must have faced cyclic changes in blood Ca levels, since bites captured by the jaw are digested after being stored in the stomach. Therefore, too large amplitudes in blood Ca levels must not

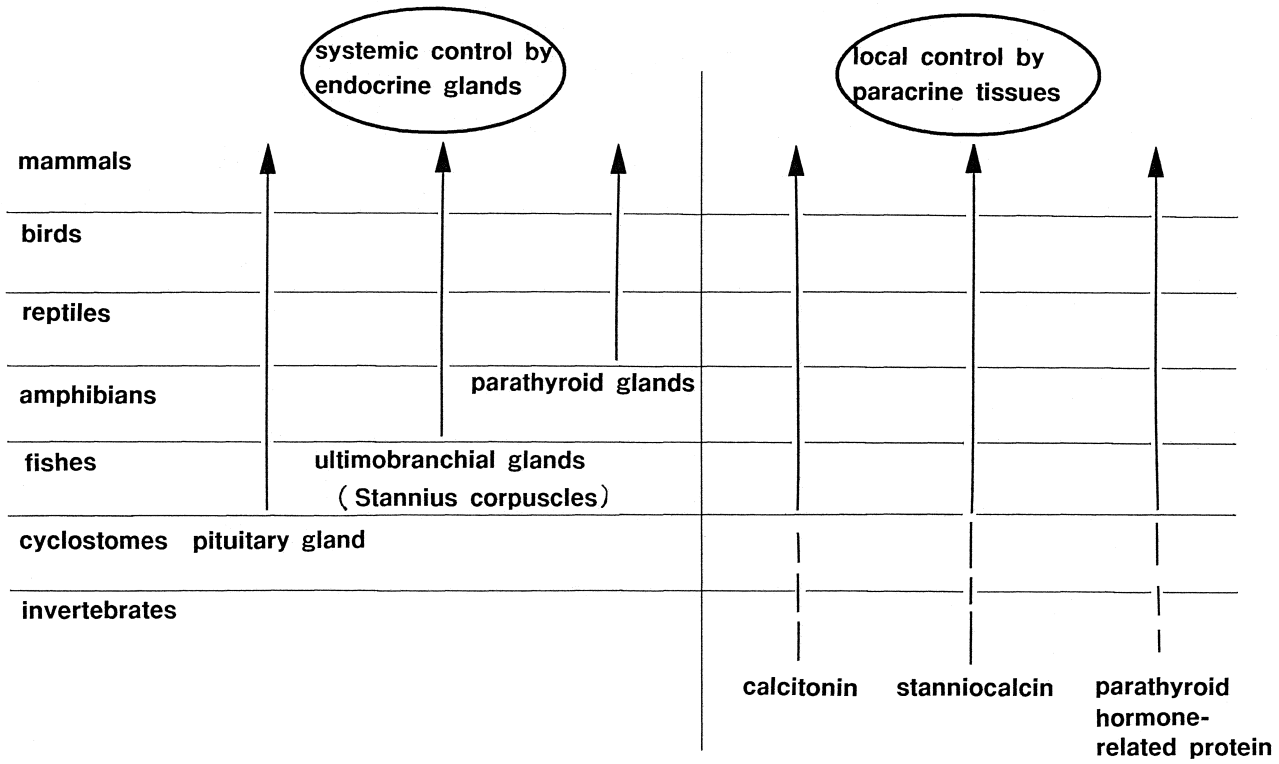

Fig. 2. The endocrine glands and the hormones involved in Ca homeostasis in vertebrates. The oldest endocrine gland is the pituitary gland, which raises blood Ca levels. All vertebrates have this gland. The older endocrine gland is the ultimobranchial gland, which lowers blood Ca levels. Jawed vertebrates have this gland. In mammals, however, the ultimobranchial gland breaks up on the processes of embryogenesis, and the parenchymal cells are incorporated into the thyroid gland as C-cells. It is only in bony fishes such as amia, garpike, and teleosts that Stannius corpuscles, which suppress the increases of blood Ca levels when exposed to a high Ca environment, are present. Hormones secreted from the ultimobranchial glands and Stannius corpuscles are calcitonin and stanniocalcin, respectively. Vertebrates other than fishes have the parathyroid gland, which raises blood Ca levels. In mammals, the parathyroid hormone-related protein has various functions. In lower vertebrates as well, this molecule may have similar functions. It may be possible to trace these hormones to invertebrates at cellular levels.

have been desirable in order to maintain blood Ca levels constant. Some organs besides the pituitary gland, such as the ultimobranchial gland, might have been necessary. In contrast, jaw-less fishes are fundamentally filtration-feeders. As they have no stomach, there may not be clear amplitudes in blood Ca levels. These circumstances might be one of the reasons why, other than the pituitary gland, no endocrine glands for controlling blood Ca levels have develop in jaw-less fishes. As mentioned later, even in these fishes, hormones concerning Ca homeostasis in jawed vertebrates may be produced at cellular levels (Fig. 2). In the brain of hagfish and lampreys, calcitonin-immunoreactive cells are present (Sasayama *et al*., 1991b).

In the Atlantic hagfish (*Myxine glutinosa*), blood and urine Ca levels were monitored after administration of salmon calcitonin (Wales, 1988). In both Ca levels, however, there was no response. On the other hand, when plasma of the Pacific hagfish (*Eptatretus burgeri*) was fractionated by high pressure liquid chromatography, a substance having an elution time similar to that of salmon calcitonin was found. When the fraction was administered to rats, serum Ca levels fell as salmon calcitonin caused (Suzuki, 1995). However, the true character of this substance in the fraction is not clear at present.

Blood Ca levels of the lamprey (*Petromyson marinus*) are maintained around 10 mg/100 ml during freshwater life, in spite of 4 mg/100 ml Ca levels in environmental water (Urist, 1963). These blood levels are almost the same as those of jawed vertebrates. Therefore, the pituitary gland must control blood Ca levels, as it does in teleosts. In the anadromous lamprey, a prolactin-like substance that reacts to the anti-mammalian prolactin antiserum is contained in the brain and pituitary gland (Write, 1984; 1986). Furthermore, immunoreactivity to the antiserum to parathyroid hormone-related protein (PTHrP) is detected in their skin (Danks *et al*., 1998). It is suggested that this PTHrP may be concerned with rises of blood Ca levels in lampreys as well. The PTHrP is will be mentioned later.

Blood Ca levels may be controlled in invertebrates

As the blood Ca levels of marine invertebrates are 35–40 mg/100 ml or 40–50 mg/100 ml, these levels appear to be almost the same as seawater levels (35–40 mg/100 ml) or somewhat higher than those. In the marine shrimp (*Palaemon serratus*), Ca levels in the hemolymph increase to about 60 mg/100 ml just before the ecdysis and decrease to 48 mg/100 ml after that. Interestingly, this shrimp has a calcitonin-like substance in the hemolymph. There is a reverse-relationship

between the substance level and the Ca level (Arlot-Bonnemains *et al.*, 1986). The terrestrial crustacean *Orchestia cavinama* has a calcitonin-like substance as well (Graf *et al.*, 1989). This substance changes quantitatively with the molting cycle, attaining its maximum level at the ecdysis. Furthermore, in the lobster from Norway, another calcitonin-like substance is found in the digestive tract and the hepato-pancreas (Fouchereau-Peron *et al.*, 1987). The molecular size is 4,500 Da, which is larger than the 3,500 Da of vertebrate calcitonins. In a binding assay, however, this molecule competes with salmon calcitonin. In the marine blue-crab (*Callinectes sapidus*), a protein of 27 kDa is present in the hemolymph, whose amino acid composition resembles that of the human pro-calcitonin (Cameron and Thomas, 1992). Just before the ecdysis, levels of the protein in the hemolymph reach their maximum. Cameron and Thomas (1992) suggest that, when it works, this substance achieves a similar size to that of vertebrate calcitonin. Immunoreactivity to this substance is the highest in the hepato-pancreas. Thus, although various calcitonin-like substances are found in crustaceans, nobody has succeeded so far in clarifying their primary structure.

On the other hand, there have been a few reports about the blood Ca levels of terrestrial invertebrates. In the past, we examined the Ca levels of the blood and coelomic fluid of a common earthworm (*Pheretima communissima*) (Oguro *et al.*, 1984). Those levels were 36–56 mg/100 ml and 16–22 mg/100 ml, respectively. Blood levels were similar to those of crustaceans. Dose this value reflect the Ca levels of seawater even in the earthworm, or does it reflect the value of soil that was swallowed? It may be interesting to examine why the coelomic Ca level has to be maintained on half of the value of the blood Ca level.

Taking these facts into consideration, it may not be an overstatement to conclude that also in invertebrates, blood Ca levels are controlled by some unknown mechanisms.

Interestingly, the unicellular ciliate, *Tetrahymena* has a calcitonin-like substance in its acid extracts (Deftos *et al.*, 1985). If this corresponds to the definition of vertebrate calcitonin, the origin of this molecule may be extremely old.

Control mechanisms of blood Ca levels in teleosts

Ca is mobilized not only from scales but also from bones

In most teleosts, osteocytes disappear from bones in adults. Such bones, which grow by accumulation of Ca on the surface, are called acellular bones (Simmons 1971; Urist 1976). Therefore, it has been thought that scales are storage sites, and that Ca is put in and mobilized for some physiological use from the scales (Dacke 1979; Simmons 1971). Recent research in tilapia, however, has made clear that mononuclear cells, which appear to be flat cells for the purpose of covering the bone surface, are present on the surface of acellular bones. These cells are activated by unknown factors, and become to osteoclastic cells, (Witten *et al.*, 1997). Furthermore, those activated cells aggregate and become large polynuclear cells resembling the osteoclasts of mammals. Therefore, it is possible to take Ca out from such acellular bones.

In the bones and scales of rainbow trout, the mRNA of the estrogen receptor has been detected (Armour *et al.*, 1997). When estrogen acts on the scales, Ca contents in the scale decrease, while Ca in the bone increases (Armour *et al.*, 1997). It seems that calcitonin is involved in this phenomenon, because estrogen receptors are present in the ultimobranchial gland of goldfish (N. Suzuki, personal communication) and of stingray as well (Yamamoto *et al.*, 1996), although the exact relationship has not been clarified yet. It is known at this point that in the female salmon, blood calcitonin levels increase with maturation, and then blood Ca levels increase as well (Watts *et al.*, 1975). This phenomenon may be explained by studying the estrogen receptor in the future, which may lead to the clarification of the development of osteoporosis in human.

Freshwater teleosts take Ca in the body from environmental water by prolactin of the pituitary gland

Prolactin increases the number of chloride cells in the gill and takes Ca in the body by activating Ca-ATPase in the chloride cell (Flick *et al.*, 1986; 1993; 1994). Therefore, the content of Ca in diet is not really important for the Ca homeostasis of freshwater fishes.

Calcitonin secreted from ultimobranchial glands suppresses excessive increases of blood Ca levels after feeding

In teleosts, ultimobranchial glands are present on the abdominal surface of the esophagus, and spread on the septum separating the pericardium and viscera (Sasayama *et al.*, 1999). In lungfish, however, the ultimobranchial gland has a close relationship with blood vessels advancing forward from the heart (Shinohara-Ohtani and Sasayama, 1998). The location of the ultimobranchial gland in urodele amphibians is similar to that in lungfish.

I would like to propose the idea that the primary role of the ultimobranchial gland is that of adjusting Ca in the inside of the body. Even in freshwater teleosts, transitory but excessive rises of blood Ca levels may take place after feeding. This is a severe problem that affects homeostasis in blood Ca levels. Recently, we carried out the following experiments. We divided freshwater eels into 2 groups, one of which was fed and the other group starved. One week later, plasma calcitonin and Ca levels were compared between those 2 groups. In the group that had been fed, both levels were significantly higher than those in the starved group (Sasayama *et al.*, 1996). In another experiment, we infused a high Ca-consommé solution into the digestive tract of goldfish and examined plasma Ca and calcitonin levels 1, 2, and 3 hrs later. Plasma Ca levels increased significantly, and plasma calcitonin levels tended to increase with the passing of time (Sasayama *et al.*, 1996). Moreover, we infused a high Ca-consommé solution into the digestive tract of freshwater eels and again examined plasma Ca and calcitonin levels from 0.5 to 5 hr. Both levels were significantly higher in the high Ca-consommé group than in the normal consommé group (Suzuki *et al.*, 1999). These results all suggest the possibility that calcitonin

was secreted against rises of blood Ca levels. In teleosts, however, it is not clear how calcitonin functions to suppress the rise. Calcitonin may suppress either the activity of osteoclastic cells as in mammals or the absorption of Ca from the digestive tract.

As well as in marine teleosts, calcitonin may function in freshwater fishes, as their blood Ca levels are not very different from those of freshwater teleosts. Among marine teleosts, some species eat algae growing on the surface of corals. As those fishes have a beak similar to that of parrots and scrape algae off by it, they are called parrotfish. These fishes eat Ca-rich corals together with algae. Therefore, it has been thought that the ultimobranchial gland of these fishes must be always activated at all times to prevent excessive rises of blood Ca levels (Dacke, 1979). However, we could not find any differences from usual marine teleosts in the ultimobranchial gland by immunohistological analyses (Sasayama *et al.*, 1999). This led us to conclude that their ultimobranchial glands may have enough activity.

Calcitonin of the primitive bony fish is conserved from fishes to birds

Calcitonin is composed of 32 amino acid residues in all jawed vertebrates, and amino acid residues located in position 1 and 7 are bound by S-S of cystein. We have so far clarified the primary structure of the calcitonin of the stingray, goldfish, sardine and bullfrog by the purification technique of peptides (Takei *et al.*, 1991; Sasayama *et al.*, 1992 and 1993; Suzuki *et al.*, 1994; Yoshida *et al.*, 1997). We have also reported the nucleotide sequence of the calcitonin of 4 species of primitive bony fishes, 6 species of amphibians, and 4 species of reptiles by PCR, RT-PCR and RACE-PCR methods (Suzuki *et al.*, 1997 and 1999; Sakamoto *et al.*, 1999). These studies have demonstrated that calcitonin of primitive bony fish such as lungfish has been conserved from fishes to reptiles and that reptile calcitonin is almost the same as that of chicken. However, mammalian calcitonin is largely different from that of lower vertebrates. The reason for this difference between mammals and non-mammals has not been clarified, as it is not so easy to find common points in the hard tissues of fishes and birds. Then, I would like to propose that the discrepancy may be due to the difference in the contents of the yolk in the egg. Usually, yolk includes a large amount of Ca. Non-mammals lay large-sized eggs. In contrast, mammalian eggs are poor in yolk and Ca. This difference might be reflected in the structure of calcitonin, if calcitonin were involved in the processes of Ca accumulation in the egg. It has been reported that during the egg maturation of salmon, blood calcitonin levels rise (Watts *et al.*, 1975).

The presence of Stannius corpuscles is limited to some groups in bony fishes

Only amia, garpike and teleosts have stanniocalcin- secreting Stannius corpuscles near the ureter of the latter half of the kidney. This hormone is composed of 231 amino acid residues in the Australian eels (*Anguilla australis*) (Butkus *et al.*, 1987) and 233 amino acid residues in salmon (*Onchorhynchus kisutch*) (Wagner, 1994).

In tilapia, stanniocalcin suppresses the excessive influx of Ca across the chloride cell of the gill (Flick *et al.*, 1993). Similar effects have been reported also in eel (Milet *et al.*, 1979) and rainbow trout (Lafeber *et al.*, 1988). Furthermore, this hormone suppresses the absorption of Ca in the intestine of cod (Sundell *et al.*, 1992), and accelerates the reabsorption rates of inorganic phosphate (Pi) in the kidney of flounder (Lu *et al.*, 1994). In flounder, stanniocalcin promotes the accumulation of Ca into hard tissues, followed by decreases in blood Ca levels. Thus, these fish groups have a dual hypocalcemic system, as both of the ultimobranchial glands and the Stannius corpuscles are present. The physiological meaning of this fact may be that the Stannius corpuscle roughly controls blood Ca levels in marine fishes, while the ultimobranchial gland is later responsible for a finer level control (Milet *et al.*, 1979). However, it seems to me that the Stannius corpuscle was an endocrine gland completed for some groups of bony fishes to advance to the sea. Therefore, I would like to regard the primary role of this organ as that of suppressing the invasion of Ca from seawater.

Why are Stannius corpuscles not present in other fish groups?

Phylogenetically, old bony fishes such as lungfish and sturgeons have no Stannius corpuscles. According to recent research, various species of early lungfish inhabited shallow seawater as well, although modern lungfish all live in freshwater (Janvier, 1996). Then, I think that lungfish could not advance greatly into the sea because their lungs, as well as their gills, were already very important organs for respiration. Although modern coelacanth inhabited the sea, the function of their lungs has been completely lost, adipose tissue having entirely taken the place of this organ. It could also be said that, as coelacanth lost the function of the lungs, they might have advanced into the sea. Therefore, amphibians, being close descendants of these primitive bony fishes, have no Stannius corpuscles.

On the other hand, the function of the lungs of amia and garpike is changing from one of respiration to one of balancing the body by acting an air bladder in water (Colbert and Morales, 1991). The ancestors of these fishes advanced into the sea with such lungs. Teleosts diverged from these ancestors, changing their lungs completely to air bladders and thus conquering the sea (Urist, 1976). Then, Stannius corpuscles must have been necessary for them to adapt the seawater. Modern freshwater teleosts have this endocrine gland, as they are descendants of teleosts which diverged into the sea. In freshwater fishes such as salmon which make a round trip between the rivers and the sea, the parenchymal cells of this gland maintain their sensibility to Ca even in freshwater life (Wagner *et al.*, 1998).

In cartilaginous fishes, Stannius corpuscles are also absent. Although this fish group initially lived in freshwater, with the reduction of the freshwater region as a result of the dry weather in the Devonian Period, they advanced into the

sea by acquiring the ureosmotic control system. Therefore, they might not have needed to differentiate Stannius corpuscles.

Various circumstances in vertebrates other than fishes

Ca homeostasis is specific in tadpoles

While tadpoles eat organic substances such as withered leaves, frogs are carnivorous. Consequently, the digestive tract of tadpoles must be remodeled, which means they can eat nothing during metamorphosis. Tadpoles change their gene expression pattern from the larval type to the adult type in the liver and produce various enzymes necessary for adult stages (Yoshizato, 1989). Therefore, amino acid residues arisen from larval tissues by the decomposition are re-utilized for the composition of adult tissues.

In view of this starvation period, then, from where do they get the necessary Ca for the formation of a skeleton? There are no bones in the tail. Tadpoles accumulate Ca in paravertebral lime sacs that enlarge into newly formed vertebral bones (Guardabassi, 1960). The lime sac is a kind of aggregate composed of many small bags formed by thin single- layer membrane. The origin of the lime sac is the endolymphatic sac of the auditory bulla (Whiteside, 1922).

In the past, we infused high Ca solution into the digestive tract of bullfrog tadpoles and determined Ca levels in the blood collected from the portal hepatic vine. Blood Ca levels were naturally high just after the infusion. At 3 hr after, however, Ca levels in the arterial blood, which had circulated many times in the body, were significantly lower than those determined just after the infusion. Subsequently, we repeated the same experiment in the ultimobranchialectomized tadpoles. This time, no significant differences were observed even after 3 hr. This result suggests that calcitonin secreted from the ultimobranchial gland deposited Ca somewhere in the body. Then, we performed the same experiment using ^{45}Ca. At 24 hr after, although radioactivity of the lime sac in the sham-operated tadpoles was extremely high, the level in the ultimobranchialectomized tadpoles was incomparably low (Sasayama and Oguro, 1985). However, Ca in the lime sac is calcium carbonate, not calcium phosphate as in bones. Can calcitonin act on calcium compounds without distinction? Then, from where does phosphate come, which is one of the important minerals for the formation of bones?

Parathyroid glands were first formed phylogenetically in amphibians

Metamorphosed amphibians can not take Ca into the body directly from environmental water, as they do not have gills. Therefore, terrestrial vertebrates turned to depositing Ca into hard tissues and mobilizing Ca into blood when necessary. Osteoclasts, which play the role of mobilizing Ca from bones, differentiate from the stem cell in the bone marrow. Kurokawa (1996) suggested that the phylogenetic maturation of the osteoclast made it possible for vertebrates to land. Then, parathyroid glands were formed as an endocrine organ to activate the osteoclast (Fig. 3). The origin of parenchymal cells of this gland is common to that of the epithelial cell of the gill (see, Srivastav *et al*., 1995a). Why do parathyroid glands differentiate from the branchial epithelium? Does it suggest a trace of the exchange of Ca between the body and environmental water? Or is the location of the parathyroid gland advantageous for monitoring blood Ca levels before blood Ca levels are modified in the periphery?

Parathyroid hormones in mammals and chicken are composed of 84 and 88 amino acid residues, respectively (Khosla *et al*., 1988). In other vertebrates, the primary structure of this hormone remains to be clarified. The active portion of the mammalian parathyroid hormone is 1–34 amino acid residues. When the activity of the same portion of the chicken hormone was compared to that of mammals, the binding activity, the potency of activating adenylate cyclase, and the relaxing activity of smooth muscle were all lower in the chicken hormone than in the mammalian hormone (Caulfield *et al.*, 1988). It is supposed that this is due to the difference in the binding site for the receptor.

The relationship between the parathyroid hormone and the receptor has not been completed in the primitive salamander

Oguro (1973) performed an interesting experiment using the American giant salamander, the most primitive species in amphibians. Even after their growth is completed, the gill crafts of this salamander are not closed as in fishes. Nevertheless, parathyroid glands are formed. For the experiment, the salamander's parathyroid glands were sectomized from the body. However, serum Ca levels did not change. Therefore, it was thought that in this species, the parathyroid gland does not produce the hormone. However, this was proved incorrect later on. When the parathyroid extract from the giant salamander was administrated to newts whose serum Ca a levels had been declined by parathyroidectomy, the serum Ca levels of the newts increased (Oguro and Uchiyama, 1975). Therefore, at least in this example, the phylogenetic maturation of the receptor that recognizes a new hormone must be retarded (Oguro *et al*., 1978).

Functions of the parathyroid gland have been gradually completed in lower vertebrates

In mammals, the parathyroid hormone accelerates the release of Ca from bones by activating osteoclasts, followed by increases of blood Ca levels. In the kidney, this hormone promotes the reabsorption of Ca and the excretion of Pi.

Parathyroidectomy in bullfrog tadpoles causes significant hypocalcemia, as seen in adult frogs (Sasayama and Oguro, 1975). In various amphibians and reptiles, except for primitive salamanders and turtles, it causes severe declines of blood Ca levels and even brings about tetanic convulsions (Srivastav *et al*., 1995a, b).

Target organs for the parathyroid hormone are mainly the bones and kidney in lower vertebrates as well. In parathyroidectomized frogs, the mobilization of Ca from the aforementioned paravertebral lime sacs becomes impossible (Robertson, 1972). As the contents of Ca and Pi in the bone

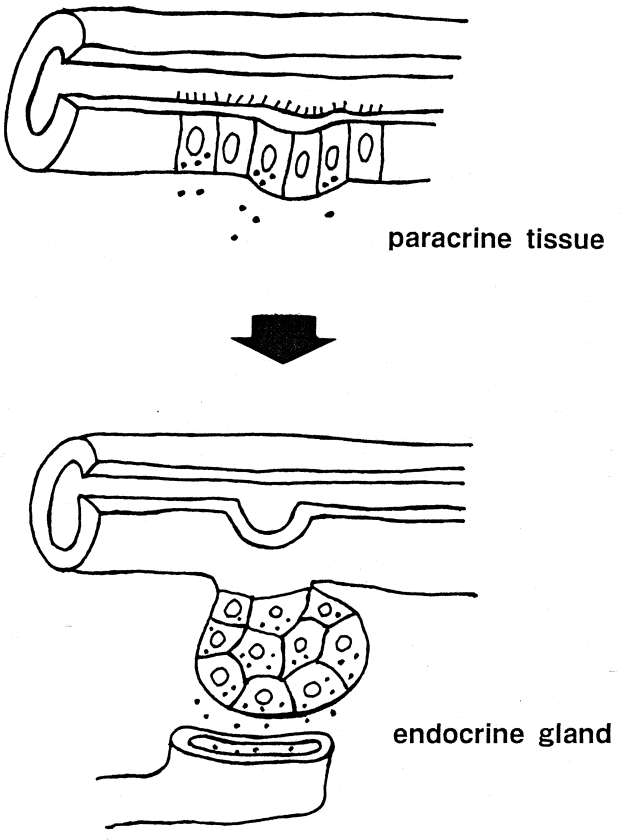

Fig. 3. A figure showing the process from the secretion of hormones at cellular levels to that at glandular levels. It has been demonstrated that ultimobranchial glands, parathyroid glands, maybe one part of the pituitary gland as well, and Stannius corpuscles are originated from tubular tissues such as the digestive tract and uriniferous tubules, respectively. It is supposed that hormone-secreting cells had originally been intermingled among the epithelial cells of those tubes and that those cells released their products to adjacent tissues or blood vessels. However, when their products became useful for not only local control but also systemic control, the proliferation of those cells accelerated. After all, the part responsible for the production of the substance became independent from each tube and was established as an endocrine gland.

increase in the parathyroidectomized snakes, bones become one of the target organs in reptiles (Singn and Kar, 1983). Although the excretion rate of Pi in the kidney is decreased in parathyroidectomized frogs, the reabsorption rate of Ca is not affected (Sasayama and Clark, 1984). Similar effects on the kidney have been obtained in some reptiles as well (Clark and Laverty, 1985). Therefore, it seems that the acceleration of the reabsorption of Ca in the kidney by the parathyroid hormone was acquired at least in birds (Clark and Sasayama, 1981).

Physiological demands for Ca homeostasis

Endocrine glands were evolved from paracrine tissues

In the central nervous system of vertebrates, calcitonin-immunoreactive cells are present. When calcitonin is administered to the brain of mammals, suppression of appetite and of feelings of pain is observed (see, Azria, 1989).

Calcitonin-immunoreactive cells are present in the nervous system in various invertebrates as well (Sasayama *et al.*, 1991a). In annelida and arthropoda, those cells are located bilaterally in the central nervous system, which suggests some physiological roles. Calcitonin-immunoreactive cells are also found in the abdominal ganglion of sea-hares (*Aplysia kurodai*). As salmon calcitonin ejected by a micro-pressure-apparatus caused marked hyperpolarization specifically in some ganglion cells, the calcitonin-like substance may function as a neuromodulator in this ganglion (Sawada *et al.*, 1993).

In goldfish, calcitonin-immunoreactive cells are also present in a certain area of the small intestine. Those cells produce the same calcitonin molecule as the ultimobranchial gland does (Okuda *et al.*, 1999). It is supposed that these

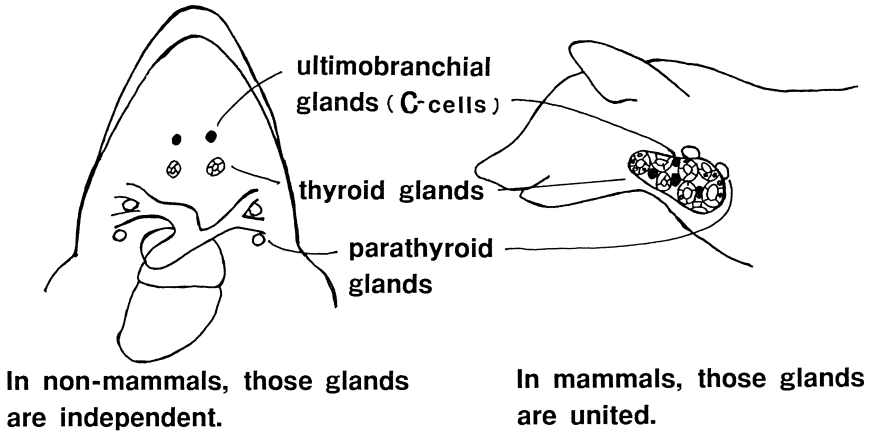

Fig. 4. A figure showing the union of the ultimobranchial glands, the parathyroid glands and the thyroid gland into a single gland in mammals. The ultimobranchial glands and parathyroid glands are originated from the 6th branchial pouches, and the 3rd and 4th branchial pouches, respectively. The thyroid gland is formed as a process of the initial part of the digestive tract to the ventral direction. In non-mammals, those endocrine glands are present in the same manner as all other independent glands. In mammals, however, the calcitonin-producing cells of the ultimobranchial gland were incorporated to the thyroid gland as C-cells. The parathyroid glands are buried in the backside of the thyroid gland and received a common blood supply with the thyroid gland. These anatomical circumstances intensely suggest that in mammals, there may be a functional relationship among those three endocrine glands.

calcitonin-producing cells might suppress acute absorption of Ca and nutrition at the small intestine. Ultimobranchial glands are embryologically originated from one part of the digestive tract. Physiological demands in the intestine might create the ultimobranchial glands (Fig. 3). Therefore, those calcitonin-producing cells in the intestine might be a remnant. Phylogenetically, the presence of calcitonin-immunoreactive cells in the digestive tract can be traced at least to sea squirts (Fritsch *et al*., 1982). Whether the origin for calcitonin- producing cells is the digestive tract or nervous tissue remains an interesting question.

In teleosts, Stannius corpuscles develop from the pronephric duct. Immunoreactive cells to the anti-stanniocalcin antibody appear at first among the epithelial cells of this duct (Kaneko *et al*., 1992). Therefore, in this case as well, physiological demands might produce the Stannius corpuscle as an endocrine organ from the pronephric duct (Fig. 3). Nevertheless, the actual functions of the Stannius corpuscle have not been determined in the kidney of teleosts. Why is the gill the target organ for stanniocalcin? How are the kidney and gills related? Since the gill is the portion exposed directly to the outer world, was the receptor for stanniocalcin created at the gill as a site for the exchange of minerals?

The ultimobranchial glands, the parathyroid glands, and the thyroid gland are united to produce physiological benefits in mammals

In lower vertebrates and birds, although the ultimobranchial glands, the thyroid gland, and the parathyroid glands are located close to each other in the neck region, there is no direct relationship. In mammals, however, although the ultimobranchial gland is once formed as an independent organ at the embryonic stage, the parenchymal cells are incorporated into the thyroid gland as C-cells. Furthermore, in mammals, the parathyroid glands are also incorporated on the surface of the thyroid gland (Fig. 4). The thyroid hormone is very important on the regulation of metabolic rates in every physiological phenomenon. The fact that the ultimobranchial gland and the parathyroid gland unite in/on the thyroid gland may reveal how important the control of blood Ca levels is.

In lower vertebrates, tissues corresponding to the cortex and medulla of the adrenal gland in mammals are separated. It has been pointed out that in mammals, norepinephrin is converted to epinephrin efficiently in the united adrenal (Hinson, 1990).

Recently, in monotremes: namely the platypus (*Orinithorhynchus anatinus*) and the echidna (*Tachyglossus acleatus*), the anatomy and morphology of the ultimobranchial glands, the thyroid gland, and the parathyroid glands were studied (Haynes, 1999). In these animals, one pair of the parathyroid glands did not connect to the thyroid gland but to the thymus, which is one of the characteristics of reptiles. Nevertheless, the histological features of those glands were similar to those of mammals. It was also determined that the ultimobranchial gland was not connected to the thyroid gland but located in the initial part of the trachea ventro-laterally. This is also one of the features of reptiles. Therefore, it seems that monotremes have common features to reptiles and mammals at least with respect to these endocrine glands.

New roles of hormones in Ca homeostasis

Calcitonin is expressed locally in various tissues

It has been reported that calcitonin is expressed in the gills of salmon that make a round trip from freshwater to seawater (Martial *et al*., 1994). Martial *et al* (1994) suggested that local expression of calcitonin in the gill might be useful for a transit suppression of Ca passing through the gill, followed by activation of the ultimobranchial gland. Recently, however, we

found that in rainbow trout kept in freshwater, calcitonin is expressed in the gill as well (Yachiguchi and Sasayama, unpublished data). Therefore, this phenomenon may not necessarily be related to seawater adaptation but to other physiological events.

On the other hand, it was determined that in rats, the glandular epithelium of the uterus expresses calcitonin when a fertilized egg is implanted (Ding *et al.*, 1994). Progesterone accelerates the production of calcitonin. It is supposed that calcitonin functions for cell adhesion in the embryo and for adjustment of environmental Ca in the cell differentiation.

Concerning other tissues, calcitonin-producing cells are found, for example, in the neuro-secreting cell mass of the prostate gland in humans (Cohen *et al.*, 1993). In this cell mass, calcitonin receptors are also expressed (Wu *et al.*, 1996). Therefore, it is suggested that calcitonin regulates the neurosecretion in the cell mass by paracrine function or autocrine function (Wu *et al.*, 1996).

Furthermore, we could amplify the cDNA coding the calcitonin region from the ovary and testis in goldfish by RT-PCR. However, its nucleotide sequence was different from that of the ultimobranchial calcitonin. The nucleotide sequence was the same as that of calcitonin produced by the brain in goldfish (Sakamoto and Sasayama, 1998). This may mean that in goldfish, what is important in gonads is not the calcitonin produced by the ultimobranchial gland but the calcitonin made by the nervous tissue. As the ends of nerve fibers distributed in the gonads terminate at steroid-producing cells (Nakamura *et al.*, 1996), calcitonin in the gonad may be necessary for the control of steroid production. It could also be that, as other ends of nerve fibers terminate at muscles in the gonad (Uematsu, 1990), calcitonin may control the contraction of muscles. The possibility can not be denied that calcitonin regulates the Ca levels of environmental fluid in the gonad for maturation of gametes.

Stanniocalcin is expressed locally in various tissues as well

Although stanniocalcin was found first as a fish hormone, some researchers thought that this hormone may be produced also in human, when considering the fact that most hormones present in lower vertebrates are also conserved in human (Wagner *et al.*, 1995). As the body organization of fish and human is rather different, stanniocalcin was searched in various tissues such as the kidney, liver, adrenal, pituitary, muscle, and urinary bladder. As a result, it was established that the kidney exhibited a positive immunoreaction. Immunohistological evidence revealed that the position was a part of uriniferous tubules (Wagner *et al.*, 1995). Furthermore, it was found that the distal segment of the uriniferous tubule in the human kidney (Koide *et al.*, 1998) and various parts of the rat kidney (Haddad *et al.*, 1996) reacts to the antibody of stanniocalcin.

Following these results, it was examined whether the genes encoding stanniocalcin are present or not in higher vertebrates. As a result, in various tissues in human, such as the ovary, prostate gland, and thyroid gland, mRNA encoding the stanniocalcin-like amino acid sequence was found (Chang *et al.*, 1995). Eventually, the nucleotide sequence encoding an amino acid sequence similar to that of salmon stanniocalcin was found from cDNA clones of the human embryonic lung tissue (Olsen *et al.*, 1996). That molecule was composed of 247 amino acid residues, and the sequence similarity from the first amino acid residue to the 204th was 92%, when amino acid residues that were similar in their biochemical characters were counted. In this region, 118 amino acid residues were completely identical (Olsen *et al.*, 1996).

The stanniocalcin-like protein expressed with recombinant DNA suppressed Ca permeability in the gill of goldfish (Olsen *et al.*, 1996). When administered to rats, the reabsorption rate of Pi was accelerated in the kidney, as observed in flounder (Wagner *et al.*, 1997). Moreover, in the digestive tract of rats, this molecule suppressed Ca absorption as fish stanniocalcin does (Madsen *et al.*, 1998). Therefore, this protein was identified as human stanniocalcin.

Therefore, all vertebrates including birds, reptiles, and amphibians may have stanniocalcin genes, although specific endocrine glands for this molecule are not formed in these animals.

Recently, the mRNA of stanniocalcin was detected in various tissues of mouse (Varghese *et al.*, 1998). Expression rates were especially high in the ovary. In addition to interstitial tissue, signals were observed in the corpora lutea and ovum in the developing follicle. Varghese *et al.* (1998) suggested that in mammals, during the processes of evolution, stanniocalcin came to be expressed in the ovary for the metabolism of minerals.

Furthermore, it was pointed out that stanniocalcin may be a regulating factor for Ca homeostasis in the nervous system as well (Zhang *et al.*, 1998). In human and mouse, stanniocalcin is expressed at a high rate in the differentiated brain, although no expression was recognized in the undifferentiated brain. Then, it would seem that stanniocalcin produced at cellular levels originally played a role in the kidney and intestine and that the local control for Ca succeeded to various tissues such as ovary and brain with the evolution of vertebrates, although some groups in bony fishes developed Stannius corpuscles.

The parathyroid hormone-related protein was found

It is currently known that in human, hypercalcemia occurs in patients suffering from malignant tumors. Therefore, it was supposed that the parathyroid hormone or a parathyroid hormone-like substance is present in the blood of such patients. Although this substance was extracted from the culture medium of the cell line of human lung cancer, it was not the parathyroid hormone itself (Moseley *et al.*, 1987). After the cDNA coding this protein was cloned, it was named as “the parathyroid hormone-related protein” (peptide in the case showing the molecule after the proteolytic splicing) (PTHrP) (Suva *et al.*, 1987). Plural forms of PTHrP are present in human. Although molecular sizes are varied among animals, about 140 amino acid residues construct this hormone. The sequence of those amino acid residues is akin to that of the

parathyroid hormone, especially to the N-terminal portion.

The origin of PTHrP is old

Contrary to the case of stanniocalcin, PTHrP was phylogenetically traced from mammals to fishes. In teleosts, various tissues were examined immunohistochemically. As a result, the salmon pituitary gland reacted to the antiserum (Fraser *et al.*, 1991). It has already been established that extracts of teleost pituitary glands sometimes bring about hypercalcemic effects. Therefore, it was supposed that the effect may be due to the presence of this molecule contained in the pituitary gland (Fraser *et al.*, 1991).

It was determined that in sea bream (*Sparus aurata*) as well, the pituitary gland contains a large quantity of the molecule reacting to the antiserum raised to the N-terminal portion of the human PTHrP (Danks *et al.*, 1993).

In shark (*Scyliorhinus canicula*) as well, PTHrP was detected in blood at a high level (Ingleton *et al.*, 1995). However, many organs such as the brain, the choroid, the saccus vasculosus (a peculiar organ for detecting water pressure), the pituitary gland, the kidney and the rectal gland showed the immunoreactivity (Ingleton *et al.*, 1993). However, using immunohistochemistry and electron microscope, Devlin *et al.* (1996) obtained results that led them to reaffirm that in the sea bream, only the saccus vasculosus produces PTHrP,.

Furthermore, in stingrays, in addition to the immunoreactivity in the choroid and saccus vasculosus, it was found that these portions were also positive to the antiserum raised to the receptor for PTHrP (Akino *et al.*, 1998). Although blood levels of PTHrP in the stingray were very high as compared to those in patients suffering from malignant tumors with hypercalcemia, the cerebrospinal fluid contained almost no PTHrP (Akino *et al.*, 1998). Therefore, the levels of PTHrP are distinctively regulated between those two portions.

Recently, PTHrP was detected immunohistochemically in lampreys and lungfish (Danks *et al.*, 1998). Immunoreaction was observed in their skin and uriniferous tubules of the kidney. In the growing lungfish, a positive reaction was detected also in the notochord, the brain and skeletal muscles.

It is suggested that PTHrP formed originally by the duplication of the ancestral gene. The ancestral molecule differentiated PTH and PTHrP (Martin *et al.*, 1991). In the process of evolution of vertebrates, PTH became a hormone for Ca homeostasis, and PTHrP came to be in charge of various functions, as mentioned below.

If PTHrP has various functions in mammals, the same must be true in non-mammals

PTHrP regulates the differentiation of keratinocytes in the epidermis of mouse (Foley *et al.*, 1998). As mammary glands are not differentiated in the mouse in which the PTHrP gene was knocked out, PTHrP may be involved in the differentiation of the integument and mesenchyme (Wysolmerski *et al.*, 1998a, b). In mammals, this hormone may be necessary when cartilages are formed and replaced by bones in embryos as well as in the process of fracture healing in adults (Ferguson *et al.*, 1998 a).

PTHrP is expressed largely in the uterus of the pregnant females of mammals and may be involved in the growth and Ca homeostasis of embryos by regulating Ca across the placenta (Curtis *et al.*, 1998). The receptor of PTHrP is expressed at the same time at the uterus and smooth muscle of blood vessels (Ferguson *et al.*, 1998b). It is supposed that this phenomenon means the prevention of excessive tension of blood vessels in the uterus. PTHrP is expressed in the endothelial cells of blood vessels themselves and may be involved in the relaxation of blood vessels (Eto *et al.*, 1998).

Vasavada *et al* (1998) explained the reason of these various functions of PTHrP by regarding this molecule as a prohormone. PTHrP becomes a family of various small peptides in proteolytic processes. These peptides function as paracrine and autocrine factors, in addition to hormonal functions. Furthermore, it is supposed that some peptides from PTHrP enter the nucleus as an intracrine factor and bring about various effects such as cell proliferation and apoptosis.

In mammals at least, the receptor for PTHrP is distributed in almost all tissues such as the kidney, bones, aorta, adrenal, urinary bladder, cerebrum, cerebellum, mammary glands, heart, digestive tract, liver, lungs, and reproductive organs etc. (Urena *et al.*, 1993). This fact is consistent with various functions of PTHrP.

Perspectives of this field

Many researchers have concentrated on the elucidation of Ca homeostasis. As a result, it has been clarified which endocrine glands are involved in it, using techniques of the experimental morphology. Recently, however, using techniques of molecular biology, it quickly became clear that calcitonin is expressed locally in some tissues and that stanniocalcin, a fish hormone, is also expressed in various tissues locally in mammals. On the other hand, it was also established that PTHrP, a mammalian hormone, can be traced to fishes. Therefore, the study of this field in the future may provide the explanation how these hormones are involved in the local regulation of the Ca at the cellular level. Although the phenomena may appear complicated, their substantial parts may be simple and universal throughout vertebrates.

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