The natriuretic peptide system in eels: a key endocrine system for euryhalinity?

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Takei, Yoshio, and Shigehisa Hirose. The natriuretic peptide system in eels: a key endocrine system for euryhalinity? Am J Physiol Regulatory Integrative Comp Physiol 282: R940–R951, 2002; 10.1152/ajpregu.00389.2001.—The natriuretic peptide system of a euryhaline teleost, the Japanese eel (Anguilla japonica), consists of three types of hormones [atrial natriuretic peptide (ANP), ventricular natriuretic peptide (VNP), and C-type natriuretic peptide (CNP)] and four types of receptors [natriuretic peptide receptors (NPR)-A, -B, -C, and -D]. Although ANP is recognized as a volume-regulating hormone that extrudes both Na⁺ and water in mammals, ANP more specifically extrudes Na⁺ in eels. Accumulating evidence shows that ANP is secreted in response to hypernatremia and acts to inhibit the uptake and to stimulate the excretion of Na⁺ but not water, thereby promoting seawater (SW) adaptation. In fact, ANP is secreted immediately after transfer of eels to SW and ameliorates sudden increases in plasma Na⁺ concentration through inhibition of drinking and intestinal absorption of NaCl. ANP also stimulates the secretion of cortisol, a long-acting hormone for SW adaptation, whereas ANP itself disappears quickly from the circulation. Thus ANP is a primary hormone responsible for the initial phase of SW adaptation. By contrast, CNP appears to be a hormone involved in freshwater (FW) adaptation. Recent data show that the gene expression of CNP and its specific receptor, NPR-B, is much enhanced in FW eels. In fact, CNP infusion increases ²²Na uptake from the environment in FW eels. These results show that ANP and CNP, despite high sequence identity, have opposite effects on salinity adaptation in eels. This difference apparently originates from the difference in their specific receptors, ANP for NPR-A and CNP for NPR-B. VNP may compensate the effects of ANP and CNP for adaptation to respective media, because it has high affinity to both receptors. On the basis of these data, the authors suggest that the natriuretic peptide system is a key endocrine system that allows this euryhaline fish to adapt to diverse osmotic environments, particularly in the initial phase of adaptation.

atrial natriuretic peptide; ventricular natriuretic peptide; C-type natriuretic peptide; membrane guanylyl cyclase; water and electrolyte balance; molecular evolution; functional evolution; volume regulation; sodium regulation; Anguilla japonica

ATRIAL NATRIURETIC PEPTIDE (ANP) was the first member of the natriuretic peptide (NP) family identified in mammals (17, 63, 69, 86). Subsequent to the isolation and sequencing of ANP from the rat atria in 1983, brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) were isolated from the porcine brain in 1988 and 1990, respectively, and they were identified as members of the NP family (Fig. 1). Whereas ANP and BNP are circulating hormones secreted from the heart, CNP is principally a paracrine factor in the brain and periphery (71). The basic structure of the NP peptides consists of an intramolecular ring with ex-
tending NH₂-terminal and COOH-terminal sequences of varying length, except for CNP that lacks the COOH-terminal “tail” sequence (Fig. 1). CNP is most conserved among the three, and all mammalian CNPs thus far sequenced are identical, except the one isolated from the venom of egg-laying platypus (18). ANP is also a conserved peptide with only two variants in mammals, but the BNP sequence is highly variable among different species (86). With respect to the receptor, two guanyl cyclase (GC)-coupled receptors (NPR-A and NPR-B) and a GC-deficient receptor (NPR-C) have been cloned in mammals (20, 54, 86). Whereas ANP and BNP bind NPR-A with high affinity, NPR-B is a selective receptor for CNP. Because NPR-C exhibits equally high affinity to all NPs, is distributed ubiquitously in various tissues in large amounts, and is internalized quickly after binding of ligands, NPR-C is believed to be a clearance-type receptor responsible for regulating plasma NP levels (54). However, recent data suggest that NPR-C is involved in the stimulation of nitric oxide production through G proteins in gastrointestinal smooth muscle cells (62). At present, therefore, the NP system consists of at least three hormones and three receptors in mammals.

Compared with ample studies in mammals, little is known about the NP system in nonmammalian species. NP proteins and/or their cDNAs have been isolated in selected species of cartilaginous fishes, bony fishes, amphibians, and birds (86). ANP, BNP, and CNP may be present in all tetrapods ranging from amphibians to mammals, although some data are still lacking in birds and reptiles (Fig. 1). BNP may be absent in teleost fish and, instead, ventricular natriuretic peptide (VNP) has been identified in the eel and two salmonid species (Fig. 1). Thus ANP, VNP, and CNP appear to be common to the teleost species (90, 94–97). VNP is characterized by its uniquely long COOH-terminal tail sequence (Fig. 2). Eel ANP is also unique in that its COOH terminus is amidated. Recent data show that tilapia (Oreochromis mossambicus) and killifish (Fundulus heteroclitus) also have amidated ANP (K. Inoue and Y. Takei, unpublished data). The COOH terminus of salmonid ANP is not amidated (90, 101), but its overall sequence is quite similar to tilapia and killifish ANP at both cDNA and prohormone levels. The VNP sequence is highly conserved in two phylogenetically distant teleost species, the eel and the rainbow trout, Oncorhynchus mykiss (Fig. 2), and is identical in two salmonid species, the rainbow trout and the chum salmon, O. keta (86). The high level of conservation of the VNP sequence is a contrast to the variability found in the BNP sequence in tetrapods. In elasmobranchs, only CNP has been identified in four species (83). Recent data further show that CNP is the only NP in this fish group (47) (Fig. 1). Thus CNP may represent an ancestral type of the NP family.

In addition to the ligands, possibly all types of NP receptors have been identified and characterized in the Japanese eel, which include NPR-A, NPR-B, and NPR-C as in mammals, and a new type named NPR-D (38) (Fig. 2). Although ANP and VNP have equally high affinity to NPR-A (45), not only CNP but also VNP exhibits high affinity to NPR-B (46). The affinity of VNP to NPR-B is 100-fold higher than that of ANP in eels. NPR-D is a new type of GC-deficient receptor with a short cytoplasmic domain similar to NPR-C (44). Unlike the dimeric NPR-C, however, NPR-D is a tetrameric receptor as observed in the GC-coupled receptors, NPR-A and NPR-B (Fig. 2). Thus HS-142-1, which was thought to be a selective blocker for GC-coupled receptors (61), blocks NP binding to NPR-D. Furthermore, NPR-D is not distributed ubiquitously in various tissues, as is the case with NPR-C, but is localized mostly in the brain. Thus NPR-D may have some function in the brain and thus serves as a good model to pursue any new biological functions of the GC-deficient receptors (62). In other nonmammalian species, NPR-B has been isolated and characterized in the spiny dogfish, Squallus acanthias, which has only CNP as a ligand (1).

**Euryhalinity and Osmotic Adaptability in Teleost Fish**

The word euryhalinity originates from the Greek eurys ( = wide) and halos ( = salt), which means an excellent adaptability to wide variations in the envi-
Fig. 2. Natriuretic peptides (A) and their receptors (B) identified to date in the Japanese eel. ANP (95), VNP (96), and CNP (97) have different numbers of NH2-terminal and COOH-terminal amino acid residues that extend from the intramolecular ring. Shaded amino acid residues are identical to those of the rainbow trout. A-type (45), B-type (46), and D-type (44) natriuretic peptide receptors (NPR-A, NPR-B, and NPR-D) are tetrameric receptors, whereas NPR-C (84) is present as a homodimer. NPR-D is identified only in eels. Affinity orders of ligands are noted above each receptor.

Environnemental salinities. Because the blood of aquatic animals is only separated from the ambient water by a thin respiratory epithelium, fishes are generally vulnerable to changes in environmental salinities (24). Among fishes that use diverse strategies for osmotic adaptation, teleost fishes maintain their plasma ionic concentrations at approximately one-third of seawater (SW), irrespective of environmental salinities. Most teleost fishes are stenohaline species that live exclusively in inland fresh water (FW) or in the sea and do not migrate between waters of different salinities during their life span. However, some are euryhaline species that live in estuaries with varying salinities or migrate between the river and the sea. Thus euryhaline fishes have been frequently used to analyze the mechanism of osmotic adaptation.

Fishes usually drink little and urinate copiously in FW to get rid of excess water that enters the gills across an osmotic gradient, whereas they actively extract Na+/H+ and Cl−/H+ from environmental FW by the gills to compensate for the loss of these ions across a concentration gradient (43). In SW, however, the regulation of water and ions is reversed; fish drink copiously and absorb water across the intestine together with Na+ and Cl− to offset an osmotic loss of water by the gills, while excess Na+ and Cl− that enter the body are actively excreted via chloride cells of the gills. The excess divalent ions (Mg2+, Ca2+, and SO42−) that are taken up from SW are actively secreted into the urine at the proximal tubules (10). Therefore, body fluid regulation can be achieved principally by oral intake, intestinal absorption, branchial fluxes, and renal excretion. Euryhalinity may be the result of integrated regulation at these osmoregulatory sites (22).

In response to changes in environmental salinities, the regulation of water and ions occurs in at least two phases with respect to time. In immediate regulation, fish start or stop drinking and increase or decrease the activity of already existing ion and water transporters/channels in the osmoregulatory epithelia on encountering a changed environmental salinity. Long-term regulation involves remodeling of osmoregulatory organs such as the gills, intestine, and kidney. At the cell and tissue levels, FW-type chloride cells disappear, whereas SW-type chloride cells newly differentiate in the gills during the course of SW adaptation (57, 67). In the intestine, epithelial cells are flattened and rich vascularization develops just beneath the epithelia to facilitate absorption of water together with Na+ and Cl− (36). At the molecular level, new transporters/channels and cell adhesion molecules may be synthesized in the epithelial cells of the gills, intestine, and kidney. It is obvious that euryhaline fishes are those that have developed mechanisms for both fast and slow phases of regulation.

The endocrine system plays a central role in homeostatic regulation such as environmental adaptation. With respect to time, the osmoregulatory hormones can also be categorized into two groups: fast-acting hormones and slow-acting hormones. The fast-acting hormones are amine or oligopeptide hormones that are secreted immediately (in seconds to minutes) after transfer of fish to different osmotic media and disappear quickly from the circulation. These hormones are involved in the fast phase of regulation mentioned above. By contrast, slow-acting hormones are polypeptide or steroid hormones that are secreted slowly (usually in days) during the course of adaptation and participate in long-term adaptation to a new environment. Among slow-acting hormones, cortisol and growth hormone are involved in SW adaptation in salmonids and tilapia (55, 72), whereas prolactin is an important hormone.
hormone for FW adaptation (13, 37). Plasma concentration of these hormones increases a few days after transfer from FW to SW or vice versa (64, 73). Many oligopeptide hormones are implicated in the initial phase of adaptation, including ANP, angiotensin II, vasotocin, urotensins, insulin-like growth factor I, and others (12, 53, 56, 73, 85, 86). Until recently, however, less was known about their roles in osmotic adaptation compared with the slow-acting hormones.

DIFFERENCE IN BODY FLUID REGULATION BETWEEN TETRAPODS AND FISH

Because life on land is characterized by the constant threat of desiccation, terrestrial tetrapods have developed mechanisms to retain water and maintain blood volume. To maintain blood volume, however, both water and osmolytes, particularly Na\(^+\)/H\(^+\) ions, have to be retained at the same time. If only water is ingested in case of hypovolemia, for example, a fall in plasma osmolality inhibits antidiuretic hormone secretion, resulting in the loss of ingested water as urine. Accordingly, both water and Na\(^+\) are usually regulated to -

On the other hand, the regulation of water and Na\(^+\) is in opposite directions in aquatic fishes. As mentioned above, teleost fish retain Na\(^+\) and excrete water in FW, whereas they retain water and excrete Na\(^+\) in SW (Fig. 3). This difference in the regulation of water and Na\(^+\) between fishes and tetrapods is important for understanding the evolution of body fluid regulation in vertebrates. More importantly, the direction of water and Na\(^+\) regulation is reversed when fish are in FW or SW. Thus we can determine which of the parameters, water or Na\(^+\), is the primary target of osmoregulatory hormones if euryhaline species that survive in both FW and SW are used for the analysis.

ANP IS A VOLUME-REGULATING HORMONE IN MAMMALS

It is evident that ANP is a hormone intimately involved in blood volume regulation in mammals (51). ANP is secreted in response to an increase in blood volume (atrial stretch) (70), and released ANP restores blood volume to normal by decreasing the uptake and increasing the excretion of both water and Na\(^+\). Regarding the uptake, ANP decreases the oral intake of water and Na\(^+\) by inhibiting thirst and sodium appetite and further inhibits the absorption of ingested water and Na\(^+\) by the intestine (86) (Fig. 4). ANP also potently stimulates the excretion of water and Na\(^+\) by the kidney. Furthermore, ANP inhibits the secretion of arginine vasopressin (AVP) and aldosterone, thereby indirectly promoting further diuresis and natriuresis (Fig. 4). Thus it is obvious that ANP is a hormone that is secreted in response to hypervolemia and acts on various organs to decrease blood volume to normal.

Concerning other members of the NP family, BNP exhibits a spectrum of biological actions similar to ANP because ANP and BNP share the same receptor, NPR-A (69). However, the differences in the major site of secretion (atrium for ANP and ventricle for BNP) and in the secretory pathway (regulatory for ANP and constitutive for BNP) produce slight differences in their functions (16). Recent studies using genetically disrupted mouse models can detect the difference (59, 99); however, this topic will not be discussed in detail here. The physiological function of CNP has not yet been fully established (29). CNP is certainly a neuropeptide that is synthesized in largest quantities in the brain, but its central actions, such as its antidiuresic effect and the inhibition of AVP secretion, are much less potent than ANP and BNP. In the periphery, CNP seems to be involved in vascular remodeling and in bone/cartilage formation through its action as an anti-growth factor (32, 39, 79). CNP may act locally in
ANP IS A SODIUM-REGULATING HORMONE IN EELS

Among teleost species, the physiological function of the NP system has been most thoroughly explored in the eel using homologous hormones and their radioimmunoassays (86). Unlike in mammals, blood volume expansion was only a weak stimulus for ANP secretion in eels (40). Instead, an increase in plasma osmolality dramatically augments ANP secretion (Fig. 4). Because ANP secretion also occurs from isolated eel atrial tissue in vitro in response to high environmental osmolality, cellular dehydration of the cardiocytes may be the major stimulus for ANP secretion (H. Kaiya and Y. Takei, unpublished data). After transfer of eels from FW to SW, plasma ANP concentration increases immediately, even though blood volume decreases after SW exposure (41). The decrease in blood volume inhibits ANP secretion in mammals. Thus the increase in ANP secretion may be induced solely by an increase in plasma Na⁺ concentration after transfer to SW. The increase in plasma ANP is probably only transient because of a short half-life of ANP in eel blood (~90 s).

Consistent with the fact that osmotic stimulus is a primary regulator for ANP secretion, the major target of ANP for its biological action is not on blood volume but on plasma Na⁺ concentration in eels (Fig. 4). When ANP is infused into SW eels at doses within a physiological range, it potently inhibits drinking without changes in arterial pressure (102). This is also in contrast to mammals, where the hemodynamic effect of ANP is much more sensitive and dominant than its osmoregulatory effects, because the genetic mouse models with disrupted ANP gene show Na⁺-sensitive hypertension but in normal Na⁺ balance (56). ANP also inhibits Na⁺ and Cl⁻ absorption by the eel intestine as evidenced by a decrease in short-circuit current in vitro (3, 52). The potency and efficacy of the intestinal effect are far greater by ANP than by any other inhibitory substances thus far reported in eels. The combined inhibitory effects on drinking and intestinal absorption profoundly decrease NaCl uptake from the environment in SW eels (Fig. 4).

ANP infusion into SW eels at physiological doses increases urine Na⁺ concentration as observed in mammals but decreases urine flow rate (92). Thus ANP acts on the kidney to excrete Na⁺ but not water (Fig. 4), although total Na⁺ excretion is unchanged due to the antiuresis. Concerning the branchial effect, ANP increases Na⁺-K⁺-ATPase activity in isolated gill cells from SW eels when added to incubation media (97). This result indicates that ANP possibly stimulates NaCl excretion from Na⁺-K⁺-ATPase-enriched chloride cells of the gill. The renal and branchial effects of ANP on Na⁺ metabolism are observed only in SW eels but not in FW eels. These effects certainly facilitate adaptation of eels to SW environments where they encounter Na⁺ concentrations greater than in the plasma.

ANP also indirectly promotes SW adaptation in eels. ANP is shown to act on the interrenal to elevate plasma cortisol concentration in SW eels (88). Cortisol acts as a mineralocorticoid in teleost fish (8) to increase the number of Na⁺-K⁺-ATPase in the chloride cells and differentiate them to an SW type in the gills (55, 57). Cortisol also acts on the intestine to reorganize the epithelia and vasculature and to facilitate absorption of water and ions in SW eels (36). These changes allow eels to survive in SW during the long-term phases of adaptation. Interestingly, similar to the renal and branchial effects, the interrenal effect of ANP is demonstrated only in SW eels.

All the biological actions of ANP mentioned above are, either directly or indirectly, to get rid of excess Na⁺ from the body and to promote SW adaptation. Consistently, ANP infusion at physiological doses de-
creases plasma Na⁺ concentration dose dependently in SW eels (92). The decrease reached up to 9.5% at 3 pmol·kg⁻¹·min⁻¹, at which dose no change in arterial blood pressure occurred. Our recent data show that most parts of the decrease in plasma Na⁺ concentration are due to the decreased drinking of SW and decreased absorption of Na⁺ by the intestine, which is demonstrated in vivo using conscious SW eels (T. Tsukada and Y. Takei, unpublished data). Again, the decrease in plasma Na⁺ concentration was demonstrated only in SW eels. Because total body sodium is strictly regulated in vertebrates (68), it is remarkable that a single hormone changes plasma Na⁺ concentration so profoundly. These results convincingly show that ANP is a hormone that extrudes Na⁺ from the body (and probably Cl⁻ also) but not water and promotes SW adaptation in eels.

**ANTIDIPSOGIC EFFECT OF ANP IN EELS**

Among ANP actions in SW eels, the antidipsogenic effect and inhibition of intestinal absorption of NaCl are seemingly inconsistent with SW adaptation, because drinking of SW and subsequent absorption of water by the intestine together with NaCl are essential for survival of teleost fish in SW (24). In fact, if ingested water is drained through an esophageal fistula after transfer of eels to SW, they suffer from gradual hypovolemia and hypernatremia and die in 5 days (98). However, it should be noted that after SW transfer, plasma ANP concentration increases only transiently and returns to the previous level in a few hours (41). This time course is a reversed pattern of the changes in drinking rate; vigorous drinking occurs immediately after SW transfer, which is suppressed for a few hours, then increases again gradually to a constant SW level (93). Therefore, it is apparent that the inhibition of drinking by ANP actually occurs after transfer of eels to SW. However, the inhibition is only transient and ANP does not inhibit drinking perpetually in SW.

What is the physiological significance of the transient inhibition of drinking after SW transfer? It is known that a burst of drinking occurs just after transfer of eels to SW in response to Cl⁻ ions in the media (35). If eels continued to drink at such a high rate after transfer, they would suffer from a sudden increase in plasma Na⁺ concentration. Thus the transient inhibition of drinking serves to alleviate the initial sudden hypernatremia. In accordance with this notion, the initial increase in plasma Na⁺ concentration is much reduced if ingested SW after transfer to SW is exteriorized by an esophageal fistula (98). Therefore, it is obvious that, under normal circumstances, ANP would transiently inhibit drinking after eels encounter SW to diminish a sudden increase in plasma Na⁺ concentration. This certainly increases the chance of survival in the initial phase of SW adaptation. It is interesting to note that the inhibition of drinking and intestinal absorption of NaCl was demonstrated in FW eels as well as SW eels (2, 88) (Fig. 4). Because they still retain physiological characteristics of FW eels just after transfer to SW, it is highly probable that increased plasma ANP could inhibit drinking in such eels.

The involvement of endogenous ANP in regulation of drinking has also been demonstrated experimentally in eels. Injection of hypertonic NaCl solution induces copious drinking in tetrapods ranging from reptiles to mammals (28). However, the similar osmotic stimulus to eels paradoxically inhibits drinking in both FW and SW fish (93). The inhibition is most likely to be caused by an increase in plasma ANP concentration as observed after osmotic stimulus (40). The osmotic stimulus also causes a slight increase in plasma ANP concentration in mammals (70), but the antidipsogenic effect of ANP is much less potent in mammals than in eels (4). Similarly, although the antidipsogenic effect of ANP is more potent than the dipsogenic effect of angiotensin II in eels (88, 102), the dipsogenic effect of angiotensin II is greater than the antidipsogenic effect of ANP in mammals. These observations, together with the fact that drinking is suppressed transiently by ANP after SW transfer, make it likely that ANP is a physiological regulator of drinking in eels.

**OSMOREGULATORY ACTIONS OF ANP IN FISH OTHER THAN EELS**

There are several studies on the osmoregulatory actions of ANP in teleost species other than eels, the results of which are not always consistent with those of eels, as summarized in a number of reviews (23, 26, 51, 86). Plasma ANP concentration is generally higher in SW fish than in FW fish or in euryhaline fish that are in SW than in FW (25, 31, 77, 105). These results differ from those of the eel, in which plasma ANP concentration increases only transiently after transfer to SW and its concentrations do not differ between FW- and SW-adapted fish (41). In regard to the stimulus for ANP secretion, volume expansion is a powerful stimulus for ANP secretion in rainbow trout, Oncorhynchus mykiss (15). This result also differs from that of the eel, in which hypervolemia is only a weak stimulus for ANP secretion compared with hypernatremia (40). The difference between the eel and other teleost species is most likely due to a species difference. However, it is also possible that the heterologous radioimmunoassay used in other species did not measure ANP but VNP or CNP because of high sequence identity of the NPs (87). In eels, a radioimmunoassay for human ANP measured VNP but not ANP (87). It should be determined whether the mechanism regulating ANP secretion differs in other teleost species by using homologous radioimmunoassay to each species. For this purpose, we recently cloned ANP, VNP, and CNP of the rainbow trout and raised an antiserum to each peptide for radioimmunoassay (K. Inoue, K. R. Olson and Y. Takei, unpublished data).

With respect to the renal action of ANP, the initial studies using mammalian ANP or homologous heart extracts resulted in potent diuretic and natriuretic effects in the rainbow trout (21, 66) and toadfish, Opsanus tau (49). Because the toadfish has an aglomeru-
lar kidney, the data provided the first direct evidence of the tubular action of ANP in vertebrates. Thus the antidiuretic effect of ANP observed in SW eels is the opposite of the results obtained from these teleost species. This difference may also be due to a species difference, but mammalian ANP may have mimicked the effect of other NP in these fishes. It is also possible that the diuretic effect is dependent on the water balance of the fish; the rainbow trout are on the edge of overhydration when they are in FW (21), and the toadfish used in the above experiment are volume expanded by the infusion of physiological saline to ensure a constant urine flow in SW (49). In fact, rat ANP was antidiuretic and antinatriuretic in the spiny dogfish, but it became diuretic and natriuretic when fish were volume expanded after transfer to dilute SW (9).

There are several pioneer studies showing an involvement of ANP in water and electrolyte metabolism in teleost fish using mammalian ANP. In the intestine, ANP potently inhibits NaCl absorption through the inhibition of Na-K-2Cl cotransporter in the winter flounder, Pseudopleuronectes americanus (65). Similar results are obtained in the goby, Gillicichthys mirabilis (50). ANP stimulates chloride secretion (short-circuit current) in the isolated opercular epithelium of killifish (75). Consistent with this result, ANP increases the excretion of $^{22}\text{Na}$ into media when injected into the circulation of three species of marine flatfish (7). It is also possible that the chloride cells are activated by cortisol, because ANP increases cortisol secretion both in vivo and in vitro in the flounder, Platichthys flesus, and rainbow trout (6). These results are consistent with those of the eel, in which ANP is an Na$^+$-extruding hormone.

Initial studies using rat ANP and homologous heart extract showed a stimulation of NaCl secretion from the rectal gland in vivo and in vitro in the spiny dogfish (78). It is now obvious that CNP is the sole NP in elasmobranchs (47) and a large amount of CNP is secreted from the rectal gland (76). There is another interesting marine vertebrate with respect to water and electrolyte regulation, the hagfish, which is thought to be the most primitive extant vertebrate and whose plasma has Na$^+$ and Cl$^-$ concentrations almost identical to SW (24). Because the presence of NP has been suggested in a species of hagfish, Myxine glutinosa (19), it is intriguing to examine how the NP is involved in Na$^+$ homeostasis in this primitive animal.

CNP MAY BE INVOLVED IN FW ADAPTATION IN EELS

CNP is principally a local paracrine factor in the brain and perihem in mammals (29). In FW eels, however, plasma CNP concentration is as high as ANP despite CNP being at a low concentration in SW eels (91). Therefore, CNP must function as a circulating hormone as well as a paracrine factor in FW eels, as does in elasmobranchs. The source of CNP in plasma may be from the heart and/or intestine in addition to endothelial cells, because the expression of CNP mRNA in these tissues is enhanced in FW eels. The expression of CNP is highest in the brain, but the amount of expressed mRNA does not differ between FW and SW eels. Furthermore, the expression of the CNP-specific receptor, NPR-B, is also enhanced in the osmoregulatory organs of FW eels, including the gills, intestine, and kidney (46). These results strongly suggest an osmoregulatory function for CNP in FW eels.

CNP infused at doses within a physiological range increased plasma Na$^+$ concentration dose dependently in FW eels without changing the arterial blood pressure (97). This increase was not detectable in SW eels.

ANP IS A Na$^+$-EXTRUDING HORMONE THROUGHOUT VERTEBRATES: A COMPARATIVE VIEWPOINT

Accumulating evidence shows that there are significant differences in the osmoregulatory actions of ANP between mammals and teleost fish (eel). This difference may originate from the difference in their habitats as illustrated in Fig. 3. Because of the regulation of water and Na$^+$ in the same direction, ANP appears to be a volume-regulating hormone in mammals. However, ANP is apparently an Na$^+$-regulating hormone in fish in which water and Na$^+$ are regulated independently in the opposite direction.

It seems that the primary target of regulation for ANP is Na$^+$ balance in mammals as in fish. However, because Na$^+$ and water are usually regulated together in the same direction in mammals, ANP is seemingly recognized as a volume-regulating hormone. It is likely that most transport epithelia of terrestrial animals are equipped with abundant water channels so that water passively moves in parallel when Na$^+$ and Cl$^-$ move across the epithelia. In teleost fish, however, transport epithelia such as the gills, which directly contact the environmental water and thus serve as a major site of water and NaCl fluxes across body surfaces, may have fewer water channels. If water channels are abundant on such epithelia, fish would suffer from overhydration in FW and dehydration in SW because of a large osmotic gradient across the epithelia.

To alleviate the potential osmotic loss of water, marine elasmobranchs accumulate urea and trimethylamine oxide in plasma and increase their plasma osmolality to SW levels (34). However, because their plasma Na$^+$ concentration is less than half of SW, marine elasmobranchs need to dispose of excess Na$^+$ from the body, which is a situation similar to that of marine teleost fish. Thus it is as anticipated that CNP, which takes the place of ANP in elasmobranchs, acts as an Na$^+$-extruding hormone by acting on the rectal gland (76).
which was expected because of the low density of NPR-B (46). The stimulatory effect of CNP in FW eels is opposite of the inhibitory effect of ANP in SW eels in terms of Na\(^+\)/H\(^+\) economy because ANP decreases plasma Na\(^+\) concentration in SW eels (92). The site of action of CNP may be the gills, because CNP treatment of isolated gill cells from FW eels increases Na\(^+\)/K\(^+\)-ATPase activity if administered together with angiotensin II (97). The presence of angiotensin II receptors has been demonstrated in the gills of European eels, *A. anguilla* (58). More recently, CNP infused into the circulation of FW eels facilitates an uptake of \(^{22}\text{Na}\) added to the environmental FW (J. C. Rankin and Y. Takei, unpublished data). These results strongly suggest that CNP stimulates an uptake of Na\(^+\) by the gills, thereby promoting FW adaptation in eels.

**NP PEPTIDES ARE A FAMILY OF NA\(^+\)-REGULATING HORMONES**

It is somewhat surprising that ANP and CNP have opposite effects on adaptation to environmental salinity in eels despite the two peptides showing \(>60\%\) sequence identity; ANP is obviously an Na\(^+\)-extruding hormone and CNP an Na\(^+\)-retaining hormone (Fig. 5). Therefore, the NP family of peptides appears to govern the Na\(^+\) economy of eels in an integrative fashion that allows the eels to invade diverse osmotic environments.

Obviously, the diverse actions of NP peptides can be accomplished by the difference in their specific receptors, NPR-A and NPR-B. However, because both receptors use cGMP as an intracellular messenger, ANP and CNP actions can be differentiated by the difference in tissue distribution of NPR-A and NPR-B, in abundance of the receptors between FW and SW fish, and in the use of signal transduction pathways (e.g., protein kinase G1 or G2). The difference in the secretory stimulus may also contribute to the opposing nature of their actions, because an osmotic stimulus enhances ANP secretion but may suppress CNP secretion, considering the high CNP concentration in FW eels (91). The difference in the effects of ANP and CNP on Na\(^+\) balance warrants further investigation.

Teleost fish have another member of the NP family, VNP, which has a unique structure with a long COOH-terminal tail sequence (Fig. 2). The biological functions of VNP are not yet fully understood in eels. However, because VNP has high affinity to both NPR-A and NPR-B (46), VNP may supplement the effects of CNP and ANP for adaptation to FW and SW, respectively (Fig. 5). VNP may be secreted via a constitutive secretory pathway so that its plasma concentration is maintained largely at a constant level irrespective of environmental conditions (41). Thus VNP may be able to ensure the effect of ANP and CNP in either FW or SW environments. VNP is as effective as ANP and much more effective than CNP in the inhibition of NaCl absorption by the intestine (52) and in the suppression of the drinking rate and plasma Na\(^+\) concentration (103) in SW eels. However, the role of VNP in FW adaptation has not yet been examined.

**Perspectives**

Accumulating evidence strongly suggests that the NP system plays a pivotal role in the adaptation of the euryhaline eel to diverse environmental salinities, particularly in the initial phase of adaptation. However, we have to admit that eels are not representative of all teleost species but are rather exceptional. In terms of osmoregulation, eels can survive direct transfer between SW and distilled water (DW) without pituitary, demonstrating that they can survive in DW without prolactin and in SW without growth hormone. These extraordinary abilities are not always shared by other teleost species. Therefore, whether the observed functions of the NP system in eels can also extend to other euryhaline fish is unknown and needs to be examined in the near future. However, because the effects of the NP peptides are so potent and consistent with respect

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**Fig. 5.** Role of natriuretic peptides in salinity adaptation in eels. Although ANP acts on various osmo-regulatory sites through NPR-A to promote SW adaptation, CNP acts on the gill through NPR-B to promote FW adaptation. VNP may act on both receptors to supplement the actions of ANP and CNP for SW and FW adaptation, respectively. For details, see NP PEPTIDES ARE A FAMILY OF NA\(^+\)-REGULATING HORMONES.
to Na\textsuperscript{+} regulation, it should be emphasized that the NP system is at least one of the key endocrine systems that are involved in the excellent adaptability of this euryhaline fish.

Because euryhalinity involves complex regulatory processes with respect to time, sites, molecules, etc., it is unlikely that this excellent ability is accounted for by a single hormonal system. Therefore, it is quite surprising that the NP system alone causes such profound effects on the adaptation to both FW and SW. It is generally accepted that the endocrine system plays a central role in homeostatic processes, such as water and electrolyte regulation. For instance, fast-acting hormones and slow (long)-acting hormones work in concert to regulate the activity and the number of various transporters in the transport epithelia of osmoregulatory sites (27, 58, 65, 75, 76, 89) (Fig. 6).

Generally, euryhaline species that can survive direct transfer from FW to SW can also tolerate concentrated SW. Thus fish that adapt well in the initial phases of salinity adjustment usually exhibit excellent euryhalinity. In this respect, it is worth pointing out that fast-acting hormones often stimulate the secretion of slow (long)-acting hormones. In fact, ANP is secreted immediately after SW transfer, which regulates drinking and preexisting transporters to cope with abrupt changes in environmental salinity. ANP itself disappears quickly from the circulation, meanwhile stimulating the secretion of cortisol, a long-acting, SW-adapting hormone. Therefore, the fast-acting hormone appears to control the whole regulatory processes, leading to a wide adaptability such as euryhalinity. From this perspective, it would be interesting to examine whether ANP stimulates the secretion of growth hormone, another long-acting, SW-adapting hormone, and whether CNP stimulates the secretion of prolactin, the most important long-acting, FW-adapting hormone. In the near future, the relationship between fast-acting and slow (long)-acting hormones (how the former passes the baton to the latter) may become an interesting area of research in the studies of environmental adaptation (Fig. 6).

The use of gene technology would define more precisely the role of NPs in salinity adaptation. Because it is impossible to apply this technique to eels whose life cycle has not yet been fully elucidated, other species that can be cultured from eggs must be chosen as an experimental species. Two closely related species of tilapia have been frequently used for studies of osmoregulation. One is the euryhaline Mozambique tilapia (Oreochromis mossambicus) that can survive in triple-strength SW, and the other is the stenohaline Nile tilapia (O. niloticus) that cannot adapt to more than half-strength SW. It is possible that the expression of the ANP or its receptor genes of Nile tilapia is not properly enhanced in response to an increase in environmental salinity. Therefore, it would be interesting to examine whether the compulsory expression of ANP gene of the Mozambique tilapia with its promoter in the Nile tilapia can produce a new euryhaline tilapia species. In the future, if an embryonic stem cell line is established in tilapia, it will also be possible to examine whether knockout of the ANP gene compromises the euryhalinity of Mozambique tilapia.

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Fig. 6. Interaction of fast-acting hormones (ANP, angiotensin II, vasotocin, etc.) and slow (long)-acting hormones (cortisol, growth hormone, prolactin, etc.) for salinity adaptation. For details, see Perspectives.
REFERENCES


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