MOLECULAR EVOLUTION OF

NATRIURETIC PEPTIDES

IN FISH

Akatsuki Kawakoshi, Susumu Hyodo and Yoshio Takei
Ocean Research Institute, the University of Tokyo
1-15-1 Minamidai, Nakano, Tokyo 164-8639 Japan
Phone: +81-3-5351-6464 Fax: +81-5351-6463
E-mail: kawakosi@ori.u-tokyo.ac.jp

EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Natriuretic peptides (NPs) comprise a hormone family that plays a pivotal role in ion and water regulation (Takei, 2000). In tetrapods, three types of NPs are identified; endocrine ANP and BNP secreted from the heart, and paracrine CNP synthesized mostly in the brain. In teleost fish, which are osmoregulators that maintain plasma osmolality at ca. 1/3 of seawater (SW) like tetrapods, three peptides are also identified, endocrine ANP and VNP and paracrine CNP. In elasmobranchs, which are partial osmoconformers that increase plasma osmolality to the SW level by urea and trimethylamine oxide, only CNP is present in the heart and brain (Kawakoshi et al., 2001). It seems therefore that CNP is the ancestral form of the NP family, which has become more complicated in vertebrate in parallel with the evolution of osmoregulatory mechanisms. If this is the case, which type of NPs is present in the hagfish, the most primitive extant vertebrate that is a full osmoconformer? It is also of interest to examine how many types of NPs exist in the sturgeon, a most primitive bony fish that is a full osmoregulator. In the present study, we cloned cDNAs encoding NPs from the heart and brain of hagfish and sturgeon to depict a clearer picture for the evolution of the NP family in fishes.

Materials and Methods

The hagfish, *Eptatretus burgeri*, were collected in Koajiro Bay, Kanagawa, and the sturgeon, *Acipenser transmontanus*, were purchased from a commercial source. For tissue sampling, each species was anaesthetized, and the heart and brain were dissected out and frozen quickly in liquid nitrogen. The rapid amplification of cDNA ends (RACE) method was performed for cloning of NP cDNAs. Five degenerate sense primers corresponding to the conserved region of known NP peptides were used for the 3'-RACE. Eel ANP, VNP and CNP were amplified by the primer pairs. For 5'-RACE, specific antisense primers were designed based on the NP cDNA sequences amplified by the 3'-RACE. In order to amplify minor NPs, ab… ased method an… evious paper (K…

```
Human     ANP    SLRRSSCFGGRMDRIGAQSGLGCNSFRY
          BNP  SPKMVQGSGCFGRKMDRISSSSGLGCKVLRRH
          CNP      GLSKGCFGLKLDRIGSMSGLGC

Eel       ANP     SKSSSPCFGGKLDRIGSYSGLGCNSRK-NH2
          VNP      KSFNSCFGTRMDRIGSWSGLGCNSLKNGTKKKIFGN
          CNP      GWNRGCFGLKLDRIGSLSGLGC

Sturgeon  ANP      RGSSGCFGSRIDRIGSMSSMGCGGSRK-NH2
          VNP       SMNGCFGNRIERIGSWSSLGCNNSRFGSKKRIF
          CNP      QQGRGCFGMKLDRIGSMSGLGC
     novel NP    NTKRYSGCFGRRLDRIGSMSALGCNGGSRLSYKRS

Dogfish   CNP      GPSRGCFGVKLDRIGAMSGLGC

Hagfish   NP     LGSTSDGCFGVKMDRIGASTGLGCRGARRRTFS-NH2
```

Figure 1. Natriuretic peptides identified in mammals (human), teleost fish (eel) and elasmobranchs (dogfish). Sequences of sturgeon and hagfish were obtained in this study. Lines show a S-S bond.

Re…

Or… duced amino aci… ig. 1). After rer… Thus, hfNP ma… identified in ela… akoshi et al., 20… ing the issue of…

Together with the present data, we can depict a new phylogenetic tree on the molecular evolution of the NP family (Fig. 2). In hagfish and elasmobranchs, only one NP, hfNP or CNP, has been identified. Hagfish has plasma ionic concentration almost identical to SW and thus need no osmoregulation and ionoregulation. Elasmobranchs has plasma isosmotic to SW but its plasma ionic concentration is much lower than SW. Therefore, they need ionoregulaton but no osomoregulation. Accumulating data indicate that the NP family is essential for adaptation of teleost fish to diverse salinity environments (Takei and Hirose, 2002). It seems therefore that the NP family is not so diversified in animals whose osmoregulatory mechanisms are not well developed. It is known that the sturgeon is a euryhaline species that lives in the estuarine area where dramatic salinity changes occur every day. It seems relevant that four NPs are present in this euryhaline species. It should be determined how many NPs are present in the lamprey, which is another cyclostome species but has highly developed osmoregulatory mechanisms.

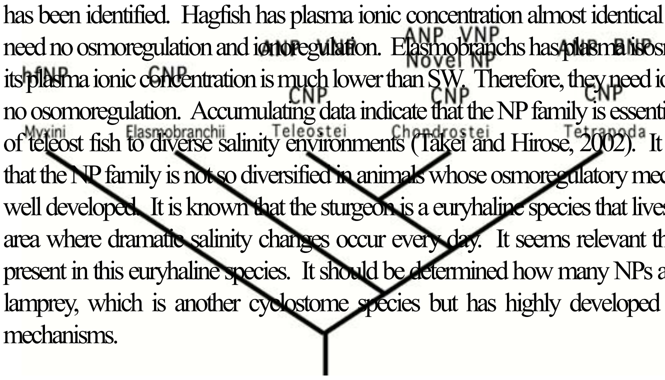

Figure 2. Phylogenic distribution of natriuretic peptides in vertebrates.

Acknowledgements

We thank Misaki Marine Biological Station, the University of Tokyo, for collecting hagfish. We are also grateful to Dr. Y. Takagi of Hokkaido University for helping us sample sturgeon tissues.

References

Kawakoshi, A., Hyodo, S. and Takei, Y. 2001. CNP is the only natriuretic peptide in an elasmobranch fish, *Triakis scyllia*. Zool. Sci. 18: 861-868

Takei, Y. 2000. Structual and functional evolution of the natriuretic peptide system in vertebrats. Int. Rev. Cytol. 194: 1-66

Takei, Y. and Hirose, S. 2002. The natriuretic peptide system in eels: a key endocrine system for euryhalinity? Am. J. Physiol. Regulatory. Integrative. Comp. Physiol. 282: R940-R951