IMMUNOHISTOCHEMICAL LOCALISATION OF NATRIURETIC PEPTIDES* IN TISSUES OF TELEOST, ELASMOBRANCH, AND CYCLOSTOME FISH

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The distribution of natriuretic peptide immunoreactivity was determined in the heart and brain of dogfish, Squalus acanthias, hagfish, Myxine glutinosa, and in the heart only of skate, Raja erinacea, flounder, Pseudopleuronectes americana and sculpin, Myoxocephalus octodecimspinosus. Heart tissues were fixed in 15% saturated picric acid and 4% formaldehyde in phosphate-buffered saline (PBS) at pH 7.4 for 16-24 h at 4 °C. Following fixation, tissues were washed in 80% ethanol to remove excess picric acid and dehydrated through an alcohol series, incubated in xylene, and rehydrated to PBS. Tissues were stored in PBS containing 20% sucrose, 3% polyethylene glycol (MW 400) and 0.1% sodium azide. Brains were removed and immersion-fixed in ice-cold 4% paraformaldehyde in 0.1M phosphate buffer at pH 7.4 for 1 h and then postfixed overnight in the same fixative. The brains were washed for 24 h in PBS containing 15% sucrose, and stored as above. Frozen sections were cut on a cryostat. Immunohistochemical staining was performed with the avidin-biotin-peroxidase complex (ABC) using an ABC kit (Vector Labs, Burlingame, CA, USA). Three antibodies were used: one raised against porcine brain natriuretic peptide (pBNP) which cross-reacts with rat atrial (rANP) and porcine C-type (pCNP) natriuretic peptides (termed natriuretic peptide-like immunoreactivity, NP-LI); the second raised against pBNP which cross reacts with CNP, but not ANP (termed pBNP-immunoreactivity, pBNP-LI); the third raised against rANP (termed rANP-like immunoreactivity, rANP-LI). The specificity of the immunohistochemical reactions was determined by incubating each antisera with either rANP (Bachem, CA, USA), pBNP (Peninsula, CA, USA), or pCNP (Peninsula, CA, USA). The antisera were incubated with 7-10 μg of each peptide at the working dilution for 24 h at 4 °C.

NP-LI and pBNP-LI, but not rANP-LI were observed in the heart of flounder and sculpin. In the shark and skate heart, NP-LI was observed in all cardiocytes in the atrium, but, only in a few cardiocytes in the ventricle, adjacent to the epicardium. No rANP-LI or pBNP-LI was observed in any cardiocytes of the shark and skate heart, and no immunoreactivity of any type in the branchial and portal hearts of hagfish. An extensive distribution of NP-LI perikarya and fibres was found in the brain of shark and hagfish (Table 1 for anatomical locations). Furthermore, in both species pBNP-LI perikarya and fibres were present in many areas which showed NP-LI but in a lower density. (Table 1). No rANP-LI immunoreactivity was found in the brain of shark and hagfish.

The observation of pBNP-LI in the heart of flounder and sculpin indicates that a pBNP/pCNP-like peptide is present in the heart, and could be released into the circulation to affect osmoregualtory tissues such as the gill, gut, and kidney. The more extensive distribution of NP-LI in the heart probably suggests the presence of an ANP-like peptide structurally different to the epitope recognised by the rANP antiserum. Since CNP has been demonstrated in the heart of Squalus (Schofield et al. 1991, Am. J. Physiol. 264: F734-F739), the presence of NP-LI in the heart shows that the antisera cross reacts with either native shark CNP or a natriuretic peptide different from rANP or pBNP. Although the chemical structure of natriuretic peptides in the brain of shark and hagfish are unknown, these observations show that a component of the natriuretic

* The term natriuretic peptide is used to describe the family of peptides which includes atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP)

peptide complement is similar to pBNP or pCNP. The presence of natriuretic peptides in the brain suggest they could be important neuromodulators and/or neurotransmitters. Furthermore, there appears to be divergence in the structural forms of natriuretic peptides in the heart and brain of shark and hagfish.

Table 1. Distribution of NP-LI and pBNP-LI in the brain of shark and hagfish*

SHARK			HAGFISH		
Brain Region	**NP-LI**	**pBNP-LI**	**Brain Region**	**NP-LI**	**pBNP-LI**
Telencephalon			**Telencephalon**		
-olfactory bulbs	++	+	-olfactory bulbs	+	-
-subpallium	++	+	-pallium (layer 2)	+	±
-area superficialis basilis	+++	++	-prim. hippocampi	+++/p	+/p
-n. septi medialis	+++/p	++/p			
-area periventricularis	+++/p	-	**Diencephalon**		
-basal forebrain bundle	+++	+	-pars ventralis th.	++/p	+/p
-n. septi caudoventralis	+++	-	-n. diffusus hypoth.	++/p	+/p
-n. septi caudodosrsalis	+++	-	-pars dorsalis th.	++/p	±/p
			-hypoth.	+	±
Diencephalon			-n. tuberculi post.	++/p	+
-preoptic area	+++/p	++/p	-n. profundus	++/p	+
-ventral/dorsal th.	++/p	+/p			
-habenula	+	±	**Mesencephalon**		
-rostral hypoth.	++	+	-tectum	++	+
-optic nerves	-	-	-tegmentum	+	±
-neurointermediate lobe	++				
-adenohypophysis	-	-	**Rhombencephalon**		
			-n. vent tegementi	++/p	+/p
Mesencephalon			-lateral fibre tracts	++	+
-tectum mesencephali.	++	±			
-tegmentum	+	±	**Spinal cord**		
			-ventral/dorsal tracts	+	±
Cerebellum	-	-			
Rhombencephalon					
-griseum centrale	++/p	±/p			
-reticular formation	++	±			
Spinal cord					
-cornu ventrale	+	±			
-funiculus dorsalis	+	±			

hypoth. hypothalamus; nucleus; post. posteriosis; prim. primordium; th. thalamus.
* - no immunoreactive structures; ±, sparse; +, sparse to moderate; ++, moderately dense; +++, highly dense; p, perikarya present.

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