The distribution and nature of natriuretic peptide receptors (NPR) in the gills of dogfish, Squalus acanthias, were examined by tissue section autoradiography, competition analysis, protein electrophoresis, guanylate cyclase (GC) assays, and molecular cloning. Specific NP binding occurred on the gill filaments, but not on the interbranchial septum or gill arch. The binding was densest on the efferent edge of the gills. Higher resolution light-microscopic examination of emulsion-coated sections showed that specific binding occurred mainly on the secondary lamellae and filament body and not on the arterial circulation. At least two types of NPR were revealed. One is linked to GC since NP binding stimulates the production of cGMP. The GC receptor may be similar to the NPR-B mammalian receptor since only pCNP stimulated cGMP production. The second receptor is not linked to GC and binds the specific ligand C-ANF [rat des(Gln18, Ser19, Gly20, Leu21, Gly22)]. The sequence of a cDNA generated using primers based on conserved regions of vertebrate NPR-C had considerable homology with mammalian and eel NPR-C and eel NPR-D. The presence of GC-linked NPR and NPR-C/NPR-D suggests that the gills are an important target organ for NP action.

The heart of mammals contains two natriuretic peptides (NPs), namely atrial natriuretic peptide (ANP; Flynn et al., 1983) and brain natriuretic peptide (BNP; Sudoh et al., 1988). The third peptide of the family, C-type natriuretic peptide (CNP; Sudoh et al., 1990), was initially thought to be a neuropeptide of the central nervous system, but recent studies have shown that it is expressed in a range of nonneural tissues of the periphery such as the rat heart (Vollmar et al., 1993) and the endothelium (Inagami et al., 1995). In contrast to the situation in mammals, CNP is the major cardiac NP in two species of elasmobranchs, Squalus acanthias (Schofield et al., 1991) and Triakis scyllia (Suzuki et al., 1991, 1992), and proCNP circulates in the plasma of T. scyllia (Suzuki et al., 1994).

There are three receptors for NPs which are variously expressed in a large range of mammalian tissues (Ruskoaho, 1992). Two NPR receptors (NPR) possess an intracellular guanylate cyclase (GC) domain, and binding of NPs stimulates the production of cGMP. However, the receptors have selective affinity for the different NPs: NPR-A is primarily activated by ANP and BNP whereas NPR-B is primarily activated by CNP. The third receptor, NPR-C, is a unique receptor that binds all NPs with high affinity but does not have an intracellular GC domain. A unique feature of the NPR-C is the ability to bind C-ANF, a truncated, ring-deleted peptide that does not bind to the GC receptors. This characteristic has been an important tool for describing NPR-C in a range of vertebrate tissues. It is generally considered that the NPR-C...
functions as a mechanism for clearing NPs from the circulation and tissues by internalization, since the receptor is distributed extensively in the vascular endothelium and most tissues in which NPR-A or NPR-B occurs. However, there is a growing database which shows that the NPR-C is coupled to the adenylyl cyclase and IP₃ signal transduction pathways in different tissues (Anand-Srivastava and Trachte, 1993). Recently, three types of NPR have been cloned from the Japanese eel, *Anguilla japonica*, an NPR-B (Katafuchi et al., 1994), an NPR-C (Takashima et al., 1994), and a new NPR which is similar to NPR-C and has been termed NPR-D (Kashiwagi et al., 1995). Similarly to NPR-C, NPR-D binds C-ANF and does not show GC activity.

In recent years, the biological role of NPs in the physiology of fish has received much attention and NPs have been shown to affect vascular smooth muscle and gill epithelial function in a variety of species (Evans and Takei, 1992). In elasmobranchs, high-affinity CNP receptors mediate vasodilatation in the branchial arteries of *Scyliorhinus canicula* (Bjenning et al., 1992) and the ventral aorta of *S. acanthias* (Evans et al., 1993) and potently stimulate chloride efflux from the rectal gland of *S. acanthias* (Solomon et al., 1992). Gunning et al. (1993) have characterized the NPR in the rectal gland of *S. acanthias* and found that the high-affinity receptor that bound CNP and stimulated cGMP was similar to NPR-B, but had some distinctive features. In addition to a GC receptor, the rectal gland contained a low-molecular-weight receptor that bound C-ANF and was concluded to be similar to mammalian NPR-C.

The control of gill blood flow and epithelial function in fish is critical in osmoregulatory and cardiovascular homeostasis since all blood pumped from the heart must pass through the gill vasculature. Many studies have shown that both neuronal and hormonal mechanisms regulate the vascular smooth muscle and the epithelium of teleost fish (Nilsson and Holmgren, 1993). In contrast, the gills of elasmobranch fish are not innervated by autonomic nerves (Nilsson and Holmgren, 1993) and, therefore, regulation of gill function must occur by humoral and/or local paracrine mechanisms alone. The presence of CNP in the heart of elasmobranchs suggests that this peptide could be an important regulator of gill function. In the present study we have examined the distribution and nature of NPR in the gills of the spiny dogfish, *S. acanthias*, to determine the role of NPs in gill function. These studies were performed using the following techniques: tissue section autoradiography to determine anatomical locations; *in vitro* receptor binding assays to determine kinetics and specificity of the sites; affinity cross-linking followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) to determine molecular weights of receptor proteins; GC assays to determine if binding sites in the gills are GC-linked receptors; and molecular analysis of putative NPR-C. In this study, iodinated porcine CNP (pCNP) was used as a ligand since it has been shown that pCNP and *S. acanthias* CNP bind to NPR in the ventral aorta and rectal gland with equal affinity (Evans et al., 1993; Gunning et al., 1993).

**MATERIALS AND METHODS**

**Animals**

Spiny dogfish *S. acanthias* (3–5 kg) were netted in Frenchman Bay, Maine, and were maintained in live cars with running sea water (12–15°) at the Mount Desert Island Biological Laboratory, Salisbury Cove, Maine. Animals were sacrificed by pithing, and the required tissues were removed and used immediately or were frozen in liquid N₂ and shipped to the University of Florida.

**Autoradiography**

For autoradiography, gills were freeze mounted in Tissue Tek (Miles Inc., Elkhart, IN) in a microtome cryostat (Minotome, IEC, Needham Heights, MA), and 20-µm sections were cut and mounted on gelatin-chromium-aluminum-coated slides and dried overnight under vacuum at 4°. The sections were used immediately or were stored in sealed boxes at −20° until used.

Preincubation of the gill sections was performed at room temperature (22–24°) for 15 min in 50 mM tris-(hydroxymethyl)aminomethane (Tris)·HCl buffer (pH 7.4), 50 mM NaCl, 5 mM MgCl₂, 0.1% bovine serum albumin (BSA), and 0.05% bacitracin. This was
followed by incubation for 60 min in the same buffer supplemented with 4 µg/ml of leupeptin, 2 µg/ml chymostatin, 2 µg/ml pepstatin, $10^{-6}$ M phenylmethylsulfonyl fluoride (PMFS), and porcine $^{125}$I-Tyr$^4$-CNP (termed $^{125}$I-pCNP here). Displacement of specific binding was examined in consecutive sections in the presence of 1 µM porcine CNP (pCNP), 1 µM Tyr$^4$-CNP (the peptide used for iodination), 1 µM rat (3–28) ANP, and 1 µM rat C-ANF, the truncated ANP that binds only to NPR-C of mammals (Maack, 1992). To test the specificity of C-ANF in S. acanthias, a set of preliminary pharmacologic experiments was performed with vascular smooth muscle of the ventral aorta. In this tissue, C-ANF did not cause vasodilatation and did not act as an antagonist to pCNP-facilitated relaxation by prior occupation of the receptor protein which mediated this response (Evans et al., 1993). Therefore, it is probable that C-ANF can be used as a NPR-C-specific ligand in the shark gill.

After incubation, the slides were washed (2 × 10 min at 4°) in preincubation buffer, fixed for 20 min in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4, 4°), washed in 0.1 M phosphate buffer (pH 7.4, 4°), and then in distilled water (1 min) and dehydrated in alcohol and dried overnight at 60°. The sections were then apposed to Hyperfilm-$\beta$max for 5–21 days at room temperature. The film was developed in Kodak GBX developer for 4 min, washed in water for 2 min, and fixed in Kodak GBX fixer for 5 min.

For higher resolution autoradiography, sections were dipped in nuclear track emulsion (Kodak NTB.2) at 43°. After drying the sections were stored at 4° for 7–14 days and then developed in Kodak D 19 for 3 min, washed in water, and fixed in Kodak Rapid Fixer diluted 1:1 for 7 min. Subsequently they were stained in 1% toluidine blue. Sections were viewed under an Olympus BH-2 microscope, and photomicrographs were taken with a Wild Leitz MPS 46 Photoautomat camera using Kodak T-max 100 black and white film. Specific binding was determined by comparing the silver grain densities of sections incubated with $^{125}$I-pCNP alone to those of sections incubated with $^{125}$I-pCNP and 1 µM cold ligand.

**In vitro NP Binding Assays**

The gill arch was dissected off and the gill filaments and interbranchial septum were placed in a 50-ml centrifuge tube in 10 vol of 50 mM Tris and 1 mM NaHCO$_3$ (pH 7.4) and homogenized with a Tissue-Tearor (Biospec, Bartlesville, OK) at setting 7 for 1 min. The homogenate was diluted 1:1 with 50 mM Tris, 1 mM EDTA, and 1 mM MgCl$_2$ (pH 7.4) and centrifuged at 800 g for 10 min at 4°. The supernatant was collected and centrifuged at 30,000 g for 20 min. The pellet was washed with 50 mM Tris (pH 7.4) and 250 mM sucrose and resuspended in the same buffer and the protein concentration was determined with a BCA protein assay kit, calibrated against BSA standards. Membranes were stored at −70° until use.

For competition binding assays, 25–50 µg protein of the gill membrane fraction was incubated in the same buffer used for the sections and 20–35 pM $^{125}$I-pCNP. Competition for the iodinated ligands was performed using the unlabeled peptides pCNP and C-ANF in increasing concentrations ($10^{-12}$–$10^{-6}$). The incubation volume was either 0.125 or 0.25 ml. Binding reactions were performed for 4 hr at 23°; preliminary experiments showed that maximal binding was achieved after 2 hr. Binding was terminated by dilution with 2 ml of ice-cold 50 mM Tris·HCl (pH 7.4) and bound ligand was separated from free ligand by filtration through 1% polyethylenimine-treated Whatman GF/C filters. Filters were washed with 5 ml of ice-cold 50 mM Tris·HCl (pH 7.4), and the radioactivity on the filter was determined in a Beckman gamma counter with 78% efficiency.

**Affinity Cross-Linking and Electrophoresis of Gill Membranes**

Membranes were isolated as previously described and incubated in binding assay buffer with $^{125}$I-CNP alone or in combination with 1 µM pCNP for 2 hr. Affinity cross-linking was performed according to Martin et al. (1989). Following incubation, the covalent cross-linking agent disuccimidyl suberate in dimethylsulfoxide was added to a final concentration of 1 mM and the incubation continued for 20 min at 4°. The cross-linking reaction was stopped by addition of an equal volume of quench buffer containing 400 mM EDTA and 1 M Tris·HCl (pH 6.8). Membranes were centrifuged in an Eppendorf centrifuge at 13,000 g for 20 min to separate unbound hormone, and the resulting pellet was suspended in 100 µl of sample buffer for SDS-PAGE, containing 62.5 mM Tris base, 2% SDS, 5%
glycerol, 0.01% bromophenol blue, 2% β-mercaptoethanol, pH 6.8, and then boiled for 3 min. The solubilized membranes were again centrifuged at 13,000g to remove particulate matter, and electrophoresis was performed on a 7.5% unidimensional polyacrylamide slab gel using a mini-gel apparatus and stained with Coomassie brilliant blue (Bio-Rad, Richmond, CA). The gels were dried and apposed to Hyperfilm MP for 1 week. Identification of the molecular weights of the labeled bands was done by use of reference standards (30,000–200,000) on the Coomassie blue-stained gel. A linear relationship between the relative mobility (Rf) and the log relative molecular mass of the standards was constructed and the unknown values were calculated.

Guanylate Cyclase (GC) Assays

Gill membranes were isolated and the protein concentration was determined. Membranes were kept on ice until needed. For determination of GC activity, 50 µg of gill protein was added to 50 mM Tris·HCl, 2 mM isobutyl methylxanthine (IBMX), 10 mM creatinine phosphate, 100 U/ml creatine phosphokinase, 4 mM MnCl₂, 1 mM GTP, and increasing concentrations of pCNP in a final volume of 100 µl. The basal rate of cGMP generation was determined in tubes without ligand. The incubations were performed for 15 min at 24°C and were terminated by the addition of 4 mM EDTA. The tubes were boiled for 3 min and centrifuged at 2300g for 15 min. The supernatant was collected and frozen until the cGMP content was determined by radioimmunoassay.

Molecular Cloning

Gill total RNA was isolated by the acid-phenol technique (Chomczynski and Sacchi, 1987), and mRNA was selected using magnetic separation (Polyattract mRNA Isolation System, Promega). Double-stranded cDNA was synthesized using the Promega Riboclon system. Primers based on conserved regions identified from aligned bovine, human, and eel NPR-C cDNA sequences were used for polymerase chain reaction (PCR), and computer analysis predicted amplification of a 470-base pair (bp) product. PCR was carried out using the following 100-µl reaction mixture: 1× PCR buffer, dNTPs, 0.8 µM forward and reverse primers, 2.5 U AmpliTaq, 10 mM MgCl₂, and 50 ng gill cDNA. An initial cycle of 3 min at 95°C, 2 min at 45°C, and 2 min at 72°C was followed by 34 cycles of 1 min at 95°C, 2 min at 45°C, and 2 min at 72°C. Following the last cycle, the PCR products were subject to blunt-ending by the addition of 2.5 U Pfu polymerase (Stratagene) and incubation at 72°C for 15 min. Ninety microliters of the PCR was run on a 1% agarose gel and an approximately 470-bp product was excised and the cDNA recovered using glass milk (Bresaclean, Bresatex). The cDNA was cloned into a pCRscript vector (Stratagene) and sequenced on an Applied Biosystems automated sequencer (Westmead Hospital, Sydney). The sequence was analyzed using BLAST (National Centre for Biotechnology Information).

Data Analysis

Binding data were analyzed using the EBDA and LIGAND computer programs (McPherson, 1985). Analysis of variance was performed using Statview SE on a Macintosh computer.

Materials

Hyperfilm-βmax, Hyperfilm MP, and the cGMP [3H] assay system were purchased from Amersham (Arlington Heights, IL). Porcine 125I-Tyr⁴-CNP (1400–1600 Ci/mmol) and porcine Tyr⁴-CNP were obtained from Peninsula (Belmont, CA). Rat ANP (3–28), porcine CNP, and C-ANF [rat des(Gln¹⁸, Ser¹⁹, Gly²⁰, Leu²¹, Gly²²)ANP-(4-23)-NH₂] were obtained from Bachem (Torrance, CA). The BCA protein assay kit was purchased from Pierce (Rockford, IL). GTP and IBMX were purchased from Sigma. All PCR reagents were purchased from Perkin–Elmer. All other chemicals were reagent grade.

RESULTS

 Autoradiographic Studies

Autoradiograms of gill sections showed high-density 125I-pCNP binding on the gill filaments (Figs. 1 and 2), but not on the interbranchial septum or gill arch. Binding was distributed over the filaments along
the entire length of each hemibranch and showed a gradation in density such that the binding was densest on the efferent (relative to blood flow) edge of the gills. The specific binding of both radioligands was displaced by incubation of sections with 1 µM pCNP (Fig. 2), 0Tyr-CNP, rANP, or C-ANF. Higher resolution light-microscopic examination of emulsion-coated sections showed that specific binding of 125I-pCNP occurred mainly on the secondary lamellae and filament body, with little, if any, specific binding being found on the afferent filamental artery, corpus cavernosum, afferent lamellar arterioles, efferent lamellar arterioles, efferent filamental artery, and the branchial arteries.

**Characterization of NP-Binding Sites**

The binding sites in the gills were analyzed by competitive radioligand binding assays, electrophoresis of membrane proteins affinity cross-linked to radioligand, and determination of GC activity.

**Competition studies.** The ability of the peptides pCNP and C-ANF to compete for the 125I-pCNP bind-
ings sites was determined with competitive radioreceptor assays. The relative IC₅₀ values for percentage displacement were as follows: 1 nM for pCNP and 0.4 nM for C-ANF. pCNP displaced all the binding; however, about 10% of residual binding was observed at 1 µM C-ANF (Fig. 3). The mean dissociation constant (Kₐ) and receptor density (Bₘₐₓ) were 1.98 ± 0.56 nM and 987.5 ± 187.1 fmol/mg protein, respectively.

**Electrophoresis.** Affinity cross-linking of ¹²⁵I-pCNP to gill membranes and subsequent SDS–PAGE showed a single band at approximately 80 kDa; this binding was displaced by 1 µM pCNP (Fig. 4).

**Stimulation of cGMP production by pCNP.** pCNP stimulated cGMP in a dose-dependent manner. Significant increases (P < 0.05) in cGMP production above basal level were observed at 0.1 and 1 µM pCNP (Fig. 5). rANP and C-ANF did not stimulate cGMP production (Fig. 5).

**Molecular Cloning**

PCR amplification using NPR-C-specific primers produced a 468-bp product as predicted; the cDNA sequence and deduced amino acid sequence are shown in Fig. 6. Computer analysis of the nucleotide sequence
showed that it was homologous to bovine NPR-C (60%), eel NPR-C (49%), and eel NPR-D (51%). Alignment of the six-frame translation of the nucleotide sequence against a protein database generated an aminosequence that was homologous to bovine NPR-C (65%), eel NPR-C (51%), and eel NPR-D (53%). The putative shark NPR-C amino acid sequence contained two cysteine (C) residues and an N-glycosylation site which are conserved in bovine and eel NPR-C and eel NPR-D (Fig. 7).

FIG. 6. Nucleotide (top line) and deduced single-letter amino acid sequence of the 468-bp PCR product generated using NPR-C specific primers; the amino acid sequences used for primer design are underlined. The nucleotides are numbered on the right side.

FIG. 7. Alignment of the putative shark NPR-C/D amino acid sequence with bovine NPR-C (bNPR-C), eel NPR-C (eNPR-C), and eel NPR-D (eNPR-D), using single-letter codes. The numbers on the left side correspond to the amino acid sequence of the complete mRNA of the bovine and eel receptors. Identical amino acids are shown by asterisks. The conserved cysteine (C) residues and N-glycosylation sites are shown in boldface and underlined respectively.
DISCUSSION

The gills are the major site of osmotic water influx in elasmobranchs because the plasma is hyperosmotic to seawater and the gill epithelium is very permeable to water (Evans, 1993). The net flux will be determined by a combination of the permeability of the gill epithelium and the blood flow profile of the gill which determines the amount of plasma available for molecular exchanges at the epithelium. The gills of elasmobranchs lack any autonomic neural elements (Nilsson and Holmgren, 1993); therefore, vascular and epithelial regulation must occur through endocrine, paracrine, or autocrine mechanisms. For example, the presence of a circulating catecholamine system in elasmobranchs is well established as are the effects of amines on the branchial circulation (Nilsson and Holmgren, 1993). It follows that CNP released from the heart (Schofield et al., 1991; Suzuki et al., 1991, 1992), or possibly the vascular endothelium (Inagami et al., 1995), could have important actions on vascular and epithelial effector tissues that affect branchial exchanges and contribute to blood volume homeostasis.

Recently, the presence of CNP in the plasma has been established in *T. scyllia* (Suzuki et al., 1994) and it is assumed that the source of this peptide is the heart and that CNP circulates in the plasma of other species of elasmobranchs. Several studies have shown that CNP has quite potent effects in organs important in salt and water balance. For example, CNP stimulates chloride secretion in the rectal gland epithelium of *S. acanthias*, indicating an epithelial action of the hormone in this tissue (Solomon et al., 1992). In addition, the vascular smooth muscle of the ventral aorta of *S. acanthias* (Evans et al., 1993) and the afferent branchial artery of *S. canicula* (Bjenning et al., 1992) is relaxed by low concentrations of CNP without prior preconstriction, suggesting that CNP is involved in the control of gill hemodynamics. Our study provides evidence that two types of CNP receptors are present in the gills of *S. acanthias*, which could be important in regulation of the gill vasculature and/or the branchial epithelium.

The 125I-pCNP binding in the gills, as shown by autoradiography, was displaced by 1 µM unlabeled pCNP, Tyr-CNP, rANP, and the NPR-C-specific ligand, C-ANF. This observation suggests that an NPR-C-like receptor is the sole NPR in the branchial tissues. However, subsequent experiments provided evidence that two types of NPR are present in the gills: one receptor linked to GC and a receptor which binds C-ANF and is homologous to vertebrate NPR-C and eel NPR-D (termed NPR-C/NPR-D hereafter).

The evidence for a GC receptor is primarily based on the experiments in which CNP stimulated the production of cGMP in gill membranes in a dose-dependent manner. In parallel assays, both rANP and C-ANF failed to stimulate cGMP, indicating the presence of a receptor whose intracellular GC domain is activated only by CNP binding. These data are very similar to the findings with rectal gland tissues which showed that only CNP stimulated the production of cGMP (Gunning et al., 1993). Further evidence for a small population of GC receptors was obtained from the competition binding assays in which CNP did not completely displace the specific binding compared to the displacement by CNP (see Fig. 5); the specific binding not displaced by C-ANF may represent GC receptors. SDS–PAGE of gill membranes affinity cross-linked to 125I-pCNP failed to show a protein of a molecular weight in the range of GC receptors (130–150 kDa), which may be due to the small number of GC receptors. It seems, therefore, that a GC receptor specific for CNP is present in dogfish tissues. However, until cloning of a GC receptor from *S. acanthias* is performed it will not be possible to conclude whether those receptors are NPR-A or NPR-B.

The anatomical location of the gill GC receptors, represented by the small proportion of binding sites that do not bind C-ANF, could not be determined with autoradiography. The autoradiographical binding data would suggest that the GC receptors reside on the filament epithelium and secondary lamellae. However, it is also possible that GC receptors occur on vascular endothelium or vascular smooth muscle cells but were not discerned with the autoradiography technique used in this study. Interestingly, a number of studies using mammalian tissues have demonstrated cGMP activity in tissues, although no binding sites for GC receptors were visualized (Leitman and Murad, 1990). In addition, the afferent branchial artery of *S. canicula* is relaxed by low concentrations of CNP (Bjenning et al., 1992), which suggests that GC receptors may reside on similar vessels of *S. acanthias*.

The second type of NPR in the shark gill is an
plasma concentration of the hormone. Interestingly, the gills could possibly influence the downstream and, therefore, the regulated expression of NPR-C in through the gills prior to reaching more distal tissues, (1993). In fish, NPs released from the heart will pass ing that NPR-C was binding the ANP (Olson and Duff, 1993). Further evidence for the presence of an NPR-C/ NPR-D in shark gills was obtained with the use of C-ANF, a truncated NP which binds to specifically to NPR-C (Maack, 1992) and eel NPR-D (Kashiwagi et al., 1995). In both autoradiography and competition displacement experiments, C-ANF displaced the majority of binding. In addition, affinity cross-linking of 125I-pCNP to gill membranes followed by SDS–PAGE under reducing conditions revealed a band of approximately 80 kDa, which parallels that of reduced mammalian and eel NPR-C and eel NPR-D. There is, therefore, strong evidence for the presence of an NPR-C/NPR-D in shark gill tissues.

Emerging evidence shows that C-ANF-binding sites are present in a range of fish tissues; in particular, they are abundant in the gills (Donald et al., 1994; Takashima et al., 1994; Toop et al., 1995). In the rectal gland of S. acanthurus, C-ANF was able to displace about 50% of CNP binding, indicating the presence of a NPR-C-like receptor in that tissue (Gunning et al., 1993). NPR-C has been cloned from the gill of the Japanese eel, A. japonica (Takashima et al., 1994), and NP ligand binding and electrophoretic studies strongly supported the presence of NPR-C-like receptors in the gills of toadfish, Opsanus beta (Donald et al., 1994). The consistent presence of NPR-C in the gills of fish raises interesting questions about their function. Mammalian NPR-C binds and internalizes NPs for metabolic degradation (Anand-Srivastava and Trachte, 1993); however, such a mechanism has not yet been demonstrated in fish. Whole animal studies using rainbow trout, Oncorhynchus mykiss, showed that the gills had an enormous capacity to bind circulating ANP and that this effect was reversed by prior treatment with C-ANF, indicating that NPR-C was binding the ANP (Olson and Duff, 1993). In fish, NPs released from the heart will pass through the gills prior to reaching more distal tissues, and, therefore, the regulated expression of NPR-C in the gills could possibly influence the downstream plasma concentration of the hormone. Interestingly, distension of the right atrium of mammals is the major stimulus for ANP release into blood flowing to the lungs, in which there is a preponderance of NPR-C on the vascular endothelium (Anand-Srivastava and Trachte, 1993). It seems, therefore, that in both fish and mammals significant changes in the plasma concentration of circulating NPs could occur prior to reaching target tissues in the periphery. In fish, such a mechanism may be present to compensate for excessive release of NPs due to large variations in the inotropic state of the heart associated with activities such as exercise (see e.g., Cousins and Farrell, 1996).

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