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The response of non-traditional natriuretic peptide production sites to salt and water manipulations in the rainbow trout

Keven R. Johnson[1,2] and Kenneth R. Olson[1,2,*]

[1]University of Notre Dame-Department of Biological Sciences, Notre Dame, IN 46556, USA and [2]Indiana University School of Medicine, South Bend Center, South Bend, IN 46617, USA

*Author for correspondence (kolson@nd.edu)

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SUMMARY

Natriuretic peptides (NPs) and their receptors (NPRs) comprise an evolutionarily conserved signaling system with profound physiological effects on vertebrate renal and cardiovascular systems. Some NPs (ANP, BNP and VNP) are primarily of cardiac origin whereas CNP is common in the brain. In mammals, non-traditional sites of NPs synthesis, BNP in brain and CNP in atrium, appear to have complementary actions. In the present study, trout were chronically adapted to freshwater (FW) (a volume-loading, salt-depleting environment), saltwater (SW) (a volume-depleting, salt-loading environment), FW and fed a high-salt diet (FW–HSD) (a volume- and salt-loading regime) or acutely volume depleted or expanded by hemorrhage or infusion with dialyzed plasma to perturb volume homeostasis. The responses of brain and atrial BNP and CNP mRNA, pro-peptide, NPR-A and NPR-B were evaluated using quantitative PCR and western analysis. Brain pro-BNP and NPR-A was increased in FW–HSD trout and decreased in SW trout. Brain pro-CNP was largely unaffected whereas NPR-B mRNA was increased in FW–HSD trout. Atrial CNP, although produced at lower levels than other cardiac NPs, was markedly elevated in chronically (FW–HSD) and acutely volume expanded trout (dialyzed-plasma infusion) whereas decreased in hemorrhaged trout. These findings indicate that non-traditional NP synthesis sites in the trout probably complement the broad hypovolemic and hypotensive actions of traditional (cardiac) NP synthesis sites in response to volume expansion but not to plasma osmolarity. This supports the hypothesis that the piscine and mammalian NP systems are fundamentally similar and appear to protect the heart from volume overload.

Key words: cardioprotection, BNP, CNP, volume regulation.

INTRODUCTION

Natriuretic peptides (NPs) are a family of potently bioactive hormones that exert physiological effects on the renal and cardiovascular systems of vertebrates (Donald and Trajanovska, 2006; Johnson and Olson, 2008; Takei, 2001; Toop and Donald, 2004). The members of the NP family common between mammals and fish are atrial natriuretic peptide (ANP), brain natriuretic (BNP) and c-type natriuretic peptide (CNP). An additional NP unique to teleosts, ventricular natriuretic peptide (VNP), has been identified in the sturgeon, bichir, eel and trout (Ventura et al., 2006). Spanning the vertebrate phyla, NPs are predominantly of cardiac origin (Inoue et al., 2005; Takei, 2000), with minor extra-cardiac expression found in the brain, CNP is perhaps the most notable exception as it is found largely in the brain and to a lesser extent in the atrium (Del Ry et al., 2008; Inoue et al., 2003; Takei et al., 2001). In addition, ANP and CNP are both found in the vasculature of rats and humans (Kelsall et al., 2006; Woodard et al., 2002). The transmembrane guanylate–cyclase-linked NP receptors (NPRs) are represented by NPR-A (the principal target for ANP and BNP) and NPR-B (the principal target for CNP) and are ubiquitously distributed throughout the cardiovascular system and central nervous system (CNS) of mammals and fish (Johnson and Olson, 2009; Potter et al., 2009).

In addition to the traditional production sites of NPs (i.e. ANP and BNP in heart, CNP in the brain), the responses of non-traditional NP production sites (i.e. ANP and BNP in the brain, CNP in the heart) generally complements the physiological response of the traditional sites. For example, cardiac NPs and NPRs have vital homeostatic roles in non-cardiovascular tissues such as the CNS. In the CNS, local synthesis of NPs is largely confined to areas involved in water and salt balance such as the hypothalamus and pituitary (Morii et al., 1987; Saper et al., 1985; Standaert et al., 1988). Ultimately, the peripheral natriuretic effects are amplified by the central inhibition of salt appetite and water drinking, which complements the renal diuretic effects of the peptide (Blackburn et al., 1995; Burrell et al., 1991). Furthermore, ANP inhibits the secretion of vasopressin and, in some studies, corticotropin through effects on the brain and pituitary (Samson, 1992). Natriuretic peptides also act in the brain stem to decrease sympathetic tone (Schultz et al., 1990; Steele et al., 1991; Yang et al., 1992). Hence, even though ANP and BNP are predominantly of cardiac origin, their actions in the CNS reinforce those in the heart. The same principle holds true for CNP, as this predominantly brain-expressed NP has limited production in the atrium, yet in a similar fashion to other cardiac NPs, CNP is released in response to cardiac stretch (Del Ry et al., 2008). Collectively it is evident that each of the physiological effects of the NP system occurs through coordinated central and peripheral actions in controlling fluid and electrolyte homeostasis in mammals.

There are many biochemical and physiological similarities between the typical mammalian NP system and the NP system described in the rainbow trout (Farrell and Olson, 2000; Johnson and Olson, 2008; Johnson and Olson, 2009), yet the potential physiological roles of non-traditional NP production sites in hydromineral balance in a teleost model have not been reported. In the present study, we used chronic volume and salt manipulations including freshwater (FW) (a volume-loading, salt-depleting

environment), saltwater (SW) (a volume-depleting, salt-loading environment), FW and fed a high-salt diet (FW–HSD) (a volume- and salt-loading regime), as well as acute volume depletion by hemorrhage or volume expansion by infusion of dialyzed plasma to examine non-traditional sites of NP production (mRNA and pro-peptide) and receptors (NPR-A, NPR-B) in the brain (BNP) and heart (CNP). Because these protocols uniquely allow the separation of water and salt homeostasis, the primary stimulus of NP secretion can be identified.

MATERIALS AND METHODS

All animal experiments were approved by the institutional animal care and use committee.

Animals

Freshwater adult rainbow trout (*Oncorhynchus mykiss* Walbaum) were used for atria, ventricle and brain quantitative PCR and western blotting applications. Freshwater trout were purchased from a local hatchery and maintained in circulating 2000-l 14°C freshwater tanks under appropriate seasonal light:dark cycles. Fish were fed a maintenance diet of commercial trout pellets (Purina Mills, St Louis, MO, USA) for up to 48 h prior to experimentation.

Chronic SW adaptation

A maximum of 10–15 FW rainbow trout were adapted to SW (1000 mosmol) at one time in a 500-l Instant Ocean Culture System (model WM-500, Aquarium Systems, Eastlake, OH, USA) using synthetic sea salts supplied by the manufacturer. The fish were initially adapted to 300 mosmol SW for 3–5 days, and the osmolarity was gradually increased to full-strength SW over the next 2 weeks. They were maintained at this salinity for a minimum of an additional 2–3 weeks before experimentation. Temperature, pH, osmolarity and total ammonia were measured daily and, with the exception of osmolarity, were not different from FW.

Chronic FW–HSD adaptation

A maximum of 10–15 FW rainbow trout were placed in a 750-l flow-through tank in aerated well water (14°C) and fed a high-salt diet (FW–HSD) containing 12% NaCl at 2% total body mass per day, for a minimum of 3 weeks before experimentation as described previously (Johnson and Olson, 2009). Briefly, the HSD was prepared by crushing commercial trout pellets and mixing the powder with 12% by weight non-iodinized Kosher® salt. A limited amount of de-ionized water was added to this mixture and the paste was then squeezed through a potato ricer and dried at room temperature overnight. This HSD feeding regime has been shown to increase gill chloride cell numbers and Na^+/K^+-ATPase activity, dorsal aortic (DA) pressure, central venous pressure and blood volume in FW rainbow trout (Chen et al., 2007; Olson and Hoagland, 2008; Salman and Eddy, 1987).

Acute volume manipulations

Methods for cannulation of the DA have been described in detail (Olson et al., 1997). Briefly, trout were anesthetized in benzocaine (ethyl-p-aminobenzoate; 1:12,000 w:v) prior to surgery. The DA was cannulated percutaneously through the roof of the buccal cavity with heat-tapered polyethylene tubing (PE 60); the gills were not irrigated during this brief procedure. The following day, trout were either volume expanded with dialyzed plasma infusion (40% of estimated total blood volume, 35 ml kg^{-1} h^{-1}, for 2 h; 80% total volume expansion), an 80% total blood volume (TBV) continuous hemorrhage (20% TBV every 30 min for 2 h) or untreated and used as a cannulated control. The dialyzed plasma was obtained from a donor fish and dialyzed overnight at 14°C in dialysis tubing with a molecular weight cut-off of 7 kDa (Pierce, Rockford, IL, USA).

Western analysis

Homologous antibody production for trout BNP and western analysis were carried out as previously described (Johnson and Olson, 2009). Heterologous polyclonal antibodies used in western detection of tCNP were generously donated by Dr Yoshio Takei, Ocean Research Institute, Tokyo, Japan, and prepared as previously described (Takei et al., 2001). These antibodies were raised against *Anguilla japonica* CNP, which bears a 95% homology to tCNP (21 of 22 a.a.).

Protein extract preparations for atrium, ventricle and brain were prepared as previously reported (Johnson and Olson, 2009). Briefly, protein extracts were prepared by homogenizing the atrium, ventricle and brain in phosphate buffered saline (PBS), pH 7.5 with 5 mmol l^{-1} EDTA and 1X HALT protease inhibitor cocktail (Pierce), 0.25% sodium deoxycholate and 1% triton X-100. The crude lysate was centrifuged at 11,500 ***g*** for 15 min. Protein concentration from the extract supernatants was determined using the Dc Protein Assay (Bio-Rad, Hercules, CA, USA). Western blots were incubated with enhanced chemiluminescence western blotting agent (Amersham, Arlington Heights, IL, USA) for 1 min and exposed to X-ray film for 1–5 min. To account for deviation in the amount of protein loaded, the same membranes were stripped with four washes of TBS–Tween and subsequently re-blotted for levels of β-tubulin to enable standardization (murine monoclonal anti-β tubulin antibody; Sigma-Aldrich, St Louis, MO, USA). Western blots were quantified by densitometry using ImageQuant software (Molecular Dynamics Inc., Sunnyvale, CA, USA).

Quantitative PCR analysis

Total RNA was extracted from atrial, ventricular and brain tissues using Trizol reagent (Molecular Research Center, Cincinnati, OH, USA), according to the protocol provided by the manufacturer. Random-primed, reverse-transcribed cDNA synthesis reactions were performed using the Promega RT System (Promega, Madison, WI, USA), according to the conditions described by the manufacturer. Forward and reverse primers for trout (t)BNP, tCNP, NPR-A, NPR-B and GAPDH were generated using MacVector software (Table 1) and were subsequently validated for use with real-time PCR by determining the optimal amplification efficiency and primer concentrations as described by the system manufacturer (Applied Biosystems, Foster City, CA, USA). Accession numbers for sequences used in generating qPCR primers are: BNP (BAE19672, Inoue and Takei, direct submission, 1994), CNP (BAC44842, Inoue, Takei and Olson, direct submission, 2001), NPR-A (DQ174276, Nankervis and Toop, direct submission, 2005), NPR-B (DQ174277, Nankervis and Toop, direct submission, 2005) and GAPDH (AF027130, Abnet and

Table 1. DNA primers used in SYBR green quantitative PCR analysis

BNP SYBR 5′	AAGCCATGGCAATGGTTGCAGAGGAT
BNP SYBR 3′	TGCATTGTATTTGCCAACCGTGGTGC
CNP SYBR 5′	GAGCAGTTTCTGGATCGCTACAACGACCTGACCC
CNP SYBR 3′	CTAGCAGCCCAGTCCACTCATTGACCCGATCCTG
NPR-A SYBR 5′	TAACCAGGAGGATGTGGAACCGCACCTACAC
NPR-A SYBR 3′	ATTGTGATGGTTCGTGCGAGGCAGGCTGGG
NPR-B SYBR 5′	AAACCAGGAATCGGGAGAGTATGGGCTTGT
NPR-B SYBR 3′	ATCCTCCACAGCATTCCAGCCAACTCCTTT
GAPDH 5′	AGCATTGACAAGGCCTCTTCCCACAT
GAPDH 3′	ATGCCGAAGTTGTCGTGGATGACCTT

Peterson, direct submission, 1997). For real-time PCR, primers were added to 25 µl total reaction volume using reagents provided in the ABgene Absolute QPCR SYBR Green Mix (ABgene, Rochester, NY, USA). Final concentrations of the sense and antisense primers were determined for each primer pair based on optimal amplification efficiency. Reactions were carried out on the ABI 7700 Thermocycler (Applied Biosystems). Conditions were set to the following parameters: 2 min at 94°C followed by 40 cycles each for 15 s at 95°C, 1 min at 60°C, 1 min at 72°C. The Ct (defined as the cycle number at which the fluorescence exceeds a threshold level) was determined for each reaction whereas quantification was accomplished using the ΔΔCt method (Livak and Schmittgen, 2001). The target Ct was determined for each sample and then normalized to the GAPDH mRNA Ct from the same sample (GAPDH mRNA Ct subtracted from the target Ct yields the ΔCt). These values were then compared with control levels using the 2-ΔΔCt method and expressed as fold difference compared with an appropriate control sample.

Data analysis

All experiments were repeated a minimum of three times, unless otherwise stated. Summarized levels of NP mRNA and pro-NPs were expressed as fold difference (means ± s.e.m.) *versus* a designated reference treatment such as FW or DA-cannulated trout (the value for the reference treatment was arbitrarily set at 1). Data were analyzed by a Student's *t*-test or by one-way analysis of variance (ANOVA) followed by the Fisher protected least significant difference multiple range test.

RESULTS

While BNP is predominantly a cardiac peptide, BNP mRNA is present, although at significantly reduced levels, in the brain of rainbow trout (Fig. 1A). Relative levels of pro-BNP peptide essentially mirror BNP mRNA levels: elevated in the atrium and present, although significantly lower in the brain (Fig. 1B).

Chronic adaptation to FW, SW or FW–HSD did not significantly affect BNP transcription levels in the brain (Fig. 2A). Brain pro-

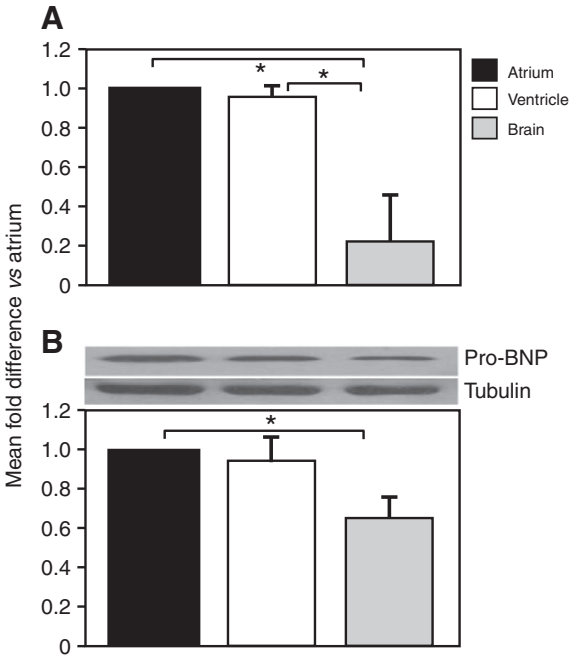

Fig. 1. Distribution of BNP in trout tissues. (A) SYBR green quantitative PCR analysis of BNP mRNA from atrial (A), ventricular (V) and brain (B) tissue of freshwater (FW) trout. BNP mRNA was present but significantly lower (N=4, *P″0.05) in the brain than either atrium or ventricle. (B) Western analysis of pro-BNP peptide in cardiac and brain tissue. Atrial pro-BNP was significantly greater (N=6, *P″0.05) than brain pro-BNP. Values normalized relative to the atrium.

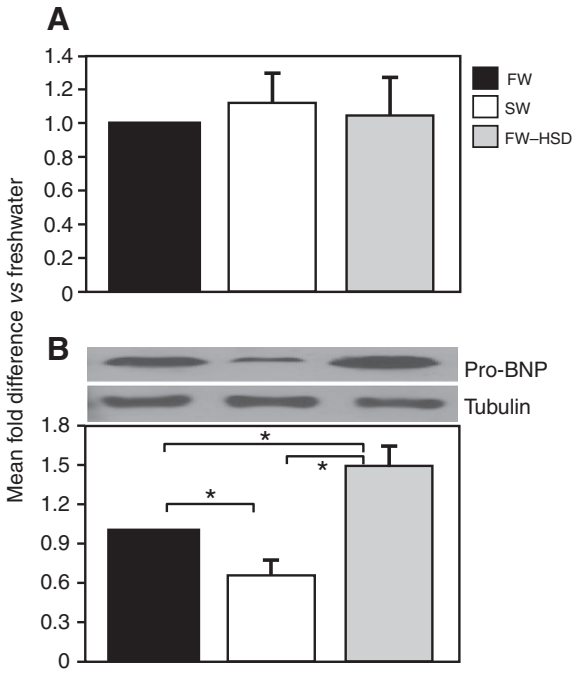

Fig. 2. Effect of freshwater (FW), saltwater (SW) and freshwater and fed a high-salt diet (FW–HSD) adaptation on brain BNP. (A) Brain BNP mRNA (SYBR green quantitative PCR) was unaffected by the different environments (N=6, *P″0.05) in FW–HSD and lower in SW trout. Values normalized relative to FW trout.

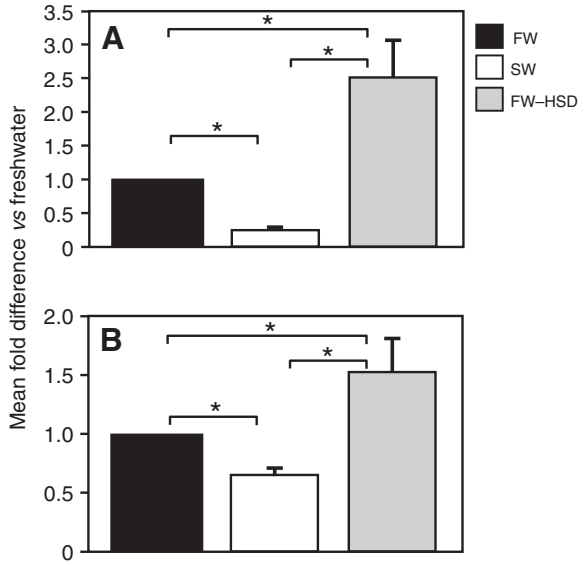

Fig. 3. Effect of freshwater (FW), saltwater (SW) and freshwater and fed a high-salt diet (FW–HSD) adaptation on brain NPR-A and NPR-B mRNA levels. (A) NPR-A mRNA was significantly higher (N=5, *P″0.05) in FW–HSD and lower in SW compared with FW trout. (B) NPR-B mRNA was significantly higher (N=5, *P″0.05) in FW–HSD and lower in SW compared with FW trout. Values normalized relative to FW trout.

BNP peptide levels, however, were significantly higher in FW–HSD trout than those in FW and SW trout whereas brain pro-BNP peptide levels in SW trout were significantly lower than FW and FW–HSD trout (Fig. 2B).

Brain expression of NPR-A, the principal associative receptor for BNP, was also affected as FW–HSD adaptation yielded significantly elevated NPR-A mRNA levels (Fig. 3A). In addition, acclimation to SW significantly reduced brain NPR-A mRNA levels (Fig. 3A). The CNP receptor, NPR-B, was significantly elevated in FW–HSD trout and significantly lower in SW trout (Fig. 3B). Brain CNP mRNA expression remained constant irrespective of adaptation environment (Fig. 4A). Brain pro-CNP peptide levels were also not significantly different among FW, SW and FW–HSD trout (Fig. 4B).

A non-traditional production site for CNP is the atrium, and as shown in Fig. 5A,B, atrial CNP mRNA and pro-peptide levels were significantly lower than those found in the brain of FW trout. Atrial CNP production was primarily responsive to blood volume as atrial CNP mRNA expression (Fig. 6A) and atrial pro-CNP peptide (Fig. 6B) levels were significantly elevated in FW–HSD trout. Atrial CNP transcriptional levels were also responsive to acute volume manipulations. Acute volume expansion by plasma infusion significantly elevated atrial CNP mRNA levels whereas constant hemorrhage reduced atrial CNP transcription (Fig. 7). Table 2 summarizes the relative changes in brain and atrial NPs.

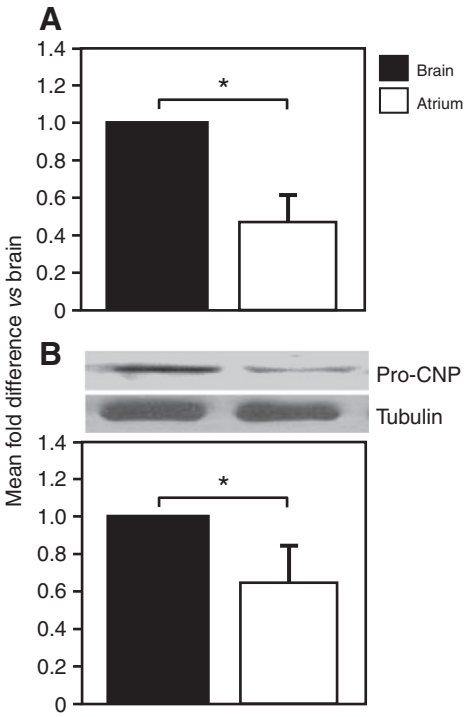

Fig. 5. Comparison of atrial and brain CNP. (A) CNP mRNA (quantitative PCR analysis) was significantly lower (N=4, *P″0.05) in the atrium than in the brain. (B) Pro-CNP peptide (western analysis) was also significantly lower (N=4, *P″0.05) in the atrium than in the brain. Values normalized relative to brain CNP in the freshwater trout.

DISCUSSION

Euryhaline fish present the unique opportunity in a vertebrate model system to analyze the cardiovascular and endocrine systems because they thrive in conditions that are potentially volume-loading and salt-depleting (FW), volume-depleting and salt-loading (SW) or volume- and salt-loading (FW–HSD). Based on the limited available studies, this seems to indeed be the case (Chen et al., 2007; Olson and Hoagland, 2008; Perry et al., 2006). The adaptation from FW to SW results in a significantly elevated plasma osmolarity, and has been reported to produce a significantly lower dorsal aortic pressure (P_{DA}), central venous pressure (P_{VEN}) and mean circulatory filling pressure (MCFP) largely *via* a reduction in blood volume. Conversely, FW trout fed a high-salt diet have a significantly elevated P_{DA}, P_{VEN} and MCFP through an increase in blood volume without a significant increase in plasma osmolarity, and the gill becomes anatomically similar to that of the salt-secreting SW fish. Through these adaptations it is possible to independently manipulate salt and water balance and observe the concomitant responses of NPs and their receptors. This provides useful insight into the physiological role(s) of the NP system (Johnson and Olson, 2008; Johnson and Olson, 2009). In the present study, these chronic adaptations were used to examine the NP system responses in non-traditional production sites, consisting of brain BNP/NPR-A, NPR-B and cardiac CNP. Results from this study indicate that the NP system in the trout brain (BNP, NPR-A and NPR-B), along with CNP produced in the atrium may be preferentially responsive to alterations in blood volume and not plasma osmolarity; a trend consistent with findings in the trout cardiac NP system (Johnson and Olson, 2008; Johnson and Olson, 2009).

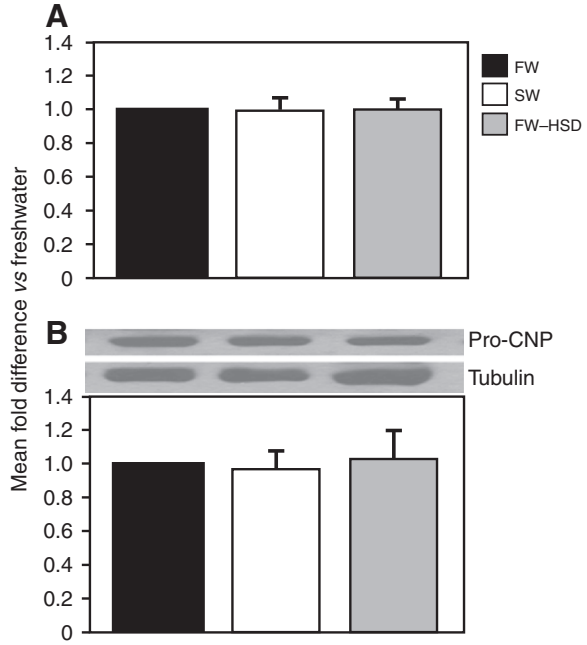

Fig. 4. Effect of freshwater (FW), saltwater (SW) and freshwater and fed a high-salt diet (FW–HSD) adaptation on brain CNP. (A) Brain CNP mRNA (qPCR analysis) was unaffected by adaptation (N=6). (B) Brain pro-CNP peptide (western analysis) was also unaffected by adaptation (N=6). Values normalized relative to FW trout.

Through the use of euryhaline teleost models in comparative studies of the NP system, two prominent hypotheses have been presented: the osmoregulatory and the cardioprotective theories. Results from eel NP studies have primarily led to the osmoregulatory theory for the NP system. The central point of inference in the NP osmoregulatory hypothesis is that an elevated plasma osmolarity acts as a stimulus for cardiac NPs (Loretz and Pollina, 2000; Takei, 2000; Takei et al., 2007). The elevated plasma NPs then act as a

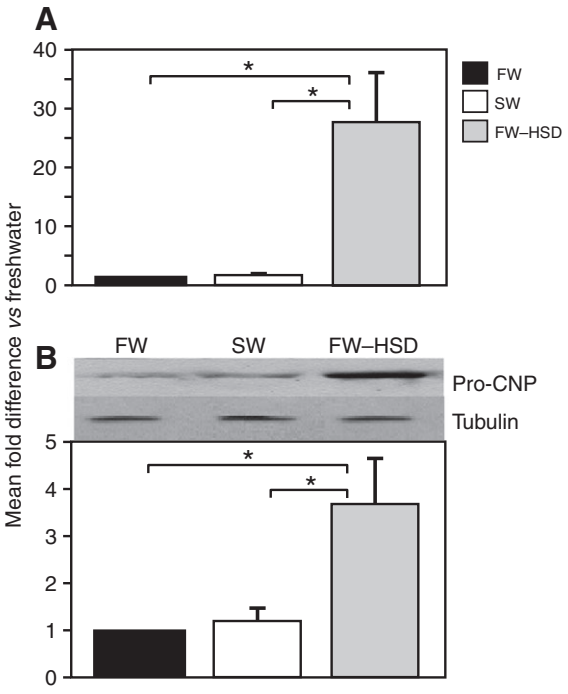

Fig. 6. Effect of freshwater (FW), saltwater (SW) and freshwater and fed a high-salt diet (FW–HSD) adaptation on atrial CNP. (A) CNP mRNA (qPCR analysis) was significantly increased by FW–HSD adaptation (N=6, *P″ 0.05). (B) Pro-CNP peptide (western analysis) was also significantly elevated (N=6, *P″ 0.05) in FW–HSD trout. Values normalized relative to FW trout.

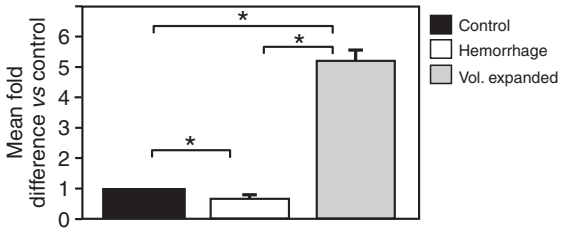

Fig. 7. Expression of atrial CNP mRNA in control trout or following acute volume expansion with dialyzed plasma or acute volume depletion by hemorrhage. Atrial CNP mRNA (quantitative PCR) was significantly elevated (N=6, *P″ 0.05) by acute volume expansion and decreased by hemorrhage.

potent antidipsogen and inhibits sodium appetite in the brain (Tsukada et al., 2007). However, an alternative interpretation of eel NP studies could also support a volume-responsive system. For example, in the euryhaline Japanese eel, transfer from FW to SW did not result in an increase in pre- or postbranchial ANP or VNP whereas plasma osmolarity was significantly elevated (Kaiya and Takei, 1996). Also, administration of ANP into conscious eels inhibits drinking and causes a reduction of plasma angiotensin II (AII), a potent dipsogen in fish (Tsuchida and Takei, 1998). While it is speculated that the NP-mediated antidipsogenic effects upon SW transfer are to reduce salt influx through drinking (Loretz and Pollina, 2000), it can be postulated that NPs are inhibiting volume uptake to protect the pumping ability of the heart in a cellular-dehydrating environment. Also, evidence of NP-mediated Na^+ extrusion from the gills and a cardiac cellular-dehydration-based release of NPs have not been reported.

By contrast, the cardioprotective hypothesis for the NP system, which is supported primarily by studies conducted in the trout as well as from the eel, claims that the NP system in teleosts functions to protect the heart from functionally-debilitating cardiodilation (Farrell and Olson, 2000). This hypothesis is supported by the findings that *in vivo* NP infusion reduces gill resistance, central venous pressure, stroke volume and subsequently cardiac output (Olson et al., 1997). Furthermore, adaptation of trout to either chronic hypervolemia (FW–HSD) or acute volume expansion resulted in elevated cardiac NP and NPR production (Johnson and Olson, 2009), a response largely consistent with that found in mammals. Evidence from the present study on the response of non-traditional NP production sites provides additional support for the cardioprotective hypothesis, and it is also consistent with results found in non-traditional NP sites in mammals.

Although rainbow trout BNP has been primarily characterized as a cardiac NP (Inoue et al., 2005), BNP has also been found in the sturgeon brain (Kawakoshi et al., 2004). Results from the present study suggest an apparent correlation between blood volume and brain BNP and NPR-A production. The significantly elevated pro-BNP levels in FW–HSD trout (and significantly lower pro-BNP levels in SW) suggest potential translational regulation, as BNP mRNA levels were unchanged between adaptations. Considered in conjunction with significantly elevated brain NPR-A expression levels in FW–HSD trout, it seems evident that BNP in the brain and its associated receptor, NPR-A, are responsive primarily to perturbations in fluid volume, and may function to complement the cardioprotective actions of cardiac NPs through anti-dipsogenesis and inhibition of sympathetic tone. This is a similar paradigm to that found in mammals.

In contrast to BNP, the traditional production site of CNP is the brain with significantly lower levels found in the atrium. While adaptation to either FW–HSD or SW yielded no significant changes in brain CNP mRNA or pro-CNP peptide levels, brain NPR-B mRNA was significantly elevated in FW–HSD and lower in SW trout. The increased production of CNP in its non-traditional synthesis site, the atrium, is consistent with other predominantly volume-responsive cardiac NPs such as ANP, BNP and VNP (Johnson and Olson, 2009). This response is also consistent with previous findings, where atrial CNP is elevated in FW eels, as compared with SW eels whereas brain CNP levels remain essentially static between FW and SW eels (Takei et al., 2001). Hence, it appears that brain CNP may function primarily as a paracrine type

Table 2. Summary of relative natriuretic peptides (NP) and NP receptors (NPR) changes in the brain and atrium of rainbow trout following transfer from freshwater (FW) to saltwater (SW) or from FW to FW plus high-salt diet (FW–HSD)

	Brain	Atrium						
	BNP mRNA	pro-BNP	NPR-A mRNA	CNP mRNA	pro-CNP	NPR-B mRNA	CNP mRNA	pro-CNP
SW	↑	↓	↓	↑	↑	↓	↑	↑
FW–HSD	↑	↑	↑	↑	↑	↑	↑	↑

neurotransmitter whereas NPR-B in the brain is specifically regulated dependent on blood volume homeostasis. Supporting the hypothesis that brain NPR-B is responsive to volume status, eel NPR-B in the brain is found at lower levels in SW eels than FW eels (Katafuchi et al., 1994). Although expression of atrial CNP mRNA in FW fish is markedly lower than other cardiac NPs (Inoue et al., 2003), the significantly elevated response of atrial CNP to chronic and acute volume expansion suggests CNP as well as NPR-B (Johnson and Olson, 2009) are vital components in circumventing detrimental cardiodilation, a likely consequence of the elevated blood volume found in FW–HSD trout (Olson and Hoagland, 2008).

In mammalian models, the responsiveness of the NP system in non-traditional production sites, such as ANP and BNP in the CNS, has been well demonstrated. Intracerebroventricular (i.c.v.) injection of BNP has been shown to attenuate AII-induced pressor response in a dose-dependent manner (Yamada et al., 1988), as well as inhibit AII-stimulated drinking with the same potency as ANP (Fregoneze et al., 1989; Itoh et al., 1988). Additionally, centrally infused ANP has been found to inhibit salt intake (Antunes-Rodrigues et al., 1985; Bastos et al., 2001), inhibit spontaneous drinking (Lappe et al., 1986; Masotto and Negro-Vilar, 1985; Yamamoto et al., 1995) and inhibit the central blood pressure response to AII (Bahner et al., 1988; Debinski et al., 1989). The central role of CNP is seemingly more region-specific; CNP mRNA in olfactory regions is significantly decreased in water deprivation states and increased in salt loading in rats (Cameron et al., 2001; Langub et al., 1995). In the medulla, water deprivation and salt loading both increased CNP mRNA but levels of CNP mRNA elsewhere in the brain were not significantly altered (Cameron et al., 2001). I.c.v. injection of AII was also found to increase CNP mRNA expression in olfactory regions of the rat brain (Cameron et al., 2001).

Contrastingly few studies have reported CNS actions for NPs in fish. In tilapia, a role for NPs in the stimulation of prolactin and growth hormone in the anterior pituitary has been established *in vitro* (Fox et al., 2007), and NPR-A mRNA has been found in most regions of the eel brain, including olfactory bulb, telencephalon, optic tectum, cerebellum and medulla oblongotta (Tsukada et al., 2007). However, the physiological role of centrally produced NPs in fish is still largely unknown.

Despite numerous questions remaining in teleosts, our studies support a commonality between the CNS NP system in fish and higher vertebrates. In the salt-induced hypervolemic FW–HSD fish, elevated BNP, NPR-A and NPR-B would seemingly be beneficial in the inhibition of drinking and salt intake similar to that observed in mammals (Bastos et al., 2001; Fregoneze et al., 1989). In the salt-rich but dehydrating SW environment, drinking is critical for survival and salt intake is necessary for water absorption. It can be reasoned in this latter state that elevation of CNS-produced NPs and NPRs would be detrimental to the survivability of the fish due to their anti-dipsiogenetic actions. Indeed, we found significantly lower pro-BNP, NPR-A and NPR-B levels in SW-adapted trout.

Extensive evidence accumulated from mammalian studies indicates integral involvement of extracardiac NP production sites such as the brain in fluid volume and electrolyte homeostasis. This study extends the paradigm for the physiological significance of peripheral or non-traditional tissue production of NPs and NPRs to trout. Clearly, further studies need to address regional NP and NPR expression patterns in the brain and locus of action for NPs and NPRs to more specifically delineate local central regulation of blood volume and electrolyte homeostasis in teleosts. However, it is evident that non-traditional NP production sites, such as atrial CNP and brain BNP, are integrally involved in teleost hydromineral balance and may complement the cardioprotective actions of cardiac NPs.

LIST OF ABBREVIATIONS

AII	angiotensin II
ANP	atrial natriuretic peptide
BNP	brain natriuretic peptide
CNP	c-type natriuretic peptide
CNS	central nervous system
DA	dorsal aorta
FW	freshwater
GAPDH	glyceraldehyde phosphate dehydrogenase
HSD	high-salt diet
i.c.v.	intracerebroventricular
MCFP	mean circulatory filling pressure
NPs	natriuretic peptides
NPRs	natriuretic peptide receptors
NPR-A	natriuretic peptide receptor-A
NPR-B	natriuretic peptide receptor-B
PBS	phosphate buffered saline
P_{DA}	dorsal aorta pressure
P_{VEN}	central venous pressure
SW	saltwater
TBV	total blood volume
VNP	ventricular natriuretic peptide

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