

HOMOLOGOUS AND HETEROLOGOUS NATRIURETIC PEPTIDE

STIMULATION OF GUANYLYL CYCLASE IN TROUT

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Introduction

Natriuretic peptides (NPs) are implicated in osmotic and cardiovascular regulation in fishes. Target organs include the gills, kidneys, brain, intestine and vasculature. The diverse physiological effects of NPs are elicited by ligand binding to receptor guanylyl cyclases and production of the second messenger cGMP. The guanylyl cyclase natriuretic peptide receptors, NPR-A and NPR-B, exhibit ligand specificity. NPR-A preferentially binds atrial natriuretic peptide (ANP) while NPR-B binds C-type natriuretic peptide (CNP). Ventricular natriuretic peptide (VNP) binds with high affinity to NPR-A and NPR-B.

In the Japanese eel, *Anguilla japonica*, eel ANP is over 100 times more potent than human ANP as a depressor of dorsal aortic pressure (Takei et al. 1989). Circulating eel ANP is amidated at the carboxyl terminal (eANP-NH₂). In contrast, salmonid ANPs are not amidated (Takei and Hirose 2002) and trout and mammalian NPs are equipotent relaxants of isolated salmonid blood vessels (Smith et al. 2000).

In *A. japonica*, NPs appear to be important for euryhalinity. ANP concentrations rise transiently upon transfer to seawater (Kaiya and Takei 1996) while plasma CNP concentration is greater in freshwater than in seawater. ANP infusion in seawater eels stimulates Na⁺ excretion while CNP infusion in freshwater eels increases plasma Na⁺ concentration (Takei and Hirose 2002). This study investigated NP stimulation of guanylyl cyclase in the gills and kidneys of rainbow trout (*Oncorhynchus mykiss*).

Materials and Methods

Cell membrane preparations were incubated with increasing concentrations of NPs in an *in vitro* guanylyl cyclase reaction 'cocktail' and cGMP was subsequently measured by radioimmunoassay (Amersham Biosciences). Data were analysed by t-test. The relative potencies of heterologous and homologous peptides were investigated, as were the effects of amidated and non-amidated eel ANPs in the gill. Guanylyl cyclase activity was compared between freshwater animals and those acclimated to 30 ppt seawater over one week.

Results

- The only difference between homologous and heterologous peptides was between trout ANP and rat ANP at $1\mu\text{mol l}^{-1}$ in seawater kidneys (Figure 1A).
- Amidated and non-amidated eel ANPs were equipotent in the gill (data not shown).
- In the gill, tCNP was the most potent peptide compared with other NPs. In both freshwater and seawater, $1\mu\text{mol l}^{-1}$ tCNP was a greater stimulant of guanylyl cyclase than was tANP (Figure 1). However, tCNP itself was equipotent in the gill and kidney.
- In the kidney, tANP was most potent. In freshwater, tANP was a greater stimulant than tCNP at $0.1\mu\text{mol l}^{-1}$ and $0.01\mu\text{mol l}^{-1}$ while in seawater, tANP was more potent than tCNP at $1\mu\text{mol l}^{-1}$ (Figure 1A), $0.1\mu\text{mol l}^{-1}$ and $0.01\mu\text{mol l}^{-1}$.
- In the gill, there was no difference between basal levels of cGMP production in freshwater or seawater-acclimated fishes. Nor was salinity a factor in natriuretic peptide-stimulated cGMP production in the gills.
- In the kidney, $1\mu\text{mol l}^{-1}$ tANP was more potent in seawater-acclimated animals than in their freshwater counterparts (Figure 2).

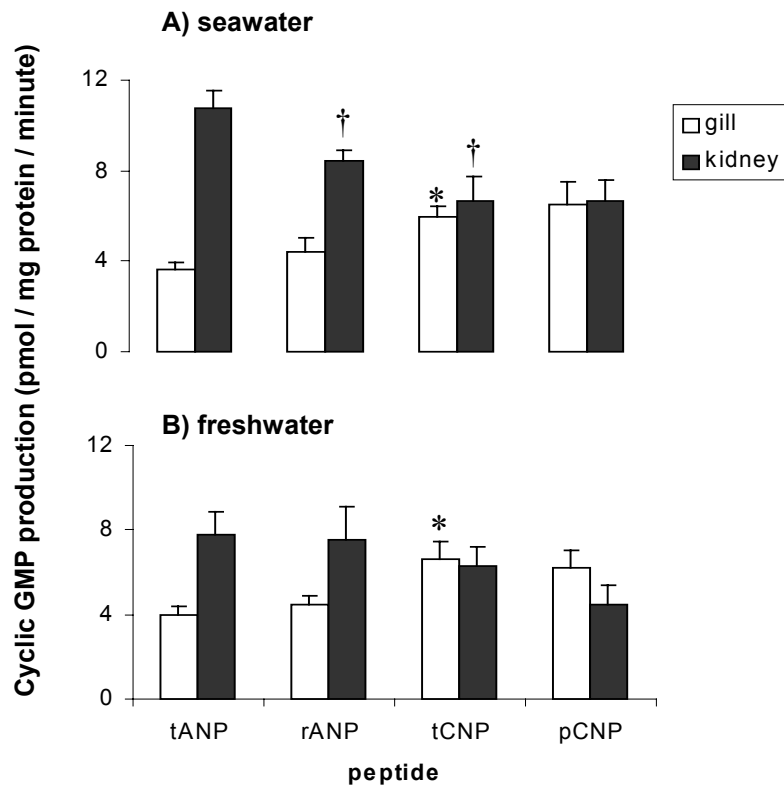


Figure 1. Natriuretic peptide-stimulated cGMP production. $1 \mu\text{mol l}^{-1}$ of trout ANP (tANP), rat ANP (rANP), trout CNP (tCNP) or porcine CNP (pCNP) were assayed in gill and kidney membrane preparations from seawater-acclimated (A) and freshwater (B) rainbow trout *Oncorhynchus mykiss*. Asterisks indicate significant differences from tANP in the gill at the same salinity, daggers indicate significant differences from tANP in the kidney at the same salinity ($p < 0.05$, $n \geq 7$)

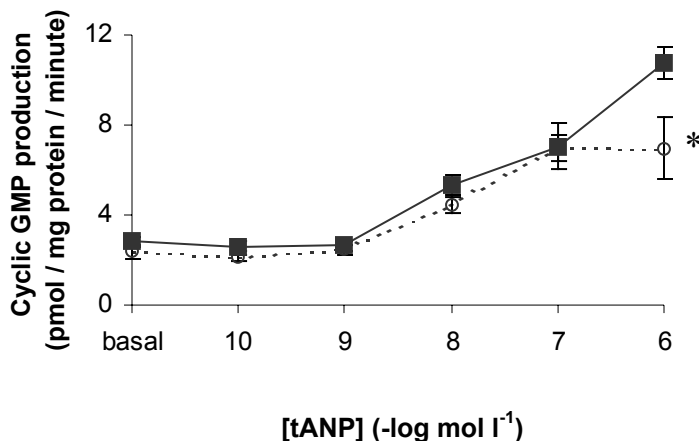


Figure 2. Trout ANP-stimulated cGMP production in membrane preparations from the kidney of freshwater (O) and seawater (!) rainbow trout *Oncorhynchus mykiss*. Asterisk indicates a significant difference from the corresponding seawater value ($p < 0.05$, $n=8$)

Discussion

Trout gill guanylyl cyclase NPRs are equally stimulated by homologous and heterologous NPs and by amidated and non-amidated eel ANP. These findings concur with the observation that trout and mammalian NPs are equipotent vasorelaxants in salmonids (Smith et al. 2000) and that salmonid ANPs are not amidated (Takei and Hirose 2002). However, stimulation of guanylyl cyclase in the seawater kidney is greater with tANP than rANP, suggesting receptor specificity for the homologous ligand. That CNP is the most potent NP in the gill while ANP is the most potent in the kidney suggests a predominance of NPR-B in the gill and of NPR-A in the kidney. Contrary to differences in cGMP production in dispersed gill cells from *O. mykiss* (Takei and Balment 1993), this study did not identify environmental salinity effects on cGMP in the gill. Salinity changes may alter circulating NP concentration without affecting receptor expression. Alternatively, if trout NPs act transiently during seawater acclimation, as seems the case with ANP in eels (Kaiya and Takei 1996), any alterations in receptor complement may have been rectified prior to sacrifice. In contrast to the case in seawater, freshwater kidney cGMP levels do not increase

between $0.1 \mu\text{mol l}^{-1}$ and $1 \mu\text{mol l}^{-1}$ tANP. This may indicate receptor saturation in freshwater and suggests a difference in NPR-A expression between salinities. Combined with the apparent specificity of kidney NPR-A for the homologous peptide in seawater, these data suggest a specific regulatory role for ANP in the trout kidney.

Acknowledgements

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