Available online at www.sciencedirect.com
SCIENCE DIRECT®
General and Comparative Endocrinology 144 (2005) 90–98
GENERAL AND COMPARATIVE ENDOCRINOLOGY
www.elsevier.com/locate/ygcen

Communication in Genomics and Proteomics

Sequence, circulating levels, and expression of C-type natriuretic peptide in a euryhaline elasmobranch, *Carcharhinus leucas*

W. Gary Anderson [a,*], Susumu Hyodo [b], Takehiro Tsukada [b], Lara Meischke [c], Richard D. Pillans [d], Jonathan P. Good [a], Yoshio Takei [b], Gordon Cramb [c], Craig E. Franklin [d], Neil Hazon [a]

[a] *University of Manitoba, Department of Zoology, Duff Roblin Building, Winnipeg, Man., Canada R3T 2N2*
[b] *Ocean Research Institute, University of Tokyo, 1-15-1 Minamidai, Nakano-ku, Tokyo 164-8639, Japan*
[c] *Bute Medical Buildings, School of Biology, University of St. Andrews, St. Andrews, Fife KY16 8LB, UK*
[d] *School of Life Sciences, Department of Zoology and Entomology, University of Queensland, Brisbane, Qld 4072, Australia*

Received 12 April 2005; accepted 26 April 2005
Available online 24 June 2005

Abstract

The present study has examined expression and circulating levels of C-type natriuretic peptide (CNP) in the euryhaline bull shark, *Carcharhinus leucas*. Complementary DNA and deduced amino acid sequence for CNP in *C. leucas* were determined by RACE methods. Homology of CNP amino acid sequence in *C. leucas* was high both for proCNP and for mature CNP when compared with previously identified elasmobranch CNPs. Mature CNP sequence in *C. leucas* was identical to that in *Triakis scyllia* and *Scyliorhinus canicula*. Levels of expression of CNP mRNA were significantly decreased in the atrium but did not change in either the brain or ventricle following acclimation to a SW environment. However, circulating levels of CNP significantly increased from 86.0 ± 7.9 fmol ml^{-1} in FW to 144.9 ± 19.5 fmol ml^{-1} in SW. The results presented demonstrate that changes in environmental salinity influences both synthesis of CNP from the heart and also circulating levels in *C. leucas*. Potential stimulus for release and modes of action are discussed.

Keywords: Euryhaline; Bull shark; *Carcharhinus*; Natriuretic peptide

1. Introduction

The euryhaline bull shark *Carcharhinus leucas*, represents one of the few elasmobranch species that inhabits both seawater (SW) and freshwater (FW) environments (Thorson, 1962; Thorson et al., 1973; Thorson and Gerst, 1972). However, our knowledge of the osmoregulatory strategy of this species of elasmobranch is limited and based largely on the work carried out by (Thorson (1962); Thorson et al. (1973); Thorson and Gerst (1972); Thorson and Lacy (1982)). In SW *C. leucas* adopts a strategy that is analogous to marine elasmobranchs. That is, plasma osmolality is maintained iso- or slightly hyper-osmotic to that of the surrounding medium through retention of comparatively high levels of sodium and chloride with respect to marine teleosts and also retention of high levels of urea (Pillans and Franklin, 2004; Thorson et al., 1973). In the FW environment *C. leucas* has an elevated plasma osmolality more than double that of most FW teleost fish and this again is achieved primarily through the retention of high levels of urea (Pillans and Franklin, 2004; Thorson et al., 1973).

* Corresponding author. Fax: +204 474 7588.
E-mail address: andersow@cc.umanitoba.ca (W.G. Anderson).

0016-6480/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2005.04.013

Key peptide systems found throughout the vertebrate phyla such as the renin angiotensin system, neurohypophysial peptides, and natriuretic peptides (NPs) have all been implicated in the control of osmoregulation in elasmobranch fish, for review see, (Hazon et al., 2003). Angiotensin II, first identified in the Japanese dogfish, *Triakis scyllia* (Takei et al., 1993) has been shown to be vasopressor (Hamano et al., 1998), dipsogenic (Anderson et al., 2001a), and antidiuretic (Wells et al., 2003) in the European lesser spotted dogfish, *Scyliorhinus canicula*. Arginine vasotocin a homologue of arginine vasopressin in mammals has been identified in all elasmobranchs examined to date (Acher, 1996) and was shown to exert an antidiuretic effect in *S. canicula* (Wells et al., 2002). The presence of NPs in elasmobranch fish was first demonstrated in *S. canicula* (Suzuki et al., 1991) and the spiny dogfish, *Squalus acanthias* (Schofield et al., 1991) and further conformation was provided in *T. scyllia* (Suzuki et al., 1992). Early efforts to isolate additional NPs from elasmobranchs were not successful (Suzuki et al., 1994; Takano et al., 1994) and more recent evidence suggests that CNP is the only NP expressed in elasmobranch fish (Kawakoshi et al., 2001). The most recognised function of CNP in elasmobranchs to date is its stimulation of the specialised salt secreting gland, the rectal gland (Anderson et al., 2002; Solomon et al., 1992). In addition, CNP is known to be vasodepressor in a number of elasmobranch species (Bjenning et al., 1992; Evans et al., 1993; Suzuki et al., 1991). Furthermore, antidiuretic (Benyajati and Yokota, 1988) and anti-dipsogenic (Anderson et al., 2001b) properties of heterologous and homologous NPs in elasmobranchs have also been reported.

By comparison, our knowledge of the control of osmoregulation in euryhaline teleost fish is much greater and to date 4 NPs, ANP, VNP, BNP, and CNP have been identified in bony fish (Inoue et al., 2003; Takei, 2000). Furthermore, ANP, VNP, and CNP have all been implicated in the adaptation of euryhaline teleosts to FW and SW environments (Takei and Hirose, 2002). To date all studies examining control of osmoregulation in elasmobranchs have involved marine elasmobranchs that can be described as partially euryhaline given their capacity to survive in varying degrees of salinity, for review see, (Hazon et al., 2003). However, there are no reports on the hormonal control of osmoregulation in fully euryhaline elasmobranchs. The present study has isolated and sequenced CNP from the heart and brain of the bull shark, *C. leucas*. Using Northern blot analysis levels of expression of *C. leucas* CNP were examined in a variety of tissues in FW and SW acclimated fish. In addition, circulating levels of CNP were assessed in *C. leucas* acclimated to FW and SW environments using a previously validated radioimmunoassay for elasmobranch CNP (Suzuki et al., 1994).

2. Materials and methods

2.1. Fish and sampling procedures

Juvenile *C. leucas* of mixed sex were caught by hook and line in FW reaches of the Brisbane River, Queensland, Australia, at least 50 km from the marine environment. The fish were immediately transferred to purpose built 10,000 L aquaria at the University of Queensland. Aquaria were supplied with re-circulating river water at ambient temperature and photoperiod.

After 2 days in FW, the sharks were transferred to tanks containing 400 mOsm kg^{-1} water for 24 h. After this period the osmolality of the water was raised 100 mOsm kg^{-1} every 24 h via the addition of seawater (approximately 1000 mOsm kg^{-1}) until the water had an osmolality of 600 mOsm kg^{-1}. The osmolality of the tank water was then increased by 50 mOsm kg^{-1} every 24 h until osmolality was 800 mOsm kg^{-1}. The tank water was then increased to approximately 1000 mOsm kg^{-1} in increments of 100 mOsm kg^{-1} every 24 h. *C. leucas* were then left to acclimate to this salinity for a period of 7 days prior to sampling. For freshwater acclimation, *C. leucas* were kept in FW, under identical holding conditions for the same period as SW acclimated sharks. *C. leucas* were fed ad libitum during the acclimation period.

Carcharhinus leucas were sacrificed following UK Home Office regulations (H.M.S.O, 1986) and a blood sample was quickly withdrawn from the caudal sinus prior to dissection and removal of tissues. An aliquot was removed for the assessment of haematocrit, and then each blood sample was centrifuged at 13,000*g* for 1 min and the plasma was removed. An aliquot of plasma was then taken and frozen at −80 °C until extraction for radio-immunoassay (RIA) of CNP. Measurement of osmolality (Knaeur semi-micro osmometer), Na, Cl, and urea (Roche Modular multiple biochemistry analyser) was carried out on the remaining plasma. Tissue samples for molecular analysis were then removed and immediately homogenised in a guanidinium thiocyanate, β-mercaptoethanol cocktail (Chomczynski and Sacchi, 1987) at a ratio of approximately 1:10 (w/v), then stored at −80 °C prior to RNA extraction. Tissues included; gill epithelial scrapings, pancreas, a section of liver, intestinal epithelial scrapings, the apex of the ventricle, the atrium, a cross section from the posterior of the kidney, the inter-renal gland, and the brain.

2.2. Cloning and sequencing procedures

Total RNA from all tissue samples were extracted by the acid guanidinium thiocyanate/phenol/chloroform method (Chomczynski and Sacchi, 1987). Poly $(A)^+$ RNA was purified from single selected brain, ventricle, and atrium samples using Oligotex-dT_{30} (Japan Syn-