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Minireview

# Fish caudal neurosecretory system: A model for the study of neuroendocrine secretion

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**Abstract**

The caudal neurosecretory system (CNSS) is unique to fish and has suggested homeostatic roles in osmoregulation and reproduction. Magnocellular neuroendocrine Dahlgren cells, located in the terminal segments of the spinal cord, project to a neurohaemal organ, the urophysis, from which neuropeptides are released. In the euryhaline flounder *Platichthys flesus* Dahlgren cells synthesise at least four peptides, including urotensins I and II and CRF. These peptides are differentially expressed with co-localisation of up to three in a single cell. Dahlgren cells display a range of electrical firing patterns, including characteristic bursting activity, which is dependent on L-type $Ca^{2+}$ and Ca-activated $K^{+}$channels. Activity is modulated by a range of extrinsic and intrinsic neuromodulators. This includes autoregulation by the secreted peptides themselves, leading to enhanced bursting. Electrophysiological and mRNA expression studies have examined changes in response to altered physiological demands. Bursting activity is more robust and more Dahlgren cells are recruited in seawater compared to freshwater adapted fish and this is mirrored by a reduction in mRNA expression for L-type $Ca^{2+}$ and Ca-activated $K^{+}$ channels. Acute seawater/freshwater transfer experiments support a role for UII in adaptation to hyperosmotic conditions. Responses to stress suggest a shared role for CRF and UI, released from the CNSS. We hypothesise that the Dahlgren cell population is reprogrammed, both in anticipation of and in response to changed physiological demands, and this is seen as changes in gene expression profile and electrical activity. The CNSS shows striking parallels with the hypothalamic-neurohypophysial system, providing a highly accessible system for studies of neuroendocrine mechanisms. Furthermore, the presence of homologues of urotensins throughout the vertebrates has sparked new interest in these peptides and their functional evolution.


*Keywords:* Caudal neurosecretory system; Urotensins; Corticotropin releasing factor; Fish; Flounder; Neurosecretion

## 1. Introduction

Studies of the fish caudal neurosecretory system (CNSS) have a history spanning nearly a century, following the first report by Dahlgren (1914) of the presence of unusually large neurons in the terminal segments of the spinal cord. Appropriately, these secretory neurons are now termed Dahlgren cells. They are found in both teleost and elasmobranch fish, but the CNSS shows more discrete organisation in the former, including a distinct neurohaemal organ, urophysis, located at the terminus of the cord (Fig. 1a; see Winter et al., 2000, for a review of CNSS structure in a range of fish species). The Dahlgren cells are magnocellular neurons, which project axons to the urophysis where their terminal swellings, packed with secretory granules, store and release neuropeptides into the circulation (Fig. 1; Arnold-Reed et al., 1991). Two apparently unique secretory products were identified from the CNSS (see Bern, 1985; Bern et al., 1985; Winter et al., 2000; for reviews). These were named urotensins (I and II), and were shown to have a number of pharmacological actions, including effects on smooth muscle and osmoregulatory epithelia (Ichikawa et al., 1986). Urotensin I and UII were first sequenced in the 1980's (Lederis et al., 1982; Pearson et al., 1980), and shown

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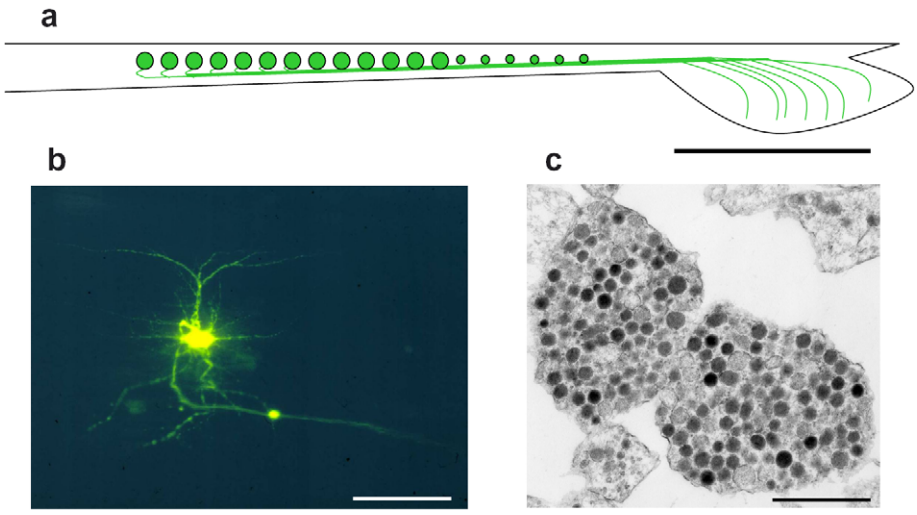


Fig. 1. (a) Schematic of the flounder CNSS showing large Dahlgren cell somata in terminal segments of the spinal cord, with axons projecting to the terminal neurohaemal swelling, urophysis. (b) Light micrograph of Dahlgren cell injected intracellularly with the fluorescent dye Lucifer Yellow, showing large cell body with extensive dendritic arborisation and caudally projecting axon. (c) Electron micrograph of terminals in the urophysis packed with electron dense peptidergic granules. Scale bars: (a) 1.5 mm, (b) 200 μm and (c) 1 μm.

to be unrelated peptides. During the last 10 years, homologues of both urotensins have been identified in the CNS and other tissues of mammals including man (Vaughan et al., 1995; Donaldson et al., 1996; Coulouarn et al., 1998; Ames et al., 1999; Balment et al., 2005), sparking new interest in these peptides and their evolution in vertebrates.

Recent studies in a number of fish species have provided further detailed information regarding the structural organisation of the CNSS (zebra fish, Parmentier et al., 2006; goldfish, Cioni et al., 1998a; Nile tilapia, Cioni et al., 2000). These confirm that, in addition to interest in urotensins and their homologues, the CNSS provides an excellent system in which to study neurosecretory mechanisms. Unlike the hypothalamic-neurohypophysial system, with which it has many organisational parallels, the CNSS is easily accessed for both *in vivo* and *in vitro* studies (e.g. Ashworth et al., 2005; Brierley et al., 2001). Owing to its location in the tail, the CNSS can be exposed in an anaesthetised fish for recording *in vivo*. Furthermore, access to the caudal vein, into which the peptides are released before reaching the general circulation, allows measurement of changes in secretory patterns over time. The entire CNSS (up to 10 caudal segments of spinal cord plus the urophysis) may be removed and maintained for many hours *in vitro*. This has enabled detailed analysis of the neurophysiology of the system, together with factors that modulate its output. Our work in Manchester has focussed on the euryhaline European flounder (*Platichthys flesus*). This species is capable of full adaptation to both seawater and freshwater and this ability supports its seasonal migratory pattern between marine and estuarine environments, which is related to reproduction. It is therefore an ideal species in which to study potential osmoregulatory and reproductive roles for the CNSS (e.g. Bond et al., 2002).

Using reverse transcriptase-polymerase chain reaction (RT-PCR) and screening of a flounder CNSS cDNA library, we have cloned cDNAs encoding a large range of genes likely to be important for CNSS function (Lu et al., 2004, 2006, submitted for publication; unpublished; Marley et al., 2007). These include neuropeptides and their receptors, receptors for other known and potential neuromodulators, and ion channels involved in generation of electrical activity patterns. This has provided us with a 'molecular toolkit' for our studies of the role of the CNSS in adaptive physiology.

This review describes recent work on the cellular physiology of the flounder CNSS in the context of its value as a model system for studies of neurosecretory mechanisms.

## 2. Neuropeptide expression in the CNSS

Urotensin I is a 41-amino acid peptide (Lederis et al., 1982) belonging to the superfamily of corticotropin-releasing factors, which also includes the mammalian UI orthologue, urocortin (Lovejoy and Balment, 1999; Lu et al., 2004). Urotensin II is a cyclic peptide found throughout the vertebrate series; flounder UII has 12 amino acids and shares 73% sequence identity with human UII (Lu et al., 2006). The CNSS is the major source of both urotensins in fish (Lu et al., 2004, 2006), though UII is also expressed in a wide range of tissues including brain, spinal cord, rectum, intestine, bladder and ovary (Lu et al., 2006).

In addition to the urotensins, we have shown that the flounder CNSS synthesises two further neuropeptides: corticotropin releasing factor (CRF, Lu et al., 2004) and parathyroid hormone related protein (PTHrP, Ingleton et al., 2002), together with significant amounts of acetylcholine (Conlon and Balment, 1996). As for the urotensins, the CNSS appears to be the major site for the production of CRF in flounder (Lu et al., 2004). Immunocytochemistry using specific antibodies has revealed co-localisation of up to three of these peptides in individual Dahlgren cell bodies,