

Seasonal morphological and biochemical changes of Dahlgren cells implies a potential role of the caudal neurosecretory system (CNSS) in the reproduction cycle of teleostean fish

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Abstract The purpose of this mini-review is to summarize recent research on the seasonal morphological and biochemical changes of Dahlgren cells in the caudal neurosecretory system (CNSS) of the freshwater teleosts carp *Carassius auratus*. The quantitative proof for these seasonal changes in the morphology and biochemistry of Dahlgren cells reflects the relationship between the CNSS and the reproduction cycle of fish and implies that the CNSS is probably involved in the reproduction process of fish.

Keywords Seasonal morphological and biochemical changes · Dahlgren cell · Caudal neurosecretory system · *Carassius auratus* · Reproduction cycle

Abbreviations

CNSS Caudal neurosecretory system
UI Urotensins I
UII Urotensins II

CRF Corticotrophin-releasing factor
Achase Acetylcholinesterase

Introduction

In addition to the hypothalamo-hypophyseal system, teleostean fishes possess another neuroendocrine structure called the caudal neurosecretory system. The caudal neurosecretory system is a neuroendocrine system found in the posterior spinal cord of teleost and elasmobranch fishes. It has been identified in most teleostean fishes and in a few dipnoans (e.g., Kobayashi et al. 1986; Onstott and Elde 1986). In 1914, Dahlgren described enlarged neurosecretory cells dispersed in the posterior region of the spinal cord in 11 species of skates (Dahlgren 1914), and subsequently Speidel named these enlarged neurosecretory cells after Dahlgren (Speidel 1922). Speidel was the first to describe the secretion of the enlarged neurosecretory cells on the basis of a large number of observations. The caudal neurosecretory system (CNSS) was first observed and investigated by Weber in 1827. However it was not until 128 years later, with the discovery of the neurosecretory activity in the caudal extremity of the spinal cord of the eel, *Anguilla japonica* that comparative anatomy of the caudal neurosecretory system (CNSS) was initially studied in various marine and freshwater fishes (Enami and Imai 1955, 1956a, b; Sano 1961; Fridberg

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1962; Holmgren 1960; Romeu 1962; Bern and Takasugi 1962). In 1959, the modern concept of CNSS was established by Enami, who further described the functional link between the neurosecretory Dahlgren cells and a secretory product storage and release organ, the teleostean urophysis (Enami 1959). Enami's modern concept of CNSS was confirmed by the investigations of Sano (1958a, b) and Holmgren (1958, 1959). The caudal neurosecretory system of teleosts has been the focus of widespread interest since Enami established the correct and systemic concept of the CNSS. In general, the CNSS is comprised of large magnocellular neuroendocrine cells (i.e., Dahlgren cells) located in the terminal vertebral segments of the spinal cord and clustered around the region of the central canal of the spinal cord, primary axons projected by Dahlgren cells and the neurohaemal release organ (i.e., urophysis) lying at the ventral side of the caudal extremity of the spinal cord (Arnold-Reed et al. 1991; Hubbard et al. 1996; Winter et al. 2000). (Fig. 1).

The caudal neurosecretory system (CNSS) produces two major caudal neuropeptides, urotensins I

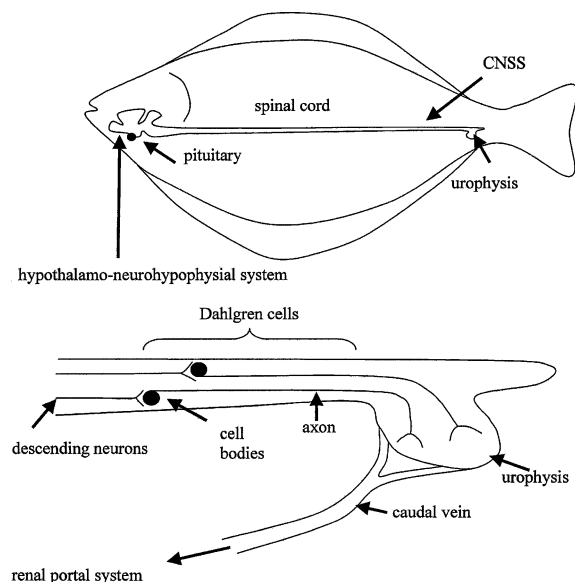


Fig. 1 Diagrammatic representation of the caudal neurosecretory system (CNSS) with enlargement of the Dahlgren cell containing posterior spinal cord and urophysis. Diagram not to scale. This figure is adapted from Winter et al. (2000) with permission from *Biochemistry and Cell Biology* © 2000 NRC Research Press. (<http://pubs.nrc-cnrc.gc.ca/>)

(UI) and II (UII). These neuropeptides have been well characterized chemically and pharmacologically and have important physiological functions. UI has proved to be similar to corticotrophin-releasing factor (CRF) and sauvagine in amino acid sequence (Lederis et al. 1982; Ichikawa et al. 1982), while UII is partially similar to somatostatin (Pearson et al. 1980; Ichikawa et al. 1984). By in situ hybridization, Ichikawa et al. (1988) have shown that all caudal neurosecretory cells (i.e., Dahlgren cells) are able to synthesize both UI and UII in the carp *Cyprinus carpio* and it was therefore concluded that the Dahlgren cells play a crucial role in CNSS.

Physiologically, the CNSS has been suggested to play a role in osmo-ionregulation, ion homeostasis, and vasopressor activities (Maetz et al. 1964; Lederis 1970b; Fryer et al. 1978; Woo et al. 1980; Bern et al. 1985; Kobayashi et al. 1986). In addition, a significant number of reports suggested that the CNSS was likely involved in reproductive functions and pheromone production (Berlind 1973; Lederis 1973; Richards 1974; Fernandes and Mimura 1983; Leonard et al. 1993; Munro 1995; Everton et al. 2000). Moreover, in vitro studies have indicated that urophysial extracts cause contractions of gonadal smooth muscle in both male and female teleosts (Lederis 1970a; Berlind 1972), but until now the precise triggering factors involved in provoking the secretory activity of the CNSS have not been elucidated, and the function of the caudal neurosecretory system of fishes has not been conclusively and definitely established.

In order to determine the relationship between the CNSS and the reproduction cycle of teleostean fish, Chen and Jiang (1998), Chen et al. (2000a, 2000b) studied cell morphology, cytochemistry and enzymic cytochemistry of Dahlgren cells. As far as we know, no quantitative study has previously been made on the seasonal changes of the caudal neurosecretory system corresponding to the reproduction cycle of fish. In previous studies on the relationship between the CNSS and the reproduction cycle of fish, attention was focused on the effect of urophysial extracts or urotensin I or II on the reproduction process of fish, but the results seemed to be unimpressive. Chen et al. demonstrated that the seasonal morphological and biochemical changes of Dahlgren cell in the caudal neurosecretory system of the freshwater teleosts carp *Carassius auratus* followed the law of cycle changes

(Chen and Jiang 1998; Chen et al. 2000a, b). This law is synchronous with the development cycle of the ovary of fish, which provides new quantitative proof for the relationship between the caudal neurosecretory system (CNSS) and the reproductive cycle of fish.

Seasonal morphological changes of Dahlgren cells and the reproductive cycle of fish

In 1998, Chen and Jiang speculated that the morphology of the Dahlgren cell probably changed synchronously with the development cycle of the ovary (Chen and Jiang 1998). In support of this speculation, they showed that the size of Dahlgren cells in the caudal spinal cord located in the vertebrate from the inverse seventh section to the inverse twelfth section changed with the seasons. From spring to winter, the Dahlgren cells become smaller. From summer to autumn, the cells do not change significantly. After winter, the Dahlgren cells begin to become larger. Conversely, the Dahlgren cells in the caudal spinal cord located in the vertebrate from the last section to the inverse third become increasingly smaller in spring and summer. After summer, these cells begin to become larger and the size difference of the cells between the two adjacent seasons is significant (Figs. 2 and 3).

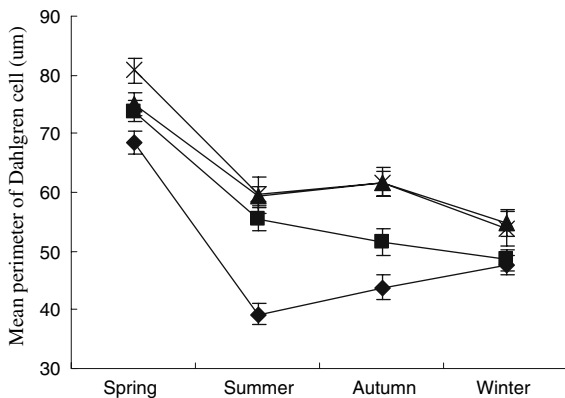


Fig. 2 Seasonal changes in the perimeter of Dahlgren cells. ◆: Dahlgren cell in caudal spinal cord located in vertebrate from the last section to the inverse third. ■: Dahlgren cell in caudal spinal cord located in vertebrate from the inverse fourth section to the inverse sixth. ▲: Dahlgren cell in caudal spinal cord located in vertebrate from the inverse seventh section to the inverse ninth. ×: Dahlgren cell in caudal spinal cord located in vertebrate from the inverse tenth section to the inverse twelfth

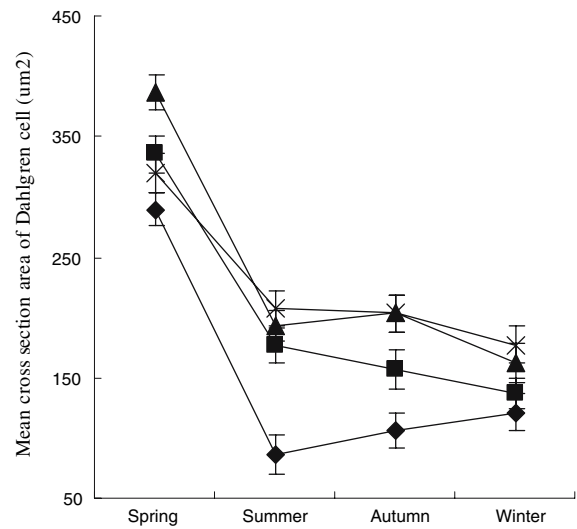


Fig. 3 Seasonal changes of the cross-sectional area of Dahlgren cells. ◆: Dahlgren cell in caudal spinal cord located in vertebrate from the last section to the inverse third. ■: Dahlgren cell in caudal spinal cord located in vertebrate from the inverse fourth section to the inverse sixth. ▲: Dahlgren cell in caudal spinal cord located in vertebrate from the inverse seventh section to the inverse ninth. ×: Dahlgren cell in caudal spinal cord located in vertebrate from the inverse tenth section to the inverse twelfth

The results from Chen and Jiang's investigations (Chen and Jiang 1998) indicate that the size of the Dahlgren cells in the caudal spinal cord located in the vertebrate from the inverse seventh section to the inverse twelfth changes regularly with the season. On the basis of synchronous observation of the development stages of the ovary, it has been shown that the regular change in size of Dahlgren cells occurs in parallel with the development cycle of the ovary. In spring, the fish is in the prophase of spawning (III–IV period), and the Dahlgren cell is largest and develops most significantly. This correlation implies that the Dahlgren cells are actively synthesizing and storing abundant biologically active substance in preparation for the spawning period. In summer, the fish is in the spawning period (V period), and the Dahlgren cells atrophy, which suggests that secretions stored in the Dahlgren cell are transported to the urophysis via its axon and ending. This facilitates the secretion by fish in their spawning period. In autumn, the fish is in the anaphase of the spawning period (VI–II period). The accretion of Dahlgren cells is not obvious, which implies that the cells start to enter into the prophase of secretion synthesis. In winter, the fish is in the

early prophase of spawning period (early III period). The Dahlgren cells are generally smaller in this season than in other seasons, which results from the diminishing feed and low metabolism of fish. Taken together, these results suggest that the size change of the Dahlgren cell in the caudal spinal cord located in the vertebrate from the inverse seventh section to the inverse twelfth with the change in seasons occurs in parallel with the development cycle of the ovary, which implies that the Dahlgren cells in these vertebrate sections are closely related to the reproductive process of fish (Chen and Jiang 1998).

Seasonal biochemical changes of Dahlgren cells and the reproductive cycle of fish

Although previous reports about the biochemical activities of Dahlgren cells have been abundant, attention has been focused on the biochemical activities of urotensin I and urotensin II produced and secreted by Dahlgren cells. Almost no cytochemical and enzyme-cytochemical studies have been done on the seasonal changes in the biochemical activities of Dahlgren cell in *Carassius auratus*. Using cytochemical and enzyme-cytochemical methods, Chen et al. reported that the protein content of the Dahlgren cells in the caudal spinal cord located in the vertebrate from the inverse fourth section to the inverse sixth decreases from spring to summer (falling to the lowest levels of the year), while it increases from summer to autumn (rising to the highest levels in the year) (Chen et al. 2000a). The difference in protein content between two adjacent seasons is significant (Fig. 4). Figure 4 indicates that the regular change of protein content of Dahlgren cells occurs in parallel to the development cycle of the ovary. In summer, the fish is in the spawning

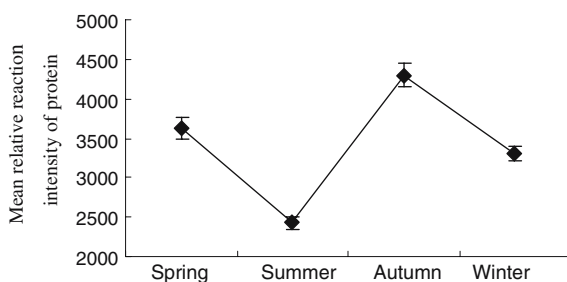


Fig. 4 Seasonal changes of protein content of Dahlgren cell

period (V period), and the protein content of Dahlgren cells is the lowest through the year, which results from the release of the secretion of protein from Dahlgren cells into the urophysis for spawning. In autumn, the fish is in the anaphase of spawning (VI–II period), and the Dahlgren cell is in the rapid recovery phase of protein synthesis and storage so that the protein content of Dahlgren cell reaches its highest at the end of autumn. After autumn, the protein content starts to diminish constantly, reaching its lowest level in summer. It is concluded that the changes in the protein content of Dahlgren cells with the seasons are in agreement with the morphological changes of Dahlgren cells with season, and furthermore, this change occurs in parallel with the development cycle of the ovary. Taken together, these results imply that the Dahlgren cell is probably involved in the reproductive process of fish (Chen et al. 2000a).

Enzyme cytochemistry in the Dahlgren cells of the caudal neurosecretory system in *Carassius auratus* demonstrates that the activity changes of cytochrome oxidase in Dahlgren cells of the caudal spinal cord located in the vertebrate from the inverse fourth section to the inverse sixth correspond to the seasonal changes in protein content of the Dahlgren cells, while the activity changes of the Achases of the Dahlgren cells is contrary to that of cytochrome oxidase (Fig. 5). As Fig. 5 shows, the activities of cytochrome oxidase and Achases in Dahlgren cells change seasonally, which implies that these two enzymes may be involved in the productive cycle of

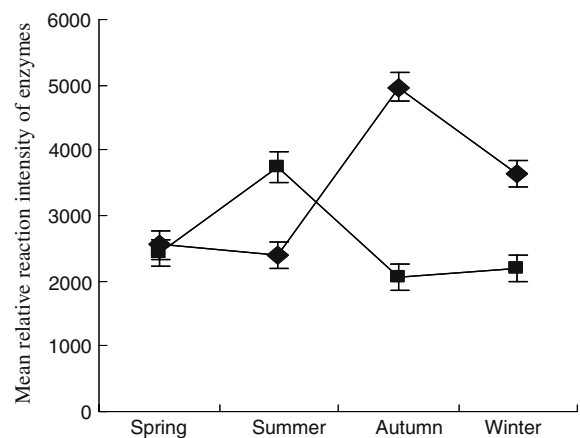


Fig. 5 Seasonal changes in Achase (■) and cytochrome oxidase (◆) activities in Dahlgren cell

fish. Cytochrome oxidase functions in the oxidation–reduction respiration chain of the cell, which supplies energy for cell activities. In autumn, the fish is in the anaphase of spawning (VI–II period) and the activity of cytochrome oxidase is the highest, which is due the significant energy requirement for the fish to actively synthesize biochemical substances in this period (Zhu and Xu 1987). Afterwards, the activity of cytochrome oxidase gradually decreases, reaching its lowest point in the summer. On the contrary, the activity of Achases is the highest in the summer (spawning or V period). Achases is one of the key enzymes involved in neurosecretory activities of fish (Conlon and Balment 1996); the summer or spawning period is the phase in which the neurosecretory activities of fish are more vigorous than in other seasons. In conclusion, the results above indicate that the seasonal activity changes in the two crucial enzymes of Dahlgren cells occur in parallel with the development cycle of the ovary, which implies that the Dahlgren cell is probably involved in the reproductive process of fish (Chen et al. 2000b).

Concluding remarks

Although previous reports have implied a relationship between the CNSS and the reproductive cycle of fish, quantitative research on the seasonal changes of CNSS in the reproductive cycle of teleost was absent from the literature. In this review, the quantitative seasonal changes of morphology and biochemistry of the Dahlgren cells were reported by Chen et al. The results primarily demonstrated that the seasonal changes of Dahlgren cells occurred in parallel with the reproductive cycle of teleostean fish, which suggested that the CNSS is probably involved in the reproductive procedure of teleostean fish. These findings provide a new understanding of the relationship between the CNSS and the reproductive cycle of fish. Even with these advances, many important questions still remain unanswered. For example, at present very little is known about the mechanism by which the CNSS regulates the reproductive procedure of fish. Although Dahlgren cells can produce UI and UII, there is no clear evidence to show that UI or UII can directly facilitate spawning and gonad development in fish. Presumably, Dahlgren cells may make another biologically active substance to function in

the reproductive system of fish so as to facilitate gonad development or spawning in fish. Thus, the seasonal changes of Dahlgren cells as presented in this review is only the first step in studying the relationship between the CNSS and the reproductive system of fish. It is anticipated that further study of the caudal neurosecretory system concomitantly with other potential secretions of Dahlgren cells in the teleost may lead to more-complete understanding of the functional significance of the CNSS in reproduction and other physiological activities of fish.

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