Mini Review

Modulation of the fish immune system by hormones

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Abstract

Immune–neuroendocrine interactions in fish, as in mammals, have become a focus of considerable interest, with the modulation of immune responses by hormones receiving particular attention. Cortisol, growth hormone (GH), prolactin (PRL), reproductive hormones, melanin-concentrating hormone (MCH) and proopiomelanocortin (POMC)-derived peptides have all been shown to influence immune functions in a number of fish species. This review summarises the known effects of these hormones on the fish immune system, as well as the often complex interactions between different hormones. The possible implications for fish health, with respect to aquaculture and the changes in immunocompetence that take place during different stages in the fish life cycle are also discussed. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

In recent years, it has become increasingly apparent that the mammalian immune and neuroendocrine systems are intimately linked and that bi-directional communication between the two is essential for the maintenance of homeostatic function. The majority of studies have concentrated on the interaction between the two systems via cytokines and neuropeptides and as a result it has become difficult to define many of these molecules simply in terms of the function with which they were originally associated. More recently, immune–endocrine interactions in non-mammalian vertebrates, especially fish, have received increased attention. The aim of this review is to summarise some of these interactions, with emphasis on the effects of circulating hormones, including cortisol, reproductive hormones, growth hormone (GH) and prolactin (PRL) and some proopiomelanocortin (POMC)-derived peptides on fish immune responses (see Table 1).
2. Immunomodulatory effects of hormones

2.1. Cortisol

The primary corticosteroid produced in the teleost interrenal gland is cortisol and, unlike in mammals, there is little evidence for significant synthesis of corticosterone or cortisone (Kime, 1987). The immunomodulatory effects of stress and corticosteroids in mammals are well documented (Ader et al., 1991; Sternberg et al., 1992; Stein and Miller, 1993) and comparable effects have been observed in fish (for a review, see Schreck, 1996). For example, a number of studies have shown that stress or cortisol administration decreases the resistance of fish to bacterial and fungal pathogens (Maule et al., 1989; Pickering and Pottinger, 1989; Wiik et al., 1989).

Most studies in fish have focused on the effects of cortisol administration on the immune system, both in vitro and in vivo (Table 2), although the release of catecholamines is also of significance, as they have immunomodulatory effects, including depressed phagocytic responses (Narnaware et al., 1994; Narnaware and Baker, 1996). Cortisol administration has been shown to reduce the number of circulating T- and B-like lymphocytes (McLeay, 1973a; Pickering, 1984; Pulsford et al., 1994), while the number of circulating phagocytes (neutrophils and macrophages) may increase (Pulsford et al., 1994). The fate of lymphocytes following stress is unclear, although re-trafficking of cells to lymphoid tissues may be involved. Thus, acute stress or cortisol treatment in juvenile coho salmon (Oncorhynchus kisutch) lowers the number of circulating leukocytes, but increases the number of these cells in the thymus and head kidney (Maule and Schreck, 1990a). Cortisol, however, has also been shown to induce apoptosis in B cells and this could be responsible for their clearance from the blood following stress (Weyts et al., 1997, 1998a; Verburg-van Kemenade et al., 1999).

Cortisol has depressive effects on a number of immune responses in fish, including phagocytosis and lymphocyte mitogenesis (Table 2). In addition, cortisol decreases the activity of antibody producing cells and circulating titres of IgM (Maule et al., 1989; Nagae et al., 1994). The effects of cortisol are mediated via the glucocorticoid receptor (GR), which has been identified in leukocytes from coho salmon and carp (Cyprinus carpio) (Maule and Schreck, 1990b; Ducouret et al., 1995; Weyts et al., 1998b).
2.2. Growth hormone and prolactin

Growth hormone and prolactin are widespread vertebrate polypeptides that are produced in the adenohypophysis of the pituitary gland and show many structural and functional similarities. In all vertebrates, GH is involved in the regulation of post-natal somatic growth, but in fish it also influences osmoregulation and stimulates gonadal steroidogenesis (Sakai et al., 1996b). Prolactin is a versatile peptide with diverse functions that may be summarised under three broad categories of growth and development, osmoregulation and reproduction (Batten and Ingleton, 1987; Sakai et al., 1996b; Prunet et al., 1989). Both hormones have also been implicated in the modulation of immune responses in vitro and in vivo. Indeed, GH is structurally similar to a number of cytokines, including interleukin (IL)-2, IL-4, IL-5, granulocyte-colony stimulating factor, granulocyte-macrophage colony-stimulating factor and the interferons (Sprang and Bazan, 1993).

In mammals, GH and PRL stimulate thymocyte maturation and differentiation and activate phagocytes (Edwards et al., 1992; Warwick-Davies et al., 1995; Ortega et al., 1996) and GH counters glucocorticoid-induced apoptosis of T cell progenitors (Murphy and Longo, 2000). Similarly, in fish, GH has been shown to stimulate lymphopoiesis and phagocytosis in gilthead sea bream (Sparus aurata) and silver sea bream (Sparus sarba) (Calduch-Giner et al., 1995, 1997; Narnaware et al., 1997). It also enhances leukocyte mitogenesis in chum salmon (Oncorhynchus keta) (Sakai et al., 1996b), phagocytosis, natural killer cell activity, antibody production and serum haemolytic activity in rainbow

### Table 2
Effects of cortisol on immune functions in fish

<table>
<thead>
<tr>
<th>Effect</th>
<th>Species</th>
<th>References</th>
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<tr>
<td>Reduced number of circulating lymphocytes</td>
<td>Oncorhynchus kisutch</td>
<td>McLeay (1973b)</td>
</tr>
<tr>
<td></td>
<td>Salmo trutta</td>
<td>Pickering (1984)</td>
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<td></td>
<td>Ictalurus punctatus</td>
<td>Ellsaesser and Clem (1986)</td>
</tr>
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<td></td>
<td>Limanda limanda</td>
<td>Pulsford et al. (1994)</td>
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<td></td>
<td>Salmo salar</td>
<td>Epselid et al. (1996)</td>
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<td></td>
<td>Oncorhynchus mykiss</td>
<td>Narnaware and Baker (1996)</td>
</tr>
<tr>
<td>Decreased lymphocyte proliferation</td>
<td>Pleuronectes platessa</td>
<td>Grimm (1985)</td>
</tr>
<tr>
<td></td>
<td>Ictalurus punctatus</td>
<td>Ellsaesser and Clem (1986)</td>
</tr>
<tr>
<td></td>
<td>Oncorhynchus kisutch</td>
<td>Tripp et al. (1987)</td>
</tr>
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<td></td>
<td>Limanda limanda</td>
<td>Pulsford et al. (1994)</td>
</tr>
<tr>
<td></td>
<td>Cyprinus carpio</td>
<td>Weys et al. (1997)</td>
</tr>
<tr>
<td>Decreased antibody production/numbers of antibody-producing cells</td>
<td>Oncorhynchus mykiss</td>
<td>Anderson et al. (1982)</td>
</tr>
<tr>
<td>Decreased phagocytosis</td>
<td>Cyprinus carpio</td>
<td>Ruglys (1985)</td>
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<td></td>
<td>Oncorhynchus kisutch</td>
<td>Tripp et al. (1987)</td>
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<td>Oncorhynchus tsawwytasa</td>
<td>Maule et al. (1989)</td>
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<td></td>
<td>Pleuronectes americanus</td>
<td>Carlson et al. (1993)</td>
</tr>
<tr>
<td>Increased apoptosis</td>
<td>Limanda limanda</td>
<td>Pulsford et al. (1995)</td>
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<tr>
<td></td>
<td>Cyprinus carpio</td>
<td>Weys et al. (1997, 1998a)</td>
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<td></td>
<td>Oreochromis mossambicus</td>
<td>Bury et al. (1998)</td>
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trout (*Oncorhynchus mykiss*) (Kajita et al., 1992; Sakai et al., 1995, 1996a; Yada et al., 1999) and respiratory burst activity of leukocytes in rainbow trout and Mediterranean sea bass (*Dicentrarchus labrax*) (Sakai et al., 1996c; Kitlen et al., 1997; Muñoz et al., 1998). Other studies have shown that the peptide enhances the resistance of rainbow trout to the bacterial pathogen *Vibrio anguillarum* in vivo (Sakai et al., 1997). In addition, transferring brown trout (*Salmo trutta*) from freshwater to seawater increases plasma titres of GH while reducing titres of thyroid hormones and this has been correlated with increased phagocytic activity of head kidney leukocytes and elevated plasma lysozyme concentrations (Marc et al., 1995). Prolactin has similar effects on fish immune responses and has been shown to stimulate leukocyte mitogenesis (Sakai et al., 1996b), respiratory burst activity (Sakai et al., 1996c) and phagocytosis (Narnaware et al., 1998) and to increase plasma IgM titres (Yada et al., 1999).

2.3. Reproductive hormones

Although in mammals androgens are predominantly associated with male and estrogens with female reproductive functions, this is not the case in fish. While many of the steroids identified in the teleost ovary are the same as those secreted in mammals, testosterone and androstenedione are major products in female fish (Scott, 1987). Steroidogenesis in the teleost testis also differs from most other vertebrates with 11-ketotestosterone or 11β-OH testosterone often being the most potent androgens (Scott, 1987).

In mammals, oestradiol enhances the activity of splenic macrophages (Schreiber et al., 1988), but inhibits the production of natural killer cells (Seaman et al., 1978). This steroid also stimulates the antibody response (Sthoefer et al., 1988) and IL-1 synthesis (Hu et al., 1988). Testosterone, on the other hand, inhibits the antibody response and interferes with lymphocyte transformation (Wyle and Kent, 1977; Sthoeger et al., 1988). Similar effects have been observed in rainbow trout with oestradiol and 11-ketotestosterone, which cause stimulation and inhibition of lymphocyte proliferation, respectively (Cook, 1994). Testosterone also reduces the number of antibody-producing cells in fish and synergises with cortisol to produce a greater inhibitory effect, suggesting that the two hormones act independently on different cells (Slater and Schreck, 1993). In addition, Slater and Schreck (1997) have demonstrated that testosterone can kill salmonid leukocytes in vitro, which may be a mechanism behind at least some of the immunosuppressive effects of the hormone. Recently, the characterisation of receptors for reproductive hormones on salmonid leukocytes has provided further evidence for an immunoregulatory role of these steroids in fish (Slater et al., 1995; Patino and Maule, 1997).

During their freshwater migration and sexual maturation, salmonids display high plasma levels of the gonadal steroids, oestradiol, testosterone, 11-ketotestosterone and androstenedione (Maule et al., 1996). Sexually mature salmonids (both male and female) demonstrate immune deficiencies at this time in their life cycle that include an inability to produce isohaemagglutinins, antibodies that are produced in immature fish (Ridgeway, 1962) and are subject to an increased frequency of ectoparasitic infestations, particularly the males (Pickering and Christie, 1980). Related to these observations is the fact that rainbow trout serum shows reduced bactericidal activity during spawning (Iida et al.,
However, Maule et al. (1996) examined specific and non-specific immune responses in Pacific salmon during their freshwater migration and sexual maturation and found positive correlations between plasma gonadal steroid levels and both serum lysozyme activity and antibody-producing cell numbers, but it is not clear if this is the result of a direct casual link, since they also correlated with the date of sampling (Maule et al., 1996).

2.4. Melanotropins and POMC-derived peptides

In teleost fish, the melanotropins α-melanocyte stimulating hormone (α-MSH) and melanin-concentrating hormone (MCH) modulate the hormonal control of skin colour. Both peptides are released from the pituitary gland and exert their effects directly on melanophores, α-MSH causes dispersion of melanin granules and thus darkening the skin, while MCH has the opposite effect, concentrating melanin granules and inducing skin pallor (Rance and Baker, 1979; Eberle, 1988). In this role, the two peptides are mutually antagonistic (Baker, 1991). Both peptides have also been implicated in a number of physiological functions in fish and mammals, including the regulation of the hypothalamo-pituitary-adrenal (HPA) axis, feeding, the response to auditory stimuli, osmoregulation and various behaviours (Miller et al., 1993; Baker, 1994; Gonzalez et al., 1996; Ludwig et al., 1998; Gilchriest et al., 1999).

In mammals, α-MSH is a potent modulator of immune responses. The peptide inhibits fever and all major forms of inflammation (Lipton and Catania, 1997), and exerts its effects both by direct interaction with immunocompetent cells in peripheral tissues and by modulation of neuronal pathways within the central nervous system (Watanabe et al., 1993; Ceriani et al., 1994). The anti-inflammatory effects of α-MSH appear to be largely directed through the modulation of cytokine synthesis, release and action. In particular, the peptide inhibits the pro-inflammatory effects of IL-1, IL-6, IL-8 and TNFα (Shih et al., 1986; Taylor et al., 1992; Ceriani et al., 1994) and stimulates the production and release of the anti-inflammatory cytokine, IL-10 (Bhardwaj et al., 1996). Alpha-MSH also inhibits a number of cellular functions in vitro, including neutrophil chemotaxis and the release of nitric oxide (NO) and neopterin from monocytes/macrophages (Star et al., 1995; Catania et al., 1996; Rajora et al., 1996). In addition, the peptide has direct antimicrobial effects against Candida albicans and Staphylococcus aureus (Cutuli et al., 2000) and enhances the production of IgE, IL-6 and TNFα by human PBMCs (Aebischer et al., 1994). The effects of MCH on immune responses in mammals have not yet been investigated, although expression of MCH mRNA has been reported in a number of immunological tissues in the rat, including the spleen (Nahon et al., 1993; Drodz and Eberle, 1995).

Early evidence for an immunoregulatory role of melanotropins in fish comes from a report by Sumner and Doudoroff (1938), who found that fish kept in dark tanks were more susceptible to infectious diseases than those kept in light tanks. Later, Bowley et al. (1983) showed that fish infected with furunculosis have increased plasma titres of α-MSH and recent studies on rainbow trout have shown that both α-MSH and MCH stimulate the proliferation of rainbow trout head kidney leukocytes in vitro (Harris and Bird, 1997). Similar investigations by Cook (1994) demonstrate that
MCH stimulates the proliferation of trout head kidney and splenic leukocytes, though in the same study α-MSH had no effect. Both peptides have also been shown to stimulate the phagocytic and respiratory burst activity of trout head kidney phagocytes in vitro (Harris and Bird, 1998, 1999; Harris et al., 1998), while α-MSH, N-des-acetyl-α-MSH and di-acetyl-α-MSH stimulate superoxide production by carp phagocytes in vitro (Takahashi et al., 1999, 2000). When cultured with supernatants derived from leukocytes that had been exposed to either α-MSH or MCH, the activity of trout phagocytes in vitro is stimulated, suggesting that the peptides trigger the release of a macrophage-activating factor (MAF) (Harris and Bird, 2000). These effects are not straightforward however, since α-MSH and MCH are mutually antagonistic on both mitogenesis and phagocytic activity when added simultaneously (Harris and Bird, 1997, 1998). The effects of α-MSH on mitogenesis appear to be strongly concentration-dependent, with inhibition at low concentrations and stimulation at higher concentrations (Harris and Bird, 1999). In view of this bi-phasic action of α-MSH, it is interesting that MCH has a similar dual action on α-MSH release from the pituitary gland of tilapia (Oreochromis mossambicus) in vitro (Gröneveld et al., 1995). MCH also reduces the inhibitory effects of cortisol on mitogenesis (Cook, 1994), although it is not clear whether this is due to antagonism between the two hormones or the result of mixed inhibitory/stimulatory signals.

The expression of the MC-1 receptor, specific for α-MSH, has been detected on human neutrophils, dendritic cells and monocytes/macrophages (Catania et al., 1996; Rajora et al., 1996; Becher et al., 1999) and on murine mast cells (Adachi et al., 1999), and the peptide itself is produced by rat splenocytes and human monocytes (Rajora et al., 1996; Jessop et al., 1994). Thus, in mammals, α-MSH may exert some of its effects in peripheral tissues through localised paracrine or autocrine release. No studies have yet determined whether fish immunocytes express MC-1 or the recently discovered MCH receptor, SLC-1 (Bachner et al., 1999; Chambers et al., 1999; Lembo et al., 1999; Saito et al., 1999; Shimomura et al., 1999).

Cleavage of the precursor molecule POMC can give rise to a number of bioactive peptides that include not only α-MSH, but also β- and γ-MSH, ACTH and β-endorphin (β-EP). In addition, a fourth MSH (termed δ-MSH) has been identified in elasmobranchs (Amemiya et al., 1999). Like α-MSH, ACTH and β-EP have been implicated in modulating immune responses in fish and all three have been detected immunocytochemically in goldfish leukocytes (Ottaviani et al., 1995). It has been shown, also, that immunoreactive ACTH is secreted by catfish blood mononuclear cells and lymphocytes (Arnold and Rice, 1997) and that immunoreactive α-MSH is released from tilapia head kidney tissue cultures (Balm et al., 1995). Since all these studies are based on the use of antibodies, they are subject to potential artifacts from cross-reactivity with other antigens and further confirmatory evidence will be required (Weyts et al., 1999).

In mammals, β-EP has been shown to augment the activation of macrophages and neutrophils (Sharp et al., 1985; Hagi et al., 1994) and stimulate T cell proliferation (Van Epps and Saland, 1984). In fish, this opioid stimulates phagocytic and respiratory burst activity of rainbow trout phagocytes in vitro, both directly and after intraperitoneal injection (Watanuki et al., 1999, 2000). Similarly, β-, γ- and δ-MSH have all been shown to stimulate superoxide production by carp head kidney phagocytes in vitro (Takahashi
et al., 1999, 2000), while ACTH decreases circulating leukocyte numbers (McLeay, 1973b), inhibits lymphocyte mitogenesis (Weyts et al., 1999) and increases phagocyte respiratory burst activity in fish (Bayne and Levy, 1991). In humans, this peptide has been shown to enhance production of IL-6 and TNFα and exert both stimulatory and inhibitory effects on IgE synthesis by PBMCs in the presence of IL-4 and anti-CD40 monoclonal antibody (Aebischer et al., 1994).

3. Concluding remarks

Fish provide a useful model for studying immune–endocrine interactions that complements our knowledge of the way these systems are linked in mammals. A comparison of the interactions in different vertebrate groups can help elucidate their physiological significance and shed light on how the association between the immune and endocrine systems has evolved.

In view of the complex mêlée of hormones present in the circulation of fish under different physiological and environmental conditions, the interplay between different hormones and the immune system in vivo is difficult to determine. Since most hormones have been shown to affect the synthesis and release of other hormones, the situation is likely to be very complex. It is already apparent, for example, that the release of MCH and α-MSH is intimately linked and that both peptides modulate the release of luteinising hormone in rats, which is itself produced by human leukocytes (Baker, 1991; Gonzalez et al., 1997; Hotakainen et al., 2000). Indeed, the effects of stress on the immune system are difficult to interpret simply in terms of cortisol/catecholamine immunosuppression, as a number of different hormones are involved via the HPA axis, including corticotrophin-releasing factor and ACTH (which, in turn, affect the release of other hormones). Thus, it is likely that immunological responses to stress are dependent on the actions of various hormones, their interactions with each other, with immunocompetent cells as well as with other endogenous factors, such as cytokines.

Many fish species go through distinct life cycle stages that are associated with changes in the levels of circulating hormones. The interactions between different hormones are often complex, but in many cases changes in plasma hormone levels correspond with changes in the immune status and health of the fish (Fig. 1). The modulatory effects of hormones on fish immune responses may have important implications for fish health and aquacultural practice and the study of these effects may lead to a better understanding of the interactions between the immune and endocrine systems in other animals, including mammals.

In fish, the potential immunomodulatory effects of other hormones associated with physiological and environmental adaptation have yet to be investigated, particularly the role of angiotensin II, the thyroid hormones, as well as other opioids, such as methionine-enkephalin, a peptide that is known to be a potent immunomodulator in invertebrates and mammals (Plotnikoff et al., 1997; Stefano and Salzet, 1999). While endocrine effects of circulating hormones may modulate general immunological competence, local paracrine and autocrine effects are likely to be more significant. This has already been shown to be the case in mammals, where local secretion of ACTH and α-MSH can have subtle
immunological actions and there is little doubt similar effects will be found to occur in fish.

Future research is likely to involve further application of mRNA probes to identify whether immunological cells are capable of synthesising specific messenger molecules. This will help establish the importance of ‘local’ secretion by different cell types while the identification of specific hormone receptors on these cells will confirm their capacity to respond to these ubiquitous messenger molecules.

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