Bacterial lipopolysaccharide (LPS) and interleukin 1 (IL-1) exert multiple physiological effects in the tilapia *Oreochromis mossambicus* (Teleostei)

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**Abstract** To gain insight in immuno-endocrine communication in teleosts the physiological effects of interleukin 1 and bacterial lipopolysaccharide in teleosts were investigated. Tilapia (*Oreochromis mossambicus*) were treated with murine interleukin 1 and *E. coli* lipopolysaccharide *in vivo*, and lipopolysaccharide was administered to pituitary lobes and head kidneys *in vitro*. The integument of the fish appeared to be a sensitive target for the preparations tested, since proliferation of chloride cells and of epidermal mucous cells was observed as well as an increase in epidermal thickness. These effects may relate to an acute phase-like reaction caused by the treatments. Lipopolysaccharide administration furthermore resulted in an increase in plasma free fatty acids levels. Lipopolysaccharide, but not interleukin 1, stimulated the interrenal axis of the fish, as judged by the increase in cortisol production measured in superfusion of head kidneys. In addition to these *in vivo* effects, lipopolysaccharide also displayed several effects *in vitro*. Pituitary adrenocorticotropic hormone, as well as α-melanocyte stimulating hormone, release was inhibited, and the head kidney responsiveness to adrenocorticotropic hormone was inhibited after pretreatment of the tissue with the *E. coli* product. This latter effect coincided with the release of an unidentified α-melanocyte stimulating hormone immunoreactive fraction by the head kidneys which could be stimulated by lipopolysaccharide. The data strongly support the notion that the immune system is involved in adaptive regulations in teleosts, and that immunoenocrine interactions are phylogenetically old mechanisms.

**Key words** Bacterial lipopolysaccharide · Interleukin 1 · Physiology · Endocrinology · Fish, *Oreochromis*

**Abbreviations** ACTH adrenocorticotropic hormone · AUC area under the curve · FFA free fatty acids · HPLC high-performance liquid chromatography · IL-1 interleukin 1 · LPS lipopolysaccharide/endotoxin · α-MSH alpha melanocyte stimulating hormone · NIL neurointermediate lobe · POMC proopiomelanocortin · RIA radioimmunoassay · RPD rostral pars distalis

**Introduction**

The importance of neuroimmunological (or immuno-endocrine) regulations during stress and disease status has been fully established in mammals (Sternberg et al. 1989; Besedovsky and Del Rey 1992) and may be phylogenetically old mechanisms (Secombes 1991). Recently teleost equivalents of IL-1 have been identified (Elsaesser and Clem 1994), and in a previous study Balm et al. (1993) demonstrated the modulation of pituitary α-MSH release by IL-1 and bacterial LPS in the freshwater teleost *Oreochromis mossambicus* (tilapia). The pituitary is unlikely to be the sole target of neuroimmunological regulations in fish, in view of the complexity of the effects exerted by LPS and cytokines as IL-1 in higher vertebrates. In fish, bacterial infections, as well as administration of LPS, have manifold immunological (Ingram and Alexander 1980), physiological (Wakabayashi and Iwado 1985; Speare et al. 1991), endocrinological (Wedemeyer 1969; White and Fletcher 1985), and histopathological (Walters and Plumb 1980) consequences. To substantiate the fundamental impact of immuno-endocrine interactions, we therefore investigated the effects of IL-1 and LPS administration on the pituitary-interrenal axis and several of its targets in fish. The adrenal axis in mammals has been demonstrated to be one of the key regulatory
systems in neuroimmunological regulations (Blalock 1989; Sternberg et al. 1989). In teleosts, the system is involved in a number of adaptive processes through the actions of cortisol, the main corticosteroid in teleosts (Sangalang et al. 1972). When fish are challenged, the hormone regulates a variety of processes, including gill and skin function (Marshall 1979), and intermediate metabolism. In the present study we investigated interrenal axis activity, plasma metabolites, chloride cells, and several skin parameters in tilapia treated with IL-1 and LPS in vivo.

The effects of these substances administered in vivo on the interrenal axis activity was studied by in vitro superfusion of pituitary and head kidney tissue, a valuable correlate of interrenal axis activity. Firstly, in vitro cortisol production is correlated with plasma cortisol levels in trout (Balm and Pottinger 1993). Secondly, in O. mossambicus in vitro hormone release is not influenced by the disturbances associated with sampling, in contrast to plasma cortisol (Balm et al. 1994). Another advantage of the in vitro approach is that modulation of ACTH sensitivity can be visualized. This parameter is regulated during immune activation in rats (Torres-Aleman et al. 1988), and is also modulated in fish under environmental challenges (Balm et al. 1987). In addition to ACTH, α-MSH and in particular di-acetylated α-MSH might also be of interest in fish, in view of the reported corticotropic potency of α-MSH forms in fish (Rance and Baker 1981; Balm et al. 1987), and because the melanotropes were found to be sensitive to IL-1 and LPS (Balm et al. 1993).

To understand the mechanisms operating in the fish treated in vivo, LPS was also administered in vitro to ACTH- and α-MSH-producing pituitary tissues, and to head kidneys. An interesting aspect of immuno-endocrine research in teleosts concerns the organization of the head kidney. This organ contains the cortisol-producing interrenal cells, which are intermingled with hematopoietic tissue, apparently in a non-compartmentalized fashion (Press et al. 1994). In the head kidneys of tilapia lymphocytes, monocytes, plasma cells and granulocytes have been identified (Sailendri and Muthukkaruppan 1975). Recent research in mammals points to the largely paracrine nature of immuno-endocrine interactions, emphasizing the role of cell-cell communication at all levels of organization including the adrenals (Gonzalez-Hernandez et al. 1994; Tilders et al. 1994). The unique organization of the adrenal homologue in fish may therefore provide an excellent opportunity to study archetypal immuno-endocrine interactions by in vitro experiments, a suggestion substantiated by the data of Schreck and Bradford (1990).

Materials and methods

Mature male tilapia (Oreochromis mossambicus) were obtained from our laboratory stock and kept in artificial freshwater at 25 °C under a 12L/12D light regime. The animals were fed with Tetramin tropical fish food daily. For the in vivo experiments, fish weighing 27 ± 3 g (average ± SEM; n = 48) were used. Animals treated with IL-1 in vivo were from the experiment described previously (Balm et al. 1993). They were injected intraperitoneally with recombinant murine IL-1α on four alternate days. Additional groups of fish (n = 12) were treated with LPS (Sigma; E. coli 0111:B4) in a similar fashion, receiving four i.p. injections (20 µl; saline or 3 mg LPS kg−1 body weight in saline each) on alternate days. One day after the final injection, fish were quickly netted and killed by spinal transection after a blood sample had been taken from the caudal vessels (Balm et al. 1994). Head kidneys containing the interrenal cortisol producing cells were removed, weighed (LPS experiment only) and superfused as described previously (Balm et al. 1987; material from two fish per chamber). Head kidneys were challenged with 10-min pulses of ACTH (1 nmol. l−1 hACTH1-39 from Peninsula). The response to ACTH is presented as AUC, superimposed on the pre-pulse production. Opercular chloride cells were quantified after DASPEI staining (Wendelaar Bonga et al. 1990). Pieces of skin were fixed in Bouin Hollande, embedded in paraffin and sectioned (10 µm). Epithelial height and superficial mucous cell numbers (IL-1 experiment only) were measured as described by Wendelaar and Meis (1980). For both groups six animals were analysed, chosen at random from the experimental groups.

In vitro effects of LPS were investigated using pituitary RPD, NIL and head kidney tissue from control animals (50 ± 4 g; n = 48). Pituitary tissues were treated with LPS (50 µg ml−1) for 90 min. After an initial period in vitro, head kidney tissue was challenged with an identical dose of LPS to test the effect on unstimulated release rates. Effects of this pretreatment on the response to a secretagogue were analysed by administering the HPLC fraction containing tilapia di-acetyl α-MSH (Lamers et al. 1991), or hACTH1-39 (Peninsula), both at 1 nmol.l−1 for 10 min. Clearance of administered α-MSH by the head kidney tissue was investigated by measuring α-MSH immunoreactivity in the supernatant; chambers without tissue (n = 6) were used as reference.

Plasma glucose (Boehringer Mannheim, Germany) and FFA (WAKO -NEFA C method, Instruchemie, Hilversum, The Netherlands) levels were measured using commercial kits. Cortisol and ACTH in superfusates were quantified by RIA as described by Balm et al. (1994). α-MSH immunoreactivity was also measured by RIA (Balm et al. 1993). To increase the sensitivity of the latter RIA, a second antibody precipitation step was introduced as described for the ACTH RIA (Balm et al. 1994). Detection limits of the assays were 1.6, 0.32, and 0.63 pg per tube for cortisol, ACTH and α-MSH, respectively. LPS at the concentration tested in vitro did not interfere in the assays.

Results are presented as means ± SEM (n = 1). Differences between groups were analysed by means of the Mann-Whitney U-test (two-tailed, unless stated otherwise); P < 0.05 was accepted as a statistically significant level of difference.

Results

Table 1 gives the results on the fish treated with IL-1 in vivo. Cytokine administration led to marked increases in the thickness of the epidermis, the number of epidermal mucous cells and opercular chloride cell density. No gross morphological disorders were observed in any of the tissues studied at the light microscope level. IL-1 did not result in changes in any of the parameters indicative of interrenal function. LPS treatment did not affect the feeding response of the animals, nor did the body weights differ between the experimental groups (not shown). However, LPS produced increases in...