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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 immuno-endocrine interactions are phylogenetically old mechanisms.

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

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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 diacetylated 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-compartimentalized 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} $hACTH_{1-39}$ 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 $hACTH_{1-39}$ (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

Table 1 Effects of *in vivo* interleukin 1 (IL-1) treatment on various parameters in tilapia (*Oreochromis mossambicus*; $n = 6$ for both groups; initial cortisol release was measured in the first 30-min fraction collected; basal release rates were quantified after 200 min *in vitro* (*AUC* = area under the curve in arbitrary units)

		Controls	IL-1	
Thickness epidermis	μm	21.0 ± 1.8	36.3 ± 3.9	$P = 0.008$
Epidermal mucous cells	$\# \cdot cm^{-1}$	6.9 ± 0.3	12.8 ± 2.1	$P = 0.004$
Opercular chloride cells	$\# \cdot mm^{-2}$	349 ± 16	834 ± 35	$P = 0.001$
In vitro cortisol production				
– initial	$pg \cdot min^{-1} \cdot g^{-1}$	13.3 ± 2.5	12.1 ± 2.4	
– basal	$pg \cdot min^{-1} \cdot g^{-1}$	1.3 ± 1.2	1.9 ± 1.1	
– ACTH	AUC	1226 ± 172	723 ± 240	

epidermal thickness and opercular chloride cell numbers (Table 2). In addition, plasma FFA, but not glucose levels were elevated in the LPS group. Although basal and ACTH-stimulated *in vitro* cortisol production rates were not significantly different between the experimental groups, LPS treatment led to increases in the head kidney somatic index and initial *in vitro* cortisol release (Table 2).

An *in vitro* challenge with LPS led to a decrease in RPD, but not in NIL ACTH production. α-MSH release from the NIL appeared slightly more sensitive to the bacterial endotoxin than ACTH release from the RPD (64 ± 6% and 45 ± 5% maximal inhibition, respectively; Fig. 1).

The *in vitro* effect of LPS ($50\ \mu g \cdot ml^{-1}$) on head kidney tissue was first analysed in relation to a challenge with a preparation containing di-acetyl α-MSH. Results indicated that LPS had no effect on unstimulated (not shown), nor on di-acetyl α-MSH-promoted, cortisol production. The area under the curves were 410 ± 39 and 469 ± 34 (arbitrary units) for control and LPS pretreated tissue, respectively. In the fractions which were collected during and after the di-acetyl α-MSH pulse, significant differences were measured in α-MSH i.r. concentrations between control and LPS-pretreated chambers. Using these values, the clearance of α-MSH immunoreactivity was calculated to be 56 ± 8% for the chambers containing control tissue, versus 95 ± 10% for the tissues which were LPS treated. In this latter group, several chambers displayed "negative" clearance rates. This prompted us to investigate the possiblity that the tissue actually released α-MSH immunoreactivity (Fig. 2a). Apparently unstimulated head kidney tissue produced low, but significant, amounts of α-MSH i.r. *in vitro*. After 300 min the immunoreactivity levels were near the

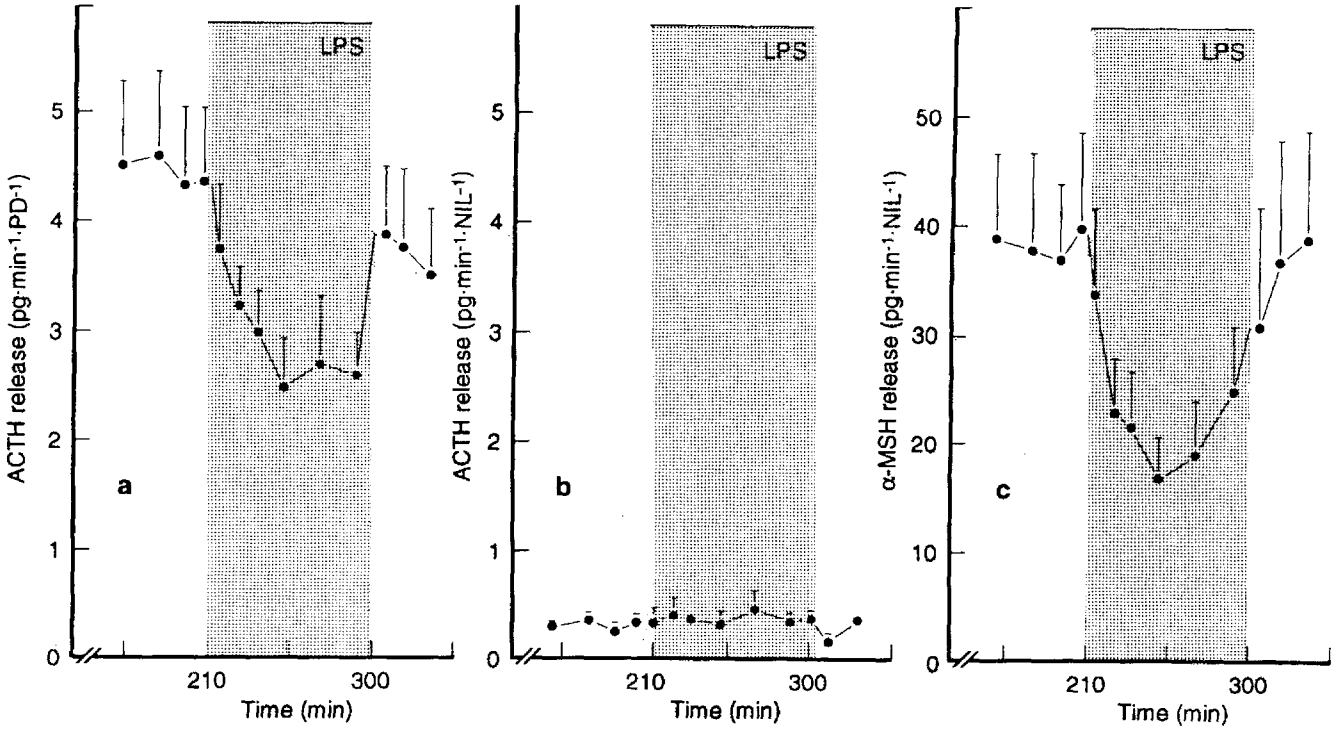


Fig. 1a–c *In vitro* effects of LPS ($50\ \mu g \cdot ml^{-1}$) on ACTH release by **a** pituitary pars distalis ($n = 6$) and **b** neurointermediate lobes ($n = 6$), and **c** on α-MSH release by neurointermediate lobes ($n = 6$). The effect of LPS was analysed using the Wilcoxon matched-pairs signed-ranks test (two-tailed). Maximal inhibition was significant ($P < 0.05$) for RPD ACTH and NIL α-MSH release

Table 2 Effects of *in vivo* administration of LPS to tilapia (*Oreochromis mossambicus*); $n = 6$ per group; head kidney somatic index: head kidney weight/body weight × 100%. Initial *in vitro* cortisol release was measured in the first 20 min fraction; basal production after 200 min *in vitro* (*AUC* = area under the curve in arbitrary units)

		Controls	LPS	
Thickness epidermis	μm	26.4 ± 0.8	47.5 ± 4.0	$P = 0.013$
Opercular chloride cells	$\# \cdot mm^{-2}$	212 ± 61	485 ± 68	$P = 0.001$
Plasma glucose	mM	4.8 ± 1.2	3.5 ± 0.3	
Plasma FFA	μM	185 ± 18	267 ± 28	$P = 0.032$
Head kidney somatic index	%	1.85 ± 0.18	2.42 ± 0.16	$P = 0.008$
In vitro cortisol production				
– initial	$pg \cdot min^{-1} \cdot g^{-1}$	16.6 ± 2.3	50.5 ± 10.1	$P = 0.004$
– basal	$pg \cdot min^{-1} \cdot g^{-1}$	1.7 ± 0.4	1.1 ± 0.3	
– ACTH	AUC	1022 ± 235	608 ± 203	

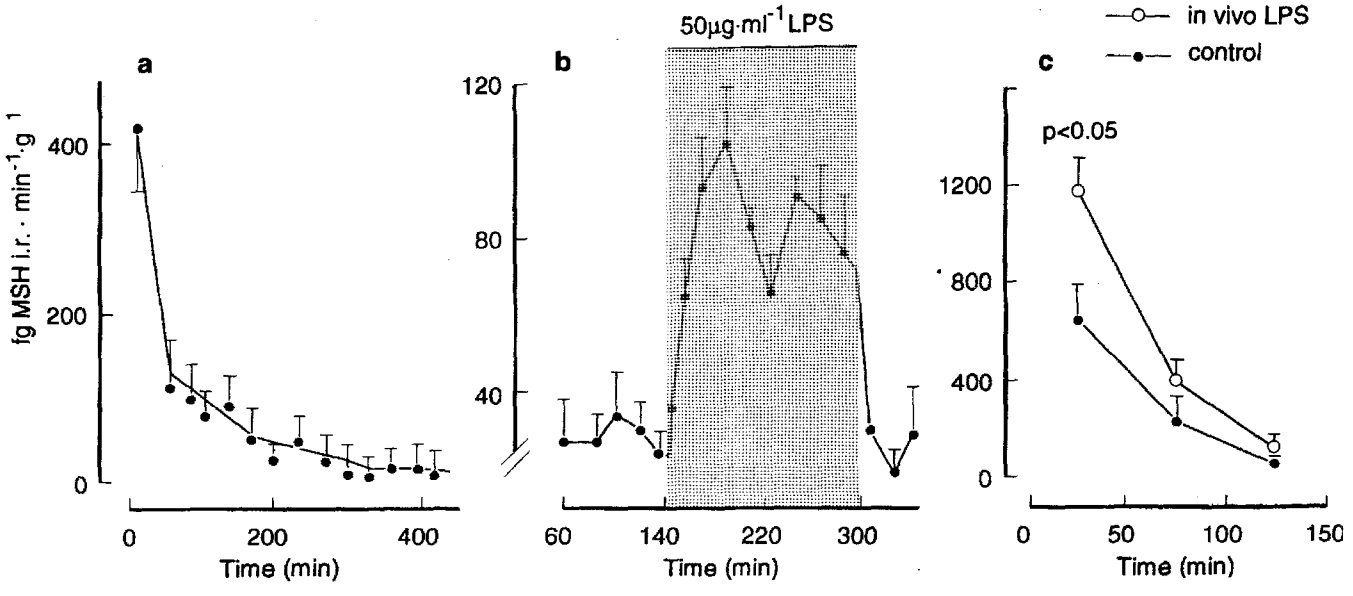
detection limit of the assay. In comparison, the NILs of these animals, which were superfused simultaneously, released 2040 ± 310 fg α-MSH $\cdot min^{-1} \cdot g^{-1}$ initially, and 1475 ± 247 fg $\cdot min^{-1} \cdot g^{-1}$ at $t = 300$ min. The release of the immunoreactive MSH from the headkidneys could be stimulated by LPS *in vitro* (Fig. 2b), and was higher in animals treated with LPS *in vivo* than in controls (Fig. 2c). No ACTH i.r. was detected in head kidney superfusates (not shown).

Fig. 2a–c *In vitro* release of α-MSH-like immunoreactivity (α-MSH i.r.) by tilapia head kidney tissue: **a** unstimulated release, $n = 6$; **b** effects of LPS *in vitro* ($n = 6$); and **c** after *in vivo* pretreatment ($n = 6$ for both groups)

Figure 3 confirms that LPS had no effect on unstimulated cortisol production, but also demonstrates that LPS pretreatment markedly reduced the ACTH responsiveness of tilapia head kidney tissue. Values for the area under the curves were 1026 ± 142 and 599 ± 132 for control and LPS treated groups, respectively (arbitrary units; $P = 0.021$). The figure also illustrates the release of α-MSH i.r. by the head kidney tissue under these conditions. Immediately upon the administration of LPS, the level of α-MSH immunoreactivity increased significantly ($P < 0.001$). This increase persisted throughout the treatment period.

Discussion

The data presented illustrate an array of physiological effects of murine IL-1 and LPS in fish. The results therefore lend support to the idea that the immune system is involved in the regulation of a wide range of physiological and endocrine regulations in fish. This conclusion is based on the idea that LPS and IL-1 exert their physiological effects via (specialized) immune cells. For mammals, emphasis is laid on the role of sessile or tissue macrophages as intermediates in the bidirectional communication between the immune and endocrine systems. This is illustrated by the mechanism of action of LPS on liver metabolism (Miller et al. 1992), on pituitary hormone release (Spangelo and MacLeod 1990) and on gonadal function (Sun et al. 1993). Fish are particularly insensitive to the toxic effects of LPS (Wedemeyer et al. 1968; White and Fletcher 1985), which therefore are an unlikely explanation for the present results. Therefore, we postulate that similar mechanisms as observed in mammals relate to



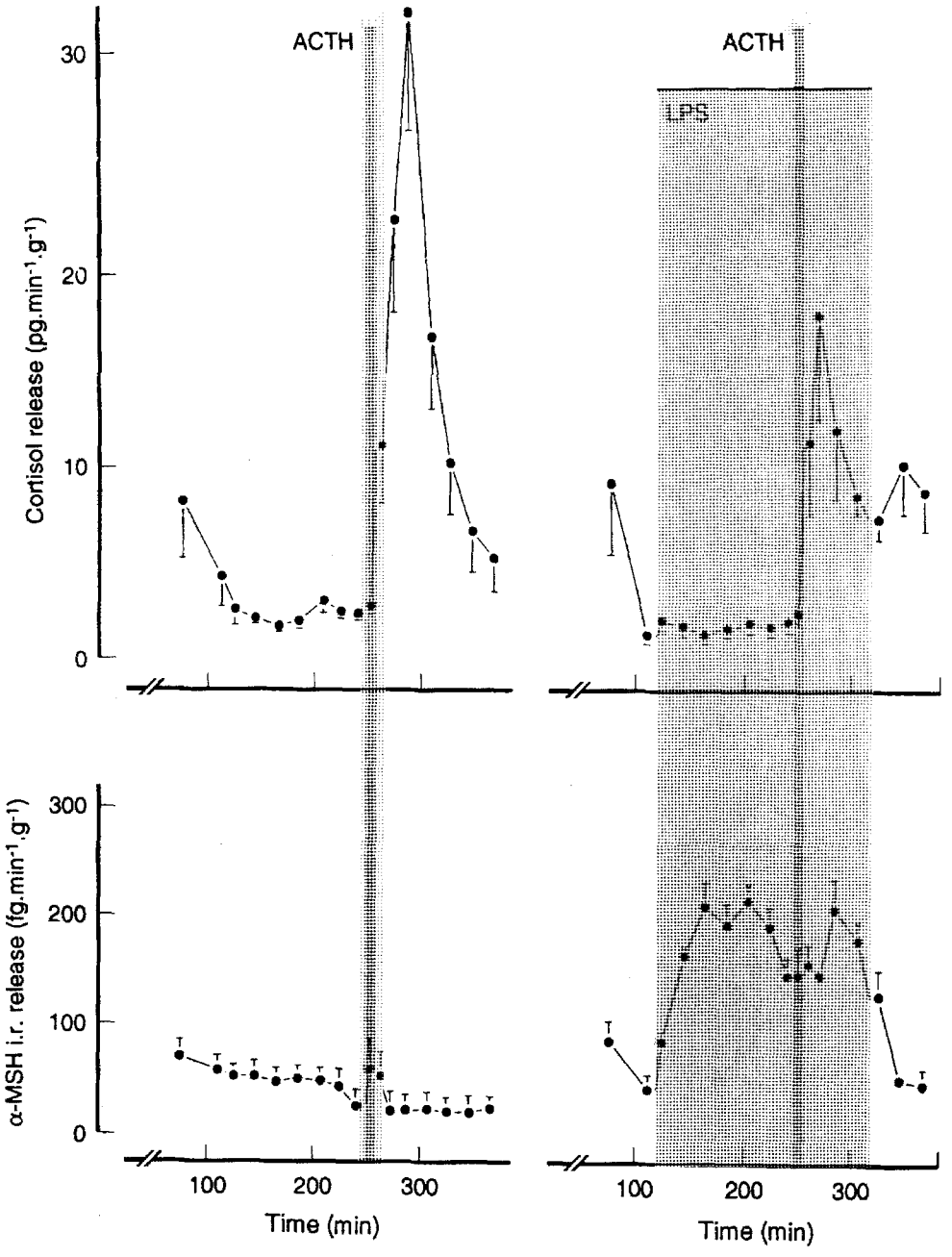


Fig. 3 *In vitro* effects of LPS on spontaneous and ACTH-regulated cortisol and α-MSH i.r. production by head kidneys from untreated control animals; $n = 6$ for both groups

the effects observed, providing evidence for the notion that immuno-endocrine communication is a phylogenetically old mechanism.

In vivo, IL-1 displayed multiple proliferative actions in the integument of tilapia which were not compensatory for tissue damage. The parameters measured in the present study have been demonstrated to be influenced by a wide variety of challenges including parasitic infestations (Urawa 1992), low environmental pH (Balm et al. 1995), salinity changes (Wendelaar Bonga and Meis 1980) and bacterial diseases (Ferguson et al. 1992). Chloride cell proliferation in freshwater fish usually is interpreted as a compensatory reaction indicating ionoregulatory disturbances induced by environmental influences (Wendelaar Bonga et al. 1990). However, the fish in this study did not display any sign of ionoregulatory discomfort (not shown), and therefore the reaction might have resulted from a non-specific, acute phase-like, reaction. In mammals this reaction is coordinated by IL-1 (Dinarello 1984). It might be argued that chloride cells might have functions other than ionic regulation. Alternatively, the results could exemplify immuno-endocrine mechanisms regulating ion transport (Cooke 1994).

The observation that the endocrine system producing cortisol – a likely candidate factor regulating the integumental changes observed (Marshall 1979) – apparently was not affected by IL-1 is consistent with the idea that the effects were mediated directly by the cytokine. Local circuits involving IL-1 have been described to regulate proliferation in the skin of mammals (Kupper 1990). Local regulations were also suggested to underly integumental effects observed in rainbow trout at low water pH (Balm and Pottinger 1993; Balm et al. 1995) and in fish acclimating to other challenges (Buchman 1993). It is unlikely that the effects of IL-1 treatment on melanotrope function described previously (Balm et al. 1993) exerted regulatory influences on the parameters presented here, since in the integument of rainbow trout at low pH similar changes occurred independently from sustained changes in circulating α-MSH levels (Balm et al. 1995).

LPS *in vivo* mimicked the integumental effects observed in the IL-1-treated fish, which is in accordance with the central role of this cytokine in endotoxin-mediated effects in mammals (Blalock 1989; Tilders et al. 1994). *E. coli* LPS, administered to fish in doses comparable to those used in tilapia in the present study, resulted in changes characteristic for the acute phase reaction, such as hypoferremia (Congleton and Wagner 1991) and an increase in C-reactive protein (White and Fletcher 1985), supporting the idea that the effects observed in tilapia could be part of a systemic acute phase reaction.

The rise in plasma FFA levels underscores the metabolic consequences of bacterial infections (Wakabayashi and Iwado 1985) and LPS (Wedemeyer et al. 1968) in fish. In rats, a rise in plasma FFA levels in animals treated with IL-1 has been reported (Argilés et al. 1989). In addition to the role of FFA in energy metabolism, the rise in plasma FFA could also serve to counteract some of the concurrent actions of LPS, since FFA inhibit the LPS-induced B-cell proliferation (Calder and Newsholme 1990).

A notable difference between the fish treated with IL-1 and LPS was the stimulation of initial *in vitro* cortisol production by endotoxin. The results corroborate the results of other studies which demonstrated the activation of the pituitary-interrenal axis in fish treated with LPS (Wedemeyer 1969; White and Fletcher 1985). *In vitro* cortisol production is a valuable index of interrenal axis activity in fish (Balm and Pottinger 1993; Balm et al. 1994). The comparison between the two experiments shows that the interrenal activation was not a requisite for the integumental effect observed, and also identifies the interrenal axis as a target of the bacterial product in tilapia. The fact that IL-1 treatment did not result in a similar activation might indicate the different mechanisms of action for integumental and endocrine effects of the cytokine preparation, which likely differs from the tilapia homologue (Ellsaesser and Clem 1994).

The stimulation of cortisol production by *in vivo* treatment with LPS may have been caused by effects of LPS at the pituitary level (Wedemeyer 1969). However, additional sites of action, such as the head kidney, might also be involved. The increase in relative head kidney weight in the LPS-treated fish likely reflects the proliferative influence of the endotoxin treatment on the hematopoetic tissue, since other tissue components constitute minor fractions of the organ (Sailendri and Muthukkaruppan 1975). These processes depend on the production of cytokines, growth factors, and other regulatory products (Blalock 1989; Secombes 1991), several of which are potential secretagogues of corticosteroid-producing cells (Torres-Aleman et al. 1987). Local immuno-endocrine regulatory circuits have also been postulated to underly the effects of LPS on pituitary hormone release (Spangelo and MacLeod 1990). To study whether these mechanisms might also operate in fish, we investigated the effects of LPS on ACTH and cortisol production *in vitro*.

The *in vitro* LPS inhibition of ACTH release by the RPD was comparable with the effects on melanotropes, and appeared to be specific for ACTH production by the corticotropes since ACTH release by the NIL was not affected by LPS. The dose of LPS tested was previously demonstrated to be maximally effective in inhibiting α-MSH release (Balm et al. 1993). The data demonstrate that the inhibition of α-MSH release does not follow from an inhibition of the processing of ACTH in the melanotropes, since in that case a stimulation of ACTH release by the NIL might have been expected. The results also argue against a cytotoxic action of LPS mediating the effects observed, since upon removal of endotoxin from the incubation medium the release of ACTH and α-MSH recovered. Alternatively, presynaptic modulation of the release of regulatory factors could explain the pituitary effects in fish as discussed previously (Balm et al. 1993).

The *in vitro* head kidney superfusions not only demonstrated that pretreatment of this tissue with LPS did not alter the sensitivity to a preparation containing tilapia di-acetyl α-MSH, but also led to the discovery of the release of α-MSH immunoreactivity from the head kidney. It is tempting to speculate that this fraction is derived from immunocyte-derived POMC (Blalock 1989), in particular since the production could be stimulated by LPS *in vitro* and *in vivo*. Alternatively, it could be produced by endocrine cells in the head kidney, since α-MSH has been demonstrated in the human adrenal medulla (Evans et al. 1983) which is practically devoid of immune tissue. In fish, however, the equivalent of medullary tissue is intermingled with interrenal and hematopoietic tissue. Data are accumulating that in teleosts POMC is produced and processed in extrapituitary tissues. ACTH-like immunoreactivity has been associated with fish neutrophils, basophils and monocytes (Ottaviani et al. 1992), β-endorphin has been detected in flounder head kidneys (Ng et al. 1991),

and more recently α-MSH immunoreactivity has been demonstrated in the ovaries of two teleostean species (Mosconi et al. 1994). Ovarian tissue levels varied markedly with reproductive status and rearing conditions, suggesting a physiological role for the peptides (Mosconi et al. 1994). In this latter study the immunoreactivity coeluted with α-MSH standards on reversed-phase HPLC. Because of the low levels produced, HPLC characterization of the α-MSH immunoreactivity released by tilapia head kidney tissue did not yield conclusive results (not shown). Immunocyte POMC-derived products, however, do not necessarily have to be identical to the forms produced in the pituitary. In mammals alternative processing of $ACTH_{1-39}$ to $ACTH_{1-24}$ has been demonstrated in lymphocytes stimulated by LPS (Smith et al. 1990). Degradation of authentic α-MSH by endopeptidase 24.11, which is present on the surface of pre-B lymphocytes, could also yield alternative immunoreactive forms of α-MSH (Deschodt-Lanckman et al. 1990). These are of interest, because regulation of interrenal cortisol production in teleosts by α-MSH appears extremely sensitive to modifications of the molecule, such as alterations of the chain length and the degree of acetylation (Rance and Baker 1981). Although the functional significance of POMC products by immune cells is still under debate (Sharp and Linner 1993), most evidence in mammals points to local regulatory actions. In the case of α-MSH, paracrine antagonistic regulation of the various biological actions of IL-1 has been proposed (Robertson et al. 1988).

The inhibition of ACTH responsiveness by LPS supports the idea that local regulatory circuits involving immuno-endocrine communications influence cortisol producing cells in the head kidney (Schreck and Bradford 1990). A similar inhibition was also suggested by the *in vivo* treatments but was not significant in these cases. The mechanism might function in the down-regulation of the activated interrenal axis, which likely involved pituitary factors such as ACTH (Wedemeyer 1969). In rats, modulation of *in vitro* ACTH sensitivity following *in vivo* LPS treatment has been reported (Torres-Aleman et al. 1988). This might be a general phenomenon for steroid-producing tissues, since the gonadotropin sensitivity of ovarian steroidogenesis is also inhibited by IL-1 (Hurwitz et al. 1991). Interestingly, the LPS effect observed in the present study was specific for ACTH, which is compatible with the idea that α-MSH and ACTH act via different mechanisms on teleost corticosteroid production, as in mammals (Kapas et al. 1992).

Although a role for the α-MSH immunoreactive fraction produced within the head kidneys in the regulation of ACTH sensitivity remains to be established, the results illustrate the release of a candidate modulatory factor by this tissue which can be regulated by LPS. Other corticosteroid secretagogues known to be produced by immune cells, or whose release is modulated by cytokines, include thymosin (Torres-Aleman et al. 1987), corticostatins (Solomon 1993) and catecholamines (Jones et al. 1993). The identification of these or other immuno-endocrine interactions in the head kidney will greatly increase our understanding of the mechanisms behind stress and disease in fish.

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