Stress-related hormones modulate cytokine expression in the head kidney of gilthead seabream (Sparus aurata)

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Abstract

Neuro-endocrine and immune systems closely interact in fish, and their regulation is crucial for the maintenance of good health of cultured fish. We have used the seabream head kidney to study whether stress-related hormones can modulate the immune response. For this purpose, the effects of adrenaline, adrenocorticotropic hormone (ACTH) and cortisol on the expression of pro-inflammatory cytokines (TNF-a, IL-1b, IL-6) and the anti-inflammatory cytokine TGF-b1 were determined by means of quantitative real-time PCR on isolated head kidney cells. ACTH (150 ng mL^{-1}) caused an acute increase of TNF-a and IL-6 mRNA levels as well as an inhibition of IL-1b expression. The expression of the anti-inflam- matory cytokine TGF-b1 was also increased, although in a lower extent. Adrenaline (1 mM) early effects were only clear inhibiting IL-1b expression but not TNF-a, IL-6 or TGF-b1 mRNA levels, while a longer exposure to the hormone inhibited all cytokines. Moreover, cortisol (50 and 100 ng mL^{-1}) reduced the expression of all cytokines in a dose-dependent manner. Bacterial lipopolysaccharide (LPS) stimulated IL-1b expression and inhibited that of the anti-inflammatory TGF-b1, although it was ineffective on TNF- a and IL-6. In addition, adrenaline and cortisol decreased the LPS-stimulated IL-1b expression, further demonstrating their previously reported anti-inflammatory effects. The combination of ACTH and LPS, on the other hand, did not affect LPS-stimulated IL-1b expression but was effective increasing TNF-a expression. Taking all these results in consideration, we conclude that the expression of pro- and anti- inflammatory cytokines in the seabream head kidney is highly influenced by stress-related hormones, thus indicating an important role for the endocrine system in the modulation of the immune response in teleost fish.

1. Introduction

In fish, as in other vertebrates, neuro-endocrine function can interact with the immune system [1,2] particularly under stress episodes that may cause many different alterations in the immune system affecting performance, health and welfare. Two different mechanisms articulate endocrine responses to stress in teleost fish: the adrenergic response (leading to an increase of adrenaline) and the hypothalamo-pituitary-interrenal (HPI) response, leading to an increase in plasma adrenocorticotropic hormone (ACTH) and cortisol levels [3]. ACTH is the major regulator of the synthesis of glucocorticoids [4–6] and its effects in fish head kidney are mediated by the MC2 receptor [7]. On the other hand, the control of chromaffin cell activity and catecholamine secretion involves neuronal signals from cholinergic and non-cholinergic transmitters and humoral signals of endocrine origin [8]. The effects of the sympathetic overactivity on the pro-inflammatory stress response are less clear: b-adrenoreceptor activation is considered as one contributor to stress-induced immunosuppression [9].

Several fish cytokine genes have been isolated and characterized in recent years and researchers have used its mRNA expression as a tool for measuring immune responses [10–14]. Although not all discovered genes by homology cloning may encode for a protein with the same function, it appears that fish would have most of the cytokines relevant for processes related to responses to bacteria, virus, inflammation or cell proliferation and chemoattraction [15]. In particular, pro-inflammatory cytokines, including interleukin-1b [16], TNF-a [17], and IL-6 [18] are commonly used immune-regulatory genes in fish. Interleukin-1b (IL-1b) is a key mediator of host response infections and a primary cause of inflammation [19], identified in 13 teleost species with a role similar to that in mammals [20]. In mammals, IL-1b is mainly found in activated macrophages, which must be cleaved by

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a converting enzyme, caspase 1 [21]. IL-1b activity occurs when it binds to its receptor on target cells. This receptor has been cloned in rainbow trout and Atlantic salmon, and its expression seems to be constitutive and up-regulated after stimulation with LPS and TNF-a in the head kidney, spleen, liver and gills, which suggests a role for the IL-1 receptor in IL-1b regulation during the inflammatory response [22,23].

TNF-a is an inflammatory cytokine as well, and, in mammals, it is produced by macrophages, neutrophils, monocytes, natural killer cells and T cells after their stimulation by bacterial lipopolysaccharide. It is probably the most studied cytokine in fish, in species such as rainbow trout [17], carp [24], catfish [25], red seabream [26], Atlantic salmon [27], Gilthead seabream [28] and the mandarin fish [29].

IL-6 is mainly secreted by T cells and macrophages to stimulate immune response to inflammation [30–32] and has been described in Fugu [33], rainbow trout [34] and in the seabream [35]. TGF-b1, on the other hand, is a multifunctional peptide controlling proliferation, differentiation, and other functions in many cell types, described in carp [36], seabream [37] and more recently in grass carp [38]. In general, fish appear to possess a repertoire of cytokines similar to that of mammals [39].

Fish lack a distinct adrenal gland and the equivalent tissue is located within the head kidney, where interrenal tissue (corticosteroidogenic cells), chromaffin cells (secreting cathecolamines), hematopoietic cells and the immune tissue (lymphocytes and macrophages) are intermingled [40,41]. The proximity of these cells in the tissue raises questions about potential paracrine interactions, and previous reports have suggested that such intra-adrenal interactions exist in fish [42,43]. Despite these results, there is relatively little information on the interaction between stress-related hormones and the immune system in fish, and more precisely the effects on cytokine expression. To this purpose, we have measured the effects of adrenaline, ACTH and cortisol, as well as their combined effects with the bacterial endotoxin LPS, on several inflammatory cytokines (IL-1b, TNF-a, IL-6) and the anti-inflammatory cytokine TGF-b1, using in vitro preparations of seabream head kidney.

2. Material and methods

2.1. Animals

Sexually immature gilthead seabream (Sparus aurata), weighting an average of 80–100 g, were obtained from a fish farm (Vilanova, Barcelona) and maintained in a semi-closed seawater flow circuit with water at a temperature of 17 °C and a salinity of 37& under a 12 h light/12 h dark cycle and a density of 7 kg m^{-3}.

2.2. Head kidney preparations

Gilthead seabream head kidney cells were isolated and cultured as previously described [44]. Briefly, head kidneys were dissected from seabream killed by over anesthetization in MS-222 (Sigma, Alcobendas, Spain), and placed in PBS. Tissues were finely minced with sterile scalpel blades, passed though a pipette to disperse into small aggregates, and filtered through a cell strainer (100 mM, Falcon # 352360). The resulting homogenate (total cell suspension) includes hematopoietic cells (lymphoid cells), immune cells (macrophages, lymphocytes), and endocrine cells (chromaffin and corticosteroidogenic cells). Cells were allowed to settle via sedimentation into culture plates and kept at 17 °C, 5% CO_2 with Dulbecco's Modified Eagle Medium (Life Technologies, #11971-025) containing high glucose.

2.3. In vitro experiments

Following isolation, cells were left undisturbed for 3 h to stabilize as previously described [44,45]. After this period, head kidney preparations were incubated with medium with or without ACTH (150 ng mL^{-1}), adrenaline (1 mM) or cortisol (50 and 100 ng mL^{-1}) (H2882, Sigma, Alcobendas, Spain) for 1 and 2 h. In addition, in order to check the effects of the interaction of LPS and stress hormones, cells were incubated with LPS alone (L8274 Sigma, Alcobendas, Spain) (10 mg mL^{-1}) or in combination with ACTH (150 ng mL^{-1}), adrenaline (1 mM) or cortisol (50 ng mL^{-1}) for 2 h. Cell viability was >90% after isolation, and remained at the same levels after treatments, as determined by Trypan Blue exclusion test. Each time condition was replicated 3 times on a 60 mm culture plate.

2.4. Analysis of cytokine gene expression

For cytokine expression analysis, total RNA was isolated from seabream head kidney using Tri Reagent (Molecular Research Center Inc, Cincinnati, OH, USA) following the manufacturer's instructions. Total RNA (2.5–5 mg) was reverse transcribed with Superscript III™ reverse transcriptase (Invitrogen S.A, Barcelona, Spain) using an oligodT15 (promega) as primer for the extension of the product, according to the manufacturer's protocol.

2.5. Quantitative real-time PCR

The cDNAs from seabream head kidney preparations were used for quantitative PCR analysis using SYBR Green PCR Supermix (Bio-Rad, Hercules, CA, USA). Results were evaluated with the ICYCLER IQ real-time detection system software (Bio-Rad, Hercules, CA, USA). The sequences of the primers used in gene expression analysis are shown in Table 1. The total volume (20 mL) of every reaction contained 500 nM of each amplification primer, 10 mL of 2× SYBR Green PCR Mix and 5 mL of a 1:50 dilution of cDNA (1:1000 for 18S determination). Products were amplified in an iCycler iQ™ Real Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). Controls lacking cDNA and controls containing RNA were included. The real-time analysis consisted of 1 cycle of 95 °C for 5 min, 40 cycles of 95 °C for 10 s and 60 °C for 30 s, 1 cycle of 95 °C for 1 min, 1 cycle of 55 °C for 1 min, and a melting curve of 81 cycles (from 55 °C to 95 °C). Quantitative PCR were performed with cell suspensions taken from the same fish, and separated in equal parts, then all PCR samples were run in triplicate and fluorescence was measured at the end of every extension step. C_T (threshold cycle) values for each sample were normalized to endogenous control 18S rRNA and expressed as percentage over untreated controls (set to 100%).

Table 1
Sequences of primers used in gene expression analysis.

Primer	DNA sequence	Direction	Size of amplicon
IL-1b Fw	5′-TCTTCAAATTCCTGCCACCA-3′	Forward	245 bp
IL-1b Rv	5′-CAATGCCACCTTGTGGTGAT−3′	Reverse	
TNF-a Fw	5′-TGAACAGAGGCGACAAACTG −3′	Forward	245 bp
TNF-a Rv	5′-GCCACAAGCGTTATCTCCAT-3′	Reverse	
TGF-b1 Fw	5′-TACCGCAGCCAAGCCAACCT-3′	Forward	184 bp
TGF-b1 Rv	5′-GCAGAGCACAGTGCCATGCT-3′	Reverse	
IL-6 Fw	5′-GCTCTGCTGCGGTGTGCTCC−3′	Forward	90 bp
IL-6 Rv	5′-GTCTCCCACTCCTCACCTG−3′	Reverse	
18S Fw	5′-CGAGCAATAACACGTCTGTG −3′	Forward	211 bp
18S Rv	5′-GGGCAGGGACTTAATCAA-3′	Reverse	

2.6. Statistical analysis

For gene expression quantification by real-time PCR on in vitro assays, treatments were performed in triplicate. Statistical significance was analyzed by one-way (ANOVA) followed by Tukey's post-hoc test, using the software package SPSS (Chicago, IL, USA) for Windows. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Effect of ACTH on cytokine expression in head kidney preparations

Primer sequences used in real-time PCR studies are shown in Table 1, and the effects of ACTH on cytokine expression in head kidney are shown in Fig. 1. Incubations with ACTH (150 ng mL^{-1}) for 1 h increased TNF-a mRNA levels (305% compared to control), IL-1b expression was clearly decreased (to 59% of control levels), while TGF-b1 and IL-6 mRNA levels increased (437% for both cytokines). On the other hand, a longer ACTH incubation for 2 h reduced TNF-a (15% of control), IL-1b (15% of control), TGF-b1 and IL-6 (to 16% and 25% of control, respectively) expression levels (Fig. 1).

3.2. Cytokine regulation by adrenaline

The effects of adrenaline on cytokine expression in seabream head kidney are shown in Fig. 2. Incubations with adrenaline (1 mM) for 1 h did not alter TNF-a mRNA levels (97% of control), TGF-b1 (95% of control) or IL-6 (106%), although IL-1b expression was clearly inhibited (69% of control, respectively). A longer adrenaline incubation for 2 h did not modify TNF-a expression (95% of control levels) and IL-1b (126%), and inhibited both TGF-b1 and IL-6 expression (68% of control expression for both cytokines) (Fig. 2).

3.3. Effects of cortisol on cytokine mRNA levels

Incubations with 50 and 100 ng mL^{-1} cortisol for 1 h inhibited the expression of all cytokines in a dose-dependent manner: TNF-a mRNA levels were reduced (to 90% and 73% of the control levels for 50 ng mL^{-1} and 100 ng mL^{-1}, respectively), and so were IL-1b (68% and 65%), TGF-b1 (55% and 56%) and IL-6 (51% and 30%) expression (Fig. 3A). With a longer incubation (2 h), cortisol effects were even stronger: TNF-a mRNA levels were

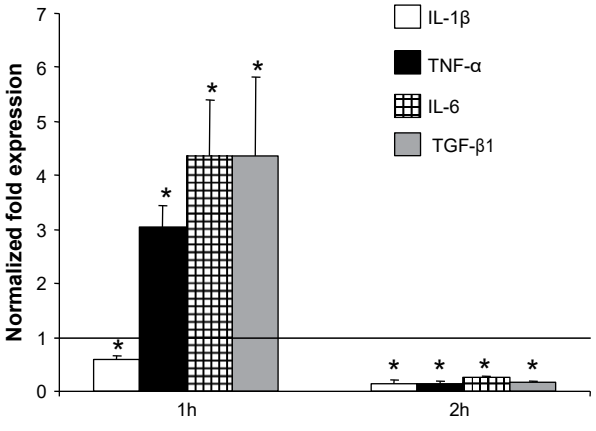

Fig. 1. ACTH effects on cytokine expression in head kidney preparations. Cell suspensions were performed as described in the Materials and methods section and incubated with 150 ng mL^{-1} ACTH. Cytokine mRNA levels were analyzed by quantitative real-time PCR and normalized to 18S rRNA. Results are expressed as mean ± SD. Asterisks indicate significant differences versus non-stimulated control (set at 1) ($P < 0.05$).

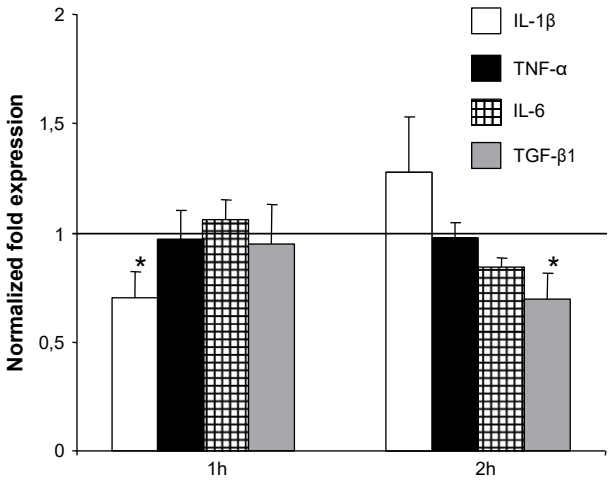

Fig. 2. Adrenaline effects on cytokine expression in head kidney preparations. Cell suspensions were performed as described in the Materials and methods section and incubated with 1 mM adrenaline. Cytokine mRNA levels were analyzed by quantitative real-time PCR and normalized to 18S rRNA. Results are expressed as mean ± SD. Asterisks indicate significant differences versus non-stimulated control (set at 1) ($P < 0.05$).

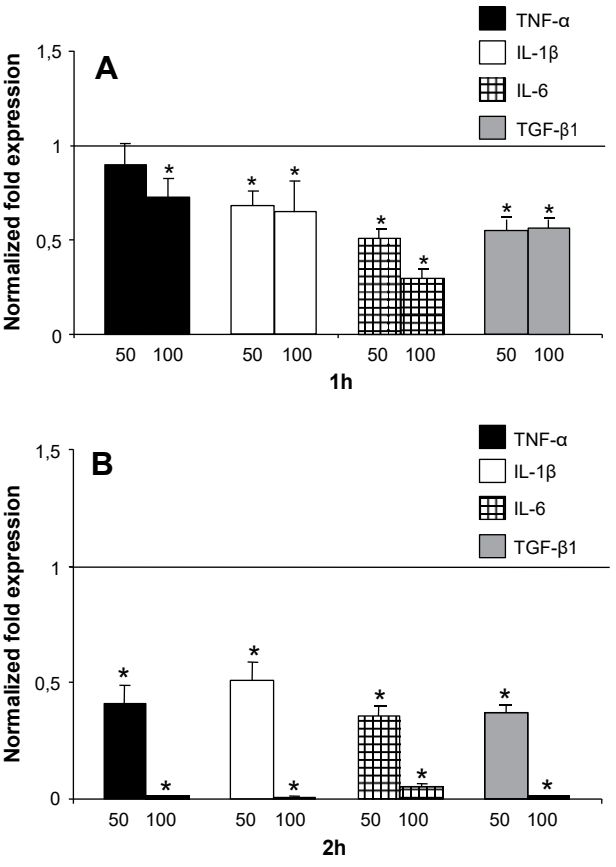

Fig. 3. Effects of cortisol on cytokine expression in head kidney preparations. Cell suspensions were performed as described in the Materials and methods section and incubated with 50 and 100 ng mL^{-1} cortisol for 1 h (A) or 2 h (B). Cytokine mRNA levels were analyzed by quantitative real-time PCR and normalized to 18S rRNA. Results are expressed as mean ± SD. Asterisks indicate significant differences versus non-stimulated control (set at 1) ($P < 0.05$).

reduced (to 41% and 1.2% of the control levels for 50 ng mL^{-1} and 100 ng mL^{-1}, respectively), and so were IL-1b (51% and 0.8%), TGF-b1 (37% and 1%) and IL-6 (36% and 5%) expression (Fig. 3B).

3.4. Effect of stress hormones on LPS-stimulated cytokine expression in head kidney preparations

When cells were incubated with 10 mg mL^{-1} LPS for 2 h, IL-1b expression was highly up-regulated (2014% versus control), although neither TNF-a nor IL-6 were significantly affected (96% of control and 149%, respectively). Moreover, the expression of the anti-inflammatory cytokine TGF-b1 was clearly inhibited (31.7% of control) by LPS (Fig. 4).

When LPS was incubated in combination with stress hormones, both adrenaline (1 mM) and cortisol (50 ng mL^{-1}) decreased the LPS-stimulated IL-1b expression (from 2014% with LPS alone to 634% and 264% in combination with adrenaline and cortisol, respectively). TNF-a expression was not significantly inhibited by the combination LPS-adrenaline (74.6% of control) but cortisol and LPS together inhibited its expression (56.18%). However, IL-6 gene expression was not affected by the combination (103% and 179% with LPS plus adrenaline and cortisol, respectively). Expression of the anti-inflammatory cytokine TGF-b1, on the other hand, was slightly recovered when combining LPS with adrenaline (from 31.7% with LPS alone to 45.7% in combination) but not when combining LPS with cortisol (31.8%) (Fig. 4).

Fig. 5 shows the effect of incubations with LPS on gene expression in combination with the pituitary hormone ACTH (150 ng mL^{-1}). ACTH did not alter the LPS-stimulated IL-1b expression (104% with ACTH plus LPS compared to LPS alone) although in combination with LPS TNF-a mRNA levels were increased (261%) and TGF-b1 expression levels were slightly recovered (from 37.7% with LPS alone to 52.3% with LPS plus ACTH). IL-6 levels, on the other hand, were not affected (80.9% of control with LPS alone and 83.2% in combination with ACTH) (Fig. 5).

4. Discussion

In this study, we show for the first time the effects of stress-related hormones (adrenaline, ACTH and cortisol) on the cytokine expression in primary cultures of head kidney from a teleost fish,

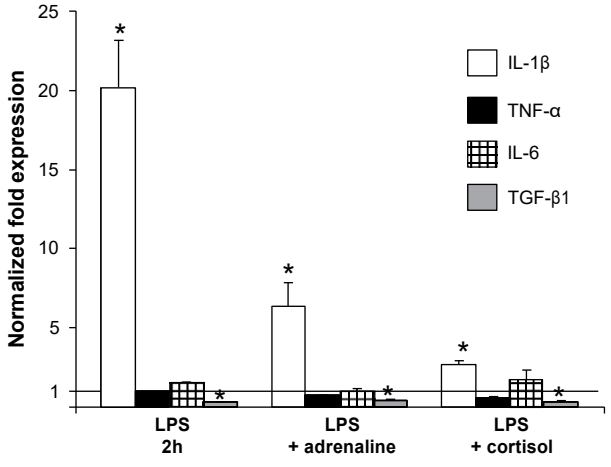

Fig. 4. Stress adrenal hormones modulate the LPS effects on cytokine expression in head kidney preparations. Cell suspensions were performed as described in the Materials and methods section and incubated with 10 mg mL^{-1} LPS alone or in combination with adrenaline (1 mM) or cortisol (50 ng mL^{-1}) for 2 h. Cytokine mRNA levels were analyzed by quantitative real-time PCR and normalized to 18S rRNA. Results are expressed as mean ± SD. Asterisks indicate significant differences versus non-stimulated control (set at 1) ($P < 0.05$).

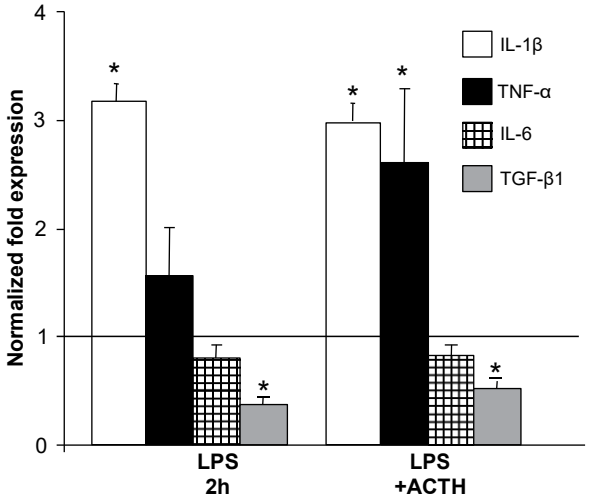

Fig. 5. The pituitary stress hormone ACTH modulates the LPS effects on cytokine expression in head kidney preparations. Cell suspensions were performed as described in the Materials and methods section and incubated with 10 mg mL^{-1} LPS alone or in combination with ACTH (150 ng mL^{-1}) for 2 h. Cytokine mRNA levels were analyzed by quantitative real-time PCR and normalized to 18S rRNA. Results are expressed as mean ± SD. Asterisks indicate significant differences versus non-stimulated control (set at 1) ($P < 0.05$).

the seabream. The effects of stress hormones on the immune system are difficult to interpret in vivo, since a number of different hormones, which, in turn affect the release of other hormones, are involved via the HPI axis. Thus, it is likely that in vivo 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. To further study the effects of stress hormones without all these interactions, we have used an in vitro model of seabream head kidney, which has been described for different fish species and has revealed as an useful tool in which to study the ''intra-adrenal'' interactions among the different cell types of the tissue with a single preparation [44,46,47]. The aim of our study was to use this model to investigate the interactions of adrenaline and cortisol (hormones related to stress and secreted in the head kidney) and the pituitary hormone ACTH on the expression of cytokines in the seabream head kidney.

Cytokine expression has been examined in the head kidney of several fish species: IL-1b mRNA has been detected in seabream [48], TNF-a has been described in many fish species, including seabream [28] and trout [17], whereas IL-6 expression was described in Fugu [33] and very recently in seabream [35]. Interestingly, some authors were unable to detect IL-6 expression in the head kidney of rainbow trout [34]. On the other hand, the anti-inflammatory cytokine TGF-b1 has been described in the head kidney of seabream [37] and trout [49], among other fish species.

ACTH, a pituitary peptide which regulates cortisol secretion by the interrenal cells, has been shown as a key hormone in stress situations in fish [50] and its receptors are present in the membranes of head kidney interrenal cells [51] but also in other tissues of fish [52]. In addition, ACTH receptors have been found in mammalian leukocyte populations, where they contribute to modulate immune responses [53,54]. The ACTH concentration used in the present study (150 ng mL^{-1}) has been shown to be effective in trout [55] and in seabream head kidney preparations, where ACTH exerts its effects through its specific receptor, which can be blocked using a specific antagonist, as we previously reported [44]. ACTH binds to MC2 receptors in the head kidney and increases cortisol production in steroidogenic cells, among other functions.

Our group has already described the effect of ACTH stimulating the expression of the steroidogenic gene StAR, which in turn is a regulatory key of cortisol secretion, in seabream head kidney suspensions [44], but so far its effects on the immune system have not been studied in this species. Here we demonstrate strong in vitro effects of ACTH on mRNA levels of pro- and anti-inflammatory cytokines in seabream head kidney. We found an important increase of the pro-inflammatory cytokines TNF-a and IL-6 mRNA levels after 1 h treatment with 150 ng mL^{-1} ACTH, although expression of these cytokines was inhibited after 2 h, indicating that their actions are rapidly regulated. Interestingly, IL-1b expression was clearly decreased by 1h incubation with ACTH, a decrease still found after 2h, indicating that the expression of this pro-inflammatory cytokine is very sensitive to an inhibition by a stress situation involving ACTH actions. On the other hand, the expression of the anti-inflammatory cytokine TGF-b1 was increased at 1 h by ACTH although after 2 h its mRNA levels were strongly down-regulated, likewise indicating the high regulation of TGF-b1 in stress situations involving ACTH release, probably counteracting the inflammatory response that takes place. We have recently reported that ACTH treatment increases cortisol secretion in seabream head kidney preparations [44], and one cannot disregard the possible contribution of this ACTH-stimulated increase of cortisol levels to the inhibitory effects on cytokine expression levels, especially after 2 h of in vitro incubation. In mammals, adrenaline has been found to suppress the expression of some inflammatory proteins, including cytokines [56,57] and to increase the production of some anti-inflammatory cytokines [58,59] suggesting that the sympathetic response to infectious challenge would dampen the robust pro-inflammatory cytokine release. However, the interaction between sympathetic stimulation and the immune system is complex, with adrenergic mechanisms having both immunosuppressive and immunoenhancing effects depending on the receptor subtype stimulated, the cytokine assessed and, often, the cell type examined [60]. In fish, stress-related cathecolamines are also involved in the depression of the immune response [61]. Here we describe that, in seabream head kidney preparations, short time incubations with adrenaline (1 mM) were unable to significantly alter TNF-a, TGF-b1 and IL-6 expression levels, and only IL-1b mRNA levels were decreased, indicating that the pro-inflammatory response of IL-1b can be inhibited by an acute stress situation. After 2h, IL-1b expression was restored to control levels, suggesting that it is rapidly regulated, TNF-a remained unchanged and IL-6 and TGF-b1 expression were inhibited. This inhibition of cytokine expression is in concordance with the described inhibition of the pro-inflammatory cytokine expression by adrenaline stress in mammalian systems [62,63] and confirms the previously described important role of immune-endocrine interactions in the head kidney of fish [64,65]. However, we cannot disregard the possible effects of different adrenaline receptors in the head kidney (already described in other species, such as catfish [66] and rainbow trout [67] where head kidney leukocytes expressed both alpha and beta adrenergic receptors) that could be exerting differential and even opposite effects and therefore counteracting the expression of cytokines.

In a previous work, we reported that a concentration of 50 ng mL^{-1} of cortisol is reached in the plasma of chronically stressed seabream, whereas 100 ng mL^{-1} cortisol is the plasma concentration found in acutely stressed seabream [44]. In the present study, we describe the effect of these two physiological cortisol concentrations on the cytokine expression in head kidney preparations; after 1 h with cortisol, all pro-inflammatory and anti-inflammatory cytokines were inhibited by both cortisol concentrations in a dose-dependent manner, and after 2 h the inhibition was even stronger. This inhibition of the immune response by cortisol is in agreement with previous studies demonstrating that glucocorticoids down regulate and affect the stability of cytokine mRNA levels [68] and confirms the previously described immunodepressive effects in fish: cortisol administration reduces the number of circulating T- and B-like lymphocytes [69–71]; also acute stress or cortisol treatment in juvenile coho salmon lowers the number of circulating leucocytes [72] and induces apoptosis of B cells following stress [73]. However, further research will increase the knowledge on the role of cortisol modulating the immune system in head kidney.

In the present study, LPS was able to increase IL-1b expression and decreased TGF-b1, without affecting TNF-a and IL-6 mRNA levels. Our results on IL-6 expression differ from a very recent work [35] where authors found that IL-6 expression in isolated acidophilic granulocytes and macrophages was up-regulated by LPS, whereas we found no changes in full head kidney preparations. These different results could be explained by the fact that our preparations consist of a mixture of head kidney cells, trying to reproduce a system closer to the in vivo situation, and therefore some interactions among different cell types may occur. In a previous report, some authors had already reported the increase of IL-1b expression in response to LPS stimulation in seabream macrophages [48]. On the other hand, the lack of effect of LPS on TNF-a expression was also described in seabream [28], where LPS failed to increase TNF-a expression in cultured macrophages. According to the authors, this lack of response could be explained because the seabream TNF-a messenger is widely and constitutively accumulated in seabream. From these data, therefore, seabream TNF-a and IL-1b expression seem to be regulated in a completely different manner.

In our experiments, both adrenaline and cortisol were able to decrease the LPS-stimulated increase of IL-1b. Regarding these data, previous reports showed that adrenaline reduces the sepsis-stimulated increase of cytokine levels [63] and cortisol has been reported to attenuate LPS-stimulated inflammation [74], indicating the ability of these hormones to regulate the cytokine response to endotoxin, and therefore having certain anti-inflammatory effects. ACTH, on the other hand, did not affect the LPS-stimulated increase of IL-1b mRNA levels, suggesting that this hormone does not possess anti-inflammatory effects by itself. It is surprising, though, that when ACTH was combined with LPS, TNF-a expression levels increased significantly (LPS alone was unable to do it, in a similar way than other authors have reported [75]), which may be due to the direct stimulatory effects of ACTH on TNF-a expression that we report in this study.

In conclusion, here we describe for the first time the effects of stress hormones on the cytokine expression in primary cultures of head kidney of a teleost fish. Adrenaline inhibited cytokine expression levels, being IL-1b the most sensitive cytokine to adrenaline in vitro. On the other hand, ACTH rapidly inhibited IL-1b and increased TNF-a, TGF-b1 and IL-6 expression. Cortisol inhibited the expression of all cytokines confirming its immunosuppressive effects. Finally, bacterial LPS induced the increase of IL-1b mRNA levels, without changes in TNF-a expression. Moreover, adrenaline and cortisol, but not ACTH, were able to reduce the LPS-stimulated expression of IL-1b in head kidney. All these data suggest that cytokine expression in head kidney is highly regulated by stress-related hormones, and it is another evidence of the existence of endocrine-immune interactions in the teleost fish. Further studies will be necessary to fully describe the paracrine interactions between these two systems in the head kidney of teleost fish.

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