

Effects of GH, prolactin and cortisol on hepatic heat shock protein 70 expression in a marine teleost *Sparus sarba*

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Abstract

Heat shock protein 70 (HSP70) expression was assessed in hepatic tissue of a marine teleost *Sparus sarba* after exogenous hormone administration. Using a PCR-amplified, homologous HSP70 cDNA clone, as a probe in Northern analysis, we detected a 2.3 kb transcript which was elevated after exposure to a temperature 7 °C above the ambient. For our studies on hormonal effects on HSP70 expression, groups of fish were administered recombinant bream GH (rbGH), ovine prolactin (oPRL) or cortisol daily over a 7-day period. Quantification of hepatic HSP70 transcript revealed that the administration of GH and PRL significantly reduced HSP70 mRNA abundance

by 42% and 54% from saline-injected fish respectively. Also hepatic HSP70 levels were reduced by 76% and 64% as determined by immunoblotting after rbGH and oPRL treatment respectively. The administration of exogenous cortisol did not alter hepatic HSP70 mRNA or protein levels in *S. sarba*. The results obtained in this study are the first evidence for hormonal modulation of heat shock protein expression in fish. The significance of these results is discussed within the context of current knowledge on the roles of these hormones in teleostean stress response.

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Introduction

The heat shock protein (HSP) group belongs to a family of proteins which are expressed in response to a wide variety of biotic and abiotic stressors (Iwama *et al.* 1998). These proteins have been isolated from evolutionary diverse organisms and are characterised on the basis of their molecular mass. Of the HSP family, it is HSP70 which has been most widely studied and is involved in multiple cellular processes such as antigen processing and presentation, protein translocation, protein folding and regulation of the stress response (De Nagel & Pierce 1992, Craig *et al.* 1994).

Most of our current knowledge on fish HSP expression has been derived from studies using established cell lines. Data from many of these studies have shown that fish HSP expression is induced by a number of stressors, such as thermal shock (Airaksinen *et al.* 1998, Chen *et al.* 1988, Mosser & Bols 1988), heavy metal exposure (Heikkila *et al.* 1982, Misra *et al.* 1989), hypoxia (Airaksinen *et al.* 1998) and exposure to infectious agents (Cho *et al.* 1997). Although fewer experiments have been performed using whole fish, elevated levels of HSP70 in different tissues from *Fundulus heteroclitus* (Koban *et al.* 1991), *Pimephales promelas* (Dyer *et al.* 1991), *Gillichthys mirabilis* and *Gillichthys seta* (Dietz & Somero 1992) after thermal shock have been described. Furthermore, the application of molecular biology techniques to fish HSP studies has been

used for several species. For example, a constitutively expressed heat shock cognate gene from *Oncorhynchus mykiss* testes (Zafarullah *et al.* 1992), two HSP70-related genes from the cell lines OLHSC70 and CEHSC70 of *Oryzias latipes* and *Oryzias celebensis* respectively (Arai *et al.* 1995), and an HSP70 from a long-term leucocyte cell line of *Ictalurus punctatus* (Luft *et al.* 1996) have been cloned and characterised. Although all of the aforementioned studies have provided valuable and interesting knowledge on the biology of piscine HSP, the endocrine regulation of HSP expression in fish remains to be elucidated. The present study investigated the expression of hepatic HSP70 of a marine teleost *Sparus sarba* after treatment with recombinant bream growth hormone (rbGH), ovine prolactin (oPRL) and cortisol.

Materials and Methods

Experimental fish and temperature stress

Silver seabream (*Sparus sarba*), weighing between 70 and 100g, were obtained from local sea cages and transferred to laboratory aquaria equipped with seawater recirculation. Fish were acclimated to these conditions for at least 4 weeks prior to experiments. For temperature stress, fish were divided into three groups 1 day before experimentation. The fish in the first group served as a control and

were maintained at an ambient temperature of 25 °C. The fish in the second and third groups were gradually exposed, at a rate of 0.2 °C/min, to a temperature of 16 °C (cold shock) and 32 °C (heat shock) respectively. In all cases, changes in temperature were achieved by using a system of immersion heaters and coolers. The fish were maintained at cold (16 °C)- and heat (32 °C)-shock experimental temperatures for 1 h, after which time they were killed and the hepatic tissue removed and quick frozen with liquid nitrogen.

Hormone treatments

Fish were randomly divided into five groups and maintained in seawater for 3 weeks. During this period, temperature was kept between 18 and 22 °C and the fish were exposed to a natural photoperiod (11 h light:13 h darkness) and fed *ad libitum* once daily with a formulated diet (Woo & Kelly 1995). During the final week of acclimation the fish received either daily intraperitoneal injections of saline (0.8% (w/v) NaCl) vehicle, rbGH (GroPep Pty Ltd, Australia; 1 µg/g), oPRL (Sigma, USA; 6 µg/g) or cortisol (Solu-Cortef; Upjohn, Belgium; 4 µg/g). Hormone doses were based on those used in previous studies (Madsen 1990, Herndon *et al.* 1991). Injections were given between 0900 and 1000 h, and the last injection was administered 24 h prior to death and removal of hepatic tissue. One group of fish was left untreated, this served as a control for saline-injected fish. No mortality was recorded throughout the experimental period, and feeding was terminated 48 h prior to death.

Total RNA extraction and first strand cDNA synthesis

Hepatic tissue was homogenised for 30 s in 2 ml lysis buffer (5 M guanidine thiocyanate, 9 mM EDTA, 45 mM Tris/HCl (pH 8.0)), using an Ultra-Turrax T25 rotor-stator homogeniser (IKA Labor Technik, Germany). Total RNA was extracted from the lysate using a Qiagen RNeasy mini kit (Qiagen GmbH, Germany), treated with DNaseI (Gibco-BRL, USA), aliquoted and stored at -70 °C. For first strand cDNA synthesis, total RNA (1 µg) was added to a reaction mix (20 µl), containing 0.5 µg oligo DT primer (Pharmacia LKB, Sweden), 2 µl dithiothreitol (0.1M), 1 µl dNTP mix (Pharmacia LKB; 10 mM), 4 µl reaction buffer and 1 µl reverse transcriptase (Gibco-BRL; 200 U/µl). First strand cDNA synthesis was allowed to proceed at 42 °C for 1 h after which time the reaction was incubated at 70 °C for 10 min and then stored at -20 °C.

Isolation and characterisation of HSP70 cDNA clone

An HSP70 cDNA clone was obtained by using PCR amplification of first strand cDNA. The oligonucleotide primers used were: 5'-CA(AG)GA(TC)TT(TC)TT(TC)

AA(TC)GGAAA(GA)GA-3' (sense) and 5'-CCCC AGCACT(TC)TG(AG)TA(ACGT)AG(TC)TT-3' (antisense). Positions of degeneracy are represented in parentheses, both primers were synthesised by Genset (Japan). Each PCR reaction (50 µl) contained 2 µl first strand cDNA, 0.2 µl Taq DNA polymerase (Promega, USA; 5 U/µl), 5 µl MgCl₂ (25 mM), 5 µl reaction buffer, 0.5 µl dNTP mix (10 mM) and 1 µl of each oligonucleotide primer (50 pM). PCR amplification was performed using a PTC-100 thermal cycler (MJ Research Inc., USA) with 45 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min and a final extension of 72 °C for 4 min. Reaction products (10 µl), were analysed electrophoretically on a 1.4% (w/v) agarose gel (Bio-Rad Laboratories, USA) and visualised by ethidium bromide staining. Putative gene fragments were subcloned into pCRscript (Stratagene, USA) and cycle sequenced using an ABI PRISM dye terminator kit with reaction products run on an ABI 310 Genetic Analyzer (Perkin Elmer, USA). The cloned fragment was sequenced on both strands and its sequence was analysed using the Basic Local Alignment Search Tool Programme (Altschul *et al.* 1990).

Actin cDNA for loading control

An actin cDNA clone was PCR amplified from first strand cDNA using primers kindly supplied by Dr K H Chu (Department of Biology, The Chinese University of Hong Kong); their sequences were: 5'-TCACCAACTGGG ATGACATG-3' (sense) and 5'-ATCCACATCTGCTG GAAGGT-3' (antisense). The PCR amplification cycle parameters and cDNA cloning methods were as previously described.

Northern and RNA dot-blot analyses

Hepatic tissue total RNA (10 µg) from individual control, cold-shocked and heat-shocked fish were adjusted to a final volume of 10 µl with RNase free water. Following the addition of 3 µl RNA loading buffer (Sigma) and incubation at 65 °C for 10 min, the RNA samples were electrophoresed under denaturing conditions through a 1.2% (w/v) agarose, 2.2 M formaldehyde, 1 × MESA buffer (Sigma) gel with running buffer (1 × MESA, 0.2 M formaldehyde). A 0.24–9.5 kb RNA ladder (Gibco-BRL) was used as molecular weight standard marker. After electrophoresis, the gel was photographed and transferred to Hybond N+ nylon membrane (Amersham International plc, UK) by capillary blotting in 20 × SSC (Sambrook *et al.* 1989). The RNA was covalently attached to the membrane using a UVC 515 multi-linker (Ultra-Lum, USA). Radiolabelled HSP70 cDNA probe was prepared using a Rediprime random labelling kit (Amersham International plc), and used for membrane hybridisation in Rapid-Hyb buffer (Amersham International plc) at 55 °C for 16 h. After hybridisation,

the membrane was washed twice with a $2 \times$ SSC, 0.1% SDS solution for 30 min at 55 °C and then once in a $0.1 \times$ SSC, 0.1% SDS solution for 30 min at 55 °C. The membrane was air-dried and then autoradiographed at -80 °C using Kodak X-OMAT AR film, with intensifying screens for 20 h. After the transcript signal was analysed, the membrane was stripped by washing at 80 °C in 0.5% SDS and then rehybridised with an actin probe as a loading control. RNA dot blots were prepared using a Bio-Dot microfiltration manifold (Bio-Rad). Hepatic tissue total RNA samples (10 µg) from individual fish were prepared and blotted according to instructions supplied with Hybond N+ membrane. Dot blots were hybridised and washed as described above and exposed to a storage phosphor screen (Molecular Dynamics, USA) for 3 h at room temperature. The plates were scanned using the Storm PhosphorImaging system with ImageQuant software (Molecular Dynamics) for quantification of mRNA transcript. The abundance of HSP70 mRNA was normalised to its corresponding actin mRNA abundance for each sample.

Protein extraction and quantification

Approximately 100 mg hepatic tissue from each fish was added to 2 ml homogenisation buffer (50 mM Tris-HCl (pH 7.2), 2% (w/v) SDS, 1 mM β -mercaptoethanol). The sample was homogenised using an Ultra-Turrax T25 rotor stator homogeniser for 30 s, centrifuged at 10 000 g for 2 min, incubated at 90 °C for 10 min and then sonicated for 5 min. Protein concentration was quantified using the dye binding method (Bradford 1976). Samples were read at 595 nm using a spectrophotometer (Milton Roy Spectronic) and protein concentration calculated from a protein standard curve of bovine serum albumin (Sigma).

Protein gel electrophoresis and immunoblotting

One-dimensional SDS-PAGE was applied to resolve proteins of different molecular size according to the method of Laemmli (1970) using a 4% (stacking) and a 12% (separating) polyacrylamide gel. Total liver protein (50 µg) and bovine HSP70 (0.1 µg; Sigma) were electrophoresed for 50 min at 160 V using a Bio-Rad mini gel kit. After SDS-PAGE, the resolved proteins were transferred to nitrocellulose membrane (Bio-Rad) using an electro-transfer cell for 1 h at 100 V. After protein transfer the membrane was rinsed briefly in 0.01M phosphate-buffered saline (PBS, pH 7.2) containing 0.05% (v/v) Tween 20 (PBS-T) and then blocked in PBS-T/3% (w/v) skimmed milk powder overnight at 4 °C. The membrane was rinsed for 1 h in PBS-T and then incubated for 1 h with antimouse HSP70 monoclonal antiserum (Sigma), diluted 1:4000 in PBS-T. The membrane was rinsed for a further 1 h before incubating with antimouse IgG horse-radish peroxidase conjugate (Sigma) diluted 1:8000. After

a final membrane rinse the bound HSP70 was visualised using an ECL development system (Amersham International plc). Protein immunodot blots were used to assess all protein samples together for hepatic HSP70. For this purpose a Bio-Dot microfiltration manifold (Bio-Rad) was used. Protein samples (20 µg) from individual fish were blotted onto nitrocellulose membrane (Gibco-BRL), probed and visualised as previously described. The relative optical density for each protein sample was measured using an image densitometer (GS-700; Bio-Rad) and these values were read against a standard curve of bovine brain HSP70 (0–200 ng) in order to determine the concentration of HSP70 per sample.

Statistical analysis

The normalised mRNA data from each group and the HSP70 sample concentrations, as determined by immunoblotting and densitometry, were subjected to a one-way ANOVA to test for significance followed by a Student–Newman–Kuels test to delineate significance (Jandel Scientific) between groups. Significant differences were accepted if $P < 0.05$.

Results

HSP70 cDNA sequence

In order to conduct Northern and dot-blot analysis we preferred to isolate a cDNA fragment containing HSP70 sequence from *Sparus sarba*. PCR amplification of single strand cDNA yielded a fragment of 794 bp which was cloned into pCRscript and sequenced. The sequence of this clone displayed 80%–92% amino acid homology with previously characterised HSP70 genes from plant (Roberts & Key 1991), bird (Morimoto *et al.* 1986), mammal (Roux *et al.* 1994) and fish (Graser *et al.* 1996). The nucleotide sequences and deduced amino acid sequences of this HSP70 cDNA clone are presented in Fig. 1.

Hepatic HSP70 expression after temperature stress

Northern blot analysis performed on total RNA from *S. sarba* hepatic tissue revealed a single HSP70 transcript of 2.3 kb (Fig. 2). Low levels of transcript were observed in control fish and the level of this transcript was elevated after 1 h of heat shock. Cold shock had no effect on the levels of HSP70 transcript.

Hormonal effects on hepatic HSP70 mRNA abundance

In order to assess the effects of hormone treatment on hepatic HSP70 expression in *S. sarba*, RNA dot-blot

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  Q   D   F   F   N   G   K   E   L   N   K   S   I
  CAG GAT TTC TTC AAT GGA AAG GAG CTT AAC AAG AGC ATC -39

  N   P   D   E   A   V   A   Y   G   A   A   V   Q
  AAT CCA GAT GAA GCT GTG GCT TAC GGC GCT GCT GTC CAG -78

  A   P   I   L   S   G   D   K   S   E   N   V   Q
  GCT CCC ATC CTG TCT GGT GAC AAG TCT GAG AAT GTG CAG -117

  D   L   L   L   L   D   V   T   P   L   S   V   G
  GAC CTG CTT TTG CTG GAT GTC ACT CCT CTG TCC GTG GGT -156

  I   E   T   A   G   G   V   M   T   V   L   I   K
  ATT GAG ACT GCT GGA GGT GTC ATG ACT GTC CTT ATC AAA -195

  R   N   T   T   I   P   T   K   Q   T   Q   T   F
  CGC AAC ACC ACC ATT CCT ACC AAG CAG ACC CAG ACT TTC -234

  P   T   H   S   D   N   Q   P   G   V   L   I   Q
  CCC ACC CAC TCT GAC AAT CAG CCT GGT GTG CTC ATC CAG -273

  V   Y   E   G   E   R   A   M   T   T   D   N   N
  GTC TAT GAA GGA GAG AGG GCC ATG ACC ACA GAC AAC AAC -312

  L   L   G   K   F   E   L   T   G   I   P   P   A
  CTG CTG GGC AAG TTT GAG CTG ACG GGC ATC CCC CCT GCT -351

  P   R   G   V   P   Q   V   E   V   T   F   D   I
  CCT CGT GGT GTT CCC CAG GTC GAG GTG ACA TTT GAC ATT -390

  D   A   N   G   I   M   N   V   S   A   V   D   K
  GAT GCC AAT GGC ATT ATG AAT GTC TCT GCT GTA GAC AAG -429

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Figure 1 (a).

analysis was used and the results are presented in Fig. 3. We observed no significant differences in abundance of HSP70 mRNA between untreated and saline-treated fish. However, HSP70 mRNA abundance after the administration of GH and PRL was significantly decreased 42% and 54% respectively when compared with the saline-injected group. Cortisol treatment did not alter hepatic

HSP70 mRNA levels. The levels of actin mRNA were not altered by hormone treatments.

Hormonal effects on hepatic HSP70 levels

Immunoblotting was used to assess hepatic HSP70 levels. A single HSP70 was detected using antimouse HSP70

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      S   T   G   K   E   N   K   I   T   N   H   N   V
AGC ACT GGC AAG GAG AAC AAG ATC ACA AAT CAC AAT GTA -468

      Q   G   S   K   E   D   I   E   R   M   V   Q   Q
CAA GGG AGT AAG GAA GAC ATT GAA CGC ATG GTT CAG CAA -507

      A   E   K   Y   K   A   E   D   D   V   Q   R   D
GCT GAA AAG TAC AAG GCT GAA GAT GAT GTC CAG CGA GAC -546

      K   V   S   A   K   N   G   L   E   S   Y   A   F
AAG GTG TCT GCT AAG AAT GGC CTG GAG TCG TAT GCT TTC -585

      N   M   K   S   S   V   E   D   E   K   L   A   G
AAC ATG AAG TCC TCT GTG GAG GAT GAA AAA CTT GCT GGC -624

      K   I   S   D   D   D   K   Q   K   I   L   D   K
AAA ATC AGT GAT GAC GAC AAG CAG AAG ATT TTG GAC AAA -663

      C   N   E   V   I   S   W   L   D   K   N   Q   T
TGC AAT GAG GTC ATC AGC TGG CTG GAC AAG AAC CAG ACT -702

      A   E   R   D   E   Y   E   H   Q   Q   K   E   L
GCC GAG AGG GAT GAG TAT GAG CAT CAA CAG AAG GAG CTG -741

      E   K   V   C   N   P   I   I   T   K   L   Y   Q
GAG AAG GTG TGC AAC CCC ATC ATC ACC AAG CTA TAC CAA -780

      S   A   G   G
AGT GCT GGG GGG GG -794

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Figure 1 (b).

Figure 1 Nucleotide and deduced amino acid sequence of *Sparus sarba* HSP70 794 base pair cDNA clone. Nucleotides are numbered on the right-hand side and the PCR primer regions are underlined.

antiserum. The molecular size of this HSP70 was identical to that of bovine brain HSP70 used as a standard (Fig. 4). The levels of hepatic HSP70 from hormone-treated fish were assessed and the results are presented in Fig. 5. No significant difference in hepatic HSP70 levels between untreated and saline-treated fish was observed. However, the administration of exogenous rbGH and oPRL significantly reduced hepatic HSP70 by 76% and 64% respectively from saline-injected controls. Cortisol treatment did not alter hepatic HSP70 levels.

Discussion

The present study was performed in order to gain new insight into the modulatory effects of hormones on HSP70 expression in a marine teleost. By using PCR amplification of single strand cDNA we have isolated a 794 base pair HSP70 cDNA clone which was used as a probe for our expression studies. Northern analysis of hepatic tissue mRNA revealed the presence of a single 2.3 kb transcript, which was elevated after acute heat shock exposure of

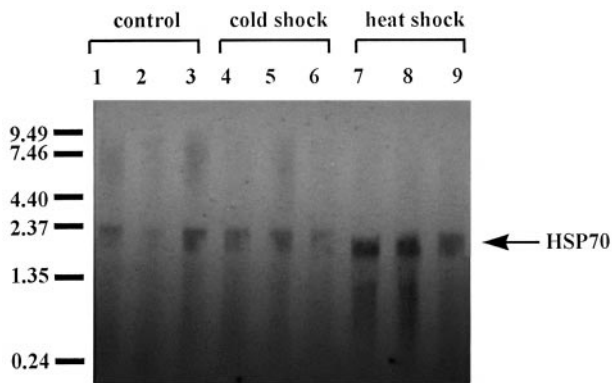


Figure 2 *Sparus sarba* HSP70 transcript analysis after temperature stress. Fish were maintained at 25 °C (control), 16 °C (cold shock) or 32 °C (heat shock) for 1 h prior to being killed. Hepatic total RNA (10 µg) from three individual fish for each temperature were analysed.

7 °C above ambient temperature and, in this respect, the HSP70 induction profile of *S. sarba* is similar to that of *Fundulus heteroclitus* hepatic HSP70 (Koban *et al.* 1991). Interestingly in *S. sarba*, a low constitutive level of HSP70 transcript was observed under ambient temperature conditions. This may suggest that HSP70 has other cellular roles apart from stress response, such as correct protein assembly and regulating interactions between hormones and their receptors (Welch 1993).

The growth rate of fish is principally influenced by food availability, appetite and consumption, intestinal processing and metabolic rate, all of which can be affected by stress (Wendelaar Bonga 1997). Fish respond to stress catabolically, utilising metabolic energy for numerous activities which together act to reduce growth rate. The administration of exogenous GH has been demonstrated to enhance growth in several fish species, including *Oncorhynchus mykiss*, *O. kisutch*, *O. tshawytscha* and *Oreochromis mossambicus* (Foster *et al.* 1991, Flik *et al.* 1993, McLean *et al.* 1997). GH-induced growth enhancement suggests a

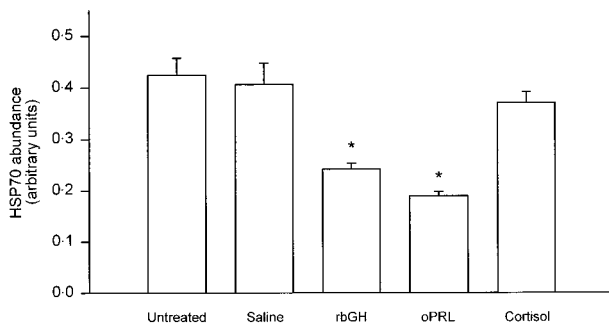


Figure 3 *Sparus sarba* hepatic HSP70 expression in untreated, saline-, rbGH-, oPRL- and cortisol-treated fish. Values are means ± S.E.M. HSP70 mRNA levels ($n=7$) normalised to corresponding actin mRNA. * $P<0.05$ compared with saline treatment.

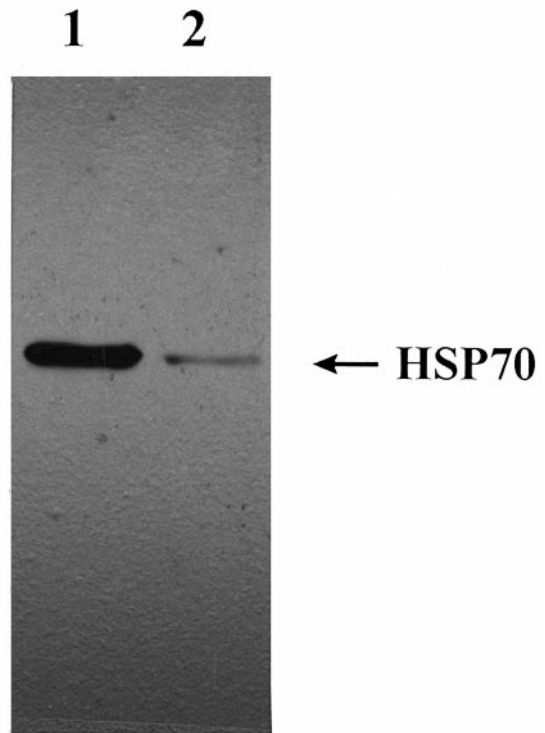


Figure 4 Immunoblot showing HSP70 from bovine brain, used as a control (lane 1) and *Sparus sarba* liver (lane 2).

reallocation of metabolic energy to anabolic processes and may be indicative of reduced stress as fish subjected to chronic stressors often exhibit compromised growth (Wendelaar Bonga 1997). Presently the role of GH in fish stress response has not been fully elucidated; however, studies on *O. mykiss* (Pickering *et al.* 1991) and *Oreochromis niloticus* (Auperin *et al.* 1997) have shown that plasma GH levels decreased significantly during confinement stress. Circulating GH returned to pre-stress levels in these fish

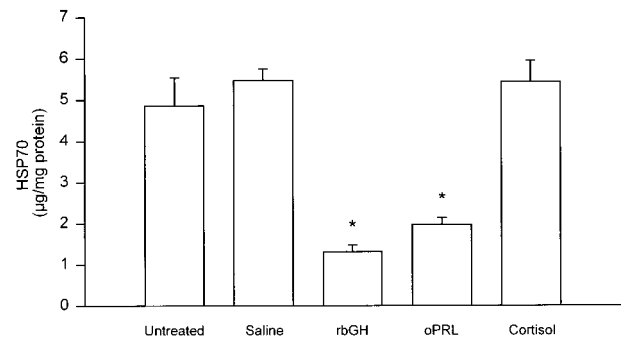


Figure 5 *Sparus sarba* hepatic HSP70 expression in untreated, saline-, rbGH-, oPRL- and cortisol-treated fish as determined by immunoblotting and densitometric analysis. Values are means ± S.E.M. HSP70 levels ($n=7$). * $P<0.05$ compared with saline treatment.

upon return to normal rearing conditions. Although both of these studies suggest that GH levels are affected during stress, no studies have addressed the effect of this hormone on HSP70 expression in fish. In the present study we have demonstrated that the administration of exogenous rbGH to *Sparus sarba* (1 µg/g for 7 days) resulted in a 42% decrease in hepatic HSP70 mRNA. From this study no firm conclusions can be made as to whether this reduction in hepatic HSP70 mRNA is a result of decreased HSP70 gene expression or reduced HSP70 mRNA stability but the parallel decrease in hepatic HSP70 as determined by immunoblotting (76% from control levels), suggests that this reduction is physiologically relevant. The effect of this downregulation of hepatic HSP70 on the overall physiological state of *S. sarba* is presently unknown. However, increased levels of renal and hepatic HSP70 in *Oncorhynchus kisutch* infected with *Renibacterium salmoninarum* (Forsythe *et al.* 1997) and elevated amounts of a novel HSP90 from a fish rhabdovirus-infected *O. tshawytscha* embryo cell line (Lee *et al.* 1996) have been reported previously. In addition, studies on *O. mykiss* (Sakai *et al.* 1995), *Sparus aurata* (Calduch-Giner *et al.* 1997) and *S. sarba* (Narnaware *et al.* 1997) have all clearly demonstrated that the administration of exogenous GH at similar doses significantly enhanced the non-specific immune response as determined by elevated macrophage phagocytosis. The relationship between HSP70 expression and the immune response in fish is presently unknown; however, establishing the specific linkage between HSP70 downregulation and non-specific immunity would be interesting for future studies.

PRL is widely regarded as a hormone involved in freshwater adaptation of fish and its role in osmoregulation has been well documented (Herndon *et al.* 1991, McCormick 1995). However, the role and importance of PRL in the stress response has yet to be established. Elevated levels of plasma PRL have been described in confined *Oncorhynchus kisutch* (Avella *et al.* 1991) while plasma PRL levels were reduced in *O. mykiss* (Pottinger *et al.* 1992). In contrast to both these studies, no change in *O. mykiss* plasma PRL concentration was observed after the application of low water level and chasing stressors (Kakizawa *et al.* 1995). *Oreochromis niloticus* subjected to confinement stress showed elevated levels of two PRL forms with kinetics similar to those of the stress hormone cortisol (Auperin *et al.* 1997). Together, these previous studies suggest that PRL has an important role in fish stress response and in this report we have demonstrated that the administration of exogenous oPRL to *Sparus sarba* (6 µg/g for 7 days) reduced hepatic HSP70 mRNA abundance and protein by 56% and 64% respectively. The effect of this oPRL-induced downregulation of hepatic HSP70 expression on the physiological state of *S. sarba* was not addressed in this study. However, it has been suggested that conditions of hyperprolactinaemia may be beneficial to *S. sarba* during stress (Kelly *et al.* 1999). Of additional

interest is the immunostimulatory effect of PRL reported by Sakai *et al.* (1995) for *Oncorhynchus mykiss* and Narnaware *et al.* (1998) for *S. sarba* using similar doses of PRL. Data from the present study suggest an oPRL-induced HSP70 downregulation may be a phenomenon that occurs in parallel to oPRL-induced immunostimulation. Clear conclusions on the effect of PRL on hepatic HSP70 cannot be made from this study, as a heterologous source of PRL (ovine) was used. Evidence suggests, based on competitive binding assays, that in *Oreochromis mossambicus*, oPRL may compete and complex with GH receptor sites as well as PRL receptor sites (Dauder *et al.* 1990). A delineation of the effects of heterologous and homologous PRLs has yet to be addressed in the seabream.

In fish, cortisol plays a major role in the regulation of hydromineral balance and metabolism, and its role in the stress response in fish has been well documented (Wendelaar Bonga 1997). Elevation in plasma cortisol concentrations is a widely used indicator of stress in fish and its levels have been shown to increase rapidly under an array of stressful conditions (Wendelaar Bonga 1997). Presently, no studies have investigated the effects of exogenous cortisol administration on HSP expression in fish and as such we attempted to address this by studying changes in the levels of hepatic HSP70 mRNA in *Sparus sarba*. Our results clearly show that there was no significant alteration in the levels of hepatic HSP70 transcript between cortisol and control fish under the previously described experimental conditions. Similarly, hepatic HSP70 levels remained unaltered in *Oncorhynchus mykiss* after handling stress despite elevated plasma cortisol levels (Vijayan *et al.* 1997). Hence, the current evidence suggests that exogenous cortisol administration or hypercortisolaemia in fish have a minimal effect on HSP70 expression. It must be emphasised however that similar doses of cortisol have been previously documented to produce hypercortisolaemia and pronounced metabolic and osmoregulatory effects in many fish species including *S. sarba* (van der Boon *et al.* 1991, Kelly 1997). However, the importance of other HSPs such as HSP90 must be considered. Among the HSP family it is the HSP90s that are most tightly coupled to the steroid transduction pathway (Smith & Toff 1993) and it has been found that rats subjected to immobilisation stress exhibited elevated levels of HSP90 in various tissues whereas the level of HSP70 remained unchanged (Vamvakopoulou *et al.* 1993). Furthermore, the levels of HSP90 have been shown to vary greatly within different rodent tissue which may suggest that its distribution and content is an important factor in corticosteroid sensitivity (Vamvakopoulou 1993). Clearly, further studies are needed in order to understand the complex interaction between cortisol and different HSPs in fish species.

In conclusion, our report is the first on the endocrine regulation of HSP70 in fish and data from this research have shown that the administration of rbGH or oPRL

reduced HSP70 expression. No alteration in hepatic HSP70 expression was found after cortisol treatment and further studies need to be undertaken in order to verify the role of other HSPs after corticosteroid administration in fish.

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