

Differences in interrenal tissue, biosynthetic capacity and ACTH sensitivity in progeny of sea bream from parents selected for high or low cortisol response

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Progeny of sea bream *Sparus aurata* from parents selected for high or low cortisol response to stress also showed similar divergent cortisol response after 48 h of handling and confinement stress. The high response progeny, however, had a lower basal unstimulated cortisol release and ACTH-stimulated cortisol output *in vitro*. © 2003 The Fisheries Society of the British Isles

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Selection for high and low cortisol response in fishes has been undertaken in salmonid and cyprinid species (Fevolden *et al.*, 1991; Tanck *et al.*, 2002). Recently, selection for divergent cortisol response after repeated stress has also been carried out in sea bream *Sparus aurata* L. (Tort *et al.*, 2001). As demonstrated by Pottinger & Carrick (2001), however, it is not clear which level of the endocrine axis is responsible for the phenotypic characters used for this differential selection. This study examined whether the observed plasma cortisol differences in sea bream selected for their cortisol response to stress arose from differences in interrenal function. The present work studied the interrenal biosynthetic capacity and the sensitivity to ACTH in first generation sea bream from parents that showed a high or low response to handling and confinement stress (Tort *et al.*, 2001).

Sea bream weighing (878 ± 127 g) were obtained from the facilities of the Instituto Canario de Ciencias Marinas (Las Palmas, Canary Islands, Spain). These fish were a first generation progeny obtained from breeders that showed either high or low cortisol levels after repeated handling and confinement stress

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(Tort *et al.*, 2001). The experiment used two groups of progeny, from high or low responding parents each in four replicate tanks containing eight fish per tank (density, 7 kg m^{-3}). Two tanks from each progeny group (16 fish) were sampled as controls and the remaining tanks were subjected to handling and confinement stress (netted and transferred to small net cages at a density of 200 kg m^{-3}) for 48 h and sampled thereafter. At sampling, all fish belonging to the same sampling group were quickly anaesthetized in 2-phenoxyethanol (1:1000 v/v; Sigma, St Louis, MO, U.S.A.) and blood was collected from the caudal vessels with a 2 ml syringe containing EDTA (1.5 mg ml^{-1} blood). Plasma aliquots were separated and frozen (-20°C) until analysis was performed. Head kidneys were quickly dissected after sampling ($n=8$ per group and treatment). The tissues were placed in superfusion chambers and superfused with a buffered HEPES Ringer solution (HEPES 15 mM; pH 7.38) containing NaCl (171 mM), KCl (2 mM), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (2 mM), 0.25% (w/v) glucose and 0.03% (w/v) bovine serum albumin. This medium was pumped through the superfusion chambers at a rate of $75 \mu\text{l min}^{-1}$ by means of a multichannel peristaltic pump (Gilson). Previous results indicated that cortisol reaches stable baseline levels after 3 h of superfusion (Rotllant *et al.*, 2000a, b). Therefore, after 3 h, tissue was stimulated at a concentration of 5 nM hACTH₁₋₃₉ (Sigma) during 20 min. For each fish the maximum cortisol release due to ACTH stimulation was compared with the baseline release in order to obtain the stimulation factor of ACTH, defined as (maximum release – baseline release) (baseline release)⁻¹. Cortisol concentrations were analysed by radioimmunoassay following the procedure described in Rotllant *et al.* (2000a).

Results are given as mean \pm S.E. One-way ANOVA was applied followed by the Student–Newman–Keuls (SNK) test to check differences between particular groups. The level for accepted statistical significance was $P < 0.05$.

The levels of plasma cortisol in progeny of high (HR) and low (LR) responding sea bream under control conditions and after 48 h handling and confinement stress are shown in Fig. 1. High response progeny had significantly higher plasma cortisol levels before and after 48 h handling and confinement stress. The kinetics of cortisol *in vitro* release from the head kidneys of HR and LR progeny, before and after handling and confinement stress, are shown in Fig. 2. These results are summarized in Fig. 3. No differences were found in the initial unstimulated cortisol release of stress and non-stress fish from both progeny groups ($P > 0.05$). Basal unstimulated cortisol release (Fig. 3) was significantly higher in control HR progeny ($P < 0.05$) than in LR progeny. An increase of the basal unstimulated cortisol release in LR progeny ($P < 0.01$), however, was found after 48 h stress. The stressor did not affect the basal cortisol release of HR progeny.

The results of this study show that the progeny of HR sea bream have higher pre- and post-stress cortisol levels. The progeny of LR fish had cortisol levels similar to previously published values for sea bream (Rotllant *et al.*, 2000b, 2001). It appears that the LR progeny were more sensitive to the 48 h handling and confinement stress: the HR progeny showed a 3.5-fold increase while the LR progeny showed a 9.7-fold increase in plasma cortisol levels. The results obtained in the levels of basal unstimulated and stimulated cortisol release from the *in vitro* superfusion clearly indicate a different response of the interrenal tissue depending on the group selected. Therefore, a different sensitivity to

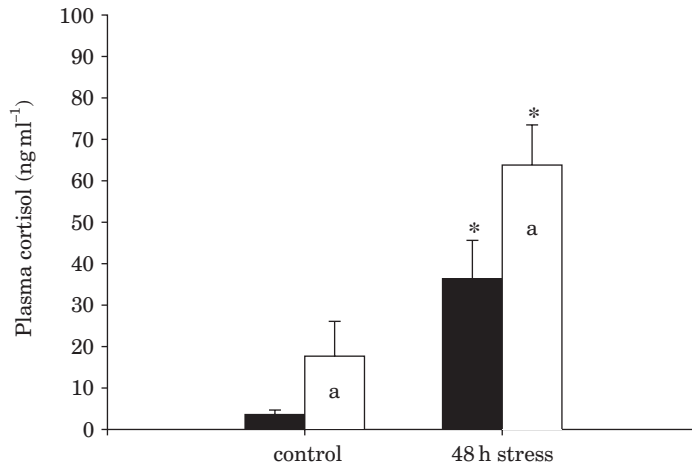


FIG. 1. Changes in mean \pm S.E. ($n = 16$) plasma cortisol levels in low response (LR, ■) and high response (HR, □) progeny of sea bream subjected to 48 h handling and confinement stress. Differences between treatments and controls (*, $P < 0.01$) and between progeny (a, $P < 0.05$) are indicated.

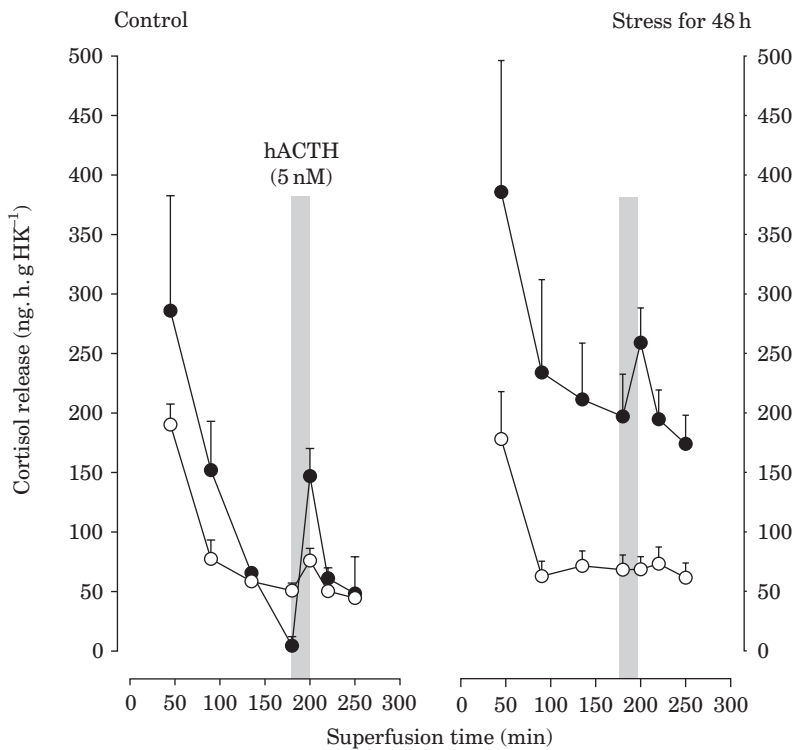


FIG. 2. Comparison of the effects of 48 h handling and confinement stress on *in vitro* head kidney cortisol release (mean \pm S.E., $n = 8$) after a pulse of 5 nM hACTH₁₋₃₉ for 20 min in progeny of low (■) and high (□) responding sea bream. Vertical bars correspond to the 20 min period of stimulation with ACTH.

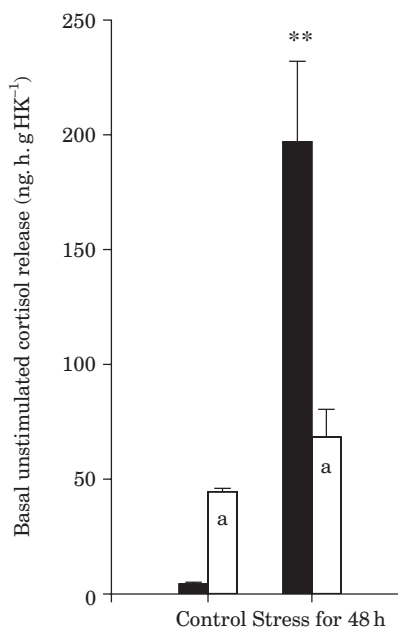


FIG. 3. Analysis of the effects of 48 h handling and confinement on *in vitro* cortisol basal release, measured in the fraction collected at 180 min in low response (■) and high response (□) progeny of sea bream. Values are mean \pm S.E., $n=8$. Differences between treatments and controls (**, $P < 0.001$) and between progeny (a, $P < 0.05$) are indicated.

ACTH and also a different biosynthetic capacity of the interrenal tissue is suggested. The response of LR progeny fish was similar to previously published data for sea bream (Rotllant *et al.*, 2000a, b), *i.e.* a low corticosteroid biosynthesis by the interrenal under control conditions and a high sensitivity when stimulated with 5 nM ACTH. Progeny of HR fish showed the opposite, *i.e.* the unstimulated tissue had a higher basal cortisol release, and after ACTH stimulation at the same concentration the response detected was not significantly different between control and stressed fish. The combination of effects observed in the non-stressed progeny of HR fish (elevated plasma cortisol levels and enhanced secretory activity of the unstimulated interrenal cells) appears to be similar to the effects observed after 23 days of crowding (Rotllant *et al.*, 2000b). So, this type of dynamic response possibly represents a characteristic response of long-term chronically stressed fish (Montero *et al.*, 1999; Rotllant *et al.*, 2000b). The progeny of HR fish had the highest cortisol levels in plasma after 48 h of handling and confinement stress, yet were unresponsive to ACTH and cortisol production *in vitro* was low. These results are in agreement with the recent results obtained in rainbow trout *Oncorhynchus mykiss* (Walbaum) by Pottinger & Carrick (2001). These authors conclude that the divergence of stress responsiveness between HR and LR rainbow trout arises from interrenal factors such as ACTH receptor density, steroidogenic capacity, interrenal tissue mass or post-interrenal factors such as the rate of cortisol metabolism and clearance. Pottinger & Carrick (2001) state that the interrenal sensitivity to ACTH may be an important factor for HR, as it is in mammals (Gómez *et al.*, 1996) and birds

(Carsia *et al.*, 1988). In these groups the difference of the corticosteroid response between strains was attributed to adrenal sensitivity to ACTH and therefore the adrenal volume was a main factor. From the results of the present work it is concluded that the differences observed in the concentrations of plasma cortisol levels between both sea bream progeny lines could be attributed, in part, to the biosynthetic capacity but not to the interrenal sensitivity to ACTH. Thus, other factors such as the density of ACTH receptors and the rate of clearance or metabolism of cortisol should be involved in the differences of the plasma cortisol stress response observed for progeny of high and low cortisol responding sea bream.

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