SHORT COMMUNICATION

DOWN-REGULATION OF RED BLOOD CELL 
\( \beta \)-ADRENORECEPTORS IN RESPONSE TO CHRONIC 
ELEVATION OF PLASMA CATECHOLAMINE LEVELS IN THE 
RAINBOW TROUT

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In many teleost fish, including the rainbow trout, *Oncorhynchus mykiss*, 
catecholamines are mobilized into the blood in situations when enhanced oxygen 
transport is necessary, such as hypoxia, anaemia, hypercapnia and strenuous exercise (see 
review by Thomas and Perry, 1992). These hormones initiate a series of integrated 
physiological responses that optimize cardiovascular and respiratory functions (see 
reviews by Perry and Wood, 1989; Randall and Perry, 1992; Thomas and Perry, 1992). 
Stimulation of red blood cell \( \beta \)-adrenoreceptors leads to the activation of a red blood cell 
membrane \( \text{Na}^+ / \text{H}^+ \) antiporter which extrudes protons in exchange for plasma \( 
\text{Na}^+ \), thereby elevating intracellular pH (pHi) (Baroin *et al.* 1984; Cossins and Richardson, 
1985; see review by Nikinmaa, 1992). The binding of catecholamines to \( \beta \)-adrenoreceptors, which are coupled to adenylate cyclase, engenders the formation of 
cyclic AMP. This second messenger, in turn, presumably initiates a phosphorylation 
cascade which ultimately activates the \( \text{Na}^+ / \text{H}^+ \) antiporter. Erythrocyte alkalization can 
increase haemoglobin oxygen-binding affinity and capacity via the Bohr and Root 
effects, thus increasing the ability of the blood to transport oxygen (Tufts and Randall, 
1989; see also reviews by Nikinmaa and Tufts, 1989; Perry and Wood, 1989; Randall and 
Perry, 1992; Thomas and Perry, 1992).

Chronic elevation of plasma catecholamine concentrations appears to attenuate the red 
blood cell adrenergic response. Rainbow trout exposed to moderate levels of hypoxia 
(water partial pressure of oxygen 6.7–9.3 kPa) for 48 h exhibited elevated plasma 
adrenaline levels, and the sensitivity of red blood cell \( \text{Na}^+ / \text{H}^+ \) exchange to *exogenous* 
adrenaline, *in vitro*, was reduced by 60–100% (Thomas *et al.* 1991). Further, the intensity 
of proton extrusion in response to adrenaline addition was found to be inversely 
correlated to the *endogenous* plasma adrenaline concentration (Thomas *et al.* 1991). 
Thomas and co-workers suggested that a reduction in functional red blood cell 

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down-regulation.
\(\beta\)-adrenoreceptors (down-regulation) was a plausible explanation of their results. Their interpretation was consistent with models, developed for higher vertebrates, of receptor desensitization caused by chronic elevation of catecholamine levels and indeed, of hormones in general (see, for example, review by Collins et al. 1991). However, they were unable to distinguish among a number of other potential explanations, including down-regulation by internalization or destruction of \(\beta\)-receptors, uncoupling of \(\beta\)-receptors from adenylate cyclase, or even the direct desensitization of the Na\(^+\)/H\(^+\) exchanger itself (Garcia-Romeu et al. 1988). The present study was undertaken to determine whether red blood cell \(\beta\)-adrenoreceptor down-regulation occurs in rainbow trout in response to persistent elevations of plasma catecholamine levels. Down-regulation is defined for the purposes of this study as a decrease in the number of red blood cell surface \(\beta\)-receptors. The results indicate that, compared with controls, fewer red blood cell \(\beta\)-adrenoreceptors are present in trout in which circulating catecholamine concentrations have been elevated for an extended period. By inference, desensitization of the red blood cell adrenergic response is likely to be mediated, at least in part, by the down-regulation of red blood cell \(\beta\)-receptors.

Plasma catecholamine levels were chronically elevated using mini osmotic pumps (Alzet, California) surgically implanted into the peritoneal cavity of fully anaesthetized (0.1 g l\(^{-1}\) ethyl-\(m\)-aminobenzoate; MS-222) rainbow trout, Oncorhynchus mykiss. Mini osmotic pumps were filled with a 140 mmol l\(^{-1}\) NaCl solution containing 100 mg l\(^{-1}\) ascorbic acid (as an antioxidant), adrenaline and noradrenaline (as their bitartrate salts). Catecholamine concentrations were determined according to the average mass of the fish (approximately 250 g), the desired catecholamine delivery rate and the pump flow rates (corrected for ambient water temperature, which was 6–12 °C over the course of the experiments). Nominal plasma concentrations of 200 nmol l\(^{-1}\) and 50 nmol l\(^{-1}\) were chosen for adrenaline and noradrenaline, respectively. These concentrations are frequently observed in stressed rainbow trout (Randall and Perry, 1992; see Table 1 in Thomas and Perry, 1992). Control fish were provided by probing the peritoneal cavity of anaesthetized trout with a cotton swab. Indwelling cannulae (Clay Adams PE 50 polyethylene tubing) were placed, using standard techniques (Soivio et al. 1975), in the dorsal aorta of both groups of fish during anaesthesia. The ability of the mini osmotic pumps to raise plasma catecholamine concentrations was assessed by monitoring plasma adrenaline and noradrenaline levels for 72 h following surgery. Blood samples (0.6 ml), obtained periodically by withdrawal from the dorsal aortic cannula, were centrifuged (12 000g for 1 min) and the resultant plasma stored at −80 °C. Catecholamine concentrations were measured on alumina-extracted plasma samples using high performance liquid chromatography (HPLC) with electrochemical detection (Woodward, 1982). 3,4-Dihydroxybenzylalanine hydrobromide (DHBA) was used as an internal standard in all analyses.

Fig. 1 demonstrates that the infusion of exogenous catecholamines using mini osmotic pumps was a suitable technique for chronically elevating plasma catecholamine concentrations. In the control fish, catecholamine concentrations (Fig. 1) remained at, or near, resting levels (Randall and Perry, 1992) throughout the experimental period. By 4 h,
the concentrations of both adrenaline and noradrenaline were significantly higher (one-way analysis of variance followed by Fisher’s LSD test for multiple comparisons; 5% level of significance) in trout implanted with pumps than in control fish. A transient decrease in plasma catecholamine levels was observed in experimental fish between 8 and 12 h post-implantation, but by 24 h (noradrenaline) to 48 h (adrenaline) the catecholamine levels of experimental fish were once again significantly higher than those of control fish (Fig. 1). It seems likely that this apparent decline in plasma adrenaline and noradrenaline levels in the experimental fish was an artefact generated by unstable pump output rates; information from Alzet indicates that miniosmotic pumps require several hours to achieve a stable output.

Having established a suitable technique for the chronic elevation of plasma catecholamine levels, receptor binding studies were used to determine whether red blood cell β-adrenoreceptors from fish implanted with mini osmotic pumps underwent down-regulation. The lipophobic β-agonist isoproterenol and the hydrophilic radioligand (±)-4-(3-tert-butylamino-2-hydroxypropoxy)-[5,7-3H]benzimidazol-2-one ([3H]CGP) have been shown to bind only to high-affinity cell surface receptors (Martilla and Nikinmaa, 1988) and, therefore, on the basis of the comprehensive study of Reid et al. (1991), isoproterenol-displaceable [3H]CGP binding was used as a measure of the number of red blood cell surface β-receptors.

Fish used for receptor assays were not cannulated: following implantation of mini osmotic pumps (experimental) or sham surgery (controls), trout were placed in large fibreglass tanks supplied with flowing, aerated water for 72 h. Blood samples obtained by caudal puncture from stunned fish were gassed with humidified oxygen for 2 min and allowed to stand in a lit room on ice for 1 h. The purpose of this treatment was to oxidize plasma catecholamines (S. G. Reid and S. F. Perry, unpublished results), thereby avoiding any competition between the radioligand and plasma catecholamines that might interfere with the binding assay. Erythrocytes were not washed or resuspended in saline.
as this was previously found both to modify the affinity and to reduce the number, of $\beta$-receptors (Reid et al. 1991).

$[^{3}\text{H}]$CGP 12177 (specific activity 2.05 TBq mmol$^{-1}$; Amersham) was prepared to yield final concentrations in the assay volume of 5, 10, 15, 20 and 40 nmol l$^{-1}$. Samples of blood (40 $\mu$l) were added to 160 $\mu$l of 140 mmol l$^{-1}$ NaCl solution containing 4% ascorbic acid into which the radioligand had been added alone or in combination with 200 $\mu$mol l$^{-1}$ (−)-isoproterenol (Sigma). The number of red blood cells added to the assay was determined by counting, with a haemocytometer, the red blood cells present in 10 $\mu$l of blood diluted in 10 ml of saline. After a 45 min incubation at room temperature, cell membranes were harvested (Brandel 24R cell membrane harvester) onto borosilicate filters (2, Mandel Scientific) and washed repeatedly (five times) with 5 ml of ice-cold 140 mmol l$^{-1}$ NaCl. Filters were placed in 8 ml of a commercial scintillation cocktail (ACS II; Amersham) and sample activity was counted (Packard TR 2500; automatic quench correction) after 24 h.

Scatchard plot analysis (Scatchard, 1949) was performed to determine the maximum number of isoproterenol-displaceable CGP binding sites (receptor density) and their apparent dissociation constant ($K_D$). Receptor densities (in moles per cell) were multiplied by Avogadro’s number to give the number of receptors per red blood cell ($B_{\text{max}}$).

The results of the receptor binding study provide evidence that down-regulation of surface $\beta$-receptors occurs in response to chronic elevation of plasma catecholamine concentrations (Fig. 2). After 72 h, the red blood cell $\beta$-adrenoreceptor density of fish implanted with mini osmotic pumps ($B_{\text{max}}=708 \pm 71$, $N=8$; mean $\pm$ standard error of the mean) was significantly lower (two-sample $t$-test, $P<0.05$) than that of control fish ($B_{\text{max}}=1064 \pm 90$, $N=8$) (Fig. 2). The apparent dissociation constants of the treated and

Fig. 2. Receptor densities ($B_{\text{max}}$, number of receptors per cell), determined as the number of isoproterenol-displaceable $[^{3}\text{H}]$CGP binding sites, for red blood cells from trout implanted with catecholamine-loaded mini osmotic pumps (diagonal striped columns) or sham-operated (open columns), 72 h post-surgery. Values are mean ± 1 S.E.M. ($N=8$). An asterisk indicates a significant difference ($P<0.05$) from the control.
control fish ($K_D=4.6\pm1.2\,\text{nmol}\,\text{l}^{-1}$ and $3.9\pm1.7\,\text{nmol}\,\text{l}^{-1}$, respectively) did not differ. Thus, the red blood cell surface $\beta$-receptors remaining in the catecholamine-treated fish continued to bind agonists with the same (high; Reid et al. 1991) affinity observed in control fish. The molecular mechanisms underlying down-regulation have yet to be clarified, even in mammalian systems where research efforts have been concentrated (e.g. Collins et al. 1991). Although it was not possible to elucidate the mechanism of down-regulation from this study, the results do add to the growing body of evidence that indicates that red blood cell surface $\beta$-receptors in some species of fish are highly mobile (Martilla and Nikinmaa, 1988; Reid and Perry, 1991).

Thomas et al. (1991) found that the sensitivity of red blood cell Na$^+$/H$^+$ antiporter activity to exogenous adrenaline was reduced following 48 h of exposure of trout to moderate levels of hypoxia, and that plasma adrenaline, but not noradrenaline, levels were significantly elevated by the hypoxia treatment. The extent of the adrenergic response in vitro was inversely correlated with the plasma adrenaline concentration at the time of sampling and directly related to the partial pressure of oxygen in the arterial blood ($P_{a\,O_2}$) for partial pressures less than 10.7 kPa; an inverse relationship was also found between plasma adrenaline levels and $P_{a\,O_2}$ (below 10.7 kPa). To explain their results, Thomas and co-workers postulated that red blood cell $\beta$-adrenoreceptors were desensitized in response to the chronically elevated adrenaline concentrations, which, in turn, reflected the intensity of the imposed hypoxia and subsequent reduction in arterial oxygen tension. They further speculated that receptor desensitization occurred via down-regulation, that is a reduction in the number of functional $\beta$-receptors. By demonstrating a relationship between elevated levels of circulating catecholamines and $\beta$-receptor down-regulation, the results of the current study support the hypotheses put forward by Thomas et al. (1991). Other possible mechanisms which could lead to a reduction in the sensitivity of the adrenergic response have not, however, been eliminated. In particular, it would be useful to examine the intermediate steps between receptor stimulation and Na$^+$/H$^+$ antiporter activation. Desensitization of the Na$^+$/H$^+$ antiporter itself has been established (Garcia-Romeu et al. 1988), although its functional significance is uncertain. Clearly, there are a variety of possible mechanisms which could be involved in the agonist-stimulated desensitization of the red blood cell adrenergic response. This study has validated one such mechanism: down-regulation of red blood cell surface $\beta$-adrenoreceptors occurs in response to chronic elevation of plasma catecholamine concentrations.

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References


