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ANTARCTIC FISHES HAVE A LIMITED CAPACITY FOR CATECHOLAMINE SYNTHESIS

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Summary

To determine whether an attenuated stress response is a general feature of Antarctic fish or is dependent on ecotype, the capacity for catecholamine synthesis within the head kidney and plasma levels of the primary stress hormones (catecholamines and cortisol) were determined in species with a range of activity patterns. Tyrosine hydroxylase (TH) activities were similar in both sluggish (*Gobionotothen gibberifons*, 153±22 nmol g^{-1} h^{-1}, mean ± S.E.M.) and active (*Notothenia rossii*, 185±39 nmol g^{-1} h^{-1}, *Dissostichus mawsoni*, 128±31 nmol g^{-1} h^{-1}) pelagic nototheniids, but only 30 % of those in Atlantic cod (*Gadus morhua*, 393±88 nmol g^{-1} h^{-1}) at the same temperature. TH activities were even lower in white-blooded channichthyids (*Chaenocephalus aceratus*, 74±16 nmol g^{-1} h^{-1} and *Champsocephalus gunnari*, 53±17 nmol g^{-1} h^{-1}), although values in *Chionodraco rastrospinosus* were similar to red-blooded species (178±45 nmol g^{-1} h^{-1}). Circulating catecholamine levels were extremely high in all species after fishing stress, with adrenaline levels 3–4 times higher than noradrenaline levels. Cortisol levels remained low, ranging from 1.33±0.58 ng ml^{-1} in *Champsocephalus gunnari* to 44.9±25.0 ng ml^{-1} in *Dissostichus mawsoni*. These data suggest that depressed catecholamine synthesis is typical of Antarctic fish regardless of life style, although they are able to release extensive stores from the chromaffin tissue under conditions of extreme trauma. Cortisol does not appear to be an important primary stress hormone in these species.

Key words: adrenaline, noradrenaline, cortisol, tyrosine hydroxylase, teleost, Antarctica

Introduction

The typical vertebrate stress response is characterised by adjustments in respiratory, circulatory, osmotic and metabolic variables mediated by the release of the primary stress hormones, catecholamines (noradrenaline and adrenaline) and corticosteroids (cortisol). In teleost fish catecholamines are released from the chromaffin tissue into the circulation in response to severe stress, such as exhaustive exercise, aerial exposure or acute hypoxia (Perry and Thomas, 1991; Randall and Perry, 1992). Under these conditions, plasma catecholamines facilitate oxygen-diffusing and oxygen-carrying capacities, and initiate mobilisation of energy reserves to meet the increase in energy demand associated with stress. The degree of stress-induced elevations in levels of plasma catecholamines are variable and depend on a number of factors, including rates of catecholamine biosynthesis, storage, secretion and degradation (Randall and Perry, 1992; Gamperl et al., 1994). Variations in experimental design, time and place of sampling are also important (Randall and Perry, 1992). In temperate species, such as rainbow trout (*Oncorhynchus mykiss*) and Atlantic cod (*Gadus morhua*), plasma adrenaline levels generally increase from resting values of less than 10 nmol l^{-1} to approximately 300 nmol l^{-1} during aerial exposure, exhaustive exercise and acute hypoxia (Wahlquist and Nilsson, 1980; Van Dijk and Wood, 1988; Perry and Reid, 1992). However, values as high as 1000 nmol l^{-1} have been recorded in sockeye salmon, *Oncorhynchus nerka* (Mazeaud et al., 1977). Plasma cortisol levels are more variable, in the range 2–42 ng ml^{-1} in resting fish and 20–500 ng ml^{-1} in fish during stress (Gamperl et al., 1994). In addition, cortisol release into the circulation from the interrenal axis occurs at a slower rate than catecholamine release from the chromaffin tissue. The effects of cortisol, however, are more prolonged and include direct and indirect changes, such as mobilisation of energy reserves and adjustments in intermediary metabolism (Vijayan et al., 1991).

Catecholamines (dopamine, noradrenaline and adrenaline) are synthesised by a series of enzymic reactions (Blaschko, 1939) in the chromaffin tissue of the head kidney (Randall and Perry, 1992). The rate-limiting step for the production of noradrenaline occurs at the beginning of the pathway, where tyrosine is hydroxylated to L-dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase (TH). TH appears to be responsible for the rapid and specific control of catecholamine synthesis as TH activity is sensitive to end-

point inhibition by noradrenaline, and can be regulated by the sympathetic nervous system and a variety of hormones (Reid et al., 1998). In contrast, the rate-limiting step for the synthesis of adrenaline occurs during the final step when noradrenaline is methylated to adrenaline by the enzyme phenylethanolamine-*N*-transferase (PNMT). Once synthesised, noradrenaline and adrenaline may be stored within the chromaffin tissue either in secretory granuoles (noradrenaline) or in storage vesicles (adrenaline). Catecholamine release is subsequently controlled by cholinergic stimulation from pre-ganglionic sympathetic fibres modulated by a variety of factors, including hormones and changes in physiological variables such as a reduction in blood oxygen content (Axelrod and Reisine, 1984; Perry et al., 1991; Reid et al., 1996).

In contrast to the general primary stress response described above, Antarctic fishes, living at the permanently low temperatures of the Southern Ocean, have low plasma catecholamine levels even after the severe stress of capture, surgery (Egginton, 1994; Davison et al., 1995) or imposed swimming activity (Egginton, 1997). The lack of any significant catecholamine release in these fish suggests a novel form of stress response, with an emphasis on cholinergic rather than adrenergic control of cardiovascular function (Egginton and Davison, 1997). However, Forster et al. (1998) have recently shown that under the extreme stress caused by heat shock, circulating catecholamine levels can be increased in the active cryopelagic species, *Pagothenia borchgrevinki*. Most Antarctic species occupy benthic or demersal niches, with the remaining pelagic species originating from either bathypelagic or mesopelagic habitats, although some coastal species have become secondarily adapted to a pelagic life style in order to exploit the open water environment (Eastman, 1993). To date nearly all physiological studies have been carried out on the relatively sluggish, inshore species, which may not be representative of the broader Antarctic fish fauna. These findings therefore raise interesting questions as to whether the normally low levels of circulating catecholamines are due to impaired synthesis or release, and whether the capacity to synthesise catecholamines is influenced by ecotype or phylogeny.

The aim of the present study was to clarify the unusual stress response of Antarctic fishes by determining whether the low catecholamine levels previously reported are (1) a general notothenioid character or a particular feature of relatively sluggish benthic fishes, and (2) correlated with a reduced ability to synthesise catecholamines in the chromaffin tissue. To this end, a range of Antarctic species were collected by benthic and pelagic trawls on a scientific cruise to the Southern Ocean to increase the number of species examined and to broaden the ecotypes represented. TH activity was determined and plasma hormone levels measured to assess interspecific differences to extreme fishing stress. Both red-blooded species, members of the Nototheniidae, and haemoglobinless icefish (Channichthyidae) were sampled to accommodate phylogenetic diversity in the study.

Materials and methods

Specimen collection

Notothenioid fish were collected around King George Island (62°S 58°W) and Elephant Island (61°S 55°W) during the XIV/2 cruise of the *RV Polarstern* (Alfred Wegner Institute, Bremerhaven). Benthic species were caught in bottom trawls lasting 30 min each, while pelagic species were caught in midwater trawls of 1 h duration. In both cases, fish were hauled on deck and sorted in air (ambient temperature <0°C). At ambient air temperatures, aerial exposure is unlikely to significantly exacerbate the stress response to capture (Egginton, 1994). Six species were collected for this study, including three icefish (channichthyids): *Champsocephalus gunnari* Lönnberg (active, pelagic), *Chaenocephalus aceratus* Lönnberg (sluggish, epibenthic) and *Chionodraco rastrospinosus* Witt and Hureau (relatively sluggish, benthic), and three Nototheniids: *Dissostichus mawsoni* Norman (active, pelagic), *Gobionotothen gibberifrons* Lönnberg (sluggish, benthic) and *Notothenia rossii* Richardson (relatively active, benthopelagic). Body mass (mean ± S.E.M.) ranged from 899±167 g (*N*=14) in *D. mawsoni* and 799±98 g (*N*=11) in *N. rossii* to 567±77 g (*N*=15) in *G. gibberifrons*, 457±88 g (*N*=17) in *C. gunnari*, 450±52 g (*N*=10) in *C. rastrospinosus* and 197±35 g (*N*=4) in *C. aceratus*. In addition, specimens of *Notothenia coriiceps* Richardson (body mass 176±17 g, *N*=5) were obtained by the British Antarctic Survey from the South Orkney Islands (60°S 45°W), and Atlantic cod (*Gadus morhua* Linnaeus; body mass 685±11 g, *N*=5) were obtained from the North Sea by CEFAS in Lowestoft. In both cases fish were kept in aquaria supplied with aerated, filtered sea water maintained at 0 °C for *N. coriiceps* and 10–12 °C for *G. morhua*. *N. coriiceps* were held for 2 weeks and *G. morhua* for 3 months before experimentation. For logistical reasons optimal assay conditions could not be established on board ship, and limited availability of frozen samples made it prudent to conduct initial assays on other species. Consequently, head kidney samples from *N. corriceps* were used to establish optimal TH assay conditions, and corresponding samples from *G. morhua* were used as controls. Although not a perciform species, *G. morhua* was used for comparative purposes as it has a large capacity for catecholamine release during stress and the pathway for catecholamine synthesis in this species has been well characterised (Jönsson and Nilsson, 1983a,b).

In all cases, fish were killed with a sharp blow to the head and blood samples quickly taken from the caudal vein into a heparinised syringe. Samples were spun at 13000 ***g*** for 3 min and the resulting plasma removed and stored with 10 % v/v antioxidant (10 mmol l^{-1} reduced glutathione and 100 mmol l^{-1} EDTA) at –30 °C. Head kidney tissue, including both the coeliac ganglion and the posterior caudal vein, was subsequently dissected out from both sides of the body and frozen at –125 °C for shipment back to the UK.

Tyrosine hydroxylase assay

Head kidney tissue (200–700 mg) was homogenised on ice in 20 volumes of 0.3 mol l^{-1} sucrose using an Ultra-Turrax

homogeniser. Crude homogenates were centrifuged at 20000 ***g*** and 4 °C for 20 min and the supernatant assayed for tyrosine hydroxylase (TH) activity using the method originally described by Nagatsu et al. (1964), and adapted for use on chromaffin and nervous tissue in *G. morhua* by Jönsson and Nilsson (1983a). This assay takes advantage of the production of tritiated water during the enzymic conversion of L-[3,5-^{3}H]tyrosine to dihydroxyphenylalanine (L-DOPA).

For each analysis, 400 µl of the supernatant was added to an incubation medium which contained: 100 µl 0.01 mol l^{-1} 'cold' tyrosine with [^{3}H]tyrosine at a specific activity of 5 µCi (185 kBq) per sample; 100 µl 0.2 mg ml^{-1} catalase; 100 µl 1 mol l^{-1} sodium acetate buffer (pH 6.0 at 20 °C); 100 µl 0.1 mol l^{-1} sodium phosphate buffer (containing 154 µg dithiothreitol, 232 µg 6,7-dimethyl-5,6,7,8-tetrahydropteridine hydrochloride and 96 µg $(NH_4)_2Fe(SO_4)_2$), 100 µl 0.3 mol l^{-1} sucrose and 100 µl deionised water. The catalase concentration and the pH level found to be optimal for TH activity in the head kidney tissue of *N. coriiceps* were the same as those for *G. morhua* (Jönsson and Nilsson, 1983a). To establish optimal incubation conditions, enzyme reactions were left for 10, 15, 20 and 30 min at 30 °C and for 15 and 30 min at 15 °C. To further validate the present assay, TH activities were estimated in head kidney homogenates of *G. morhua* after 10, 15, 20 and 30 min incubation at 30 °C to compare results with published values.

To determine absolute values of TH activity, samples were pre-incubated with 3-iodo-L-tyrosine at a concentration of 5 mmol l^{-1} before addition to the incubation medium. This substance is an effective inhibitor of TH activity in mammals, and has also been shown to inhibit [^{3}H]water formation in head kidney homogenates of *G. morhua* (Jönsson and Nilsson, 1983a). In addition, two different blanks were included in preliminary assays, consisting of the incubation mixture plus 100 µl 20 % trichloroacetic acid (TCA), and the incubation mixture plus 400 µl 0.3 mol l^{-1} sucrose instead of the enzyme preparation. In both cases apparent TH activities were consistently low, while background counts were notably lower (×300–3000) than those measured in the enzyme preparations, indicating that this technique was an effective method of analysing TH activity.

Each enzymic reaction was stopped by the addition of 100 µl 20 % TCA and the samples spun at 2000 ***g*** for 5 min. The supernatant was passed through a Dowex 50W X4 ion-exchange resin (0.5 cm×3.0 cm) to remove excess radiolabel, L-DOPA and tyrosine from the [^{3}H]water. The remaining protein pellet was washed with 1 ml deionised water, which was subsequently passed through the column. The combined effluent was added to 15 ml scintillant (Cocktail EX, BDH) and counted in a scintillation counter (Packard Auto-Gamma 5650). Recovery of [^{3}H]water through the columns was 98 %.

Each sample was run in triplicate to include duplicate assays and one blank, which had been treated with the TH inhibitor 3-iodo-L-tyrosine. Total counts (counts min^{-1} of [^{3}H]tyrosine added to each sample) were measured directly on samples containing incubation mixture plus 400 µl 0.3 mol l^{-1} sucrose without passage through an ion-exchange column. TH activity was calculated from the following equation (Nagatsu et al., 1964): (counts min^{-1} of sample – counts min^{-1} of sample treated with inhibitor) × [standard] / total counts, where [standard] is the concentration of tyrosine in the incubation mixture (1000 nmoles). Results are expressed as nmol g^{-1} h^{-1}.

Hormone assays

Catecholamines were extracted from plasma samples using alkaline alumina in the presence of an antioxidant (Hugh et al., 1987). Catecholamine levels were determined by high-performance liquid chromatography (HPLC), with reversed-phase ion-pair chromatography and electrochemical detection in the Department of Medicine (Clinical Biochemistry), University of Manchester, UK. The HPLC system was sensitive enough to detect 0.1–0.5 nmol l^{-1} catecholamines. Internal standards were included to test the accuracy of the HPLC system and the data adjusted accordingly. Cortisol levels were measured in 25 µl plasma samples using radioimmunoassay (ICN Biomedicals). This cortisol assay is accurate over a broad range of cortisol values as verified by comparisons against another well-established cortisol radioimmunoassay.

In all cases, data are given as means ± S.E.M. (*N*), with statistical differences between group means tested using single-factor analysis of variance (ANOVA) and Fisher's PLSD statistic. The significance level was set at $P<0.05$.

Results

The relationships between incubation time and TH activity in head kidney homogenates of *G. morhua* and *N. coriiceps* are shown in Fig. 1. In *G. morhua*, optimal activities were obtained after 15 min of incubation at 30 °C when values reached 393±88 nmol g^{-1} h^{-1} (N=5). Apparent activity subsequently decreased to 125±38 nmol g^{-1} h^{-1} (N=4) by 30 min of incubation. Analysis of TH activities at the lower

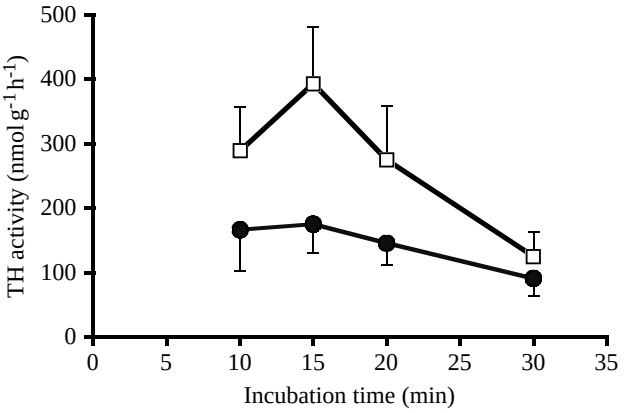

Fig. 1. Relationship between incubation time at 30 °C and apparent tyrosine hydroxylase (TH) activity in the head kidney tissue of *G. morhua* (open squares, N=5) and *N. coriiceps* (filled circles, N=4). Values are means ± S.E.M.

temperature of 15 °C gave a value of 211±130 nmol g^{-1} h^{-1} (N=5) after 15 min (not shown), resulting in a Q_{10} of 1.52. A similar response was observed in head kidney homogenates from *N. coriiceps*: TH activities were 166±63 nmol g^{-1} h^{-1} (N=4) after 10 min and 175±45 nmol g^{-1} h^{-1} (N=4) after 15 min at 30 °C. By 20 min incubation TH activities had fallen to 146±34 nmol g^{-1} h^{-1} (N=4) followed by a further decrease to 91±27 nmol g^{-1} h^{-1} (N=4) after 30 min. Incubation at 15 °C gave a value of 78±36 nmol g^{-1} h^{-1} (N=4) after 15 min (not shown), resulting in a Q_{10} of 1.75 between 15 and 30 °C in *N. coriiceps*.

Tyrosine hydroxylase activities in the head kidney tissue of all six species of Antarctic fish sampled after trawling are presented in Fig. 2, along with corresponding data for *N. coriiceps* taken from aquaria. TH activity levels were lowest in the icefishes, *C. aceratus* and *C. gunnari*, at 74±16 nmol g^{-1} h^{-1} (N=3) and 53±17 nmol g^{-1} h^{-1} (N=7), respectively. In contrast, TH activity in the other channichthyid, *C. rastrospinosus*, was significantly higher at 179±45 nmol g^{-1} h^{-1} (N=10). TH activities in the notothenioids showed a more modest range from 128±31 nmol g^{-1} h^{-1} (N=9) in *D. mawsoni* to 184±39 nmol g^{-1} h^{-1} (N=8) in *N. rossii*.

Mean values for adrenaline and noradrenaline levels in the plasma of fish caught by trawling are shown in Fig. 3. Adrenaline concentrations were generally 3–4 times higher than that of noradrenaline in each species, with the exception of *N. rossii* where the adrenaline:noradrenaline ratio was 2. For example, mean plasma adrenaline levels in *C. gunnari* were 826±255 nmol l^{-1} and mean noradrenaline levels were 238±63 nmol l^{-1} (N=6). In *C. rastrospinosus* plasma, the corresponding values were 694±101 nmol l^{-1} and 167±24 nmol l^{-1} (N=5), respectively, and in *D. mawsoni* mean adrenaline levels were 2601±517 nmol l^{-1} and mean noradrenaline levels were 841±217 nmol l^{-1} (N=5). In all three species intraspecific differences between adrenaline and noradrenaline means were significant. Interspecific comparisons showed that plasma catecholamine levels were significantly higher in the active pelagic species, *D. mawsoni*, compared to the icefishes *C. gunnari* and *C. rastrospinosus*, and the benthic notothenid *G. gibberifrons* (Fig. 3).

Plasma cortisol levels for each species are given in Fig. 4. Mean values were lowest in the icefish, *C. gunnari* and *C. rastrospinosus*, at 1.33±0.58 (N=7) and 1.56±0.96 ng ml^{-1} (N=7), respectively, and highest in *D. mawsoni* at 44.85±25.00 ng ml^{-1} (N=5). Cortisol levels in *G. gibberifrons*, *N. rossii* and *C. aceratus* were intermediate, but intraspecific variability was such that none of the pairwise differences among the means were significant.

Discussion

Out of the two temperatures used in the initial study, TH activities in *N. coriiceps* were higher after 15 min at 30 °C. Consequently, 30 °C was considered to be a suitable temperature at which to make interspecific comparisons in TH activities, as 30 °C was also the optimal temperature for the measurement of TH activities in the head kidneys of Atlantic cod, *G. morhua* (Nagatsu et al., 1964; Jönsson and Nilsson, 1983a). This suggests that no major differences in metabolic control were found in Antarctic fish species, despite the fact

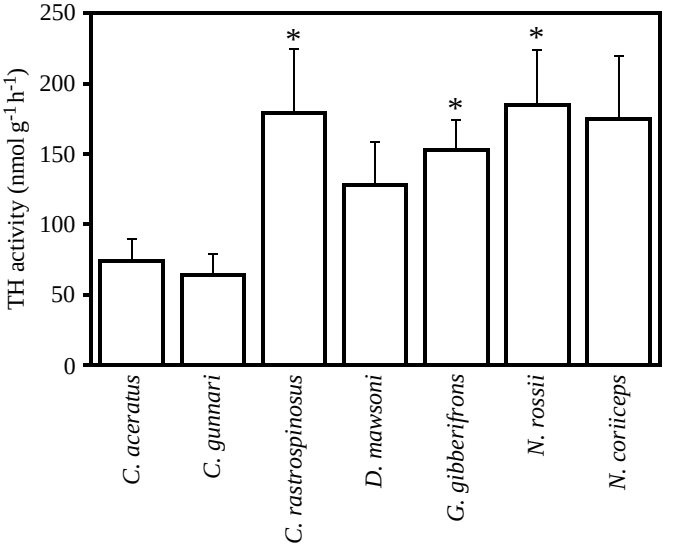

Fig. 2. Interspecific differences in tyrosine hydroxylase (TH) activities of head kidney tissue between Antarctic fishes under optimal in vitro conditions (30 °C for 15 min). Significant differences (P<0.05) from the mean values obtained in *C. gunnari* are indicated with an asterisk. Values are means + S.E.M.; for *C. aceratus*, N=3; *C. gunnari*, N=7; *C. rastrospinosus* and *G. gibberifrons*, N=10; *D. mawsoni*, N=9; *N. rossii*, N=8, *N. coriiceps*, N=4.

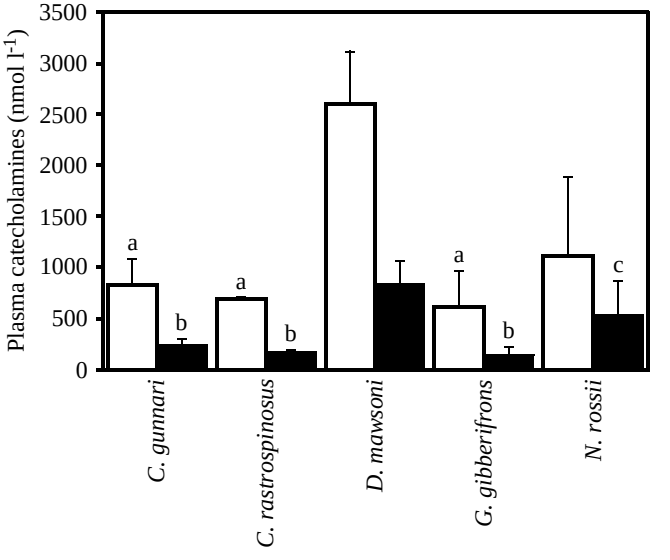

Fig. 3. Catecholamine levels in the plasma of five species of Antarctic fish after capture by trawling. Values given for adrenaline (open bars) and noradrenaline (filled bars) are means + S.E.M. Significant differences from the means in *D. mawsoni* are indicated by a for adrenaline and b for noradrenaline. Significant differences from mean noradrenaline values in *G. gibberifrons* are indicated by c. (N=5 in all species apart from *C. gunnari*, where N=6).

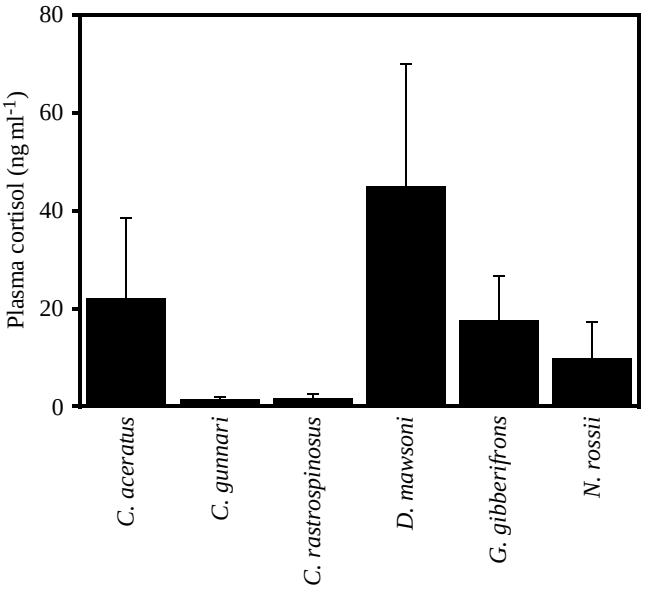

Fig. 4. Plasma cortisol levels (mean + S.E.M.) in six species of Antarctic fish after capture by trawling. *N*=7 in *C. gunnari*, C. *rastrospinosus*, and *G. gibberifrons; N*=5 in *D. mawsoni* and *N. rossii; N*=3 in *C. aceratus*.

that these animals live in a highly stenothermal environment at temperatures close to 0 °C. Similar thermal stabilities have been reported for other enzymes from Antarctic fish, indicating that acute temperature maxima are not compromised in fish living at continuously low temperatures (Hochachka and Somero, 1984; Ciardiello et al., 1997; Clarke, 1997). Rather, these authors suggest that changes in kinetic properties at the level of the enzyme, such as changes in the balance between enthalpic and entropic contributions to the overall free energy change at low ambient temperatures, are more important in maintaining enzyme function in these fishes. A direct comparison between TH activities in *G. morhua* and *N. coriiceps* after incubation for 15 min at 30 °C demonstrated that TH activities were 50 % lower in the head kidneys of Antarctic species, indicating a reduced capacity for catecholamine synthesis at a common temperature. However, extrapolation of the data obtained from *N. coriiceps* down to 0 °C at a Q_{10} of 1.75 gives an extremely low TH activity of approximately 32 nmol g^{-1} h^{-1}. Consequently, TH activities in *N. coriiceps* at 0 °C were six times lower than those recorded in *G. morhua* at 15 °C, so that Antarctic fish operating at normal in situ temperatures have much reduced capacities for catecholamine synthesis. As Antarctic fish have a long evolutionary history in the Southern Ocean, undergoing endemic speciation over the last 30 million years (Eastman, 1993), the overall reduction in TH activities may represent the result of progressive adjustments in enzyme abundance due to changes in gene expression (Blum et al., 1987; Fukamauchi et al., 1997). This may reflect an apparent shift in cardiovascular control from an adrenergic to predominantly cholinergic system (Egginton and Davison, 1997), but it remains unclear whether this is a consequence of cold adaptation or an ancestral trait of the notothenioid stock.

TH activities varied among Antarctic species and revealed some interesting differences between notothenioid families. TH activities in both sluggish (*G. gibberifrons*) and active pelagic (*N. rossii, D. mawsoni*) notothenioids were similar, at about 30 % of the values obtained from *G. morhua*. The lowest activities were found in two of the Channichthyidae, *C. gunnari* and *C. aceratus*, being only 16–19 % of those measured in *G. morhua*. These differences may be related to the phylogenetic diversity of the notothenioids as the Channichthyidae are exclusively Antarctic, whereas the notothenioidae diverged before the Channichthyidae and have a more broad general geographical distribution (Eastman, 1993). Interestingly, values for *C. rastrospinosus* were similar to those found in the red-blooded notothenioids. Closer examination of *C. rastrospinosus* reveals that this species has maintained myoglobin expression in the ventricle and represents a transitional stage in the evolution of the Antarctic ichthyofauna (Sidell and Vayda, 1997). Thus, low TH activity may be a derived characteristic. The contrast in catecholamine synthetic capacity between haemoglobin-producing fish and some haemoglobinless fish may be related to one of the primary roles of catecholamines, which involves modulation of respiratory function and maintenance of oxygen delivery to the tissues during periods of stress. The absence of red blood cells and respiratory pigments may negate the need for elevated levels of circulating catecholamines, leading to a reduction in TH levels in the chromaffin tissue of channichthyids.

No clear relationship between TH activity and circulating catecholamine levels was evident, and plasma catecholamine levels were high, regardless of the presence or absence of facilitated oxygen transport by respiratory pigments. However, values were particularly high in the active pelagic piscivore, *D. mawsoni*, compared to the benthic and benthopelagic species. *D. mawsoni* is more likely to benefit from the role of elevated catecholamine levels in mobilising energy reserves and enhancing gas exchange across the gills in order to support the increased metabolic demands of exercise (Randall and Perry, 1992). In the present study, adrenaline and noradrenaline levels were considerably higher than those previously reported for the benthic and epibenthic species, *N. coriiceps*, *C. aceratus* and *Tremotomous bernacchii*, after stress imposed by capture, surgery, enforced exercise and handling (Egginton, 1994, 1997; Davison et al., 1995). Rather, values were more characteristic of temperate fish after acute stress (Gamperl et al., 1994). Values were also higher than those obtained from the active cryopelagic species, *Pagothenia borchgrevinki*, after heat stress, which may reflect differences in either the level of routine activity or the nature of the imposed stress (Forster et al., 1998). In the present study, fish were severely traumatised as they were captured by trawl, landed on deck and exposed in air for sorting. Blood samples were taken after the fish had been passed down a chute to the decks below. Although each species experienced similar capture and sampling conditions, and therefore experienced a

similar degree of stress, individuals caught at different times during the trawl would have been subjected to a variable duration of stress. While this is unlikely to affect the interspecific comparisons, it may be a cause of the high intraspecific variability. In contrast, other studies of Antarctic species have measured catecholamine levels in stressed fish after a prolonged entrapment in nets or after being held in aquaria. Although most indices of physiological disturbance (blood pH, haematocrit, plasma ion concentrations) stabilised by 2-3 days recovery from capture stress in previous studies, the imposition of any subsequent stress may be inadequate to stimulate further release of catecholamines due to slow turnover rates.

With the exception of *N. rossii*, circulating adrenaline levels were 3–4 times higher than noradrenaline levels, consistent with the adrenaline:noradrenaline ratios that are more characteristic of teleosts (Randall and Perry, 1992). Previous experiments on the biosynthesis of [^{3}H]catecholamines from [^{3}H]tyrosine in cod chromaffin tissue demonstrated that adrenaline was the predominant catecholamine, even though formation of adrenaline was slow, suggesting low turnover rates of stored catecholamines (Jönsson and Nilsson, 1983b). A similar situation is likely to exist in Antarctic fish, with higher adrenaline levels indicating storage of catecholamines in the chromaffin tissue as adrenaline after conversion from noradrenaline by the enzyme, phenylethanolamine-*N*-methyl transferase (PNMT), the rate-limiting step in the synthesis of adrenaline. The previously reported dominance of noradrenaline (Egginton, 1994; 1997) may then represent a consequence of depleted catecholamine stores.

The elevated catecholamine levels measured in the present study demonstrate that Antarctic fish are capable of storing and subsequently releasing high levels of catecholamines into the circulation under conditions of extreme trauma. The turnover of catecholamines within the chromaffin tissue is likely to be slow because of the low temperature and reduced capacity to synthesise noradrenaline, and hence subsequent recovery of stored catecholamine levels after stress-induced depletion may be slow and minimise further adrenergic responses to acute stress. Circulating catecholamine levels remain low during moderate stress, and indicate that recovery from capture may have been incomplete in such studies and/or that other factors, such as those that regulate storage and release, may play a more important role in the adrenergic control of secondary stress responses in Antarctic fishes. The large differences between the present catecholamine values and those obtained previously could be due to variations in previous stressful experiences, with species in the present study being newly exposed to fishing stress and consequently able to release accumulated levels of catecholamines into the circulation. Previous studies have been conducted on fish caught by netting or hook and line and subjected to handling, transportation and maintenance in holding tanks before experimentation. All of these processes have the potential to stress the fish and cause release of catecholamines from head kidney tissue and hence result in low circulating levels of catecholamines during experimental manipulation. The slow turnover of noradrenaline in Antarctic species further supports the view that the autonomic cholinergic system is more important than blood-borne catecholamines in regulating the stress response in Antarctic fish (Axelsson et al., 1994; Egginton and Davison, 1997).

Plasma cortisol levels ranged from 1–64 ng ml^{-1} among the six species of Antarctic fishes after trawling and handling. These values are similar to those considered to be resting levels in temperate fish at 2–42 ng ml^{-1} (Gamperl et al., 1994). Previous measurements of plasma cortisol levels in Antarctic fish during stress are scarce, but demonstrate that the nature of the stressor is important in determining circulating levels. For example, exercise in *P. borchgrevinki* decreased circulating cortisol levels (Lowe and Wells, 1997), while chronic heat shock induced an increase in cortisol concentration from 15 to 70 ng ml^{-1} (Ryan, 1995). The relatively low cortisol levels recorded in the present study suggest either that cortisol is not an important stress hormone in Antarctic fish, or that stress-induced elevations in cortisol are lower than those experienced in temperate species. There is also some evidence to suggest that circulating cortisol levels vary with ecotype, as cortisol measurements in the relatively active, pelagic species, *D. mawsoni* and *P. borchgrevinki* (Ryan, 1995), tended to be higher after extreme stress than those recorded in more sluggish, benthic and benthopelagic species. However, there were no significant differences in cortisol levels between the species sampled; catecholamine levels were significantly higher in *D. mawsoni* compared to all the other species with the exception of *N. rossii*, which is described as a relatively active species.

In summary, these data suggest that depressed catecholamine synthesis is typical of Antarctic fish, and that release of primary stress hormones may be more closely related to life style rather than to the presence or absence of respiratory pigments. The differences in synthetic activity in the head kidney cannot explain the observed differences in circulating catecholamine levels during acute stress, indicating that other factors such as those regulating storage and release of catecholamines are more important in Antarctic fish during stressful situations.

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