J. exp. Biol. ***181****, 27–47 (1993)*
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THERMAL ACCLIMATION ALTERS BOTH ADRENERGIC SENSITIVITY AND ADRENOCEPTOR DENSITY IN CARDIAC TISSUE OF RAINBOW TROUT

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Accepted 15 April 1993

Summary

We examined the effect of temperature acclimation on the sensitivity of the rainbow trout heart to adrenaline and on the density of β-adrenoceptors. The sensitivity of the heart was assessed using *in situ* working perfused heart and *in vitro* isometric ventricular strip preparations. When tested *in situ* and at acclimation temperature, hearts from fish acclimated to 8˚C were approximately 10-fold more sensitive to adrenaline-supplemented perfusate than were hearts from fish acclimated to 18˚C. The concentrations required for half-maximal stimulation (EC_{50}) of myocardial power output were 1.9×10^{-8} mol l^{-1} adrenaline and 1.7×10^{-7} mol l^{-1} adrenaline for hearts acclimated to 8˚C and 18˚C, respectively. *In vitro*, isometric ventricular strip preparations demonstrated a similar increase in adrenergic sensitivity with cold-acclimation. The EC_{50} values for maximal tension development were 2.7×10^{-7} mol l^{-1} adrenaline (8˚C-acclimated) and 1.1×10^{-6} mol l^{-1} adrenaline (18˚C-acclimated) when tested at acclimation temperature. This shift in adrenergic sensitivity was a function of the temperature acclimation because changes in bath temperature *per se*, either from 8˚C to 18˚C for 8˚C-acclimated hearts or from 18˚C to 8˚C for 18˚C-acclimated hearts, had no significant effect on the concentration–response curve for adrenaline.

We conducted radioligand binding studies with [^{125}I]iodocyanopindolol and propranolol to quantify the β-adrenoceptor density (B_{max}) of both homogenates and isolated sarcolemmal fractions of ventricles from rainbow trout acclimated to either 8˚C or 18˚C. The B_{max} for isolated sarcolemmal fractions was significantly higher in the 8˚C-acclimated group, but the B_{max} of ventricular homogenates was not significantly different in the two acclimation groups. No significant differences in dissociation constant (K_d) were apparent in either the homogenates or sarcolemmal fractions. These results suggest that cardiac tissue from rainbow trout acclimated to 8˚C has a greater cell surface

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Key words: *Oncorhynchus mykiss*, rainbow trout, temperature, acclimation, heart, adrenergic, β-receptor.

adrenoceptor population available for β-antagonist binding. This might explain the heightened cardiac sensitivity to adrenaline observed *in situ* and *in vitro* in 8˚C-acclimated fish.

Introduction

Adrenergic regulation of the fish heart is involved in the maintenance of tonic levels of cardiac stimulation in resting animals (Graham and Farrell, 1989; Axelsson *et al.* 1987), in protecting cardiac performance during periods of stress (Gesser *et al.* 1982; Farrell, 1984) and possibly in stimulating maximum cardiac performance during exercise (Farrell and Jones, 1992). The effects of adrenergic agents, however, are temperature-dependent and are particularly affected by acclimation temperature. Peyraud-Waitzeneger *et al.* (1980) found that the eel (*Anguilla anguilla*) heart was influenced solely by α-adrenoceptors in the winter, but that summer acclimation resulted in the development of a β-adrenergic control. Similarly, in the carp (*Cyprinus carpio*), intravenous injection of adrenaline produced bradycardia at cold temperatures (1–8 ˚C) but tachycardia at warm temperatures (9–20˚C; Laffont and Labat, 1966). Wood *et al.* (1979) reported that in rainbow trout (*Oncorhynchus mykiss*) adrenergically mediated tachycardia became quantitatively more important at warm temperatures. Graham and Farrell (1989) observed a 10-fold increase in the sensitivity of an *in situ* rainbow trout heart preparation to adrenaline in cold-acclimated animals. Because α-adrenoceptors are regarded as being functionally unimportant in terms of cardiac contractility in the rainbow trout (Ask *et al.* 1981; Farrell *et al.* 1986), the temperature-related shift in cardiac adrenergic sensitivity in the rainbow trout probably reflects changes in functions mediated by β-adrenoceptors.

Although thermal modification of β-adrenergic sensitivity has been observed in a number of fish species, its cellular basis is unknown. The present series of experiments examined the effect of thermal acclimation on adrenergic sensitivity of the trout heart at a number of organizational levels, ranging from the intact organ to subcellular fractions. We have used the results of Graham and Farrell (1989) as a starting point, and have re-examined the adrenergic sensitivity of trout hearts from 8˚C-acclimated and 18˚C-acclimated individuals using an improved *in situ* heart preparation. This preparation, unlike that used in the earlier study, leaves the pericardium intact (Farrell *et al.* 1988*b*). In this improved preparation, the input pressure which supports resting cardiac output is routinely negative and atrial filling is achieved *vis a fronte.* Additionally, the positive filling pressure required to produce maximum myocardial power output is significantly reduced and the potential problems associated with atrial over-distension are largely obviated. Because maximum myocardial power output generated *in situ* with this heart preparation (Milligan and Farrell, 1991; Keen, 1992) is similar to that estimated from fish swimming at U_{crit} (maximum prolonged aerobic swimming speed; Kiceniuk and Jones, 1977), greater confidence is afforded for extrapolation of *in vitro* results to *in vivo* occurrence. We therefore felt it was important to use this preparation to corroborate the shifts in adrenergic responsiveness found in the earlier study.

Studies using live animals and isolated heart preparations have not always clearly

distinguished whether observed changes in adrenergic sensitivity are caused by thermal (acute) or acclimation-induced (chronic) effects on the properties of the receptor. Such a distinction is difficult to resolve *in situ*, primarily because inotropic stimulation by adrenaline is influenced by the accompanying chronotropic changes, which are themselves dependent on temperature (Ask *et al.* 1981). A distinction can be made, however, by measuring the isometric force developed by paced ventricular strips in response to adrenergic agonists. Therefore, our second objective was to distinguish between the direct influence of temperature and thermal acclimation by measuring the adrenergic sensitivity at both 8˚C and 18˚C of ventricular strips from 8˚C-acclimated and 18˚C-acclimated rainbow trout.

Our third objective was to determine whether a change in adrenergic sensitivity was associated with differences in adrenoceptor density and/or affinity, because thermally induced shifts in adrenergic sensitivity of cardiac performance in fish must be correlated with an alteration in at least one cellular component. These alterations could include changes in the number of receptors, in their affinity for the agonist or in the steps involved in the transduction of the signal into an intracellular response. However, none of these effects has been detailed in the cardiac tissue of fish. Accordingly, we examined the binding of the radioligand (−)-[^{125}I]iodocyanopindolol (ICYP; a hydrophobic β-antagonist) to homogenates and to suspensions enriched in sarcolemmal membrane from ventricles of 8˚C-acclimated and 18˚C-acclimated rainbow trout in order to quantify both total and cell surface β-receptor densities.

Materials and methods

Yearling rainbow trout [*Oncorhynchus mykiss* (Walbaum); 290–670g] of undetermined sex were purchased from a local supplier (West Creek Trout Farms; Aldergrove, British Columbia) and held indoors in 2000l fibreglass tanks supplied with aerated, dechlorinated tap water. Fish were put into tanks of either cold water (8˚C) or warm water (18˚C). Cold water temperature was maintained by a Min-O-Cool cooling unit (Frigid Units Inc.; Blissfield, Michigan), whereas warm water temperature was maintained by a countercurrent heat exchanger of local construction. Neither temperature varied by more than 1˚C from its set point. Fish were held at the experimental temperature for a minimum of 3 weeks under a 12h:12h light:dark photoperiod. Experiments were performed throughout the year. Fish were fed daily *ad libitum* with commercial trout pellets.

After acclimation, fish were used in *in situ* working perfused heart (WPH) preparations, in *in vitro* isometric ventricular strip (IVS) preparations or in *in vitro* radioligand binding (RLB) studies. All experiments were performed in accordance with the guidelines of the Canadian Council on Animal Care.

Working perfused heart preparations

In situ WPH preparations were prepared as previously described (Farrell *et al.* 1988*b*). In brief, fish were anaesthetized (MS222, 1:5000 w/v), transferred to an operating sling and the gills were superfused with a chilled, buffered and aerated 1:10000 (w/v) MS222

solution. 75i.u. of sodium heparin in 0.5ml of 0.85% saline was injected into the caudal vessels. Input and output cannulae were constructed from stainless-steel chromatography columns [i.d. 1.9mm (input) and 1.5mm (output)]. The input cannula was introduced into the sinus venosus *via* a hepatic vein and the output cannula was inserted into the ventral aorta, to a point confluent with the bulbus arteriosus. Silk ligatures were used to prevent backflow in the remaining hepatic veins. The ducts of Cuvier were ligated, thereby crushing the cardiac branches of the vagus nerve; heart rate was maintained by the sinoatrial pacemaker rhythm. The pericardium was not disturbed and the heart received saline (described below) at a constant pressure throughout surgery once the input cannula had been inserted.

Following surgery, the fish were submerged in a saline-filled bath, and the connection on the input cannula was switched from the temporary surgical reservoir to a constant pressure head, which delivered perfusion saline to the heart. Both immersion bath and perfusion saline reservoirs were water-jacketed and temperature was controlled by a Lauda cooling unit (Brinkmann Instruments; Rexdale, Ontario) set at the acclimation temperature of the fish in use (i.e. either 8°C or 18°C).

Cardiac output ($\dot{Q}$ in $\mathrm{ml\,min^{-1}}$) was measured by an electromagnetic flow probe (Zepeda Instruments; Seattle, Washington) in the output line. Filling (P_i) and output (P_o; diastolic afterload) pressures were measured using Micron pressure transducers (Narco Life Sciences; Houston, Texas). Filling pressure and diastolic afterload (i.e. the resistance against which the heart pumped) were referenced to the saline level in the immersion bath. Diastolic afterload was set at 4.9kPa and filling pressure was adjusted to set mass-specific $\dot{Q}$ at the basal level of approximately 10–12$\mathrm{ml\,min^{-1}\,kg^{-1}}$bodymass (8°C) or 18–20$\mathrm{ml\,min^{-1}\,kg^{-1}}$bodymass (18°C), thereby approximating *in vivo* resting conditions (Kiceniuk and Jones, 1977). Pressure and flow signals were amplified and displayed on a chart recorder (Gould model 2400; Cleveland, Ohio). Input pressures were routinely negative, showing that the heart was in good condition and the pericardium was intact. Signals were also fed into an Apple II+ microcomputer *via* an analog-to-digital interface for subsequent analysis (Farrell and Bruce, 1987).

Mass-specific myocardial power output (power; in $\mathrm{mW\,g^{-1}}$ventricularmass) was calculated from these variables as:

$$\text{Power} = [\dot{Q} \times (P_\mathrm{o} - P_\mathrm{i}) - k]/VM,$$

where $\dot{Q}$ is in $\mathrm{ml\,min^{-1}}$, P_o and P_i are in kPa, VM is the ventricular mass in g, and k is $0.00167\,\mathrm{min\,mW\,ml^{-1}\,kPa^{-1}}$ (a constant for conversion to mW).

Adrenaline ($5\times10^{-9}\,\mathrm{mol\,l^{-1}}$) was added to all salines used in WPH experiments. This level, which approximates *in vivo* resting levels (Milligan *et al.* 1989), provides a tonic cardiac stimulation and improves preparation stability (Graham and Farrell, 1989).

After stabilization of the preparation (at the basal level of cardiac output), the concentration–response protocol was started by switching the connection on the input line from a saline containing $5\times10^{-9}\,\mathrm{mol\,l^{-1}}$ adrenaline to one containing $5\times10^{-10}\,\mathrm{mol\,l^{-1}}$ adrenaline. This removed the stimulatory effects of $5\times10^{-9}\,\mathrm{mol\,l^{-1}}$ adrenaline, which otherwise would have attenuated the response to further adrenaline additions, particularly in cold-acclimated fish (Graham and Farrell, 1989). Small

increases in filling pressure were then used to elevate $\dot{Q}$ by 1.5-fold to twofold to better simulate the situation in an exercising fish. Plasma catecholamine levels increase as U_{crit}, and thus maximum $\dot{Q}$, is approached (Priede, 1974; Kiceniuk and Jones, 1977; Farrell and Jones, 1992). To ensure that the preparation did not deteriorate during the course of the experiment (which took up to 1h to complete), we did not raise $\dot{Q}$ to its maximum (which is approximately three times greater than the resting level). After stabilization (2–3min), a recording was taken and the adrenaline concentration was increased. After restabilization of the preparation (2–3min), another recording was taken and the next adrenaline concentration was added. Cumulative additions of adrenaline to the perfusing saline were made such that final concentrations ranged from 5×10^{-10} mol l^{-1} to 5×10^{-5} mol l^{-1}.

Isometric ventricular strip preparations

Isometric ventricular strips were prepared as previously described for skipjack tuna atrial strips (Keen *et al.* 1992). To summarize, trout were killed by a sharp blow to the head and the heart was quickly excised and placed in ice-cooled saline. Ventricular strips were dissected using two parallel razor blades. Thin silk threads (5-0) were tied to both ends of the strip, which was mounted in a saline-filled, O_2-aerated and water-jacketed organ bath (20ml volume). Bath temperature was initially either 8°C or 18 °C, depending upon the temperature at which each fish had been acclimated. One end of the muscle strip was attached to a fixed post and the other to a Metrigram isometric force transducer (Gould; Cleveland, Ohio). Signals from the force transducer were amplified and displayed on a chart recorder (Gould model 3400; Cleveland, Ohio). Strips were electrically paced by a Grass SD9 Student stimulator which delivered 10ms current pulses *via* flattened platinum electrodes positioned longitudinally on both sides of the strip. The voltage (10.3±0.6V) was 1.25 times greater than the level required to produce maximal tension development. Muscle strips were stretched until active tension (developed tension) reached a peak and were then allowed to equilibrate for 1h at a stimulation rate of 0.2Hz. Bath saline was replaced with fresh saline followed by a 15 min equilibration period, prior to starting the adrenaline concentration–response trials.

A protocol similar to that used for the WPH studies was used to examine the effect of adrenaline on isometric tension development in ventricular strips. Adrenaline was cumulatively added to the bathing medium surrounding the strips to generate a concentration range of 10^{-8}–10^{-4} mol l^{-1}. Maximal responses typically occurred 10min after addition of adrenaline, although this depended upon incubation temperature, with somewhat longer periods required at high concentrations (up to 30min). Trials for both acclimation groups were performed at a stimulation rate of 0.2Hz and incubation temperatures of both 8°C and 18°C. Control ventricular strips from the same heart as trial strips were run in parallel and were subjected to the same stimulation and temperature regimes, but without adrenaline addition. Changes in peak developed tension (as a percentage of pre-trial values) in control strips were subtracted from trial results to account for the slow, modest deterioration of the preparations (typically <5% decline in developed tension over an 8h period).

Sarcolemmal isolation and radioligand binding studies

Homogenization of ventricles, and sarcolemmal isolation procedures were conducted as previously described for rainbow trout (Tibbits *et al.* 1990) with minor modifications. For each isolation, 13–15 fish were killed by a sharp blow to the head and their ventricles removed and placed in an ice-cooled, buffered homogenization medium consisting of 280mmol l^{-1} sucrose and 20mmol l^{-1} *N*-tris[hydroxymethyl]methyl-2-aminoethanesulphonic acid (Tes; pH7.7 at 21°C). After removal of fat and connective tissue, the ventricles were minced with scissors in 10 volumes of ice-cooled homogenization medium. Homogenization was completed by three 3s bursts of a Tissumizer (Tekmar, Cincinnati, Ohio) set at 40. The homogenate was then passed through two layers of stainless-steel mesh (numbers 28 and 40). A 1ml sample of the filtered homogenate was removed at this point for use in marker and radioligand binding analyses (described below).

Sarcolemmal enrichment of the remaining homogenate (approximately 50ml) was conducted either on ice or under refrigeration (4°C). Contractile proteins were solubilized by adding KCl and $Na_4P_2O_7$ to the homogenate (final concentrations of 100 and 25mmol l^{-1}, respectively). The suspension was then centrifuged at 180000***g*** for 1h. The supernatant was subsequently discarded and the pellet resuspended in fresh homogenization medium. All resuspensions were conducted using a motor-driven Teflon pestle (10 repetitions) at low speed. The suspension was re-centrifuged at 2000***g*** for 10min. The supernatant was then retained and re-centrifuged at 180000***g*** for 1h. The resultant pellet was resuspended in 5ml of 45% (w/v) sucrose and a discontinuous sucrose gradient was constructed by sequential addition of 5ml each of 32%, 30% and 28% (w/v) sucrose solutions to the suspension. The gradient was completed by addition of a top layer of 5–7ml of 8% (w/v) sucrose and was then centrifuged at 122000***g*** in a swinging bucket rotor for 15–16h. The gradient was separated into four fractions designated F1–F4 in order of increasing density. 5ml of a medium consisting of 560mmol l^{-1} NaCl and 80mmol l^{-1} Tes (pH7.7 at 21°C) was added to each fraction and the tubes were incubated for 1h on ice. Following this, 5ml of 280mmol l^{-1} NaCl + 40mmol l^{-1} Tes solution was added and the samples were incubated on ice for 30min. Tubes were then brought to a final volume of 28.5ml with 140mmol l^{-1} NaCl + 20mmol l^{-1} Tes and centrifuged for 1h at 180000***g***. Pellets from each fraction were resuspended in 1.0–1.5ml of 140mmol l^{-1} NaCl + 10mmol l^{-1} Tes solution. Samples were frozen in liquid nitrogen for subsequent marker and radioligand binding analyses.

Protein concentrations in homogenates and isolated fractions were determined by the method of Bradford (1976) using bovine serum albumin as a protein standard. Sarcolemmal enrichment was estimated by measuring the activity of two sarcolemmal enzymes, potassium-stimulated *p*-nitrophenylphosphatase (K^+-*p*NPPase, EC 3.1.3.16) and adenylyl cyclase (EC 6.6.1.1), in both homogenates and isolated fractions.

K^+-*p*NPPase activity was determined by the method of Heller and Hanahan (1972) with minor modifications. In brief, total phosphatase activity was determined by adding of 10–100 μg of protein to tubes containing 200 μmol l^{-1} EGTA, 1mmol l^{-1} $MgCl_2$, 20mmol l^{-1} Tes (pH7.6 at 37°C), 5mmol l^{-1} *p*-nitrophenylphosphate and 50mmol l^{-1}

KCl. Non-K^+-stimulated phosphatase activity was measured by equimolar substitution of NaCl for KCl in a separate series of tubes. All assays were performed in triplicate. Sample dilutions, when needed, were made using a 140mmol l^{-1} NaCl + 20mmol l^{-1} Tes solution (pH7.6 at 37˚C). Test tubes were incubated for 20min at 37˚C and the reaction was terminated by adding 2ml of 100mmol l^{-1} NaOH. Absorbance was measured at 415nm on a Novaspec spectrophotometer (LKB Biochrom, Cambridge, England) and the values were compared with those obtained using *p*-nitrophenol standards. K^+-*p*NPPase activity was calculated from the difference between total and non-K^+-stimulated phosphatase activities.

Adenylyl cyclase activity was measured as described by White and Zenser (1971) with minor modifications. A reaction medium containing 1 mmol l^{-1} 3-isobutyl-1-methyl xanthine (IBMX), 10 mmol l^{-1} $MgCl_2$, 25 mmol l^{-1} Tes (pH7.1 at 22˚C), 10 mmol l^{-1} phosphocreatine, 500 units ml^{-1} creatine phosphokinase, 0.1% (w/v) bovine serum albumin, 500 μmol l^{-1} ATP and 0.53 μmol l^{-1} [^{32}P]ATP (25 Cimmol^{-1}) was added to protein samples which ranged from 20 μg (F2 fraction) to 300 μg (homogenate). The final reaction volume was 150 μl. Tubes were incubated with gentle agitation at room temperature (22˚C) for 30min. The reaction was terminated by adding 100 μl of 100mmol l^{-1} EDTA (pH7.6 at 22˚C) and 100 μmol l^{-1} cyclic AMP and immediately placing the tubes on ice. A 50 μl recovery standard of 0.333 μmol l^{-1} ^{3}H-labelled cyclic AMP (30 Cimmol l^{-1}) was added to each tube. Following this, 2.7ml of 50 mmol l^{-1} imidazole buffer (pH7.6 at 22˚C) was then added and the tube contents were immediately applied to pre-washed alumina columns for separation of ^{32}P-labelled cyclic AMP from other adenylates. Columns were prepared as follows: 1cm of glass wool was packed into 5ml polypropylene disposable syringes to which 1–1.2g of neutral alumina was then added. The columns were washed by passage of 10ml of 1 mol l^{-1} and 20ml of 50mmol l^{-1} imidazole buffer (pH7.6 at 22˚C). After application of the test tube contents to the columns, an additional 15ml of 50 mmol l^{-1} imidazole buffer was applied. The eluent was collected from each column and divided into three 1ml samples, which were used for determination of radioactivity. 10ml of scintillation cocktail was added to each 1ml sample and ^{3}H and ^{32}P radioactivity were determined using standard scintillation counting techniques. Blanks were obtained by incubation in the presence of EDTA. Recovery from columns, estimated from ^{3}H-labelled cyclic AMP counts, typically exceeded 95%. ^{3}H-labelled cyclic AMP and [^{32}P]ATP counting standards were run during each determination.

The enrichment index (EI) of isolated fractions over homogenate was determined using the following equation:

$$EI_{Fi} = (SA_{Fi})/(SA_{HMG}),$$

where specific activities (SA) of either K^+-*p*NPPase (in μmol mg^{-1} protein h^{-1}) or adenylyl cyclase (in nmol mg^{-1} protein h^{-1}) from isolated fractions and homogenate are indicated by subscripted F*i* (where F*i* is fraction number from 1 to 4) and HMG, respectively. The percentage recovery (Rec) of surface membrane (sarcolemma) from the homogenate was calculated from:

$$Rec_{Fi} = ([SA_{Fi} \times Protein_{Fi}]/[SA_{HMG} \times Protein_{HMG}]) \times 100,$$

where Protein is total protein and was calculated from the product of the protein concentration and total volume of either the homogenate or each isolated fraction.

Radioligand binding studies of homogenates and enriched sarcolemmal fractions were conducted using the hydrophobic β-antagonists (−)-[^{125}I]iodocyanopindolol and propranolol, in order to assess the densities of total and surface β-adrenoceptor pools. Total adrenoceptor density was determined from homogenate adrenoceptor binding, whereas the fraction most highly enriched in sarcolemma (F2) was used to measure surface adrenoceptor density. The intracellular pool of adrenoceptors was calculated from the difference between total and surface adrenoceptor populations. Ten-point binding assays were performed in duplicate. Total binding in homogenate (100–300 μg protein per assay) or F2 (20–50 μg protein per assay) was determined in a reaction medium containing 10mmol l^{-1} $MgCl_2$, 100 μmol l^{-1} GTP, 20mmol l^{-1} Tes (pH7.7 at 22°C) and 20–250pmol l^{-1} ICYP. Non-specific binding was assayed by addition of 0.2–2.5 μmol l^{-1} propranolol to a second series of reaction tubes (ICYP:propranolol ratio of 1:10000 in all instances). Final reaction volume was 300 μl. Binding was carried out in a shaking water bath at room temperature for 3h. In pilot trials, this period was determined to be sufficient for binding equilibrium at all radioligand concentrations used. After incubation, 2.2ml of ice-cooled buffer (10mmol l^{-1} $MgCl_2$ + 20mmol l^{-1} Tes) was added to each tube (total volume 2.5ml) and a 1ml sample from the tube was then applied to a Whatman GF/C filter under vacuum filtration. The filter was immediately rinsed with three 4ml washes of ice-cooled buffer and put into a scintillation vial. This was then repeated with a second sample (i.e. two determinations per assay tube). After allowing the filters to dry, 10ml of scintillation cocktail was added to each vial and radioactivity was determined using standard scintillation counting techniques. Specific binding is defined as total binding in the absence of a competing ligand (propranolol) minus the amount bound in the presence of propranolol. The density of binding sites and affinity for ICYP were determined by Scatchard plot analysis (Scatchard, 1949). Assays were performed in duplicate and the K_d and B_{max} were calculated from the mean of the two determinations.

Salines and drugs

Salines used for WPH (surgical, bath and perfusion) and IVS (incubation) preparations contained the following common elements (in mmol l^{-1}): NaCl, 124.1; KCl, 3.1; $CaCl_2$, 2.5; $MgSO_4$, 0.9; dextrose, 5.0. Salines were buffered with 20mmol l^{-1} Tes (sodium salt and free acid combinations) and were gassed with 100% O_2. Saline pH was 7.90 at 8°C and 7.74 at 18°C, approximating *in vivo* blood values at these temperatures (Howell *et al.* 1970; Randall and Cameron, 1973; Railo *et al.* 1985).

All chemicals and drugs other than radioactive compounds were purchased from either Sigma (St Louis, Missouri) or BDH (Toronto, Ontario). Radiolabelled ICYP and cyclic AMP were purchased from Amersham (Oakville, Ontario) and [^{32}P]ATP was purchased from ICN (Costa Mesa, California).

Statistical analyses

Statistical comparisons were made using non-parametric Mann–Whitney *U*-tests and differences were considered significant when $P<0.05$. Data are presented as means ±1 S.E.M.

Results

Ventricular mass, expressed as a percentage of body mass, was significantly greater in 8 ˚C-acclimated fish (0.097±0.002; *N*=63) than in 18˚C-acclimated fish (0.089±0.001; *N*=70). An increase in relative ventricular mass with seasonal acclimation to cold temperatures has previously been demonstrated for rainbow trout (Farrell, 1987; Farrell *et al.* 1988*a*). This result therefore indicates that the 3 week (minimum) holding period used in this study was sufficient to stimulate acclimatory responses.

Concentration–response curves for working perfused hearts

Adrenergic sensitivity of *in situ* WPH preparations, as indicated by the minimum adrenaline concentration producing a response (threshold concentration) and the concentration estimated to produce a half-maximal response (EC_{50}), was clearly temperature-dependent. Hearts from 8˚C-acclimated fish tested at 8˚C were significantly more sensitive to adrenergic stimulation (threshold of 5×10^{-9} mol l^{-1}) than were hearts from 18˚C-acclimated trout tested at 18˚C (threshold of 5×10^{-8} mol l^{-1}; Fig. 1). More specifically, stroke volume, cardiac output, myocardial power output and heart rate of 8 ˚C-acclimated hearts all had adrenaline EC_{50} values approximately 10-fold lower than corresponding values from 18˚C-acclimated hearts (Table 1).

The effect of acclimation temperature on the factoral scope of adrenergic stimulation of cardiac performance was assessed by dividing the maximum value for each variable by its minimum value. Factoral scope of stroke volume, $\dot{Q}$ and power output were all significantly greater in hearts from 8˚C-acclimated fish, but the factoral scope of heart rate was not significantly different between the two acclimation groups (Table 1). Therefore, in addition to the shift in sensitivity, a relatively greater increase in adrenergic stimulation of cardiac performance was possible after cold-acclimation.

Concentration–response curves for ventricular strips

In vitro tension development by IVS preparations also showed a shift in adrenergic sensitivity with temperature acclimation (Table 1, Fig. 2). In this case, strips from 8˚C-acclimated hearts tested at 8˚C had a fourfold greater adrenergic sensitivity (estimated from EC_{50} values) than did strips from 18˚C-acclimated hearts tested at 18˚C (Fig. 2C). However, there was no significant difference in the threshold values. Importantly, the differences in responsiveness of ventricular strips to adrenaline were not a direct consequence of incubation temperature *per se* because there were no significant differences in EC_{50} estimates between 8˚C-acclimated strips tested at 8˚C and 18˚C (Table 1, Fig. 2A) or between 18˚C-acclimated strips tested at 8 and 18˚C (Table 1, Fig. 2B). Therefore, the shift in adrenergic sensitivity resulted from acclimation to environmental temperature and was not simply a response to environmental temperature itself. Adrenaline increased the maximum isometric tension by approximately 2.6-fold in 8 ˚C-acclimated animals and by approximately threefold in 18˚C-acclimated animals, but the values were not significantly different (Table 1).

Sarcolemmal isolations and radioligand binding

A summary of the sarcolemmal isolations from ventricles of 8˚C-acclimated and

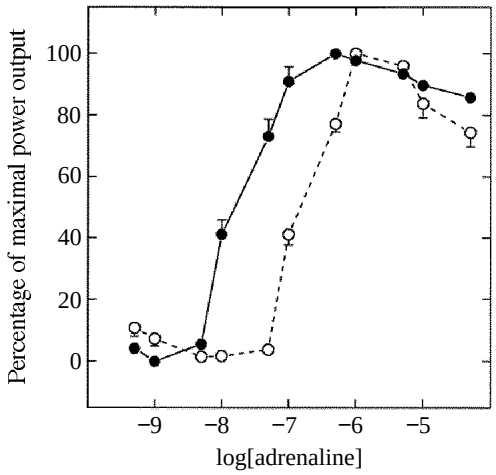

Fig. 1. Sensitivity of mass-specific myocardial power output of *in situ* WPH preparations from 8 ˚C-acclimated (filled symbols) and 18˚C-acclimated (open symbols) trout as a function of adrenaline concentration. Power output is expressed as a percentage of maximum and was calculated as $(Power_{dose}-Power_{min})/(Power_{max}-Power_{min})\times100$, where $Power_{dose}$ is the power generated at each particular adrenaline dose and $Power_{min}$ and $Power_{max}$ are the minimum and maximum power outputs achieved. Values are means ±1 S.E.M. Some error bars are smaller than the symbol. *N*=5 (8˚C) or 6 (18˚C).

18˚C-acclimated trout is presented in Table 2. The lightest fraction collected from the discontinuous sucrose gradient, F1, typically possessed little or no detectable protein and was therefore discarded. The F2 fraction was the most highly enriched in sarcolemma, as assessed from K^+-*p*NPPase and adenylyl cyclase activities. No significant differences in the recovery of F2 fraction, yield or K^+-*p*NPPase activities of homogenates and F2 fractions were found in isolations conducted on 8˚C-acclimated and 18˚C-acclimated ventricular tissues. The combined homogenate yield for both acclimation groups was 98.37±3.28mgprotein g^{-1} wetmass and the combined K^+-*p*NPPase activity was 0.27±0.02 μmolmg^{-1} protein h^{-1}. The combined sarcolemmal (F2) recovery was 4.5±0.2% and the combined yield was 0.54±0.02mgprotein g^{-1} wetmass. The combined K^+-*p*NPPase activity averaged 2.18±0.15 μmolmg^{-1} protein h^{-1}. The combined enrichments of this fraction were 8.3±0.3 (K^+-*p*NPPase) and 7.8±0.3 (adenylyl cyclase). These variables are in general agreement with previous isolations conducted on ventricles from winter-acclimated trout (Tibbits *et al.* 1990), although our K^+-*p*NPPase activities of homogenate and F2 are somewhat depressed. The underlying reasons for the reduced enzyme activity are not apparent. Interestingly, adenylyl cyclase activity was significantly higher in both homogenate and F2 fractions from 8˚C-acclimated cardiac tissue than from 18˚C-acclimated cardiac tissue (Table 2). This difference is a consequence of a significantly higher basal adenylyl cyclase activity in 8˚C-acclimated fish hearts (Keen, 1992) but does not affect the use of the enzyme as an indicator of enrichment.

Pilot studies were performed to determine the time required to reach equilibrium between radioligand and receptor. Studies were conducted on both homogenates and

Table 1. *Effect of acclimation temperature and trial temperature on the sensitivity (EC_{50} expressed as −log[adrenaline]) and stimulatory scope of* in situ *WPH and* in vitro *IVS preparations to applied adrenaline*

Variable	Acclimation temperature (°C)	Trial temperature (°C)	EC_{50} −log[adrenaline]	Factoral scope
In situ WPH				
Stroke volume	8	8	7.82±0.08[a]	1.23±0.04[b]
	18	18	6.81±0.10[a]	1.13±0.02[b]
Cardiac output	8	8	7.71±0.06[c]	1.37±0.02[d]
	18	18	6.79±0.04[c]	1.26±0.02[d]
Power output	8	8	7.71±0.06[e]	1.37±0.03[f]
	18	18	6.78±0.04[e]	1.27±0.02[f]
Heart rate	8	8	7.65±0.10[g]	1.14±0.03
	18	18	6.93±0.07[g]	1.14±0.02
In vitro IVS				
Tension	8	8	6.57±0.06[h]	2.55±0.33
Tension	8	18	6.45±0.03	2.57±0.24
Tension	18	8	5.92±0.03	3.08±0.28
Tension	18	18	5.96±0.03[h]	2.94±0.26

Factoral scope was calculated by dividing the maximum value achieved for each variable by its minimum value.

Values are means ± 1 S.E.M. The *N* value for each acclimation group is as follows: *in situ*, 8°C (5), 18°C (6); *in vitro*, 8°C (6), 18°C (6).

Statistically significant differences between acclimation groups tested at their acclimation temperature are indicated by paired superscripted letters.

isolated F2 fractions using three ICYP concentrations (25, 125 and 250pmol l^{-1}) which spanned the K_d for trout heart β-adrenoceptors. Equilibrium binding was slower in homogenate samples than in F2 fractions. Homogenates reached equilibrium with the radioligand after approximately 2h, as opposed to the approximately 1h required for binding in F2 fractions (data not shown). In order to ensure achievement of equilibrium, subsequent studies employed a 3h incubation period.

Saturation binding curves established saturable and non-saturable radioligand binding components (Fig. 3). Specific binding, calculated from the difference between total and non-specific binding, represented more than 85% of total ligand binding at 125pmol l^{-1} while less than 10% of the total available ligand was bound. Scatchard plot analysis (see Fig. 3) of homogenate fractions from 8°C-acclimated and 18°C-acclimated trout hearts revealed no significant differences in receptor density (B_{max}) or dissociation constant (K_d) (Fig. 4). However, ICYP binding was significantly greater in F2 fractions derived from 8°C-acclimated hearts than in F2 fractions from 18°C-acclimated hearts. B_{max} of the F2 fraction was 116.6±26.8fmol mg^{-1} protein in 8°C-acclimated hearts and 39.7±2.2fmol mg^{-1} protein in 18°C-acclimated hearts (Fig. 4). No significant differences in K_d were observed (Fig. 4).

Calculated sarcolemmal, intracellular and total β-adrenoceptor populations per cell

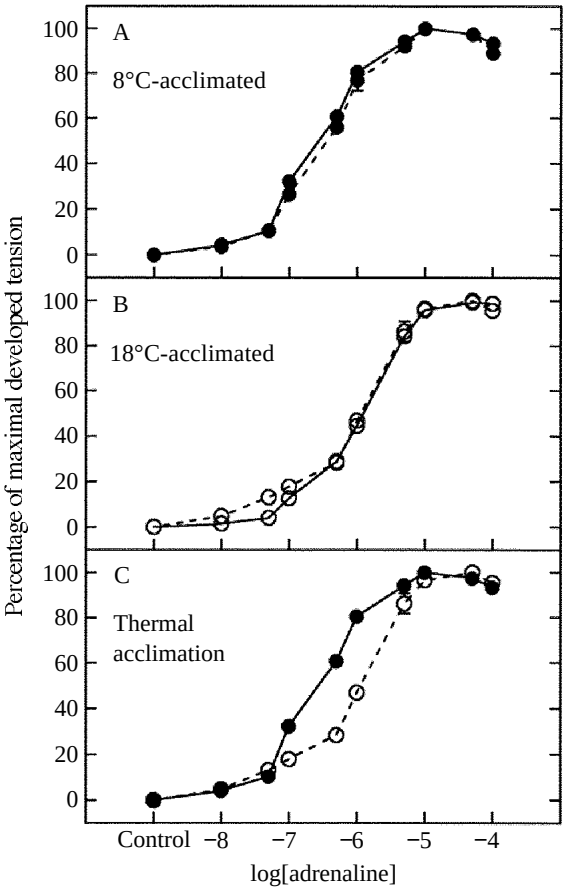

Fig. 2. Effect of trial temperature on maximum isometric tension in ventricular strips from 8 ˚C-acclimated (filled symbols) and 18˚C-acclimated (open symbols) trout. Incubation temperatures are indicated by solid (8˚C) and broken (18˚C) lines. Percentage maximum tension was calculated in the same manner as described for power in the caption for Fig. 1. The data for an acute change in bath temperature are presented in A and B to highlight that there was no direct effect of temperature on adrenergic sensitivity. C compares the data for 8 ˚C-acclimated and 18˚C-acclimated ventricular strips tested at their respective acclimation temperatures and highlights the acclimatory change in adrenergic sensitivity to adrenaline. N=6 for each point. Values are means ±1 S.E.M. Most error bars are smaller than the symbol.

were all greater in ventricles from 8˚C-acclimated trout than in those from 18˚C-acclimated trout, but only the sarcolemmal populations were significantly different (Fig. 5).

Discussion

The increased sensitivity to applied adrenaline in 8˚C-acclimated trout hearts is in agreement with the results of Graham and Farrell (1989), who used a similar preparation. Heart rate, stroke volume, $\dot{Q}$ and power output were all significantly more responsive to

Table 2. *Summary of sarcolemmal isolations from ventricles of trout acclimated at either 8 or 18°C*

		Acclimation temperature	
Variable	Fraction	8 °C	18°C
Yield	HMG	101.62±4.52	95.21±5.43
	F2	0.54±0.05	0.55±0.03
Specific activity			
K^+-*p*NPPase	HMG	0.26±0.03	0.28±0.03
	F2	2.10±0.30	2.27±0.15
Adenylyl cyclase	HMG	0.59±0.04[a]	0.36±0.03[a]
	F2	4.56±0.45[b]	2.79±0.22[b]
F2 SL enrichment			
K^+-*p*NPPase		8.2±0.7	8.3±0.3
Adenylyl cyclase		7.8±0.6	7.8±0.5
F2 recovery			
K^+-*p*NPPase		4.3±0.3	4.8±0.2
Adenylyl cyclase		4.2±0.4	4.5±0.3

Yield, mgprotein g^{-1} wetmass; specific activity, μmolmg^{-1} protein h^{-1} (K^+-*p*NPPase) or nmol mg^{-1} protein h^{-1} (adenylyl cyclase); enrichment in number-of-fold-increase over homogenate value; recovery, percentage of homogenate values.

Homogenate, HMG; Fraction 2, F2.

Values are means ± 1S.E.M. of four isolations.

Statistically significant differences are indicated by paired superscripted letters.

adrenaline in 8˚C-acclimated hearts than in 18˚C-acclimated hearts. A more detailed concentration–response curve was constructed in the present study, but the 10-fold shift in sensitivity is essentially equivalent to that found by Graham and Farrell (1989). In their study, the threshold adrenaline concentrations were 10×10^{-9} mol l^{-1} and 10×10^{-8} mol l^{-1} for 5˚C-acclimated and 15˚C-acclimated hearts, respectively. These values are only slightly higher than the threshold concentrations found in our study (5×10^{-9} mol l^{-1} at 8˚C and 5×10^{-8} mol l^{-1} at 18˚C). Furthermore, because the EC_{50} values indicated a similar 10-fold shift in sensitivity, the change in sensitivity of the *in situ* heart was not a consequence of a ‘broadening’ of the range of concentrations producing a stimulatory effect.

The factoral scope of adrenergically mediated increases in $\dot{Q}$, stroke volume and power output were all significantly greater in 8˚C-acclimated than in 18˚C-acclimated WPH preparations. Cardiac output of 8˚C-acclimated trout hearts was increased by 37% when maximally stimulated by adrenaline, compared to a corresponding increase of only 26 % in 18˚C-acclimated hearts. These values agree qualitatively with those of Graham and Farrell (1989), but are quantitatively slightly different. In their study, maximal stimulation by adrenaline increased $\dot{Q}$ by 63% in trout hearts acclimated to 5˚C but by only 38% in hearts acclimated to 15˚C. The disparity between the two studies may, in part, simply reflect a combination of the 3˚C difference in acclimation temperatures and the fact that factoral scope is greater with cold-acclimation. Thus, fish acclimated to 5˚C

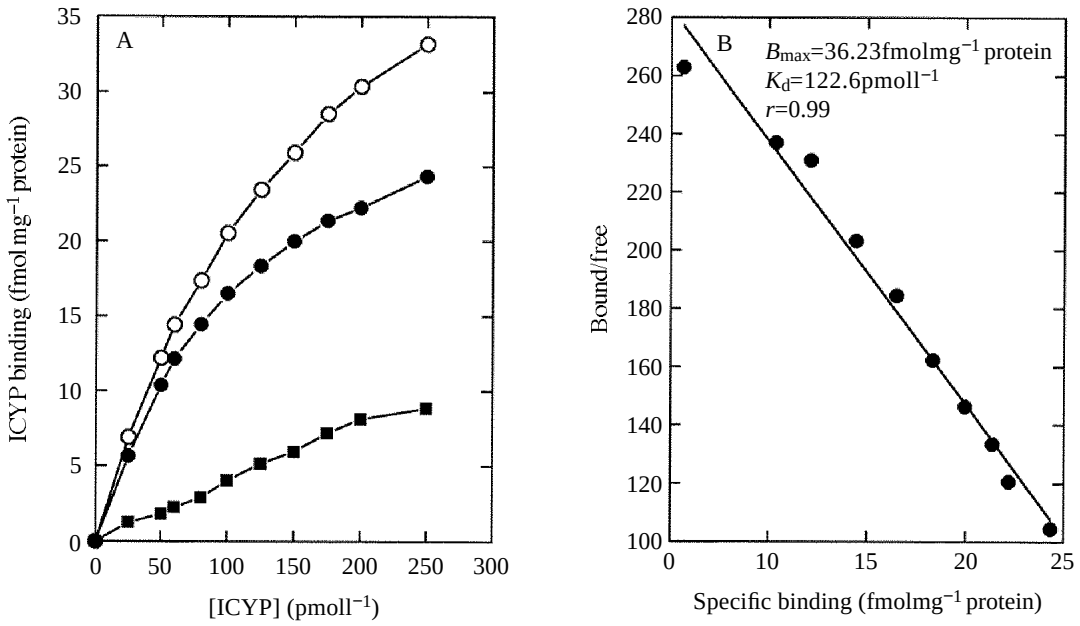

Fig. 3. Typical saturation binding plot (A) and Scatchard plot (B) of ICYP binding to homogenate. These particular examples are from one of the two replicates performed on a homogenate of 18˚C-acclimated tissue. Saturation binding curves depict total (open circles), specific (filled circles) and non-specific (filled squares) binding. The *r* value for linear regressions of Scatchard plot data exceeded 0.90 in all instances.

are expected to have a greater factoral scope in cardiac performance than fish acclimated to 8˚C, as was the case. In addition, basal heart rate was lower in the study of Graham and Farrell (1989), possibly because of the absence of an intact pericardium and the effect this may have on the interaction between stroke volume and heart rate (Farrell and Jones, 1992).

Studies conducted using isolated ventricular strips found that the EC_{50} for adrenergic stimulation of active tension development decreased with cold-acclimation and clearly demonstrated that this shift was acclimation-dependent and not simply an effect of acute temperature change. In this regard, the IVS experiments complemented the findings of the WPH preparations. The absolute value and magnitude of the shift in adrenaline EC_{50} values, however, differed in the two preparations. The EC_{50} values for IVS preparations were approximately 10-fold lower than those for perfused hearts (Table 1). Furthermore, the 10-fold difference in EC_{50} values recorded from 8˚C-acclimated and 18˚C-acclimated WPH preparations was substantially greater than the fourfold difference found between ventricular strips from 8˚C-acclimated and 18˚C-acclimated rainbow trout when tested at acclimation temperature. The reasons for this are unclear, but the fact that differences exist is not surprising considering the vast disparity between the two techniques. The more important consideration is that in both preparations differences in EC_{50} value were found and the direction of change was consistent.

In both WPH and IVS preparations this leftward shift in sensitivity following 8˚C-

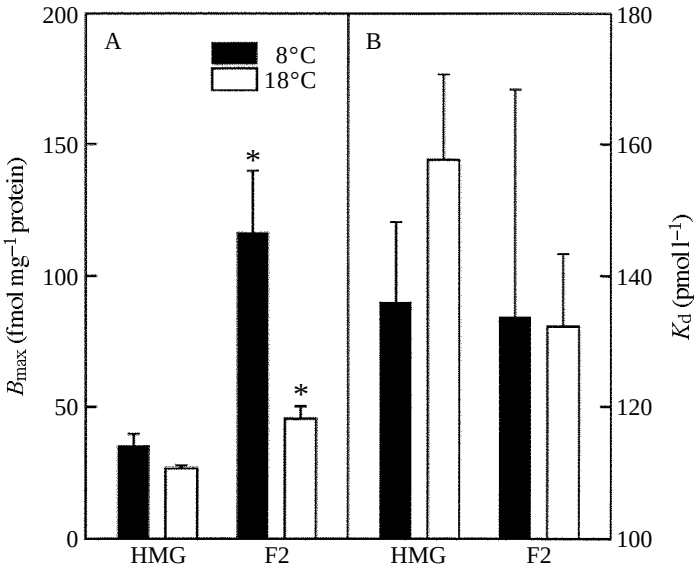

Fig. 4. B_{max} (A) and K_d (B) values for homogenate and enriched sarcolemmal fraction (F2) from 8°C-acclimated (filled bars) and 18°C-acclimated (open bars) trout ventricular tissue. Values are means +1 S.E.M. of four preparations. Significant differences between acclimation temperature groups are indicated by an asterisk.

acclimation was also correlated with an increase in specific binding of ICYP in sarcolemmal fractions. As outlined in Tibbits *et al.* (1990), it is possible to use the yield, recovery and B_{max} to calculate the number of binding sites per milligram of sarcolemma (F2 fraction) and the number of binding sites per milligram protein (HMG; recovery=1.00). Assuming a cell mass of 5.3pg and surface area of 3550 μm^2 (Farrell *et al.* 1988*a*), we can estimate the number of binding sites per cell and site density (per μm^2 of sarcolemma). The surface β-adrenoceptor density thus calculated from ICYP binding was 1.29±0.23sites μm^{-2} for ventricular tissue from 8°C-acclimated trout; a value almost three times higher ($P<0.05$) than that calculated for 18°C-acclimated heart tissue (0.47±0.02sites μm^{-2}). It should be noted that the present calculation of ventricular cell adrenoceptor density assumes no change in myocyte size with thermal acclimation, an assumption that has yet to be clearly resolved because cardiac growth at cold temperatures is achieved through both hyperplasia and hypertrophy (Farrell *et al.* 1988*a*). However, even if the relative increase in ventricular mass is only caused by myocyte hypertrophy, the increase in ventricular mass of around 10% observed here for 8°C-acclimated trout would increase cell surface area to approximately 4000 μm^2 and would reduce the surface adrenoceptor density to approximately 1.1sites μm^{-2}, a value still substantially higher than the estimate of 0.47sites μm^{-2} estimated for 18°C-acclimated ventricular tissue.

Our calculations of adrenoceptor number and density can be compared with earlier estimates from other tissues and species. The total adrenoceptor population of 8000–12000 sites per cell for the rainbow trout ventricle (this study) is an order of magnitude less than for rainbow trout erythrocytes (Reid and Perry, 1991) and for rat

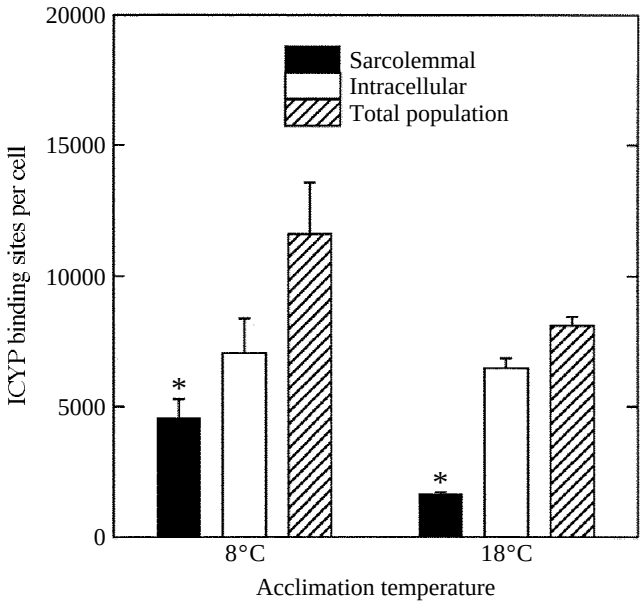

Fig. 5. Calculated density of cellular ICYP receptors in ventricular myocytes from trout acclimated to 8˚C and 18˚C. Densities are divided into surface (sarcolemmal; filled bars), intracellular (unavailable for binding; open bars) and total (hatched bars) populations. Calculations were conducted as described in the Discussion. Values are means +1 S.E.M. of four preparations. Significant differences between acclimation temperature groups are indicated by an asterisk.

cardiomyocytes (Moustafa *et al.* 1978; Buxton and Brunton, 1985). Similarly, we estimated from the data of Reid and Perry (1991) a red blood cell surface density of approximately 20–30adrenoceptorsites μm^{-2}, which is at least an order of magnitude greater than our estimate of approximately 1.0sites μm^{-2} sarcolemma. Our estimate is also lower than the surface density of 33sites μm^{-2} for rat cardiomyocytes, but is similar to values of 3sites μm^{-2} determined for S49 lymphoma cells and approximately 1 site μm^{-2} for pigeon red blood cells (Buxton and Brunton, 1985). These comparisons clearly indicate considerable variability in surface receptor densities as a function of tissue and species.

The increase in surface β-adrenoceptor density in ventricular tissue from 8˚C-acclimated trout is in agreement with the greater sensitivity observed in the working heart and ventricular strip preparations. Receptor occupancy by adrenaline activates a GTP-binding protein (G protein) intermediary, which then catalyses adenylyl cyclase to convert ATP to cyclic AMP (Lefkowitz *et al.* 1983). L-type calcium channel current can be increased both directly, through interaction with the stimulated G protein (Yatani *et al.* 1987), and indirectly, through phosphorylation by a cyclic-AMP-dependent kinase (Osterrieder *et al.* 1982; Kameyama *et al.* 1985). Force development is directly related to the amount of calcium available for interaction with the contractile apparatus (Wier and Yue, 1986; Yue, 1987). An adrenergically stimulated increase in calcium-channel-mediated calcium influx across the sarcolemma should be manifest as an increase in force

produced. A greater surface density of β-receptors in cold-acclimated hearts permits a greater likelihood of receptor occupancy and thus stimulation at lower concentrations of adrenaline. This has been demonstrated previously in dog hearts by comparing the responsiveness of neonate and adult tissues to applied adrenaline and determining the number of surface receptors (Rockson *et al.* 1981). Neonates possess 50% more surface β-adrenoceptors than do adults and this difference was correlated with a decrease in the concentration of isoproterenol required to half-maximally activate adenylyl cyclase.

Although the calculated sarcolemmal β-adrenoceptor density was significantly higher in 8˚C-acclimated trout hearts than in 18˚C-acclimated ones, the corresponding increase in total β-adrenoceptor population size and intracellular population size were not significantly different (Fig. 5). We are therefore unable to determine whether the differences in the surface adrenoceptor population stem from an increase in the total available receptor pool or from a shift in receptor trafficking. The mechanisms responsible for stimulation of adrenoceptor synthesis and routing of β-adrenoceptors have not been examined in fish. Heightened cortisol levels, however, have been correlated with an increase in β-adrenoceptor number and with the routing of internal receptors to the surface in trout red blood cells (Reid and Perry, 1991) under a wide variety of stressors, including hypoxia (Fievet *et al.* 1987), hypercapnia (Perry *et al.* 1989) and strenuous exercise (Primmett *et al.* 1986). The action of cortisol represents a short-term adaptation to an imposed stress, but the mechanisms utilized may be related to the long-term adaptive change in surface receptor density observed in this study. In mammals, long-term increases of β-adrenoceptor levels in a number of tissues have been demonstrated to occur in response to stimulation by glucocorticoids (Collins *et al.* 1989, 1991) through increases in transcription of the β-adrenoceptor gene. Whether such a mechanism exists in fish has yet to be determined. The link between cold-temperature acclimation and the increase of ventricular surface β-adrenoceptors also needs further study.

The differences in surface receptor density in sarcolemmal fractions from 8˚C-acclimated and 18˚C-acclimated trout hearts have been ascribed to temperature acclimation, but it is possible that they could arise from differences in the stability of adrenoceptors within the membrane during the extensive isolation procedure. Reid *et al.* (1991) found that trout red blood cell adrenoceptors were extremely labile and were even affected by ‘gentle’ procedures such as erythrocyte washing and resuspension. Although comparable studies have not been made in cardiac tissues, differences in tissue type could influence the relative susceptibility of the adrenoceptors to perturbations arising from homogenization and isolation procedures. An observation that supports the idea of tissue-specific differences can be found by comparing Scatchard plots derived from experiments in which antagonists such as propranolol were used as the displacing ligand. In this situation, Scatchard plots for both trout (Reid *et al.* 1991) and turkey (Andre *et al.* 1981) erythrocytes were curvilinear, indicating the presence of at least two adrenoceptor populations that differ in accessibility or affinity for propranolol. In the present study, Scatchard plot correlation coefficients were always greater than 0.90, indicating the presence of only a single adrenoceptor population in trout cardiac tissue (or at least adrenoceptors which could not be distinguished by propranolol interaction).

The increase in cell surface β-adrenoceptors with cold-acclimation is likely to be only one of a suite of responses which improve cardiac performance in cold-acclimated rainbow trout, some of which are intrinsic to the heart. Decreased temperature causes a large reduction in pacemaker frequency, thus lowering maximum $\dot{Q}$ (Farrell, 1984; Farrell and Jones, 1992). This thermally mediated decrease in heart rate, however, produces an increase in the filling time of the atrium and thus an increase in stroke volume, which partially offsets the thermal dependency of beat frequency (Keen, 1992). At the myofilament level, exposure to cold temperature decreases maximum force development which, in part, reflects a decrease in the calcium sensitivity of ventricular tissue (Harrison and Bers, 1989, 1990) and further reduces maximum cardiac performance. This reduction in calcium sensitivity may be intrinsically offset, in part, by the concomitant increase in intracellular pH associated with a drop in temperature. In mammals, a decrease in intracellular proton load has been demonstrated to increase the calcium sensitivity of the myofilaments (Fabiato and Fabiato, 1978; Gulati and Babu, 1989). Although these (and other) intrinsic mechanisms ameliorate the effects of an acute temperature decrease, compensation is incomplete and performance at cold temperatures remains reduced.

Chronic exposure to low ambient temperature (i.e. acclimation) stimulates the development of elements and processes that augment existing intrinsic mechanisms designed to enhance performance at cold temperatures. For example, the high temperature-dependency of heart rate is reduced following acclimation to cold temperatures such that the Q_{10} for heart rate is less than 2.0 (Priede, 1974; Graham and Farrell, 1985, 1989). In the present study, the decrease in EC_{50} for adrenaline observed *in situ* indicates an increase in adrenergic sensitivity at cold temperature, and the *in vitro* ventricular strip results demonstrate that at least part of this is due to thermal acclimation. These observations, in addition to the demonstration of an increase in adrenoceptor density of isolated sarcolemmal fractions from cold-acclimated hearts, suggest that acclimation-induced changes in adrenergic sensitivity of the heart may further reduce the deleterious effects of cold temperature on cardiac performance. Because adrenaline is considered to be approximately 10 times more effective than noradrenaline as a β-adrenergic agonist in rainbow trout (Ask *et al.* 1981; Farrell *et al.* 1986), it is only necessary to consider the effect of circulating adrenaline on cardiac performance. Given the similarities in performance (as outlined in the Introduction), it is possible to extrapolate the results of the *in situ* WPH preparation to the condition *in vivo* with some measure of confidence. Circulating levels of adrenaline have been determined for winter-acclimated (5°C) and summer-acclimated (17°C) rainbow trout at both rest (5nmol l^{-1} at 5°C, 11nmol l^{-1} at 17°C) and under conditions of extreme stress (248nmol l^{-1} at 5°C, 186nmol l^{-1} at 17°C) (Milligan *et al.* 1989). Resting blood catecholamine levels in cold-acclimated rainbow trout are therefore poised precisely at the threshold for adrenergic stimulation as determined *in situ* (5nmol l^{-1}) and may underlie the importance of a tonic adrenergic stimulation of the heart at cold temperatures, as previously reported by Graham and Farrell (1989). In contrast, resting blood adrenaline levels in warm-acclimated trout (11nmol l^{-1} at 17°C) are well below the threshold level of 50nmol l^{-1} (18°C) found *in situ*. In both cold-acclimated and warm-acclimated fish, peak blood-

borne adrenaline levels released during periods of extreme stress (248nmol l^{-1} at 5˚C, 186nmol l^{-1} at 17˚C) are expected to stimulate the heart maximally. The results from *in situ* WPH preparations suggest that only the 8˚C-acclimated hearts would be fully stimulated by this level of circulating adrenaline, whereas hearts from 18˚C-acclimated trout would be stimulated to only 60–70% of the maximum possible (Fig. 1). However, these experiments do not take into account the potential contribution that adrenaline released from nerve endings terminating in the myocardium might make to adrenergic stimulation of the heart (Farrell and Jones, 1992; Taylor, 1992). An increase in adrenoceptor density with cold-acclimation, in conjunction with a putative increase in dihydropyridine-sensitive voltage-gated calcium channels (G. F. Tibbits, personal communication) and temperature-dependent increases in action potential duration (Moller-Nielsen and Gesser, 1992) and mean open-state probability (Cavalie *et al.* 1985), should promote an increased trans-sarcolemmal influx of calcium. Given that force generation by the myofilaments is directly related to calcium availability, maximum developed force should be increased after acclimation to the cold above that achievable after acute exposure. Thus, against a background of reduced contractility at lower temperature, adrenergic stimulation would produce a relatively greater increase of cardiac performance in cold-acclimated rainbow trout.

In conclusion, *in situ* heart preparations and *in vitro* ventricular strips from rainbow trout acclimated to 8˚C had a greater sensitivity to adrenaline than did their 18˚C-acclimated counterparts. The effect was clearly demonstrated in ventricular strips to be an acclimatory response and not a direct function of temperature *per se*. Furthermore, binding studies conducted on isolated sarcolemmal fractions indicated a significant increase in surface β-adrenoceptor density in 8˚C-acclimated fish hearts. It is suggested that the increase in surface β-adrenoceptor density is at least partially responsible for the increased adrenergic sensitivity found in the cardiac tissue of cold-acclimated rainbow trout and that this process represents a compensatory response to reductions in ambient temperature.

We thank the two anonymous referees for comments on the manuscript. The technical assistance and expertise of Mr Jeff A. Johansen, Ms Haruyo Kashihara and Ms Kathy L. Cousins was greatly appreciated. This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada to A.P.F. and G.F.T. and by research awards (M. Fretwell/J. Abbott Graduate Fellowship; Petro-Canada Graduate Scholarship in Science; President's Research Stipend; SFU Graduate Fellowships) to J.E.K.

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