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The effect of temperature acclimation on myocardial β-adrenoceptor density and ligand binding affinity in African catfish (*Claris gariepinus*)

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Abstract

This study assessed the effects of temperature acclimation on myocardial β-adrenoceptor density (B_{max}) and binding affinity (K_d) in African catfish (*Claris gariepinus*) acclimated to 15, 22 and 32 °C. B_{max} values were not significantly different ($P>0.05$) among the three acclimation groups. Conversely, the K_d value of the 32 °C acclimation group ($K_d=0.88$) was significantly higher ($P=0.002$) than both the 15 °C ($K_d=0.48$) and 22 °C ($K_d=0.46$) acclimation groups. In addition, K_d of rainbow trout (*Oncorhynchus mykiss*) was significantly lower ($P<0.001$) and B_{max} significantly higher ($P<0.05$) than that of African catfish at all three acclimation temperatures. These results contrast with those reported previously for temperate species, in which B_{max} is inversely related to acclimation temperature, and counter a previous suggestion that B_{max} is higher in tropical versus temperate species.

Keywords: Acclimation; African catfish; β-adrenoceptor (β-AR); Binding affinity (K_d); Cardiac; *Claris gariepinus*; Temperature

1. Introduction

The β-adrenoceptor (β-AR) signaling pathway is known to mediate important cardiac inotropic and chronotropic actions of adrenaline and noradrenaline in fish (Ask et al., 1981; Temma et al., 1986; Gamperl et al., 1994). The main β-AR subtype mediating these cardiac actions in rainbow trout (*Oncorhynchus mykiss*) is a β_2 subtype (Ask et al., 1980, 1981; Keen et al., 1993; Gamperl et al., 1994), although other sub-types have been identified in other fish species (see Nilsson, 1983; Farrell and Jones, 1992). Temperature acclimation can alter the response of the rainbow trout heart to adrenaline (Ask et al., 1981; Farrell et al., 1996) and some of this change has been attributed to a temperature-dependent change in cell surface β-AR density (B_{max}) (Keen et al., 1993; Shiels et al., 2002). For example, the increase in adrenergic sensitivity with cold acclimation in rainbow trout hearts is associated with an increase in β_2-AR density (Keen et al., 1993; Gamperl et al., 1998) and this adrenergic stimulation is extremely important in preserving the L-type Ca^{2+} current in isolated trout myocytes exposed to cold temperature (Shiels et al., 2000, 2003). The objective of this study was to determine whether the tropical species African catfish (*Claris gariepinus*), exhibits a similar intraspecific plasticity in β-AR density in response to acclimation temperature as rainbow trout. It was expected that β-AR density would be inversely related to acclimation temperature.

2. Materials and methods

2.1. Fish

African catfish (*C. gariepinus*) were originally obtained from a live fish supplier in Singapore and held at National University of Singapore for >6 months (with temperature exhibiting the normal diurnal fluctuation between 23 and 28 °C). For the temperature acclimation experiment, fish were transferred into small fiberglass aquaria (2 per temperature

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group) that were then gradually adjusted to the three experimental temperatures (15, 22, 32 °C) over a period of 2–6 days (i.e. approximately 1 °C change per day). Fish in all three acclimation groups were then held at experimental temperature for 28 days before sampling. The fish were quickly killed by a blow to the head, the ventricle was excised, rinsed in a 0.8% NaCl solution and frozen in liquid nitrogen. Ventricles were frozen at −80 °C before being shipped on dry ice to Simon Fraser University, where they were stored at −80°C for no more than 2 months prior to analysis. Body mass ranged from 296 to 707 g and relative ventricular mass from 0.05% to 0.11%.

Earlier studies of cardiac β-AR in fish have included rainbow trout, therefore they were used as a reference species to ensure consistency of experimental techniques. Rainbow trout (*O. mykiss*) were purchased from Sun Valley Trout Farms (Langley, BC, Canada) and held in an outdoor tank (14 °C) for at least 3 weeks prior to sampling. The fish were killed by a quick blow to the head after which the cardiac ventricle was quickly excised, rinsed in saline (composition in mM: NaCl, 124.1; KCl, 3.1; $MgSO_4$, 0.90; $CaCl_2$, 2.5; TES free acid, 7.0; TES Na salt, 3.3; pH 7.85 at 15 °C), gently massaged to remove blood and frozen in liquid nitrogen. Tissues were stored at −80 °C for no longer than three months prior to analysis. Body mass ranged from 338 to 476 g and relative ventricular mass from 0.08% to 0.13%.

2.2. Quantification of β_2-adrenoceptors

Cell surface β_2-adrenoceptor density (B_{max}) and binding affinity (K_d) were determined for ventricular punches, using the tritiated ligand technique of Watson-Wright et al. (1989), as modified for fish hearts by Gamperl et al. (1994). Frozen ventricles were first rinsed in saline (see above) several times to remove any remaining blood. Ventricles were then partially refrozen at −80 °C before being sliced (350 μm thickness) with a McIlwain tissue chopper (Brinkman, Rexdale, ON, Canada). Tissue slices were placed in ice-chilled saline while ventricular tissue punches (2 mm diameter by 350 μm thickness) were taken. For rainbow trout, the punches were taken almost exclusively from the compact myocardium, while for African catfish the compact myocardium was not as well defined and punches were taken from the outer regions of the ventricular wall. Gamperl et al. (1998) showed that there was no significant difference between K_d for the compact and spongy myocardium of rainbow trout, but that B_{max} was 14% higher in spongy myocardium. Single punches were placed in separate wells of a 24-well tissue culture plate (Flow Laboratories, McLean, VA), with each well containing 500 μL of saline and various concentrations (0.05–3.5 nM) of the hydrophilic β_2-adrenoceptor ligand [^{3}H] CGP-12177 (CGP specific activity 55 and 52 Ci/mol; Amersham Life Science). To determine nonspecific binding, separate tissue punches were incubated at each concentration with the competitive β_2-adrenoceptor antagonist timolol (10 μM). All tissue punches were incubated at 0 °C for 2 h, which was sufficient for equilibrium binding to occur (Gamperl et al., 1994). The culture plates were agitated frequently during incubation to prevent the formation of a depletion layer. Following incubation, punches were washed twice in ice-chilled saline, placed into 8 ml scintillation vials containing 2 ml of Ecolite

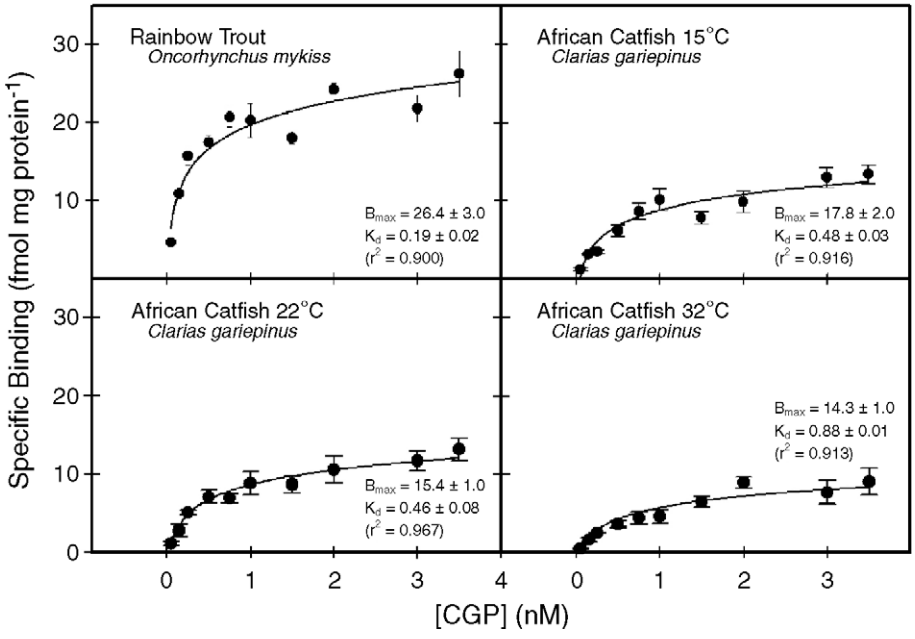

Fig. 1. Specific binding of [^{3}H]CGP-12177 to ventricular β-adrenoceptors. Due to the small size of some African catfish ventricles, they were sometimes pooled for a single binding curve. The number of fish used and the number of binding assays performed are as follows: rainbow trout (N=6 binding assays, 6 fish), African catfish 15 °C (N=3 binding assays; 5 fish), African catfish 22 °C (N=3; 5 fish), and African catfish 32 °C (N=3; 5 fish). The β-adrenoceptor density (B_{max}, fmol mg protein^{-1}), [^{3}H]CGP-12177 dissociation constant (K_d, nM) and r^2 values for each graph are indicated. Values are mean ± SEM.