A COMPARISON OF MYOCARDIAL ß-ADRENORECEPTOR DENSITY AND LIGAND BINDING AFFINITY AMONG SELECTED TROPICAL FISHES

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EXTENDED ABSTRACT ONLY – DO NOT CITE

In fish, the β-adrenoreceptor (β-AR) signaling pathway is known to mediate the cardiac actions of adrenaline primarily via receptors of the β_2 subtype (Ask et al., 1980; Gamperl et al., 1994). Temperature acclimation can alter the response of the heart to adrenaline and some of this change has been attributed to a temperature-dependent change in cell surface β-AR density. Olsson et al. (2000) found that B_{max} and K_d differed among a limited number of tropical, temperate and Antarctic teleost fish species alluding to the possibility that intrinsic differences in β-AR density and binding affinity may exist among species adapted to different temperatures. The purpose of this study was to examine interspecific variation in myocardial β-adrenoreceptor density (B_{max}) and binding affinity (K_d) for ventricular tissue in 7 previously unstudied species of tropical fish.

Materials and Methods

Quantification of ß-Adrenoreceptors

Cell surface ß-adrenoreceptor density and binding affinity were determined for ventricular punches, using a tritiated ligand technique (Watson-Wright et al., 1989; Gamperl et al., 1994). Ventricular tissue punches were incubated with various concentrations (0.05-3.5nM) of the hydrophilic $ß_2$-adrenoreceptor ligand $[^3H]$ CGP-12177.

The mass of individual ventricles determined the degree of replication for the binding assays. Assays were replicated up to six times and consisted of 6-8 replicates for each ligand concentration.

Data Analysis

Where only one binding curve was performed, mean values ± SEM are presented for the replicates at each ligand concentration. Where more than one ligand binding curve was performed, mean values ± SEM are presented for all specimens. Binding parameters were determined using a Scatchard plot (Zivin and Waud, 1982). Protein content of representative punches was determined using a Bradford protein assay so that B_{max} could be expressed as fmol mg protein^{-1}.

Results

B_{max} values ranged from 19.5 to 52.8 ± 8.0 fmol mg protein^{-1} (Figure 1). The highest B_{max} values were observed in marble goby, blue spotted fantail ray, sleeper, and snakehead. B_{max} was significantly higher than rainbow trout in blue spotted fantail ray and marble goby (Figure 2).

Ligand binding affinity (K_d) varied from 0.19 ± 0 .02 to 1.05 ± 0.11 nm among the 8 species (Figure 1). K_d of both African catfish and Blue spotted fantail ray was significantly higher when compared with rainbow trout (p < 0 .05, Figure 2).

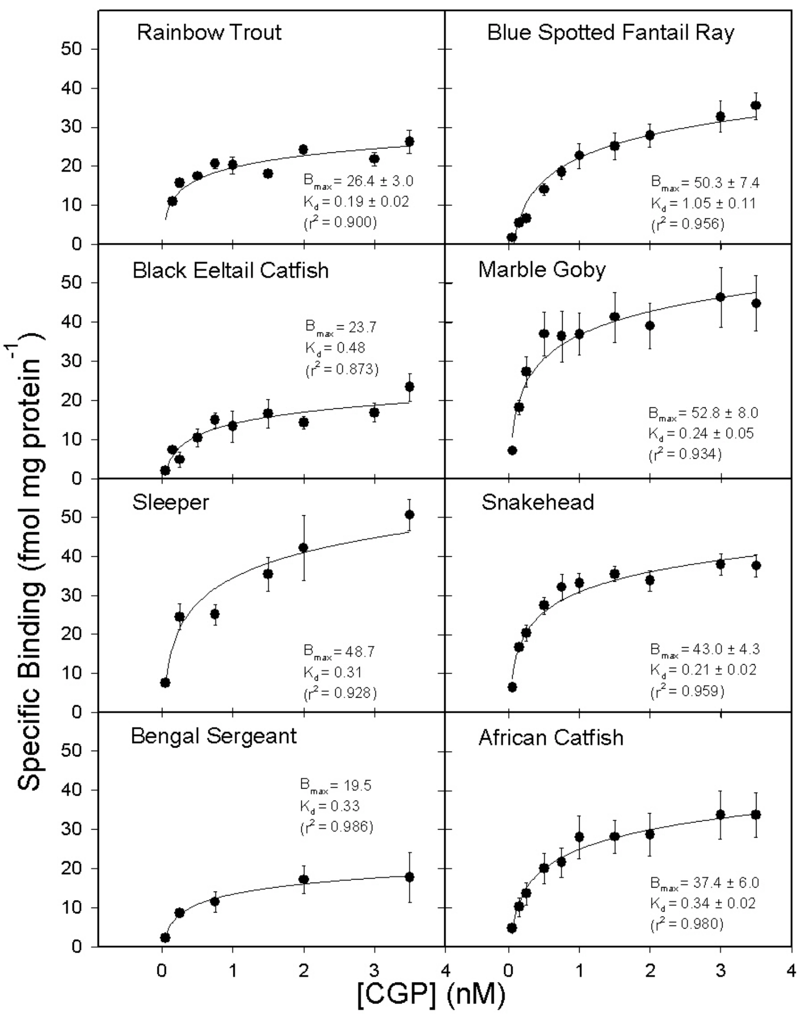

Figure 1 - Specific binding of [^{3}H]CGP-12177 to ventricular β-adrenoreceptors in rainbow trout (N=6), blue spotted fantail ray (N=6), black eeltail catfish (N=1, 10 hearts pooled), marble goby (N=6, 19 hearts), sleeper (N=1, 10 hearts),

snakehead (N=6, 17 hearts), Bengal sergeant (N=1, 10 hearts) and African catfish (N=6, 15 hearts). The B_{max} (fmol mg protein^{-1}), K_d (nM) and r^2 values for each graph are indicated. Values are mean ± SEM.

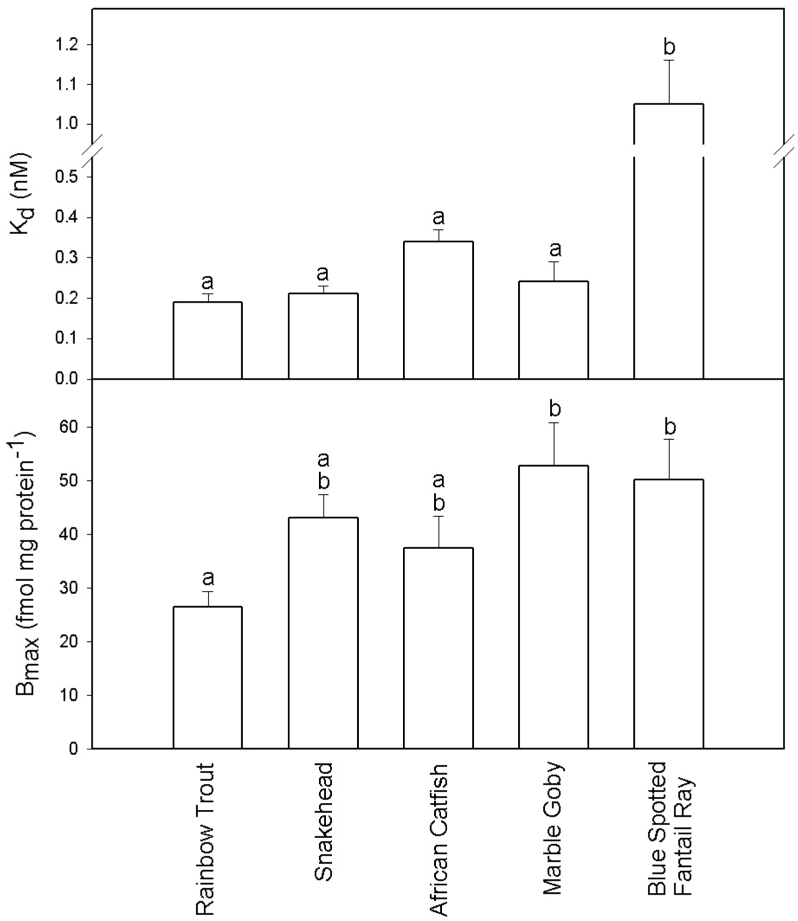

Figure 2 - [3][H]CGP-12177 dissociation constant (K_d) and ventricular β-adrenoreceptor density (B_{max}). Values are presented as mean ± SEM. Dissimilar letters denote significant differences at $P<0.05$. N=6 for all species.

Conclusions

We demonstrated variation between species of over twofold in B_{max} and almost sevenfold in K_d. Our results for rainbow trout compare favorably with earlier studies giving us confidence in the methods utilized to characterize myocardial β-AR density and binding affinity.

Tropical Marine Elasmobranchs

Blue spotted fantail ray is the first elasmobranch in which β-AR density and binding affinity have been characterized. The B_{max} value for this species was similar to those of the other tropical species we studied. This significantly lower binding affinity observed may be due to variation in β-AR subtypes between teleosts and elasmobranchs.

Tropical Marine Teleosts

Due to the small size of the tropical marine ventricles we were only able to generate 1 binding curve for each species. In this regard the results can only be considered a guideline as to the true values of β–AR density and binding affinity. Neither B_{max} or K_d differed significantly from rainbow trout.

Tropical Freshwater Teleosts

β-AR densities for snakehead, African catfish, and marble goby were all observed to be greater than the rainbow trout. Nevertheless, due to the large standard errors present in the values of the tropical species, the difference was only statistically significant in the case of the marble goby ($P < 0.05$).

The present results suggest that B_{max} is higher in freshwater, but not marine, tropical species. However, Olsson et al. (2000) reported high B_{max} values for both marine tropical species (mahimahi = 46.9, skipjack tuna = 41.3) and marine temperate species (sockeye salmon = 47.5). As of yet, no clear phylogenetic or environmental pattern of β-AR values is evident.

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