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Cardiovascular and respiratory reflexes of the gulf toadfish (*Opsanus beta*) during acute hypoxia

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ABSTRACT

The acute cardiovascular and respiratory responses of the gulf toadfish, *Opsanus beta*, to acute hypoxia or exposure to the O_2 chemoreceptor stimulant, sodium cyanide (NaCN) were characterized and the role of serotonin type 2 (5-HT_2) receptors in mediating these responses was investigated. Toadfish responded to hypoxia or NaCN exposure with a decrease in heart rate (fH) and an increase in breathing amplitude (V_{AMP}) but no change in breathing frequency (fR). The bradycardia appeared to be mediated to some extent by 5-HT_2 receptors, as methysergide, a non-selective 5-$HT_{1/2}$ receptor antagonist, and ketanserin, a 5-HT_2 receptor antagonist, attenuated the response. Injection of α-methyl-5-HT, a 5-HT_2 agonist, also resulted in bradycardia that was inhibited by ketanserin, lending further support for 5-HT_2 receptor involvement, possibly 5-HT_{2A} or 5-HT_{2C}, in the regulation of fH. External NaCN exposure resulted in a significant decrease in caudal arterial blood pressure (P_{CA}) that was attenuated by methysergide. In contrast, injection with α-methyl-5-HT resulted in a substantial increase in P_{CA} that was not affected by ketanserin, suggesting the possible involvement of 5-HT_{2B} or 5-HT_{2C} receptors. These data are the first to suggest a unique distribution of 5-$HT_{2B/2C}$ receptors may be involved in mediating vasoconstriction of the systemic vasculature of toadfish. These data also provide mechanistic support for why pulsatile urea excretion, believed to be regulated by 5-HT via the toadfish 5-HT_{2A} receptor, is not triggered by hypoxia or external chemoreceptor activation.

1. Introduction

Gulf toadfish, *Opsanus beta*, frequently experience hypoxia in their natural environment (Lapointe and Clark, 1992; Serafy et al., 1997; Hopkins et al., 1997; Hall et al., 1999) and are able to tolerate periods of hypoxia, anoxia and other environmental perturbations in a laboratory setting (Hall, 1929; Ultsch et al., 1981; Wang and Walsh, 2000; Veauvy et al., 2005, 2009; McDonald et al., 2007). However, there are no published data on their cardiovascular responses to hypoxia or how these responses might be regulated. The typical teleost fish responds to acute hypoxia with a substantial decrease in heart rate but an increase in stroke volume that allows cardiac output to be maintained (Holeton and Randall, 1967b; Wood and Shelton, 1980; Short et al., 1979; Fritsche and Nilsson, 1989; reviewed by Farrell, 2007; Gamperl and Driedzic, 2009). They also respond with an increase in systemic and gill vascular resistance and an increase in breathing frequency and/or amplitude (Randall et al., 1965; Holeton and Randall, 1967a,b; reviewed by Randall, 1982; Shelton et al., 1986; Fritsche et al., 1992; Burleson et al., 1992; Fritsche and Nilsson, 1993; Sundin et al., 1995, 1998; Gilmour and Perry, 2007; Perry et al., 2009). These changes, together with altered branchial blood flow distribution, allow for ventilation–perfusion matching at the gill, optimizing gas exchange (reviewed by Nilsson and Sundin, 1998).

The monoamine neurotransmitter, serotonin (5-hydroxytryptamine; 5-HT) influences cardiorespiratory physiology in vertebrates. In mammals, 5-HT has been shown to elicit bradycardia or tachycardia, hypotension or hypertension, and vasodilation or vasoconstriction depending on the species, the vascular bed, the dose of 5-HT, the experimental conditions and the 5-HT receptor type (reviewed by Villalón and Centurión, 2007). In teleost fish, 5-HT is a potent vasoconstrictor of the branchial vasculature and is present in both neuronal processes and epithelial cells in fish gills. Serotonergic nerve fibers run to the efferent filamental artery (EFA) sphincter, which is believed to be a major site of blood flow control in the branchial vasculature (reviewed by Nilsson and Sundin, 1998). Serotonin is also intimately associated with branchial neuroepithelial cells (NECs), which are believed to act as O_2-sensitive chemoreceptors, homologous to the glomus cells of the mammalian

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carotid body (Gonzalez et al., 1994; Jonz and Nurse, 2003; Jonz et al., 2004). In zebrafish (*Danio rerio*), branchial neuroepithelial cells in culture respond to hypoxia with a dose-dependent decrease in K^+ current and a membrane depolarization (Jonz et al., 2004). In rainbow trout (*Oncorhynchus mykiss*) exposed to severe hypoxia, 5-HT-containing vesicles within NECs appear to degranulate suggesting the release of 5-HT from these cells in response to hypoxia (Dunel-Erb et al., 1982).

The role of 5-HT in vasoconstriction suggests that its release from NECs during hypoxia may contribute to the redistribution of the branchial blood flow (reviewed by Nilsson and Sundin, 1998). While arterial injection of exogenous 5-HT results in the predicted increase in gill vascular resistance and ventral aortic blood pressure (Fritsche et al., 1992; Sundin et al., 1995, 1998), this treatment surprisingly impairs gas exchange (Fritsche et al., 1992), possibly owing to vasoconstriction of the distal portion of the filament leading to a reduction in the number of perfused lamellae (Sundin et al., 1995). These findings support the likelihood that 5-HT exerts a paracrine action when released from NECs that are distally located on the EFA (reviewed by Nilsson and Sundin, 1998). The serotonin type 2 (5-HT_2) receptors, of which there are three subtypes, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}, are believed to mediate the gill vascular response to 5-HT injection, because arterial injection of the 5-HT_2 receptor agonist, α-methyl-5-HT, elicits effects similar to injection of 5-HT (Sundin et al., 1998).

The 5-HT_2 receptor, and specifically 5-HT_{2A}, is particularly significant in gulf toadfish as it is believed to play a role in the regulation of the pulsatile urea excretion mechanism found in the gill (McDonald and Walsh, 2004). Specifically, within an acute time frame (<5 min), the toadfish 5-HT_{2A} receptor is believed to mediate the activation of the toadfish urea transporter, tUT (McDonald and Walsh, 2004). Interestingly, the increase in arterial blood pressure observed in the teleost response to hypoxia (Holeton and Randall, 1967a) also occurs in toadfish prior to urea pulses (Gilmour et al., 1998). Given the apparent involvement of 5-HT in both hypoxic responses and urea pulses, and the possible association with changes in arterial blood pressure, it is tempting to speculate that a link exists between chemoreceptor activation (leading to 5-HT release and changes in vascular resistance) and pulsatile urea excretion (via the toadfish 5-HT_{2A} receptor; GenBank accession # FJ611960.1). However, an earlier study designed to test this hypothesis failed to find an association between acute hypoxia or acute external chemoreceptor activation and the activation of tUT (McDonald et al., 2007). A remaining question is whether 5-HT_{2A} receptors in toadfish contribute to cardiovascular or respiratory control mechanisms as well as pulsatile urea excretion. We hypothesize that the lack of an association between pulsatile urea excretion and acute hypoxia or external chemoreceptor activation might reflect a reduced involvement of 5-HT_{2A} receptors in mediating the response to hypoxia, in particular, the cardiovascular response. To address this question, we documented the cardiovascular and respiratory responses of the toadfish to acute hypoxia and to administration of the O_2 chemoreceptor stimulant sodium cyanide (NaCN). Selective 5-HT receptor agonists and antagonists were then used to elucidate the relative roles of 5-HT_2 receptor subtypes, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}, in mediating these responses.

2. Methods

2.1. Experimental animals

Gulf toadfish [*O. beta*; mean mass 83.4 ± 5.9 (SEM) g (N = 18)] were captured with a roller trawl by commercial shrimpers in Biscayne Bay, Florida in November 2003. The toadfish were held in an outdoor tank at the shrimpers' holding facility with running seawater (ambient seasonal conditions) for no longer than 24 h following capture, then transferred to the laboratory where they were held for up to 1 month. Fish were treated with malachite green (final concentration 0.05 mg L^{-1}) in formalin (15 mg L^{-1}) (AquaVet, Hayward, CA, USA) on the day of transfer to the laboratory to reduce infection by the ciliate, *Cryptocaryon irritans* (Stoskopf, 1993). Fish were kept in 50 L glass aquaria with flowing, aerated seawater at a temperature of 24–26 °C. Fish were fed weekly with squid up until the time of surgery.

2.2. Experimental protocol

As outlined by Wood et al. (1997) and McDonald et al. (2000), caudal artery and/or vein catheterizations were performed on fish anaesthetized by immersion in a solution of MS-222 (1 g L^{-1}; Argent Chemical Laboratories, Redmond, WA, USA) and then wrapped with towels soaked in anaesthetic solution. Fish were fitted with a buccal catheter (Clay-Adams PE 160 tubing; Becton Dixon, Franklin Lakes, NJ, USA) to facilitate injections of sodium cyanide (NaCN) over the gills (McDonald et al., 2007) and an opercular catheter (Clay-Adams PE 160 tubing; Becton Dixon, Franklin Lakes, NJ, USA) to record pressure changes in the opercular cavity associated with breathing. Fish were left to recover undisturbed overnight in individual shielded chambers (1.5 L volume) containing an opaque PVC pipe shelter and supplied with flowing seawater.

Cardiovascular or respiratory parameters [caudal artery blood pressure (P_{CA}; mmHg), heart rate (fH; beats min^{-1}), ventilatory amplitude (V_{AMP}; mmHg) or ventilatory frequency (fR; min^{-1})] were monitored in toadfish exposed to a variety of conditions and pharmacological agents. Arterial blood pressure was measured by connecting the saline-filled caudal artery cannula to a Bell + Howell pressure transducer that was interfaced with an amplifier and data acquisition system (Biopac Systems Inc., Goleta, CA, USA). Data were captured (50 Hz sampling frequency) and processed using the associated commercial acquisition software (AcqKnowledge 3.1; Biopac Systems Inc., Goleta, CA, USA). Cardiac frequency was determined automatically from the pulsatile blood pressure trace using a built-in rate detector. V_{AMP} and fR were monitored by connecting the seawater-filled opercular catheter to the data acquisition system via a second pressure transducer. The difference between the minimum and maximum opercular pressures during any breathing cycle was used as an index of V_{AMP}. fR was determined automatically from the pulsatile opercular pressure trace using a built-in rate detector. The pressure transducers were calibrated daily against a static column of water.

Once stable ventilatory and cardiovascular readings were obtained, the impact of acute exposure to low environmental O_2 gas tensions on the cardiovascular and respiratory parameters outlined above was determined. Toadfish were monitored for a 1-min control period prior to a 14-min exposure to conditions of hypoxia (final Pw_{O_2} = 20–30 mmHg) achieved by vigorous bubbling, with appropriate gas mixtures provided by a Cameron GF-3 gas mixing flowmeter, of a water equilibration column supplying the fish chamber. Water P_{O_2} was monitored using an O_2 fiber optic electrode and associated hardware and software (Ocean Optics Inc., Dunedin, FL, USA) that was calibrated daily using zero solution (2 g L^{-1} of sodium sulphite) and air-saturated water. Cardio-ventilatory parameters were monitored for an additional 8-min post-exposure.

To determine the cardiovascular and respiratory responses to the stimulation of externally oriented (water sensing) O_2-sensitive chemoreceptors, toadfish in a second group were given a bolus injection of NaCN (0.2 ml kg^{-1} using a solution of 0.1 mg ml^{-1}) into the buccal cavity over approximately 5 s. The buccal catheters were then flushed with an equivalent volume of seawater (0.2 ml kg^{-1}). A separate group of toadfish was injected with seawater alone to