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Adenosine/nitric oxide crosstalk in the branchial circulation of *Squalus acanthias* and *Anguilla anguilla*☆

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Abstract

The potent vasomodulator adenosine (AD), thanks to the interaction with by A_1 and A_2 receptors, dilates systemic, coronary and cerebral vasculatures but exert a constrictor action in several vessels of respiratory organs. Recent investigations suggest that nitric oxide (NO) contributes to AD effects. In fish, both NO and AD induce atypical effects compared to mammals. Since there is very little information on the role of NO and its involvement in mediating the actions of AD in fish, we have analysed this question in the branchial vasculature of the elasmobranch *Squalus acanthias* and the teleost *Anguilla anguilla* using an isolated perfused head and a branchial basket preparation, respectively. In both dogfish and eel, AD dose–response curves showed a biphasic effect: vasoconstriction (pico to nanomolar range) and vasodilation (micromolar range). Both effects were abolished by the classic xanthine inhibitor theophylline (Theo) and also by specific antagonists of A_1 and A_2 receptor subtypes. To analyse the involvement of the NO/cGMP system in the AD responses, we tested a NOS inhibitor, L-NIO, and a specific soluble guanylate cyclase (sGC) blocker, ODQ. In both dogfish and eel preparations L-NIO abrogated all vasomotor effects of AD, whereas ODQ blocked the AD-mediated vasoconstriction without affecting the vasorelaxant response. This indicates that only AD-induced vasoconstriction is mediated by a NO–cGMP-dependent mechanism. By using the NO donor SIN-1, we showed a dose-dependent vasoconstrictory effect which was completely blocked by ODQ. These results provide compelling evidence that the vasoactive role of AD in the branchial circulation of *S. acanthias* and *A. anguilla* involves a NO signalling.

Keywords: Adenosine; Nitric oxide; Branchial circulation; Vascular tone; Teleosts; *Anguilla anguilla*; Elasmobranchs; *Squalus acanthias*

1. Introduction

The role of AD as a universal negative feedback modulator of cell energy demand and consumption (Newby, 1984) has been very recently extended to the metabolic protection of hypoxia–anoxia tolerant-animals (Buck, 2004). The heart and the vasculature are major targets of the regulatory actions of AD (Mubagwa et al., 1996), particularly during metabolic stress and hypoxia, as epitomized by the well-known hypoxia-evoked vasodilation attributed to AD (Berne et al., 1983). In mammalian and non-mammalian vertebrates, AD is known to induce vasodilation in systemic, coronary and cerebral vessels (Mubagwa and Flameng, 2001; Lutz and Reiners, 1997). It is also known to constrict the vasculatures of the respiratory organs (lung: Lippton et al., 1982; Broadly and Maddock, 1996; fish gills: Colin and Leray, 1979; Okafor and Oduleye, 1986; Sundin and Nilsson, 1996, and references therein). In mammals, in which four AD receptor subtypes (A_1, A_{2A}, A_{2B} and A_3) have been identified

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(Shryock and Belardinelli, 1997), the cardiovascular effects are primarily mediated by A_1 and A_2 receptors, the former eliciting vasoconstriction, the latter vasodilatation (Tabrizchi and Bedi, 2001). Although this paradigm of AD cell–tissue interactions with receptor subtypes offers an insight in the mechanism by which AD affects different vascular districts, some aspects remain controversial. To this regard, the finding that in some vascular beds the AD dose–response curve induces a biphasic response has been interpreted based on either multiple sites of AD actions or different involvement of signal-transduction systems (Harrison et al., 1996).

Recent studies have implicated a close and complex relationship between AD and NO in modulating both oxygen consumption and vascular resistance. For example, it has been shown that NO, through its inhibition of mitochondrial cytochrome oxidase in competition with O_2, induces AD release, which in turn partially causes vasodilatation by releasing NO from the endothelium (Ray et al., 2002; Edmunds et al., 2003 and references therein). This implies the hypothesis that endothelium, through NO-modulated cytochrome oxidase, acts as an oxygen sensor, releasing AD in response to moderate falls in O_2 (Edmunds et al., 2003). One of the most complex and versatile vascular designs present in vertebrates is found in the fish gills. This multifunctional organ is able to sense several environmental changes including oxygen level fluctuations. Conceivably, in fish gills, the vascular endothelium acting as an oxygen sensor could trigger, under hypoxic conditions, an autocrine–paracrine AD–NO mechanism able to achieve multilevel coordinated compensations, including vascular adjustments, metabolic reduction and decrease of ion fluxes (Buck, 2004). This mechanism could be crucial for many fish species, which, being exposed in their life to frequent dramatic environmental fluctuations of temperature and oxygen levels, represent remarkable examples of hypoxia- and anoxia-tolerant vertebrates. Therefore, they are of interest for studying, at the cardiovascular level, the repertoire of AD-induced homeostatic responses (Buck, 2004) and the underlying biochemical mechanisms. However, as in mammals, the few vascular studies available indicate variable vasomotive actions of AD with scarce information on the underlying transduction mechanisms. For example, the AD-dependent vascular effects range from vasodilation in hagfish (*Myxine glinosa*) gills (Axelsson et al., 1990) to vasoconstriction in the gills of *Oreochromis niloticus* (Okafor and Oduleye, 1986) and rainbow trout (*Oncorhynchus mykiss*) (Sundin and Nilsson, 1996). In the swim bladder vessels of *Anguilla anguilla* (Schwerte et al., 1999) and in the intact coronary tree of the trout (*O. mykiss*) (Mustafa and Agnisola, 1998), AD exerts vasodilation. In ventral aorta ring preparations of the dogfish *Squalus acanthias*, in which both receptors have been detected, A_1 elicited contraction while A_2 mediated relaxation (Evans, 1992).

Apart from the AD/NO crosstalk, identified in trout coronaries (Mustafa and Agnisola, 1998), there is no information on the putative involvement of an AD–NO signal-transduction pathway in fish vasculature. The aim of this work was to verify the presence of an AD–NO signal-transduction mechanism in fish gills by analysing the vasomotive responses of the isolated and perfused branchial preparations of the elasmobranch *S. acanthias* and the teleost *A. anguilla* to AD both alone and in presence of NO donors and inhibitors.

2. Materials and methods

2.1. Animals

Dogfish, *S. acanthias*, ($n=52$) of both sexes, weighing 1.37 ± 0.06 kg (mean value ± SE) were used. The experiments were conducted at the Fisheries and Oceans laboratory, West Vancouver, British Columbia, Canada. The fish were caught by trawl and held in a large sea water tank for 4 to 5 days at approximately 12 °C prior to experimentation.

Fresh-water eels, *A. anguilla,* ($n=56$) of both sexes, weighing between $125.55+2.56$ g, were kept for one week prior to experimentation in fresh-water tanks at a mean water temperature of 17 °C in the laboratories of the Department of Cell Biology, University of Calabria, Italy.

Animal care, sacrifice and experiments were supervised under the European Community guiding principles in the care and use of animals and the projects supervised by the local ethical committee.

2.2. Preparations

The perfused head preparation: *S. acanthias*, anaesthetised with ethyl-aminobenzoate (Tricaine, 2 g/L), were pithed, the tail cut off, and the caudal vein cannulated and perfused with aerated elasmobranch physiological saline containing sodium heparin (5000 UI/kg), which drained from the exposed caudal artery at the cut end of the trunk. The perfusion was continued for 10–15 min until the gills were cleared of blood. The fish were opened ventrally and the ventral aorta was cannulated with polyethylene tubing. In order to preserve the elasmobranch branchial anatomy, the gill basket was dissected together with the head (from the transverse septum). The preparation was placed in a perfusion chamber filled with circulating sea water and the ventral aortic inflow cannula was connected with a perfusion apparatus. Perfusion was started immediately via a constant flow-pulsatile pump whose rate was adjusted to the value of resting cardiac output of the intact fish of about 18 mL/min/kg (the input pressure and the flow being regulated by speed variation of the pump). A compliant system (syringe) ensured a differential input pressure of about 5–7 cm H_2O. The bubbled (0.5% CO_2), non-recirculating physiological saline had the following composition (g/L): NaCl 16.363,