

EFFECTS OF ADENOSINE IN THE BRANCHIAL CIRCULATION OF

***A. anguilla* AND *S. acanthias*: INVOLVEMENT OF NO**

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EXTENDED ABSTRACT ONLY: DO NOT CITE

Adenosine (AD) is a potent vasoactive modulator that in mammals dilates systemic, coronary and cerebral vasculatures (Collis, 1989), whereas can exert a constrictor actions in several lung vessel preparations (Lippton et al., 1982). In fish, the scant data indicate variable vasomotive actions of AD, ranging from vasodilation in hagfish (*Myxine glutinosa*) 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 (*Oncorhynchus mykiss*) (Mustafa and Agnisola, 1998), AD exerts vasodilation. In mammals, the cardiovascular effects of AD are primarily mediated by A₁ and A₂ receptors, the former causing vasoconstriction and the latter inducing vasodilatation (Ralevic et al., 1998). In the ventral aorta of the dogfish *Squalus acanthias*, in which both receptors have been detected, the A₁ elicited contraction while the A₂ mediated relaxation (Evans, 1992).

Endothelial cells modulate vascular tone via the release of many substances such as nitric oxide (NO), the typical vasodilator in mammals. 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*.

An isolated perfused head (*S. acanthias*) and a branchial basket preparation (*A. anguilla*), set up according to the method described by Perry et al. (1982) were used. The preparations were perfused with physiological saline using a peristaltic pump with pulsatile flow and a compliant system (syringe). Afferent perfusion pressure was measured via a pressure transducer connected through a T-tube placed immediately before the input cannula, and its output displayed on a rectilinear pen recorder.

Infusion of the dogfish preparation with saline containing increasing concentrations of adenosine (AD) induced a biphasic effect: AD at lower doses (nano-micromolar range) caused a vasoconstrictory response while at higher doses (millimolar range) caused vasodilation. The curve generated in the eel preparation showed the same profile. However, in the eel the vasoconstrictory response started at much lower concentrations (picomolar range), while the vasodilatory response appeared at the same concentration range observed in the dogfish. In both preparations, the vasoconstrictory and vasodilatory effects were abolished by theophylline (theo). Theo is the classic xanthine inhibitor but it shows weak affinity and subtype selectivity for the AD receptors. We have also used three antagonists of A₁ and A₂ receptors subtypes that in mammals have shown high degree of potency and selectivity, i.e. CPT and DPCPX (antagonists of A₁ receptors) and DMPX (antagonist of A₂ receptors). In the dogfish preparation, exposure to either DPCPX or DMPX elicited a significant vasoconstriction, suggesting removal of an AD-mediated vasodilatory tone. In contrast, in the eel preparation all antagonists had no effect on input pressure. In both preparations, all antagonists (10⁻⁵ M) blocked both the vasoconstrictory and the vasodilatory effects of AD.

To study the putative involvement of the NO/cGMP system in the AD responses, we tested a specific inhibitor of NOS, 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. These data indicate that the vasoconstrictory response of AD is mediated by a NO-cGMP-dependent mechanism, while the vasorelaxant response is cGMP-independent. In agreement with this, when both preparations were exposed to a

stable analogue of cGMP, 8-Br cGMP, they exhibited a dose-dependent vasoconstriction. Finally, by using the NO donor SIN-1 to test the effect of exogenous NO, we showed a dose-dependent vasoconstrictory effect that in turn was completely blocked by ODQ.

On the whole, these results confirm the branchial NO-cGMP vasoconstrictory tone previously detected in the eel by us (Pellegrino et al., 2002) and provide compelling evidence that the vasoactive role of AD in the branchial circulation of *S. acanthias* and *A. anguilla* involves a NO-cGMP signalling,

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