

**THE INOTROPIC INFLUENCE OF ANGIOTENSIN II ON
THE WORKING HEART OF THE EEL (*Anguilla anguilla*):
PARACRINE ASPECTS AND SUBCELLULAR MECHANISMS**

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EXTENDED ABSTRACT ONLY- DO NOT CITE

Angiotensin II (Ang II) not only is a pluripotential hormone but is also an autocrine-paracrine cardiac factor modulating short- (i.e. inotropism) and long-term (i.e. hypertrophy) adaptations of the heart. While a wide range of species-dependent variations is apparent in the inotropic effects of Ang II in mammalian myocardia, there is a lack of information regarding the direct influence of this hormone on the mechanical performance of the fish heart.

Therefore, experiments were carried out in fresh water eels (*Anguilla anguilla*) using an isolated working heart preparation, previously set up in our lab (Imbrogno et al., 2001), to explore whether Ang II exerted direct inotropic effects and to clarify the underlying mechanisms. Ang II ($10^{-11} \div 10^{-7}$ M) elicited negative chronotropic influence and caused on the electrically paced preparations a cardio-suppressive action, decreasing stroke volume (SV) and

stroke work (SW). This negative inotropic effect was abrogated by a selective AT₁ subtype antagonist, CV 11974 (10⁻⁷ M), specific for non mammalian vertebrates, while it was not antagonized by the mammalian AT₁ antagonist losartan or the AT₂ antagonist CGP42112. The Ang II-mediated inotropism was abolished by exposure to either pertussis toxin (PTx; 10⁻¹¹ M) or atropine (10⁻⁶ M), indicating an involvement of the Gi protein system and the muscarinic cholinergic receptors, respectively. In contrast, it was not affected by pre-treatment with the adrenergic receptor antagonists propranolol, sotalol and phentolamine.

Nitric oxide (NO) is an important modulator of the mechanical performance in the eel heart (Imbrogno et al., 2001). Using donors (L-arginine) and inhibitors (L-NMMA, L-NIO) of nitric oxide synthase (NOS), as well as hemoglobin as NO scavenger, we have demonstrated the obligatory role of the nitrenergic signalling in mediating the Ang II response. Pretreatment with either ODQ (10⁻⁶ M), a specific inhibitor of soluble guanylate cyclase (GC) or the inhibitor of the cGMP-activated protein kinase (PKG) KT5328 (10⁻⁷ M) abolished the Ang II-mediated inotropism, indicating that the cGMP-PKG component is a crucial target of the NO signal-transduction pathway.

Interestingly, while NO remarkably modulates the Frank-Starling response of the eel heart (Imbrogno et al., 2001), Ang II *per se* did not affect this mechanism. This stresses the importance of the *local* cardio-suppressive action of Ang II in regulating cardiac performance in the eel heart without deterioration of its intrinsic eterometric modulation.

In the eel heart, the endocardial endothelium (EE) is a major source of NO (Imbrogno et al., 2001). The functional integrity of the EE is a prerequisite for mediating intracavitary Ang II-mediated inotropic signals, since these are abolished by pre-treatment of the EE with Triton X-100. This emphasises the autocrine-paracrine role of the EE in the control of fish heart function.

In conclusion, these results provide the first evidence in fish that endoluminal Ang II exerts a direct cardio-suppressive action on the mechanical performance of the heart via an EE interaction, thereby activating G protein coupled-AT₁ type receptors which trigger a NO-cGMP-protein kinase G signalling transduction pathway. The cardio-depressive action of Ang II does not influence the Frank-Starling response of the heart. These data, together with the involvement of the muscarinic receptors in mediating the Ang II inotropic stimulation, suggest that the EE caveolae (i.e. the plasma invaginations located at or near the plasma

membrane) can be attractive candidates as the domains in which the tonic-phase Ang II-NO signalling is generated.

Reference

Imbrogno, S., L. De Iuri, R. Mazza and B. Tota. 2001. Nitric oxide modulates cardiac performance in the heart of *Anguilla anguilla*. *J. Exp. Biol.* 204:1719-1727

