

**NITRIC OXIDE INFLUENCES THE FRANK-STARLING RESPONSE  
IN THE ISOLATED HEART OF THE EEL (*Anguilla anguilla*)**

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

Teleost hearts are very sensitive to the Frank-Starling mechanism. The isolated and perfused eel heart, in which filling pressure is the primary determinant of cardiac output, displays the typical Frank-Starling response (Davie et al., 1992), allowing adaptation to the remarkable haemodynamic challenges experienced by the animal during its life cycle.

Recent studies in mammalian heart preparations have indicated that nitric oxide (NO), released by either the cardiomyocytes and /or the endothelial cells of both the coronary microvasculature and the endocardium, can influence the Frank-Starling response (Prendergast et al., 1997). Since no information is available in fish, we have investigated the interaction between NO and the Frank-Starling response in the isolated and perfused eel heart. An in vitro preparation of the working heart of *Anguilla anguilla*, electrically paced, able to generate physiological values of output pressure, cardiac output, ventricle work and power, was used (Imbrogno et al., 2000).

Fresh-water *Anguilla anguilla* (n= 44) of both sexes, (weighing  $96.55 \pm 4.56$ ), were used. Each eel was anaesthetized in benzocaine (0.2g/l) for 15 min. The animals were ventrally opened behind the pectoral fins. The hearts were removed without the pericardium and cannulated. The isolation time was 15-20 min. The cannulated heart was transferred in a perfusion chamber filled with saline and connected with a perfusion apparatus. The heart received saline from an input reservoir and pumped against an afterload pressure given by the height of an output reservoir. The saline composition contained the following in g/l: NaCl

6.68, KCl 0.15, KH<sub>2</sub>PO<sub>4</sub> 0.05, MgSO<sub>4</sub> 0.35, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.05, CaCl<sub>2</sub> 0.14, glucose 1, Na<sub>2</sub>HPO<sub>4</sub> 0.227 (Davie et al., 1992); pH was adjusted to 7.7-7.9 by adding NaHCO<sub>3</sub> (about 1g/l). The saline was equilibrated with a mixture O<sub>2</sub>:CO<sub>2</sub> 99.5:0.5%. A Grass stimulator was used to electrically stimulate the hearts. Data were expressed as mean  $\pm$  SE of percent changes from individual experiments. Comparisons within groups were made by paired Student's t-test on absolute data; comparisons between groups were made by two-way ANOVA test. Significant differences from the time control group were detected by Duncan's multiple range test.

The cardiac preparations (constant afterload and heart rate) were studied before and after intervention. Three steps of input pressure elevation, from 0.2 to 0.8 kPa, increased cardiac output. At these conditions, a Starling curve was generated (baseline condition). After baseline assessment, the input pressure was returned to 0,2 kPa and a second Starling curve (untreated time-control) was regenerated (Fig.1 left panel). These time-control curves were then compared with Starling curves obtained in presence of the authentic NO donor, L-arginine (10<sup>-7</sup> M), or the nitric oxide synthase (NOS) inhibitor, L-NIO (10<sup>-5</sup> M) (Fig. 1 right panel). The treatment with L-arginine did not influence the Frank-Starling response, while L-NIO treatment induced significant reductions in the Frank-Starling response.

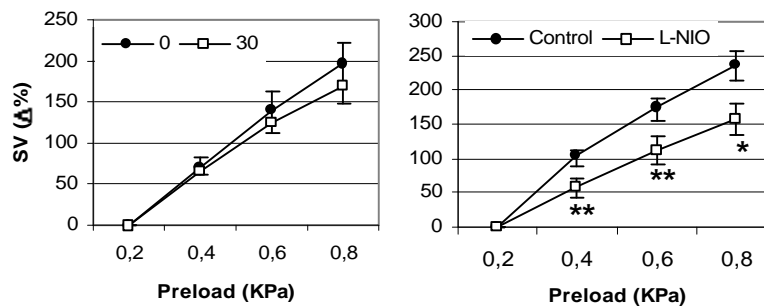


Figure 1. Left panel: effects of preload elevation in control (untreated) hearts at baseline (●) and 30 minutes later (□). Note absence of significant changes with time at equivalent preloads. Right panel: effects of preload elevation before (●) and after (□) L-NIO treatment (10<sup>-5</sup>M). Data are means  $\pm$  SE of 4 experiments (\*=p<0.05; \*\*=p<0.01).

The results obtained were consistent with NO-induced changes in the homeometric regulation of the eel heart. The basal nitregeric tone of the eel heart is also affected by several donors and inhibitors of the NO-cGMP pathway (Fig.2). The results obtained with Triton X-100 suggests a nitregeric modulatory role of the endocardial endothelium (Imbrogno et al., 2000).

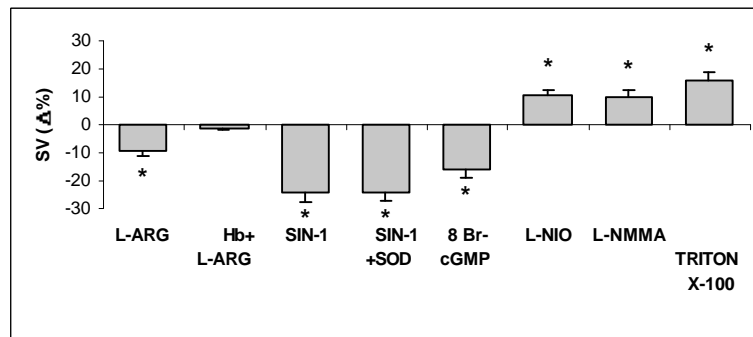


Figure 2. Effects of L-arginine ( $10^{-7}$ M) before and after pretreatment with Hb, SIN-1 ( $10^{-9}$ M) before and after pretreatment with SOD, 8 Br-cGMP ( $10^{-7}$ M), L-NIO ( $10^{-5}$ M), L-NMMA ( $10^{-4}$ M), Triton X-100 (0.05%) (n=4; \*=p<0.05).

Taken together, these data demonstrate for the first time in fish that NO exerts a major role in the modulation of myocardial performance.

## References

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