

EFFECTS OF NITRIC OXIDE

ON THE BRANCHIAL CIRCULATION OF THE EEL

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EXTENDED ABSTRACT ONLY – DO NOT CITE

In mammals, nitric oxide (NO) released by vascular and cardiac endothelium is an important modulator of vascular biology. The principal target of NO is the soluble guanylate-cyclase (sGC) with consequent release of cGMP (Moncada and Higgs, 1995). In fish, the role of NO in the control of vascular tone remains unclear, the contradictory results depending either on different organ-tissue preparations used or possible species-specificities (McGeer and Eddy, 1996). Some authors presented evidence against the presence of a NO system in the teleost systemic circulation (Olson and Villa, 1991), whereas others suggested that NO-releasing compounds cause vasodilatation in rainbow trout *in vivo* by activation of sGC (McGeer and Eddy, 1996). In this study we have analyzed the putative involvement of the NO/cGMP system in the control of the branchial circulation of the teleost *Anguilla anguilla*.

Fresh-water *Anguilla anguilla* (n= 50) of both sexes, (weighing 125.55 ± 2.56), were used. A branchial basket preparation, set up according to the method described by Perry et al. (1982) for the holobranch preparation, was used. The eels were anaesthetized with benzocaine (0.2 g/l). The physiological saline (bubbled 0.5 % CO₂) had the following composition (g/L) : NaCl 6.68, NaHCO₃ 2.20, Na₂HPO₄ 0.227, KCl 0.15, KH₂PO₄ 0.05, MgSO₄ 0.35, (NH₄)₂SO₄ 0.05, glucose 1.00, CaCl₂ 0.14, pH 7.8 Pressure measurements were expressed as mean \pm SE of percent changes from individual experiments; statistical significance of differences was evaluated on absolute values using paired Student's t-test.

Dose-response curves in presence of either L-arginine, the substrate of nitric oxide synthase (NOS), or two NO donors with different chemical properties, SIN-1 and SNP, were performed to test the effects of endogenous and exogenous NO, respectively. L-arginine from 10^{-11} to 10^{-6} M induced a dose-dependent vasoconstriction. The NO donors, SNP and SIN-1, exerted significant vasoconstrictory effects already at very low concentrations (from 10^{-13} M, fig. 1). Vasoconstrictory effects exerted by SIN-1 can be attributed to the generation of NO. In fact, in the presence of superoxide dismutase (SOD), SIN-1 effects were notably potentiated, while in the presence of the NO scavenger hemoglobin, SIN-1 effects were completely blocked. SIN-1 and SNP acted by stimulation of cGC, since their effects were abolished by pre-treatment with the specific inhibitor of sGC, ODQ. The 8 Br-cGMP, a stable cGMP analogue, induced a dose-dependent vasoconstriction, which is in agreement with the vasoconstrictory effects of the NO donors (Fig. 2).

We have also tested three inhibitors of NOS (L-NAME, L-NMMA and L-NIO). All these competitive antagonists caused a mild vasoconstriction. This might be caused by the lack of basal vasodilatory effect of peroxynitrite.

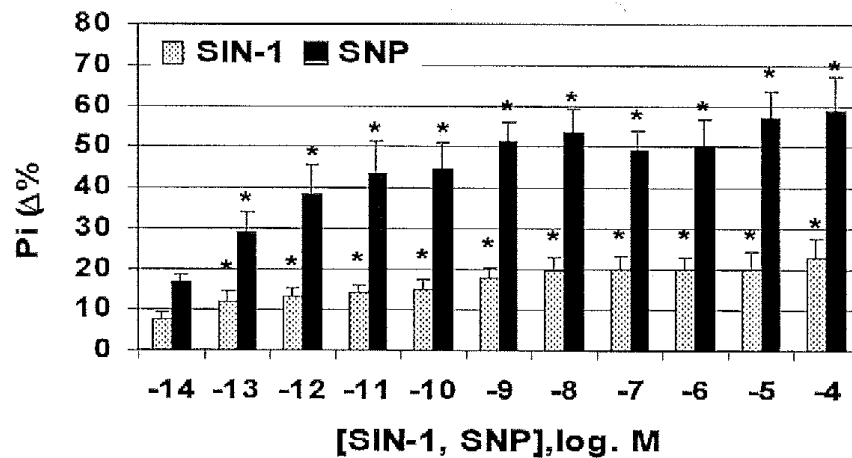


Figure 1. Dose-response curves for SIN-1 and SNP on Pi in isolated and perfused gill preparation. Results are expressed as percent changes (%) from control value; data are means \pm SE of 4-6 experiments in each group (*= $p < 0.05$ vs control value).

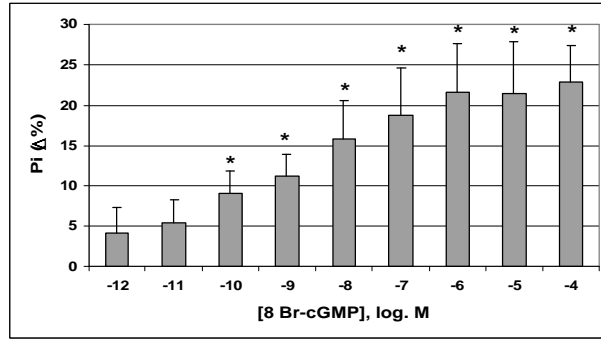


Figure 2. Dose-response curve for 8 Br-cGMP on Pi in isolated and perfused gill preparation. Results are expressed as percent changes ($\Delta\%$) from control value; data are means \pm SE of 4 experiments in each group (*= $p < 0.05$ vs control value).

The present investigation demonstrates an involvement of the NO/cGMP system in the control of the branchial circulation of eel. Endogenous and exogenous NO induce a vasoconstrictory effect on the eel branchial circulation through sGC stimulation. The results obtained with inhibitors of NOS might suggest an involvement of peroxynitrite in the maintenance of vascular tone. These data are in agreement with the hypothesis that also in fish there is a basal release of NO in the vascular endothelium.

References

- McGeer, J.C. and F.B. Eddy 1996 Effects of sodium nitroprusside on blood circulation and acid-base and ionic balance in rainbow trout indications for nitric oxide induced vasodilation. *Can. J. Zool.* 74: 1211-1219
- Moncada, S., and E.A. Higgs 1995 Molecular mechanisms and therapeutic strategies related to nitric oxide. *FASEB J.* 9: 1319-1330
- Olson, K.R., and J. Villa 1991 Evidence against nonprostanoid endothelium-derived relaxing factor(s) in trout vessels. *Am. J. Physiol.* 260: R925-R933
- Perry, S.F., P.S. Davie, C. Daxboeck, A.G. Ellis and D.J. Randall 1982 A comparison of CO₂ excretion in a spontaneously ventilating blood-perfused trout preparation and saline-perfused gill preparations: contribution of the branchial epithelium and red blood cell. *J. Exp. Biol.* 101: 47-60

