BRANCHIAL SEROTONERGIC VASOCONSTRICTION IN THE EEL:

INVOLVEMENT OF NITRIC OXIDE SYNTHASE

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Since the work of Östlund and Fänge (1962) and Reite (1969), which first showed the vasoconstrictory action of serotonin on gill vasculature in fish, there has been no substantial progress in our understanding of the mechanisms involved in this response. It is known in mammals that the vascular endothelium, in response to neuro-humoral subtances such as acetylcholine, can release nitric oxide (NO) with consequent vasodilation. There is an expanding role of NO as a bi-directional messenger between the chemical stimuli acting on the luminal endothelial surface of the vessels and the subjacent smooth muscle. In fish, NO-dependent vasodilation has been shown in the brain of crucian carp superfused with acetylcholine (Hylland and Nilsson, 1995), but has not yet been explored in the gill vasculature. The aim of this study was to verify whether a NO-cGMP signal-transduction pathway is involved in serotonergic branchial vasoconstriction in the European eel (*Anguilla anguilla*) .

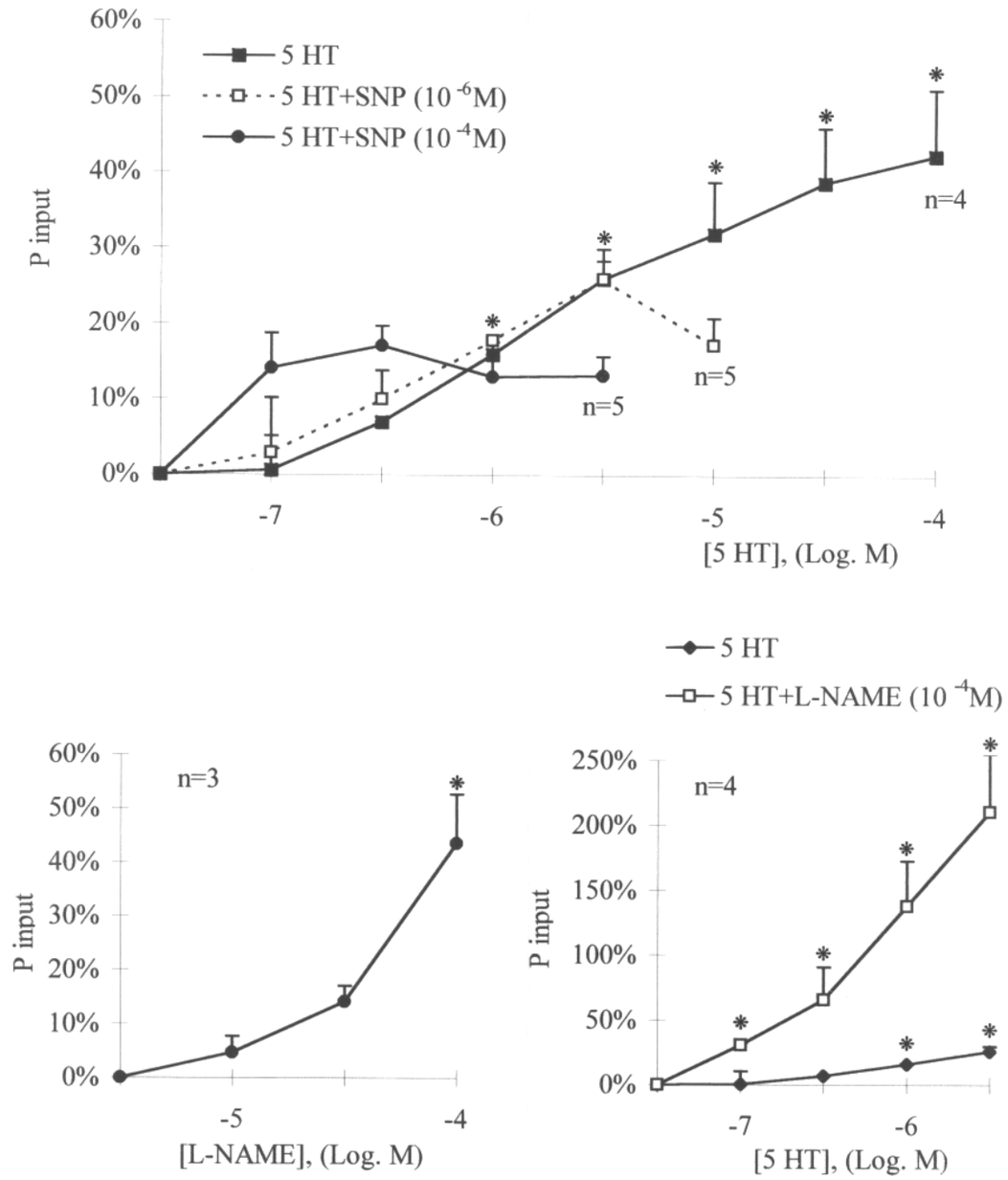

Fig. 1. Effects of 5-HT on the input pressure (expressed as % of P_{input} in control) before and after treatment with SNP (10^{-6} and 10^{-4} M) or L-NAME (10^{-4} M).
The response to L-NAME per se is indicated on the left panel; (*=$p<0.05$).

A basket gill preparation was performed in order to evaluate the response of the whole branchial vasculature, following the method described by Perry *et al.* (1982) for the holobranch preparation. Eels (195 ± 24 g) were anaesthetized in Ethyl p-Amino-Benzoate ($0.1 g.L^{-1}$) and heparinized. The ventral aorta was cannulated and the animal decapitated by cutting behind the opercular openings. Gills were quickly cleared of blood with heparinized saline and dissected free by cutting the arches near the roof of the mouth. The isolated gill basket was then connected to the perfusion system via the aortic cannula and placed in a chamber (60 ml)

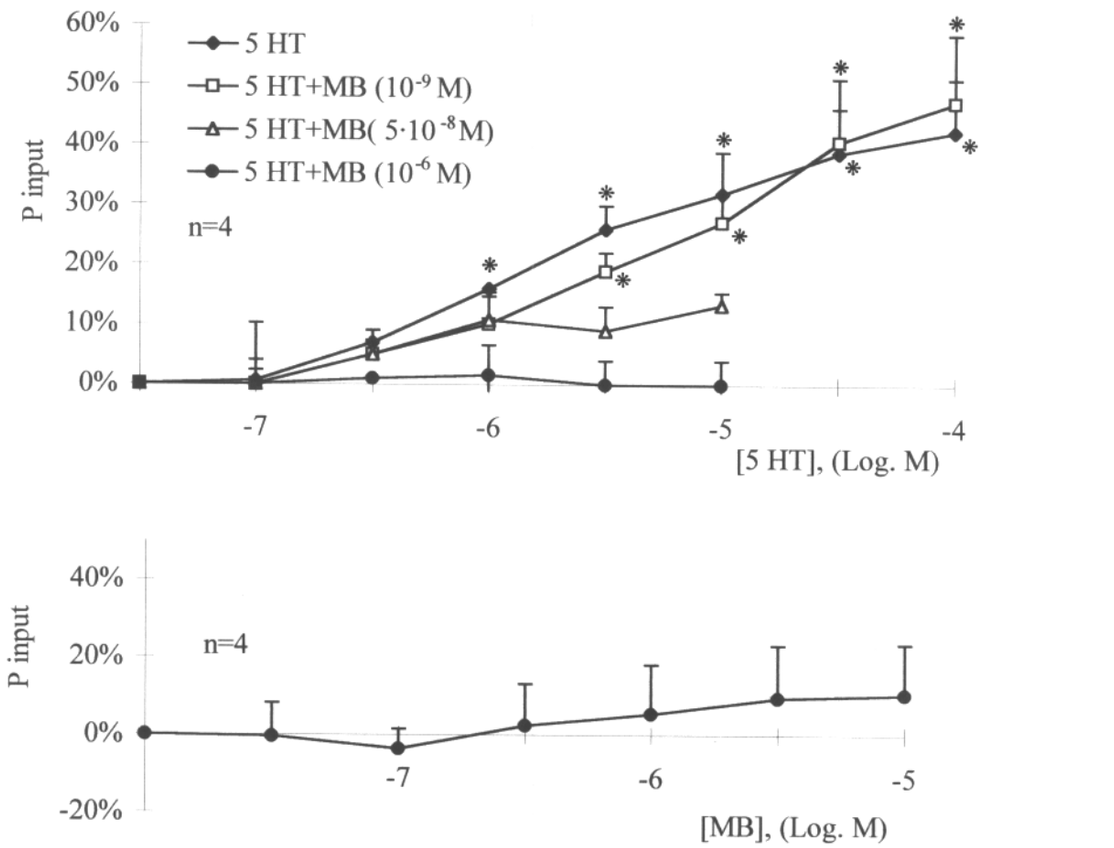

Fig. 2. Effects of 5-HT on the input pressure before and after treatment with an inhibitor of guanylate cyclase, methylen blue. The response to methylen blue per se is indicated in the lower panel; (*=$p<0.05$).

containing bubbled (0.5% CO_2) saline whith monitored pH. The external media was mixed by a stirrer to ensure saline flow over the lamellae and to avoid formation of dead spaces. The preparation was perfused with a pulsatile flow using a peristaltic pump and a compliant system (syringe) to ensure a differential input pressure of about 5-7 cm H_2O. The outflow was determined by collecting the total effluent from the experimental chamber and adjusted to the values of cardiac output previously reported in the European eel (18-20 $ml.min^{-1}.kg^{-1}$, Janvier *et al.* 1996). Input pressure was monitored with a Harvard pressure transducer and displayed on a chart recorder. Experiments started when input pressure was stabilized (usually 20-30 min). We used the following Ringer ($g.L^{-1}$): NaCl 6.68, $NaHCO_3$ 2.20, Na_2HPO_4 0.227, KCl 0.15, KH_2PO_4 0.05, $MgSO_4$ 0.35, $(NH_4)2SO_4$ 0.05, glucose 1.00, $CaCl_2$ 0.14.

The vascular responses, evaluated as means ± SEM of input pressure changes (absolute values), using a Student's *t* -test ($p<0.05$), are represented as percent of control values.

The drugs used were serotonin creatinine sulfate complex (5-HT), methysergide maleate, acetylcholine chloride, atropine sulfate, pirenzepine dihydrochloride, sodium nitroprusside (SNP), N_W-nitro-L-arginine methyl ester hydrochloride (L-NAME), methylen blue (MB), heparin sodium salt. They were dissolved in physiological saline just prior to experiments.

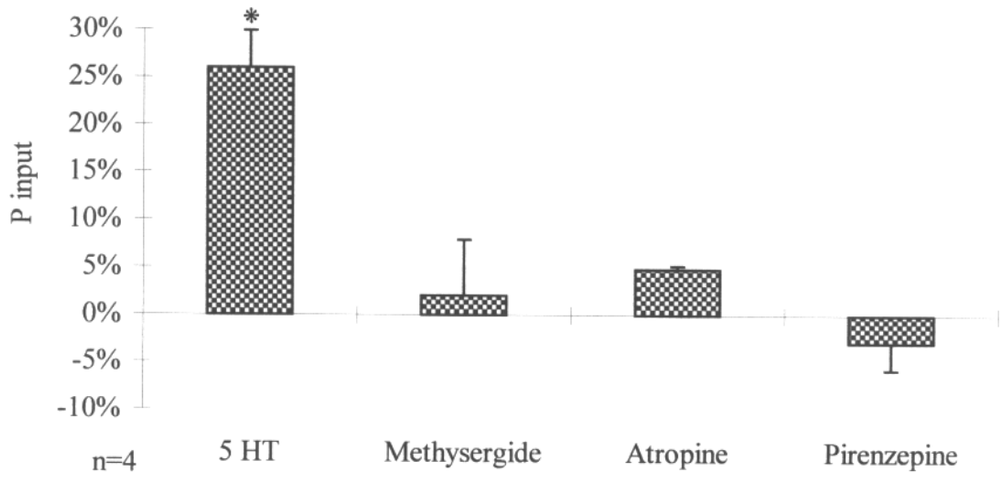

Fig. 3. Effects of methysergide (10^{-5} M), atropine (10^{-5} M) or pirenzepine (10^{-5} M) on the vasomotor response to 5-HT (5.10^{-6} M) in gill; (*=$p<0.05$).

The whole gill basket vasoconstricted in a dose-dependent manner to exogenous 5-HT. This effect was inhibited by the 5-HT-receptor antagonist methysergide (Fig. 3), as previously described in vivo by Fritsche et al. (1992).

When pre-treated with the nitric oxide donor SNP (10^{-4} M), the serotonergic vasoconstriction was abolished. On the other hand, pre-treatment with the nitric oxide synthase (NOS) inhibitor L-NAME (10^{-4} M) greatly potentiated the 5-HT-induced vasoconstriction. Perfusion with L-NAME alone induced a dose-dependent vasoconstriction, consistent with a putative NO-basal tone (Fig. 1).

NO is known to stimulate guanylate cyclase, thereby increasing intracellular levels of cGMP. Accordingly, we tested the effects of the guanylate cyclase inhibitor MB. Methylen blue caused per se a slight, non-significant increase in P_{input}, while remarkably potentiated 5-HT-induced vasoconstriction at 5.10^{-8} and 10^{-6} M (Fig. 2). Taken together, these results indicate that in the eel NOS activity may participate in the basal tone of the branchial vasculature and can constitute part of the signal-transduction pathway interposed between the serotonergic receptors at the luminal endothelial surface and the smooth muscle effector.

In the last series of experiments, in which we confirmed the well known vasoconstrictory action of exogenous acetylcholine (10^{-8} to 10^{-4} M), it was found that either atropine (10^{-5} M) or the M1-M3-muscarinic receptor antagonist pirenzepine (10^{-5} M) greatly reduced the 5-HT-induced vasoconstriction (Fig. 3). This suggests that a cholinergic mechanism could partly account for the serotonergic control of gill vascular resistance in eel.

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