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VASODILATION OF SWIMBLADDER VESSELS IN THE EUROPEAN EEL (*ANGUILLA ANGUILLA*) INDUCED BY VASOACTIVE INTESTINAL POLYPEPTIDE, NITRIC OXIDE, ADENOSINE AND PROTONS

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Summary

The effects of β-adrenergic stimulation, vasoactive intestinal polypeptide (VIP), adenosine, the nitric oxide (NO)-releasing agent sodium nitroprusside and of metabolic end-products of gas gland cell metabolism on swimbladder blood flow were investigated using saline- or blood-perfused swimbladder preparations of the freshwater European eel *Anguilla anguilla*. While β-adrenergic vasodilation was not detectable, a bolus injection of adenosine (100 μl, 10^{-7} mol l^{-1}) and application of VIP (10^{-7} mol kg^{-1}) caused a significant decrease in perfusion pressure in saline-perfused swimbladder preparations. Immunohistochemical analysis revealed the presence of VIP-immunoreactive nerve fibres in the swimbladder artery and in the swimbladder vein (seawater-adapted eels were used for immunohistochemical studies). Application of sodium nitroprusside also elicited a small, but significant, decrease in perfusion pressure in saline-perfused swimbladder preparations, while preincubation of swimbladder tissue with N^{ω}nitro-L-arginine, a non-selective inhibitor of nitric oxide synthase, significantly enhanced the flow-induced increase in perfusion pressure. Lactate, the major metabolic end-product of gas gland cell metabolism, had no effect on perfusion pressure. In contrast, an increase in proton concentration in both saline- and blood-perfused preparations induced a vasodilation, as indicated by a significant decrease in perfusion pressure. The results demonstrate that VIP, NO, adenosine and protons may induce a vasodilation of swimbladder blood vessels. None of these effects, however, compares in time span with the previously described immediate, short-lasting vasodilation of swimbladder vessels elicited by pulse stimulation of the vagus nerve.

Key words: vasoactive intestinal polypeptide, nitric oxide, adenosine, H^+, vasodilation, swimbladder, eel, *Anguilla anguilla*.

Introduction

Teleosts using a gas-filled swimbladder as a hydrostatic organ have evolved a sophisticated control system to regulate gas reabsorption and gas deposition to keep the swimbladder volume constant despite changes in hydrostatic pressure encountered during vertical migrations. Afferent information is apparently provided by stretch receptors in the swimbladder wall or by effects on the balance organs (Qutob, 1962; Tytler and Blaxter, 1973; Fänge, 1983). The efferent part of the system consists of nervous control of the relative surface areas of the resorbent and the secretory parts of the swimbladder wall (Fänge, 1983; Nilsson, 1983), control of blood flow through the swimbladder and regulation of metabolic activity of swimbladder gas gland cells (Pelster and Scheid, 1992).

Evidence for the importance of blood flow for swimbladder function was initially indirect. Bohr (1894) reported that vagotomy resulted in a decrease in the rate of gas deposition, and Hall (1924) and Fänge (1953) observed that swimbladder blood vessels became more dilated during periods of gas deposition. Concomitant measurements of blood flow and gas deposition in the swimbladder of the European eel indeed proved that there is a significant correlation between these two parameters (Pelster and Scheid, 1992).

Studies analyzing the regulation of swimbladder perfusion mainly resulted in a description of mechanisms causing a constriction of swimbladder blood vessels. Electrical stimulation of the splanchnic nerve or the vagosympathetic trunk resulted in a vasoconstriction (Nilsson, 1972; Wahlqvist, 1985). McLean and Nilsson (1981) reported that both nerves stain for catecholamines. Humoral application of adrenaline in saline-perfused swimbladder preparations, and also *in situ*, provoked a vasoconstriction mediated by α-adrenergic receptors (Stray-Pedersen, 1970; Nilsson, 1972; Wahlqvist, 1985; Pelster, 1994). These results suggest that, in a stressful situation, when catecholamines are released from chromaffin tissues, swimbladder perfusion is largely reduced as a result of α-adrenergic vasoconstriction to the benefit of other tissues.

Given the possibility of reducing swimbladder blood flow, there should also be a way to increase blood flow in situations where gas deposition is required. Information about mechanisms resulting in a vasodilation of swimbladder vessels is scarce. Only recently, Schwerte et al. (1997) reported that a vagal tonus controls the resistance of the swimbladder circulation and may provoke a significant vasodilation, while the absence of this tonus results in a shut-down of swimbladder perfusion. This effect, however, could be blocked by neither cholinergic nor adrenergic antagonists, indicating that other mediators must contribute to the control of swimbladder blood flow. In the cod *Gadus morhua*, vasoactive intestinal polypeptide (VIP) also induced a vasodilation of swimbladder vessels (Lundin and Holmgren, 1984), but application of VIP did not stimulate gas deposition (Lundin and Holmgren, 1991), as one would expect from the positive correlation between blood flow and gas deposition (Pelster and Scheid, 1992).

The present study therefore set out to identify possible agonists causing a vasodilation of eel swimbladder vessels. The addition of VIP, NO, adenosine and an increase in proton concentration all provoked a decrease in vascular resistance in the swimbladder vessels and thus in an increase in swimbladder blood flow.

Materials and methods

Specimens of the European eel *Anguilla anguilla* L. (body mass 350–500 g) were obtained from a local supplier and kept either in fresh water (for physiological studies; performed at the Zoology Department in Innsbruck) or in 30 ‰ sea water (for immunohistochemistry; performed at the Department of Zoophysiology in Göteborg) at 12 °C until used in the experiments. All experiments were performed at room temperature (20–22 °C).

Animal preparations and perfusion experiments

Anaesthetized animals were quickly immobilized by penetrating the skull with a fine needle and spinal pithing. The animals were placed into an eel-holder (Schwerte et al., 1997), and the gills were irrigated with well-aerated water (20–22 °C) at a constant flow rate of approximately 1.5–2 l min^{-1}. The body wall was opened ventrally. The swimbladder was carefully exposed and freed of connective tissue. Blood vessels from other tissues entering the vein leaving the retia were ligated.

Artificially perfused swimbladder preparations were obtained by occlusive cannulation of the swimbladder artery (PE 20 cannula) and of the swimbladder vein (PE 50). Catheter tubing was heparinized with 100 i.u. ml^{-1} saline. The arterial catheter was connected to a pressure transducer (Gould, Statham, BD 23 ID) and to a peristaltic pump. The swimbladder was perfused with saline of the following composition (concentrations in mmol l^{-1}): NaCl, 135; KCl, 5.1; $CaCl_2$, 1.1; $MgSO_4$, 0.93; $NaHCO_3$, 10; glucose, 5, at pH 7.8. Depending on the dimensions of the swimbladder, the perfusion rate was adjusted to a flow rate of 0.1–0.4 ml min^{-1} so that the perfusion pressure was in the range 2.5–3.5 kPa, which is the pressure range previously recorded in the swimbladder artery of the European eel (Pelster, 1994). For blood perfusion studies, the dorsal artery was non-occlusively cannulated (PE 50). Blood was drawn from the dorsal artery and infused into the swimbladder artery at a constant flow rate (see Pelster, 1994). The pressure transducer was calibrated using a static water column. The pressure signal was continuously recorded on a computer system at a sampling rate of 20 Hz and using the software package LabView (National Instruments, Vienna).

Agonist and antagonist applications

The possible existence of β-adrenergic vasodilation was tested by adding adrenaline (E4375, Sigma Chemical Company), isoproterenol (I5627, Sigma) and/or the β-adrenergic anatagonist propranolol (P0884, Sigma) to the saline solution described above. The influence of vasoactive intestinal polypeptide (VIP) on swimbladder blood flow was tested using commercially available VIP (V6130, Sigma). The effect of NO on swimbladder perfusion was tested by application of the artificial NO donor sodium nitroprusside (S0501, Sigma). In addition, swimbladder preparations were preincubated with N^{ω}nitro-L-arginine (L-NNA; N5501, Sigma), an irreversible inhibitor of the neuronal NO synthase (nNOS) and a competitive inhibitor of the inducible NO synthase (iNOS). Adenosine (A9251, Sigma) was applied as a bolus injection into the catheter loop (100 µl, 10^{-7} mol l^{-1}), and theophylline (10^{-4} mol kg^{-1}; T1633, Sigma) was used as a purinoceptor antagonist. All solutions containing agonists and/or antagonists were freshly prepared prior to use, and the pH was carefully adjusted to 7.8.

Immunohistochemistry

For immunohistochemical analysis, small sections of swimbladder vessels (whole mounts) were dissected from various locations in the vascular bed and fixed in 4 % paraformaldehyde in 0.1 mol l^{-1} phosphate-buffered saline (PBS). The tissue sections were preincubated with normal donkey serum (Jackson, West Grove, USA, 1:10) for 1 h, followed by an incubation for single labelling with the primary antisera listed in Table 1 in a moist chamber at room temperature for 20–72 h. The preparations were rinsed in PBS for up to 20 h with three changes of saline (for the thicker whole mounts) and incubated with species-specific secondary antibodies for 2–20 h (depending on the thickness of the tissue section). The secondary antibodies (affinity-purified donkey IgG; Jackson, West Grove, USA) were conjugated to dichlorotriazinyl aminofluorescein (DTAF, 1:100), Cy3 (indocarbocyanine, 1:800) or Texas Red (1:100). All antibodies were diluted in hypertonic PBS to reduce non-specific binding to the tissue sections (see Grube, 1980). The preparations were once again rinsed three times in PBS before being mounted in carbonate-buffered glycerol, pH 8.6.

The specificity of the immunoreactivity was tested by preabsorptions with the respective antigens for the antiserum

Table 1. *Primary antibodies used in the study*

Antibodies raised against	Code/Source	Raised in	Dilution	Reference
NO synthase	N31030/Affinity	Rabbit	1:100	Karila and Holmgren (1997)
VIP	B34-1/Milab	Rabbit	1:1000	Olsson and Holmgren (1994)
VIP	B-GP 340-X/ Euro-Diagnostica	Guinea pig	1:400	Olsson and Holmgren (1994)

VIP, vasoactive intestinal polypeptide.

not previously used in fish species (see Table 1). No inappropriate binding was detected. Because we employed antibodies raised against mammalian antigens, we use the terms VIP-like immunoreactivity or NOS-like immunoreactivity when referring to the immunohistochemical localization of the neuropeptides.

Data analysis

Statistically significant differences were evaluated using a one-way analysis of variance (ANOVA), followed by a multiple-comparison procedure (Bonferroni, SigmaStat). Significance was accepted when $P<0.05$. Data are presented as means ± S.E.M.

Results

A classical pathway inducing vasodilation is β-adrenergic stimulation. In saline-perfused swimbladder preparations, the presence of 10^{-7} mol kg^{-1} isoproterenol, however, did not reduce perfusion pressure (Fig. 1). Adrenaline at a concentration of 10^{-8} mol kg^{-1} provoked a significant increase in perfusion pressure, and similar results were obtained during adrenaline application in the presence of the β-adrenergic inhibitor propranolol (6×10^{-8} mol kg^{-1}; Fig. 1).

Vasoactive intestinal polypeptide at a concentration of 10^{-7} mol kg^{-1} induced a small, but significant, decrease in perfusion pressure (Fig. 2). Starting at a perfusion pressure of 2.6±0.5 kPa, addition of VIP reduced the perfusion pressure by approximately 0.5 kPa (N=5) within 5 min.

Immunohistochemical staining for VIP revealed the presence of VIP-like immunoreactivity in nerve fibres innervating the swimbladder artery (Fig. 3A) and the swimbladder vein (Fig. 3B). VIP-reactive nerve fibres were also found in the initial branches of the rete mirabile and parallel to blood vessels on the secretory part of the swimbladder (Table 2).

The influence of nitric oxide on swimbladder blood flow was tested in swimbladder preparations perfused with saline at a perfusion pressure of 2.5±0.2 kPa. Addition of the NO donor sodium nitroprusside (10^{-7} mol kg^{-1}) to the perfusate reduced the perfusion pressure to 2.1±0.25 kPa (N=6) (Fig. 4). In the endothelium and in nerves, NO is produced from arginine by the action of nitric oxide synthase (NOS), and N^{ω}nitro-L-arginine (L-NNA) can be used as a competitive inhibitor of this enzyme. *In vivo*, shear stress appears to be important for the production of NO. The influence of L-NNA

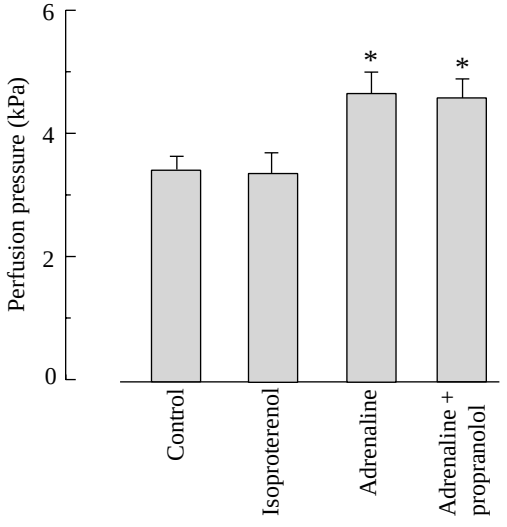

Fig. 1. Perfusion pressure in saline-perfused swimbladder preparations under control conditions and following application of isoproterenol (10^{-7} mol kg^{-1}), adrenaline (10^{-8} mol kg^{-1}) or adrenaline (10^{-8} mol kg^{-1}) in the presence of propranolol (6×10^{-8} mol kg^{-1}). An asterisk indicates a significant difference from the control value; values are means + S.E.M., N=6.

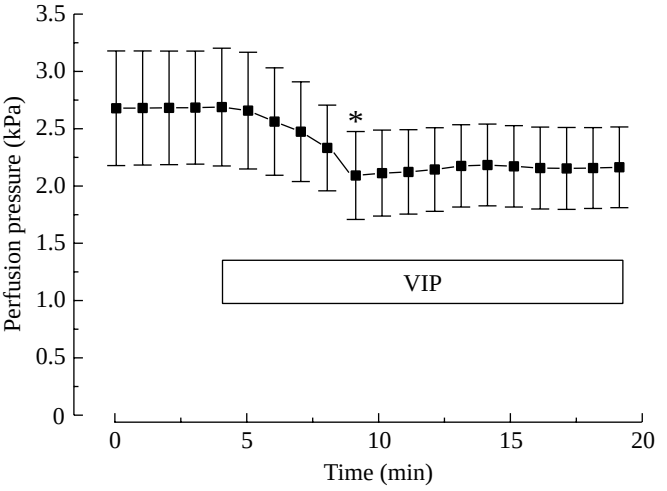

Fig. 2. Changes in perfusion pressure in saline-perfused swimbladder preparations following application of vasoactive intestinal polypeptide (VIP; 10^{-7} mol kg^{-1}). The asterisk indicates the first value significantly different from the control value; values are means ± S.E.M., N=5.

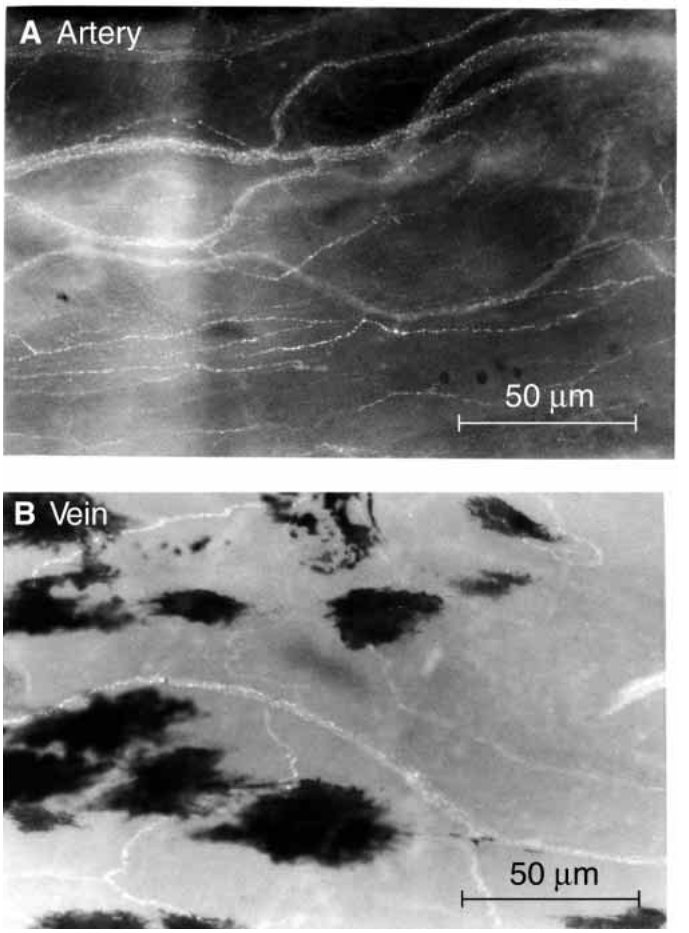

Fig. 3. Using specific antibodies raised against vasoactive intestinal polypeptide (VIP), the presence of VIP-like immunoreactivity was detected in nerve fibres innervating the swimbladder artery (A) and in the swimbladder vein (B). VIP-like immunoreactivity was also found in the initial branches of the rete mirabile and parallel to blood vessels on the secretory part of the swimbladder.

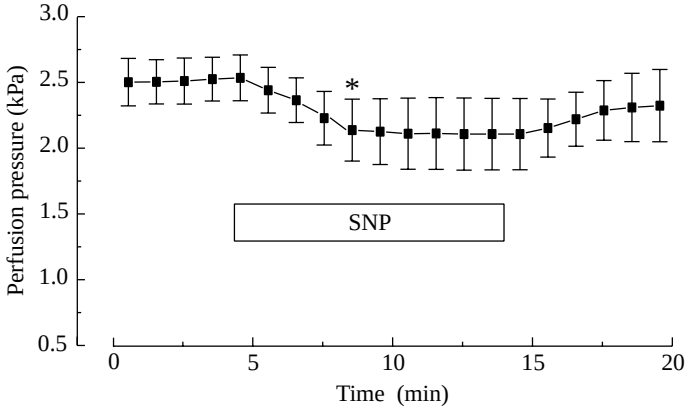

Fig. 4. Changes in perfusion pressure in saline-perfused swimbladder preparations following application of the artificial NO donor sodium nitroprusside (SNP; 10^{-7} mol kg^{-1}). The asterisk indicates the first value significantly different from the control value; values are means ± S.E.M., *N*=6.

Table 2. *VIP-like immunoreactivity and NOS-like immunoreactivity at sites I–VI in the swimbladder tissue*

Antiserum	I	II	III	IV	V	VI
VIP	+	+	+	(+)	+	+
NOS		+		+		

I, swimbladder artery and vein; II, arterial vessels at the entrance to the rete mirabile; III, arterial vessels at the exit from the rete mirabile; IV, large vessels on the secretory part of the swimbladder; V, epithelium of the secretory part of the swimbladder; VI, resorbing part of the swimbladder; (+), immunoreactivity was not found in all preparations analyzed.

VIP, vasoactive intestinal polypeptide (*N*=5); NOS, nitric oxide synthase (*N*=2).

was therefore tested in a dynamic preparation, in which the increase in perfusion pressure induced by an increase in perfusion rate from 0.1 to 0.2 ml min^{-1} was compared with and without preincubation with L-NNA (10^{-6} mol kg^{-1}). The results clearly show that, after preincubation of the swimbladder with L-NNA for 2 h, the flow-induced increase in perfusion pressure was significantly larger than under control conditions (Fig. 5).

Immunohistochemical analysis using specific antibodies against NO synthase (NOS) revealed weak NOS-like immunoreactivity in arterial vessels at the entrance to the rete mirabile and occasionally in arterial vessels distal to the rete mirabile (Fig. 6). Because of the low reactivity, however, a complete identification of the structures or cell types (e.g. nerve, endothelium) that showed a positive response was not possible, but the pictures reveal no indication of a staining of

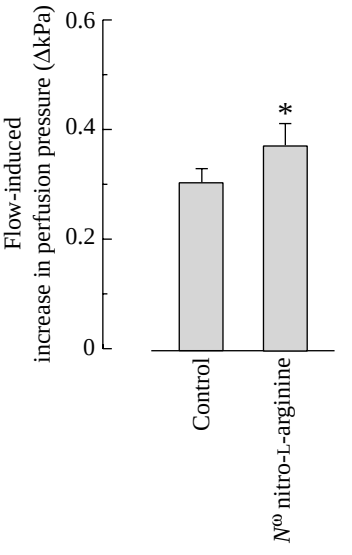

Fig. 5. Changes in perfusion pressure induced by an increase in perfusion rate from 0.1 to 0.2 ml min^{-1} in saline-perfused swimbladder preparations with and without preincubation of the tissue with the competitive inhibitor of nitric oxide synthase N^{ω}nitro-L-arginine (L-NNA; 10^{-6} mol kg^{-1}). An asterisk indicates a significant difference from the control value; values are means + S.E.M.; *N*=5.

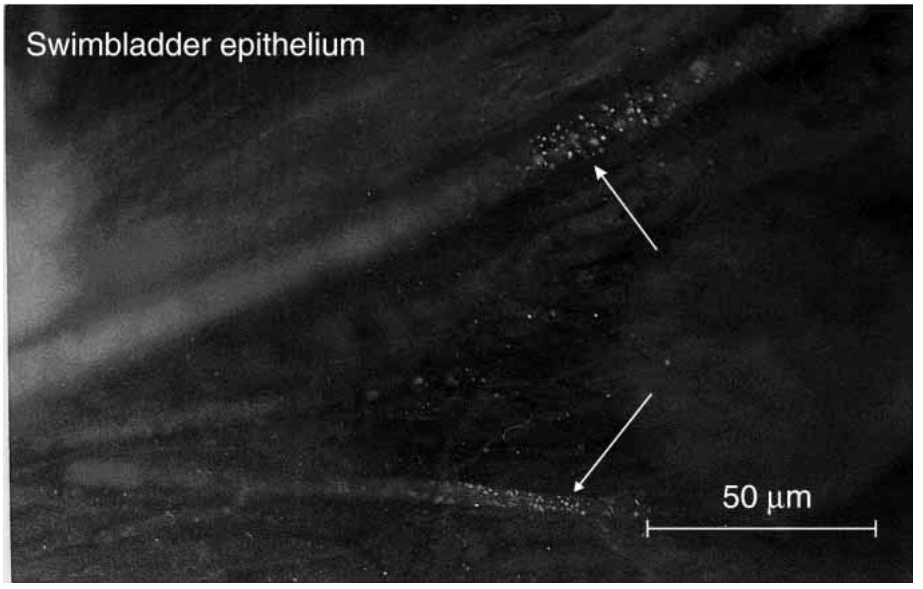

Fig. 6. Using specific antibodies against nitric oxide synthase (NOS), NOS-like immunoreactivity (arrows) was detected in arterial vessels distal to the rete mirabile. The low intensity of the staining precludes a complete identification of the structures, but the immunoreactivity does not appear to be located in nerve fibres.

nerve terminals. No NOS-like immunoreactivity was found in venous vessels (Table 2).

Adenosine applied as a bolus injection into the perfusion loop (100 μl of a stock solution, 10^{-7} mol l^{-1}) induced a rapid decrease in perfusion pressure of approximately 14 %, which then slowly returned to control levels (Fig. 7). After perfusing the swimbladder tissue with saline containing theophylline (10^{-4} mol kg^{-1}) for 30 min prior to the application of adenosine, the effect of adenosine was completely abolished (data not shown).

Lactic acid is the major end-product of gas gland cell metabolism and is released into the bloodstream, but 20 mmol l^{-1} lactate had no effect on perfusion pressure in a saline-perfused swimbladder preparation (data not shown).

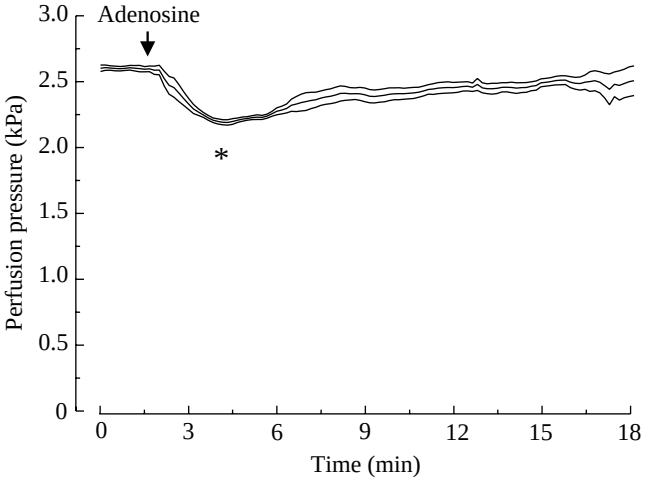

Fig. 7. Changes in perfusion pressure in saline-perfused swimbladder preparations following a bolus injection of adenosine (100 μl of a stock solution, 10^{-7} mol l^{-1}). Thick line, mean value; thin lines, standard error of the mean. An asterisk indicates a significant difference from the control value; *N*=6.

Protons, however, significantly reduced the perfusion pressure. In saline-perfused swimbladder preparations, acidification of the saline from pH 8.0 to pH 6.5 reduced the perfusion pressure by approximately 20 % (Fig. 8A). After a return to the alkaline pH, the perfusion pressure increased again to the original level, indicating that this effect was reversible. The perfusion pressure was higher in blood-perfused swimbladder preparations than in saline-perfused preparations because of the higher viscosity of the blood; nevertheless, qualitatively similar results were obtained on acidification. In the control period with air-equilibrated blood, blood pH was 7.62±0.22 and perfusion pressure was 3.47±0.16 kPa (*N*=6). For acidification, 8 % CO_2 was added to the equilibrating air, so that the pH dropped to 7.31±0.09. This decrease in pH significantly reduced the perfusion pressure by approximately 25 %. As in the saline-perfused preparations, the effect was fully reversible. After a return to air-equilibrated blood and a pH of 7.58±0.19, perfusion pressure also returned to the control level (Fig. 8B).

Discussion

The results show that swimbladder blood flow can be increased by VIP, NO, adenosine and an increase in proton concentration, while lactate concentration and β-adrenergic stimulation have no effect.

β-*Adrenergic stimulation*

While the presence of an α-adrenergic vasoconstriction close to the rete mirabile is well documented (Pelster, 1994), evidence for the presence of β-adrenergic receptors is not conclusive. The co-existence of α- and β-adrenergic receptors is common among vertebrates, and this is also true for fish (Nilsson, 1984). Sundin and Nilsson (1992) nicely demonstrated that adrenaline may provoke vasoconstriction as well as vasodilation in the gills of Atlantic cod *Gadus*

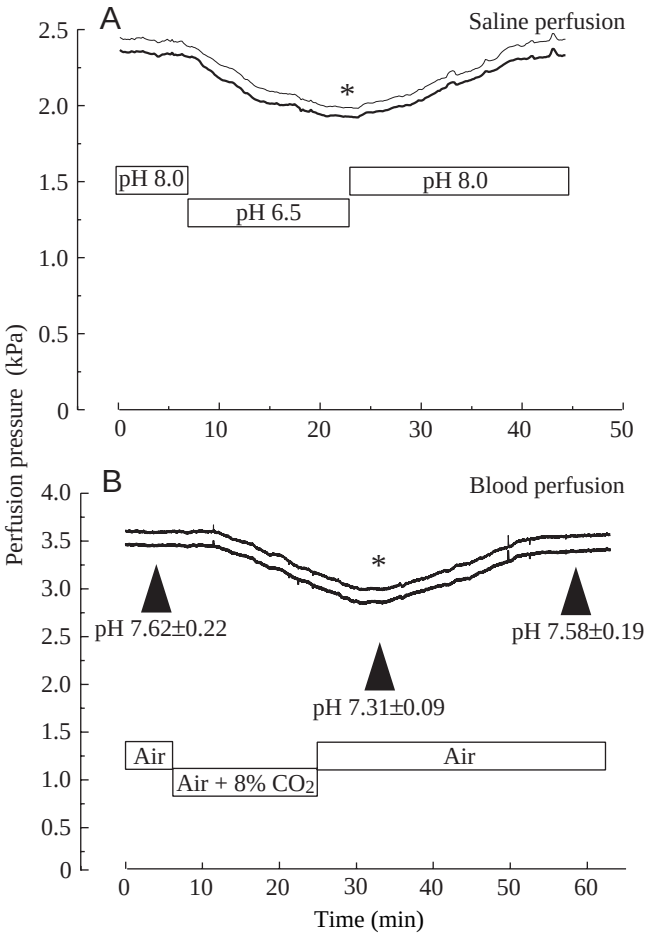

Fig. 8. (A) Perfusion pressure measured in the swimbladder artery of a saline-perfused swimbladder preparation while the pH of the perfusate was reduced from pH 8.0 to pH 6.5 and during the subsequent recovery period. (B) Perfusion pressure measured in the swimbladder artery of a blood-perfused swimbladder preparation while the pH of the blood was reduced from pH 7.62 to pH 7.31 and during the subsequent recovery period. Thick line, mean value; thin line, standard error of the mean. An asterisk indicates a significant difference from the control value; *N*=6.

morhua. Following adrenergic stimulation, Pelster (1994) observed a slight initial increase in swimbladder blood flow in the European eel prior to a profound α-adrenergic vasoconstriction, causing a reduced blood flow. At the same time, however, cardiac output was elevated, and an increase in cardiac output following adrenergic stimulation has also been demonstrated in the Australian eel *Anguilla australis* (Hipkins, 1985) and in the American eel *Anguilla rostrata* (Oudit and Butler, 1995). The results of the present study using specific β-adrenergic stimulation as well as a specific β-adrenergic antagonist did not provide any indication of a β-adrenergic vasodilation in eel swimbladder blood vessels. It must therefore be concluded that the initial increase in swimbladder blood flow following adrenergic stimulation is a passive effect induced by an increase in cardiac output.

Vasoactive intestinal polypeptide

Although VIP has been shown to increase vascular resistance in spiny dogfish *Squalus acanthias* (Holmgren et al., 1992), in the teleosts analyzed so far it provoked a vasodilation, as shown, for example, for small arteries of the proximal intestine region of rainbow trout *Oncorhynchus mykiss* (Kågström and Holmgren, 1997). In that study, the VIP-induced vasodilation was not dependent on the presence of the endothelium and did not include NOS activity, while in some mammalian vessels the effect is dependent on the presence of an intact endothelium.

The small VIP-induced (19 %) vasodilation observed in the swimbladder vessels in the present study confirms the results of Lundin and Holmgren (1984), who reported an increase in perfusion rate in the cod swimbladder following application of VIP. Similarly, the presence of nerve fibres showing VIP-like immunoreactivity, especially in the swimbladder artery, is in accord with the results obtained for the cod (Lundin and Holmgren, 1984). Most of the VIP-immunoreactive nerve fibres detected were associated with vessels such as arterial vessels close to the rete mirabile and the swimbladder vein. Positive staining in the secretory part of the swimbladder, however, could not be attributed to perivascular fibres of blood vessels. It therefore appears quite possible that these nerve fibres were located in the muscularis mucosa, where the presence of VIP-like immunoreactivity has been established by Lundin and Holmgren (1984). VIP-like immunoreactivity thus appears to be localized particularly to arterial, but also to venous, vessels anterior to the swimbladder, and VIP could therefore contribute to a vasodilation.

The vasodilation induced by VIP is not pronounced and appears not to be of importance for rapid and major changes in blood flow; however, it should increase swimbladder blood flow and thus enhance gas secretion into the swimbladder (Pelster and Scheid, 1992). The question of why VIP application decreased the rate of gas deposition in the study of Lundin and Holmgren (1991) remains unresolved.

Nitric oxide

Another potent vasodilator produced from the endothelium or from nerves is NO, and evidence has recently been presented that NO may decrease arteriolar resistance in trout *Oncorhynchus mykiss* (McGeer and Eddy, 1996; Olson et al., 1997), although this appears not to apply to all arteries. Olson and Villa (1991) reported that trout endothelium does not release NO, and Kågström and Holmgren (1997) could not detect NOS immunoreactivity in small arteries in the intestine of rainbow trout. Because of the instability of NO, sodium nitroprusside is often used experimentally as an artificial NO donor, and application of nitroprusside induced a vasodilation of swimbladder vessels. While this result indicates the presence of the receptor for NO-mediated vasodilation, i.e. soluble guanylate cyclase, it does not necessarily imply that the reaction is of physiological relevance. However, the additional experiments showing that unselective blockade of NOS activity with L-NNA resulted in a significantly larger increase

in perfusion pressure in response to an increase in perfusion rate suggest that, at least in a situation of increased shear stress, NO synthase activity does contribute to a vascular relaxation. A further characterization of the NOS isoform was beyond the scope of the present study. Preliminary observations suggested that subsequent application of arginine reduced the effect of L-NNA, which would indicate that the inducible isoform (iNOS) is present.

Furthermore, NOS-like immunoreactivity in the vicinity of the swimbladder artery and of larger arterioles on the secretory part of the swimbladder was demonstrated immunohistochemically. Taken together, these results suggest that NO may induce a vasodilation in the swimbladder vasculature of the eel and that this is a physiological response.

Adenosine

In recent years, it has been demonstrated that purine derivatives occur in the extracellular space and may have a signalling function (Burnstock, 1972; Gordon, 1986; Mione et al., 1990). Prominent examples for the modification of blood flow by adenosine are a hypoxia-induced stimulation of brain blood flow (Hudetz, 1997; Lutz and Nilsson, 1997) and the modification of cardiac activity (see Nilsson and Holmgren, 1992). In fish, the effect of adenosine on the systemic vasculature appears to be quite variable, ranging from vasodilation in hagfish (*Myxine glutinosa*) gills (Axelsson et al., 1990) to vasoconstriction in the gills of *Oreochromis niloticus* (Okafor and Oduleye, 1986). In the eel swimbladder, adenosine induced a rapid and reversible vasodilation, which was mediated by purinoceptors, as demonstrated by the inhibitory effect of theophylline. No information, however, is available on the possible presence of adenosine in the extracellular space in swimbladder tissue. Hypoxia-induced release of purines from swimbladder tissue appears to be most unlikely, because this tissue is typically in a hyperoxic state (Pelster, 1997). It also is possible that adenosine does not affect vascular smooth muscle cells directly, but induces a release of NO (endothelium-derived relaxing factor, EDRF) from the vascular endothelium (Nilsson and Holmgren, 1992).

Metabolites

The metabolism of gas gland cells produces and releases CO_2 and lactic acid (Pelster, 1995, 1997), and both protons and lactate have been shown to be vasoactive in various tissues (Tian et al., 1995; McKinnon et al., 1996; Aalkjaer and Poston, 1996). While lactate at a concentration of 20 mmol l^{-1} had no effect on perfusion pressure, an acidification clearly induced a vasodilation. The highest lactate levels recorded in swimbladder blood so far are in the range 10–20 mmol l^{-1} (see Pelster, 1995). In the physiological concentration range, therefore, lactate has no regulatory effect on swimbladder blood flow. Increased proton concentrations, however, induce a vasodilation, and a relaxation of vascular smooth muscle induced by an extracellular acidosis is a common observation (Tian et al., 1995). In the swimbladder, the acidification of blood therefore appears to be of dual importance: acidification reduces the gas-carrying capacity of the blood and thus induces an increase in gas partial pressure, but it also provokes a vasodilation and thus stimulates blood flow through the swimbladder.

Physiological significance

The results of the present study reveal vasodilatory effects of VIP, NO and adenosine. In a recent study (Schwerte et al., 1997), we were able to demonstrate that the tonus of the vagus nerve is crucial for swimbladder blood flow, and that vagotomy or stimulation of the vagus nerve provokes marked changes in swimbladder perfusion. The action of the vagus nerve, however, apparently did not involve the classical α- and β-adrenergic receptor or the muscarinic cholinoceptor functions. The reduction in perfusion pressure observed in the present study following application of VIP, nitroprusside or adenosine was comparable with, but much slower than, the reduction in perfusion pressure induced by vagal stimulation (Schwerte et al., 1997). The maximum effect of vagal stimulation occurred after approximately 30 s, while the maximum response in the present study was only observed several minutes after application of the agonist. Furthermore, a bolus injection of VIP or sodium nitroprusside in an *in vivo* preparation did not induce a significant increase in swimbladder blood flow (T. Schwerte, unpublished results). The results of the present study therefore provide no conclusive evidence that VIP, NO or adenosine is responsible for the effect of vagal activity, but the possibility that a synergistic effect of various components is involved cannot be excluded.

The results of the present study, together with our previous studies on the importance of the vagus nerve (Schwerte et al., 1997) and on adrenergic control (Pelster, 1994), suggest that the most dominant components controlling swimbladder perfusion in the eel are the vagus tonus and a pronounced α-adrenergic vasoconstriction. In a stressful situation, when adrenaline is released, swimbladder blood flow is severely reduced to the benefit of other organs as a result of α-adrenergic stimulation of resistance vessels at the entrance to the rete mirabile, supported by a reduction in the vagal tone. This will essentially shut down gas deposition into the swimbladder (Pelster and Scheid, 1992). Reactivation of gas deposition is probably induced by an increase in the vagal tonus, which may also stimulate the metabolic activity of gas gland cells (Fänge et al., 1976; McLean and Nilsson, 1981). VIP and NO may enhance vasodilation.

The onset of acid release by the gas gland cells will cause a relaxation of vascular smooth muscle and therefore further enhance blood flow to the swimbladder. It appears, therefore, that control of blood flow and control of gas gland cell metabolism are achieved by the combined action of nervous and humoral control pathways. Furthermore, a modification of one parameter will always occur in conjunction with a modification of the other, and this is related to the fact that gas gland cell metabolism and blood flow both appear to be under vagal control. A decrease in blood flow, for example, will also decrease glucose supply to the gas gland cells, and acid

production by these cells largely depends on extracellular glucose (Pelster and Niederstätter, 1997). A decrease in acid secretion, however, will result in an alkalization of the blood and thus cause a constriction of vascular smooth muscle (see Fig. 8), which in turn enhances the decrease in blood flow. An increase in swimbladder blood flow will have exactly the opposite effect.

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