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SHORT COMMUNICATION

EVIDENCE OF REGULATORY MECHANISMS FOR THE DISTRIBUTION OF BLOOD BETWEEN THE ARTERIAL AND THE VENOUS COMPARTMENTS IN THE HAGFISH GILL POUCH

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Of the extant vertebrate animals, the hagfishes are generally considered to be the group which diverged first from the ancestral vertebrate lineage, although molecular sequence analysis has recently suggested that they form a monophyletic group with lampreys (Stock and Whitt, 1992). The circulatory system of hagfishes has features that have been described as 'primitive' (Burggren *et al.* 1985), but their gills are effective gas exchangers. The gills are contained within discrete muscular pouches, and the anatomy of the blood system and ventilatory ducts has an ideal countercurrent arrangement (Mallatt and Paulsen, 1986; Elger, 1987).

Reite (1969) first reported effects of catecholamines and other drugs on the branchial vasculature of hagfish. Recent studies of both perfused gills *in situ* and of blood flow *in vivo* have suggested that blood flow through the gills of hagfish is under tonic control by catecholamines (Axelsson *et al.* 1990; Forster *et al.* 1992). In teleosts, several studies have shown that adrenergic control mechanisms are involved in the distribution of blood between the arterio-arterial and the arterio-venous pathways of the gill vasculature (see Nilsson, 1983). Anatomical and ultrastructural studies have demonstrated the existence of similar pathways in hagfish (Cole, 1925; Mallatt and Paulsen, 1986; Elger, 1987). The experiments reported here demonstrate that, in the hagfish gill pouch, both adrenaline and isoprenaline can increase the proportion of fluid leaving *via* the efferent arterial route, at the expense of the venous outflow.

Hagfish (*Eptatretus cirrhatus* Forster) were collected off Motunau, North Canterbury, New Zealand, and held in seawater aquaria until used. The masses of the 11 animals used in these experiments ranged from 680 to 1720 g with a mean of 1140±110 g (S.E.M.). Animals were anaesthetized in a 0.4 % solution of benzocaine in sea water. The hagfish

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were opened ventrally to expose the gills and their blood supply. Individual gill pouches were prepared for perfusion studies.

The afferent and efferent water ducts of the fourth, fifth or sixth gill pouch of either side were cannulated (Portex PP120). The cannulae were tied into place close to the insertions of the water ducts into the gill pouch. The afferent branchial artery was cannulated (Portex PP90) at its origin from the lateral ventral aorta. A cannula (Portex PP50) was introduced into the efferent branchial circulation through the lateral dorsal aorta and moved through an efferent branchial artery until it came to lie close to the gill pouch itself. Latex casts of the efferent branchial circulation revealed collateral vessels in some gill pouches. Once the efferent artery cannula had been tied into place, it was further secured by a ligature around the afferent water duct, which ensured that all the fluid leaving the efferent arterial system entered the cannula.

At the time of implantation, the arterial cannulae were filled with a heparinised physiological saline (Davie *et al.* 1987). The water duct cannulae were filled with sea water. Once cannulated, the gill pouch was removed from the animal and submerged in an organ bath filled with physiological saline. The afferent cannulae were connected to the pumps and perfusion was started. The venous outflow passed into the organ bath and overflowed through tubing to drip onto a strain gauge (Ugo Basile), which acted as a drop counter. The venous outflow was also logged on a computer interfaced with an electronic balance. The afterload on the efferent arterial cannula was set at 0.8–1.3 kPa. The outflow was dripped onto another strain gauge drop counter and the fluid was collected into vials and weighed. Previous experiments on isolated gill pouches had demonstrated that there was no leakage of dye from the sea water to blood compartments, indicating that the two compartments are functionally separated (M. E. Forster and J. C. Fenwick, in preparation).

A pneumatically driven syringe pump was used to perfuse the vascular pathway at flows adjusted according to the size of the gill pouch, but averaging $1\,\mathrm{ml\,min^{-1}}$. The saline was gassed with a mixture of air, CO_2 in N_2, and N_2 delivered by a Wöstoff pump. The gas mixture contained 6.3 % O_2 and 0.5 % CO_2. A peristaltic pump (One-Pump, Narco Systems) delivered aerated sea water to the afferent water duct at a constant flow rate of approximately $1.5\,\mathrm{ml\,min^{-1}}$. Pressures in the afferent cannulae were monitored using Bell & Howell pressure transducers and care was taken to ensure that hydrostatic pressures remained within the range of those recorded *in vivo* (Forster *et al.* 1988). The temperature of the bath containing the gill pouch was controlled using a waterbath and varied from 17 to 19 °C, which is equivalent to the summer seawater temperatures at Motunau.

For at least the first 10 min, the gill blood vessels were perfused with saline alone. When pressures and flows had stabilized, perfusion was switched to saline containing $10^{-8}\,\mathrm{mol\,l^{-1}}$ adrenaline or isoprenaline (L-isomers, Sigma Chemical Co., St Louis). The concentration of the drug was increased at 10 min intervals to a maximum of $10^{-4}\,\mathrm{mol\,l^{-1}}$. Each gill pouch was tested with a single drug. Branchial vascular resistance was calculated by dividing the pressure differential by the weight of fluid perfusing that route. Total vascular resistance R_g was calculated as $1/R_g=1/R_a+1/R_v$, where R_a and R_v are the resistances of the arterio-arterial and arterio-venous pathways respectively. The dose–response curves were generated from the values taken during the tenth minute of each treatment.

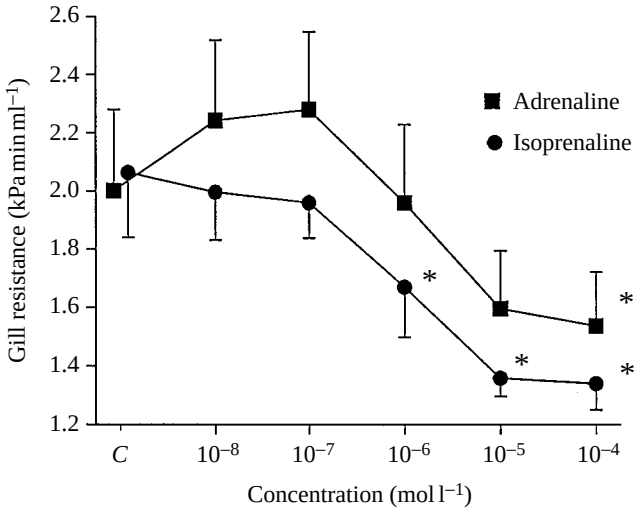

Fig. 1. The effects of adrenaline and isoprenaline on the vascular resistance of isolated gill pouches of the hagfish *Eptatretus cirrhatus*. Values are given as means ± S.E.M., N=9. * significantly different from the respective control value (P<0.05), using analysis of variance (ANOVA) and *post-hoc* Duncan's multiple-range test. *C*, control value.

Adrenaline at low concentrations had a pressor effect, but as its concentration was increased a depressor effect became dominant (Fig. 1). When a biphasic response was obtained, the pressor effect preceded the depressor effect. For example, when the gill pouch was exposed to an adrenaline concentration of 10^{-6} mol l^{-1}, afferent arterial pressures peaked at a mean value of 2.65±0.26 kPa, a 14.6 % increase from the pre-dose value (P<0.05, N=9). With continued exposure to this same concentration of adrenaline, a depressor effect became evident and within 10 min pressure fell to be little different from the initial control value. The β-adrenoceptor agonist isoprenaline had only a depressor effect (Fig. 1). We therefore assume that the pressor action of adrenaline was due to α-adrenergic vasoconstriction. Both catecholamines caused a switch of flow from the venous to arterial outflow (Fig. 2). In the absence of drugs, the flow of saline to the efferent artery decreased over time, and afferent arterial pressures rose. This explains the non-significant reduction in flow to the arterial route that is apparent at the lowest concentrations of adrenaline and isoprenaline tested (Fig. 2).

The actions of serotonin hydrochloride (5-hydroxytryptamine, Sigma) and cholecystokinin (CCK-8 sulphated, Cambridge Research Biomedicals) were tested at a single concentration. Both drugs significantly increased flow to the arterial route, although their actions on the branchial vasculature differed (Table 1). The biphasic response to serotonin at 10^{-6} mol l^{-1} was very similar to that of adrenaline at the same concentration, with an initial pressor response giving way to a fall in pressure resulting from a decreased vascular resistance by the tenth minute of exposure. A similar biphasic response was also noted by Reite (1969), who found that it could be abolished by α- and β-adrenoceptor blocking agents, but not by the serotonergic receptor antagonist

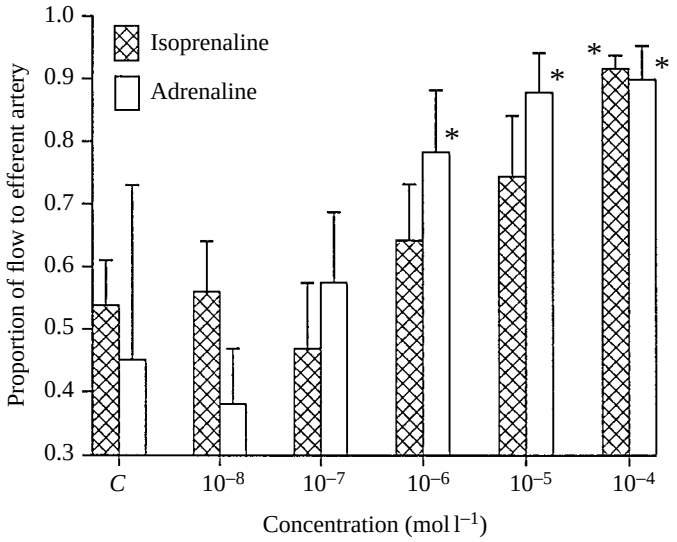

Fig. 2. The effects of adrenaline and isoprenaline on the proportion of the total vascular outflow from isolated hagfish gill pouches that leaves by the efferent branchial artery. The balance of the fluid is thought to leave by the venous route. * significantly different from the control value (P<0.05), using ANOVA and *post-hoc* Duncan's multiple-range test. Values given as means ± S.E.M., N=9. *C*, control value.

Table 1. *Effects of serotonin and cholecystokinin on gill pouch efferent flow distribution and vascular resistance*

	Gill pouch resistance (kPa min ml^{-1})	Proportion of flow to efferent artery
Control	2.28±0.28	0.409±0.092
Serotonin (10^{-6} mol l^{-1})	1.84±0.18*	0.656±0.074*
Control	2.03±0.20	0.593±0.090
Cholecystokinin (10^{-7} mol l^{-1})	2.26±0.22*	0.821±0.039*

*Significantly different from control value P<0.05, paired Student's *t*-test.
Values are mean ± S.E.M., N=9.

methysergide, suggesting that serotonin acted *via* adrenergic receptors. CCK at 10^{-7} mol l^{-1} (dissolved in 0.9 % NaCl from a stock solution containing 0.002 % merthiolate and 1 mg ml^{-1} albumin) produced a pressor response that was sustained for the 10 min of exposure to the drug. The vehicle alone was without effect. In the Atlantic cod *Gadus morhua*, CCK caused a similar increase in branchial vascular resistance *in vivo* and in perfused gills *in situ* (Sundin and Nilsson, 1992).

All four drugs that we tested increased the proportion of the perfusate leaving by the efferent branchial artery. *In vivo* the venous outflow from the branchial vasculature would enter the venous sinus and the peribranchial sinus (Elger, 1987), the latter being connected to the subcutaneous sinus (Cole, 1925). The turnover time of the subcutaneous sinus is slow (Forster *et al.* 1989). We must assume that *in vivo*, when the animal is in normoxic sea water, little blood leaves from the gill pouch by the venous route and that most enters the efferent branchial arteries. The observation that a significant proportion of the fluid outflow entered the venous outflow *in vitro* suggests that, in this isolated preparation, haemodynamic constraints are not as they would be *in vivo*.

Despite the possible artefacts resulting from isolating the gill, the preparation clearly illustrates the potential for vasoactive agents to change the distribution of blood flow in the hagfish gill in a manner analogous with that found in the teleost fish gill. For example, in the Atlantic cod *Gadus morhua*, catecholamines, acting at α-adrenoceptors, constrict the arterio-venous pathway and so direct blood away from branchial veins, favouring the arterio-arterial pathway (Nilsson and Pettersson, 1981; Sundin and Nilsson, 1992). The arterio-arterial flow is increased by a β-adrenoceptor-mediated dilatation (Wahlqvist, 1980; Nilsson and Pettersson, 1981). The observation of similar responses in a hagfish indicates that an adrenergic control of gill blood flow must have appeared early in the evolution of vertebrate animals.

These results also confirm and extend previous work, supporting the contention that catecholamines are involved *in vivo* in the maintenance of the branchial vascular tone of hagfish. Studies on *Myxine glutinosa* gills perfused *in situ* indicated that both α- and β-adrenoceptors are present in the vasculature (Axelsson *et al.* 1990). In addition, the β-adrenoceptor antagonist propranolol increased branchial resistance in live hagfish (Forster *et al.* 1992). There is no evidence that autonomic fibres innervate the gills of hagfish (Nilsson, 1983), so these findings and the reports of an adrenergic tonus on the gill vasculature (Axelsson *et al.* 1990; Forster *et al.* 1992) indicate that any tonic control must be achieved by blood-borne catecholamines. The hearts of hagfish contain subendocardial stores of adrenaline and noradrenaline (see Forster *et al.* 1991), which might exert actions downstream, at the gills, and there are other potential sources of catecholamines (Giacomini, 1902). However, it is a cause for concern that relatively high doses of catecholamines were necessary to evoke a full response. Resting concentrations of catecholamines in the blood of teleost fish lie in the range 1–5 $\mathrm{nmol\,l^{-1}}$ (Randall and Perry, 1992), and the highest concentrations measured in the blood of the hagfish *Myxine glutinosa* by Perry *et al.* (1993) were only approximately $2\times10^{-8}\,\mathrm{mol\,l^{-1}}$. Perhaps perfusion with the physiological saline rather than blood desensitises the catecholamine receptors.

Our data suggest that the vasoregulatory drugs used have at least two sites of action, because the diversion of perfusate to the efferent arterial route could accompany either a rise or a fall in afferent arterial pressure. Afferent arterial pressures decreased in response to isoprenaline and to high doses of adrenaline. Dilatation of the efferent arterial vasculature would explain both the fall in pressure and the increased proportion of perfusate flowing to the arterial route. Raised blood pressures *per se* might be expected to increase outflow from the branchial arteries to the peribranchial sinus *via* the papillae

(Cole, 1912; Elger, 1987) and from the afferent and efferent circular and radial arteries to the venous sinus (Elger, 1987). The observation that afferent arterial blood pressure increased as flow was directed to the arterial route in response to low doses of adrenaline suggests either that the vasoconstrictor response is upstream from the sites of egress into the venous space and peribranchial sinus and/or that the venous pathway vasoconstricts.

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