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# THE EFFECT OF CATECHOLAMINES, SUBSTANCE P AND VASOACTIVE INTESTINAL POLYPEPTIDE ON BLOOD FLOW TO THE GUT IN THE DOGFISH *SQUALUS ACANTHIAS*

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## Summary

The effects of adrenaline, noradrenaline, substance P and vasoactive intestinal polypeptide (VIP) on dorsal aortic blood pressure, coeliac artery blood flow and heart rate were studied in unrestrained dogfish, *Squalus acanthias*. Changes in the coeliac vascular bed were calculated from these variables. Perfused tail preparations were used to study the effects of the various drugs on the somatic vascular bed. Corrosion casts were made to study the gross architecture of the coeliac vascular bed.

Adrenaline and noradrenaline increased dorsal aortic pressure and reduced the coeliac artery blood flow. Adrenaline caused a small increase in heart rate while noradrenaline caused a small decrease. Both drugs increased the resistance in the coeliac vascular bed. VIP increased dorsal aortic pressure, heart rate and resistance in the coeliac vascular bed. Substance P caused an increase in heart rate, cardiac output and, in particular, in coeliac artery blood flow; dorsal aortic pressure was simultaneously reduced. Voluntary swimming or fright immediately caused a pronounced reduction in blood flow in the coeliac artery, even though cardiac output and dorsal aortic pressure increased.

It is concluded that adrenergic mechanisms and possibly VIP are involved in reducing the blood flow to the gut, while substance P increases the flow to the gut.

## Introduction

Redistribution of blood to or from the gut normally occurs after feeding (prostprandially) and during exercise, respectively (Fara, 1984; Rushmer *et al.* 1961; Axelsson *et al.* 1989; Axelsson and Fritsche, 1991). A number of intrinsic and extrinsic factors, such as nervous and humoral tone, smooth muscle metabolism, venous pressure, oxygenation of the blood and extravascular com-

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pression, determine the resulting blood flow to the gut (Fara, 1984). The release of transmitters from perivascular neurones permits a rapid control of local vascular beds, while hormonal control essentially gives a more general, diffuse effect, which is commonly more long-lasting. The bulk of studies in mammals show that, in addition to adrenergic and cholinergic mechanisms, there are several neuropeptides involved in the nervous control of the blood flow to the gut (Dahlström *et al.* 1988). Amongst these, vasoactive intestinal polypeptide (VIP) and substance P (tachykinins) are well established as vasodilators in the gut (Owman, 1988; Edvinsson and Uddman, 1988; Dahlström *et al.* 1988; Mione *et al.* 1990).

In fish, little is known of the mechanisms behind the control of blood flow to the gut. A persistent increase of flow after feeding has been observed in the sea raven *Hemitripterus americanus* and the cod *Gadus morhua* (Axelsson *et al.* 1989; Axelsson and Fritsche, 1991), while exercise caused a reduction of blood flow to the gut in the cod. The reduced blood flow during the exercise period was not completely explained by an $\alpha$-adrenoceptor-mediated vasoconstriction and could possibly involve peptidergic or cholinergic mechanisms (Axelsson and Fritsche, 1991). In cod, as in mammals, the neuropeptides/gut hormones substance P and VIP increase the flow to the gut. This is achieved, in part, by a decrease in vascular resistance in the coeliac and mesenteric vascular beds (Jensen *et al.* 1991). Previous histochemical studies have demonstrated the presence of catecholamines and VIP-like immunoreactive material in perivascular nerves of the coeliac and mesenteric arteries of the spiny dogfish (Holmgren and Nilsson, 1983), while substance-P-like immunoreactivity mainly occurs in endocrine cells and non-vascular gut neurones (Holmgren, 1985).

The aim of the present study was to investigate the effects on coeliac artery blood flow *in vivo* of catecholamines, substance P and VIP in an elasmobranch, the spiny dogfish *Squalus acanthias*.

## Materials and methods

### *Fish*

Fifteen spiny dogfish, weighing 500–1500 g, were used. The fish were caught (line and hook) in English Bay outside West Vancouver, BC, Canada, and were kept in aerated, running sea water from the same area. The water temperature during the experiments was 8–10 °C. The animals were fed once a week. The experiments were performed in March–April.

### *Surgical procedure for* in vivo *studies*

The fish were anaesthetized in MS 222 (tricaine methane sulphonate 100 mg $l^{-1}$, Sigma) until breathing movements ceased. They were transferred to an operating table, and the gills were then continuously irrigated with aerated sea water containing MS 222 (50 mg $l^{-1}$) throughout the operation. A polyethylene cannula (PE50) filled with heparinized (100 i.u. $ml^{-1}$) 1.3 % NaCl was inserted non-occlusively in the dorsal aorta 10 cm anterior to the tail end of the fish and was

pushed forward approximately 10 cm. This cannula was used for measurement of dorsal aortic pressure and heart rate and for injection of drugs.

Cuff-type Doppler flow probes (single crystal, P. Pohl International Inc.) were fitted on the ventral aorta and coeliac artery, essentially as described for the Atlantic cod (Axelsson and Fritsche, 1991). A flow probe was placed around the ventral aorta (3–5 mm i.d.) between the third and fourth/fifth afferent branchial arteries. The coeliac artery branches from the dorsal aorta. It is a large vessel that runs freely in the abdominal cavity for 5–10 cm before giving off branches to the stomach and liver region (see Fig. 1). The second flow probe (2–3 mm i.d.) was fitted on the vessel about 2 cm from the point where it branched off the dorsal aorta.

The two flow probes were connected to a Doppler flow meter (Iowa University). In this study no attempt was made to calibrate the flow probes and only relative changes in flow are reported. Since it is practically impossible to place a flow probe around the ventral aorta posterior to the fourth/fifth afferent gill arteries, the recorded flow in this study represents partial cardiac output ($\dot{Q}_{part}$).

The dorsal aortic cannula was attached to a Micron pressure transducer. The pressure was calibrated against a static column of water. The pressure and flow probe signals were suitably amplified and displayed on a Grass Polygraph recorder system model 7D. The signals were also simultaneously sampled by a data acquisition software program (Labtech Notebook) using an IBM-compatible computer. Sampling frequency was set to 2 samples $s^{-1}$, and on-line mean value calculation over 5 s periods was performed.

After surgery, the animals were transferred to the experimental chambers and left to recover for between 20 and 70 h before injections of any drugs, to allow the effects of anaesthesia and handling to wear off (see Smith *et al.* 1985).

### *Experimental protocol for* in vivo *studies*

Drugs were injected into the fish in boluses of 0.1 ml $kg^{-1}$ body mass. The injection catheter was flushed with 0.4 ml of saline after each injection, and again after 10 min or when the peak effect of the drug had been obtained. The drugs were given in random order and not until the variables recorded had returned to pre-injection values. Each experiment normally lasted 2 days.

### *Perfused tail preparation*

To study the effects of the various drugs on the somatic vascular bed, five tail perfusion experiments were performed. Each animal was killed by a sharp blow to the head and rapidly injected with approximately 1000 i.u. of heparin intracardially. After approximately 5 min, the tail was cut off and two polyethylene cannulae were implanted, one in the dorsal aorta and one in the vein. Both cannulae were secured and the dorsal aortic cannula was connected to a constant-flow peristaltic pump. An elasmobranch Ringer's solution (Nilsson *et al.* 1975) bubbled continuously with a mixture of $O_2/CO_2$ (97 %/3 %, pH 7.7) was used to perfuse the preparation. The preparation was immersed in saline in an organ bath

at the same temperature as was used in the rest of the experiments. The drugs were injected in bolus doses while the input pressure was continuously recorded.

### *Drugs used*

The following drugs were used: L-adrenaline bitartrate, atropine sulphate, DL-noradrenaline hydrochloride, synthetic substance P, synthetic porcine vasoactive intestinal polypeptide (VIP). All drugs were purchased from Sigma. Peptides (substance P and VIP) were dissolved in distilled water and subsequently diluted in 1.3 % NaCl containing 1 mg $ml^{-1}$ of bovine serum albumin. The other drugs were dissolved and diluted in 1.3 % NaCl.

### *Calculations and statistics*

Heart rate ($f_H$) was derived from the phasic blood pressure signals by the software program, and expressed as beats $min^{-1}$.

Vascular resistance was calculated by the software program at each sampling point as the pressure drop (see below) over the vascular bed divided by the blood flow in the same vascular bed. The pre-injection value was set to 100 %, and changes were related to this value. Three assumptions were made for the calculations of vascular resistance: (1) the blood pressure in the coeliac artery equals that in the dorsal aorta, (2) central venous pressure equals zero without any major changes during the different drug injections, and (3) blood viscosity remains the same during the experiment (Kiceniuk and Jones, 1977; Greenway, 1982). Capra and Satchell (1977) have shown that adrenaline and noradrenaline affect the central venous pressure in the dogfish; adrenaline produces an increase and noradrenaline a decrease. The effects are very small compared to the recorded changes in dorsal aortic pressure and blood flow in the coeliac artery in the present study. The error in the calculation of coeliac vascular resistance caused by assuming a zero pressure in the central venous system is, therefore, going to be negligible, and will not affect the conclusions.

Data are presented in graphs as means±S.E.M., where each point represents a mean value over 30 s. Wilcoxon's signed-ranks test for paired samples (two-tailed) was used to determine the statistical significance of observed effects of the drugs. The statistical tests on coeliac artery blood flow, cardiac output and systemic and coeliac vascular resistance were performed before the data were transformed to percentage changes. The level of significance was set to $P \leq 0.05$. In the case of repeated testing, a sequentially rejective Bonferroni test (Holm, 1979) was used to eliminate, as far as possible, any type I error.

### *Vascular casts*

Corrosion casts of the vasculature were made in two animals. They were killed and heparinized in the same way as described for the tail perfusion experiments, a PE100 cannula was secured in the conus and the vascular system was then flushed with saline for 15 min. 40 ml of the casting material (Mercox, Ladd Research Ind.

Inc.) was injected slowly and allowed to polymerize. The tissue was then digested in 30 % KOH over 48 h.

## Results

### *Anatomy of the gut circulation*

The corrosion casts showed that the coeliac artery branches off the dorsal aorta as a distinct vessel approximately 1 cm posterior to the last efferent gill arteries. The mesenteric and splanchnic arteries each branch off as major separate vessels. Smaller segmental arteries branch off the dorsal aorta along its length (Fig. 1). The coeliac artery carries blood to the liver and stomach regions.

### *Resting values and effects of voluntary movements and fright*

Resting values for dorsal aortic pressure ($P_{DA}$; 2.02±0.2 kPa; $N$=8) and heart rate ($f_H$; 18.8±1.3 beats min$^{-1}$; $N$=8) compare well with previously recorded

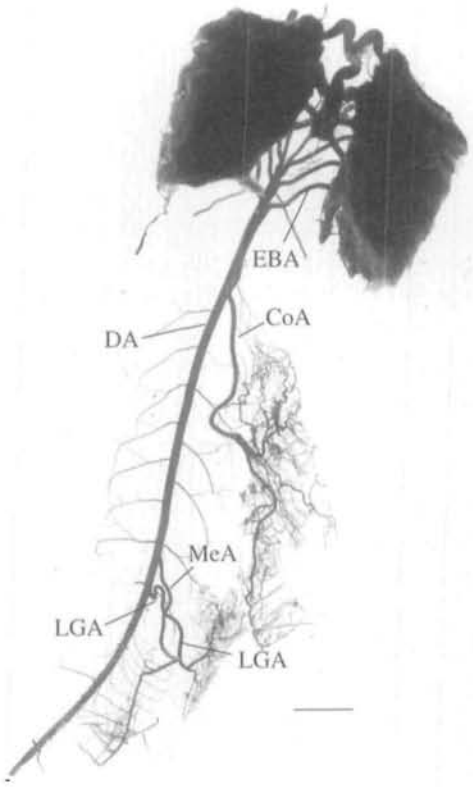


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Fig. 1. A vascular cast from the spiny dogfish *Squalus acanthias*. Note the three large arteries leaving the dorsal aorta as distinct vessels well separated from the efferent gill arteries. EBA, efferent branchial arteries; DA, dorsal aorta; CoA, coeliac artery; LGA, lienogastric artery; MeA, mesenteric artery.

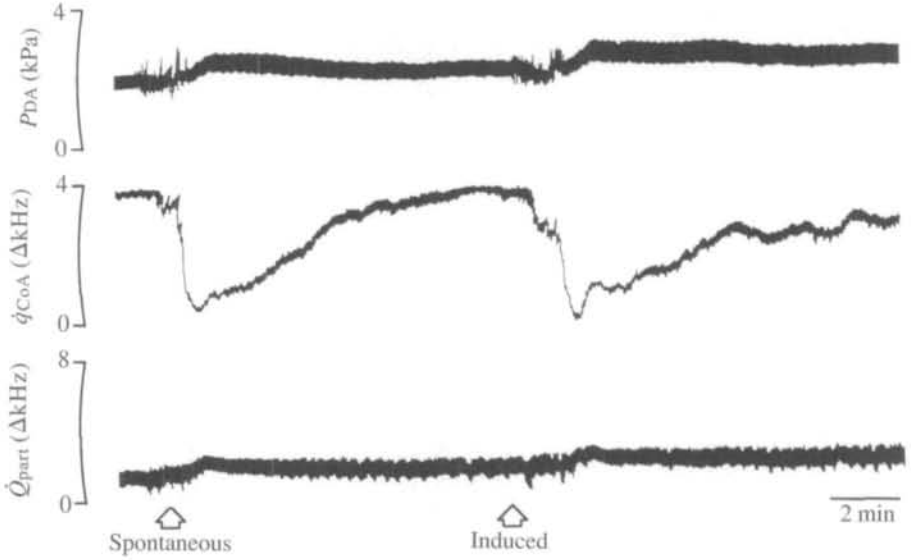


Fig. 2. Recording showing the effects of spontaneous activity and 'fright' (tapping the tank) on dorsal aortic pressure ($P_{DA}$), coeliac artery blood flow ($\dot{q}_{CoA}$) and cardiac output ($\dot{Q}_{part}$).

values of these variables in the spiny dogfish (Capra and Satchell, 1977; Taylor and Butler, 1982).

Voluntary swimming/struggling caused a rapid decrease in flow in the coeliac artery, while blood pressure and cardiac output increased. A similar response was obtained when the animal was disturbed by tapping the tank (Fig. 2).

### In vivo *effects of adrenaline, noradrenaline, substance P and VIP*

After initial testing, doses of adrenaline at 1 nmol $kg^{-1}$, noradrenaline at 1 and 10 nmol $kg^{-1}$, substance P at 0.1 nmol $kg^{-1}$ and VIP at 0.1 nmol $kg^{-1}$ were chosen for further studies, since these produced consistent responses that declined to control levels within 1 h. Peak responses to adrenaline (Fig. 3) and noradrenaline (Fig. 4A,B) were obtained within 2–3 min, to substance P within 6 min (Fig. 5) and to VIP within 3–4 min (Fig. 6) of injection.

Both adrenaline and noradrenaline significantly increased dorsal aortic pressure ($P_{DA}$) and coeliac vascular resistance ($R_{CoA}$). At the same time, blood flow in the coeliac artery ($\dot{q}_{CoA}$) was significantly reduced. Cardiac output was unaffected by 1 nmol $kg^{-1}$ adrenaline and noradrenaline, but was reduced by 10 nmol $kg^{-1}$ noradrenaline (Fig. 4B). In 1 nmol $kg^{-1}$ doses, adrenaline caused a small increase in heart rate ($f_H$). The effects of adrenaline were more pronounced and long-lasting than those of noradrenaline of the same concentration.

Substance P (0.1 nmol $kg^{-1}$; Fig. 5) produced a 100 % increase in coeliac artery blood flow with a corresponding decrease in coeliac vascular resistance. At the same time as the coeliac artery blood flow increased there was an increase in

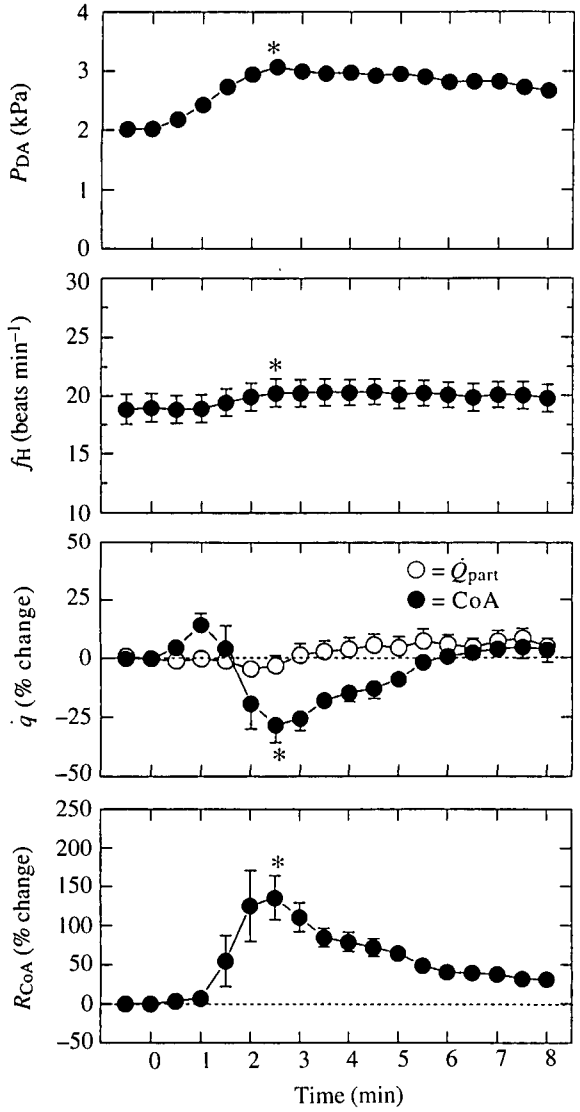


Fig. 3. A summary of the cardiovascular response to adrenaline (1 nmol kg$^{-1}$, injected at time zero) in the spiny dogfish; mean values±S.E.M.; $N$=8. $P_{DA}$, dorsal aortic blood pressure; $f_H$, heart rate; $\dot{q}$, blood flow; CoA, coeliac artery; $\dot{Q}_{part}$, partial cardiac output; $R_{CoA}$, coeliac artery vascular resistance. Asterisks indicate statistically significant differences compared to pre-injection values. In this and other figures statistical significance was only calculated for the maximum or minimum values of variables.

cardiac output and a small tachycardia and a drop in dorsal aortic pressure were seen.

VIP (0.1 nmol kg$^{-1}$; Fig. 6) produced a small increase in dorsal aortic pressure

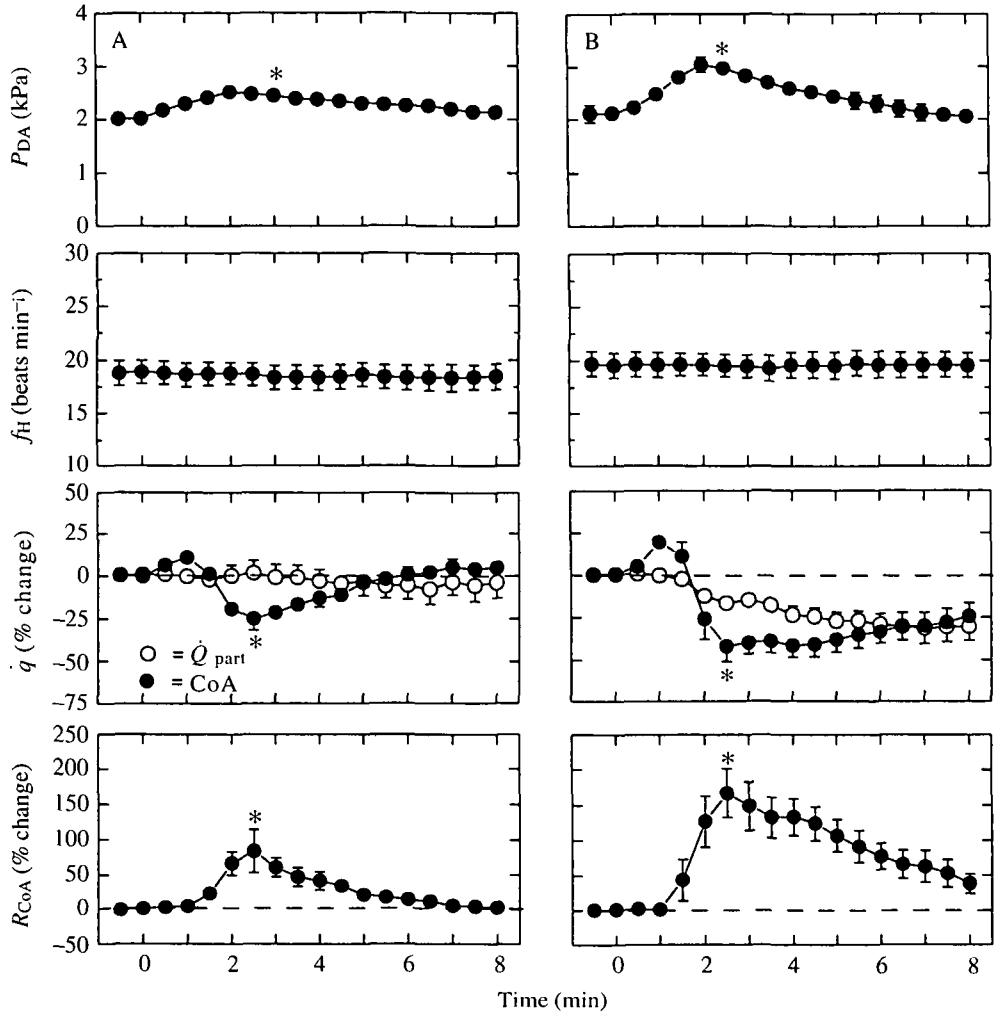


Fig. 4. A summary of the cardiovascular response to two doses of noradrenaline (A, 1 nmol kg$^{-1}$; B, 10 nmol kg$^{-1}$) in the spiny dogfish; mean values±S.E.M.; $N$=8. Details as in Fig. 3.

and an increase in coeliac vascular resistance. Cardiac output and heart rate were unaffected. In five out of eight animals, the coeliac flow was reduced (by 8–41 %).

In the perfused tail preparations, both adrenaline (Fig. 7) and noradrenaline produced an increase in perfusion pressure, indicating a vasoconstriction of the somatic vasculature. Substance P caused a small decrease in perfusion pressure, indicating a vasodilatation of the somatic vasculature. VIP had no significant effect on the somatic vasculature in any of the preparations tested.

## Discussion

The corrosion casts show that the coeliac artery supplies most parts of the

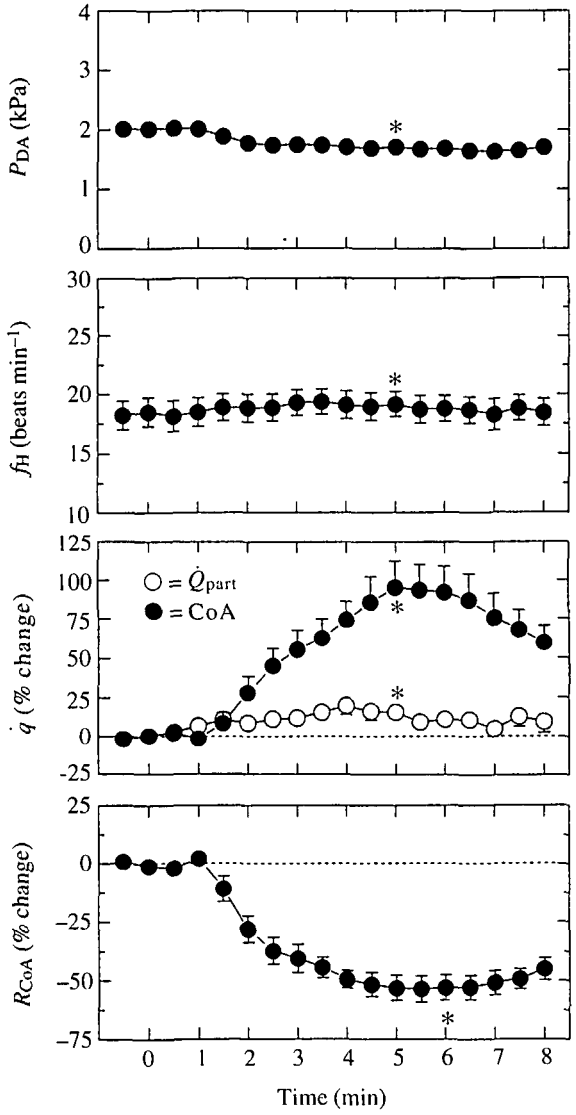


Fig. 5. A summary of the cardiovascular response to substance P (0.1 nmol kg$^{-1}$) in the spiny dogfish, mean values±S.E.M; $N$=8. Details as in Fig. 3.

stomach, and the effects obtained with the test substances may reflect their general influence on the blood flow through the stomach wall. The casts also show that the coeliac, mesenteric and splanchnic arteries branch from the dorsal aorta as major distinct arteries and are well separated from the last efferent gill arteries. This is in contrast to the condition often encountered in teleost species, where the common coeliaco-mesenteric artery originates as a single vessel, often in very close association with the fourth efferent gill arteries (Farrell, 1980; H. Thorarensen, personal communication; M. Axelsson, unpublished results). In some species it

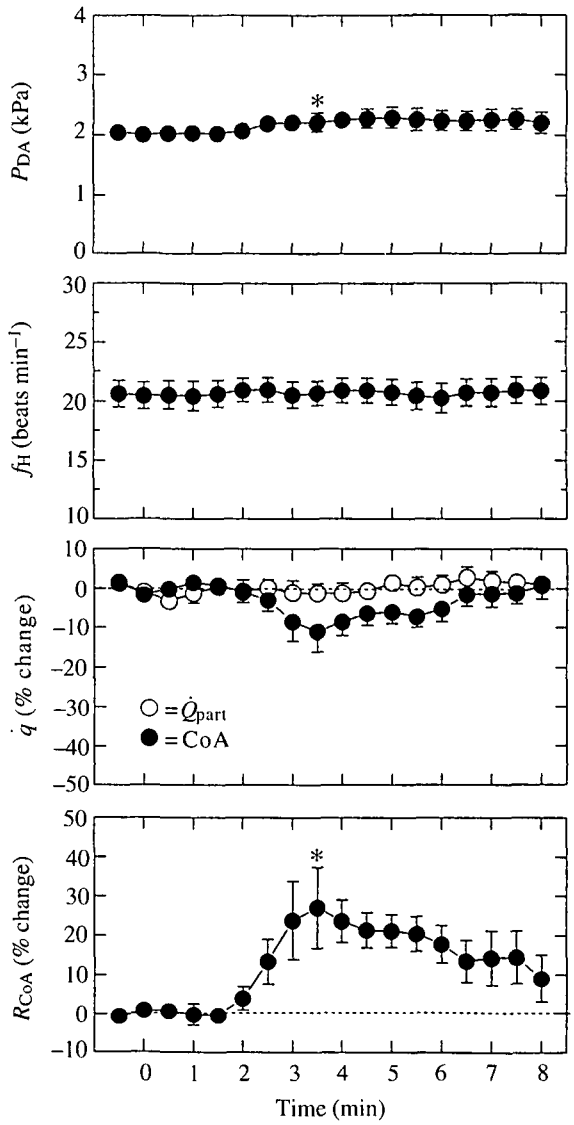


Fig. 6. A summary of the cardiovascular response to VIP (0.1 nmol kg$^{-1}$) in the spiny dogfish; mean values S.E.M.; $N$=8. Details as in Fig. 3.

appears that the coeliaco-mesenteric artery is largely fed from one of these most posterior gill arteries. The reason for this proximity is not known, but it leaves a potential for redistributing blood away from, or towards, the gut by redirecting blood through the anterior or posterior gill arteries.

During periods of spontaneous movements/struggle and immediately after disturbing the animals, $P_{DA}$ and $\dot{Q}_{part}$ increased. This response to exercise/stress is common and has been reported for many species of mammals and fish (for references see Rowell *et al.* 1964; Satchell, 1991). A prominent reduction in $\dot{q}_{CoA}$

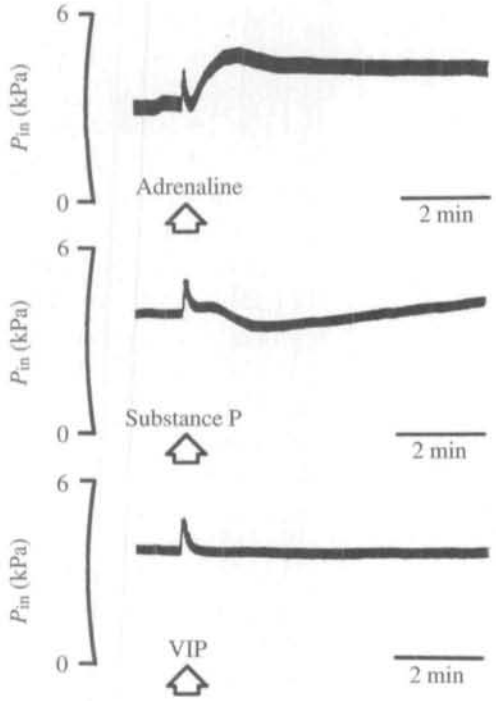


Fig. 7. Recording from an *in vitro* tail perfusion experiment showing the effect on perfusion pressure ($P_{in}$) of bolus injections of (A) adrenaline (0.1 ml of $10^{-5}$ mol $l^{-1}$), (B) substance P (0.1 ml of $10^{-6}$ mol $l^{-1}$) and (C) VIP (0.1 ml of $10^{-6}$ mol $l^{-1}$).

was seen simultaneously. Again, this is a well-documented response to exercise/stress in mammals and has also been reported in two species of teleost fish (Axelsson *et al.* 1989; Axelsson and Fritsche, 1991). In the Atlantic cod *Gadus morhua*, both the coeliac and mesenteric arterial blood flow is reduced during even moderate exercise and may be almost completely shut down during periods of stress or hypoxia (Axelsson and Fritsche, 1991).

In the present study, we have recorded effects resulting from the injection of substances into the bloodstream. The effects may, therefore, primarily mimic the effects of circulating substances (hormones), or of substances released from the endothelium, rather than the effects of neuronally contained transmitters. The presence of vasa vasorum in the larger vessels allows a shorter diffusion distance to the perivascular nerve net, which increases the possibility of an effect on neuronal receptors. Histochemical studies in *Squalus acanthias* indicate the presence of extraneuronal sources of catecholamines (chromaffin tissue; see Nilsson, 1983), substance P (endocrine cells; El-Salhy, 1984; Holmgren, 1985) and VIP (endocrine cells; Reinecke *et al.* 1981; El-Salhy, 1984), but also of an innervation of the major systemic arteries by catecholamine-containing and VIP-immunoreactive fibres (Nilsson *et al.* 1975; Holmgren and Nilsson, 1983). There are, to our knowledge, no reports on endothelial sources of these substances in the fish gut. Whether the

injected substances reach innervated receptors (junctional receptors) needs further testing involving nerve stimulation, the use of specific antagonists and receptor binding studies, which is beyond the scope of the present study. Further studies are also needed to determine whether the injected substances act directly on the vascular muscle or *via* endothelial factors (see Burnstock, 1990).

The responses to adrenaline and noradrenaline are very similar, except that adrenaline causes a small tachycardia, which agrees with the positive chronotropic effect reported by Capra and Satchell (1977). Although the effect of adrenaline and noradrenaline on the reduction of flow is the same (indicating a similar vasoactive effect), there is a slight difference in the response of the coeliac vascular resistance to these catecholamines: adrenaline (at 1 nmol $kg^{-1}$) produces a larger increase in resistance, and this may be correlated with the more pronounced increase in dorsal aortic blood pressure following the adrenaline injection.

Substance P produces a 'classical' response reducing the coeliac artery vascular resistance in the spiny dogfish. A small vasodilatation occurs in the somatic vascular bed, in addition to the more pronounced effect on the coeliac artery. Numerous mammalian studies report a vasodilator action of substance P and related tachykinins (Mione *et al.* 1990), and in the Atlantic cod, *in vivo*, substance P increases the flow in both the coeliac and the mesenteric arteries (Jensen *et al.* 1991). Also, although less pronounced than that seen in mammals, the tachycardia observed in dogfish is similar to the effect of substance P on the mammalian heart. In contrast, heart rate is unaffected by substance P in the cod (Jensen *et al.* 1991).

It has been argued, in mammals, that the vasodilation of arteries produced by tachykinins is not correlated to the density of perivascular nerves, but is dependent on an intact endothelium, and may thus be a humoral or local endothelial effect rather than a neuronal effect (Furchgott, 1983; D'Orleans-Juste *et al.* 1985; Edvinsson *et al.* 1985). This may also be the case in *Squalus acanthias*, where, so far, no perivascular nerve fibres immunoreactive to substance P have been found in the gut (Holmgren, 1985, and unpublished results). It is also possible that tachykinins are not endogenous vasodilators in *Squalus acanthias*.

The increase in resistance of the coeliac artery vascular bed after injection of VIP is an unusual observation. In mammals, VIP is established as a dilator of most vascular beds, including that of the gut (see, for example, Fahrenkrug, 1991). The vasoconstrictor effect may be specific to the gut vascular bed in the dogfish, since the perfused somatic bed (tail) did not show the same response, and in the rectal gland of *Squalus acanthias* VIP causes the same effect as has been reported from studies of mammalian exocrine glands: an increase in perfusion flow in combination with an increased secretion (Solomon *et al.* 1984; Thorndyke *et al.* 1989). Few studies have been performed on the effect of VIP on the gut of fish or other non-mammalian vertebrates, but one study in the catfish *Ictalurus melas*, where an intestinal loop was perfused with porcine VIP as well as with VIP extracts from catfish and trout, demonstrated a dilatory effect of VIP (Holder *et al.* 1983). In the cod *Gadus morhua*, vascular perfusion of the gas gland and swimbladder with VIP produces a long-lasting decrease in vascular resistance, probably caused by a

vasodilation of the mesenteric and swimbladder arteries (Lundin and Holmgren, 1984). Similarly, in the cod *in vivo*, injection of VIP causes a reduced resistance in the vascular bed of the coeliac artery, while in these experiments the increase in flow in the mesenteric artery was due to the increase in cardiac output only (Jensen *et al.* 1991).

Other details are similar in the teleosts and elasmobranchs investigated; in both the cod and the spiny dogfish, VIP-immunoreactive perivascular fibres have been demonstrated in the vessels leading to the gut (Holmgren and Nilsson, 1983, 1991; Lundin and Holmgren, 1984). VIP peptides from the dogfish *Scyliorhinus canicula* and from the cod have been sequenced and differ from each other at just two positions; also, both peptides differ from porcine VIP in only five positions (Dimaline and Thorndyke, 1986; Dimaline *et al.* 1987; Thwaites *et al.* 1989). It is, therefore, conceivable that the VIP-induced vasoconstriction in the gut of *Squalus acanthias* indicates a true species difference in the function of VIP. It may, however, be possible that, by acting *via* the endothelium rather than directly on the smooth muscle, VIP produces the opposite effect, although there are no reports of this in any other species.

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