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VASCULAR RESISTANCE RESPONSES OF AN EEL TAIL PREPARATION: ALPHA CONSTRICTION AND BETA DILATION

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SUMMARY

1. An isolated, saline-perfused, eel tail preparation is described. This preparation was easily prepared and did not suffer from progressive deterioration, as indicated by stable baseline resistance with time and maintained responsiveness to drugs.

2. Elevation of caudal venous pressure caused a reduction in resistance of about 10% per 0·5 kPa. Addition of 3% or 5% human serum to the perfusate increased baseline resistance and imparted a degree of autoregulation to the preparation.

3. Full dose–response curves for the preparation's resistance responses to adrenaline (AD), noradrenaline (NA), and isoprenaline are presented.

4. AD and NA increased resistance, indicating the presence of alpha adrenergic receptors. AD was more potent than NA; the mean potency ratio was 2·78 : 1·00 (AD : NA).

5. Isoprenaline decreased resistance, indicating beta adrenergic receptors. From the relative potencies of isoprenaline $\ll$AD $<$ NA in stimulating these receptors, they are classified as beta two adrenergic receptors.

6. The possible physiological functions of alpha and beta responses of the eel tail are discussed.

INTRODUCTION

The adrenergic pharmacology of teleost systemic vascular beds has been intensively studied in only one species – the rainbow trout *(Salmo gairdneri)* (see Wood & Shelton, 1975; Wood, 1976). However, information is available from studies of isolated coeliac artery strips from the cod *(Gadus morhua)* (Holmgren & Nilsson, 1974), and various eel preparations (see Forster, 1976*a*). All studies thus far have demonstrated systemic alpha adrenergic constriction in response to adrenaline and noradrenaline, whereas few workers have reported significant beta adrenergic dilator responses to isoprenaline (Chan, 1967; Helgason & Nilsson, 1973; Holmgren & Nilsson, 1974; Holmgren, 1978). Beta dilator responses could be elicited from perfused trout trunk preparations only after vascular tone had first been elevated by adrenaline (Wood, 1976).

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The post-vent eel tail was chosen because it is a relatively homogeneous piece of tissue. In contrast to the perfused trout trunk (Wood & Shelton, 1975) it excludes the viscera, which in mammals have different vascular responses to skeletal muscle (Mellander & Johansson, 1968). A preparation including these organs may be complicated by responses such as redistribution of perfusate. However, the presence of red and white muscle (Hulbert & Moon, 1978) and their totally different vasculatures (Gorkewicz, 1948) indicates that the eel tail may be less homogeneous than some mammalian skeletal muscle preparations.

Many vascular preparations assume very low levels of tone when isolated (Waugh & Shanks, 1960; Bohr & Johansson, 1966), and consequently further dilation has been difficult to elicit. In about half of the preparations to be described here, 3 or 5% human serum was added to the perfusate to create vascular tone. Increases in vascular tone which result from addition of serum to the perfusate are purported to be non-neurogenic and non-humoural (Bohr, Verrier & Sobieski, 1971).

The objectives of this study were to record vascular resistance responses to adrenaline, noradrenaline and isoprenaline from an isolated, saline-perfused, eel tail preparation. Adrenergic receptors were then classified according to the relative potency of these sympathetomimetic drugs (Arnold, 1972; Wood, 1976). These results are presented so that they are easily compared to the results from rainbow trout (see Wood, 1974; Wood & Shelton, 1975; Wood, 1976).

EXPERIMENTAL ANIMALS

Immature, short-finned eels (*Anguilla australis schmidtii* Phillips) used in this study were trapped in fyke nets in the Selwyn River, Canterbury, New Zealand. Eels were maintained for at least 2 weeks after capture in an indoor tank (1600 l) under conditions of low illumination. Experiments were performed within 6 weeks of capture, during which time the fish were not fed.

METHODS

(*a*) *Preparation of the tail for perfusion*

Short-finned eels between 270 and 719 g (461·8 ± 5·7 g; mean ± S.E.M.; $n = 86$) were anaesthetized in 0.04% aqueous benzocaine (Wedemeyer, 1970). Heparin (ammonium salt, Sigma Chemical Co.) was administered at 500 i.u. per 100 g body weight in 1·0 ml of saline via the bulbus arteriosus and allowed to circulate for 5 min. A ventral incision about 30 mm long was made in the body wall to the left of the midline, about 20 mm caudal of the vent. The kidney tissue was carefully dissected from the connective tissue attaching it to the body wall and the caudal artery and vein exposed to their points of entry into the haemal arch. The front of the eel was then cut from the tail just caudal of the vent. Ligatures were placed about the caudal artery and vein before cannulation. Portex pp160 cannulae (120 mm long) were used to cannulate both vessels. Cannulae were prefilled with heparinized saline (100 i.u. per ml). The cannulae were inserted to about 10 mm beyond the first haemal arch tied, and a further ligature tied about the caudal vein and kidney.

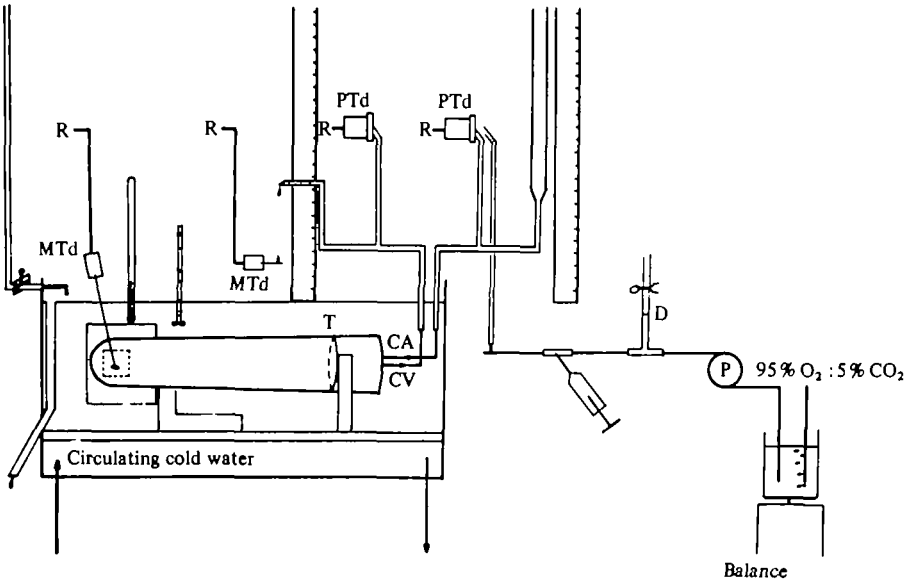


Fig. 1. Diagram of experimental apparatus used for perfusion of the eel tail. CA, Caudal artery cannula; CV, caudal vein cannula; D, depulsator; MTd, mechanotransducer; PTd, pressure transducer; P, peristaltic pump; R, to recorder; T, tourniquet. The mechanotransducer which extends to the tip of the tail was used to record caudal lymphatic heart rate. Data from these records will be presented at a later date.

After wighing, the tail was placed in a constant-temperature saline bath (10 ± 1 °C) (see Fig. 1). Preparation time was around 30 min. No preparation was experimented with for more than 6 h. After the end of all experiments the preparation was reweighed.

(*b*) *Saline*

The saline used throughout this study was freshwater eel Ringer solution (Rankin & Maetz, 1971). Glucose and polyvinyl pyrrolidone (PVP) were omitted from the bathing saline. All salines used for perfusion were filtered through Watman GFC filter paper immediately before use.

The viscosity of the saline was increased with PVP (360,000 average molecular weight, Sigma Chemical Co.) to a value near halfway between whole blood and plasma viscosities (see Whittaker & Winton, 1933; Wood, 1974). Blood was collected in dried heparinized syringes from the hearts of nine eels, and pooled for three pairs, thus obtaining six samples. The mean haematocrit of the six samples was 40·15 ± 0·93 (mean ± S.E.M.) – slightly higher than reported by McArthur (1977) for this species. A large-bore (1·0 ml) Ostwald viscometer was used to measure viscosity. The mean relative viscosity of whole blood was 6·05 ± 0·37. The mean relative viscosity of plasma was 2·15 ± 0·11 ($n = 4$). When half the difference between the relative viscosities of blood and plasma is added to the plasma viscosity it gives a vaule of 4·1, which is very close to that of the 10 g l^{-1} PVP solution. Consequently the basic saline was freshwater eel Ringer solution plus 10 g l^{-1} PVP. Addition of 5% human serum

(supplied by the Haematology Laboratory, Christchurch Public Hospital) to the saline did not significantly alter the viscosity.

(*c*) *Perfusion methods*

Two perfusion methods were employed. The first consisted of a vertical tube of known volume per unit height (1·11 ml per 10 mm), filled with saline and connected to the arterial cannula (see Nichol *et al.* 1951). Data from this method allowed construction of flow/pressure profiles.

The second method employed a peristaltic pump (Cole Parmer Masterflex) to perfuse the preparation.

Administration of drugs and blanks was either as a constant concentration in the perfusate (infusion), or as a 0·1 ml bolus (injection).

(*d*) *Measurement of perfusion parameters*

Flow: vertical tube perfusion. A tangent to the curved pressure record was drawn at the desired pressure. Flow was calculated from the slope of the tangent, since this is directly related to the rate of pressure fall.

Pump perfusion. Change in weight of the reservoir with time gave the pump perfusion flow rate. This flow was set to 0·5 g of saline per 100 g of tail tissue weight.

Outflow. Outflow was recorded by a drop counter.

Pressure. Both caudal artery and caudal venous pressures were measured with Bell and Howell 4–327–0010 pressure transducers. Pressure calibration was against the internal standard in the recorder and a column of saline connected to the other port of the transducer head.

(*e*) *Units*

Pressures are in kilopascals (kPa), flows in ml min^{-1} and resistances (R) in kPa ml^{-1} min, (1 kPa = 7·5 mmHg = 10·2 cm H_2O, 1 kPa ml^{-1} min = 7·5 P.R.U.). Changes in resistance are indicated by delta ($\triangle$), or as a percentage of baseline resistance $\triangle R\%$. All values are given as means $\pm$ sample standard error.

ANALYSIS OF DOSE–RESPONSE CURVES

Adrenaline (AD) and noradrenaline (NA) both increased vascular resistance, while isoprenaline decreased resistance which suggests the presence of both alpha and beta adrenergic receptors (see Results). Thus the classical single receptor model (see Waud, 1968, 1975) was not applicable and an empirical approach was applied. A logistic equation was chosen because it provides a good fit, is easily calculable and is reasonably flexible (Waud, 1975). The equation used is as follows:

$$E = M \frac{A^p}{A^p + K^p},$$

where E = the response, M = the maximum response, A = the concentration of the drug, K = the half maximal dose, and p reflects the slope of the curve.

The form of the equation contains elements of the curve that provide useful information about the response. Curves were fitted using the least-squares method [redacted] an iterative process. The search procedure for the parameter values was that of

Powell (1964). The mean responses for each concentration of the drug were calculated and used to fit the equation. Since responses are measured as changes from baseline resistance, all data points have equal weights. In addition to these points the curves were constrained to pass through the origin. The dose and response were given very small values and the weights were increased to 100 times that of the other points. Curves fitted to the data had multiple correlation coefficients of greater than 0·97 ($0{\cdot}988 \pm 0{\cdot}004$; $n = 10$), indicating the propriety of the model for the data. The standard error of the response estimate (multiplied by 1·96) gave the 95% confidence limits and allowed comparison of the responses to equal doses of different drugs. Confidence limits for dose, the independent variable, were taken as horizontal excursions within the 95% confidence limits of the response estimate. Because of the shape of the confidence interval and the logarithmic dose scale, ED 50 confidence limits are not equidistant from the ED 50.

DRUGS

Drugs used in experiments on these preparations were: L–adrenaline, free base (Sigma Chemical Co.); L–noradrenaline, free base (Sigma Chemical Co.); L–noradrenaline bitartrate (Koch-Light Laboratories); L–isoprenaline bitartrate (Sigma Chemical Co.); phentolamine mesylate (CIBA); propranolol (ICI Ltd, Parmaceuticals Division); dichloroisoproterenol hydrochloride (Eli Lilly and Co. Ltd.). Drug concentrations are expressed in molar (M) concentrations or doses.

EXPERIMENTAL PROTOCOL

Flow/pressure profiles were measured before any drugs were introduced. Preparations were then pump perfused for a 20-30 min stabilization period. Blank saline samples were prepared and administered before each set of drugs and intermittently between various concentrations. None of the blanks produced a significant response. Dose–response curves were prepared by the cumulative addition procedure (van Rossum, 1963). In no case was a dose–response curve constructed from a preparation treated with any other drug.

After all experiments were completed, input and output cannulae were dissected free with ligatures still in place and connected by a short piece of tubing (short-circuit perfusion). Flow was not altered, and the resistance of the delivery and collection system was measured. The system resistance was $0{\cdot}62 \pm 0{\cdot}08$ kPa ml^{-1} min ($n = 86$), equivalent to about 40% of the mean baseline tail resistance value.

RESULTS

Ninety-eight isolated saline-perfused eel-tail preparations were prepared. Twelve preparations were discarded because venous outflow was less than 80% of the inflow (efficiency of perfusion). Mean efficiency of perfusion of the 86 satisfactory preparations was $87{\cdot}2 \pm 2{\cdot}1$%.

The tail preparation amounted to $45{\cdot}2 \pm 1{\cdot}5$% ($n = 86$) of the total body weight of the fish. This includes the small amount of tissue at the anterior end of the tail which was excluded from perfusion by the tourniquet.

All tails gained weight during perfusion; the mean increase was $12.5 \pm 1.6\%$ ($n = 86$). Perfusion stimulated mucus production, and upon wiping off the excess mucus the weight gain was $7.6 \pm 1.5\%$ ($n = 11$).

Mean measured flow rate, when corrected for the specific gravity of the saline (1·0090), was 0.499 ml min^{-1}. Because the reservoir was continuously bubbled with 95% O_2/5% CO_2, evaporation may have been significant.

Although baseline resistance ($R_{Initial}$) regressed against initial tail $weight^{-1}$ produced a significantly linear relationship, a better fit was obtained when natural logs of both resistance and weight were used. The regression line using all data was

$$R_{Initial}\,(\text{kPa ml}^{-1}\,\text{min}) = 251{\cdot}0\ \text{wt}\,(\text{g})^{-0.806};\quad F = 47{\cdot}6 \text{ for } 1, 82 \text{ D.F.}$$

The points from eel tails perfused with 5% human serum added to the saline showed greater scatter than those perfused with the basic saline. When regressions were performed separately, different lines resulted. Because the two groups of data using the two perfusates must, if the regression analysis is accepted, be transformed differently to correct for weight, data are presented as kPa ml^{-1} min rather than kPa ml^{-1} min 100 g^{-1}.

Flow-versus-pressure-differential profiles and the effect of venous pressure in baseline resistance

The vertical-tube method of perfusion was used on 15 preparations. In each preparation at least two profiles were constructed: one at a venous pressure of zero and one at a venous pressure of 1·33 kPa. Nine of the preparations were perfused with the basic saline. Six had 3% human serum added to the perfusate.

Preparations perfused with the basic saline showed significantly greater flows at all pressure differentials when venous pressure was raised. Lack of convexity over most of the pressure range suggests significant vascular tone (Kuida, 1965).

Profiles from preparations perfused with 3% serum added to the perfusate at the same venous pressures had similar shapes to those perfused with the basic saline. Flow rates were not significantly different when venous pressure was raised in preparations perfused with 3% serum in the saline.

In eight preparations, pump-perfused with the basic saline, venous pressure was raised in 0·5 kPa steps from zero to 4·5 kPa. Resistance fell by approximately 10% per 0·5 kPa venous pressure increment.

Baseline resistance

Pump perfusion of 50 tails with the basic saline was performed at venous pressures of zero and 1·33 kPa. Thirty-six preparations were perfused with 5% human serum added to the perfusate at venous pressures of 1·33 kPa. The results from these experiments are given in Table 1.

Table 1. *Baseline resistance of isolated saline-perfused eel tail preparations, in kPa ml^{-1} min (mean* ± S.E.M.)

Venous pressure (kPa)	Basic saline	5% serum saline
Zero	$1{\cdot}83 \pm 0{\cdot}08$ ($n = 50$)	$2{\cdot}09 \pm 0{\cdot}45$ ($n = 8$)
1·33	$1{\cdot}12 \pm 0{\cdot}06$ ($n = 50$)	$1{\cdot}75 \pm 0{\cdot}09$ ($n = 36$)

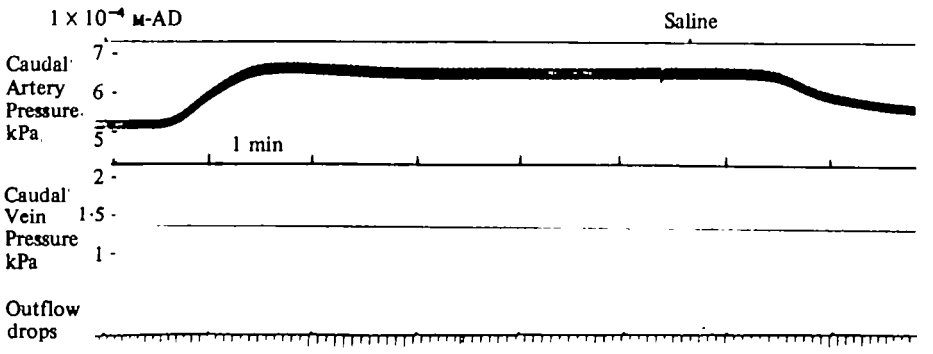


Fig. 2. Record of a response to infusion of 1×10^{-4} M adrenaline into a perfused eel tail. Tail resistance change = + 129·87%.

When the venous pressure was raised from zero to 1·33 kPa there was a significant drop in resistance of 38·6% ($n = 50$; $P < 0{\cdot}0001$, Student's t test). Addition of 5% serum to the perfusate increased resistance by 44·6% which was significant at the $P < 0{\cdot}0001$ level (Student's t test).

DOSE–RESPONSE CURVES FOR ADRENALINE, NORADRENALINE AND ISOPRENALINE

Cumulative dose–response curves were prepared for three sympathetomimetic drugs: adrenaline (AD), noradrenaline (NA) and isoprenaline. Adrenaline and noradrenaline both caused increases in resistance, and isoprenaline caused decreases in resistance at concentrations below 1×10^{-4} M. Threshold concentrations for AD and NA were between 1×10^{-10} and 1×10^{-9} M. The threshold for isoprenaline was between 1×10^{-11} and 1×10^{-10} M. A typical response to AD is presented in Fig. 2.

All doses of AD and NA injected as boluses caused resistance increases. All doses of isoprenaline tested in this manner caused decreases in resistance. It was not possible to prepare full dose–response curves for the bolus method since doses of 10 and 100 nmol of all drugs often initiated violent muscular contractions of the tail suspended in the bath. Skeletal muscle contraction in response to high concentrations and doses of drugs was noted throughout this study. Catecholamines can elevate the general level of excitability of the vertebrate nervous system, which could offer an explanation in terms of spontaneous motor discharges (see Vogt, 1973).

Dose–response curves for AD (Fig. 3), NA (Fig. 4*a*) and isoprenaline (Fig. 4*b*) below 1×10^{-4} were sigmoid. Adrenaline was 1·14–4·43 times more potent a vasoconstrictor than NA (see Table 2). Isoprenaline decreased resistance at concentrations below 1×10^{-4} M. Addition of 5% human serum to the perfusate had little effect upon the ED 50 of drugs.

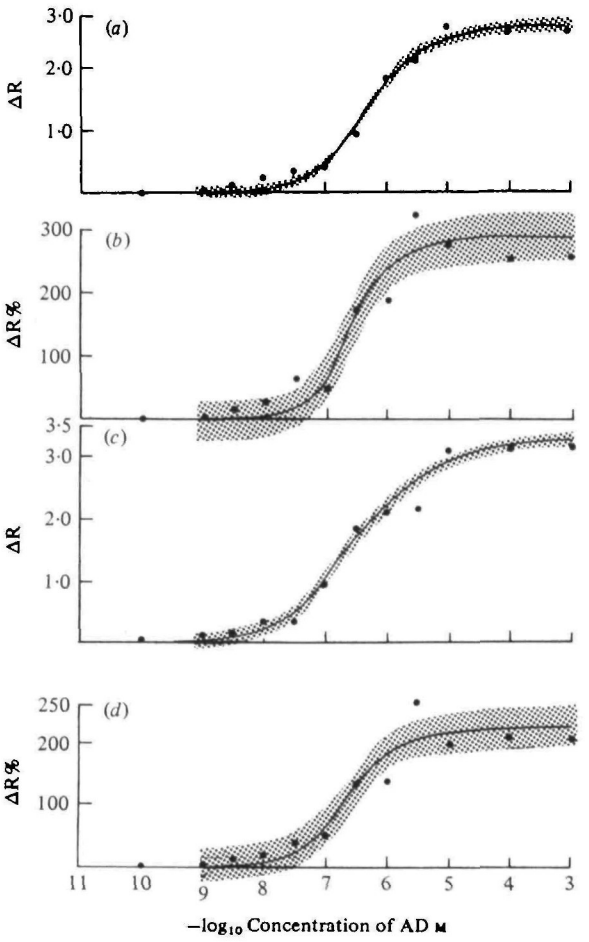


Fig. 3. Dose–response curves constructed from peak responses to infusion of 1×10^{-11} to 1×10^{-3} M adrenaline. Data from these curves are summarized in Table 2. All responses were recorded while perfusing at caudal venous pressures of 1·33 kPa. Stippled area indicates the 95 % confidence limits of the response estimates. (*a*). Responses expressed as kPa ml^{-1} min; no serum in the perfusate ($n = 6$). (*b*). Responses expressed as % changes in resistance over baseline resistance, $\Delta R\%$; no serum in the perfusate ($n = 6$). (*c*). Responses expressed as kPa ml^{-1} min. 5 % human serum added to the perfusate ($n = 12$). (*d*). Response expressed as % change in resistance over baseline resistance; 5 % human serum added to the perfusate ($n = 12$).

SYMPATHETIC ANTAGONISTS

To further elucidate the adrenergic control of the eel tail vasclature three adrenergic blocking agents were employed. Phentolamine and propranolol were the principal alpha and beta blockers respectively. Dichloroisoproterenol was occasionally used for beta blockade. Each of these antagonists acts in a competitive manner.

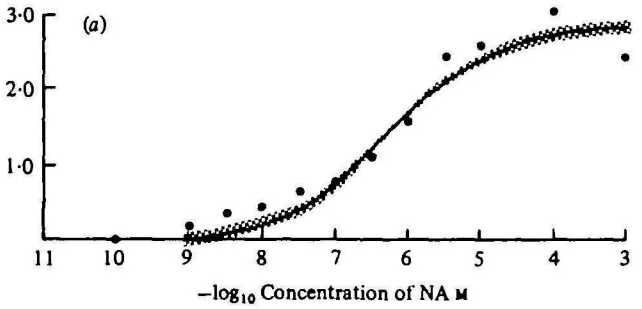
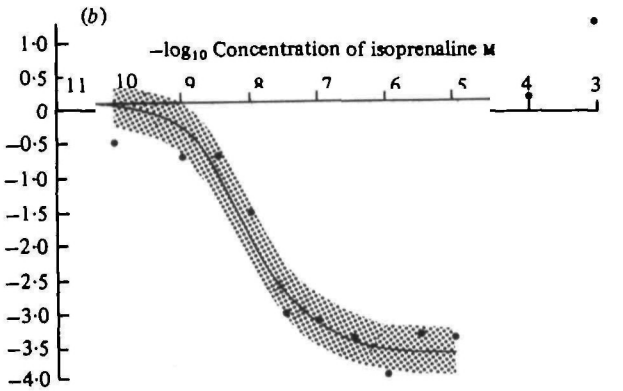


Fig. 4(*a*). Dose–response curves constructed from peak responses to infusion of 1×10^{-11} to 1×10^{-3} M noradrenaline. Data from this curve are summarized in Table 2. All responses were recorded while perfusing at caudal venous pressures of 1·33 kPa and without serum in the perfusate. Stippled area indicates the 95 % confidence limits of the reponse estimate. Responses expressed as kPa ml^{-1} min. ($n = 6$).



(*b*) Dose–response curve constructed from peak responses to infusion of 1×10^{-11} to 1×10^{-3} M isoprenaline. Data from this curve are summarized in Table 2. All responses were recorded while perfusing at caudal venous pressures of 1·33 kPa. Stippled area indicates the 95 % confidence limits of the response estimates. Responses expressed as kPa ml^{-1} min with 5 % serum added to the perfusate ($n = 9$).

Table 2. *Summary of results from dose–response curves.*

Drug	$\Delta R/\Delta R\%$	Serum no Serum	Maximum	ED 50 95 % confidence limits	Slope
AD	ΔR	no serum	2·640	$5{\cdot}152 \times 10^{-7}$ / $4{\cdot}16–6{\cdot}02 \times 10^{-7}$	9·935
	$\Delta R\%$	no serum	287·170	$2{\cdot}602 \times 10^{-7}$ / $1{\cdot}78–3{\cdot}09 \times 10^{-7}$	13·297
	ΔR	serum	3·285	$5{\cdot}157 \times 10^{-7}$ / $3{\cdot}71–7{\cdot}58 \times 10^{-7}$	6·733
	$\Delta R\%$	serum	225·966	$3{\cdot}042 \times 10^{-7}$ / $1{\cdot}99–3{\cdot}24 \times 10^{-7}$	10·577
NA	ΔR	no serum	2·942	$5{\cdot}887 \times 10^{-7}$ / $1{\cdot}12–6{\cdot}30 \times 10^{-7}$	6·172
	$\Delta R\%$	no serum	285·280	$1{\cdot}348 \times 10^{-6}$ / $1{\cdot}00–1{\cdot}90 \times 10^{-6}$	4·984
Isoprenaline	ΔR	no serum	−0·171	$1{\cdot}218 \times 10^{-8}$ / $1{\cdot}04–1{\cdot}44 \times 10^{-8}$	5·776
	$\Delta R\%$	no serum	−18·760	$8{\cdot}549 \times 10^{-9}$ / $6{\cdot}30–11{\cdot}21 \times 10^{-9}$	5·878
	ΔR	serum	−0·367	$9{\cdot}079 \times 10^{-9}$ / $6{\cdot}76–12{\cdot}58 \times 10^{-9}$	5·949
	$\Delta R\%$	serum	−22·018	$8{\cdot}194 \times 10^{-9}$ / $5{\cdot}12–12{\cdot}02 \times 10^{-9}$	6·584

ED 50 ratios	AD:NA	Potency ratio at 1×10^{-5} M	AD:NA
ΔR	1·00:1·141	ΔR	1·00:1·040
$\Delta R\%$	1·00:4·431	$\Delta R\%$	1·00:0·723

Resistance in kPa ml^{-1} min, concentrations in mol l^{-1}.

Phentolamine blockade of the response to adrenaline

Eight preparations, each of which had previously shown a decreased resistance response to isoprenaline, were given three low doses of AD (50, 100 and 200 pmol). Control responses were compared to the responses to the same doses of AD plus phentolamine. AD : phentolamine concentration ratios were varied between 1 : 1 and 1 : 1000 (Fig. 5). After blockade experiments, the control doses of AD were again administered and the responses were always diminished by 30–50% compared to the initial controls. This could result from either incomplete washout of phentolamine or the loss of sensitivity of the receptors to AD. Loss of sensitivity to AD was not apparent in the absence of phentolamine, especially at the low doses chosen specifically to avoid this problem. The other reason for using low doses of AD was that, in order to obtain AD to phentolamine concentration ratios of up to 1 : 1000, even at moderate doses, the large amounts of phentolamine necessary caused muscular contractions.

The increase in resistance caused by AD was diminished by phentolaime. At each of the three doses of AD used, the null response occurred at a concentration ratio of AD to phentolamine of 1 : 35 to 1 : 45, suggesting that this was the level of effective alpha blockade. At higher agonist to antagonist ratios, the response changed from an increase in resistance to a decrease. The magnitude of the falls in resistance in response

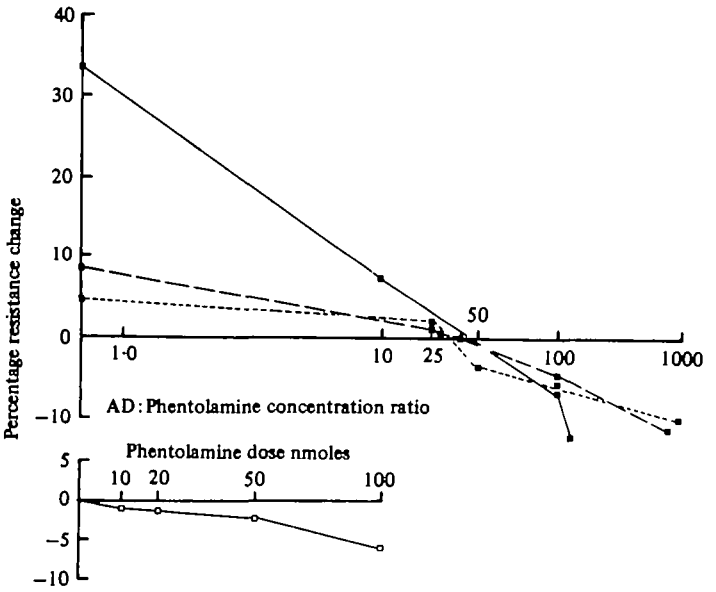


Fig. 5. Effects of increasing concentration ratios of phentolamine on the % resistance responses to 50, 100 and 200 pmol adrenaline. Responses to control doses of phentolamine are presented on the same graph. The constrictory responses to adrenaline at these doses were reversed to dilation at a concentration ratio of about 1:40 adrenaline: phentolamine. Dilator responses to adrenaline in the presence of the alpha blocker phentolamine may be partly due to the antagonist alone. —■—, 200 pmol AD; —■—, 100 pmol AD; - -■- -, 50 pmol AD; —□—, phentolamine.

to phentolamine alone were too small to account for the falls in resistance in response to AD plus phentolamine at concentration ratios of 1:45 or greater (see Fig. 5). These results demonstrate AD mediated vasodilation during alpha blockade.

Propranolol blockade of the response to isoprenaline

Propranolol diminished the size of the resistance fall elicited by isoprenaline in all eight preparations tested. Administration of a bolus containing 200 pmol of isoprenaline plus propranolol at concentration ratios of 1:1–1:20 reduced the magnitude of the responses by 40–70%. The residual dilator responses at high concentrations of propranolol could have been due to propranolol alone, since control doses of propranolol elicited small falls in resistance. The small falls in resistance caused by propranolol alone rapidly returned to the baseline resistance values (< 5 min). Injection of propranolol at 10 min before injections of isoprenaline (200 pmol) reduced the magnitude of the dilator responses to isoprenaline by 90–97% at concentration ratios of 1:20. Propranolol had a greater inherent dilator activity than dichloroisoproterenol in this preparation, but was the more potent antagonist.

Propranolol and the response to adrenaline

The response to AD is assumed to be the sum of opposed alpha (constrictor) and beta (dilator) responses. Blockade of the beta component should cause enhanced constrictor responses to AD.

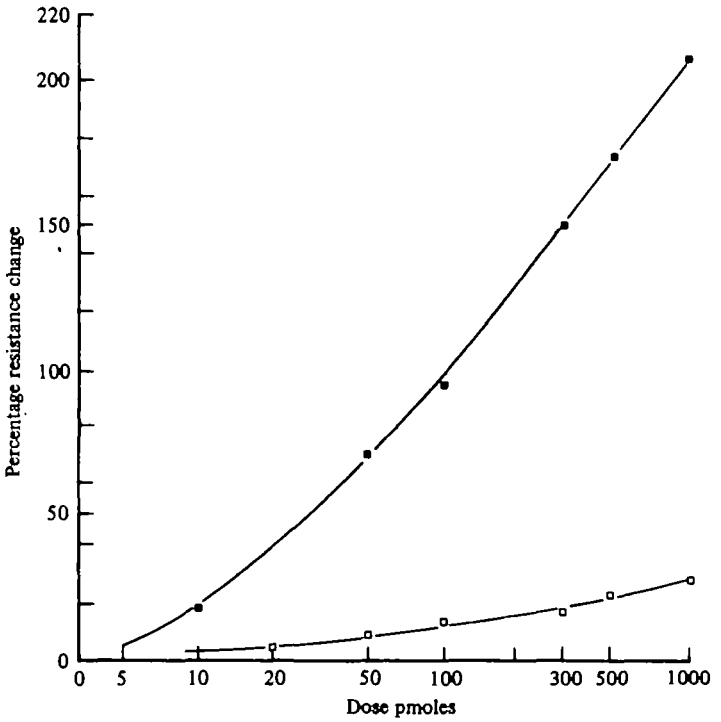


Fig. 6. Effects of propranolol, a beta antagonist, on the responses to adrenaline. Propranolol diminished the constrictor responses to adrenaline when compared to the control responses from the same preparations. Propranolol was administered at 100 times the concentration of adrenaline simultaneously with adrenaline. —■—, Control responses; —□—, AD plus propranolol.

Nine preparations were used to study the effects of propranolol on the responses of the eel tail to AD. Propranolol and AD were administered either as a single bolus or the propranolol bolus preceded AD injection by 5 min, the time taken for recovery from the response to propranolol alone. These methods always produced a smaller rather than larger increase in resistance. The dose of AD was varied between 10 pmol and 1 nmol at concentration ratios of AD to propranolol from 1 : 5 to 1 : 100, yet the nature of the response remained the same, namely a depression of the resistance increase caused by AD. Fig. 6 illustrates blockade of the constrictor responses to AD at a concentration ratio of 1 : 100, AD: propranolol.

These results are at variance with the proposed hypothesis for the action of AD in this vascular bed and will be discussed later.

To examine these responses further, five preparations were perfused with 5×10^{-7} M AD for 10 or 20 min. After this period the perfusate was immediately changed to the following solutions, each perfused for 10 min:

5×10^{-7} M AD plus 5×10^{-7} M propranolol (1 : 1),
5×10^{-7} M AD plus $2{\cdot}5 \times 10^{-6}$ M propranolol (1 : 5),
5×10^{-7} M AD plus 5×10^{-6} M propranolol (1 : 10),
5×10^{-7} M AD.

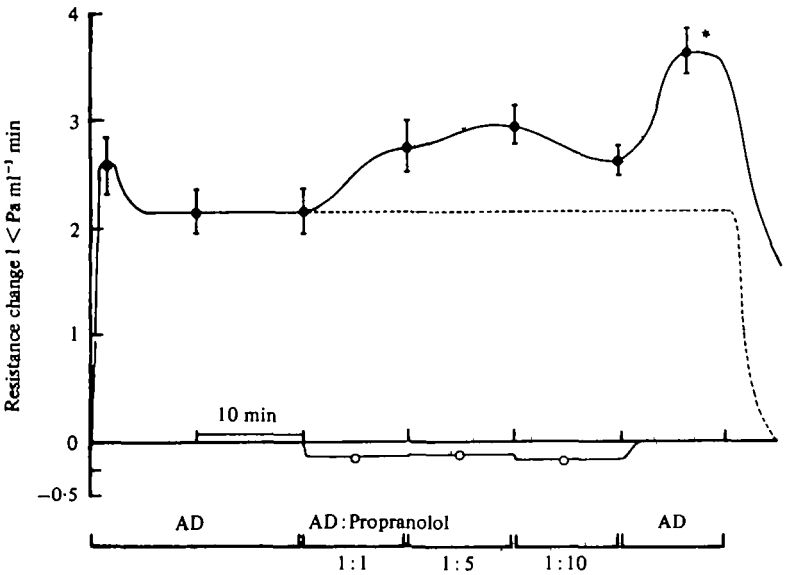


Fig. 7. Mean resistance responses to infusion of 5×10^{-7} M adrenaline followed by 5×10^{-7} M adrenaline plus propranolol at concentrations of 5×10^{-7} M, $2{\cdot}5 \times 10^{-6}$ M and 5×10^{-6} M followed by 5×10^{-7} M adrenaline again. Responses to propranolol at the concentrations used are displayed on the same figure. Error bars are ± 1 S.E.M. ($n = 5$). Student's t test between mean responses to each drug solution combination and the initial control response to adrenaline alone showed that only during infusion of adrenaline (*) after infusion of adrenaline with propranolol was there a significant increase in the response ($P < 0{\cdot}01$). —●—, Resistance responses throughout experimental series of drugs; - - -, represents the expected response in the absence of propranolol; —○—, resistance responses to control doses of propranolol.

The elevated resistance level caused by AD rose higher in the presence of 1 : 1 and 1 : 5 AD to propranolol. However, when 1 : 10 AD to propranolol was infused, resistance fell to a level near that of the response to 1 : 1 AD to propranolol (see Fig. 7). The increased constrictor responses to AD in the presence of propranolol are against the inherent dilator activity of propranolol controls (see Fig. 7). When perfusate with only AD was infused, the greatest resistance increase was observed. This peak was significantly higher than the control resistance increase ($P < 0.01$, Student's t test). This response peaked at about 330 s after propranolol was removed.

DISCUSSION

The eel tail was chosen for this study for many reasons. The tail consists mainly of muscle and excludes the viscera. It represents about 45% of the total body weight of the animal, and because of its bulk is likely to have significant effects upon the circulation of blood in the animal as a whole. This eel-tail preparation was easily repared and remained responsive for more than 6 h. The eel-tail preparation was well perfused as indicated by an X-ray study and had a circulation time of 60–90 s.

Pressure differential/flow profiles

A progressive fall in vascular resistance was observed for the first 5–10 min of perfusion, as evident from the downturn of the profiles at high initial pressures. This effect was observed in all profiles when a period of low flow intervened. It was, however, much smaller at the start of the second profile, which was usually performed at an elevated venous pressure. The initial high resistance of the eel tail is interpreted as the result of near zero luminal pressures during preparation of the tail for perfusion when vessels could have collapsed. When perfusion was resumed the vasculature was in a state of constriction or collapse which was rapidly dispelled in the presence of 'normal' luminal pressure.

Flow was significantly greater ($P < 0.005$-0.001; Student's t test) when venous pressure was raised to 1·33 kPa in preparations perfused with the basic saline. In the presence of serum none of the differences in flow at any pressure differential was significant. Addition of 3% human serum to the perfusate resulted in smaller changes in flow when venous pressure was raised.

There was no significant difference between the resistance calculated from vertical tube perfusion at the flow rate later used for pump perfusion of the same preparations, and baseline resistance for pump perfusion. Addition of serum to the perfusate reduced the effects of different methods of perfusion upon estimates of vascular resistance in this preparation. Addition of serum to the perfusate imparted a degree of vascular autoregulation to this preparation that was otherwise absent.

Baseline resistance and venous pressure

Mean pressure differentials across eel tails were between 1·75 and 2·17 kPa (see Table 1). Davie & Forster (1980) found tail pressure differentials (dorsal aorta–caudal vein) of 1·97–2·29 kPa in unrestrained unanaesthetized short-finned eels of slightly larger size. Martel & Cech (1978) reported tail pressure differentials (caudal artery–caudal vein) of 2·0 and 2·8 kPa in anaesthetized and conscious winter flounders (*Pseudopleuronectes americanus*). Wood, McMahon & McDonald (1977) found mean tail pressure differentials of 2·17 kPa in starry flounders (*P. stellatus*). Caudal venous pressures recorded from live fish provide an *a posteriori* rationale for setting the caudal venous pressure of the preparations to 1·33 kPa for the pharmacological studies. Tail-pressure differentials from perfused eel tails reported here are in good agreement with those from live intact fish. This is in distinct contrast to results from perfused trout-trunk preparations (Wood & Shelton, 1975), where the pressure differential across the trunk of the trout was much lower than that measured *in vivo*.

The fall in resistance associated with increased venous pressure was probably due to distension of the vessels by increased luminal pressure (Folkow & Löfving, 1957; Wood, 1974).

Adrenergic receptors of the eel tail

The alpha adrenergic receptors of the eel-tail show classical potencies to sympathetomimetic drugs of AD> NA≫ isoprenaline (Ahlquist, 1948; Furchgott, 1967). The mean potency ratio of AD to NA of 2·78:1·00 is close to that reported for mammalian

alpha receptors of 3·2 : 1·0 (Furchgott, 1967) and to those reported from rainbow trout (see Wood & Shelton, 1975; Wood, 1976).

Data points of the NA curves show a marked deviation from sigmoidicity at higher concentrations (see Fig. 4*a*). At 1×10^{-4} M the response is above the estimated asymptotic maximum, while at 1×10^{-3} M it is below the asymptotic maximum. This behaviour suggests that only a small proportion of beta receptors were stimulated by NA at concentrations below 1×10^{-3} M, and that the response was almost entirely due to alpha stimulation. At higher concentrations (1×10^{-3} M) some beta stimulation is evident. When this is superimposed upon maximal alpha stimulation, the result is a downturn in the dose–response curve. Adrenaline, on the other hand, elicited significant beta activity at much lower doses, certainly at 5×10^{-7} M, causing the dose–response curve to be depressed along its entire length. The downturn at high concentrations of AD is not so evident but is still discernible (see Fig. 3 *b d*). This indicates that AD is a more potent beta agonist than NA in this preparation, therefore the beta receptors, by definition, must be of the beta-two variety (Ahlquist, 1948; Furchgott, 1967; Arnold, 1972; Wood, 1976).

The greater beta activity of AD seems likely to be the cause of the lower maximum constriction when compared to NA (2·64 kPa ml^{-1} min (AD); 2·94 kPa ml^{-1} min (NA)). Despite the greater AD response depression by beta stimulation, the ED 50 of AD is lower than that of NA.

Examination of the ED 50 concentrations for AD and NA from Wood & Shelton (1975) shows that around ten times the ED 50 of the eel tail was required to elicit half-maximal responses from the perfused rainbow trout trunk. That the eel tail is an order of magnitude more sensitive to both AD and NA is of interest. Helical strips of coeliac artery from rainbow trout gave ED 50s of $1{\cdot}1 \times 10^{-8}$ M (AD) and $2{\cdot}2 \times 10^{-8}$ M (NA) (Holmgren & Nilsson, 1974), which are about one-fifteenth of the ED 50 concentrations for the perfused trout trunk. Holmgren & Nilsson (1974) also gave ED 50 concentrations for cod coeliac artery strips of $3{\cdot}7 \times 10^{-7}$ M (AD) and $8{\cdot}4 \times 10^{-7}$ M (NA), which are close to those from the perfused eel tail. The poor agreement of data from the rainbow trout, outlined above, could simply be the result of the different preparations, although cumulative addition procedures were used in both studies. Restraint, therefore, should be exercised when comparing the results from different preparations from the same species, let alone different species.

Eel-tail alpha receptors exhibited significant activity in response to isoprenaline at high concentrations (see Fig. 4b). The change of direction of the response (to constriction), at isoprenaline concentrations above 1×10^{-4} M in the eel tail, agrees with concentrations at which similar changes in direction have been observed in mammalian preparations (Jenkinson, 1973). Systemic responses to isoprenaline during beta blockade by propranolol were reported by Helgason & Nilsson (1973) in the cod and by Chan & Chow (1976) in Asiatic eels. No alpha activity in response to isoprenaline was described from the trout trunk by Wood & Shelton (1975) or Wood (1976), despite very high concentrations of isoprenaline (up to 1×10^{-1} M). It is clear that the trout systemic alpha receptors described by Wood & Shelton (1975) and Wood (1976) are sufficiently different from other teleost and mammalian receptors that only limited comparisons should be made.

As no isoprenaline dose–response curves from teleost systemic preparations have

been described, it is difficult to compare effective doses of isoprenaline. Previous reports of systemic beta activity have indicated effective doses of tens or hundreds of micromoles (Chan, 1967; Chan & Chow, 1976), or concentrations of about 1×10^{-5} M (Stray-Pedersen, 1970; Holmgren & Nilsson, 1974; Wood, 1976). It is possible that these workers used supramaximal doses of isoprenaline, thereby masking the dilator responses.

Comparison of ED 50 and maximum resistance changes in response to isoprenaline from trout gills (Wood, 1974) with the data presented here shows that effective doses are similar but that trout gills have a greater inherent dilator potential.

Antagonists

Phentolamine was a highly specific alpha antagonist in the eel tail. Propranolol was a potent beta antagonist. Effective blockade of the alpha responses to AD by phentolamine was achieved with agonist to antagonist ratios of around 1 : 40, which is similar to effective ratios in other teleost systemic preparations (see Forster, 1976 *b*). Propranolol produced effective beta blockade at ratios of isoprenaline to propranolol of around 1 : 20, which is similar to ratios for mammalian beta two receptors (Osnes, 1976).

The response to AD is assumed to be the sum of opposed alpha (constrictor) and beta (dilator) responses. Blockade of the alpha component by phentolamine failed to reveal substantial beta activity. Blockade of the beta component of the AD response was expected to reveal enhanced vasoconstriction. The results from administration of AD and propranolol in single boluses (see Fig. 6) showed diminished, rather than enhanced, constriction at concentration ratios of between 1 : 1 and 1 : 1000. Clearly either propranolol elicited large beta responses or it blocked the alpha responses. The falls in resistance in response to propranolol controls were too small to account for the reduced responses to AD. Furthermore the qualitative features of the responses to propranolol controls (time course; shape of the trace) suggested that the response was due to alpha blockade rather than beta stimulation. Kirby & Burnstock (1968) and Holmgren & Nilsson (1974) have reported significant alpha blockade by propranolol in teleosts. Thus in the eel tail, propranolol shows partial alpha as well as beta antagonism.

When propranolol was administered in the perfusion line with AD, constriction was enhanced. The nature of the results portrayed in Fig. 7, however, requires further explanation. The increase in the constrictor response to AD in the presence of 5 and 25×10^{-7} M propranolol (1 : 1 and 1 : 5 AD : propranolol) can be interpreted in terms of the above outlined hypothesis. However, at 1 :10 AD : propranolol, propranolol may displace some AD from the alpha sites, causing a smaller response than would be expected if propranolol showed no alpha antagonism. The time to peak response of 5×10^{-7} M AD was around 145 s and that for 5×10^{-7} M isoprenaline was around 300 s. Thus during perfusion with propranolol-free 5×10^{-7} M AD, any propranolol occupying alpha or beta sites was being washed out. As propranolol left the receptors, alpha or beta, it was replaced by AD. But because the constrictor response was more rapidly developed than the dilator response, there was a rise in resistance until the beta response developed. This explanation suggests that there is

a potential for AD mediated vasodilation in the eel tail of 40-50% of the magnitude of the alpha response to AD at this concentration.

Effects of serum on the dose–response curves for adrenaline and isoprenaline

Addition of 5% human serum to the perfusate enhanced the absolute responses to both constrictor (AD) and dilator (isoprenaline) sympathetomimetic drugs. This enhanced responsiveness is probably due to one or several of the actions of serum on vascular beds. For a discussion of the action of serum and serum proteins on vascular beds see Waugh & Shanks (1960) and Johnson (1964).

Without serum in the perfusate, preparations failed to develop their full alpha or beta potential to exogenous catecholamines, despite supramaximal doses. This questions the concept that the development of responses to active agents introduced via the perfusion line is limited by the rate of delivery or perfusion, rather than by diffusion across the capillary-cum-interstitial barrier (Mellander & Johansson, 1968).

The use of $\Delta R\%$ instead of ΔR appears unwarranted since error estimates for $\Delta R\%$ curves are proportionately larger than those of the ΔR curves (see Fig. 3).

Alpha and beta receptor functions

The hypothesis that teleost cardiovascular systems are controlled principally by alterations in concentrations of circulating catecholamines (Randall & Stevens, 1967: Burnstock, 1969) has received criticism in the light of reports of significant autonomic control of the heart (Campbell, 1970; Holmgren, 1977), blood pressure (Smith, 1978), and gill and systemic resistance (Holmgren & Nilsson, 1974; Wood, 1976; Pettersson & Nilsson, 1979). Although catecholamines alter vascular resistance, their actions may also be directed at other targets such as blood levels of free fatty acids and glucose (Mazeaud, 1972; Mazeaud, Mazeaud & Donaldson, 1977), trans-capillary exchange (Lundvall & Jarhult, 1976) and retention of toxic metabolites (Wardle, 1978). It is clear, however, that one of the principal effects of circulating catecholamines is to alter vessel calibre.

The function of the alpha constrictor response in the teleost systemic vasculature has been discussed by Wood & Shelton (1975) and Wood, McMahon & McDonald (1978). Systemic constriction elevates dorsal aortic blood pressure thereby increasing perfusion of the gills. Thus in stressful situations when plasma catecholamine concentrations rise (Nakano & Tomlinson, 1967; Nilsson, Abrahamsson & Grove, 1976; Mazeaud *et al.* 1977; Butler *et al.* 1978) cardiac output increases (beta-one stimulation), gill resistance decreases (beta-one stimulation), and dorsal aortic pressure rises, thereby increasing perfusion of the gills to meet the increased metabolic demands of stress. Each of these responses is aimed at increased gill perfusion.

The beta dilator response to catecholamines in the eel tail may simply be regulatory 'escape' from prolonged vasoconstriction (Folkow, 1964; Viveros, Garlick & Renkin, 1968; Rengo *et al.* 1976). However, within the eel tail there could be differential distribution of alpha and beta receptors as seen in the dogfish (Capra & Satchell, 1977). If alpha receptors were primarily located in the vasculature of white muscle, and beta receptors in the red muscle vasculature, then increases in plasma catecholamine levels could redirect blood from white to red muscles. Stevens (1968) reported no significant

redistribution of blood volume in swimming trout. However, Daxboeck (1978) has found significant increases in blood flow to the red muscles of trout during exercise.

Increases in systemic vascular resistance of teleosts by alpha adrenergic stimulation elevates dorsal aortic pressure and may serve to decrease branchial resistance. Decreased systemic resistance caused by beta-two stimulation may represent regulatory 'escape' from prolonged vasoconstriction. It is possible, however, that the beta-two dilator response, as observed in the eel tail, redirects blood flow to the red muscles in the tail.

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