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VASOCONSTRICTORY EFFECTS OF ADRENALIN ON THE PERFUSED HEAD OF THE EEL (*ANGUILLA ANGUILLA* L.)

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Abstract—1. Adrenalin (5.0×10^{-5} M) increases gill vascular resistance up to $23.2 \pm 6.8\%$ in the isolated and perfused head of the european eel (*Anguilla anguilla* L.).

2. This vasoactive response is nearly abolished by the α -adrenergic blocking agent phentolamine mesylate (5.0×10^{-5} M), while it is increased by the β -blocker propranolol (5.0×10^{-4} M).

3. From these results it may be inferred that, in our experimental conditions, the major response to the catecholamine is a vasoconstriction mediated via α -adrenoceptors.

INTRODUCTION

In fish gills are the main junction between the external and internal environment, allowing the achievement of two discrete functions: the exchange of respiratory gases, and the maintenance of osmotic balance. As a result, the control of blood flow through the branchial vascular district must be, at the same time, effective and flexible, capable of adapting, as far as possible, the external conditions to the changes of the external environment (Johansen, 1971; Potts, 1977).

The contribution of catecholamines to gill vessels homeostasis has been extensively investigated, and some of their physiological roles assessed (Wahlquist, 1980). Adrenalin is synthesized in chromaffin tissue in the head kidney and can be released into the circulation by the stimulation of adrenergic sympathetic fibres (Nilsson *et al.*, 1976). A large number of studies show its vasodilatory effect on the branchial vascular bed, mediated *via* β -receptors, opposite to the α -adrenergic constrictory one, showed in the systemic circulation (Pettersson and Johansen, 1982).

The presence of adrenoceptors of the α -type in the branchial vascular smooth muscle is also reported (Wood, 1974; Smith, 1977; Pettersson and Nilsson, 1979), and is suggested to produce vasoconstriction. Besides, a slight increase in vascular resistance has been sometimes reported to precede the stronger and longer lasting dilatation (Wood, 1975; Payan and Girard, 1976; Wahlquist, 1980).

In contrast with these data, Wahlquist (1980) observed an appearance of occasional constrictory responses to adrenalin in the cod (*Gadus morhua*); more complete evidence of this uncommon phenomenon has been given by Pärt *et al.* (1982) on the rainbow trout (*Salmo gairdneri* Rich). These conflicting results prompted this study, carried out on the european silver eel (*Anguilla anguilla* L.).

MATERIALS AND METHODS

Male specimens of *A. anguilla* L., aged 8–14 years and weighing between 75 and 120 g, were obtained in November

1982 from local dealers. The fishes were kept in circulating tap water (water temp. 16–21°C) until used.

The isolated and perfused head was prepared according to Payan and Matty (1975), with minor modifications.

The ventral body wall was opened and the heart exposed; heparin (1000 U.I.) was injected into the ventricle and let flow through the gills. The fish was decapitated behind the pectoral fins and a polyethylene catheter inserted into the ventral aorta through the bulbus arteriosus.

The head was put in a plastic box filled with circulating water: during preparation and experiment gills were ventilated with tap water (temp. 16–21°C; pH 6.8; flow 600 ml/min) continuously bubbled with O₂ 95% + CO₂ 5%.

The perfusion was carried out by means of a peristaltic pump with a steady flow of 3.5 ml/min/100 g fish, resulting in a perfusion pressure close to that reported by Mott (1950).

Freshwater Telecost Ringer solution (NaCl 0.11 M; KCl 0.0018 M; NaHCO₃ 0.0002 M, CaCl₂ 0.0010 M) was used as perfusion medium (temp. 19–20°C pH 7.35–7.50) containing glucose (1 g/l) and heparin (1000 U.I.).

Adrenalin tartrate (ISM), phentolamine mesylate and propranolol HCl, obtained from usual commercial sources, were dissolved in saline, and added to the flow entering the gills by means of a micropump (flow rate 0.06 ml/min); preliminary experiments have showed that no detectable changes in perfusion pressure were caused by the additional flow.

The pressure in the ventral aorta was measured by a pressure transducer connected to a polygraph Beckman.

The gill resistance was calculated according to Pärt *et al.* (1982) from

$$R_g = \Delta P / Q,$$

where R_g = gill resistance; ΔP = drop in blood pressure through the gills and Q = perfusion flow (ml/min/100 g of fish). ΔP was made equal to ventricular pressure (P_v), since no systemic counter-pressure was applied in our system. P_v was calculated according to Pärt *et al.* from

$$P_v = P_v(\text{syst}) + 2/3 P_v(\text{diast}).$$

Experimental protocol

After cannulation, gills were perfused for an equilibration period of about 30 min before experiments were begun. In all experiments the following schedule has been followed: adrenalin (5.0×10^{-5} M), 15 min; recovery, 15 min; phentolamine (5.0×10^{-5} M) or propranolol (5.0×10^{-4} M),

Fig. 1. Effects of adrenalin perfusion (5.0×10^{-5} M for 15 min) on branchial vascular resistance of the eel *Anguilla anguilla* L. During the initial period (not shown) the head was perfused with Ringer solution only. Significance for paired comparisons: * $P < 0.05$, ** $P < 0.01$.

10 min; phentolamine or propranolol plus adrenalin, 10 min.

RESULTS AND DISCUSSION

In our experimental conditions the basal values of gill vascular resistance (R_g) showed marked variation, with a mean value of 33.7 ± 7.38 ($n = 12$). R_g increased by 27.2 ± 6.8 ($n = 12$) within the first 5 min of perfusion with 5.0×10^{-5} M adrenalin (Fig. 1). Later R_g decreased and established at a value 30.0 ± 7.5 ($n = 10$) above the basal level. Previous experiments performed with a lower adrenalin concentration (5.0×10^{-6} M) have occasionally shown analogous results, but more frequently, neither vasoconstriction, nor dilatation occurred.

One case differed from all others, resembling the constriction-dilatation pattern already observed by several Authors (see Introduction).

During administration of the α -blocker phentolamine mesylate (5.0×10^{-5} M), the response to adrenalin was almost abolished (Fig. 2A) suggesting an involvement of α -adrenoreceptors in the evocation of the effect. The marked increase in R_g following perfusion of adrenalin with the β -antagonist propranolol (5.0×10^{-4} M plus 122% of the response to adrenalin alone) showed an implication of the β -receptors also (Fig. 2B).

These data oppose the major part of the results earlier observed, while agreeing with those obtained by Pärt *et al.* (1982). Pärt *et al.*, using an experimental system very similar to ours, in the study of rainbow trout (*Salmo gairdneri* Rich.) gills have found an analogous increase in R_g after administration of adrenalin (1.0×10^{-6} M). Discussing the results they have suggested "the time of the year, winter, and the water temperature" (10°C) being an explanation of their data.

It is worthwhile to stress the differences between

thermic and seasonal effects: in the former the variation in reactivity would be ascribable to a direct action of the environment, i.e. a change in temperature, while in the latter it would be due to endogenous rhythms, not directly dependent on external influence.

Moen *et al.* (1981) have found that the systemic α -response of *S. gairdneri* Rich. is highest at the normal habitat temperature of the fish ($5-8^\circ\text{C}$): this may support the hypothesis of a dependence on temperature of α - or β -adrenergic prevalence in branchial vascular control.

Some objections might be raised: Pettersson and Johansen (1982) have given one more evidence of the vasodilatory properties of adrenalin in rainbow trout gills, at the temperature of 10°C (season not reported), namely the same used by Pärt *et al.* (1982) with contrasting results.

One of the classical models of temperature dependent β - α conversion has been, in the past years, the adrenoceptor population of frog myocardium (Buckley and Jordan, 1970; Harry, 1972; Kunos and Nickerson, 1976; Nickerson and Kunos, 1977). But this hypothesis has been doubted by a growing series of findings (Stene-Larsen and Helle, 1979; Ask *et al.*, 1981; Ask, 1983). The lacking of a model of temperature-dependent conversion is of importance. On the other hand, the idea of seasonal, temperature-independent variations is sustained by experimental facts.

In several works, Peyraud-Waitzenegger *et al.*, using animals taken at constant temperature (Waitzenegger and Serfaty, 1967; Peyraud-Waitzenegger, 1972; Peyraud-Waitzenegger *et al.*, 1980) have given evidence of season-dependent changes in adrenergic ventilatory control in the carp (*Cyprinus carpio*) and in the eel (*Anguilla anguilla* L.); according to these Authors "L'effet α caractérise les périodes d'hypoactivité métabolique", winter and autumn (Peyraud-Waitzenegger, 1972).

Fig. 2A.

Fig. 2B.

Fig. 2. Comparison of the vasoactive effects of adrenalin perfusion (5.0×10^{-5} M for 15 min) alone and in the presence of the α -blocker phentolamine (5.0×10^{-5} M) (A) and of the β -blocker propranolol (5.0×10^{-4} M) (B). Propranolol alone gave an increase in R_g up to 31.7 ± 11.3 ($n = 5$).

Table 1. Effects of adrenalin, phentolamine and propranolol

Adrenalin		Phentol.		P vs Cont.		P vs Aadr.		P vs cont.		P vs Aadr.		P vs Phent.	
Time	mean ± SEM	n	mean ± SEM	n	mean ± SEM	n	mean ± SEM	n	mean ± SEM	n	mean ± SEM	n	
5'	27.2 ± 6.8	5	9.4 ± 3.3	5	NS	NS	9.2 ± 3.2	NS	NS	NS	NS	NS	
10'	30.0 ± 7.5	5	5.6 ± 4.6	5	NS	NS	5.8 ± 5.1	NS	NS	NS	NS	NS	
Adrenalin		Propranolol		Prop. ± Adren.		Prop. ± Adren.		Prop. ± Adren.		Prop. ± Adren.		Prop. ± Adren.	
mean ± SEM		mean ± SEM		mean ± SEM		mean ± SEM		mean ± SEM		mean ± SEM		mean ± SEM	
5'	16.8 ± 3.7	7	31.7 ± 11.3	7	<0.05	NS	63.7 ± 10.5	<0.01	<0.01	<0.05	<0.05	<0.05	vs prop.
10'	32.1 ± 7.4	7	42.1 ± 14.5	7	<0.01	NS	64.1 ± 13.0	<0.01	<0.01	<0.05	<0.05	NS	

Significance has been calculated by means of the Student's *t* test for paired data.

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