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NERVOUS CONTROL OF THE BLOOD PRESSURE IN THE ATLANTIC COD, *GADUS MORHUA*

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SUMMARY

Dorsal (PDA) and ventral aortic blood pressure (PVA) and heart rate (HR) were measured in conscious resting cod, *Gadus morhua* L., which has been allowed 24 h recovery from surgery. Plasma adrenalin and nonadrenalin concentrations in these fish were 3·4 and 2·2 nmol l^{-1} respectively, and thus lower than previously reported values from partially recovered cod. Twenty-four hours after treatment with the adrenergic neurone blocking agent bretylium, PDA was significantly reduced by 17 % compared to sham-injected controls, although PVA and heart rate were unaltered. Subsequent α-adrenoceptor blockade by phentolamine produced no further fall in PDA and no changes in PVA or HR, provided a 5-h period was allowed to overcome the acute toxic side effects of phentolamine. The effectiveness of the bretylium or phentolamine blockade was confirmed by noting the absence of any vasoconstrictor response during sympathetic nerve stimulation in perfused tails from fish used in the *in vivo* experiments.

Bretylium had no significant effect on the sensitivity of the isolated coeliac artery to adrenalin, but effectively blocked the adrenergic innervation of this artery or the vasculature of the tail. Evidence for a non-selective blockade of non-adrenergic nerves to the heart was also obtained.

It is concluded that the adrenergic tonus affecting the dorsal aortic blood pressure in resting cod that have recovered for 24 h following surgery is due solely to an adrenergic nervous tone.

INTRODUCTION

The importance of autonomic nerves in controlling the vascular resistance in fish has only recently become apparent. In his review, Campbell (1970) noted that there

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was 'still no report of a direct effect of autonomic nerve stimulation on the resistance of any vascular bed in fishes'. The consensus at that time was that generalized control of the vascular resistance was brought about by the actions of catecholamines released from adrenal chromaffin tissue (Randall & Stevens, 1967; Campbell, 1970), a view that contrasted sharply with that accepted for mammals (e.g. Folkow & Neil, 1971).

There are now numerous reports of vasomotor responses to autonomic nerve stimulation in many species of fish (see review by Nilsson, 1983) but these are mostly restricted to investigations of the *in vitro* responsiveness of various isolated perfused vascular beds: few attempts have been made to assess the relative importance of nervous factors (such as an adrenergic vasomotor innervation) compared to humoral factors (such as circulating catecholamines) in the control of arterial blood pressure in conscious fish, or to determine how regional redistributions of blood flow are achieved.

In the rainbow trout, Wood & Shelton (1975, 1980) and Smith (1978) have concluded that the arterial blood pressure is elevated by the tonic influence of adrenergic vasomotor fibres. In the cod, on the other hand, a pharmacological analysis of the nature of the adrenergic tonus affecting the blood pressure was interpreted in favour of an influence of circulating catecholamines (Wahlqvist & Nilsson, 1977). In the eel (*Anguilla australis*), finally, it appears that neither neural nor humoral catecholamines are used to maintain the systematic or branchial vascular tonus (Hipkins, Smith & Evans, 1985).

The interpretation of the previous experiments is difficult because the specific action of the drugs ('chemical tools') used in the studies has not been sufficiently established in the animal studied. The interpretation of pharmacological actions *in vivo* is particularly hazardous, and careful studies of the effects of new drugs both *in vitro* and *in vivo* must be made to establish the specificity and selectivity of the drug actions.

Further complications for a discussion about the nature of the adrenergic vasomotor tonus in fish come from obvious species differences, but also from differences in the experimental protocol between different investigations. The time elapsed from the surgical anaesthesia to the experiment appears to be of particular importance in this respect, and it is also important to control the levels of 'stressful stimuli' (sound, light, water movemtents, O_2-level, temperature, etc.).

The present investigation was designed to clarify the nature of the adrenergic tonus affecting blood pressure in resting chronically-catheterized cod that had been allowed 24 h recovery from anaesthesia. The experimental design involved the comparison of the blood pressure reduction obtained with bretylium, a drug known from mammalian studies to impair the function of adrenergic neurones, with that obtained with the α-adrenoceptor antagonist phentolamine, which could be expected to block the vasoconstrictor effect of *both* adrenergic nerves and circulating catecholamines. Since no critical examination of the mechanism of action of bretylium in fish exists, a number of experiments were designed to clarify the acute and chronic effects of bretylium *in vitro* and *in vivo*, particularly the ability of this drug to select between a nervous and a humoral adrenergic tonus.

MATERIALS AND METHODS

The experiments were performed on Atlantic cod, *Gadus morhua*, caught off the Swedish west coast. The animals were of either sex with a body weight of 500–1100 g.

Before the experiments the fish were kept without feeding in large tanks containing filtered, recirculating sea water at 10 °C.

In vitro *experiments*

Coeliac artery preparations

Longitudinal preparations of the cod coeliac artery were suspended in organ baths in an O_2/CO_2 (97:3 %) bubbled cod Ringer's solution at 10 °C for recording of tension changes as described by Holmgren & Nilsson (1974).

The effect of bretylium on the adrenalin concentration/response curve was studied by obtaining cumulative concentration/response curves for adrenalin and adding bretylium (10^{-5} mol l^{-1}) 20 min before the second curve (cf. Holmgren & Nilsson, 1974).

In some experiments the coeliac artery was dissected out with its splanchnic nervous supply attached as described by Holmgren (1978). The longitudinal tonus changes of the artery produced by electrical stimulation of the splanchnic nerve with 1-min trains of pulses (10 Hz, 1 ms duration and 8 V) at 8-min intervals were recorded with a Grass FT 03 isometric transducer connected to a Grass Polygraph Model 7 (cf. Holmgren, 1978). To assess the effect of bretylium on the electrically stimulated preparation, the drug was added to the organ bath to give final bath concentrations of 10^{-6} or 10^{-5} mol l^{-1}.

Perfused innervated tail preparations

The isolated cod tail preparation was prepared for perfusion at room temperature (20–22 °C) from a constant pressure head as described by Wahlqvist & Nilsson (1981). The sympathetic chain was stimulated with 30-s trains of pulses at either 10 or 20 Hz, 1 ms duration and 15 V, and the changes in flow monitored as drop rate by a Grass PTTI photoelectric transducer/tachograph recording system (cf. Wahlqvist & Nilsson, 1981).

Isolated perfused heart preparation

The spontaneously beating cod heart was perfused at room temperature (20–22 °C) *in situ* as described by Holmgren (1977), and the heart rate recorded by a Grass tachograph/polygraph recorder system (cf. Holmgren, 1977). The right cardiac branch of the vagus ('vago-sympathetic trunk'), which runs along the duct of Cuvier, was stimulated by a Grass SD9 square wave stimulator *via* two platinum hook electrodes. The effect of vagal stimulation on the heart rate was studied by stimulating the nerve with 30-s trains of pulses (1 ms duration, 10 V) with 8-min intervals. The frequency of stimulation was chosen to produce a reduction in the heart rate by about 25–30 beats min^{-1}; this could be obtined with a frequency of 5–8 Hz. The effect of bretylium (10^{-6}–10^{-4} mol l^{-1}) on the cholinergic vagal inhibitory innervation of the heart was studied by dissolving the drug in the perfusion fluid.

Chronic in vivo *experiments*

Dorsal (P_{DA}) and ventral aortic blood pressure (P_{VA}), heart rate (HR) and transbranchial pressure difference (ΔPg) were recorded from conscious cod for up to 5 days following surgery. Throughout this period the fish were housed in a darkened, covered chamber supplied with aerated, recirculated sea water at 10–11 °C.

Implantation of catheters was made in the following manner. Fish were anaesthetized with tricaine methane sulphonate (MS 222-Sandoz; 80 mg l^{-1} sea water) until breathing movements had just ceased. They were then transferred to an operating table and artificially ventilated with sea water containing MS 222 (40–55 mg l^{-1}). The depth of the anaesthesia was such that occasional shallow breathing movements occurred in the fish during surgery. All fish recovered rapidly (< 5 min) when transferred to the experimental chamber.

P_{VA} was recorded from a catheter implanted occlusively in the afferent branchial artery of the 2nd or 3rd gill arch (Wahlqvist & Nilsson, 1977). The catheter was secured with a ligature around the arch. The efferent branchial artery of the same arch was exposed and catheterized occlusively to measure P_{DA}. Each catheter was secured with double skin sutures to prevent dislodgement during occasional struggling movements. The catheters were connected to Statham P23 BB pressure transducers calibrated against a static column of water. Pressures were displayed on a Grass Model 79 Polygraph. HR was derived from the pulsatile P_{VA} signal *via* a Grass 7P44 tachograph amplifier. ΔPg was estimated from $\bar{P}_{VA} - \bar{P}_{DA}$ where $\bar{P}$ was given by $(P_{systolic} + 2P_{diastolic})/3$.

Blood samples were drawn *via* the VA catheter and analysed for plasma catecholamines as detailed below. Drugs were dissolved in 0·9 % NaCl and injected *via* the DA catheter. At the end of the experiment the tails of some fish were prepared for perfusion as described above.

In order to determine the effectiveness of bretylium as an adrenergic neurone blocker when injected *in vivo*, a series of experiments was performed in which fish were anaesthetized and the VA catheterized as described above. The fish was allowed to recover for at least 1 h, and then injected *via* the VA catheter with bretylium (10 mg kg^{-1}). After 10 min (establishment of Phase I of the bretylium response, i.e. a marked hypotension, see Results section) the fish was killed and the tail prepared for perfusion as described above.

A second group of fish was treated with bretylium in a similar fashion and killed 24 h after the bretylium injection (Phase III in the bretylium response, a sustained hypotension, see Results section) and the tails prepared for perfusion as described. During these experiments the conscious fish were housed in dark chambers supplied with filtered recirculated sea water at 10 °C. Similar experiments were performed with fish that had been treated 5 h previously with phentolamine (2 mg kg^{-1}).

Data from fish treated with bretylium were compared with data from sham-injected fish at equivalent times following surgery. Comparisons were analysed by Student's *t*-tests for independent samples. Homogeneity of variances was checked by means of Cochran's test (F-ratio: Clarke, 1980). In sequential drug tests, where phentolamine was injected 24 h after a single dose of bretylium, the 'bretylium + 24 h' values of each variable were used as controls, against which the 'phentolamine + 5 h' value was compared. Because each fish formed its own control in this case, a 1-tailed *t*-test for paired data was used.

Analysis of the plasma catecholamines

Blood samples, taken at times indicated by points *A*, *B* and *C* in Fig. 5 (0·5 ml each), were drawn into a non-heparinized syringe and rapidly transferred to a glass

tube containing a 50 μl mixture of anticoagulant (EGTA; 90 mg ml^{-1}) and antioxidant (glutathione; 60 mg ml^{-1}). The tube was stoppered, inverted twice and rapidly transferred to a refrigerated centrifuge (+ 2 °C; Beckman Model J-21C) for centrifugation at 3000 rev. min^{-1} for 10 min. At least 250 μl of plasma was transferred to a clean sample tube, stoppered and stored at −70 °C until analysed. Analysis of adrenalin (A) and noradrenalin (NA) was performed by the radio-enzymatic method of Peuler & Johnson (1977) as modified by Eriksson (1981). This technique as used could detect A and NA concentrations down to 20 pg ml^{-1} and required only 100 μl plasma per sample.

Drugs

The drugs used in this study were adrenalin bitartrate, bretylium tosylate and phentolamine methanesulphonate.

RESULTS

In vitro *experiments*

The adrenalin concentration/response curve for the coeliac artery was not significantly affected by bretylium (10^{-5} mol l^{-1}; Fig. 1). However, the contractions of the coeliac artery induced by electrical stimulation of the splanchnic nerve were markedly reduced (mean reduction 75 ± 13 %; $N = 6$) by bretylium at 10^{-6} mol l^{-1} and

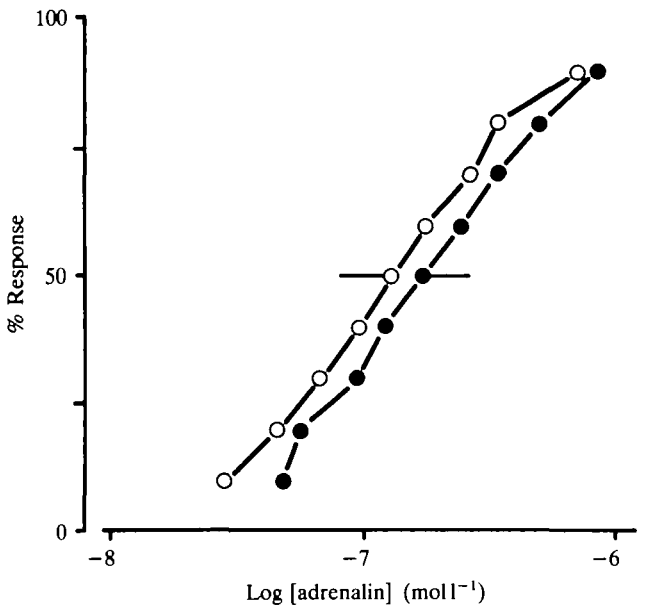

Fig. 1. Concentration-response curves for adrenalin acting on isolated coeliac artery strip preparations from the cod, *Gadus morhua*. ○, Adrenalin control curve; ●, adrenalin in the presence of bretylium (10^{-5} mol l^{-1}). The curves represent mean individual sensitivity (Ariëns & Simonis, 1961) from five preparations. Horizontal bars at the EC_{50} values indicate S.E.M.

invariably abolished if the bretylium concentration was raised to 10^{-5} mol l^{-1} ($N = 6$).

In the perfused tail preparation, stimulation of the sympathetic chain invariably caused a marked reduction in the perfusion flow (cf. Wahlqvist & Nilsson, 1981). The vasoconstriction was completely abolished by the addition of either phentolamine (10^{-7}–10^{-6} mol l^{-1}) or bretylium (10^{-6} mol l^{-1}) to the perfusion fluid. The vasoconstrictor response was also completely absent in fish pretreated *in vivo* with bretylium for 10 min ($N = 2$) or 24 h ($N = 4$) or phentolamine (up to 5 h; $N = 3$) as described.

The heart rate of the perfused heart preparation was 52 ± 1 beats min^{-1} ($N = 4$) under the conditions of the experiment. By stimulating the vagal supply to the heart a bradycardia was obtained which could be adjusted to reduce the heart rate by 25–30 beats min^{-1}. Addition of bretylium (10^{-6}–10^{-4} mol l^{-1}) produced a partial blockade of the bradycardia caused by nerve stimulation (Fig. 2).

In vivo *experiments*

In order to study the cardiovascular correlates of recovery from MS 222 anaesthesia and confinement for up to 5 days, cardiovascular variables were sampled from five fish at set intervals commencing 10 min after placing the fish in the experimental chamber.

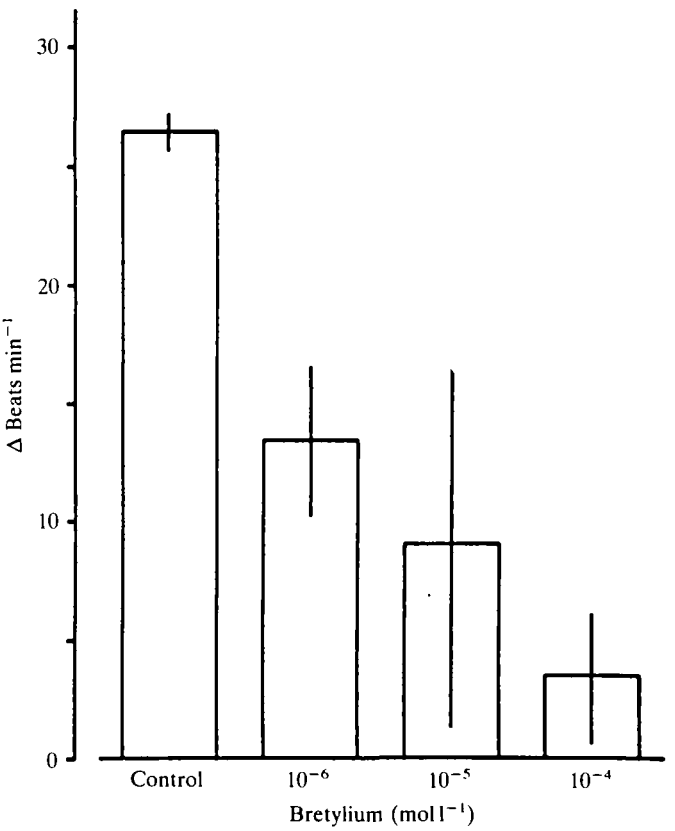

Fig. 2. Effect of bretylium (10^{-6}–10^{-4} mol l^{-1}) on the bradycardia (Δ beats min^{-1}) caused by electrical stimulation of the right vagus to the cod heart at 5–8 Hz, 1 ms pulse duration and 10 V. Note the concentration-dependent depression of the nerve response. Means ± S.E.M. ($N = 5$).

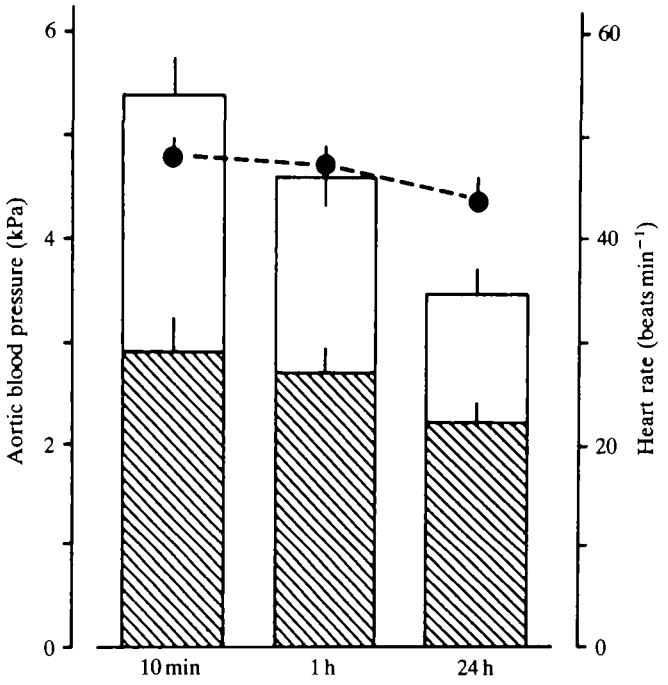

Fig. 3. Stabilization of ventral (PVA, clear bars) and dorsal (PDA, hatched bars) aortic blood pressures and heart rate (HR, broken line) following MS 222 anaesthesia and surgery in the cod, *Gadus morhua*. The figure indicates means ± S.E.M. of 11 fish. Blood pressures are expressed in kPa and heart rate in beats min^{-1}.

Initial blood pressures and heart rates were high, declining over the next 6–12 h to reach steady values (Fig. 3). The 24 h values for blood pressure and heart rate were then essentially unchanged over the next 4 days. Withdrawal of $3 \times 0{\cdot}5$ ml blood samples with saline replacement occasionally caused a small and transient (< 10 min) increase in PDA and sometimes in HR.

At the beginning of recording each morning, the initial pressures were somewhat higher than the maintained values from the previous day: this slight hypertension declined during the first 2 h and steady values were maintained for the rest of the daylight period. Even very slight disturbance of the fish, e.g. removal of the lid of the experimental aquarium, caused rather dramatic (although transient) cardiovascular effects (Fig. 4E). Sham injections had no effects on any of the variables recorded.

Effect of bretylium

There were no differences in the control values between the sham and experimental group for any of the variables recorded ($P > 0{\cdot}05$; 2-tailed t-test).

Following the injection of bretylium (10 mg kg^{-1}), most fish showed a characteristic sequence of changes in PDA and PVA (Figs 4, 5). Many fish were greatly distressed by the injection of bretylium, so the required dose was injected slowly over 5 min. Within 10 min of the injection, both blood pressures began to fall rapidly and the variability in the HR trace (though not HR itself) was greatly reduced. There followed

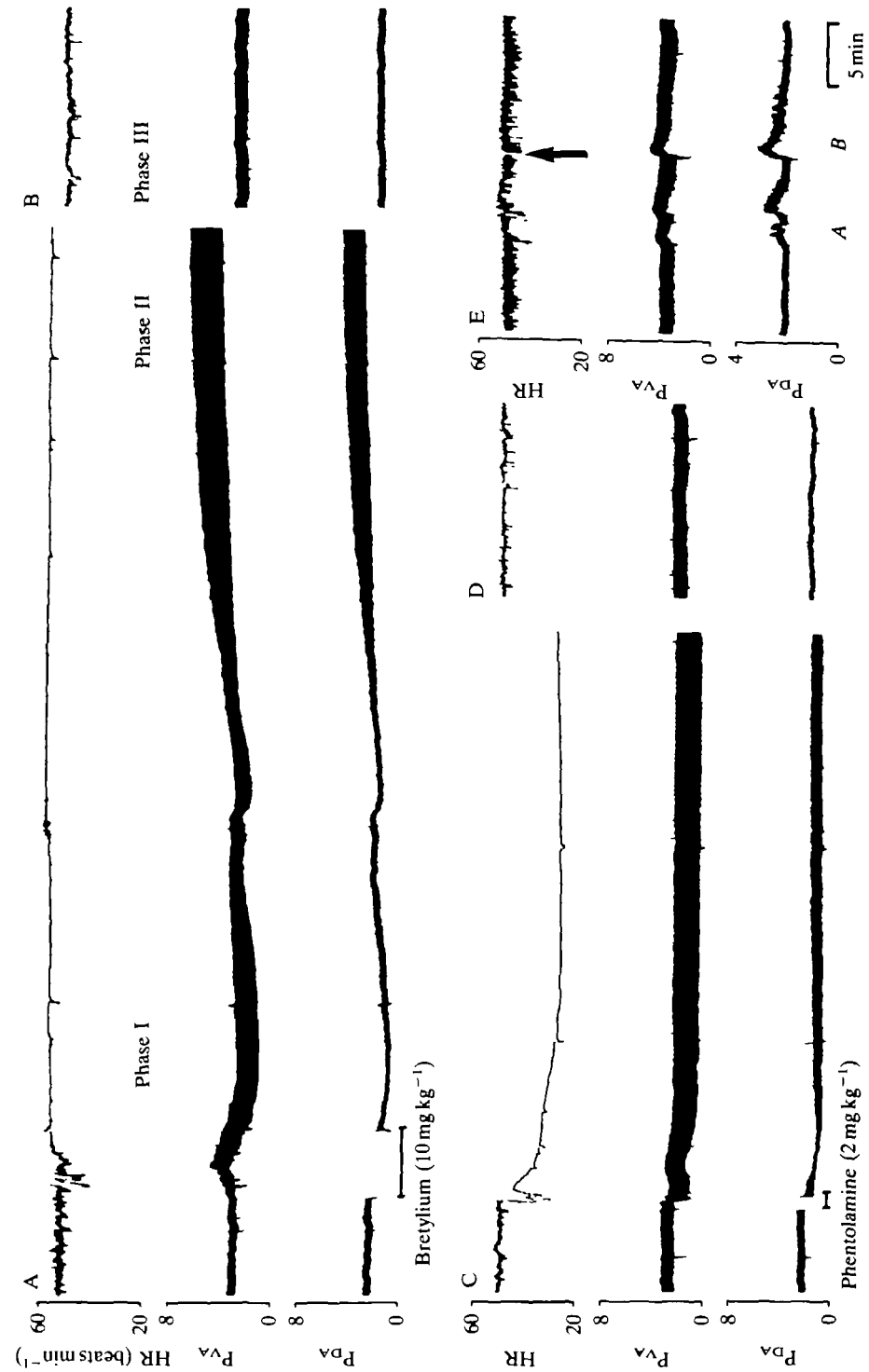

Fig. 4. Continuous recordings of P_{DA}, P_{VA} (kPa) and HR (beats min^{-1}) from conscious cod, *Gadus morhua*. (A) Injection of bretylium (10 mg kg^{-1}) 24 h after the end of surgery. Note the initial hypotension about 10 min after the bretylium injection (Phase I), and the secondary hypertension about 60 min after the injection (Phase II). (B) 22 h after the end of A (Phase III), a stabilization of the blood pressure below the pre-bretylium control values. (C) Same fish as in A and B, 24 h after the bretylium injection. Injection of phentolamine (2 mg kg^{-1}). Note the initial hypotension and marked bradycardia, which are likely to be due to a non-specific 'histamine-like' effect of phentolamine. (D) 5 h after the phentolamine injection, there is a stabilization of the blood pressures at a level similar to that prior to the phentolamine injection. (E) Transient changes in blood pressures (P_{DA} and P_{VA}) and heart rate (HR) caused by mild disturbance of a cod, *Gadus morhua*, 20 h post-surgery. At *A* the experimental aquarium was touched and the lid lifted and at *B* the water was gently stirred. Note the brief cardiac arrest arrowed in *B*.

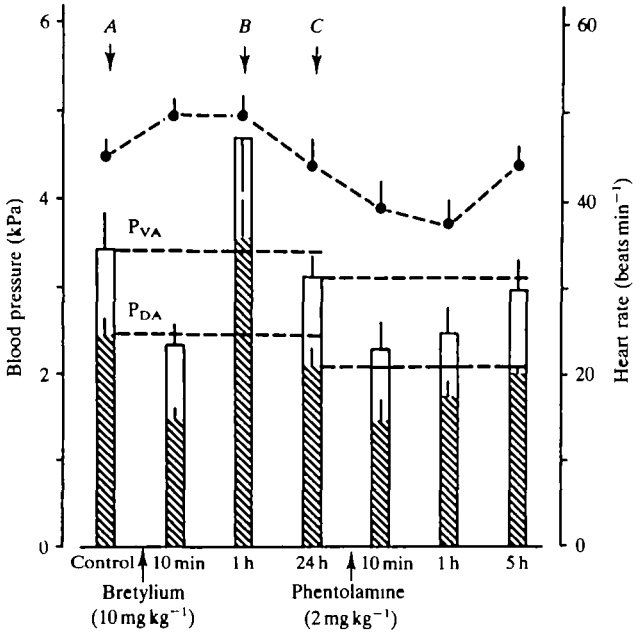

Fig. 5. Summary of the effects of bretylium (10 mg kg^{-1}) and phentolamine (2 mg kg^{-1}) injected into conscious cod, *Gadus morhua*, at points indicated on the figure. Note the initial hypotension after bretylium (Phase I) at 10 min, the secondary hypertension (Phase II) at 1 h and the stabilized hypotension at 24 h (Phase III). Plasma samples for catecholamine analyses were taken at points indicated by *A*, *B* and *C* in the figure. The broken line shows mean values for heart rate. P_{DA}, but not P_{VA} or HR, was significantly lower ($P < 0{\cdot}01$) 24 h after bretylium treatment to control values. There was no significant reduction in any of the parameters (P_{DA}, P_{VA} or HR) 5 h after phentolamine injection compared to the 'bretylium + 24 h' values. The figure shows mean values ± S.E.M. from six cod. Blood pressures are expressed in kPa and heart rate in beats min^{-1}.

a slow increase in both pressures, reaching a peak some 1–2 h after the injection. This hypertension persisted for up to 6 h, declining steadily until hypotensive values were recorded 8–12 h after the injection. Hypotensive values persisted until at least 24 h after the injection, at which time phentolamine (2 mg kg^{-1}) was injected (see below). The three phases of the bretylium response will be referred to here as Phase I (initial, transient hypotension), Phase II (secondary hypertension) and Phase III (maintained hypotension). As indicated in Fig. 5, P_{DA} in Phase I was 36 % below that in untreated

Table 1. *Adrenalin and noradrenalin concentrations in blood plasma from chronically-catheterized cod which were either sham-injected or injected with bretylium (10 mg kg^{-1})*

	Sham-injected		Bretylium-injected	
	Adrenalin	Noradrenalin	Adrenalin	Noradrenalin
(A)	4·3 ± 1·4	2·8 ± 0·9	2·6 ± 0·3	1·7 ± 0·2
(B)	4·2 ± 0·9	3·3 ± 0·8	9·6 ± 5·9	3·7 ± 1·9
(C)	4·4 ± 1·8	3·2 ± 0·9	2·8 ± 0·6	1·5 ± 0·3

Letters refer to sampling points as indicated in Fig. 5, i.e. (A) Control values, 24 h post-surgery; (B) Phase II of the bretylium response (secondary hypertension) 1 h after bretylium injection; (C) Phase III of the bretylium response (sustained hypotension) 24 h after bretylium injection.

The values indicate means ± S.E.M. of four (shams) or five (bretylium) animals indicated as nmol l^{-1}.

fish at the same time, in Phase II 56% above untreated controls, and in Phase III 17% below untreated controls ($N = 6$). All of these differences were statistically significant ($P < 0{\cdot}01$). HR was not greatly affected by bretylium (Fig. 5). A similar, although less pronounced, triphasic response to bretylium has been reported for *Salmo gairdneri* (Smith, 1978).

Plasma catecholamine values in 24-h recovered fish were lower than previously reported, with adrenalin being $3{\cdot}4 \pm 1{\cdot}9$ nmol l^{-1} and noradrenalin $2{\cdot}2 \pm 1{\cdot}4$ nmol l^{-1} ($N = 9$; Table 1). Catecholamine concentrations sampled from Phase II and Phase III are also summarized in Table 1. There was no significant difference between 24-h recovered and Phase II (peak hypertension) values.

Effect of phentolamine

In fish injected with phentolamine (2 mg kg^{-1}) after 24 h recovery from the anaesthesia, a powerful and rapid fall in PDA, PVA and HR was observed. This effect, which has been ascribed to a direct, unspecific effect of the drug in man (Taylor *et al.* 1965), wore off over the next 3 h, leaving stable hypotensive values that were maintained from 5–24 h after injection. At 5 h, chosen because heart rate had returned to control value, PDA and PVA were 10% and 9% below the control, respectively ($N = 4$). This clearly demonstrates the existence of an adrenergic tonus affecting the vasculature and confirms the observation of Wahlqvist & Nilsson (1977) using another α-adrenoceptor antagonist, yohimbine.

In order to test for a residual tonus due to circulating catecholamines, phentolamine was injected into fish that had previously been exposed to bretylium for 24 h: there was a similar transient fall in blood pressures (Fig. 4), but no change in HR over the next 5 h. Neither PVA, PDA nor HR was significantly different at 5 h post-phentolamine compared to the 24-h post-bretylium value ($N = 6$; Fig. 5).

Stimulation of the sympathetic chain in tails from fish treated with bretylium plus phentolamine (5 h) was ineffective in eliciting vasoconstriction ($N = 4$).

DISCUSSION

In this study, several *in vitro* experiments were performed in an attempt to assess the usefulness of bretylium as a chemical tool in the differentiation between a neural and a humoral tonus affecting the circulatory system in the cod. From the experiments with the isolated coeliac artery preparations it is clear that bretylium produces a blockade in the nerve response at a concentration (10^{-5} mol l^{-1}) which does not affect measurably the α-adrenoceptor mediated response to adrenalin. The effect of bretylium on the inhibitory cholinergic innervation of the heart shows, however, that bretylium (10^{-6}–10^{-4} mol l^{-1}) will reduce the cholinergic nerve response and that bretylium therefore is not specific for the adrenergic nerves.

From the above experiments it can be concluded that bretylium is potentially useful for the differentiation between the effects exerted by adrenergic nerves on the one hand and circulating catecholamines on the other, but also that a 'non-specific' blockade of non-adrenergic nerves cannot be ruled out. This latter observation is in agreement with the findings of Boyd *et al.* (1963) in a number of preparations.

In some experiments bretylium (and/or phentolamine) was injected *in vivo* and the

responsiveness of the tail vasculature to sympathetic chain stimulation was investigated after several hours. From these studies it is possible to conclude that either bretylium or phentolamine, administered by intravascular injection *in vivo*, is capable of blocking the effects of the adrenergic nerves up to at least 24 h (bretylium) or 5 h (phentolamine) after the injection. This means that recordings of the cardiovascular variables can be made at times when any non-specific ('toxic') effects of the drugs will have disappeared, and only the adrenergic neurone blocking capacity (bretylium) or adrenoceptor blockade (phentolamine) is likely to remain.

Injection of bretylium *in vivo* into 'resting fish' (> 24 h post-surgery) produced a triphasic response in which an initial hypotension (possibly due to a direct 'toxic' effect of the drug) is followed by a secondary hypertension of unknown origin. Since it is known that bretylium can produce a release of catecholamines from adrenergic nerve endings in mammals (Koch-Weser, 1979), an attempt was made to correlate the Phase II hypertension with the plasma levels of catecholamines. However, no such correlation could be demonstrated in the present experiments. Indeed there was no significant increase in either plasma adrenalin or noradrenalin during Phase II (Table 1).

The Phase III hypotension following bretylium injection is interpreted as a sustained (chronic) blockade of the adrenergic nerves, since stimulation of the sympathetic chain failed to elicit vascular responses in perfused tail preparations from bretylium-treated cod. The drop in P_{DA} compared to control values (−17 %) is somewhat smaller than the 47 % fall seen with yohimbine in previous experiments with 'acute fish' (< 6 h post-surgery) (Wahlqvist & Nilsson, 1977), which may in part be due to the 'resting' state of the cod in the present experiments. The Phase III hypotension caused by bretylium is thus interpreted as a blockade of a nervous adrenergic tonus affecting the vascular system of the cod. Since phentolamine produced no further reduction in blood pressure in the present experiments, it appears that the cause of the adrenergic tonus in these experiments with undisturbed (> 24 h post-surgery) fish is due solely to adrenergic nerves. During 'stress', induced by handling, surgery, anaesthesia or other causes, it is likely that the importance of circulating catecholamines increases. A possible effect of circulating amines on the branchial vasculature during 'stress' has been postulated in both cod and rainbow trout (Wahlqvist & Nilsson, 1980; Nilsson, 1984).

In this analysis there is a tacit assumption that changes in arterial pressure reflect changes in vascular resistance. In the case of changes in P_{DA} this assumption is justified by the fact that there were no significant changes in HR following treatment with either bretylium or phentolamine. Thus, unless stroke volume changes were occurring, which is unlikely since HR and stroke volume are normally positively correlated in a wide range of circumstances in cod (Pettersson & Nilsson, 1980), it is likely that we are dealing with an essentially constant flow situation in which changes in P_{DA} will be a good indicator of changes in systemic vascular resistance.

The importance of keeping the fish in a stable environment during experiments of this kind is illustrated by the rather drastic effect on blood pressure elicited by removing the dark lid of the fish chamber, or gently stirring the water (Fig. 4). It is concluded that the tonic influence on the cardiovascular control exerted by various factors is susceptible to rapid changes at very slight stimuli, and that great care must be taken if 'undisturbed fish' are to be studied.

In conclusion, the present experiments show that bretylium is a potentially useful drug for the differentiation of effects exerted by adrenergic nerves on the one hand and circulating catecholamines on the other. The fact that bretylium, injected *in vivo* in undisturbed, conscious fish, causes a reduction in arterial blood pressure which is not further enhanced by phentolamine argues for an exclusively neural control of arterial pressure in undisturbed, conscious cod.

An important consequence of allowing 24 h recovery from surgery was that the levels of plasma catecholamines fell to values that were lower than those reported earlier from 'partially recovered' cod (e.g. adrenalin 29·8 nmol l^{-1}, noradrenalin 13·2 nmol l^{-1}; Wahlqvist & Nilsson, 1980). These lower values (adrenalin 3·4 nmol l^{-1}; noradrenalin 2·2 nmol l^{-1}; $N = 9$) are probably closer to those that will be maintained in unrestrained, unstressed fish. These concentrations will have little effect on any of the target organs yet studied in cod, where the threshold of responsiveness of isolated organs is usually encountered at concentrations of about 1 nmol l^{-1} (Wahlqvist, 1980). Thus it appears that circulating catecholamines in cod will become important under conditions of stress, at which time they may over-ride the adrenergic nervous cardiovascular control, just as occurs in mammals (e.g. Folkow & Neil, 1971).

Finally, it seems that, whereas *Salmo gairdneri* (Smith, 1978) and *G. morhua* are similar in making use of such an adrenergic nervous control of PDA at rest, the eel *Anguilla australis* differs in using neither neural nor circulating catecholamines in this way even though the eel has a potentially powerful adrenergic constrictor innervation of vessels in the tail (Hipkins *et al.* 1985). These findings indicate the importance of studying a far wider range of fish before attempting to make generalizations on the nature of their systems for blood pressure regulation.

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