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THE IMPORTANCE OF NERVOUS AND HUMORAL MECHANISMS IN THE CONTROL OF CARDIAC PERFORMANCE IN THE ATLANTIC COD *GADUS MORHUA* AT REST AND DURING NON-EXHAUSTIVE EXERCISE

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Summary

The nervous regulation of heart rate and stroke volume in the Atlantic cod *Gadus morhua* was investigated both *in vivo*, during rest and exercise, and *in vitro*. The cholinergic and adrenergic influences on the heart were estimated in experiments with injections of atropine and sotalol. At rest the cholinergic and adrenergic tonus on the heart were 38 % and 21 %, respectively (ratio 1·81:1). At the end of an exercise period, the cholinergic tonus had decreased to 15 % but the adrenergic tonus had increased to 28 % (ratio 0·54:1). The results suggest that variation of the cholinergic tonus on the heart is a major factor in the regulation of the heart rate.

In one group of fish, cardiac output was also measured, allowing calculation of stroke volume. Cardiac output increased significantly during exercise, and this effect persisted in the presence of both atropine and sotalol, although the increase in heart rate was reduced or abolished. The persisting increase in cardiac output during exercise is due to an increase in stroke volume, reflecting a Starling relationship.

In the presence of the adrenergic neurone-blocking agent bretylium, a positive inotropic effect on isolated, paced atrial and ventricular strips was observed. In the atrial preparations the effect persisted after 24 h. The effect was prevented by pretreatment with sotalol or cocaine, but potentiated by phentolamine pretreatment. This shows that bretylium exerts its neurone-blocking action after being taken up into the adrenergic nerves, and suggests that the positive inotropic effect of bretylium observed *in vivo* is due to release of endogenous catecholamines.

The concentration–response curves for adrenaline on isolated spontaneously beating atrial preparations showed that the concentrations of catecholamines necessary to produce appreciable effects on the heart are higher than the concentrations found in cod plasma during 'stress' situations (handling and exhaustive swimming).

Key words: *Gadus morhua*, heart, autonomic nervous system, exercise.

Introduction

A large number of histochemical and physiological investigations concerned with the innervation of the fish heart have been carried out during the past 20 years (for references see Sætersdal, Justesen & Krohnstad, 1974; Holmgren, 1977; Short, Butler & Taylor, 1977; Jones & Randall, 1978; Ask, Stene-Larsen & Helle, 1980; Donald & Campbell, 1982; Taylor & Butler, 1982; Laurent, Holmgren & Nilsson, 1983; Nilsson, 1983, 1984). However, relatively few attempts have been made to elucidate in more detail the mechanisms behind the integrated nervous and humoral control of heart rate (f_H) and cardiac output ($\dot{Q}$) in fish at rest and during exercise.

The innervation of the teleost heart resembles that of higher vertebrates, and comprises (in most cases) vagal cholinergic inhibitory nerve fibres acting *via* muscarinic receptors on the heart, and spinal autonomic ('sympathetic') adrenergic excitatory nerve fibres acting *via* β-adrenoceptors, or in some instances a combination of α- and β-adrenoceptors (Laurent *et al.* 1983; Nilsson, 1983, 1984). In some teleosts the adrenergic nerves enter the heart along the vagus (vagosympathetic trunk), and in other species in separate nerves (Holmgren, 1977; Nilsson, 1983).

A few exceptions to the general rule are known; the pleuronectids studied seem to lack an adrenergic innervation of the heart, but these species do have receptors for catecholamines which mediate excitation when stimulated (Falck, von Mecklenburg, Myhrberg & Persson, 1966; Cobb & Santer, 1973; Donald & Campbell, 1982).

It has been concluded that the modulation of vagal cholinergic influence is of great importance, being responsible for the bradycardia seen during hypoxia and fright, and suppression of the heart rate at rest. Thus the exercise tachycardia seen in most species studied is, at least in part, due to a withdrawal of this inhibitory vagal tonus (Wood, Pieprzak & Trott, 1979; Cameron, 1979; Randall, 1982; Axelsson, Ehrenström & Nilsson, 1987). The role of the adrenergic innervation and the circulating catecholamines in modulating f_H and $\dot{Q}$ during rest and exercise is not yet clear.

Cameron (1979) estimated the relative importance of the cholinergic and adrenergic tonus on the heart of the goldfish (*Carassius auratus*) at rest and during exercise, and found that the cholinergic tonus decreased while there was an increase in the adrenergic tonus on the heart during enforced swimming periods. However, no attempt was made to discriminate between the actions of adrenergic nerves and circulating catecholamines (total adrenergic tonus), and no measurements of cardiac output were made.

In other studies it has been shown that the catecholamine levels in blood plasma do not increase substantially during non-exhaustive exercise in rainbow trout (*Salmo gairdneri*) (Ristori & Laurent, 1985; Butler, Metcalfe & Ginley, 1986; Primmett, Randall, Mazeaud & Boutilier, 1986) and Atlantic cod (*Gadus morhua*) (Axelsson & Nilsson, 1986). In these species there is, however, a substantial increase in f_H and $\dot{Q}$ during exercise, but no indication of the factors responsible.

One of the aims of this study was to assess the cholinergic and adrenergic influences on heart rate and cardiac stroke volume (SV) *in vivo*, at rest and during moderate exercise, in an attempt to explain the importance of the cholinergic and adrenergic tonus in the regulation of cardiac output.

The second objective was to study further (cf. Smith, Wahlqvist, Nilsson & Eriksson, 1985) the effects of the adrenergic neurone blocker bretylium, to test its usefulness in differentiating between humoral and neuronal components in heart regulation.

Materials and methods

Atlantic cod, *Gadus morhua*, of either sex and with a body mass of 350–730 g and a length of 35–43 cm were used in this study. The fish were kept in well-aerated recirculated sea water at 10–12 °C, and were either used within a week of capture or fed until 1 week before surgery. The study was performed in December–June.

Surgical and preparative procedure

In vivo *experiments*

To investigate the adrenergic and cholinergic influence on the heart rate, one group of fish (GI) ($N = 7$) was anaesthetized in MS 222 (tricaine methanesulphonate; 100 mg l^{-1}) until breathing movements ceased. The fish were then transferred to the operating table, and sea water containing the anaesthetic (≈50 mg l^{-1}; individual correction in the concentration was made to keep the animal in the desired depth of anaesthesia) was continuously pumped over the gills during the operation.

A catheter (PE 50) was implanted in the afferent artery of the third gill arch for recording of ventral aortic (prebranchial) blood pressure (P_{VA}) and f_H. The catheter was passed through the upper part of the operculum and secured with a skin suture. The catheter was filled with heparinized (approx. 50 i.u. ml^{-1}) 0·9 % NaCl, and attached during the experiment to a Statham P23 pressure transducer connected to a Grass polygraph recorder system, model 7D. Calibration of the pressure transducer was made against a static water column.

To assess the adrenergic and cholinergic influence on $\dot{Q}$, fish in a second group (GII) ($N = 7$) were similarly equipped with a ventral aortic catheter and, in addition, an electromagnetic flow probe (Biotronex BLI) was placed around the ventral aorta for recording of cardiac output (for details, see Axelsson & Nilsson, 1986). After surgery the fish were transferred to a Blazka-type water channel (see Axelsson & Nilsson, 1986). The water in the experimental chamber was continuously replaced at a rate of 2 l min^{-1} from the departmental seawater system. The temperature was 10–12 °C during all experiments. Drugs were diluted in 0·9 % NaCl and injected through the ventral aortic catheter in volumes not exceeding 0·5 ml. Each fish was allowed to recover in the swim tunnel for ≥24 h before the

experiments to let the effects of surgery and anaesthesia wear off and the cardiovascular parameters stabilize (see also Smith *et al.* 1985).

The same experimental protocol was used for both groups of animals tested (GI) and (GII). Resting values for P_{VA}, f_H and, in GII, $\dot{Q}$ were recorded, and then the water flow through the swim tunnel was started and adjusted to 2/3 body length s^{-1} ($L\,s^{-1}$) (uncorrected for body-/tube-area ratio). Except for a few animals (which were discarded from the study), the fish would swim at the onset of water flow without further stimulus. The water speed of $2/3\,L\,s^{-1}$ was used since it has been shown to be in the range of sustained swimming speed for this species (M. Axelsson, P. J. Butler, J. D. Metcalfe & S. Nilsson, in preparation).

After 10–12 min, exercise values of all variables were recorded and the water flow was stopped. The fish was allowed to rest for 30–120 min, until the cardiovascular variables had returned to the pre-exercise values. Atropine ($1{\cdot}2\,mg\,kg^{-1}$), a muscarinic receptor blocker, was then injected and about 30 min later resting and exercise recordings were again made as described above. After another recovery period the fish was injected with the β-adrenoceptor blocker sotalol ($2{\cdot}7\,mg\,kg^{-1}$), and about 30 min later a third recording of resting and exercise values was made.

Heart rate was derived from the pulsatile blood pressure signals *via* a Grass 7P44 tachograph. It is expressed as beats min^{-1}, and $\dot{Q}$ and SV as $ml\,min^{-1}\,kg^{-1}$.

In situ *perfusion*

The fish ($N = 6$) were killed in the same way as described below and the ducts of Cuvier were exposed on both sides. The left duct of Cuvier was ligated dorsally and then freed down to the heart for later electrical stimulation, while the right duct of Cuvier was catheterized (PE 160) towards the heart. The catheter was connected to a thermostatically controlled perfusion funnel (10–11 °C) for constant pressure perfusion (for details, see Nilsson & Grove, 1974). An inflow pressure of 1·0–2·0 kPa was used. Gas-bubbled (97 % O_2/3 % CO_2) cod Ringer's solution (pH 7·4) was the perfusion fluid.

The bulbus arteriosus was exposed and catheterized (PE 160). The catheter was connected to a three-way stopcock with one arm attached to a Statham P23 pressure transducer connected to a Grass recorder, model 7D. The outflow pressure was kept between 1·0 and 1·5 kPa. The unphysiologically high inflow pressure and low outflow pressure were necessary to keep the *in situ* preparation working for at least 4 h, which was the duration of the experiments. The fish was then placed on its right side and two platinum hook electrodes were placed around the left duct of Cuvier. The preparation was left until a steady heart rate was achieved (20–90 min). Recordings were derived from the pulsatile pressure *via* a Grass tachograph (7P44).

An initial electrical stimulation (20–30 s) of the left duct of Cuvier was made as a control (8 V, 10 ms, 20 Hz), and then atropine was added to the perfusion fluid to a final concentration of 10^{-6} mol l^{-1}. After 30–60 min another stimulation was made (50–70 s). To test the response to exogenous catecholamines, adrenaline was

injected *via* the lower funnel in the perfusion apparatus to give a (1 ml) bolus with a concentration of approx. 10^{-7} mol l^{-1}. When the effect of adrenaline had worn off, bretylium was added to the perfusion fluid to a final concentration of 10^{-5} mol l^{-1} and the effects of electrical stimulation and adrenaline were retested 60 min later (Fig. 6).

In vitro *preparations*

The cod was killed by a blow to the head and heparin was then injected into the caudal vein ($\approx$1000 i.u. kg^{-1} body mass). The heart was then excised and placed in cold cod Ringer's solution (Holmgren & Nilsson, 1974).

For studies of the effects of bretylium, the atrium and ventricle were separated and the sinus venosus and bulbus arteriosus removed. The atrium and ventricle were both divided longitudinally into two equal parts and mounted in 50-ml thermostatically controlled (10–11 °C) organ baths containing gas-bubbled (97 % O_2/3 % CO_2) cod Ringer's solution (pH 7·4). Isometric tension in the preparations was recorded *via* Grass FT03 transducers connected to a Grass polygraph, model 7D. The preparations were electrically paced (8 V, 10 ms, 0·4 Hz) *via* a Grass stimulator (model SD 9) using platinum hook electrodes. The paced cardiac strip preparations were individually preloaded (0·005 N) and left for 60–120 min to equilibrate. Bretylium (10^{-5} mol l^{-1}) was then added to one of the atrial and one of the ventricular strip preparations. The other two preparations were used as controls. The inotropic effect was recorded after 15 min and 24 h and expressed as a percentage of the control value.

In another series of experiments, sotalol (10^{-6} mol l^{-1}), a β-adrenoceptor antagonist, the α-adrenoceptor antagonist phentolamine (10^{-6} mol l^{-1}) or the neuronal catecholamine uptake inhibitor cocaine (10^{-6} mol l^{-1}), was added to all four preparations 30 min before addition of bretylium to one of the atrial and one of the ventricular preparations, and again the effects were recorded after 15 min and 24 h.

To investigate the concentration–response relationship for adrenaline on the atrium, the ventricle was removed and the atrium dissected out either (in half the preparations) attached to part of the sinus venosus, or (in the rest of the preparations) without the sinus venosus. The atrium was cut open longitudinally and mounted in a 50-ml organ bath. Those preparations with parts of the sinus venosus left intact were spontaneously beating (used for the chronotropic response study) and the other preparations were electrically paced (used for the inotropic study) as described above. The tension was recorded *via* FT03 transducers connected to a Grass recorder, and the rate of beat of the spontaneously beating atrial preparations was recorded *via* a tachograph (model 7P44). The preparations were left until stable recordings were obtained. Adrenaline was added cumulatively (see e.g. Holmgren & Nilsson, 1974), and the chronotropic and inotropic responses were recorded.

All drugs were dissolved in cod Ringer's solution and added to the organ bath to the final concentration.

Chemicals used

The following drugs were used: adrenaline bitartrate (Sigma), atropine sulphate (Sigma), bretylium tosylate (a gift from Wellcome Foundation Ltd), cocaine chloride, phentolamine methanesulphonate (Sigma) and sotalol hydrochloride (Hässle AB). They were dissolved in 0·9 % NaCl or Ringer's solution.

Statistics and calculations

Means ± S.E.M. are presented and the number of experiments is indicated by N. Wilcoxon's sign rank test for paired samples was used (two-tailed). The level of significance was set to $P \leq 0{\cdot}05$ (*). Nonparametric tests were used since there were indications of a non-normal distribution in the material.

The cholinergic and adrenergic tonus on the heart during rest and exercise was calculated essentially as described by Cameron (1979) as follows: the change in fH induced by atropine (fH_{chol}) and additional sotalol (fH_{adr}) were expressed as a percentage of the 'intrinsic fH' (fH_{int} = fH after injection of both atropine and sotalol). For estimation of the cholinergic tonus, fH_{chol} was calculated as fH after atropine minus control fH, and for estimation of the adrenergic tonus fH_{adr} was calculated as fH after atropine minus fH_{int}. 'Control fH' (fH_c) is defined as heart rate before injection of drugs.

To express the sensitivity of the strip preparations to the drugs used, pD_2 values are given. The pD_2 value is defined as $-\log EC_{50}$, where EC_{50} is the agonist concentration at which 50 % of the maximum effect is seen (cf. Ariëns & von Rossum, 1957).

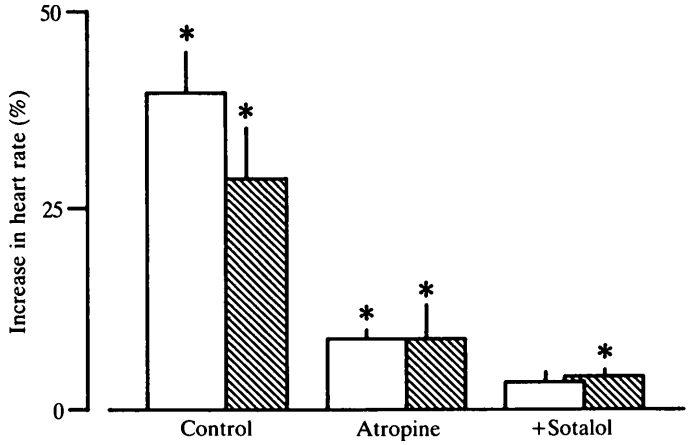

Fig. 1. Histogram showing increase in heart rate during exercise expressed as a percentage of resting heart rate in the two experimental groups (GI, clear bars, $N = 7$; GII, hatched bars, $N = 7$). The exercise-induced increase in heart rate was statistically significant (indicated by *) ($P < 0{\cdot}05$; Wilcoxon sign rank test for paired samples) in all cases except in GI after the combined atropine/sotalol (+Sotalol) treatment. Means ± S.E.M.

Results

In vivo *experiments*

Values of control heart rates at rest and during exercise in both experimental groups (GI, GII) were comparable to those observed in the cod in previous studies (Smith *et al.* 1985; Axelsson & Nilsson, 1986).

In GI, atropine significantly increased the resting f_H compared with the control (from $30{\cdot}5 \pm 0{\cdot}9$ to $44{\cdot}3 \pm 1{\cdot}1$ beats min^{-1}), and at the same time abolished the irregular heart beat seen in the resting untreated animals. After the subsequent injection of sotalol there was a significant lowering of f_H in the resting animals down to $36{\cdot}6 \pm 0{\cdot}3$ beats min^{-1} (Fig. 1; Table 1).

During exercise there was a significant increase in f_H by 12 beats min^{-1} (40 %) compared with rest values (Fig. 1; Table 1). After atropine injection, there was still a significant increase of 4 beats min^{-1} (9 %) during exercise; after the subsequent injection of sotalol there was a non-significant increase in f_H during exercise of 1 beat min^{-1} (3 %).

Estimations of the cholinergic and adrenergic tonus on the heart were made as described in the Materials and methods section. The cholinergic tonus on the heart at rest was 38 % and this was reduced to 15 % during exercise, whereas the resting adrenergic tonus was 21 % and increased to 28 % during exercise (Table 1).

In GII, resting f_H was higher than in GI ($40{\cdot}4 \pm 3{\cdot}9$ compared with $30{\cdot}5 \pm 0{\cdot}9$ beats min^{-1}), and during exercise f_H increased to $42{\cdot}7 \pm 2{\cdot}4$ (40 %) in

Table 1. *Heart rate (f_H) in six cod during rest and exercise, and calculated cholinergic and adrenergic tonus*

Rest	
Control (f_{H_c})	$30{\cdot}5 \pm 1{\cdot}0$
f_H after atropine ($1{\cdot}2$ mg kg^{-1}) ($f_{H_{atr}}$)	$44{\cdot}3 \pm 1{\cdot}1$
Change	+13·8
f_H after sotalol ($2{\cdot}7$ mg kg^{-1}) ($f_{H_{int}}$)	$36{\cdot}6 \pm 0{\cdot}33$
Change	−7·7
Cholinergic tonus	37·7 %
Adrenergic tonus	21·0 %
Ratio	1·87
Exercise	
Control (f_{H_c})	$42{\cdot}7 \pm 2{\cdot}4$
f_H after atropine ($f_{H_{atr}}$)	$48{\cdot}3 \pm 1{\cdot}1$
Change	+5·6
f_H after sotalol ($f_{H_{int}}$)	$37{\cdot}8 \pm 0{\cdot}6$
Change	−10·5
Cholinergic tonus	14·8 %
Adrenergic tonus	27·7 %
Ratio	0·54

Values are means ± S.E.M.
Heart rates are measured in beats min^{-1}.

GI and 51·1 ± 3·4 (26 %) in GII. After the atropine treatment, the difference in resting f_H between the two groups was less pronounced, as was the increase of f_H during exercise after atropine and the combined atropine/sotalol treatments (Fig. 1).

Cardiac output, recorded in GII, showed significant increases during exercise, from 19·2 ± 0·9 to 30·2 ± 1·7 ml min^{-1} kg^{-1} (65 %), in the control fish, from 21·0 ± 1·4 to 34·2 ± 2·5 ml min^{-1} kg^{-1} (70 %) after treatment with atropine, and from 17·0 ± 2·3 to 24·2 ± 2·3 ml min^{-1} kg^{-1} (50 %), after the combined treatment with atropine and sotalol. SV also increased during exercise from 0·49 ± 0·04 to 0·61 ± 0·06 ml min^{-1} kg^{-1} (12·9 %) in the control fish, from 0·45 ± 0·05 to 0·66 ± 0·07 ml min^{-1} kg^{-1} (52·3 %) after atropine treatment, and from 0·47 ± 0·10 to 0·64 ± 0·11 ml min^{-1} kg^{-1} (55·8 %) after a combined blockade with atropine and sotalol (Fig. 2).

In situ *perfusion*

The initial electrical stimulation of the nerves running along the left duct of Cuvier inhibited the heart; this response was reversed after atropine treatment and the ensuing positive chronotropic effect could be mimicked by adrenaline (10^{-7} mol l^{-1}) (Fig. 6). Perfusion with bretylium (10^{-5} mol l^{-1}) for 60–90 min elevated f_H, and the positive chronotropic response to electrical stimulation seen after atropine was weak or absent (<9 % of the control), although the effect of adrenaline was unaffected or only slightly reduced (>90 % of control response).

In vitro *preparations*

The presence of bretylium in the bathing solution enhanced the contractility of the strip preparations (Fig. 3). There were mean increases of 31·3 ± 4·0 % after 15 min and 15·5 ± 6·1 % after 24 h in the atrial strips ($N = 41$) and 1·4 ± 1·1 % in the ventricular preparations ($N = 7$) after 15 min but no remaining effect was seen after 24 h in these preparations.

Sotalol (10^{-6} mol l^{-1}) and cocaine (10^{-6} mol l^{-1}) reduced the effect seen after bretylium treatment in the atrial preparations to 14·6 ± 5·2 % and 12·2 ± 6·7 %, respectively, after 15 min. Phentolamine potentiated the effect of bretylium treatment by 37·4 ± 25·1 % compared with the control ($N = 7$) (Fig. 4).

Concentration–response curves for the excitatory effect of adrenaline on isolated paced (to assess inotropic response) or spontaneously beating (to assess chronotropic response) atrial preparations were constructed. The maximal chronotropic response was an increase in f_H of 10·5 ± 3·2 beats min^{-1}. The pD_2 value for the positive chronotropic response was 7·05 ± 0·40 ($N = 9$) whereas the pD_2 value for the positive inotropic response was 6·45 ± 0·28 ($N = 9$) (Fig. 5).

Discussion

To obtain cardiovascular variables that are as close to 'normal' as possible, it is vital to limit the surgical treatment to a minimum, and to allow the animals to

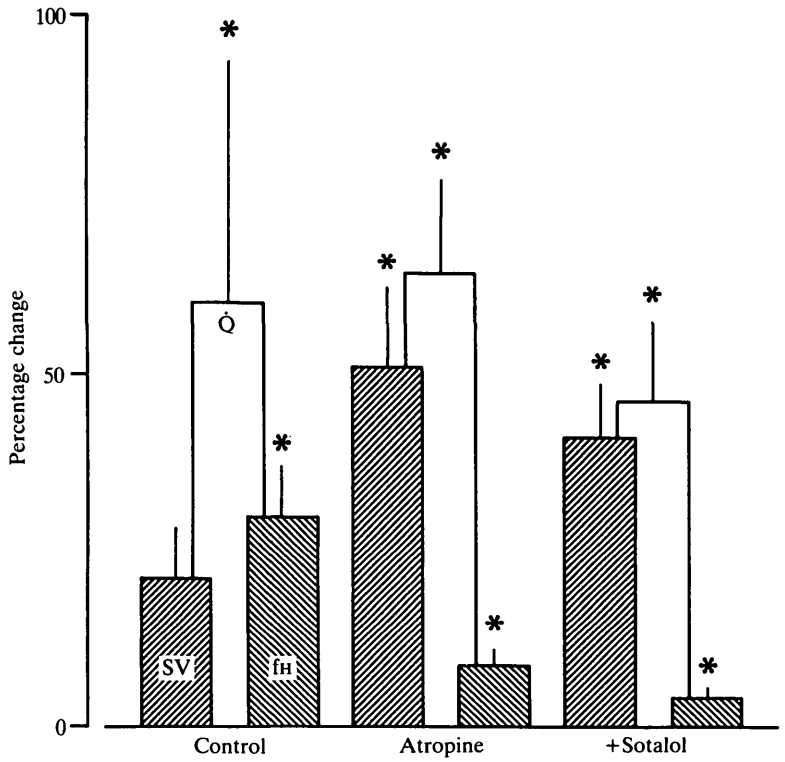

Fig. 2. Percentage increase during exercise compared with resting animals in GII showing stroke volume (SV, left-hand hatched bars), cardiac output ($\dot{Q}$, clear bars) and heart rate (f_H, right-hand hatched bars), before (Control) and after treatment with atropine (Atropine) and the subsequent sotalol treatment (+Sotalol). Note the relative similarity in the response of cardiac output to exercise in the three cases, and the transition from heart rate control to stroke volume control when the autonomic nervous input to the heart is blocked by atropine or the combined atropine/sotalol treatment. Significant ($P < 0{\cdot}05$; for method see Fig. 1) effects of exercise are indicated by *. Means ± S.E.M. ($N = 7$).

recover fully before experiments (Wood *et al.* 1979; Smith *et al.* 1985). Therefore, the *in vivo* experiments were made with two different groups: in the first group only f_H was recorded, and in the second group $\dot{Q}$ was also recorded.

Clear differences in f_H between the two groups were observed: both resting and exercise f_H were lower in the group where only a ventral aortic catheter had been implanted (GI) than in the group with an additional ventral aortic flow probe (GII). After atropine and sotalol injection these differences persisted, although they were less pronounced. Since the experiments followed the same protocol for both groups, the differences in f_H probably reflect effects of the extended surgery used to measure cardiac output (cf. Woakes & Butler, 1986, working on ducks). For this reason the f_H data from GI were used to calculate the cholinergic and adrenergic tonus affecting f_H. The resting f_H in this group ($30{\cdot}5 \pm 0{\cdot}9$ beats min^{-1})

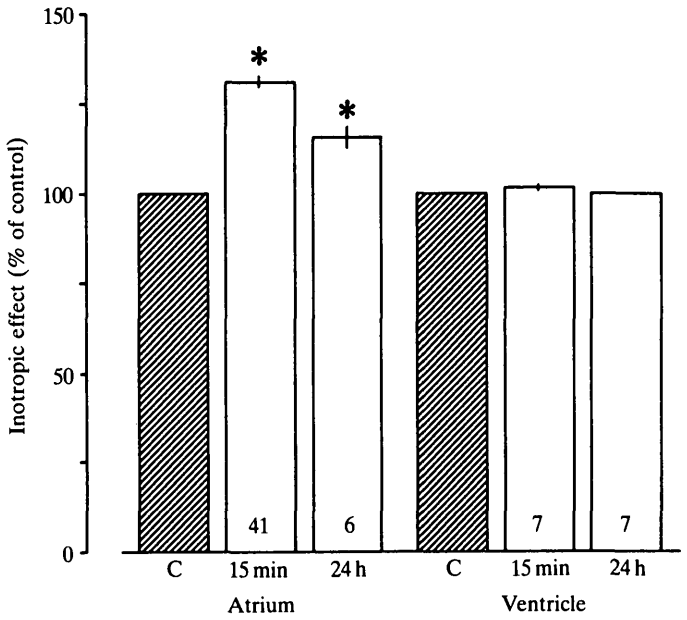

Fig. 3. Inotropic effect of bretylium on paced atrial and ventricular strips after 15 min and 24 h, expressed as a percentage of the control preparation (C, hatched bars). In the atrial strips there is a statistically significant (indicated by *) ($P < 0{\cdot}05$; for method see Fig. 1) increase both at 15 min and 24 h, but in the ventricular preparations no significant increase could be demonstrated. Means ± S.E.M.; number of samples is indicated inside the bars.

was somewhat lower than previously found in the cod in laboratory experiments (Pettersson & Nilsson, 1980; Wahlqvist & Nilsson, 1980), and can be compared with the lowest recorded resting f_H in free-swimming Atlantic cod (Wardle, 1974), where the ECG recordings were made with ultrasonic telemetric equipment. Such telemetric studies are likely to come as close as possible to 'normal' values for the recorded variables, and it is encouraging that the experimental conditions of the present study provide very similar values in the laboratory.

The cholinergic tonus in the control fish at rest was higher than the adrenergic tonus (37 % and 20 %, respectively) and during exercise there was a decrease in cholinergic tonus and a small increase in adrenergic tonus. The results suggest that variation in the cholinergic tonus is a major factor in the control of heart rate in cod, at rest as well as during exercise. This conclusion was also reached for the goldfish, *Carassius auratus* (Cameron, 1979), and the ballan wrasse, *Labrus berggylta*, but not for a number of other teleost species in which the adrenergic tonus appears to exert the greater influence (Axelsson *et al.* 1987).

The inhibitory cholinergic tonus is present in most fish species studied and seems to be a general feature among all vertebrates except cyclostomes. Among the fish studied there is a great variation in the cholinergic tonus (Cameron, 1979; Axelsson *et al.* 1987), and it is difficult to compare the different studies because of

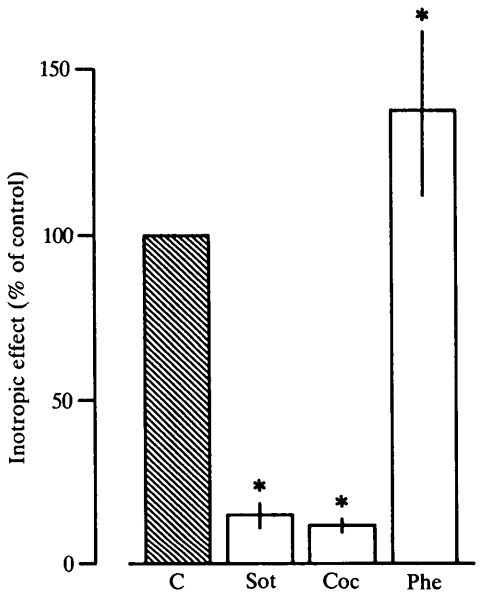

Fig. 4. Inotropic effects on electrically paced atrial strip preparations 15 min after addition of bretylium, expressed as a percentage of the control preparation (C, hatched bar). The preparations had been pretreated with sotalol (10^{-6} mol l^{-1}, Sot), cocaine (10^{-6} mol l^{-1}, Coc) or phentolamine (10^{-6} mol l^{-1}, Phe). Sotalol and cocaine both significantly (indicated by *) ($P < 0{\cdot}05$, for method see Fig. 1) inhibit the response to bretylium, but phentolamine significantly potentiates the response. Means ± S.E.M. ($N = 7$).

variations in the experimental conditions. For instance, it appears that the importance of the cholinergic control of the heart decreases with elevated temperature, whereas the adrenergic influence increases (Laffont & Labat, 1966; Priede, 1974; Wood *et al.* 1979).

In GII, cardiac output was also measured using the same experimental protocol as in the first group. During exercise, $\dot{Q}$ and SV increased significantly, both before and after injection of atropine and sotalol. In the atropine-treated animals the elevation of resting f_H was compensated for by a decreased SV, maintaining a near-constant $\dot{Q}$. During exercise there was only a small increase in f_H after atropine, while stroke volume increased more than in the exercising control fish, again maintaining $\dot{Q}$ comparable to that in the control fish during exercise.

The data suggest that the regulation of SV is little affected by cholinergic and adrenergic influences, and the major factor controlling it could be an increase of the venous return in the exercising animals due to muscle pumping (Starling relationship).

The effect of bretylium on adrenergic neurones is known from studies on mammals (Boura & Green, 1959; Kirpekar & Furchgott, 1963; Ledsome & Linden, 1964; Markis & Koch-Weser, 1971; Namm *et al.* 1975). In the study of Smith *et al.* (1985) on the Atlantic cod, it was shown that bretylium tosylate

blocked the transmitter release from adrenergic vasomotor nerves without affecting the sensitivity of the α-adrenoceptors. They concluded that bretylium could be used in the cod to distinguish between the effects of adrenergic vasomotor nerves and circulating catecholamines.

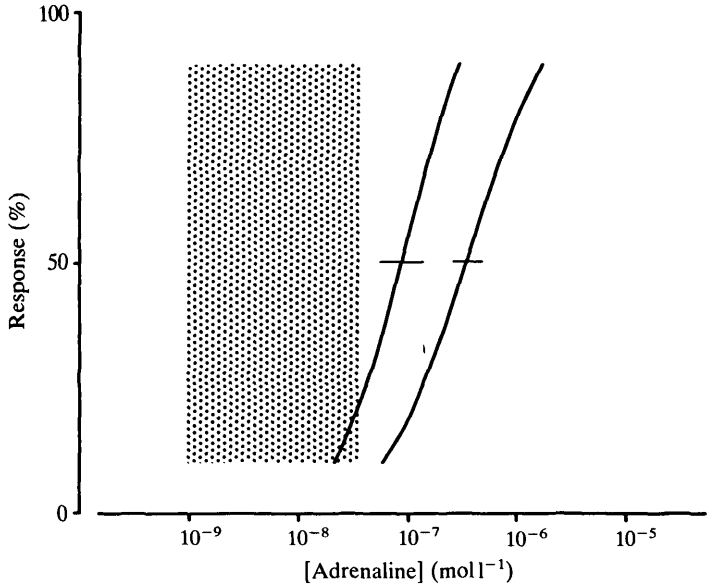

Fig. 5. Concentration–response curves for adrenaline on atrial strip preparations showing the chronotropic response of spontaneously beating preparations (left-hand curve) and the inotropic response of the paced preparations (right-hand curve). The pD_2 value ($=-\log EC_{50}$) for the chronotropic response is $7{\cdot}05 \pm 0{\cdot}40$, and for the inotropic response $6{\cdot}45 \pm 0{\cdot}28$. Means ± S.E.M. at 50% response; $N = 9$ for both curves. The dotted area represents the level of circulating catecholamines found *in vivo*, with the lowest concentrations seen at rest and the highest during stress (Axelsson & Nilsson, 1986).

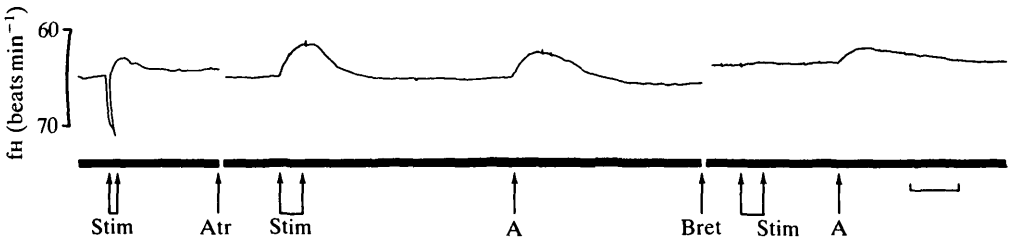

Fig. 6. Recording from an *in situ* heart perfusion experiment showing the effect of electrical stimulation (8 V, 20 Hz and 10 ms pulse duration for 15–60 s) of the left vagosympathetic trunk on heart rate (f_H). The experiment compares the effect of electrical stimulation (Stim) or adrenaline (A; bolus injection of 1·0 ml, 10^{-7} mol l^{-1}) before and after atropine (Atr; 10^{-6} mol l^{-1}) and bretylium (Bret; 10^{-5} mol l^{-1}). Note the increased basal f_H and the very weak response to electrical stimulation after bretylium ($N = 7$).

In the *in situ* perfusion experiments, bretylium was shown to block the effect of the adrenergic neurones innervating the heart, without affecting the influence of externally applied adrenaline. The capacity of this drug to block selectively the function of adrenergic nerves (Boura & Green, 1959; Donald & Campbell, 1982; Smith *et al.* 1985; Axelsson & Nilsson, 1986) therefore appears to be useful in separating the effect of adrenergic nerves and circulating catecholamines in the cod heart.

In the study on blood pressure regulation during exercise in the cod (Axelsson & Nilsson, 1986), it was found that bretylium caused a decrease in f_H compared with the control, both at rest and during exercise. Furthermore, resting SV was elevated after injection of bretylium. To elucidate further the mechanisms of these actions of bretylium on the cod heart, the effect of the drug was investigated on isolated strip preparations *in vitro*. Bretylium produced positive inotropic effects in the heart strip preparations: the response was small and transient in the ventricular preparations, but the atrial preparations showed a significant increase in the force of contraction 15 min after the application of bretylium, an effect that persisted after 24 h. The acute effects are known from studies in other animals, but no long-term effects of bretylium are described for *in vitro* preparations (Boura & Green, 1959; Ledsome & Linden, 1964; Markis & Koch-Weser, 1971).

The inotropic response in the presence of bretylium could be blocked by sotalol. This suggests that the effect could be mediated by released catecholamines which act *via* β-adrenoceptors. The effect was potentiated by the α-adrenoceptor antagonist phentolamine; this could be due to inhibition of the negative feedback of catecholamine release mediated *via* presynaptic α-adrenoceptors. This effect of phentolamine has been described previously in cod spleen preparations (Nilsson & Holmgren, 1976). The difference in the response between the two heart chambers probably reflects a difference in innervation density and thus the amount of catecholamines available for release by bretylium (Sætersdal *et al.* 1974; Laurent *et al.* 1983).

Cocaine, which is known to inhibit the neuronal uptake of amines into the adrenergic nerve terminals (uptake$_1$; Iversen, 1967; Nilsson & Holmgren, 1976; Ask *et al.* 1980), impaired the effect of bretylium. This suggests that bretylium has to be taken up neuronally in order to release the endogenously stored catecholamines, a mechanism of action that has been described in mammals (Kirpekar & Furchgott, 1963; Markis & Koch-Weser, 1971).

It has been shown that the levels of circulating catecholamines do not increase to any large extent during non-exhaustive exercise in teleosts (*Gadus morhua*, *Salmo gairdneri*, *Pollachius pollachius*, *Labrius mixtus*) (Primmett *et al.* 1986; Butler *et al.* 1986; Axelsson & Nilsson, 1986; Axelsson *et al.* 1987). The *in vitro* experiments on the effects of adrenaline on the chronotropic and inotropic response in atrial preparations show that the level of adrenaline necessary to cause major effects on the heart were higher than the plasma catecholamine levels found *in vivo* during non-exhaustive exercise. Only during 'stress' or exhaustive exercise may the levels rise enough to cause some effect on the heart (Fig. 5; Axelsson & Nilsson, 1986;

P. J. Butler, M. Axelsson, F. Ehrenström, J. D. Metcalfe & S. Nilsson, in preparation). The above findings are compatible with the view that the adrenergic tonus on the heart both at rest and during exercise is nervous (cf. Axelsson & Nilsson, 1986).

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