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THE CARDIAC CYCLE AND THE EFFECTS OF NEUROHUMORS ON MYOCARDIAL CONTRACTILITY IN THE ASIATIC EEL *ANGUILLA JAPONICA*, TIMM. & SCHLE.*

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Abstract—1. Intracardiac pressures were above atmospheric level. Intrapericardial pressure level was subambient only for 50% of the cycle.

2. *Vis a tergo* was found to be an important cardiac filling mechanism.

3. The sino-auricular valve was competant because no regurgitation of blood took place during auricular systole.

4. Auricular contraction was the sole contributor to ventricular filling.

5. Acetylcholine produced a marked bradycardiac effect. The peak systolic pressure developed was higher than the control level although the rate of pressure build up was lowered (in the auricle) or remained unchanged (in the ventricle).

6. Noradrenaline had no effect on heart rate but greatly increased the rate to peak pressure in the ventricle and auricle.

INTRODUCTION

The fish heart typically consists of four chambers arranged in series: sinus venosus, auricle, ventricle and bulbus (teleost) or conus (elasmobranch) arteriosus. The cardiac cycle begins with the filling and contraction of the sinus venosus and ended when the bulbus arteriosus ejects blood into the ventral aorta. Myocardial contraction produces a sequence of changes in the pressures and volumes of blood in the heart chambers. Intracardiac pressures, especially ventricular pressure, have been measured in various piscine species under different experimental and physiological conditions. Simultaneous recordings of blood pressures from all the heart chambers has only been reported for the smooth dogfish *Mustelus canis* (Sudak, 1965). Data on changes in cardiac volumes is lacking. Changes in the sizes of the heart chambers have been measured in a radiological examination of the cardiovascular system of *Anguilla anguilla* (Mott, 1951). Subatomospheric intracardiac pressures have been demonstrated in elasmobranchs and is attributable to the subambient intrapericardial pressure (Johansen & Martin, 1965; Sudak, 1965). Reported values of intracardiac pressures in teleosts are always above ambient levels.

Autonomic parasympathetic regulation of fish heart function has generally been recognized. Negative inotropic and negative chronotropic effects of acetylcholine have also been consistently observed in both elasmobranchs and teleosts (Burnstock, 1969; Johansen, 1971). Although adrenergic nerve endings have been found in the trout (Gannon & Burnstock, 1969), it has not been demonstrated in other species (von Mecklenberg, 1966, cited in Falck *et al.*, 1966; Gannon *et al.*, 1972; Santer, 1972). Catecholamines have been shown to enhance contractility in fish heart (Randall, 1970) but their effect on heart rate is not consistent (Johansen & Martin, 1965; Johansen, 1971).

In this paper, the events of mechanical changes in the heart of the Asiatic eel will be described. Blood pressures from the sinus venosus, auricle, ventricle and bulbus arteriosus were recorded within very short intervals from the *in situ* perfused heart-gill preparation. The effects of noradrenaline and acetylcholine on heart rate and cardiac contractility have also been studied.

MATERIALS AND METHOD

A total of 25 Asiatic eels weighing from 200 to 300 g were examined. They were purchased from local dealers and transported to the laboratory unanaesthetized. The animals were kept in well-aerated tap water at 20–25°C in glass tanks measuring 47 × 25 × 20 in. They were unfed and were acclimated for at least 2 weeks before use. The eels were anaesthetized with MS222 (0·1%, Tricaine Methane Sulphonate, Sandoz). A mid-ventral incision was made at the level of the heart and extended caudally for about 6 cm. The dorsal aorta and bulbus arteriosus were cannulated (Chester Jones *et al.*, 1969). The sinus venosus was cannulated by direct puncture through the hepatic vein. The fish was then fixed on its dorsal surface with the ventral side uppermost and out of water. Wet paper towels were wrapped around the animal to keep it moist. Respiratory gas exchange was provided for by perfusing the gills through the mouth with a constant supply of air-saturated tap water containing MS222 at a concentrati[illegible] 100 mg/l.

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Experiments were conducted at a room temperature of 20 ± 0·5 C. Blood pressure measurements were obtained directly from the heart chambers, pericardial cavity and the right posterior cardinal vein by direct punctures using hypodermic needles (gauges 20–22) fitted with polyethylene cannulae of appropriate sizes. The latter were attached to physiological pressure transducers (Sanborn model 267B and 268B). The hydraulic portion of the system was filled with degassed 0·9% saline. The transducers were arranged in such a way that all pressure measurements were relative to atmospheric pressure (0 mmHg) at the level of the auricle. The outputs of the transducers were displayed on a Sanborn 2-channel recorder (Hewlett Packard) equipped with Sanborn Carrier Preamplifiers (model 350–3000) at a paper speed of 20 cm/sec. Records were made in pairs: pericardial and dorsal aorta; sinus venosus and dorsal aorta; right posterior cardinal vein and dorsal aorta; sinus venosus and ventricle; auricle and ventricle; auricle and bulbus arteriosus; right posterior cardinal vein and ventricle. Heparin (200 IU) was injected intravenously to prevent blood clotting before the experiment started. Following placement of the needle catheters, pairs of records were obtained in rapid succession by operating appropriate three-way taps. The entire recording procedure was completed within 90 sec. It was considered that these records were *obtained simultaneously*. Acetylcholine (Acetylcholine chloride, British Drug House) and noradrenaline (L-arterenol, Sigma Chemical Company) were administered at a dosage of 0·1 μg/kg via the pneumatic duct vein.

Blood pressure tracings were superimposed on the same time base. The dorsal aortic and ventricular pressure tracings, together with the zero ordinates, were used as references. Finally, these were retraced on the same pressure scale.

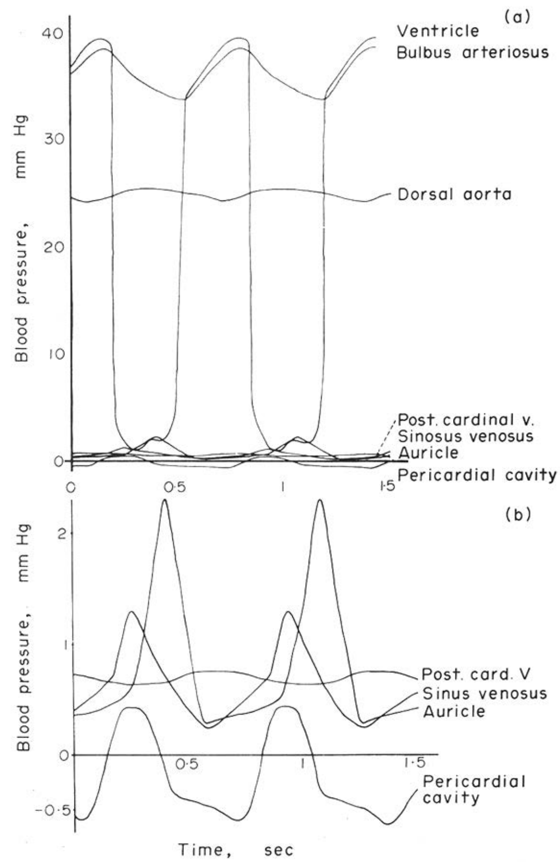

Fig. 1(a) *Anguilla japonica*: the sequence of events during the cardiac cycle. This has been retraced from original physiograph tracings expressed on the same pressure scale. 1(b). Portion of Fig. 1(a) magnified to show the relation of pressure patterns in the low pressure chambers.

RESULTS

The cardiac cycle in the Asiatic eel started with the filling of the sinus venosus and ended when the bulbus arteriosus expelled its contents into the ventral aorta. The typical sequence of events which occurred during the filling and emptying of the various cardiac chambers was illustrated in Fig. 1. On the average, the heart beat 70·9 times/min with each cardiac cycle lasting 0·86 sec. (Tables 1 and 2). Since it was quite impossible to determine accurately the details of different phases of contraction and relaxation from pressure traces alone, only the total contraction and total relaxation times were calculated.

Intrapericardial pressure was above atmospheric level during late auricular diastole and early ventricular diastole, but remained subatmospheric for the remainder of the cardiac cycle. Its peak pressure corresponded to the beginning of ventricular isovolumetric relaxation and started to decrease when both the auricle and the bulbus arteriosus were contracted. A further drop occurred during ventricular isovolumetric contraction and reached a lowest value at the end of ventricular ejection.

Central venous pressure and blood pressure in the sinus venosus did not appear to fall below atmospheric pressure level in all the specimens studied.

The magnitude of auricular pressure was always above atmospheric pressure level. The pressure pat-

Table 1. Effects of noradrenaline on the events of the cardiac cycle in *Anguilla japonica*. Number of animals = 5. Values presented as mean ± 1 S.E.

Treatment	Ventricle			Auricle			Sinus Venosus		Heart	Duration of
	total diastole sec.	total systole sec.	dP / dt mmHg / sec.	total diastole sec.	total systole sec.	dP / dt mmHg / sec.	filling time sec.	emptying time sec.	rate Beats/min.	the cardiac cycle sec.
Control	0.38±0.09	0.45±0.04	273±78.5	0.47±0.11	0.28±0.06	9.85±2.63	0.45±0.16	0.36±0.1	67.9±8.98	0.90±0.12
Noradrenaline 0.1 µg	0.32±0.08	0.55±0.04	434±128	0.41±0.1	0.39±0.06	14.7±3.63	0.46±0.1	0.35±0.08	68.0±9.17	0.90±0.13
P	N.S.	<0.1	<0.01	N.S.	<0.1	N.S.	N.S.	N.S.	N.S.	N.S.

Table 2. Effects of acetylcholine on the events of the cardiac cycle in *Anguilla japonica*. Number of animals = 5. Values expressed as mean ± 1 S.E.

Treatment	Ventricle			Auricle			Sinus Venosus		Heart	Cardiac
	total diastole sec.	total systole sec.	dP / dt mmHg / sec.	total diastole sec.	total systole sec.	dP / dt mmHg / sec.	filling sec.	emptying sec.	rate Beats/min.	cycle duration sec.
Control	0.32±0.05	0.45±0.1	311±85.8	0.49±0.08	0.28±0.03	11.2±1.55	0.54±0.09	0.26±0.04	77±12.0	0.80±0.12
Acetyl-choline 0.1 μg	0.67±0.16	0.49±0.11	342±89.1	0.82±0.17	0.36±0.08	6.76±1.9	0.79±0.15	0.34±0.12	53.8±11.1	1.17±0.32
P	<0.05	N.S.	N.S.	<0.01	<0.01	<0.01	<0.01	N.S.	<0.001	<0.05

tern recorded showed two pulses, one corresponding to auricular filling and the other to auricular systole. The auricular systolic pressure trace crossed that of the ventricular diastolic phase twice, corresponding to the opening and closing of the auriculo-ventricular valves respectively. The ventricular pressure curve was characterized by a steep slope during ventricular systole followed by a more gentle rise after ventricular pressure exceeded the pressure in the bulbus arteriosus, indicating the commencement of ventricular ejection. Immediately after attaining the peak pressure, ventricular pressure began to drop sharply, crossing the pressure curve of the bulbus arteriosus, corresponding to the closure of the ventriculobulbar valves and the end of ventricular ejection. Ventricular isovolumetric relaxation immediately followed, until the pressure reached its lowest value somewhere in the middle of the diastolic phase. The values of pressure measurements during the latter phase were always found to be above atmospheric pressure level. The ventricular pressure record displayed two pulses, the smaller one of which corresponded to auricular systole. The pressure in the bulbus arteriosus was always above the ventricular pressure level except during ventricular ejection. The small incisura of the pressure trace after closure of the ventriculobulbar valves marked the relaxation of the muscles between the bulbus arteriosus and the ventral aorta, or the beginning of bulbal ejection.

In general, noradrenaline elevated the blood pressure but practically had no effect on the heart rate. The rate of pressure rise in both ventricular and auricular contractions were accelerated. Ventricular relaxation time was significantly decreased (Table 1; Fig. 2). Acetylcholine lowered blood pressures of the posterior cardinal vein, the sinus venosus, ventricle during the end diastolic phase and the dorsal aorta to various degrees, yet its bradycardiac effect was most remarkable (Table 2; Fig. 2).

DISCUSSION

The cardiac cycle of the Asiatic eel begins with the contraction of the sinus venosus, forcing the two flaps of the sino–auricular valves to open and subsequently filling the auricle. The auricle then begins to contract, building up enough pressure to exceed ventricular end diastolic pressure. Then the three-cusps of the auriculo–ventricular valves open, allowing blood to gush into the ventricle. Ventricular systole squeeze the blood into the elastic bulbus arteriosus. The cardiac cycle is completed when the bulbus arteriosus ejects blood into the ventral aorta. In as much as the mechanical changes taking place at different phases

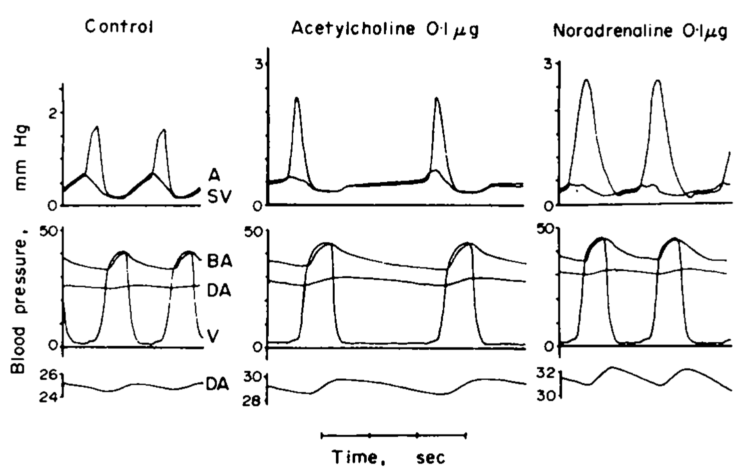

Fig. 2. *Anguilla japonica*: effects of neurohumors on cardiac pressures. A = auricle; BA = bulbus arteriosus; DA = dorsal aorta; SV = sinus venosus; V = ventricle.

of the cardiac cycle, the Asiatic eel is quite different from that of the smooth dogfish *Mustelus canis* (Sudak, 1965), the only available report on a study of similar nature. The subambient intrapericardial pressure of the elasmobranch necessarily alters the filling mechanism of the cardiac chambers.

The present study shows that cardiac pressure remains above atmospheric level throughout the entire cardiac cycle. Since central venous pressure is always higher than the sinus venosus blood pressure during the filling phase of the latter, *vis a tergo* appears to be an important mechanism in cardiac filling. Filling of the auricle occurs when the sinus pressure exceeds that of the auricle, forcing the sino auricular valves to open. The positive intrapericardial pressure at this point may help to squeeze blood from the sinus venosus into the auricle. Blood pressure in the sinus venosus is elevated during its contraction. This is in contrast to the situation found in the elasmobranchs (Sudak, 1965; Johansen & Martin, 1965). This observation supports our conclusion that sinus venosus actually fills the auricle actively instead of just merely serving as a venous reservoir (Johansen, 1965; Randall, 1970). In the smooth dogfish, Sudak (1965) observed that blood was being aspirated into the auricle throughout the entire auricular diastolic phase. This is not the case in the Asiatic eel because auricular pressure is higher than that of the sinus venosus in early auricular diastole. Auricular filling takes place in the latter half of auricular diastolic period only. The auricular pressure pattern crosses that of the ventricle twice. It is only during this brief interval that auricular pressure exceeds that of the ventricle. This can be referred to as the auricular ejection period and has been observed in other fishes (Johansen, 1965; Sudak, 1965; Satchell & Jones, 1967; Randall, 1968; Johansen, 1971). Randall (1968) also remarked that the auricular and ventricular volumes are equal in fishes. Thus, auricular ejection may be the sole contributor to ventricular filling. This is in marked contrast to the mammalian situation where most of the ventricular filling is accomplished passively during early and mid diastolic phase when the empty ventricle actually created a suction to mobilize blood from the venous system. Rapid active filling by virtue of auricular contraction completes ventricular filling only during the brief presystolic phase. In the smooth dogfish, Sudak (1965) observed a simultaneous drop of intrapericardial pressure during auricular systole. This indicates that regurgitation of blood from the auricle occurs. In the Asiatic eel, similar pericardial pressure drop has not been observed showing that the sino-auricular valves are competent. This observation is also supported by radiological study of the European eel (Mott, 1950) and physiological studies on other species (Mott, 1950; Satchell & Jones, 1967).

Duration of the cardiac cycle in the Asiatic eel is much shorter than that of the smooth dogfish (Sudak, 1965). Total ventricular and auricular systole is longer than the corresponding diastolic period in the dogfish, but in the Asiatic eel, ventricular and auricular contractions are either longer than or equal to the corresponding diastolic period.

The role played by pericardium in assisting cardiac filling in the eel is by no means comparable with that in elasmobranchs. Peak pericardial pressure occurs immediately after peak ventricular systolic pressure, during onset of bulbal ejection and lasting through ventricular relaxation; at the same time, auricular filling and the brief auricular isovolumetric contraction have also been completed. This is to be expected because part of the blood from the previous cardiac cycle is still retained within the bulbus arteriosus while another full volume of blood is gushing into the cardiac chambers. The end of pericardial pressure plateau is marked by the onset of auricular ejection or ventricular filling. During this interval, the pressure of the bulbus arteriosus further decreases as blood is being squeezed into the ventral aorta. Pericardial pressure gradually decreases as ventricular systole proceeds and auricular isovolumetric relaxation takes place until the next auricular filling. Thus the pressure pattern of the pericardial cavity reflects the volume changes in the various chambers at different phases of the cardiac cycle. However, the fact that it resembles the pressure curve of the sinus venosus does not exclude the possibility that the pericardium facilitates the filling of the sinus venosus.

The physiological response of most vertebrate hearts to noradrenaline is increased force of contraction and elevated heart rate (Prosser & Brown, 1965). This is generally associated with an increase in the intensity of the active state of muscle fibres, enhancing the speed of intrinsic shortening and reducing the duration of activity. Positive inotropism induced by noradrenaline has also been reported for various piscine species (Randall, 1970). This is also true in the *in situ* Asiatic eel heart. The rate of rise of ventricular pressure is significantly accelerated ($P < 0.01$). Although the tachycardiac effect of catecholamines on fish heart has been observed in some cases, yet it is not of universal occurrence (Johansen, 1971). Our present study indicates that the change in cardiac rhythm is insignificant. In fact, in most of the animals studied, heart rate remains unchanged. In mammals, the noradrenaline-induced increase in cardiac contraction frequency is associated with a reduction of the duration of total systole, with shortening of both the isovolumetric phase and the ejection period (Wallace *et al.*, 1963). The present investigation using pressure changes alone without corresponding cardiac phonographic and volume measurements is insufficient for us to clearly define the details taking place at various stages of mechanical activity of the heart. Nevertheless, a striking difference from the mammalian situation has been noticed. Ventricular diastolic period is significantly decreased while ventricular contraction time is lengthened. Auricular systolic period is also lengthened. Filling and emptying times of the sinus venosus are not affected although filling pressure is definitely increased. Acetylcholine produces the typical negative chronotropic effect on the eel heart. As a result, the duration of the various events of the cardiac cycle, especially diastole, is prolonged. However, the intracardiac pressures, especially the auricular and the ventricular pressures, have not been lowered. Characteristically, acetylcholine mimics the autonomic parasympathetic effect and inhibits the development of inotropism during contraction. This apparent lack of negative inotropism can be explained by the Frank Starling mechanism. The prolonged diastolic phase allows more complete relaxation of the cardiac muscles as well as adequate filling of the cardiac chamber resulting in increases in the end diastolic volume and consequently the resting length of the cardiac muscle fibres. Increased end diastolic volume leading to augmented contractility as a result of vagal stimulation has been demonstrated in marine teleosts (Jullien & Ripplinger, 1950). Indeed, Sonnenblick (1962), found that increases in resting muscle fibre length augments the development of tension without a proportionate increase in the maximal shortening velocity. This fits in well with our present observation that in spite of the raised peak pressure level, the rate of rise of pressure is either decreased (in the auricle) or remains unchanged (in the ventricle). The difference in the responses of auricle and ventricle may be attributed to a difference in their sensitivity to the neurohumors.

Nervous control provides a quick means of readjustment for the living organisms, but just how important it is for cardiac regulation in fish is questionable. In fish, the density of neural elements is highest in the sino auricular region and is very sparse in the ventricle (Yamauchi & Burnstock, 1969; Santer, 1972). Pace-makers are known to be present in the sino auricular regions. Excitatory adrenergic innervation of the fish heart (Gannon & Burnstock, 1968) does not appear to be of universal occurrence in fish. It is absent in the port jackson shark (Gannon *et al.*, 1972), the plaice (Santa, 1972) and the Asiatic eel (Chow, unpublished observations). Slowing of the cardiac pace is under parasympathetic control but the mechanism of cardio-acceleration is not known. One possibility is by lowering the level of cardiac vagal tone. Moreoever, stroke volume, rather than heart rate has been speculated to be a more important determinant of cardiac output readjustment, as for instance, during exercise (Randall & Stevens, 1967; Stevens & Randall, 1967). Haemodynamic factors altering venous return and circulating hormones may have more profound influnece on cardiac performance of fish.

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Key Word Index Teleost; *Anguilla japonica*; heart-gill preparation; cardiac cycle; intracardiac pressure; pericardial pressure; heart rate; adrenaline; acetylcholine.