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Cardiac morphodynamic remodelling in the growing eel (*Anguilla anguilla* L.)

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Summary

The morphodynamic changes occurring during growth were evaluated in the eel (*Anguilla anguilla* L.) heart. Using an *in vitro* working heart preparation, cardiac performance of small (body mass 96.76±27.49 g; mean ± S.D.) and large (body mass 656±12 g; mean ± S.D.) eels was compared under basal conditions and under loading (i.e. preload and afterload) challenges. A parallel morphometric evaluation of the ventricle was made using light and transmission electron microscope images.

The small eel hearts show a basal cardiac output lower than their large counterparts (heart rate f_H, 38.93±2.82 and 52.7±1.8 beats min^{-1}, respectively; stroke volume V_S, 0.27±0.017 and 0.37±0.016 ml kg^{-1}, respectively; means ± S.E.M.). The two groups show similar responses at increasing preload, but differ remarkably at increasing afterload. Small eel hearts decreased V_S at afterload greater than 3 kPa, in contrast to larger hearts, which maintained constant V_S up to 6 kPa. These changes in mechanical performance are related to structural differences.

Compared with the small eels, the large eels show an increase in the compacta thickness and in the diameter of the trabeculae in the spongiosa, together with reduction of the lacunary spaces. The increased compacta thickness is attained by enlargements of both the muscular and vascular compartments and reduction of the interstitium; consequently, this layer appears more compacted. Both compacta and spongiosa show higher number of myocytes together with reduced cross-sectional area and myofibrillar compartment. The compacta also shows an increased mitochondrial compartment. Our results document a cardiac morphodynamic remodelling in the growing eel.

Key words: fish, *Anguilla anguilla*, myocardial growth, cardiac performance, ventricular ultrastructure, compacta, spongiosa.

Introduction

The vertebrate heart grows to accommodate increasing circulatory demands by adjusting its mass in response to pressure and volume loading. These adjustments are determined by the principle of Laplace, which states that the major determinants of heart mass are the ventricular radius and blood pressure. Accordingly, the cardiac wall changes in thickness to maintain wall stress within a relatively narrow range of variations, as documented, for instance, in a comparative survey of birds and mammals across a broad range of body size (Seymour and Blaylock, 2000, and references therein).

An extensive amount of data from both human and experimental animal cardiology has detailed the main aspects of ventricular remodelling of the mammalian heart in response to volume and pressure loading. Ventricular adaptation to volume loading involves the enlargement of the cavity volume by increasing myocardial fibre length, with a parallel increase in the thickness of the ventricular wall. On the other hand, ventricular adaptation to pressure loading is matched by wall thickening through the parallel addition of myofibrils, without a corresponding increase in luminal volume (Braunwald, 1984). This is illustrated by the paradigm of right and left ventricles of the mammalian heart working as volume and pressure pumps, respectively. The morpho-dynamic design of the right ventricle is well suited for ejecting relatively large volumes of blood against relatively low blood pressure, while that of the left ventricle is better suited for ejecting relatively low blood volumes against higher blood pressure (Rushmer, 1972).

The fish heart is capable of impressive morpho-functional rearrangements to match the variable hemodynamic challenges resulting from developmental and eco-physiological changes of the animal, such as changes in body size and shifts in lifestyle patterns. A notable aspect of this cardiac flexibility is evident in the close relationship between the structural organization of the ventricular pump and the mechanical performance of the heart, evaluated in terms of the relative contribution of pressure and volume work to the stroke work (Tota and Gattuso, 1996). In many fish, this relationship allows a distinction between ventricles producing mainly volume work and those producing mainly pressure work (Tota and Gattuso, 1996). This picture provides an insight into how the internal construction of the ventricular chamber is adapted to its functional performance. On the other hand, many fish species experience remarkable

changes in cardiac mechanical performance associated, for instance, with thermal acclimation (Rodnick and Sidell, 1997, and references therein), or with changes in locomotive habits and/or body growth, or both (Poupa and Lindstrom, 1983). However, the mechanisms underlying these structural readjustments are mostly unexplored, both phylogenetically and ontogenetically. Studies on the parallel morphological and functional changes of the heart experienced by a fish species during its life cycle could help to elucidate the relationship between functional flexibility and bio-constructional constraints of cardiac remodelling in fish.

The European eel *Anguilla anguilla* L. represents an appropriate model on which to study the morpho-functional changes that may occur in the fish heart in association with body growth and changes in life style. The eel has a complex life cycle which, following metamorphosis, includes a spawning migration requiring high levels of swimming performance and elevated metabolic demands (van Ginneken and van den Thillart, 2000; Ellerby et al., 2001). Therefore, it may be expected that cardiac adaptation plays a crucial role in these organism changes. The aim of this study was to evaluate the relationship between changes in cardiac mechanical performance and the structural organisation of the ventricle relative to body growth in the European eel *Anguilla anguilla* L. Preliminary results of this study have been reported in abstract form (Cerra et al., 2002).

Materials and methods

Animals

We used two stocks of freshwater European eels *Anguilla anguilla* L., small and large, weighing 96.76±27.49 g (N=22) and 656±12 g (N=22), mean ± S.D., respectively. Both groups were provided by the same hatchery where fish were kept at 15–18°C for 1–2 months. Subsequently they were transferred to the tanks in the laboratory and maintained at room temperature (18–20°C) for 5–7 days. All animals were in the yellow-phase. Experiments were performed from November to April. Each eel was anaesthetised in benzocaine (0.2 g l^{-1}) for about 15 min, weighed and ventrally opened behind the pectoral fins. The heart was immediately removed and processed for the specific protocol.

Isolated and perfused in vitro *working heart preparations*

The hearts of seven small and seven large eels were removed from the animals and placed in a dish of saline for cannulation. Two polyethylene cannulae were secured in the ventral aorta and in the atrium (at the junction with the sinus venosus), respectively. Once cannulated, hearts were connected to a perfusion apparatus, as described by Tota et al. (1991).

Briefly, the atrial input cannula was connected to an input reservoir, while the ventral aortic cannula was connected to an output reservoir. Input and output pressures were regulated by varying the height of reservoirs with respect to the level of Ringer solution in the perfusion chamber containing the heart.

The Ringer's solution contained the following components in g l^{-1}: NaCl 6.68, KCl 0.15, KH_2PO_4 0.05, $MgSO_4$ 0.35, $(NH_4)_2SO_4$ 0.05, $CaCl_2$ 0.14, glucose 1, Na_2HPO_4 0.227; pH was adjusted to 7.7–7.9 by adding $NaHCO_3$ (about 1 g l^{-1}); the Ringer's solution was equilibrated with a mixture of O_2:CO_2 at 99.55:0.5%. Experiments were carried out at room temperature (18–20°C).

Measurements and calculations

Pressure was measured through T-tubes placed immediately before the input cannula and after the output cannula, and connected to two MP-20D pressure transducers (Micron Instruments, Simi Valley, CA, USA) in conjunction with a Unirecord 7050 (Ugo Basile, Comerio, Italy). Pressure measurements (input and output) were expressed in kPa and corrected for cannula resistance. Heart rate (f_H) was calculated from pressure recording curves. Cardiac output ($\dot{Q}$) was collected over 1 min and weighed; values were corrected for fluid density and expressed as volume measurements. The afterload (mean aortic pressure) was calculated as two-thirds of the diastolic pressure plus one-third of the maximum pressure (Tota et al., 1991). Stroke volume (V_S; cardiac output/heart rate, ml kg^{-1}) was used as a measure of ventricular performance; changes in V_S were considered to be inotropic effects (i.e. changes in the developed force at a given resting fibre length). $\dot{Q}$ and V_S were normalized per kilogram of wet body mass. Ventricular stroke work [W_S; (afterload – preload) × stroke volume/ventricle mass, mJ g^{-1};] served as an index of systolic functionality (Imbrogno et al., 2001).

Experimental protocols

Basal conditions

Isolated perfused hearts were stabilized at the basal condition for 15–20 min. For the two eel groups, afterload was set to 3 kPa. $\dot{Q}$ was set to about 10 ml min^{-1} kg^{-1} body mass for the small eels and 20 ml min^{-1} kg^{-1} body mass for the large eels, by appropriately adjusting the filling pressure. Cardiac parameters were simultaneously measured during the experiments. Hearts that did not stabilise within 20 min from the onset of perfusion were discarded.

Physiological experimental protocols

To evaluate the Frank–Starling response of the heart, after the stabilization period (15–20 min), starting from basal conditions, filling pressure was increased until there was no further discernible increase in $\dot{Q}$. For each filling pressure increase, the variables of cardiac performance were measured after a 5 min perfusion with saline. Each increment was 0.5 cmH_2O. The output pressure was stable at 3 kPa.

To examine the ability of the heart to adjust pressure development in response to increased peripheral resistances, starting from maximum $\dot{Q}$, the afterload was raised (about 5 cmH_2O for each step) until cardiac pumping was sufficiently compromised.

Ventricular gross morphometry

The hearts of six small (heart mass 0.14±0.012 g; mean ±

S.E.M.) and six large (heart mass 0.67±0.5 g; mean ± S.E.M.) eels were weighed immediately after sacrifice. The ventricle was then separated from the atrium and the bulbus arteriosus, and weighed to determine the relative ventricle mass (M_{RV}: ventricle mass × 100/body mass).

Morphometric analysis

The isolated hearts of six small and six large eels were blocked in diastole with an excess of KCl, and fixed in Bouin's fixative or in 2.5% glutaraldehyde in phosphate-buffered saline (PBS). Samples were then processed for light or transmission electron microscopy (TEM), according to conventional procedures.

For light microscopy, Bouin's-fixed samples were dehydrated in graded ethanol, embedded in Paraplast, and serially sectioned at 10 μm thickness. Selected sections were stained with Hematoxylin and Eosin.

For semithin sections and TEM, small cubes of tissue were taken from the middle anterior wall of the glutaraldehyde-fixed hearts. The pieces were then dehydrated in graded acetone and propylene oxide, and embedded in Araldite (Fluka, Chemie GmbH, Buchs, Switzerland). Semithin sections were cut with a LKB III ultratome, stained with 1% Toluidine Blue and inspected to determine orientation. Ultrathin sections were cut with a Leica Ultracut UCT, stained with uranyl acetate and lead citrate, and examined with a Zeiss ME 10C microscope. Micrographs of near-perfect cross-sections of ventricular myocytes were then obtained.

Images were digitalized by using Olympus Camedia Z200 (GmbH, Hamburg, Germany) connected with a Zeiss III photomicroscope (thick and semithin sections), or by using a scanner Umax IIc (UMAX Systems GmbH, Willich, Germany; TEM sections). Morphometrical evaluations were obtained on 256 grey value images using NIH Image 1.61 for Macintosh computer. Geometrical scaling was performed prior to start measurements on both light and TEM images. Each morphometric parameter was measured using at least 12 images for each animal. The thickness of the compacta was quantified by measuring the distance from the border between the epicardium and endocardium. To determine the cross-sectional area of myocytes and myofibrils, and the surface area of the mitochondria, the structures were outlined with the use of a specific software (i.e. lazo tool), which permitted selection of parts of an image in order to measure or modify them. Vascular and trabecular diameters were measured only when the structure possessed a maximum diameter/minimum diameter ratio of approximately 1. The percentage of surface area occupied by both the lacunary spaces and the myocardium was calculated by thresholding random images of different transverse sections of the whole ventricle in order to differentiate the lacunary spaces from the myocardium. The resulting area (in pixels) was subtracted from the total area of the section, thus obtaining the myocardial surface (in pixels).

Six additional hearts (three small, three large) were processed for scanning electron microscopy (SEM). Ventricle samples were dehydrated in graded acetone, dried by the critical point method, and gold-sputter-coated. Observations were made using a Philips SEM 501 scanning microscope.

Statistics

Values are presented as means ± S.E.M. We used Student's *t*-test on absolute values for within-group comparison of the curves ($P<0.05$ was taken as significant). Comparison between groups were made using two-way analysis of variance (ANOVA). Significant differences were detected using Duncan's multiple-range test ($P<0.05$).

Results

Cardiac allometry

In both eel groups, the ventricle represents the same percentage of the wet heart mass (59.7% and 54.32%, $P>0.05$ in small and large eels, respectively). Heart and ventricle mass increased proportionally to body mass. In fact, for a fourfold increment of body growth (from 138.53±7.45 g to 565.43±51.57 g, small and large eels, respectively), there was a similar increase of absolute ventricular mass (from 0.084±0.0077 g to 0.358±0.043 g, small and large eels, respectively). This was confirmed by the comparable values of the relative ventricular mass M_{RV} (0.061±0.0036 and 0.062±0.0036 for small and large eels, respectively).

Isolated and perfused in vitro *working heart preparations*

The *in vitro* isolated and perfused whole heart preparations work at physiological loads (i.e. preload, 0.15 kPa; afterload, 3 kPa) and generate values of output pressure (P_O), $\dot{Q}$, V_S, W_S and power that mimic the physiological values of the animal, as previously described (Imbrogno et al., 2001). As indicated in Figs 1 and 2, the isolated and perfused heart of the large eels showed an improved basal performance with respect to their smaller counterparts. This is exemplified by the increased basal f_H (38.93±2.82 and 52.7±1.8 beats min^{-1} for small and large hearts, respectively) and V_S (0.27±0.017 and 0.37±0.016 ml kg^{-1} for small and large hearts, respectively).

When exposed to preload increases (up to 0.65 kPa), small and large hearts revealed a similar Frank–Starling response (Figs 1, 2), being very sensitive to filling pressure increases, ranging from 0.15 to 0.6 kPa. In the two groups, the maximum $\dot{Q}$ (29.29±2.12 and 37.51±1.05 ml min^{-1} kg^{-1} body mass for small and large hearts, respectively) and the maximum V_S (0.65±0.05 and 0.66±0.008 ml kg^{-1} body mass for small and large hearts, respectively) were obtained at an input pressure of 0.55 kPa.

In the small hearts, increases of the output pressure P_O reduced $\dot{Q}$ and V_S starting from the first increment. In contrast, the large hearts were able to sustain increases of P_O up to 6 kPa without any significant decrease in V_S. Further increases of afterload significantly compromised cardiac function (Figs 1, 2). Gradual increase of either preload or afterload did not significantly modify f_H in either of the two heart groups (Figs 1, 2).

Morphology and morphometry

Both small and large eels showed the typical mixed type of ventricle consisting of an outer compact layer and an inner spongy layer (Fig. 3). Eel ventricular growth was achieved by increasing both the compacta thickness and the trabecular diameters in the spongiosa (Fig. 3, Table 1). This occurred without a parallel increase in free ventricular lumen. On the contrary, the surface area of the lacunary spaces was

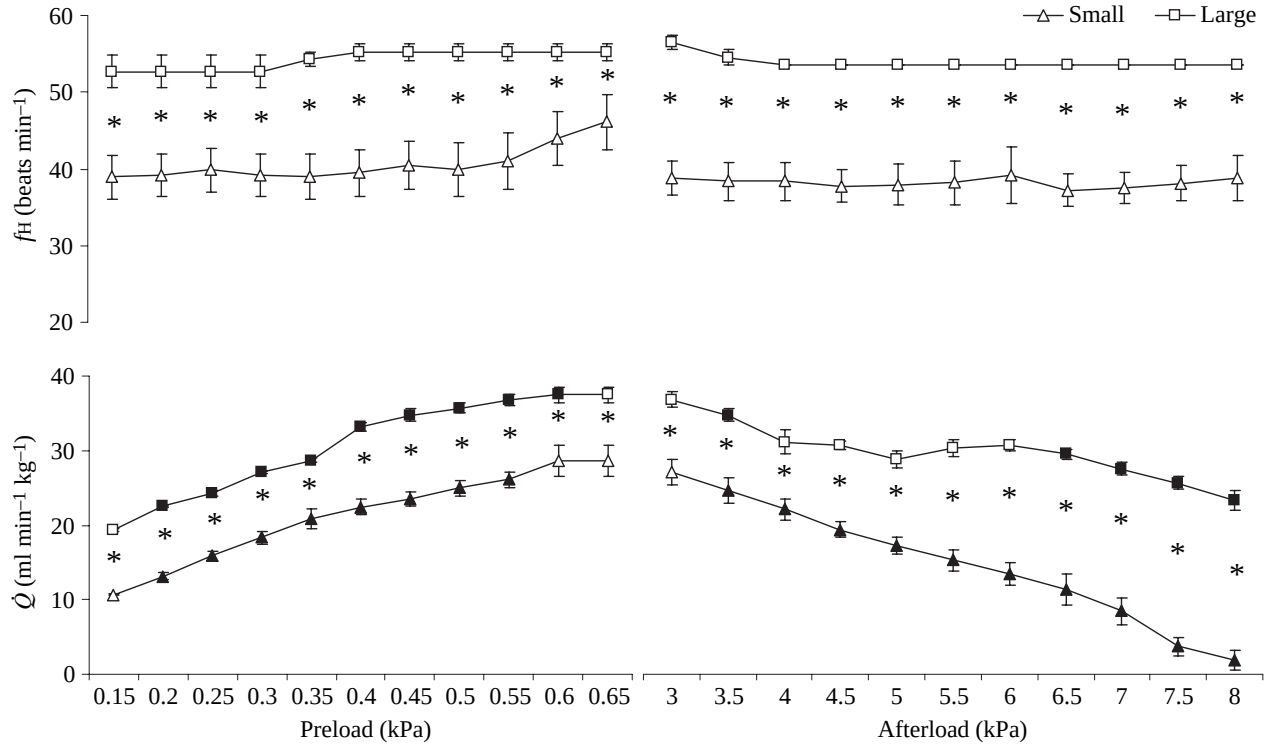

Fig. 1. Effects of preload and afterload elevation on f_H and $\dot{Q}$ in isolated and perfused small (triangles; N=7) and large (squares; N=7) eel hearts. Values are means ± S.E.M. Comparison within group: open symbols, not significant; closed symbols, P<0.05. Comparison between groups: *P<0.05.

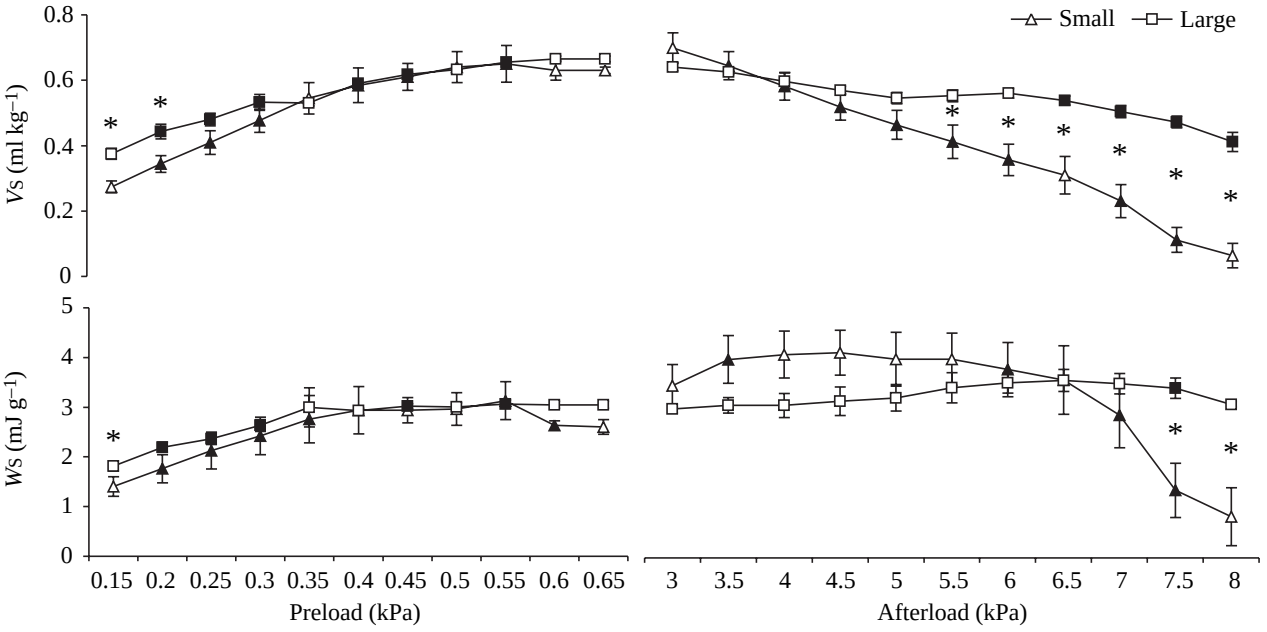

Fig. 2. Effects of preload and afterload elevation on V_S and W_S in isolated and perfused small (triangles; N=7) and large (squares; N=7) eel hearts. Values are means ± S.E.M. Comparison within group: open symbols, not significant; closed symbols, P<0.05. Comparison between groups: *P<0.05.

significantly reduced with growth (Table 1), the ventricle appearing more ‘muscularized’. The increase in thickness of the compacta was accompanied by an increase in the number of vascular profiles, and in the surface area occupied by vessels, as indicated by a significant lower myocardium/vessel ratio (Fig. 3, Table 1). In addition, analysis of the percentage of the surface area occupied by myocardium, vessels and interstitium in the compacta revealed that myocardium and vessels undergo a significant increase with growth. However, the interstitium undergoes a significant decrease (Table 1). In the spongiosa, due to the absence of vessels, it is only possible to measure the percentage of the surface area occupied by the myocardium and the interstitium, and we did not find significant modifications in either of the two parameters with growth, although a similar trend in increasing the surface area occupied by the myocardium and in decreasing that of the interstitium was detected (Table 1).

In the compacta, the number of myocytes increased with

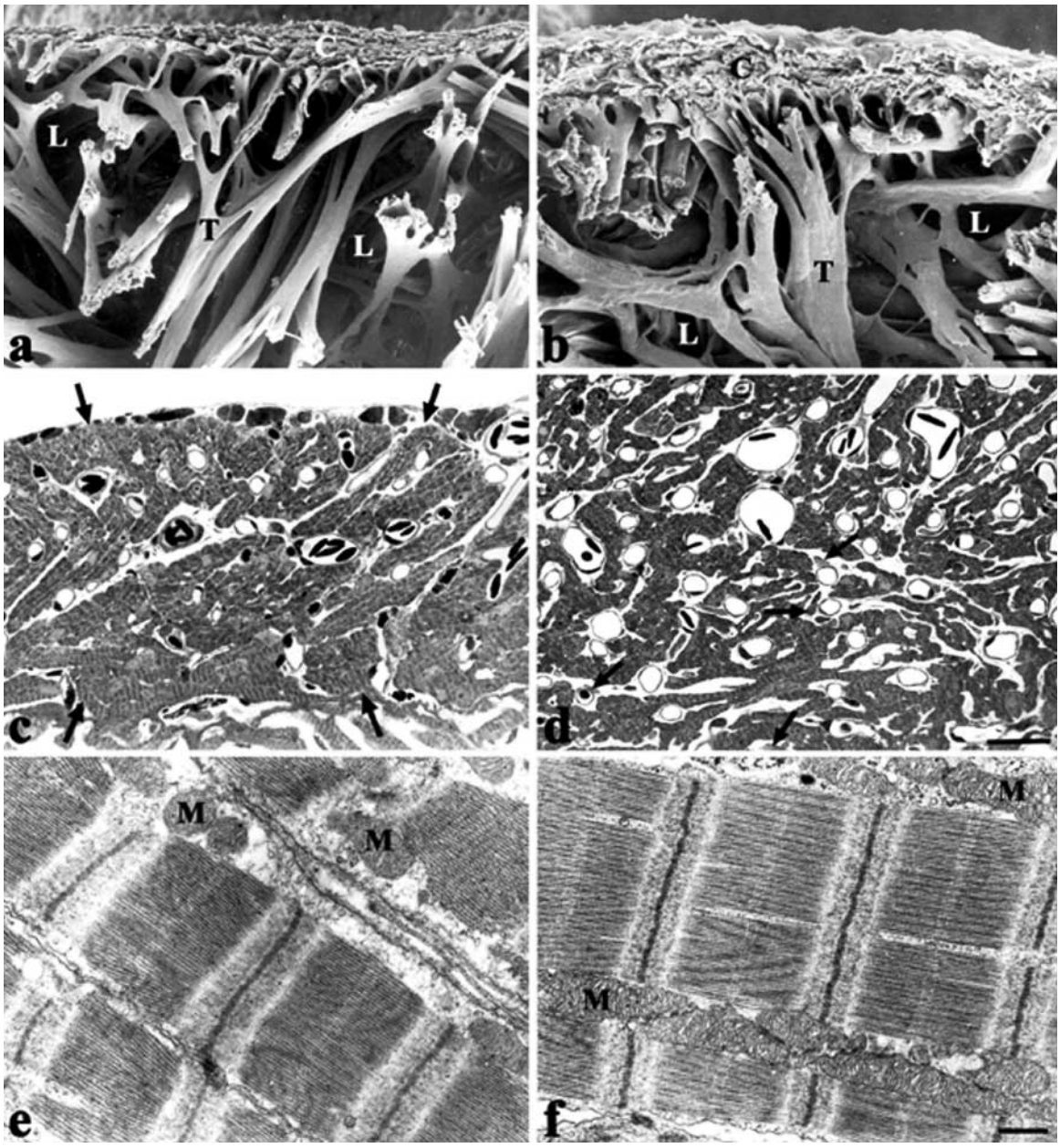

Fig. 3. Morphological changes that occur in the eel ventricle during growth. (a,b) Scanning, (c,d) light and (e,f) transmission-electron microscope images of small (a,c,e) and large (b,d,f) eels. (a,b) The thickness of the compacta and that of the trabeculae increases with growth. (c,d) The entire compacta thickness (arrows) of the small eel is included in c. At the same magnification, only part of the compacta appears in d. Note the increased vascularization of the latter. Arrows in d indicate accumulations of collagen; no such accumulations appear in c. (e,f) Myofibrils are developed at both ages. Note in f the alignment of the mitochondrial and myofibril axis. L, lacunary spaces; T, trabeculae; M, mitochondria. Bars, 25 μm (a,b); 25 μm (c,d); 600 nm (e,f).

Table 1. *Morphometric parameters obtained from the ventricles of small and large eels (*Anguilla anguilla *L.)*

Morphometric parameters	Small eel (*N*=6)	Large eel (*N*=6)
Lacunary spaces (% of section surface)	63.25±0.7	28.03±2.23***
Myocardium (% of section surface)	36.74±0.55	71.96±4.52***
Compacta		
Thickness (mm)	0.0977±0.0019	0.9927±0.0166***
Vessels (number mm^{-2})	986.84±62.6	1692.8±73.97***
Vessels [diameter (mm)]	0.0059±0.00008	0.009±0.00026***
Myocardium (% of section surface)	7.07±0.56	11.06±1
Vessels (% of section surface)	70.49±2.08	76.92±1.8
Interstitium (% of section surface)	22.44±2.01	12.02±1.29
Myocardium/vessels ratio	9.98	6.95**
Cell number (number/0.01 mm^2)	215.7±22.8	377.6±20.3***
Cellular cross-section (μm^2)	37.25±1.08	18.3±0.302***
Myofibril (% of cell surface)	16.5±2.36	11.65±1.43*
Mitochondria (number in each cell)	10±1.87	17±1.34*
Mitochondria size (μm^2)	0.174±0.017	0.107±0.006***
Mitochondria (% of cell surface)	4.7±0.8	9.91±1.78**
Spongiosa		
Trabecular diameter (mm)	0.03±0.00097	0.04±0.0013***
Myocardium (% of section surface)	73.52±3.81	78.91±1.9
Interstitium (% of section surface)	26.48±3.81	21.09±1.97
Cell number (number/0.01 mm^2)	174.8±8.2	196.6±11.4*
Cellular cross-section (μm^2)	29±0.56	25±0.48***
Myofibril (% of cell surface)	24.06±3.79	5.81±0.44**
Mitochondria (number in each cell)	13.2±2.17	15±1.5
Mitochondria size (μm^2)	0.209±0.016	0.197±0.0087
Mitochondria (% of cell surface)	9.43±1.61	10.26±1.4

Morphometric parameters were measured on light and transmission EM (TEM) images (256 grey values).

Values are means ± S.E.M. of 12 determinations in triplicate for each animal. The compacta thickness was calculated on 12 images for each animal and the values represent the means ± S.E.M. of 10 measurements for each image. Statistical significance of the differences was assessed using the paired Student's *t*-test (***P<0.0005; **P<0.005; *P<0.05).

growth, while the cellular cross-sectional area decreased, the myofibrillar compartment decreased, and the mitochondrial compartment increased. In the spongiosa, there was also an increase in cell number with growth, and a decrease in the cross-sectional area and in the myofibrillar compartment. However, the mitochondrial compartment of the spongiosa did not show any significant modification with growth.

When the two eel groups were compared, the compacta of the small eels contained larger myocytes, a smaller myofibrillar compartment, and a smaller mitochondrial compartment than the spongiosa. In the large eels, however, the compacta contained smaller myocytes than the spongiosa, a larger myofibrillar compartment, and a similar mitochondrial compartment (Table 1). Thus, compacta myocytes, despite becoming smaller in size than the spongiosa myocytes with heart growth, contain a higher amount of myofibrillar material.

Discussion

The comparative dynamic analysis between the hearts of small and large eels in the present study illustrates how the ventricle pump is able to operate in a flexible manner in relation to growth. Furthermore, changes of cardiac mechanical behaviour occurring during growth positively correlate with structural modifications of the heart ventricle, providing a dynamic perspective to its myoarchitectural heterogeneity (i.e. compacta *vs* spongiosa) (Tota and Gattuso, 1996).

The relationship between heart and ventricular growth *vs* body growth, not previously recorded in *A. anguilla*, shows that heart growth is linearly related to body growth and that in the two eel groups the ventricle mass represents the same percentage of the total body mass. Although a complex network of factors such as cold acclimation, relative cardiac work, sexual maturity, etc., may modify the rate of ventricular growth in teleosts (Houlihan et al., 1988; Graham and Farrell, 1989; Clark and Rodnick, 1998), the linear increase in ventricle mass during growth of the animal is well documented (Poupa et al., 1981; Franklin and Davie, 1992, and references therein).

The growing eel ventricle shows enhanced hemodynamic performance, both under basal and load-stimulated conditions. This is expressed by the increased basal f_H and V_S, and by the better capacity shown by the heart of the large eels to maintain

work against higher P_O. As the animal (and, thus, the heart and the ventricle) grows, f_H significantly increases from 38.93±2.82 to 52.7±1.8 beats min^{-1}. The f_H values in the isolated cardiac preparation fall within the range usually reported in anguillidae *in vivo (A. japonica*: Chan and Chow, 1976; *A. australis schmidtii*: Davie and Forster, 1980; *A. anguilla* L.: Soulier et al., 1988) and *in vitro* (*A. dieffenbachii*: Davie et al., 1992; *A. anguilla*: Imbrogno et al., 2001, and references therein). Although the importance of f_H as an index of cardiac performance is well known, there is scant information concerning the ontogenetic changes of f_H in the eel, or in other teleosts. In particular, as extensively reviewed by Farrell and Jones (1992), while in mammals an allometric relationship exists between f_H and body mass, so that resting and maximal f_H values are higher in smaller mammals, there is no evidence for a similar allometric relationship in teleosts. In juvenile and adult trout, the increased body mass relates positively with an increased cardiac performance, expressed by enhancement of both f_H and V_S (Mirkovic and Rombough, 1998). We observed here that, in the large eels, the higher f_H is associated with a higher V_S obtained at the same filling pressure. Taken together, these variations represent the physiological evidence of an increased basal cardiac performance in the large eels.

In fish, the control of $\dot{Q}$ or, more precisely, the increase in V_S, is chiefly achieved by increasing the filling pressure (Starling's law) (Farrell and Jones, 1992). Our data indicate that there is no variation of cardiac mechanical behaviour in response to increments in the filling pressure in either of the two eel groups. However, large eels possess an enhanced cardiac ability to work against high P_O. In fact, the heart of the small eels fails when P_O values higher than 3 kPa are applied.

The dynamic data correlate with modifications of the ventricular architecture observed during growth. In large eels, the ventricle becomes more 'muscularized', by increasing both the thickness of the compacta and the diameter of the trabeculae in the spongiosa, together with a reduction of the lacunary spaces. The increased muscularization and the reduction of the lacunary spaces resemble the situation described in the compact mammalian ventricle, where pressure overload is counterbalanced by wall thickening and reduction of the lumen (Braunwald, 1984). The increase in thickness of the compact myocardium during cardiac growth is common to many fish species (*Ciprinus carpio*: Bass et al., 1973; *Salmo salar*: Poupa et al., 1974; *Thunnus thynnus*: Poupa et al., 1981; *Salmo gairdneri*: Farrell et al., 1988). In the eel, the enlargement of the epicardium–endocardium distance found in the large animals is paralleled by an increase in vascularization of the compacta. Clearly, thickening of the compacta increases the diffusion distances from the blood perfusing the ventricle and from the subepicardial vessels. In mammalian and non-mammalian growing hearts, hypoxia appears to be the trigger for an increased capillarization of the compact myocardium through tightly controlled local mechanisms (Poupa and Lindstrom, 1983; Tomanek et al., 1999). These include mechanical (e.g. myocardial stretch), metabolic and growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) (Tomanek and Ratajska, 1997, and references therein). The role of both lower P_{O_2} and stretch in increasing VEGF levels, which in turn stimulates endothelial growth, is well acknowledged (Hudlicka and Brown, 1993). For example, in the developing rat heart, hypoxia raises VEGF levels by increasing the stability of its mRNA and by enhancing its mRNA transcription rate (Luscinskas and Lawler, 1994). Moreover, in their study on the trout (*Oncorhynchus mykiss*) heart ventricle, showing that the rate of protein synthesis was higher in the coronary-perfused compacta than in the spongiosa, Houlihan et al. (1988) suggested an intimate link between force of ventricular contraction, coronary perfusion and protein synthesis in the compacta. On the basis of these considerations, we suggest that in the growing eels, the enlarged vascular supply of the compacta is designed to cope with the metabolic and energetic demands of the deeper myocardial cells.

It should be emphasised that the increase in thickness of the compacta is accompanied by an increase in the muscular and vascular components per area, and by a reduction of the interstitium. A similar trend is observed in the spongiosa. Although the reduction of the interstitium in the compacta is an overestimation due to the increase of the vascular compartment, our data indicate that, with growth, the compacta not only increases in thickness but becomes more compacted. This occurs together with an increase in the amount of collagen, as it can be observed in semithin sections (Fig. 3) and after Syrius Red staining (own unpublished observations). The increase in the amount of collagen increases stiffness of the compacta, adds structural resilience, and should contribute to the increased mechanical performance (for an example, see Weber, 1989).

At the ultrastructural level, the ventricular myocardial modifications occurring during growth are characterised by an increased cellularity (expressed as number of cells/ units of surface area) and by a decrease in the cross-sectional area of the myocytes. This indicates, albeit indirectly, that the increase in mass of the eel ventricle occurs through hyperplasia. Hyperplasic ventricular growth is common to many fish species, although hypertrophy has also been shown to play an important role (Farrell et al., 1988; Bailey et al., 1997; Clark and Rodnick, 1998). In fact, it has been reported that myocyte proliferation not only plays a substantial role in the increase in myocyte mass during fish heart growth, but also that the role of hyperplasia has been underestimated (Clark and Rodnick, 1998). The importance of cardiac hyperplasia in teleosts is highlighted by the recent findings in adult zebrafish heart ventricle, which indicate extensive cardiomyocyte proliferation able to regenerate the injured outer compact myocardium (Poss et al., 2002). Notably, the growth mode of the two layers, compacta and spongiosa, appears to differ slightly. Compacta and spongiosa myocytes become smaller during growth, reducing their myofibrillar compartment. However, this reduction is threefold smaller in the compacta than in the spongiosa. In addition, the compacta shows an

increase in the mitochondrial compartment that is not observed in the spongiosa. The decrease in cellular cross-sectional area observed in both compacta and spongiosa during ventricular growth may represent an advantage to counteract the constraints imposed by the low area-to-volume ratio, which comes from the absence of a transverse tubule network (Santer, 1985; Rodnick and Sidell, 1997; Harwood et al., 2002, and references therein). Thus, in the growing eel ventricle, the strategic decrease of the diffusion distances for small molecules could probably help to cope with the increased mechanical efficiency of the ventricular wall. In this perspective, the increment of the mitochondrial compartment, which occurs in the compacta during growth, is important. Since mitochondria, in association with myofibrils, represent a basic index of the potential cardiac work (Kayar et al., 1986; Barth et al., 1992), we suggest that, in the presence of both an accelerated contractile rhythm and a higher pumping capacity, the enlargement of the mitochondrial compartment provides the myofibrillar apparatus with an adequate amount of energetic compounds. A number of signalling mechanisms and transcription factors are known to contribute to the coordinated increase in mitochondrial content that is associated with cardiac growth in response to increased afterload (Leary et al., 2002, and references therein). The increase in the mitochondrial compartment of the compacta myocytes with growth, together with the smaller decrement of the myofibrillar compartment, suggests that the compacta may sustain a higher workload than the spongiosa.

In conclusion, the cardiac ventricle of the eel undergoes important morphodynamic changes during ontogenetic growth. The ventricle of small eels, with its limited response to pressure overload and large lacunary spaces, appears better adapted to produce volume work. In contrast, the ventricle of the large eels is better suited to produce pressure work. These changes in heart performance are accompanied, both in the compacta and in the spongiosa, by an increase in cell number per unit area and a decrease in cellular cross-sectional area. The compacta of the large eels also exhibits an increased vascularization, probably matching the enhanced contractile demands.

Although the intimate molecular mechanisms underlying this cardiac morphodynamic remodelling were not directly addressed by this study, our results strongly suggest that the growing eel heart represents a useful model system for investigating basic aspects of ventricular plasticity from organ to cellular, subcellular and molecular levels.

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