Overcontraction and excess actin filaments

Basic elements of hypertrophic cardiomyopathy

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SUMMARY Endomyocardial biopsies were taken from the right ventricular aspect of the interventricular septum in three patients with hypertrophic cardiomyopathy and were subjected to electronmicroscopical examination. Longitudinal sections confirmed already well-established findings. In the transverse sections disarray in the arrangement of the actin filaments and expansion of the myosin lattice, indicating clear overcontraction, were observed. The number of actin filaments varied from seven to 14 per hexagon; a number exceeding 12, however, was found in only one case. From our findings we conclude that overcontraction leads to a progressive deviation of the actin filaments during systole caused by double overlap. The interplay of these mechanisms results in a "self-impeding contraction" of the fibres. Functionally the excess of actin filaments may provide a balance between the unequal forces of contraction.

The pathological characteristics of hypertrophic cardiomyopathy were described by Teare in 1958 as asymmetric hypertrophy of the interventricular septum together with a bizarre arrangement of muscle fibres.¹ As a consequence of these findings, no less than 58 synonyms, based on functional and/or morphological criteria, have been used for this disease.²⁻⁴ Because of the wide range of symptoms, a comprehensive definition is lacking at present so that the general term hypertrophic cardiomyopathy has found wide acceptance.

Several morphological investigations⁵⁻¹² have been carried out in an attempt to determine the cause of hypertrophic cardiomyopathy. Quite apart from the thickness of the septum and the free posterior wall of the left ventricle,¹³ a peculiar septal form,¹⁴ the small size of the left ventricle itself¹⁵ as well as isometric contraction,¹⁵ have been considered to explain the cause of the disease. Microscopical studies^{5,7,9} have repeatedly shown disarray of muscle fibres, and their abnormal shortness and width. Sarcomere length, which can be measured from the illustrations provided in the cited studies, showed a pronounced decrease, but no reference to this was made in those papers. Angiocardiographic studies have shown a remarkably small left ventricular volume in end-systole while diastolic

volume is normal or nearly normal¹⁶; this can only be achieved by intensified shortening of the sarcomeres.

The key to an understanding of the pathological anatomy and pathophysiology of hypertrophic cardiomyopathy clearly must lie in a distinct behavioural pattern of the sarcomeres. It is the purpose of this study to define the correlation that exists between overcontraction of the sarcomeres, the ensuing arrangement of actin filaments, and the effect on muscle fibres. Furthermore, an increase in the number of actin filaments found in one case will be discussed.

Patients and methods

Three male patients, F, L, and Ro (aged 40, 48, and 49, respectively) were examined. Their complaints ranged from dizziness, chest pain, or dyspnoea on exertion, to recurrent syncopal attacks. No members of the families of the three men have been examined. In each case, an apical systolic murmur, grade 2-3/6 was heard extending along the left sternal edge to the third intercostal space.

The electrocardiogram was abnormal in every case. The pathological Q waves followed by positive T waves in patient F; delta waves, a slurred R, and signs of left ventricular hypertrophy in patient

L; and slight excitation delay in the left ventricle in patient Ro. All patients were in sinus rhythm and had a normal PR interval. The cardiac silhouette and size were within the normal range. On cardiac catheterisation, increased filling pressure of the left ventricle was noted in two cases; patient Ro showed a left atrial a wave of 20 and patient L a left atrial a wave of 13 mmHg. There were no left-sided pressure gradients at rest but after stimulation with isoprenaline, or after postextrasystolic augmentation, gradients of up to 85 mmHg were recorded. At rest, patient F showed a gradient of 8 mmHg across the outflow tract of the right ventricle, while patient L showed a gradient of 6 mmHg. The cardiac indices for the three patients were 5.66 (F), 3.0 (L), and 3.72 (Ro) (l/min per m²). Endomyocardial biopsies were performed with the informed consent of the patients.

Two to three biopsy specimens were taken from the right ventricular aspect of the interventricular septum using a Konno biopsy forceps. The endomyocardial biopsies measuring about 2 mm in length were immersed immediately after their collection in a fixative solution containing 2 per cent glutaraldehyde, 2 per cent formaldehyde, and 0.1 M cacodylate buffer pH 7.2 to 7.4. After two hours' fixation and successive washing in the same buffer for one hour, the specimens were postfixed in 1 per cent OsO₄, dissolved in phosphate buffer,¹⁷ dehydrated in graded ethanols, and embedded in Epon. Thin sections cut with a LKB-4800 III Ultratome were collected on formvar-carbon membranes,¹⁸ stained with uranyl acetate¹⁹ and lead citrate,²⁰ and examined in a Siemens Elmiskop Ia electron microscope.

Fifty or more longitudinal and cross-sections were studied in each patient. Centre-to-centre spacing from one myosin filament to the next can be calculated according to the formula:

$$d = \sqrt{\frac{2}{\sqrt{3} \times n}}$$

where "n" equals the number of filaments in 1 μm²; such a formula is derived from the hexagonal lattice of the myosin filaments (Fig. 1). There is

one myosin filament per rectangle (dashed lines), that is per area d × a. Since $a = \frac{\sqrt{3}}{2} \times d$ in an equilateral triangle, there is one myosin filament per $\frac{\sqrt{3}}{2} d^2 \mu\text{m}^2$ and therefore

$$1/n = \frac{\sqrt{3}}{2} d^2; d = \sqrt{\frac{2}{\sqrt{3} \times n}}$$

The relation of (d) to the sarcomere length has been studied by Matsubara and Millman²¹ by means of x-ray diffraction in cat myocardium. Their curve descended as far as 2.1 μm (sarcomere length) where it intersected a value of (d) of 395 Å. We extended the curve in order to obtain our values for (d).

Results

LONGITUDINAL SECTIONS

Our findings confirm those of Ferrans *et al.*⁷ Disorganisation of the myofibrils, increased number of mitochondria, and enlarged nuclei are the striking features. Some Z bands may be wavy which alters the length of the sarcomeres by up to 15 per cent, with a range of 1.9 to 1.6 μm (Fig. 2). Other Z bands show widening.⁷

CROSS-SECTIONS IN THE A BAND

Allowing for minor deviations, the lattice of the myosin filaments is regular in the three cases. Centre-to-centre spacing (d) from one myosin filament to the next was calculated in patient Ro (Fig. 3a) by adopting the formula:

$$d = \sqrt{\frac{2}{\sqrt{3} \times 580}} = 0.446 \mu\text{m}.$$

In patients L and F, illustrated in Fig. 3b and c, cross-sectional areas of good quality were too few to apply morphometry effectively or accurately. Measuring (d) directly showed values in the same range as case Ro (Fig. 3a). As (d) is directly related to the sarcomere length,²¹ a value of d=446 Å corresponds to a 1.65 μm value of sarcomere length; these results indicate overcontraction.

During the state of overcontraction, the actin

Fig. 1 Illustration of the hexagonal arrangement of myosin filaments. The centre-to-centre spacing (d) can be calculated from the number of filaments (n) in 1 μm² by the formula

$$d = \sqrt{\frac{2}{\sqrt{3} \times n}}$$

filaments are irregularly distributed within a given hexagon; in some instances they are arranged in a helix around the central myosin core. The number of actin filaments varies from seven to 14 per hexagon. A number exceeding 12, however, was established only in patient Ro. The total number of actin filaments per μm^2 is 1901 (calculated in Fig. 3a), producing a ratio of 3.27 actin filaments to 1 myosin filament.

Discussion

Shortening of sarcomeres to less than $1\ \mu\text{m}$ results in overlap of actin filaments in the centre²²; this was clearly shown by Sonnenblick *et al.*²³ in their study on the intact left ventricle in which post-extrasystolic contraction shortening of up to $1.6\ \mu\text{m}$ occurred. These authors pointed out that double overlapping of thin filaments in the centre of the sarcomere may occur during potentiated contractions.

As we observed in our study of hypertrophic cardiomyopathy, such overlap produces disarray of actin filaments, the number of which varies from

one hexagon to another. Instead of 12 actin filaments per hexagon as would be expected in over-contraction, numbers between seven and 12 were found. These findings could be firstly, the result of the presence of wavy instead of normal straight Z bands (longitudinal section) (Fig. 2), and, secondly, of asymmetry of the waves and valleys of adjacent Z bands, leading to a varying length of contact between myosin filaments and actin filaments. We cannot, however, rule out a real difference in the length of the filaments themselves. Taking the relative length of the actin filaments into account, though present they may not necessarily appear in a particular cross-section. This would explain the varying numbers of actin filaments within each hexagon provided that they do not exceed 12 in number. In contrast, a quantity of 13 or 14 as found in patient Ro can only be explained if a real increase of actin filaments occurs.

This spatial and numerical arrangement of the actin filaments is of importance as to the function of the sarcomeres. Within a sarcomere myofilaments may contract at different points of the length-tension curve because of varying lengths of contact

Fig. 2 Longitudinal section of a biopsy specimen taken from the right ventricular portion of the septum (patient Ro) by means of a Konno biptome. The sarcomeres are overcontracted, their length varies between 1.4 and $1.8\ \mu\text{m}$. The Z bands are wavy. The waves of the two adjacent Z bands do not correspond (*). Stained with uranyl acetate and lead citrate. (Original magnification $18\ 400\times$.)

between each other. Consequently, the force of contraction within a single sarcomere varies. This mechanism generates forces within a single myofibril which branches into different directions deviating from the regular shortening axis. These forces are non-synergistic in character and influence each other in such a way that the resulting direction is a curve rather than a straight line; this results in a meshwork type of arrangement and whorls²⁴ of competing muscle fibres. Net shortening of the fibres can be impaired to such a degree that only low amplitude is detectable

wherever maximal disarray is located. This, in the final stage of the disease, results in a thick, poorly contractile muscle.

Apart from the consequences of spatial and numerical disarray, it is probable that during systolic overcontraction progressive deviation of actin filaments from their original direction occurs, as a result of mutual encroachment. Such a mechanism could bring the systole to an abrupt end.

The interplay of the above mechanisms produces a form of contraction for which we would like to propose the term "self-impeding contraction"; this

Fig. 3 (a) Patient Ro. Cross-section of the specimen of Fig. 2. The myosin filaments are distributed regularly; the centre-to-centre spacing (d) is $0.0446 \mu\text{m}$, indicating that the myosin lattice is expanded by overcontraction. The actin filaments are irregularly arranged. In part they follow the figure of a helix or two helices (*) around a central myosin. Their number varies between 7 and 14 (right hexagon). Stained with uranyl acetate and lead citrate. (Original magnification 156 000.) (b) Cross-section of specimen from patient L. The lattice of the myosin filaments is not as regular as in Fig. 2, (d) varies but is in the range of that in Fig. 2. The number of actin filaments ranges from 7 (▲) to 9 (lower left hexagon). (Original magnification 156 000.) (c) Cross-section of specimen from patient F. The myosin filaments are distributed fairly regularly. (d) is in the range of that in Fig. 2. The actin filaments are again arranged irregularly; their number per hexagon varies between 7 and 12 (hexagon with helix). (Original magnification 156 000.)

may correspond to a distinct form of isometric contraction postulated by Wigle and Silver.¹²

Several authors⁵⁻⁷ have investigated the structure of hypertrophic cardiomyopathy. Ferrans *et al.*⁷ recognised the abnormal architecture of the heart muscle cells as being the basic morphological feature of "hypertrophic subaortic stenosis". They did not comment on average sarcomere length; analysing their illustrations values of 1.15 up to 1.9 μm are found, that is values within the range of overcontraction. These calculated lengths confirm our own findings. A cross-section in the A bands (Fig. 3a, b, and c) clearly shows the irregular distribution of the actin filaments. On the basis of theoretical considerations, Sonnenblick²⁵ assumed that in overcontraction there was a lateral displacement of the filaments since the volume of the sarcomere remained constant. This assumption is confirmed in our findings. A helical arrangement of actin filaments around the central myosin filaments results from the unequal distribution of a great quantity of these filaments in a confined space. An excess of actin filaments could only be observed in a single case (Ro). S Page (personal communication) noted in the I bands of kitten papillary muscles small regions in which many fibrils contained more than six actin filaments around a myosin filament. These recent findings are difficult to explain. It is none the less highly probable that the additional actin filaments serve by way of compensation. The distribution of fibre disarray was investigated by Maron *et al.*⁸ They found the most pronounced structural changes in the interventricular septum of patients with hypertrophic cardiomyopathy with obstruction, while changes to a lesser extent were seen in the left and right ventricular walls in the same cases. In contrast, widespread distinct changes were noted in seven out of eight cases of hypertrophic cardiomyopathy without obstruction involving left and right ventricular walls. From right ventricular biopsies, Alexander and Gobel⁹ were able to confirm typical changes of hypertrophic cardiomyopathy. The specificity of fibre disarray, in hypertrophic cardiomyopathy, was questioned when it was described in pulmonary disease, coronary heart disease, and even in normal hearts,¹⁰⁻¹¹ though it is less frequent in these.¹⁰

We therefore conclude that overcontraction of the sarcomeres is the basic element of fibre disarray and that an excess of actin filaments acts in compensation. Neither overcontraction nor fibre disarray is specific for hypertrophic cardiomyopathy. Based on Van der Bel-Kahn's findings¹⁰ we consider the extent of the areas with overcontraction is the decisive factor in the pathogenesis of dis-

array. Bulkley *et al.*,¹⁵ however, believe that bizarre septal architecture may be secondary to a small systolic cavity and late systolic isometric contraction. Hutchins and Bulkley¹⁴ proposed two possible causes for "hypertrophic subaortic stenosis". These are isometric contraction and a catenoid-shaped septum. Isometric contraction, first described in connection with hypertrophic cardiomyopathy by Criley *et al.*,²⁶ is in our opinion "self-impeding contraction". A small ventricular cavity as well as a catenoid-shaped septum are more likely the results and not the causes of bizarre septal architecture. The actual cause of hypertrophic cardiomyopathy is in our opinion the stimulus to overcontraction which up to the present time has remained unknown.²⁷

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