

Specific Regional and Directional Contractile Responses of Aortic Cusp Tissue

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Background and aim of the study: The control of valve size and function is a dynamic process that may be modulated by vasoactive factors. The exact response of different regions of the cusp tissue with regard to extent and direction could influence valve shape, function and response to stress.

Methods: Porcine aortic valve cusps were cut into either circumferential (basal, belly and coapting edge) or radial (left, center and right) strips. Together with an intact cusp orientated circumferentially, specimens were set up in isolated organ baths.

Results: In response to 90 mM KCl, the belly of the cusp (0.66 ± 0.05 mN; $p < 0.05$) was significantly more responsive than either the basal region (0.41 ± 0.06 mN) or the coapting edge (0.31 ± 0.03 mN) and all three regions of radially orientated strips (left: 0.13 ± 0.02 mN; center: 0.23 ± 0.04 mN; right: 0.11 ± 0.03 mN). All strips showed contraction to endothelin-1 (10^{-9} to

10^{-7} M). When corrected for weight, the responses of the basal (15.2 ± 1.8 mN/g) and belly (11.3 ± 1.5 mN/g) regions were significantly greater than that of the coapting edge (8.4 ± 1.0 mN/g; $p < 0.05$) and the intact cusp (7.1 ± 1.9 mN/g, $n = 7$, $p < 0.05$). In the radially orientated tissue, responses to endothelin-1 were similar in all three regions (left: 3.4 ± 1.0 mN/g; center: 3.2 ± 0.5 mN/g; right: 2.3 ± 0.9 mN/g).

Conclusion: The contractile ability of valve cusps occurs preferentially in the circumferential direction. The enhanced contraction of the basal region may have important implications for the management of stresses experienced by the hinge of the valve. In addition, these findings may be relevant in designing tissue for valve repair by cusp extension or for the tissue engineering of a whole valve.

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Aortic valve cusp tissue exhibits an extensive range of biological mechanisms that contribute to the coordinated function of the valve by regulating both its size and shape (1-3). These mechanisms are similar to those that regulate the diameter of blood vessels - a process that is controlled by a balance between contractile and dilatory forces (4). While variations in the receptor mechanisms related to the regulation of the diameter of blood vessels are predominantly related to their anatomical position, there has been no study that has addressed the concept of regional variations in the receptor systems present on valve cusps.

It has been shown however, that there are directional and regional differences in the structure and

mechanical properties of cusp tissue (5,6). Studies have shown that collagen synthesis is highest in the basal region of the valve cusps, while glycosaminoglycan production is greatest in the area of attachment, suggesting that the different regions of the cusps, which experience different mechanical forces, exhibit variations in certain biological mechanisms (7). In addition, the contractile cell phenotypes found in valve cusps are not distributed ubiquitously, and there is a reduction in the density of nerves across the valve from the hinge region towards the coapting area (8,9). This raises the possibility that the contractile responses of cusp tissue may show regional and directional variations that allow the regulation of cusp tension.

One of several mediators that have the potential to regulate valve tone is the endothelium-derived vasoconstrictor, endothelin-1 (ET-1). This peptide is found in the valve endothelial cells, and produces an efficacious and potent contraction of cusp tissue (10), which is in excess of that produced by other mediators such as 5-hydroxytryptamine (5-HT), thromboxane A₂, noradrenaline, adrenaline, histamine and angiotensin

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II (11). It has been shown that both ET-1A and ET-1B receptors mediate the contractile effect of endothelin-1 on aortic cusp tissue (10). The aim of the present study was to determine whether the contractile responses of cusp tissue showed regional or directional variations. The contractility of cusp tissue in the circumferential and radial directions in response to a non-specific stimulus was examined, and compared to the effect mediated by endothelin-1. An understanding of the directional and regional contractile responses of cusp tissue will assist in understanding how vasoactive mediators may play a role in regulating valve function.

Materials and methods

Tissue collection

Porcine hearts were collected from a commercial

abattoir. Immediately after the animals were sacrificed, the hearts were placed into fresh Hank's solution (Sigma) and transported on ice packs to the laboratory. All tissue was used within 24 h of removal from the animals. Aortic valves were dissected free of connective tissue and opened longitudinally between the left and non-coronary cusps. Each cusp was carefully removed with a scalpel blade, placed into fresh sterile Hank's solution, and then set up in an isolated tissue bath.

Experimental procedure

The cusps were allocated to three different groups. Radial and circumferential contraction was tested using the right and left cusps respectively, while the non-coronary cusp was tested as a whole cusp with a non-specific orientation that allowed a contribution of

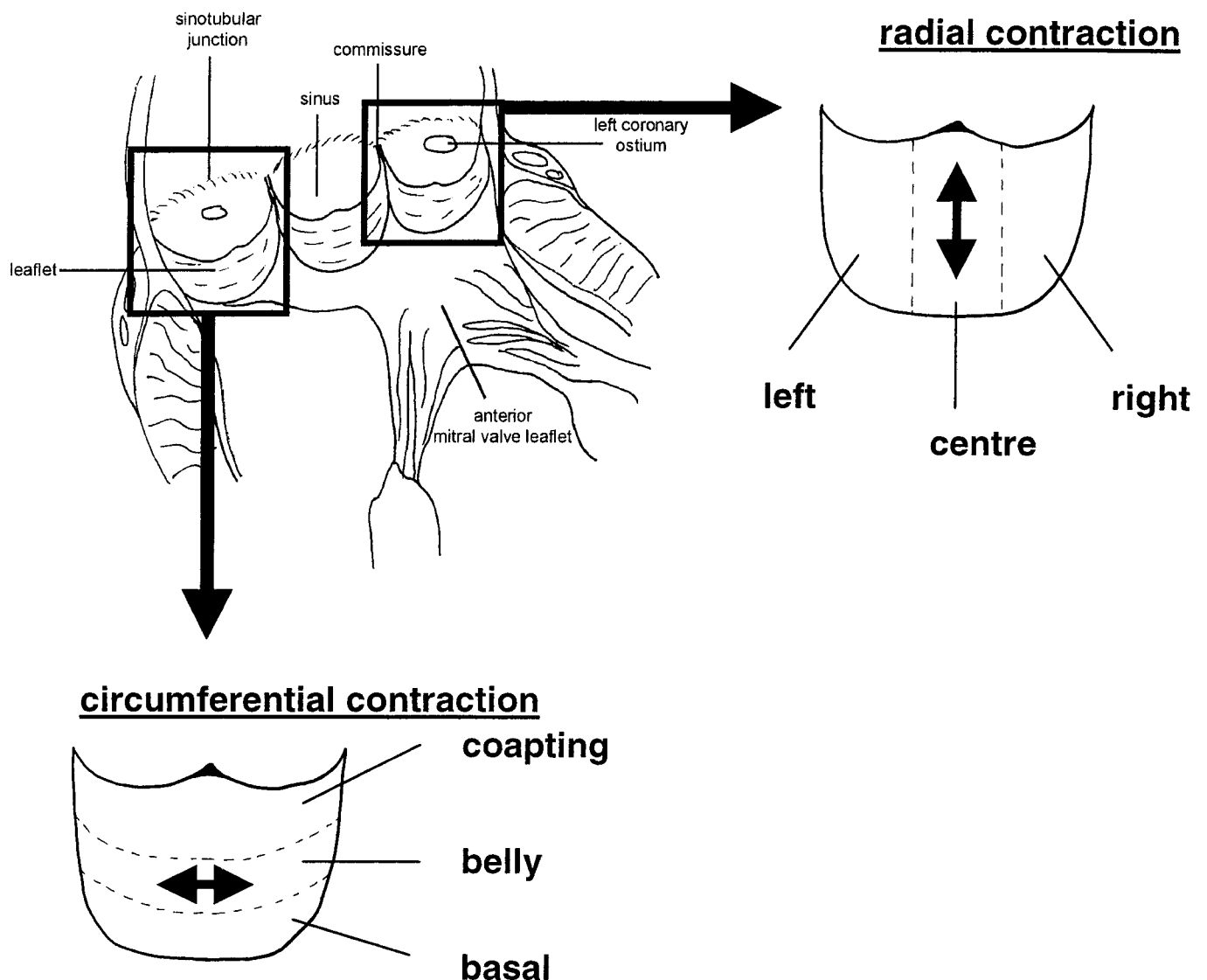


Figure 1: Diagrammatic representation of dissection of valve cusps into circumferentially and radially orientated groups. The third cusp from each valve was set up as the 'whole cusp'.

radial and circumferential contraction to the response. This group was directly comparable with the way in which previous studies on valve cusp contraction had been performed, and served as a positive control for the viability of the tissue (3,12). Cusps in the circumferential and radial groups were dissected into three separate sections: right edge, center and left edge for the radial contraction, and basal region, belly and coapting edge for the circumferential contraction (Fig. 1).

Following dissection, one end of each tissue section was attached to the strain gauge force transducer (Grass), and the other end to a static steel rod by a length of cotton string. The force displacement transducer was connected to a Grass polygraph, which allowed continuous monitoring of changes of force in each specimen. The tissues were placed in organ baths containing Krebs solution (NaCl 136.9 mM, NaHCO₃ 11.9 mM, KCl 2.7 mM, NaH₂PO₄ 0.4 mM, MgCl₂ 2.5 mM, CaCl₂ 2.5 mM, glucose 11.1 mM, and disodium EDTA 0.04 mM), maintained at 37°C and continuously gassed with 95% O₂/5% CO₂. Samples were allowed to stabilize within the organ baths for 10 min before being loaded with the pre-tension of 3 mN.

After being allowed to relax to a stable baseline over a period of 40 min, tissue viability was tested with 90 mM KCl. The contractile response to KCl was allowed to reach a stable plateau before washing the tissue. Once a stable baseline had been re-established, the tissues were challenged with increasing concentrations of endothelin-1 (10⁻¹⁰ to 10⁻⁷ M) in half-log₁₀ increments. Each concentration was allowed to attain a stable level of tension before the next concentration of endothelin-1 was added in cumulative fashion. At the end of the experiment, the valve specimens were removed from the tissue bath, blotted dry and weighed.

Data analysis

Responses were measured in mN increase in tension. Valves were normalized for each specimen to mN/g tissue and expressed as mean ± SEM. Values of *N* refer to the number of pigs from which valves were obtained, and statistical analyses was made with an ANOVA followed by a Bonferroni *t*-test. Concentration-response curves were constructed by fitting the data to a logistical sigmoidal dose-response equation using the curve fitting function in the graph-plotting program Fig P (BioSoft). The potency of endothelin-1 was measured by calculating the pEC₅₀, which is defined as the negative logarithm of the concentration required to produce 50% of the maximum response.

Results

The contractility studies with 90 mM KCl revealed that the samples from circumferential and radial orien-

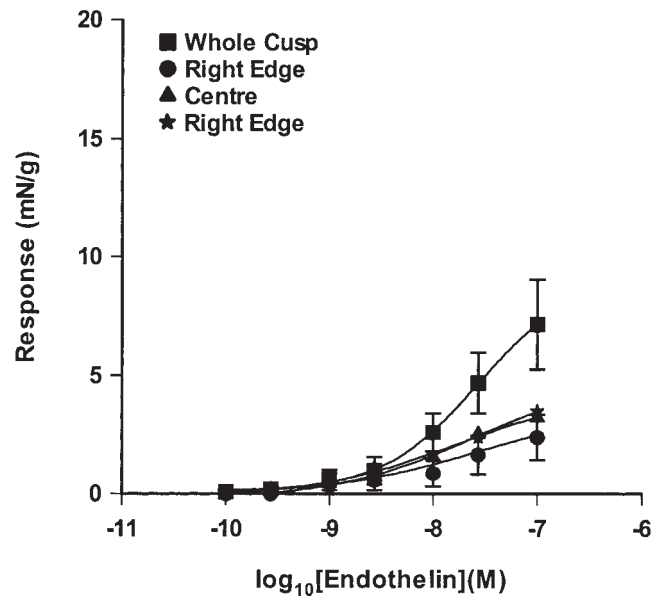


Figure 2: Circumferential contraction of aortic valve cusp regions (basal region, belly and coapting area) in response to increasing concentrations of endothelin-1. Values for each group are mean ± SEM (n = 7).

tations were consistent with the strongest response occurring in the center and belly regions for radial and circumferential directions, respectively. There was a significant difference between the radially and circumferentially orientated cusps. The largest response was seen in the belly region of the circumferentially orientated cusps, and was significantly greater than that seen in the basal region, the coapting edge or all three radially orientated strips (Table I). By comparison, the whole cusp achieved a maximum response of 0.61 ± 0.08 mN, which was similar to that seen in the belly region.

Radial responses to endothelin-1 (Fig. 2) showed a significant reduction in contraction compared to the whole cusp (p<0.05), which showed a maximum response of 7.1 ± 1.9 mN/g, while the right edge achieved a maximal response of 2.3 ± 0.9 mN/g, the center achieved a response of 3.2 ± 0.5 mN/g, and the left edge a contraction of 3.4 ± 1.0 mN/g. All three groups were not significantly different from each other or the whole cusp (p <0.05). The potency values for endothelin-1 in each of the regions were similar, with pEC₅₀ values of 7.95, 8.02 and 8.22 in the right, center and left regions, respectively.

Circumferential samples showed an increased contraction to endothelin-1 compared to the whole cusp (Fig. 3). This difference was only significant for the basal region (p <0.05), which achieved the largest response of 15.2 ± 1.2 mN/g. The basal region contraction was also significantly greater than the coapting edge (8.4 ± 1.0 mN/g), but not when compared to the

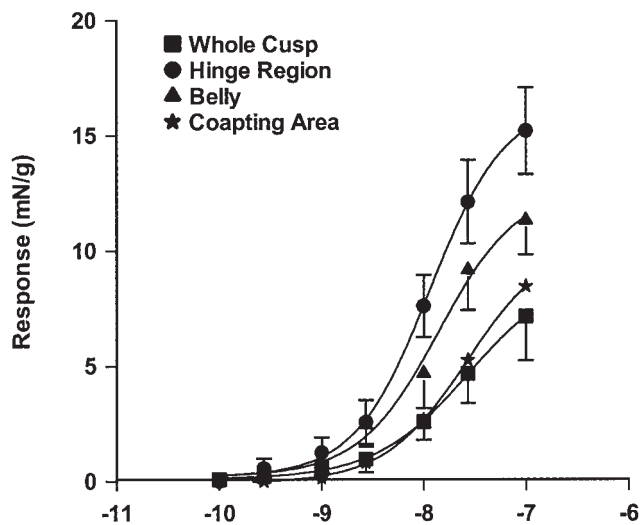


Figure 3: Radial contraction of aortic valve cusp regions (left, center and right) in response to increasing concentrations of endothelin-1. Values for each group are mean \pm SEM ($n = 7$).

belly of the cusp (11.3 ± 1.3 mN/g). The pEC_{50} values of endothelin-1 in the basal (7.91) and belly (7.83) regions of the cusp were up to six-fold greater than in the coapting area (7.18).

In order to establish whether there was any variation in the contractile response to endothelin-1 between the individual cusps, each whole cusp was tested in the non-specific orientation. This yielded no significant difference between the cusps (data not shown).

Discussion

The results of the present study showed that the contractile capacity of aortic valve cusps resides primarily in the circumferential direction. The observed radial contraction to KCl was only about one-third of that seen in the circumferential direction. In addition, it was shown that endothelin-1 exhibits regional variations in the function of its receptors with respect to circumferential contraction.

It is of interest that this pattern of contractility does not match that of the distensibility of cusp tissue in the

radial and circumferential directions. It has been shown that in the radial direction, the aortic valve cusps are up to six-fold more distensible for the same degree of force (5). This finding relates to the difference in the collagen orientation in the cusps, which occurs primarily in the circumferential direction. The present findings suggest that the contractile mechanisms in the cusp, which can be activated by a range of vasoactive agents (3,10,12), may serve to limit distensibility of the cusp in the circumferential direction, while having a relatively minor effect on distensibility in the radial direction. Influencing distensibility in the circumferential direction may still have the capacity to regulate changes in the size and shape of the valve that occur during the cardiac cycle. Additional studies are warranted to examine how the degree of contraction in the fresh cusp can affect the overall shape of cusp tissue and the stress-strain relationship, as described by Christie and Barratt-Boyes (5). The ability of contractile agents to influence the stress distribution in the cusp may serve as an important physiological mechanism which, if disrupted, could precipitate valve dysfunction. Investigations to assess the effect of endothelin-1 on the mechanical properties of cusp tissue will be able to determine how this endothelium-derived peptide may modulate valve function.

Endothelin-1 was originally described by Yanigasaawa as a potent and efficacious vasoactive peptide released by the vascular endothelial cells (13). Since its original discovery, other cells types have been shown to release the peptide, various converting enzymes and receptor subtypes have been characterized, and a number of other biological actions have been identified (14,15). With respect to cusp tissue, the presence of endothelin-1 has been demonstrated in the endothelial cells covering both sides of the aortic valve cusps (10). Endothelin-1 has been shown to be released preferentially towards the underlying tissue rather than into the blood, and as such it could act locally on the valve interstitial cells at high concentrations (16). It has been shown previously that endothelin-1 is the most powerful constrictor of cusp tissue, when compared to 5-HT, catecholamines, thromboxane, angiotensin II and histamine (3). In addition, the

Table I: Maximum responses to 90 mM KCl of the contractile effect in circumferentially and radially orientated regions of the aortic valve cusp.

Circumferential contraction		Radial contraction	
Valve region	Response (mN)	Valve region	Response (mN)
Basal	$0.41 \pm 0.06^{\ddagger}$	Left	0.13 ± 0.02
Belly	$0.66 \pm 0.05^{\ddagger, \S}$	Center	0.23 ± 0.04
Coapting area	$0.31 \pm 0.03^{\S}$	Right	0.11 ± 0.03

$^{\ddagger}p < 0.05$ versus all other regions; $^{\S}p < 0.05$ versus all radial regions; $^{\S}p < 0.05$ versus the right radial region.

effects of endothelin-1 have shown to be mediated by both ET-1_A and ET-1_B receptor subtypes (10). In the present study, it was found that the contraction elicited by endothelin-1 was not homogeneous across the whole cusp, but occurred preferentially in the circumferential direction. The regional effects caused by endothelin-1 suggest that it can affect cusp function in a specific manner. The relative lack of effect and the reduced potency of endothelin-1 in the coapting area suggest that it cannot influence greatly this area of the cusps during valve closing. However, the preferential effect in the hinge and belly region suggests that the peptide could be capable of affecting the tension of the cusp in load-bearing regions. In addition, significant circumferential contraction would then restrict outward movement of the annulus.

The limitation of these observations is the difficulty in translating them directly to valve function *in vivo*. Investigations are required to examine how the movement and function of the valve cusps are influenced by endothelin-1. These experiments would need to be carried out *ex-vivo* in a mock circulation that closely mimics hemodynamic conditions.

The results for the effect of endothelin-1 in the present study were normalized for the weight of tissue. This differed from previous studies of this nature, and was carried out in order to allow for the variation in amount of tissue used from each of the regions tested (3,12). The data obtained with KCl showed a variation in the contractility of each region, and was therefore a reflection of the contractile capacity of each specimen of tissue. In contrast, the endothelin-1 responses - when normalized to wet weight - provided a comparative measure of the function of the endothelin-1 receptors in each region. The apparent reduction of receptors, from the hinge region towards the center of the cusp, mirrored what has been reported for the density of nerves observed in human valve tissue (8). Interactions between locally released agents and neurotransmitters thus remain a possibility for the modulation of valve tone. Recently unpublished observations from the present authors' laboratory have identified an association between endothelin-1 receptors and neuronal structures.

It has been reported that levels of circulating endothelin-1 were raised in patients with aortic valve disease (17), but the higher levels did not correlate with variations in blood pressure. In addition, raised levels of endothelin-1 have been demonstrated in patients with mitral valve disease, which increased upon trauma to the valve during balloon dilation (18). It is difficult to translate the increased circulating levels of endothelin-1 seen in these studies with that which would be present locally at the receptors in the cusp. The observation that trauma to the valve is associated with increased levels of endothelin-1 suggests

that the endothelium present on the cusps is capable of releasing significant amounts of the peptide. Studies are warranted that will investigate the effect of changes in hemodynamic parameters and risk factors for valve disease on endothelin-1 production, as well as changes in receptor profile and number.

The role of endothelin-mediated contraction in the regional and directional contraction of cusp tissue requires further investigation. The presence of these mechanisms in aortic valve cusps highlights the complex nature of how the size and shape of the valve may be regulated. The precise role of valve contraction - and more specifically that mediated by endothelin-1 - in the physiology and pathophysiology of heart valve function requires further investigation, and should yield results that will aid in the understanding of the complex function of these structures.

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Meeting discussion

DR. ROBERT FRATER (New York, USA): Can you tell us the nature of the contraction?

DR. ADRIAN H. CHESTER (London, UK): We measure the contraction as an increase in tension in terms of millinewtons as an isometric contractility.

DR. FRATER: Can you suggest a mechanism for it? Why is there an increase in that tension? What is happening in the leaflet?

DR. CHESTER: The receptors present must have signaling mechanisms linked to the smooth muscle alpha-actin elements in the tissue, which then produce contraction in a similar way that they would in a vascular segment.

SIR MAGDI M. YACOUB (London, UK): Can I step in here? If one wants to wildly theorize and speculate, because the contraction is different in different directions, it would change the shape. It is different - circumferentially it is more, so you have more in the radial position, and that will increase coaptation. Equally, the contraction peripherally is higher than in the center - again suggesting that the endothelin-1 will reduce the size of the peripheral region and make more tissue available for coaptation. endothelin-1 is secreted very slowly and cannot be responding to different stimuli during the cardiac cycle. Yet, in blood vessels it

is known to be very important in maintaining long-term vascular tone.

DR. JOHN PEPPER (London, United Kingdom): The porcine valve is asymmetric, and you have shown in human valves a very different innovation pattern in the non-coronary and coronary leaflets. Can you comment about your findings with respect to each leaflet? Does each leaflet behave the same as in a porcine valve?

DR. CHESTER: In these experiments, they do. Initially, we performed the experiments in the same cusp each time, with radial and circumferential strips. But when we switched that, the response didn't change. So with respect to endothelin-1, I don't think there is an asymmetric pattern of contraction. Our long-term plan is to stimulate these cusps with an electrical field. One of the aims of the present study was to define a region with the most sensitive or increased contractility. We believe we have found that region in the attachment edge, so we will now go ahead and perform neuronally mediated responses on that tissue.

DR. MICHAEL SACKS (Pittsburgh, USA): It's nice to see the circumferential preference in contraction - I think that fits in with what we find with cell orientation, that the dominant direction was clearly the circumferential direction, and that's very consistent. However, while I think your forces are clearly measurable and significant, what is their role? They seem to be involved more as the valve opens and closes, but at diastolic closure the passive-induced tensions by the transvalvular pressure will be orders of magnitude larger than the forces you are measuring. Can you comment on how these forces relate to the closed position of the valve?

DR. CHESTER: I don't think that the mechanisms I have described influence the moment-to-moment opening and closing of the valve. I think they confer a change or a modulation on the stress-strain properties of the valve cusps, and thereby regulate in a long-term manner how they behave. With regard to the size of the forces, it is obviously difficult to extrapolate from an in-vitro experiment to an in-vivo situation. But we have performed some experiments with intact perfused porcine aortic roots in a mock circulation where we have attached a combination of ultrasonic crystals to the aortic root. We then measured the distance between the commissures and between different regions in the annulus, and showed that - not with endothelin-1 but with 5-HT - you can change the dimensional changes with each cardiac cycle compared to control valves. So although the contractile forces seem to be small, they may influence the dimensions of the root when exposed to hemodynamic conditions.