Local serotonin mediates cyclic strain-induced phenotype transformation, matrix degradation, and glycosaminoglycan synthesis in cultured sheep mitral valves

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Lacerda CM, Kisiday J, Johnson B, Orton EC. Local serotonin mediates cyclic strain-induced phenotype transformation, matrix degradation, and glycosaminoglycan synthesis in cultured sheep mitral valves. Am J Physiol Heart Circ Physiol 302: H1983–H1990, 2012. First published February 17, 2012; doi:10.1152/ajpheart.00987.2011.—This study addressed the following questions: 1) Does cyclic tensile strain induce protein expression patterns consistent with myxomatous degeneration in mitral valves? 2) Does cyclic strain induce local serotonin synthesis in mitral valves? 3) Are cyclic strain-induced myxomatous protein expression patterns in mitral valves dependent on local serotonin? Cultured sheep mitral valve leaflets were subjected to 0, 10, 20, and 30% cyclic strain for 24 and 72 h. Protein levels of activated myofibroblast phenotype markers, α-smooth muscle actin (α-SMA) and nonmuscle embryonic myosin (SMemb); matrix catabolic enzymes, matrix metalloprotease (MMP) 1 and 13, and cathepsin K; and sulfated glycosaminoglycan (GAG) content in mitral valves increased with increased cyclic strain. Serotonin was present in the serum-free media of cultured mitral valves and concentrations increased with cyclic strain. Expression of the serotonin synthetic enzyme tryptophan hydroxylase 1 (TPH1) increased in strained mitral valves. Pharmacologic inhibition of the serotonin 2B/2C receptor or TPH1 diminished expression of phenotype markers (α-SMA and SMemb) and matrix catabolic enzyme (MMP1, MMP13, and cathepsin K) expression in 10- and 30%-strained mitral valves. These results provide first evidence that mitral valves synthesize serotonin locally. The results further demonstrate that tensile loading modulates local serotonin synthesis, expression of effector proteins associated with mitral valve degeneration, and GAG synthesis. Inhibition of serotonin diminishes strain-mediated protein expression patterns. These findings implicate serotonin and tensile loading in mitral valve degeneration, functionally link the pathogeneses of serotoninergic (carcinoid, drug-induced) and degenerative mitral valve disease, and have therapeutic implications.

valvular heart disease; myxomatous mitral valve disease

DEGENERATIVE VALVULAR HEART disease is an increasingly important public health problem in industrialized countries, particularly as the population ages (23, 33). Calcific aortic valve disease and myxomatous mitral valve disease (MMVD) are the most common manifestations of degenerative valve disease and together account for a majority of valvular heart disease overall (23, 43). The initiating mechanisms and signaling pathways that mediate these apparently diverse pathologies are not known.

Pathologic mechanical loading of heart valves is postulated to play an initiating role in degenerative valvular heart diseases (16, 34, 35, 38). Heart valves are subjected to three principal loading forces: tension, shear, and flexure (38). Any or all of these forces, if inappropriate, could play a role in degenerative valvular heart disease. Hypertension is a reported risk factor for development of age-related mitral regurgitation (41). Increased systolic blood pressure has been shown to increase tensile strain on mitral valve leaflets (39). Heritable connective tissue disorders such as Marfan’s syndrome are also known to predispose MMVD (7, 45). Such disorders can also be reasoned to increase strain on valve interstitial cells because of inherent weakness in their supporting matrix. Thus abnormal tensile strain on mitral leaflets could play an initiating or propagating role in the pathogenesis of MMVD. This hypothesis is supported by recent in vitro studies (1–3, 31) of cyclically stretched porcine aortic valves demonstrating up-regulation of catabolic enzymes, cell proliferation, induction of bone morphogenic proteins (BMP) and transforming growth factor-β (TGFβ), calcification, collagen synthesis, and proteoglycan (PG) expression in response to tensile strain. Similar in vitro studies of cyclically strained mitral valves have not been reported.

Serotonin is well known to mediate valvulopathy in a variety of clinical settings including carcinoid tumors (19) and serotonergic drugs (36, 37, 46). Further, exogenous administration of serotonin induces valvulopathy in rats (13). Currently, degenerative and serotonergic valvulopathies are regarded as unrelated pathologies. We (10, 12) have reported increased abundance of the key serotonin synthetic enzyme tryptophan hydroxylase 1 (TPH1) increased in strained mitral valves. Further, we (12, 40) have reported increased phosphorylated ERK1/2 in canine degenerative mitral valves. These findings have led us to the hypothesis that local serotonin synthesis plays a mediating role in MMVD.

In this study, we addressed the following questions: 1) Does cyclic tensile strain induce protein expression patterns consistent with myxomatous degeneration in cultured mitral valves? 2) Does cyclic strain induce local serotonin synthesis in cultured mitral valves? 3) Are cyclic strain-induced myxomatous gene expression patterns dependent on local serotonin synthesis?

MATERIALS AND METHODS

Sheep mitral valves and bioreactor. Hearts were collected from young adult sheep (18–24 mo) within minutes of slaughter and transported from the abattoir to the laboratory on ice. Whole mitral
septal (anterior) leaflets were harvested aseptically under a laminar flow hood, mounted for radial stretch onto custom-built devices, and placed into a custom-built culture chamber designed to hold four mounted valves in separate wells (Fig. 1). Tissue was mounted for application of radial strain by clamping the leaflet annulus and leaflet free edge at the attachment of primary chordae tendineae into the mounting device.

Valves were cultured in a defined serum-free medium consisting of DMEM:F-12 (10–092; Cellgro Mediatech, Manassas, VA) supplemented with 1% serum replacement (purified bovine transferrin, albumin, and insulin; S9388; Sigma-Aldrich, St. Louis, MO) and 1% antibiotics and antimycotics (10,000 U/ml penicillin G, 10 mg/ml streptomycin sulfate, and 25 µg/ml amphotericin B; A5955; Sigma-Aldrich). Valves were incubated at 37°C in 5% CO2 for 24 or 72 h.

Mitral valves were strained using a small axial loading machine housed in a standard incubator and capable of applying axial deformations as small as 1 µm (15). Cyclic loads were applied by the machine pushing downwards on the carriage and the chamber lid, which in turn caused the space between the clamps of the mounting device to proportionally increase (Fig. 1). Experiments used a sinusoidal dynamic cycling protocol at 0.5 Hz. Applied strain levels were 0% (control), 10% (low physiologic strain), 20% (high physiologic strain), and 30% (pathologic strain; Refs. 2, 26, 39). Applied tensile load was measured every 30 min during each experiment.

Serotonin inhibition experiments. In separate experiments, sheep mitral valves were subjected to 10 and 30% strain for 72 h with and without serotonin inhibition. Inhibition of serotonin signaling was accomplished pharmacologically with the tryptophan hydroxylase inhibitor 4-chloro-DL-phenylalanine (C6506; Sigma-Aldrich) at 250 µM (28) or the serotonin type 2B/2C receptor (5HT2B/2C-R) blocker SB-206553 (S180; Sigma-Aldrich) at 10 µM (24).

Viability assay. Cellular viability of cultured mitral valves was compared with fresh mitral valves by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay (CGD1; Sigma-Aldrich). Equal weight tissues were washed twice with PBS containing antibiotics and placed in a 24-well plate containing 200 µl/well of MTT solution. After 3 h of incubation at 37°C and 5% CO2, tissues were carefully removed, blotted dry, and transferred to a new well containing 1 ml/well of extraction solution. The plates were incubated for 1 h at 37°C and 5% CO2 in the dark. After incubation, the plate was thoroughly mixed and tissue samples discarded. Two-hundred-microliter aliquots from each sample were transferred to a 96-well plate for absorbance readings. Absorbance was measured at 570 nm. Background absorbance was measured at 690 nm and subtracted from 570-nm readings. Viability of cultured mitral valves was expressed as a percentage of average viability of fresh mitral valves.

Histology and immunohistochemistry. Tissues were fixed with 10% formalin, embedded in paraffin, cut into 4-µm sections, and mounted on glass slides. Each valve was stained with modified Movat pentachrome stain for histopathologic evaluation. Valves were evaluated for morphologic changes including PG/glycosaminoglycan (GAG) accumulation, disruption of collagen bundles, elastin fragmentation, and loss of normal valve architecture.

Immunohistochemistry was performed to determine myofibroblast activation, indicated by α-smooth muscle actin (α-SMA) staining. Immunohistochemistry staining was performed as per previously published methods (9). Briefly, slides underwent deparaffinization and rehydration. TBS (pH 7.8) was used to dilute enzymes, sera, and antibodies. Sections were incubated with 10% goat serum followed by 0.03% hydrogen peroxide for 15 min before incubation with the primary antibody (40 min). The primary anti-mouse monoclonal antibody and titer was α-SMA (1:500; Santa Cruz Biotechnology, Santa Cruz, CA); primary antibody was revealed with biotinylated antimouse antibody (1:500; Vector Laboratories, Burlingame, CA) and peroxidase-conjugated streptavidin (1:50; Sigma-Aldrich). Staining was visualized using nickel-enhanced diaminobenzidine (DAB) as a chromogen.

Fig. 1. Apparatus for applying cyclic strain to cultured mitral valves. A miniaturized axial loading machine capable of delivering precise tensile cyclic strain and measuring tensile load on cultured mitral valves was housed within a standard incubator (A). Main components of the machine were as follows: axial linear stepper motor (a); bearing/carriage assembly (b); precision displacement transducer (c); and culture chamber (d). Valve leaflets were mounted onto custom-built devices with clamps to fix the leaflets and placed into a 4-well culture chamber (B). Drawing of custom-built culture chamber with sliding lid and 4 valve mounting devices (C). Compression of the chamber lid by the axial loading machine increased the distance between clamps of the mounting device resulting in tensile strain on the mounted valve leaflets (D and E).

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Santa Cruz, CA). The anti-mouse peroxidase-labeled polymer (Envision+; Dako, Carpinteria, CA) was then applied for 30 min, and peroxidase activity was visualized with 3,3-diaminobenzidine.Slides were rinsed in TBS containing 0.01% Tween-20 after each incubation step. Sections were counterstained with Mayer’s hematoxylin (Dako), dehydrated, and mounted.

**GAG assay.** Sulfated glycosaminoglycan content was quantified using the Blyscan assay kit (Biocolor, Belfast, UK). Samples were digested for 4 h at 60°C in 1% papain (Sigma-Aldrich). After digestion, GAG quantification was performed according to the recommended manufacturer’s protocol. GAG is quantified based on 1,9-dimethylmethylene blue colorimetry.

**Serotonin assay.** ELISA was performed to determine the concentration of serotonin secreted into the media of each mitral valve culture well. The ELISA was performed according to the manufacturer’s instructions (ADI-900–175; Enzo Life Sciences, Plymouth Meeting, PA). Standard curves were generated using the above defined serum-free culture media. Serotonin concentrations represent conditioning of 50 ml of culture medium by valve sections of 0.15 ± 0.01 g wet weight.

**Immunoblotting.** Valve samples were weighed, manually homogenized, and rinsed with PBS. Each gram of homogenized tissue was then dispersed in 10 ml of ice-cold protein extraction buffer (10 mM Tris-HCl pH 7.8, 10 mM dithiothreitol, 2 mM MgCl₂, 1 M urea, and 1% CHAPS; all Sigma-Aldrich) and 1% halt protease inhibitor cocktail (Thermo Scientific, Rockford, IL). Samples were vortexed with glass beads for several cycles of 30 s and then incubated for 1 h at 4°C. Finally, samples were centrifuged at 22,000 g at 4°C for 20 min. Protein concentrations of the supernatant were evaluated using the BCA assay (Pierce/Thermo Scientific).

Immunoblotting was used to determine abundance of α-SMA, nonmuscle (embryonic) myosin heavy chain B (SMemb), matrix metalloproteinases 1 and 13 (MMP1 and MMP13), cathepsin K and TPH1, SERT, and monoamine oxidase A. Equal amounts (40 μg/lane) of protein extract from each valve were separated by one-dimensional electrophoresis on mini-gradient gels (4–12% Tris-glycine polyacrylamide; Invitrogen, Carlsbad, CA) and transferred to nitrocellulose membranes (Invitrogen). Membranes were blocked with 1% BSA for 30 min and then incubated in 1% nonfat dry milk in TBS overnight, along with the following mouse monoclonal antibodies: α-SMA (1:400, sc-32251; Santa Cruz Biotechnology), SMemb (1:200, ab684; Abcam, Cambridge, MA), MMP1 (1:200, sc-21731; Santa Cruz Biotechnology), MMP13 (1:200, sc-80200; Santa Cruz Biotechnology, Santa Cruz, CA), cathepsin K (1:200, sc-48353; Santa Cruz Biotechnology), TPH1 (1:100, T0678; Sigma-Aldrich), SERT (1:400, AB-N09; Advanced Targeting Systems, San Diego, CA), monoamine oxidase A (1:200, sc-271123; Santa Cruz Biotechnology), and α-tubulin (1:200, sc-8035; Santa Cruz Biotechnology) loading control. Membranes were rinsed three times with TBS-Tween 20 and incubated with goat anti-mouse IgG (H + L) conjugated with horseradish peroxidase (Jackson ImmunoResearch, West Grove, PA) for 1 h. Membranes were further rinsed, and blotted proteins were visualized by using a chemiluminescence detection kit (Millipore, Billerica, MA) and Chemidoc XRS imager (Bio-Rad Laboratories, Hercules, CA). Protein band densities were quantified on Image J (image processing and analysis in Java by NIH Image, NIH, Bethesda, MD) and normalized to the density of α-tubulin.

**Statistical analysis.** Differences in serotonin concentration or relative optical density between treatment groups were determined by two-way ANOVA for the effects of time and strain. One-way ANOVA with pair-wise comparisons (Tukey’s honestly significant difference) was used to determine differences between strain levels within each time point. Differences in serotonin inhibition experiments determined by Student’s t-test. A P value <0.05 was considered significant.

**RESULTS**

**Tensile load, cell viability, and morphology.** The measured tensile load necessary to maintain strain on mitral valves decreased exponentially over the first 30 h of cyclic strain (Fig. 2). The magnitude of decrease in tensile load was greater at higher strain levels consistent with greater weakening of the extracellular matrix (ECM) with higher strain levels. Mitral leaflets cultured for 72 h at 0 and 30% strain did not demonstrate a decrease in cell viability compared with fresh uncultured mitral leaflets (Fig. 2). Qualitative differences in Movat histologic staining of mitral leaflets were suggestive of increased GAG deposition throughout the valve leaflet and disruption of elastin fiber organization with increasing levels of cyclic strain (Fig. 3).

![Fig. 2. Representative curves of measured tensile load necessary to maintain 10, 20, and 30% strain on cultured mitral valves over 30 h (A). Cellular viability (%) normalized to viability of fresh tissue samples for cultured mitral valves (n = 4) subjected to 0 and 30% strain for 72 h (B). Data are means ± SD.](http://ajpheart.physiology.org/)
Phenotype markers, catabolic enzymes, and GAG. Cells from fresh mitral valves were negative for α-SMA on immunohistochemistry except for a thin layer of immunopositive cells just beneath the endothelial layer on the atrialis side of the leaflet. Most cells (>95%) in cultured mitral valves showed positive staining for α-SMA on immunohistochemistry regardless of level of cyclic strain or time in culture (Fig. 4). Based on immunoblot analysis, protein levels of α-SMA in cultured mitral valves increased significantly with each level of cyclic strain at both culture times but were not different between culture times (Fig. 5). Protein levels of SMemb were increased in cultured mitral valves subjected to 24 h of 20 and 30% strain and 72 h of 30% strain. Sulfated GAG content of cultured mitral valves was increased with 30% strain after 24 h and 20 and 30% strain after 72 h (Fig. 5). Protein levels of MMP1 were increased in mitral valves subjected to 10 and 30% strain for 24 h and 30% strain for 72 h (Fig. 6). MMP13 showed a nonsignificant trend towards increased levels with increased strain after 24 h of culture and was significantly increased at all levels of strain compared with unstrained mitral valves after 72 h. Protein levels of cathepsin K were significantly increased by level of cyclic strain in mitral valves cultured for 24 and 72 h. Both MMP13 and cathepsin K protein levels were independently and significantly increased by time in culture.

Serotonin and TPH1. Serotonin was not present in unconditioned culture media, but was present in culture media of cultured mitral valves at all strain levels. Serotonin concentrations in the culture media of cultured mitral valves increased with increased cyclic strain level (Fig. 7). Protein levels of the serotonin synthetic enzyme TPH1 were increased in cyclically strained cultured mitral valves compared with unstrained cultured mitral valves after 24 h of culture and showed a nonsignificant trend toward increase after 72 h of culture. Protein levels of SERT and monoamine oxidase, both important in the metabolism of serotonin, were not different in cultured mitral valves subjected to different levels of cyclic strain.

DISCUSSION

Role of tensile strain in mitral valve degeneration. Degenerative disease of the mitral valve is characterized by gross thickening and weakening of the valve apparatus leading to progressive valve prolapse and regurgitation (i.e., floppy mitral valves). Valve thickening results primarily from exuberant deposition of PG core proteins and sulfated GAG (formerly mucopolysaccharide) synthesis within the leaflets and chordae (16, 18). This change is responsible for the pathologic description of degenerative mitral valves as “myxomatous” or “myxoid.” Mechanical testing of myxomatous mitral valves demonstrates significant weakening of the valve apparatus consistent with net degradation of the ECM (4, 5). Increased expression...
of catabolic enzymes (collagenases, elastases, and gelatinases) without net increases in matrix synthesis in myxomatous mitral valves likely mediates loss of mechanical strength (35, 42). It is widely accepted that pathologic change in degenerative mitral valves is accompanied by transformation of valve interstitial cells to an “activated myofibroblast” phenotype identified by expression of α-SMA or SMemb, or both (10, 35). While the effector proteins that mediate pathologic change in degenerative mitral valves have been identified, the specific inciting mechanisms and signaling pathways that activate these effector proteins are less well understood.

Pathologic mechanical loading, including high tensile loading when the valve is closed, is postulated to be an inciting cause of both aortic and mitral degenerative disease (34, 35, 38). Recent studies of cyclically stretched porcine aortic valves in culture have demonstrated acute activation of punitive effector genes and proteins in response to pathologic levels of tensile stretch. These punitive effectors include proteins associated with phenotype transformation, catabolic enzyme expression, sulfated GAG synthesis, PG expression, and calcification (1–3, 31). An in vivo study of the sheep mitral valve anterior leaflet reported peak radial strains of 16% at 90 mmHg systolic pressure and 22% at 150 mmHg systolic pressure (39). Based on this study, radial strains of 10 and 20% on mitral leaflets could be considered at the low and high ends of the physiologic range. By extrapolation radial strains of 30%
would likely be pathologic corresponding to systolic pressures of just over 200 mmHg. In this study, we demonstrated that cyclic tensile strain, particularly 30% (pathologic) strain, acutely modulates proteins that have been associated with MMVD including myofibroblast phenotype transformation (α-SMA and SMemb), catabolic enzymes (MMP1, MMP13, and cathepsin K), and synthesis of sulfated GAG. These results support a hypothesis that tensile loading, particularly pathologic loading, can initiate protein expression and synthetic patterns that have been associated with degenerative mitral valves.

Role of local serotonin in mitral valve degeneration. Serotonin is well known to mediate valvulopathy in a variety of clinical settings including carcinoid tumors (19) and serotoninergic drugs (36, 37, 46). Further, exogenous administration of serotonin induces valvulopathy in rats in a dose-dependent manner (13). Several lines of evidence have implicated the 5HT2B receptor as the molecular target for serotoninergic valvulopathy (22). Upregulation of 5HT2B and downregulation of SERT are reported in rats administered serotonin (14). Currently, serotoninergic and degenerative valvulopathies are regarded as unrelated pathologies with serotonin playing no known role in the pathogenesis of degenerative disease. We (10, 12) have reported several-fold increase in the level of the key serotonin synthetic enzyme TPH1 in canine and human degenerative mitral valves. Further, we (12, 40) have reported increased abundance of 5HT2B, decreased abundance of SERT, and increased phosphorylation of ERK1/2 in canine degenerative mitral valves. These findings have led us to the hypothesis that local serotonin synthesis plays a mediating role in degenerative mitral valve disease.

In this study, we found serotonin in the conditioned serum-free media of cultured mitral valves and thus provide first direct evidence that mitral valves are capable of local serotonin synthesis. Importantly we also found that serotonin concentrations in the media of cultured mitral valves were influenced by increased levels of cyclic tensile strain. Lastly, we demonstrated with that pharmacologic inhibition of TPH1 (serotonin synthesis) or 5HT2B/2C-R diminishes strain-mediated increases in the levels of proteins associated the myofibroblast phenotype and matrix catabolic enzymes. These findings are first evidence of a functional link between tensile strain on valve leaflets, local serotonin synthesis, and expression of proteins associated with myxomatous degeneration in mitral valves. These results also support a possible functional link between the pathogeneses of serotoninergic valvulopathy and degenerative mitral valve disease. Interestingly, a similar functional link has been found between serotoninergic (drug-induced) and other forms of pulmonary hypertension (29, 32). Differences between the pathology of serotoninergic valvulopathy and degenerative mitral valve disease might be explained by different cellular influences of exogenous vs. local serotonin. They might also be explained by activation of other signaling pathways in degenerative valve disease, most notably developmental signaling pathways (8, 27, 30).

Fig. 7. Concentration of serotonin (5HT; ng/ml; A) in the serum-free culture media and immunoblot analysis of tryptophan hydroxylase 1 (TPH1; B) of mitral valves subjected to 0, 10, 20, and 30% cyclic strain for 24 and 72 h. Densitometry plots of TPH1 immunoblot results of 3 biological replicates normalized against α-tubulin loading controls. *Significant difference (P < 0.05) from 0% strain. **Significant difference from *. Data are means ± SD.

Fig. 8. A: immunoblot analysis of α-SMA, SMemb, MMP1, MMP13, and catK in cultured mitral valves subjected to 30% cyclic strain for 72 h with and without TPH1 inhibition by 4-chloro-DL-phenylalanine (250 μM). Concentrations of 5HT in the culture media with and without TPH1 inhibition also shown (C). Densitometry plots of immunoblot results of 3 biological replicates (B) normalized against α-tubulin (α-Tub) loading controls. *Significant difference (P < 0.05) with or without inhibition. Data are means ± SD.
The results of this study differ somewhat from the results of other studies. Barzilla et al. (6) demonstrated myxomatous plaque formation in response to exogenous serotonin added to mitral valves cultured in a splashing bioreactor. They further found qualitative decreases in MMP9 and MMP13 in response to exogenous serotonin based on immunohistochemistry. However, this study differs from our study in several important ways. The Barzilla study was conducted using culture media containing serum and therefore was conducted under conditions that are presumably different in a splashing bioreactor (flextensions of a significant serotonin background. Biomechanical loading is presumably different in a splashing bioreactor (flexure and shear), and the effect of altered loading was not studied.

Several lines of evidence implicate the 5HT2BR as the mediator of serotoninergic valvulopathy in humans and rats (19, 21). In this study, the 5HT2BR antagonist SB-206553 diminished strain-mediated myxomatous effector protein expression and thus was consistent with in vivo evidence implicating the 5HT2BR in serotonin-mediated valvulopathy. However, studies in cultured sheep aortic valve interstitial cells implicate the 5HT2AR as central to serotonin-ERK1/2 signaling (25, 44). The possible role that the 5HT2BR plays in strain-mediated myxomatous effector protein expression was not determined in this study. Future experiments should make use of selective inhibitors for each receptor type (5HT2A, 5HT2B, or 5HT2C). Given the low expression of the 5HT2C in mitral valves, we predict that the 5HT2BR is central to strain-induced myxomatous protein expression due to the similarities between the results of TPH1 inhibition and 5HT2BR blockade. No major conclusion can be drawn for a role of 5HT2AR in strain-mediated myxomatous-like changes as of yet.

Activation of ERK1/2 signaling by serotonin signaling pathways has been previously identified in heart valves (12, 44). ERK1/2 signaling has also been implicated in regulation of VIC phenotype (17). We suspect that ERK1/2 signaling is activated in the in vitro valve strain model; however, this remains to be demonstrated. Future studies demonstrating activation of downstream signaling including phosphorylation of ERK1/2, Smads, and protein kinases by tensile strain will strengthen the hypothesis that local serotonin mediates strain-induced myxomatous protein expression in mitral valves.

Limitations. While this study identifies new mechanisms and potential therapeutic targets for MMVD, an important limitation is that mechanisms elucidated by this model system represent acute responses to cellular strain, whereas degenerative valve disease is a chronic condition that develops over much longer periods. Another limitation is that applied tensile strain in this model system was uniaxial (radial), whereas physiologic tensile strain on heart valve leaflets is biaxial (radial and circumferential). These limitations highlight the difficulty of studying chronic degenerative diseases in a biologically meaningful way. Thus it is important to note this model system does not fully mimic valve degeneration; however, it may prove useful in elucidating mechanosignaling mechanisms that contribute to the degenerative process.

Summary and conclusions. In summary, this study demonstrates that acute cyclic strain induces activated myofibroblast phenotype, increased catabolic enzyme expression, and GAG synthesis in mitral valves that are dependent on local serotonin synthesis. We conclude that these mechanisms could play an important role in chronic mitral valve degeneration and thereby provide a mechanistic link between serotoninergic valvulopathy and degenerative mitral valve disease. Serotonin could be a therapeutic target for slowing progression of degenerative mitral valve disease.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: C.M.R.L., J.K., and E.C.O. conception and design of research; C.M.R.L. and B.J. performed experiments; C.M.R.L. analyzed data; C.M.R.L. and E.C.O. interpreted results of experiments; C.M.R.L. prepared figures; C.M.R.L. drafted manuscript; C.M.R.L. and E.C.O. edited and revised manuscript; E.C.O. approved final version of manuscript.
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