

Mitral Valve Hemorrhage and Mitral Annulus Shrinkage in Rabbits with Transient Ventricular Bigeminies Induced by Vagal Stimulation

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Background and aim of the study: Cervical vagal stimulation in rabbits frequently induces transient ventricular bigeminies, followed by mitral regurgitation lasting a few days, and development of peculiar lesions of the mitral complex. The present study investigated early lesions of the mitral valve and subsequent deformation of the mitral annulus associated with ventricular bigeminies in this animal model

Methods: A 1-min period of right cervical vagal electrical stimulation was repeated in anesthetized rabbits under ECG monitoring. Animals were sacrificed at 1 h after stimulation to investigate early mitral valve lesions (n = 71; group A), or after one week for measurement of mitral annulus area, using photographic planimetry (n = 56; group B).

Results: Ventricular bigeminies were recorded during vagal stimulation in 72% of group A (n = 51), and 73% of group B (n = 41). The most prominent early mitral valve lesion comprised widespread punctate

hemorrhages over the chordae and leaflets, which developed in most animals with ventricular bigeminies (88%), but rarely in those without ventricular bigeminies (5%). This inter-group difference was highly significant (p <0.001). Gross examinations performed at one week after vagal stimulation revealed a deformed mitral annulus resulting from stiff, edematous periannular myocardial tissues. Animals with ventricular bigeminies displayed a significantly smaller mitral annulus area than those without ventricular bigeminies (26.8 ± 1.0 versus 31.7 ± 1.2 mm²; p <0.01).

Conclusion: Early mitral valve hemorrhage and later mitral annulus shrinkage occur following vagus-induced transient ventricular bigeminies. This may result from unusual mechanical stress on the mitral complex secondary to distorted ventricular contraction in the context of ventricular bigeminies.

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Cervical vagal stimulation in rabbits frequently induces ventricular bigeminies that are followed by systolic murmur. Although these bigeminies disappear within a few minutes, the systolic murmur persists for a few days (1,2). Peculiar lesions of the mitral valves, mitral annulus and papillary muscles are frequently found at autopsy after one week (1-4). Color Doppler echocardiography has shown that the transient systolic murmur is attributable to mitral regurgitation (MR) (5), and that mitral valve prolapse might be the cause of MR (6), though the initial changes to the mitral com-

plex after cervical vagal stimulation are unclear. Moreover, whether the mitral annulus is enlarged or shrunken remains unknown. In the present study, the mitral complex lesions were examined immediately after cervical vagal stimulation, and the mitral annulus area measured at one week after stimulation.

Materials and methods

Female White rabbits (body weight ~2 kg) were anesthetized using pentobarbital sodium (20 mg/kg, intravenous) and placed in a supine position. Anesthesia was adjusted to the depth required to maintain spontaneous regular respiration. The right cervical vagus nerve was exposed and gently dissected free from the sympathetic and depressor nerves. Electrical stimulus comprised 50 Hz square-wave pulses, 1 ms in duration (0.3-0.8 V), given for 1 min under electrocardiographic monitoring. Stimulation was repeated at 2-min intervals for 20 min. Color

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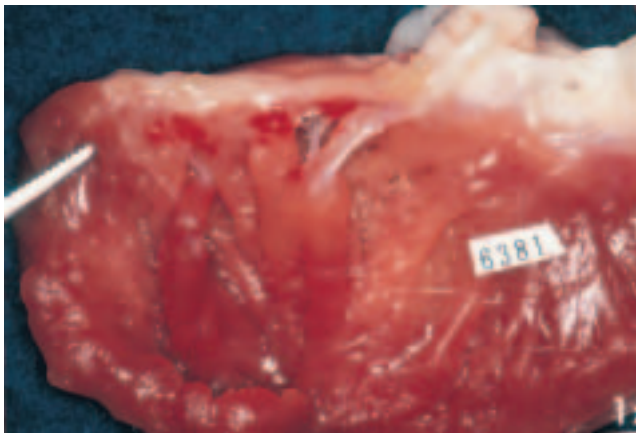


Figure 1: Macroscopic findings of the left ventricle at 1 h after vagal stimulation. Widespread fresh punctate bleedings in the anterior and posterior leaflets of the mitral valve and mitral annulus were observed.

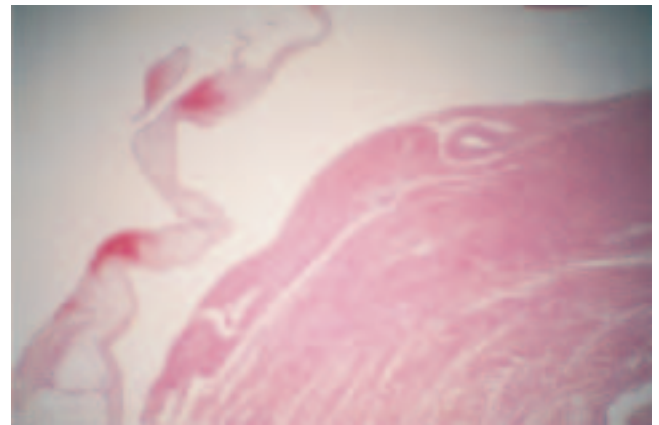


Figure 2: Microscopic findings of the mitral leaflet at 1 h after vagal stimulation. Multiple fresh bleedings in the atrial aspect of the anterior leaflet of the mitral valve were observed.

Doppler echocardiography and phonocardiography were performed before, immediately after, and at one week after vagal stimulation.

A total of 71 animals (group A) was sacrificed at 1 h after vagal stimulation. Early changes in the mitral complex were compared between animals that showed ventricular bigeminy and those that did not, either during or after vagal stimulation. Another 56 animals (group B) were sacrificed at one week after vagal stimulation. Deformation of the mitral annulus was compared between animals which showed ventricular bigeminy and those which did not, either during or after vagal stimulation. After removal of the left atrium, the mitral annulus was photographed its area measured using a computer cooperating area curvemeter (X-PLAN360C+, Ushikata, Tokyo).

Results

Electrocardiography before vagal stimulation revealed a normal sinus rhythm with a heart rate of about 300 beats per minute. Immediately after initiating vagal stimulation, 51 animals in group A (72%) and 41 in group B (73%) displayed ventricular bigeminy, which disappeared within a few minutes after ending stimulation. Furthermore, 35 group A animals (49%) and 23 group B animals (41%) showed a systolic murmur which lasted for a few days. Phonocardiography indicated holosystolic or late systolic murmur and systolic click. A color Doppler parasternal long-axis view of the left ventricle often showed marked MR immediately after vagal stimulation. At one week after vagal stimulation, 51 group B animals (73%) showed peculiar lesions in the mitral valve, mitral annulus and papillary muscle on gross examination.

At 1 h after vagal stimulation, macroscopic observa-

tion of the left ventricle revealed widespread fresh punctate bleedings in the anterior and posterior leaflets of the mitral valve and mitral annulus (Fig. 1). Also at 1 h after vagal stimulation, microscopic examination demonstrated multiple fresh bleedings in the atrial aspect of the anterior leaflet of the mitral valve (Fig. 2). Fresh bleedings in the mitral complex were found almost exclusively (88%; 45 of 51 animals) in animals that had displayed ventricular bigeminy during vagal stimulation, but were minimal (5%; 1 of 20 animals) that had not shown ventricular bigeminy ($p < 0.001$). The effect of ventricular bigeminy and systolic murmur on the incidence of fresh bleeding in the mitral complex is shown in Figure 3. Among rabbits that had shown ventricular bigeminy during vagal stimulation, fresh bleedings were observed in all 28 animals (100%) with moderate systolic murmur, in all seven (100%) that had shown mild systolic murmur,

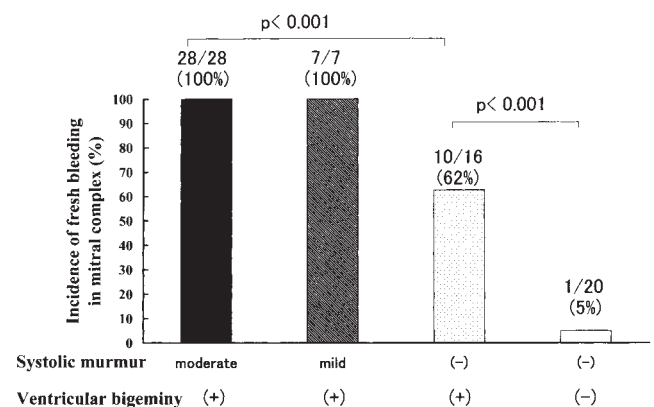


Figure 3: Effect of ventricular bigeminy and systolic murmur on the incidence of fresh bleeding in the mitral complex.

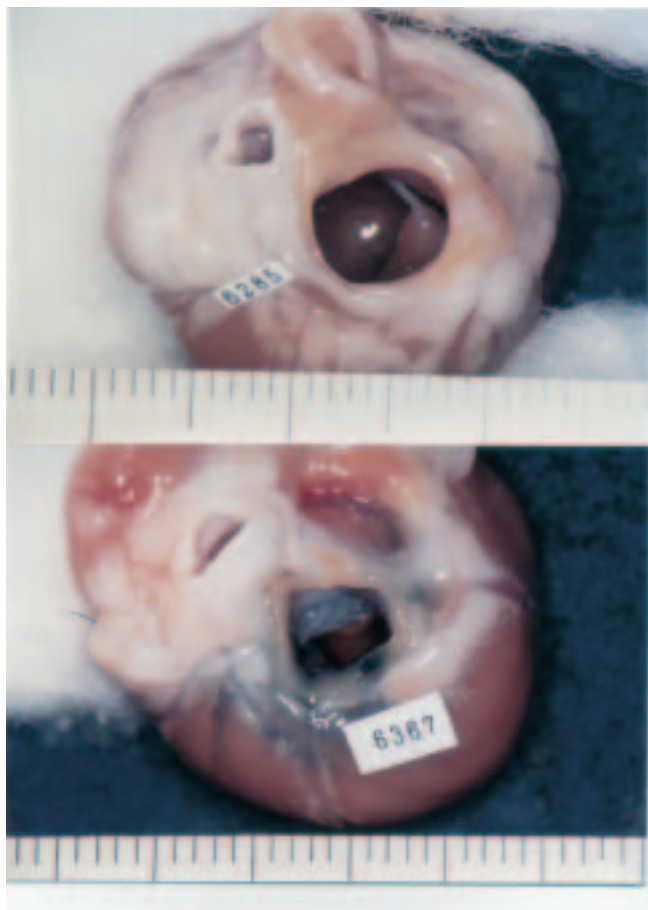


Figure 4: The mitral annulus at one week after vagal stimulation. Upper panel: Mitral annulus of a rabbit that did not show premature ventricular contractions (PVCs) during vagal stimulation; the annulus area (36 mm^2) was very similar to that of a normal rabbit. Lower panel: Mitral annulus of a rabbit that displayed ventricular bigeminy during vagal stimulation; the annulus area was only 24 mm^2

and in 10 of 16 animals (62%) that had shown no systolic murmur ($p < 0.001$) (Fig. 3). Some animals that had shown fresh bleedings of the mitral complex also showed fresh bleedings in the histology of the anterior or posterior papillary muscles. However, no animals showed fresh bleedings in the histology of the free wall of the left ventricle.

Immediately after vagal stimulation, 1 ml of carbon particulate suspension was injected into the ear vein of each animal. Macroscopic findings in the heart at one week after vagal stimulation included swelling and carbon deposition in the mitral periannular cardiac muscle and papillary muscle. The mitral annulus at one week after vagal stimulation is shown in Figure 4. The mitral annulus area of a rabbit that did not show premature ventricular contractions (PVCs) during vagal stimulation was 36 mm^2 (Fig. 4, upper panel),

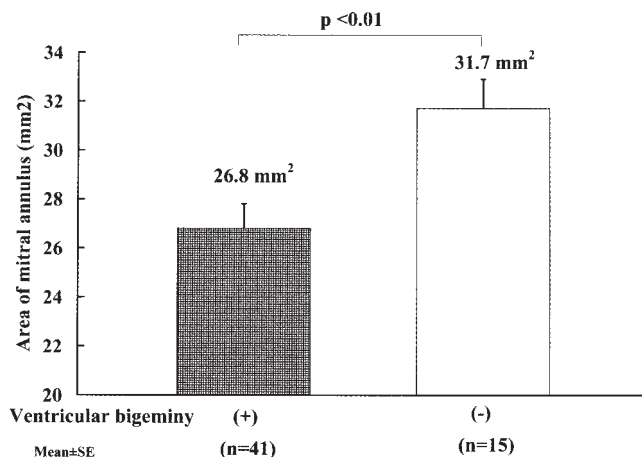


Figure 5: Effect of ventricular bigeminy during vagal stimulation on mitral annulus area after one week. Annulus area in rabbits showing ventricular bigeminy was significantly smaller than in rabbits not showing bigeminy.

and very similar to that of a normal rabbit. By contrast, the mitral annulus area of a rabbit that displayed ventricular bigeminy during vagal stimulation was only 24 mm^2 (Fig. 4, lower panel). At one week after vagal stimulation, the mitral annulus area was significantly smaller in rabbits that had shown ventricular bigeminy ($26.8 \pm 1.0 \text{ mm}^2$) than in those that had not ($31.7 \pm 1.2 \text{ mm}^2$) ($p < 0.01$) (Fig. 5).

Discussion

Cervical vagal stimulation in rabbits frequently causes transient ventricular bigeminy and systolic murmur. In the present study, color Doppler echocardiography was used to confirm that the systolic murmur was due to MR (5). Moreover, a close relationship was also demonstrated between the direction of the MR jet and the site of mitral complex lesions, suggesting that mitral valve prolapse (MVP) might be the cause of MR (6). The results of the present study revealed widespread punctate bleedings in the mitral valve and mitral annulus from immediately after cervical vagal stimulation in rabbits. The mechanisms underlying this bleeding are unclear, though the findings of the present investigations suggested that ventricular bigeminy was involved in the pathogenesis of bleeding, as mitral bleedings were predominantly observed in rabbits that had shown ventricular bigeminy. PVCs of this type are characterized by a long coupling time and superimposition on subsequent P waves. When PVCs occur on P waves, the atrium and ventricle contract simultaneously, and distorted contractions arise in the left ventricle. Both of these events may produce abnormal tension on the mitral valve and papillary muscles. Direct effects of vagal nerve stimulation may

also be involved. Human cardiac valves display distinct patterns of innervation that comprise both primary sensory and autonomic components (7). The presence of these distinct nerve terminals suggests a neural basis for interactions between the central nervous system and the mitral valve (mitral valve brain-heart interactions) (8), notably as the subendocardial surface on the atrial aspect at the middle portion of the mitral valve is rich in nerve endings.

The mitral annulus in humans (9,10) and other mammals (11) comprises a discontinuous fibrous ring with variable insertions of atrial and ventricular myocardial fibers. Both experimental (12,13) and clinical (14,15) studies have shown that the mitral annulus has a sphincteric function during valve closure, facilitating leaflet coaptation by reducing the mitral valve area. Atrial myocardial fibers have been shown to possess dense neural innervation (7), suggesting a neuromuscular role in annular and leaflet dynamics, the significance of which is yet to be defined. Heerregods et al. (16) reported that patients with severe MR experienced a significant increase in dimensions of the mitral valve annulus on echocardiography. Timek et al. (17) reported that myocardial fibers on the atrial side of the valve influence the shape of the mitral annulus. In the present study, shrinkage of the mitral annulus was observed at one week after cervical vagal stimulation, but the mechanism of this reduction in area remains unclear. However, the results of the present experiments showed that mitral annulus shrinkage was observed predominantly in rabbits that had displayed bigeminy. The present authors have reported previously (3) that periannular cardiac muscle lesions in MR rabbits from these experiments are characterized by swelling and increased stiffness of the muscle, appearing as swelling and degeneration of myocardial cells and interstitial fibrosis on microscopic observation, and as increased amounts and dimensions of collagenous tendons, strands, and struts on scanning electron microscopy. It has also been shown (4) that swelling and degeneration of the myocardial cells is temporary, and that the increase in interstitial collagen had regressed at four weeks after vagus stimulation. The relationship between MR and mitral complex lesions is not clear, but the earliest change may be mitral valve bleeding. These hemorrhages may lead to dysfunction of the mitral valve, resulting in heart murmurs immediately after vagal stimulation. Mid- to late-systolic heart murmurs were occasionally accompanied by a systolic click that resembled mitral valve prolapse syndrome in humans. Echocardiography sometimes showed mild MVP in the present study. Coghlan et al. (18) reported an increased prevalence of vagotonia in MVP. Papillary muscle lesions characterized by swelling and increased stiffness may be secondary to

acute mitral valve regurgitation. MR might also have disappeared, due in part to the swelling and increased stiffness of the mitral complex, as the present results showed a reduction in area of the mitral annulus at one week after vagal stimulation (Figs. 4 and 5). The possible clinical impact of the changes identified in the present study might be that transient ventricular bigeminy may induce mitral complex lesions including mitral valve hemorrhage, and that these lesions might be both subclinical and reversible.

In conclusion, the present results suggest that early lesions, including bleeding in the mitral leaflet and chordae tendineae immediately after vagal stimulation, are involved with MR and lesions of the mitral complex after vagal stimulation. There is also a possibility that the reduction in mitral annulus area at one week after vagal stimulation might be involved in the formation of mitral complex lesions and the disappearance of MR.

Acknowledgements

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