Experimental Production of Papillary Muscle and Mitral Valve Lesions by Vagal Manipulations in Rabbits

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The present paper describes peculiar lesions in papillary muscles and mitral valves produced by vagal manipulations. In the 109 rabbits killed one week after clipping or crushing of their cervical vagi, papillary muscle and mitral valve lesions were found in 53 (48.6%) and 52 (47.7%) hearts respectively. The lesions were identified by visible deposits of colloidal carbon which had been injected intravenously during the first 3 postoperative days. The left ventricular free wall, interventricular septum and right ventricle were free of carbon deposits. The papillary muscles involved were characterized by swelling and increased stiffness which corresponded to degeneration of the myocardial cells and interstitial fibrosis on microscopic observation. Hydroxyproline content of the papillary muscles involved was 1.4 times more than that of normal hearts, while there was no significant difference in hydroxyproline content of either interventricular septum or left ventricular free wall between the manipulated and the normal preparations. Heart murmurs were heard in 23 animals studied. Phonocardiograms revealed mid to late systolic murmurs with or without midsystolic click.

These results indicate that the vagus nerve plays an important role in the pathogenesis of some forms of papillary muscle and mitral valve lesions.

NEUROGENIC mechanisms can be involved in the pathogenesis of some forms of clinical heart disease. Experimental investigations have revealed that electrical stimulation of various brain structures or the stellate ganglion produces cardiac lesions in animals¹⁻³. These lesions consist of necrotic foci and subendocardial bleedings which are widely distributed over ventricular myocardium and papillary muscles. They are known to be histologically not specific and similar to lesions produced by intravenous infusion of catecholamines. On the other hand, little attention has been paid to cardiac lesions of

vagal origin. As far as we know, only Manning et al⁴ and Groover and Stout⁵ produced cardiac lesions by electrical stimulation of the vagus nerve. However, it remains unclear whether or not the vagal cardiac lesions are identical to the sympathetic ones. The present paper describes peculiar cardiac lesions induced by simple manipulations on the vagus nerve which were localized in the papillary muscles and mitral valves. We are not aware of any previous studies on these characteristic cardiac lesions except for our preliminary reports^{6,7}.

Key Words:

Vagus nerve
Mitral valve prolapse
Papillary muscles
Hydroxyproline

METHODS

Male rabbits weighing 1.8–2.5 kg were used. Under pentobarbital sodium anesthesia (30 mg/kg, iv) the left cervical vagus nerve was exposed

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Fig.1. A representative papillary muscle lesion one week after clipping of cervical vagus nerve. Visible deposits of carbon were seen on mitral valves, chordae tendineae and papillary muscles, but not on ventricular septum and free wall. Both papillary muscles were swollen and stiff.

and gently dissected free from connective tissues and the carotid artery. Animals were divided into 3 groups according to the type of manipulation on the vagus nerve. Preliminary investigation revealed that both clipping and crushing of the vagus nerve had almost similar effect on the development of cardiac lesions, namely papillary muscle lesions were found in 56 (62.5%) of 90 animals after clipping and in 19 (54.2%) of 35 animals after crushing. In the 109 animals (Group 1), the left cervical vagus was loosely clipped by a silver clip of 0.7 mm in internal diameter and 3.0 mm in width ($n = 15$), moderately crushed by forceps ($n = 39$) or treated with both procedures ($n = 55$). During the vagal manipulation, ventricular premature beats were frequently observed on monitor electrocardiogram. Colloidal carbon (Pelikan C 11/1431a, 1 ml/kg) was injected into the marginal vein of the ear lobe once a day for 3 consecutive days after the operation in order to identify cardiac lesions on gross examination. The animals were killed one week after operation. Thirty-three other animals (Group 2) which served as the controls were unoperated or sham-operated upon.

Colloidal carbon was also injected and the animals were killed in a manner identical to that described in Group 1. In another 11 animals (Group 3), the left cervical vagus was cut immediately after intravenous injection of colloidal carbon, and then the peripheral cut nerve was stimulated electrically for 10 sec with square wave pulses of 1 msec duration, 50 Hz and 0.3–0.8V. The voltage was set at a level enough to produce marked sinus bradycardia. The stimulation of 10 sec period was repeated 10 times. The animals were killed 3 hours later.

In all 3 groups, the hearts were excised and the endocardial surface was exposed by a longitudinal incision. Special attention was directed to papillary muscles, mitral valves and chordae tendineae on gross examination. Lesions were identified by visible deposits of carbon particles. Specimens were weighed and fixed by 10% formalin or 2.5% glutaraldehyde solutions for light and electron microscopic examinations.

Hydroxyproline Content: Left ventricles were analysed in 9 unoperated controls and 8 typically manipulated hearts to examine if there were an increase of hydroxyproline content in

Fig.2. Incidence of carbon deposits in parts of ventricle. Almost all cardiac lesions introduced by vagal clipping or crushing were limited to mitral valves, chordae tendineae and papillary muscles.

Fig.3. Transverse sections of papillary muscles obtained from involved (left) and control (right) hearts (original magnification $\times 200$). A large interstitial space shows massive accumulation of edema fluid with fibrosis, and myocardial cells show degenerative changes.

the fibrous tissue concerned. After the removal of both the atria and the right ventricle, the remainder of the heart was divided into the papillary muscles and the left ventricle. The anterior and posterior papillary muscles, the specimens resected from interventricular septum and left ventricular free wall were weighed, dehydrated in graded ethanol solutions, and subjected to critical point drying. Then fatty substances were removed by pure acetone. After weighing these dehydrated specimens, hydroxyproline was measured in samples of approximately 10 mg tissue weight (dry weight) by the

method of Prockop and Udenfriend.⁸

Phonocardiogram: Phonocardiogram was recorded just before, immediately after and 2 days after vagal manipulations in 109 animals of Group 1.

RESULTS

Morphological Findings

The gross findings of typical cardiac lesions were as follows: First, marked carbon deposits were seen on the mitral valves, chordae tendineae and papillary muscles, while the remainders of

Fig.4. Electron micrographs of involved (left) and control (right) papillary muscles. Enlarged interstitial space containing massive amount of collagen fibrils and degenerative changes in the myocardial cells. Original magnification $\times 1600$.

TABLE I WEIGHT AND HYDROXYPROLINE CONTENT FOR CONTROL AND INVOLVED ANIMALS

	Weights (mean $\pm$ SE)			Hydroxyproline mg/g $\cdot$ dry weight (mean $\pm$ SE)		
	Control (C) n = 9	Involved (I) n = 8	I/C ratio	Control (C) n = 9	Involved (I) n = 8	I/C ratio
Whole Body	2150 $\pm$ 50 g	2340 $\pm$ 90 g	1.09			
Whole Heart	4.12 $\pm$ 0.10 g	4.80 $\pm$ 0.48 g*	1.17			
LV Free Wall				6.47 $\pm$ 0.71	6.04 $\pm$ 0.62	0.93
Ventricular Septum	2.57 $\pm$ 0.06 g	2.97 $\pm$ 0.08 g*	1.16	5.01 $\pm$ 0.71	4.86 $\pm$ 0.58	0.97
Anterior Papillary Muscle	64.3 $\pm$ 6.7 mg	105.6 $\pm$ 7.9 mg*	1.64	7.36 $\pm$ 0.37	12.56 $\pm$ 0.90*	1.71
(dry weight)	(10.5 $\pm$ 1.0 mg)	(15.3 $\pm$ 1.3 mg)*	(1.46)			
Posterior Papillary Muscle	64.7 $\pm$ 3.3 mg	108.0 $\pm$ 4.6 mg*	1.67	7.39 $\pm$ 0.74	10.66 $\pm$ 0.78*	1.44
(dry weight)	(10.8 $\pm$ 0.5 mg)	(15.7 $\pm$ 0.9 mg)*	(1.45)			

* $p < 0.05$

the heart such as interventricular septum, left ventricular free wall, tricuspid valves and right ventricle were free from carbon deposits. Second, the papillary muscles involved were swollen and their stiffness was increased (Fig. 1). The incidence of carbon deposits is shown in Fig. 2. In Group 1, definite carbon deposits on papillary muscles and mitral valves were found in 53 (48.6%) and 52 (47.7%) of the 109 animals, respectively. In the left ventricular free wall or the ventricular septum, carbon deposits were seen only in 4 (3.7%) of the 109 animals. Control animals (Group 2) revealed low incidence of carbon deposits on papillary muscles, mitral valves and other parts of left ventricle in 2 (6.1%), 4 (12.1%) and one (3.0%) of the 33 animals, respectively.

In Group 3, the incidence of carbon deposits on mitral valves or chordae tendineae was as high as 82% (9 out of 11 animals). Bleedings were also seen in the mitral apparatus in 5 animals. Carbon deposits on papillary muscles were seen in 4 of the 11 animals, but the size and the stiffness of these muscles were not different from those of the controls. This may be due to the fact that the interval between electrical stimulation and sacrifice was too short to induce interstitial fibrosis or edema.

Localization of carbon particles and structural changes in cellular elements were studied by light and electron microscopic examinations in animals of Group 1. The aggregations of carbon particles were mainly seen in subendothelial layers of the mitral valve and chordae tendineae,

Fig.5. Typical example of phonocardiogram after vagal manipulations. Mid to late systolic murmurs with systolic click are recorded.

but carbon particles were diffusely deposited on interstitial spaces and vascular walls of the papillary muscles. Vacuolar or hyaline droplet degenerations of myocardial cells and fibrosis of interstitium were also seen in the papillary muscles (Fig. 3). Electron microscopy of papillary muscles revealed cellular enlargement, nuclear degenerations and increased collagenous fibers in interstitial spaces more distinctly (Fig. 4).

Hydroxyproline Content

Table I shows the detailed results of weight and hydroxyproline measurements. The weights of the whole body, the whole heart and the sum of the left ventricular free wall plus the ventricular septum in animals with cardiac lesions were only 1.09, 1.17 and 1.16 times greater than those of the controls, respectively. But the weights of both anterior and posterior papillary muscles of the manipulated hearts were 1.64 and 1.67 times more than those of the control hearts. The increase in weight of these papillary muscles was associated with an increase in dry weight. Hydroxyproline contents of both anterior and posterior papillary muscles of the manipulated hearts were 1.7 and 1.4 times greater than those of the control hearts, which was significant, while hydroxyproline contents of both the ventricular free wall and the ventricular septum of the manipulated hearts showed insignificant differences from those of the control hearts.

Phonocardiographic Findings

After clipping or crushing of the vagus nerve, heart murmurs appeared in 23 of 109 animals

(21%). Phonocardiograms revealed mid to late systolic murmurs which were occasionally accompanied by systolic clicks. A representative record is shown in Fig. 5. Postmortem examination showed that the animals with postoperative systolic murmurs had typical cardiac lesions in both papillary muscles and mitral valves and were frequently complicated by pericardial effusion and ascites.

DISCUSSION

The present study showed that simple manipulations such as clipping or crushing of the cervical vagus produced peculiar lesions confined to mitral valves, chorda tendineae and papillary muscles in about half of the animals. These lesions were quite different in their localization from those of cardiac lesions produced by electrical stimulation of stellate ganglion or intravenous injection of norepinephrine which are seen everywhere in the wall of the left ventricles. The real mechanisms responsible for the development of these peculiar lesions remain as yet obscure, although some kinds of changes in vagal tone are probably related to the genesis of the lesions. Ventricular arrhythmias were often observed during the operative procedures on the vagus nerve. These arrhythmias cannot be ruled out as a contributory factor, because dysynergic ventricular contractions may exert mechanical stress on the papillary muscles.

The terminal parasympathetic innervation of the ventricle has been a subject of controversy,⁹⁻¹³ but the protective effect of vagus stimula-

tion against ventricular tachycardia or other ventricular arrhythmias have recently been reported by some investigators.¹⁴⁻¹⁶ Myocardial damage following vagus nerve stimulation was clearly demonstrated in dogs by Manning et al.⁴ They observed myocardial lesions, including infarcted areas in some of the papillary muscles, and congestion and hemorrhages in the atrio-ventricular valves. Although they used a stimulation which was far beyond the realm of physiologic limits, their experiments provided some clues for the elucidation of the role played by autonomic nervous factors in the development of myocardial diseases. Moreover, in baboons Groover et al.⁵ reported that by using more physiologic electrical stimulation of the vagus nerve there occurred streaky fibrosis confined to the anterior half of septum and adjacent ventricular wall including the anterior papillary muscle. But these studies are contrary to the observations of Corley et al.¹⁷ who reported that secondary sympathetic activation *via* vagal afferents to the central nervous system plays a dominant role in myofibrillary degeneration, and pure parasympathetic stimulation appears to be incapable of producing morphological myocardial alterations. In our experiments, vagal stimulation produced definite carbon deposits in mitral valves and papillary muscles. These results reassured the findings of Manning et al.

Colloidal carbon is biologically inactive, and it deposits in perivascular and interstitial tissues as a result of increased vascular permeability or tissue damage as shown in the papillary muscles involved. On the other hand, carbon deposits might result from an increase in the endothelial phagocytotic activity evoked by vagal manipulations. But in anycase, this colloidal tracer technique obviously increased the sensitivity for detecting cardiac lesions when compared with the studies of earlier investigators.

Also our study may provide some insights regarding the pathogenesis of mitral valve and papillary muscle diseases. Despite extensive clinical investigations, the pathogenesis of the mitral valve prolapse syndrome remains to be explained. Recently, some investigators proposed the possibility of the presence of abnormal autonomic neural function in this syndrome.^{18,19} Late systolic murmurs with or without clicks were heard in a substantial number of animals after clipping or crushing of their cervical vagi. These findings indicate that the cardiac lesions demonstrated in the present study may be an

experimental model for mitral regurgitation, mitral valve prolapse or papillary muscle dysfunction in man.

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