MORPHOLOGICAL STUDY ON VAGAL INNERVATION IN HUMAN ATRIOVENTRICULAR VALVES USING HISTOCHEMICAL METHOD

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To demonstrate innervation in human atrioventricular valves, we examined the tricuspid and mitral valves of apparently normal autopsied hearts of four men (ages ranging from 50 to 74 years). Whole valve tissues were stained for acetylcholinesterase by a histochemical method. Acetylcholinesterase-positive nerve fibers with a diameter of 2 to 5 μm were distributed widely in the deep atrialis of the atrioventricular valves and partly in the fibrosa. The nerve fibers formed a network or plexus from the base to the anatomical edge of the valves. Meshes of the nerve fiber network were more dense towards the base and at the commissure than either towards the edge or at the body. Thicker nerve fibers, which were interspersed coarsely in the leaflets, were intercalated by special varicose apparatuses at a few sites in their long running course. On the contrary, thinner nerve fibers which were distributed abundantly, ended, as a rule, in small dotor brush-like formations. Approximately half of the chordae tendineae were innervated by the nerve fibers. The mode of vagal innervation suggests that the nerve system may assist valve movement by moderating myocyte contraction in the valve base and change valve structure by sensing a stress in the valves.
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MORPHOLOGICAL and physiological evidence of the relative richness of innervation in the atrioventricular (AV) valves of the mammalian heart has been well documented by Smirnow, Michailow², Woollard³ and Nettleship⁴. However, the classical technique using a silver impregnation method did not readily differentiate between the sympathetic and parasympathetic nerves, and showed incomplete discrimination of the nerve fibers from fine connective tissue fibers. Recent advances in histochemical staining have afforded a clear identification

and differentiation of the autonomic nerve fibers. The aim of this paper is to demonstrate a morphologically detailed distribution pattern of vagal innervation in human AV valves using a cholinesterase stain and to compare the pattern between human and other mammalian hearts if any differences exist. Only two papers so far, by Ferreila Rossi⁵ and De Biasi et al⁶ have referred to innervation in the human AV valves. Characterization of the morphological features of the vagal nerve in the valve will allow consideration of the possible role of the nerve in valve function, thus providing useful information to cardiologists and surgeons treating valve diseases.

Key words:

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Fig.1. Photomicrograph of the anterior leaflet of the mitral valve, 30 μ m thick section. $\times 40$.

Arrow marks show the nerve plexus located in the deep atrialis of the leaflet.

MATERIALS AND METHODS

Four apparently normal autopsied hearts obtained within 2 h after death were used for the study. The cases were all males with ages in the range of 50–74 years (64 years on average). The weight of the hearts ranged from 250 to 300 g. The hearts were removed from the chest, opened by the routine method, and the entire AV valves were dissected free from the AV rings. After being rinsed in physiological saline solution, the valves were fixed in Zamboni's fixative for approximately 24 h, then, washed in 0.1 M cold phosphate buffer saline at 4°C and pH 7.2. After the valve tissue had been frozen in liquid nitrogen, then repeatedly bathed in hot water at 40°C to facilitate diffusion of acetylcholine, it was placed in medium contained the following: acetylthiocholine iodide 20 mg, 0.1 M sodium citrate 2 ml, 30 mM CuSO_4 4 ml, 10^{-3} M tetraisopropylpyrophosphoramidate 4 ml, 5 mM potassium ferricyanide 4 ml, and 0.1 M sodium hydrogen malic acid buffer 26 ml according to Karnowsky-Roots' formula⁷. The valves were soaked in this medium at 4°C for three days after incubation at 37°C for 30 minutes. After the completion of staining, the valves were examined under a light microscope on a glass slide, then part of the valve was embedded in paraffin in a routine manner and cut into sections of 30 μ m thickness to identify the exact locations of the nerves in the valves.

Fig.2. Photomicrographs of the tricuspid valve base.

A. Arrow marks show nerves distributed along with atrial myocytes. $\times 100$. Myo (RA): myocytes of the right atrium overhanging to the valve base.

B. Nerves are distributed at myocyte-free areas of the valve base. Arrow marks show a thin nerve fiber with a diameter of 5 μ m. Check mark shows a thick nerve fiber with a diameter of 30 μ m. $\times 200$.

RESULTS

Acetylcholinesterase-positive nerve fibers measuring approximately 2 to 50 μ m in diameter were distributed widely and diffusely in the deep atrialis (proximalis) of the AV valves (Fig. 1) and in the subepicardial space outside of the valve rings. In addition, some thin nerve fibers were demonstrated sparsely at limited locations in the fibrosa. There was significant difference between the mitral and tricuspid valves nor among the four cases with respect to the distribution pattern of the nerves. At the base of the AV valves, approximately half of the nerve fibers intermingled with the atrial myocyte group which overhung into the valve tissue beyond the annuli fibrosi (Fig. 2-A). The other half of the nerve fibers with considerably varied

Fig.3. Photomicrographs of the commissures of the mitral valve. $\times 100$. Nerves form dense networks.

A: anterior commissure of the anterior mitral leaflet

B: posterior commissure of the anterior mitral leaflet

C: anterior commissure of the posterior mitral leaflet

D: posterior commissure of the posterior mitral leaflet

AML=anterior mitral leaflet PML=posterior mitral leaflet

Fig.4. Photomicrograph of the tricuspid valve body. $\times 100$. Nerves of various diameters form a network. Several intercalated varicose apparatuses exist in the course of nerve fibers.

Fig.5. Photomicrograph of thick nerves at the tricuspid valve body. $\times 200$. Arrow marks show a high magnification view of the varicose apparatus.

diameters overran the annuli irrespective of the atrial myocytes (Fig. 2-B). At the commissure sites in the AV valve, thin nerve fi-

bers with a diameter of approximately $5\ \mu\text{m}$ were distributed diffusely, forming a fine network (Fig. 3). The mesh of the nerve

Fig.6. Photomicrograph of thin nerves at the tricuspid valve body. $\times 400$.
Arrow marks show dot and brush-like nerve endings.

Fig.7. Photomicrograph of the tricuspid valve edge. $\times 100$.
Thin nerves run straight and parallel. An arrow mark shows a thick nerve taking a U-shaped turn.

network was more dense in these commissure sites than in the rest of the valve tissue. At the body of the valves, nerve fibers of varied diameters were distributed and interconnected to form a coarse mesh (Fig. 4). Thick nerve fibers with a diameter greater than $30\ \mu\text{m}$ in the body were intercalated in places by varicose apparatuses (Fig. 5). On the other hand, thin nerve fibers with a diameter less than $5\ \mu\text{m}$ in the body ended, as a rule, in small dot- or brush-like formations (Fig. 6). At the marginal area close to the anatomical edge of the valves, nerve fibers, which either ran straight to the chordae tendineae or ran with a U-shaped turn to back into the body, had fewer both varicose

Fig.8. Photomicrograph of a chorda tendinea attached to the tricuspid valve. $\times 40$.
Nerve networks are extending into the chorda.

apparatuses and dot- or brush-like formations than in the body (Fig. 7). Half of the chordae tendineae of the valves, which were inserted to the anatomical edge and to the body, contained thin nerve fibers passing through to reach the papillary muscles of the ventricles (Fig. 8), and the other half of the chordae had no nerve tissue at all. Fig. 9 illustrates a variety of vagal innervation patterns by locations of the AV valves.

DISCUSSION

Since Smirnow¹ first identified the nerve networks in the mammalian AV valves in 1895, several investigators²⁻⁴ have studied the mode of innervation in various animals, but only two papers⁵⁻⁶ have described innervation in human AV valves. Controversy still remains as to whether or not the mode of innervation is identical in animals and humans. In 1990, Williams et al⁸ compared innervation of the AV valves in four mammalian species, i.e., mouse, rat, guinea pig, and opossum, and found some variations among them. Our results, though limited to the vagal innervation, suggest considerable variation in the distribution pattern between human and other species of mammals in following points: the sparse, but positive nerve distribution at the free edge of the valve, a U-shaped turn of the nerve fibers towards the valve base at the marginal area of the valves, and the most dense innervation is in the commissure sites of the valves, are all special features of humans. We do not know

Fig.9. Schematic illustration of the vagal nerve distribution in the AV valves. Area maps of A, B, C, and D correspond to the A, B, C, and D pictures, respectively.

whether the differences arise from the different sizes of the bodies or from the different grades of evolution among the mammalian species, but it should be worth mentioning that careful consideration is necessary when generalizing the results of animal experiments to humans.

The acetylcholinesterase stain⁷ used in this study is an established technique used to demonstrate the whole cholinergic nerve, i.e., vagal nerve system, but strictly speaking, it also gives positive results in a sensory nerve connecting with or contained within the vagal nerve. Therefore, both efferent and afferent (including sensory) nerve components of the vagal nerve are discussed in this paper.

On comparison with the previous studies of the geometrical distribution pattern of the vagal nerve, Smith⁹ reported that the AV valves contain numerous nerve fibers connecting with the subendocardial nerve plexuses of the atria, and that some of the nerve fibers seem to be attached to the myocytes in the valve base, thus allowing them to play a role in valve movement per se or its modulation. On the contrary, Cooper et al¹⁰ insist that the intravalvular and intrachordal nerves are unrelated to valve function, because the nerves merely traverse the leaflets and chordae in their course to or from papillary muscle. From the electrophysiological

standpoint, Sonnenblick et al and other investigators suggested that the nerves and myocytes play some form of active role in the valves,¹¹⁻¹⁴ i.e., maintenance of the form of the valve during diastole, prevention of excessive ballooning of the valve into the atrium during early systole, and alteration of the leaflet size according to the cardiac contraction cycle. And they also demonstrated that acetylcholine gives a negative inotropic effect on the valve motion. Hibbs and Elisson^{15,16} reported that the nerve terminals in the distal third of the leaflets are completely devoid of muscle fibers and blood vessels, suggesting that the function of the nerve is likely to be sensory or afferent. We demonstrated a rich nerve distribution in the bodies of the AV valves, and detected intercalated varicose apparatuses along some of the thicker nerve fibers which have already been described in the animal hearts by Miller et al²⁰. The morphological appearance suggests that the function of the apparatus is to act as a sensory receptor.¹⁷ From the intercalated position between the relatively thick nerve fibers, the apparatus could be functioning as a sensor for stretch stress of the valve. It has been recognized by Okada²¹ that valves undergo morphological change in accordance with hemodynamic stresses, which can be more easily explained if an active receptor for stretch stress exists

in the valves. The mechanism might involve a responsive increase in the efferent nerve tonus, which stimulates connective tissue proliferation through secretion of active substances from the nerve endings, according to increased afferent (sensory) nerve tonus. On the other hand, the dot- or brush-like endings of thinner nerves, which are found abundantly in the valve bodies, are identical to those described by De Biasi et al⁶ and were assumed to be sensory receptors by Hibbs et al¹⁶. The smallness of the endings suggests that it may be a sensor of local change in the valve structures such as degenerative change of connective tissue fibers, which is needed for the repair process. The reason why the distal part close to the anatomical edges of the valves has only a few nerve endings remains speculative, but incessant minor injury in the line of closure might gradually reduce the preexisting nerve endings, or congenital inhibition of development of the nerve endings at the periphery of the valve might occur. In either case, the shortage of the nerve endings seems to result in spongiosa proliferation as a primitive reaction of the connective tissue corresponding to pressure or impact hemodynamic stress at the valve tip, instead of the regular fibrosing process in the spongiosa adjacent to the deep atrialis which is assumed to be under nerve control, corresponding to volume or flow hemodynamic stress¹⁸.

One of the characteristic distribution patterns of the vagal nerve in the AV valves, which was clarified first by the authors, was the presence of the most dense meshes of the nerve network at the commissure sites. Though most of the nerve elements at the commissures consisted of thinner nerve fibers, the nerve network also included long nerve fibers bridging over the valve base, chordae tendineae and tips of the papillary muscles. The thin nerve fibers were ramified, interconnecting with others in the valve body, and a half of them terminated to the atrial myocytes at the valve base. Williams^{19,20} demonstrated that vagotomy causes degeneration of nerves in the chordae tendineae. Groover et al²¹ and Imataka et al²² reported that vagal nerve manipulation leads to pathologic lesions such as necrosis, hemorrhage, and fibrosis in the AV valves and papillary muscles. These findings sug-

gest that the bridging long vagal nerves play a role as efferent fibers to regulate cardiac performance through adjustment of the contraction timing of the papillary muscle. From this point of view, the relationship between the long vagal nerve and the mitral valve prolapse syndrome²³ is worthy of future study. The basal nerve fibers terminating in the atrial muscle around the commissure are assumed to play a role in reduction or fixation of the AV ring size to assure valve coaptation as commented previously. The significance of the dense nerve network in the myocyte-free commissural valve body still remains obscure. If the assumption that nerve tissue has an organizing or guiding capacity to remodel the AV valves in embryological development is supported, the nerve plexus might be a remnant of primitive valve construction, but this hypothesis seems to be too imaginative to be widely acceptable.

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