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Characteristics of distribution of peptide-containing nerve fibres in the atrioventricular valves of the rat

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Summary. The distribution of vasoactive intestinal polypeptide-, neuropeptide Y-, and calcitonin gene-related peptide-immunoreactive nerve fibres was investigated in the atrioventricular valves of the rat. These nerve fibres were visualized by immunostaining of whole-mount preparations by the avidin-biotin-peroxidase complex method. Vasoactive intestinal polypeptide-immunoreactive nerve fibres were observed mainly in the anterior cusp of the mitral valve and, to a lesser extent, in the medial cusp of the tricuspid valve. Numerous neuropeptide Y-immunoreactive nerve fibres were found covering all of the cusps. Both types of peptidergic nerve fibre formed dense networks that consisted of interlacing and anastomosing nerve fibres. Calcitonin gene-related peptide-immunoreactive nerve fibres were seen in every cusp, but did not form a fine network. These results provide detailed anatomical information for evaluation of the possible roles of each type of peptide-containing nerve fibre in the function of atrioventricular valves.

Key words: Vasoactive intestinal polypeptide, Neuropeptide Y, Calcitonin gene-related peptide, Atrioventricular valve, Rat

Introduction

It has been reported that the nerves in the atrioventricular valves of several mammals contain substance P (SP; Papka et al., 1981, 1984), vasoactive intestinal polypeptide (VIP; Della et al., 1983; Weihe et al., 1984), calcitonin gene-related peptide (CGRP; Kawano et al., 1989) and neuropeptide Y (NPY; Gu et al., 1984; Sternini and Brecha, 1985; Dalsgaard et al., 1986; Kawano et al., 1989; Zhang et al., 1993; Tsumori et al., 1994). However, these studies do not provide any detailed information about the distribution of these peptide-containing nerve fibres in the whole cusps of the tricuspid or mitral valves.

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Kawano et al. (1989) evaluated the relationship between mitral valve prolapse and nerve elements within the valve cusps from a clinical point of view. They showed that the normal human mitral valve received a rich supply of nerves including NPY- and CGRP-immunoreactive (IR) nerve fibres, and that these nerve elements disappeared in the degenerated portion of the cusp in the prolapsed mitral valve (Kawano et al., 1989). This evidence implies the crucial roles of the peptide-containing nerve fibres in the valvular function. However, the possible roles of each peptide-containing nerve fibres in the valve are still unknown.

Investigations on the detailed distribution of each peptide-containing nerve fibre throughout whole cusps of the valve are likely to help us to understand the possible roles of these nerve fibres in the valves. In the present study, therefore, we used an immunohistochemical method to examine the presence of VIP-, NPY-, CGRP- and SP-IR nerve fibres in the atrioventricular valves of the rat and determined their characteristic patterns of distribution in the tricuspid and mitral valves.

Materials and methods

Twelve female Wistar rats (250-300 g) were used. The animals were sacrificed by administration of an overdose of ether. The hearts were removed and immersed in Bouin's solution for 5 min. The mitral and tricuspid valves were carefully dissected out together with the annulus fibrosus and the chordae tendineae and fixed in the same solution for a further three hours. A whole mount technique (Costa et al., 1980) was employed for immunostaining in order to obtain an overall view of the valvular innervation. Each set of valves, including both the tricuspid and mitral valves, was immunostained by the avidin-biotin-peroxidase complex (ABC) method (Hsu et al., 1981) with rabbit antisera raised against VIP (Incstar), NPY (Incstar), CGRP (Cambridge Research Biochemicals), and SP (Incstar). The tissue was incubated successively with primary antiserum (1:400) at 4 °C for 48 h, with biotinylated antibodies raised in pigs, against rabbit IgG

(1:100; DAKO) for 3 h at room temperature, and with ABC (1:100; DAKO) for 60 minute at room temperature. After visualization of immunoreactivity by the diaminobenzidine reaction, postintensification was performed by Gallyas' method (Gallyas et al., 1982). Finally, the whole-mount specimens were mounted onto glass slides coated with gelatin, and air-dried. Slides were dehydrated in a graded alcohol series, cleared in xylene and coverslipped with Entellan (Merck).

Results

VIP-IR nerve fibres

The tricuspid valve received a poor to moderate supply of VIP-IR nerve fibres. In the medial cusp, an interlacing network of varicose fibres and small bundles was found at the base and in the body of the cusp (Fig. 1A,B).

The mitral valve received a dense plexus of VIP-IR nerve fibres, predominantly in the anterior cusp (Fig. 1C-G). A mesh-like network was formed by loosely-arranged bundles of varicose fibres or by a single delicate fibre in the body of the cusp (Fig. 1D,E). Near the side of attachment of the chordae tendineae, numerous fibres were seen to leave the network and run towards the free edge. However, they terminated before actually reaching it (Fig. 1F,G).

NPY-IR nerve fibres

In both the tricuspid and the mitral valves, NPY-IR nerve fibres formed an extensive, dense, net-like plexus that covered the entirety of all cusps (Fig. 2A-E). The networks were composed of interlacing and anastomosing varicose fibres, which were mostly delicate, single fibres (Fig. 2B,C). Loose bundles of varicose fibres were also found in the network in the body of the mitral valve (Fig. 2F, G). Near the free edge of all cusps, some fibres leaving the network terminated before reaching the edge (Fig. 2D,H).

CGRP-IR nerve fibres

CGRP-IR nerve fibres were formed in all the cusps of the valves examined (Fig. 3). However, the network of such fibres in each cusp appeared to be less dense than that of NPY-IR nerve fibres. In the tricuspid valve, several varicose fibres pursued an irregular course from the base towards the free edge of the cusp (Fig. 3A-D). The mitral valve contained a network, composed of single varicose fibres, that was conspicuous in the body of the anterior cusp (Fig. 3E-G). Most CGRP-IR fibres did not form bundles and were visible as single varicose fibres. The fibres ended before reaching the free margin and the chordae tendineae (Fig. 3C,F,G).

SP-immunoreactive nerve fibres

No SP-IR nerve fibres were observed in any cusps of the valves examined.

Discussion

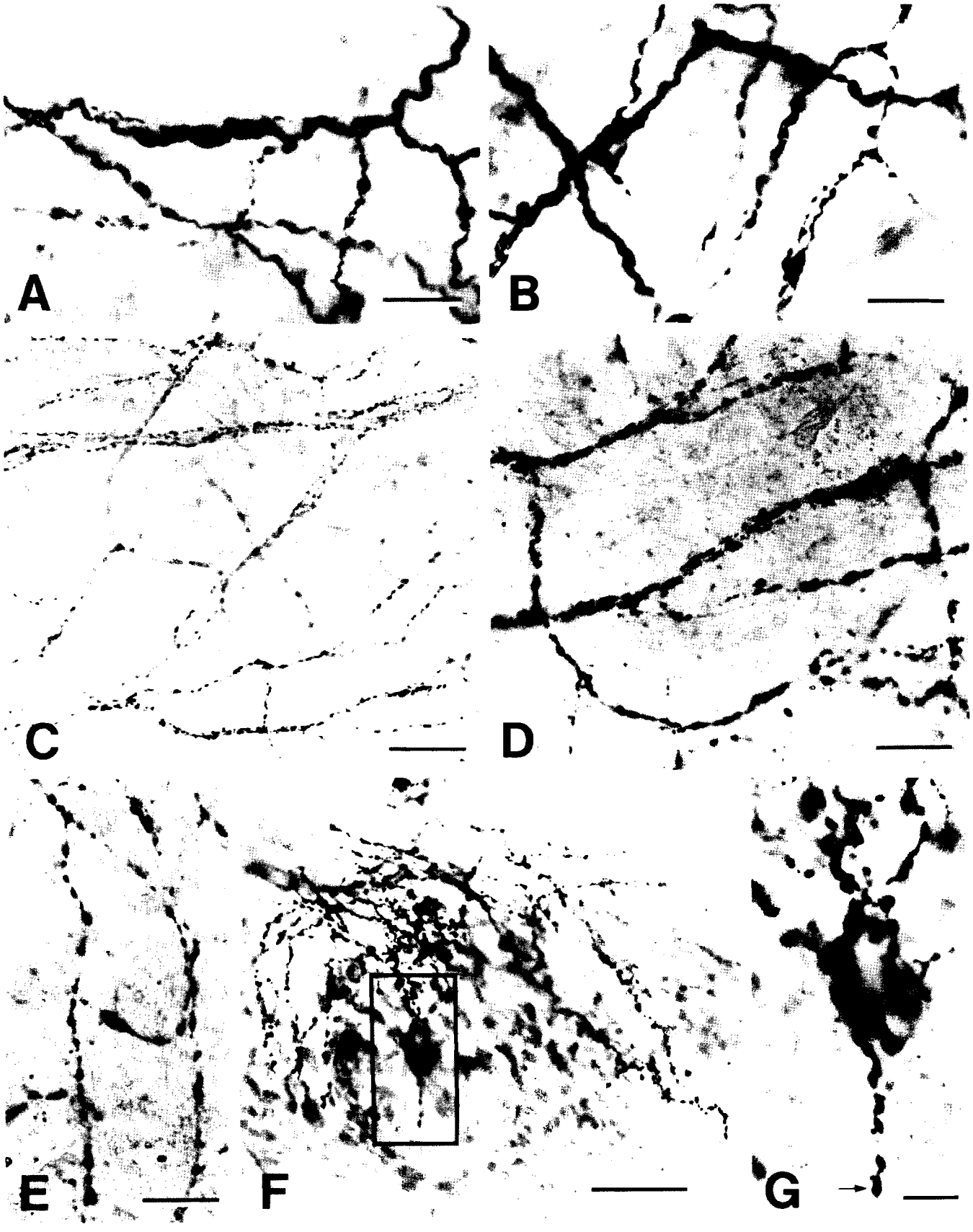
In the present study, detailed mapping of the distribution of peptide-containing nerve fibres in atrioventricular valves was achieved by immunostaining of whole-mount preparations. Our results clearly demonstrate that VIP-, NPY- and CGRP-IR fibres have distinct and characteristic patterns of distribution in the mitral and tricuspid valves. Therefore, each of these types of fibres may play a separate role in valve functions.

In the rodent heart, densely-distributed NPY-IR nerve fibres have been demonstrated in the nodal tissues, around the blood vessels, and within the myocardium (Gu et al., 1984; Sternini and Brecha, 1985). These studies also indicated that the atrioventricular valves contained NPY-IR nerve fibres. NPY innervation in the heart is likely to be derived from extrinsic sympathetic ganglia (Sternini and Brecha, 1985; Dalsgaard et al., 1986). However, some intrinsic cardiac neurons contain NPY (Hassall and Burnstock, 1984), the origins of NPY-IR nerve fibres in the valves remains to be well characterized.

In the present study, the valvular NPY-IR nerve fibres were characterized by their extremely dense, overall distribution throughout every cusp. NPY has potent vasoconstrictor properties (Lundberg et al., 1982), acting directly on vascular smooth muscles (Lundberg and Tatemoto, 1982). However, innervation of smooth muscles has not been confirmed structurally in the valves (Hibbs and Ellison, 1973; De Biasi et al., 1984). Our previous study provided ultrastructural evidence that NPY-IR nerve fibres terminate in the interstitial cells within the valve in the monkey (Tsumori et al., 1994). Thus, the NPY-IR nerve fibres might play a significant role in valvular function via the interstitial cells.

With respect to the valvular interstitial cells, Filip et al. (1986) reported that such cells had morphological and functional characteristics similar to those of the smooth muscle cells, forming a complex cellular framework that spans the entire valve. These observations led them to conclude that the valvular interstitial cells might have

Fig. 1. Distribution of VIP-immunoreactive nerve fibres in the tricuspid valve **(A,B)** and in the mitral valve **(C-G)**. Varicose fibres form a mesh-like network at the base (A) and in the body (B) of the medial cusp of the tricuspid valve. A dense plexus is observed in the body of the anterior cusp of the mitral valve (C). D and E show enlarged views of the plexus. Note the network that consists of loosely-arranged bundles of varicose fibres (D) and single delicate fibres (E). Some varicose fibres, leaving the network, run toward the free margin (lower side of the photograph) of the anterior cusp of the mitral valve (F). The area enclosed by a rectangle in F is enlarged in G. An arrow shows a presumptive nerve ending. Bar= 10 μm in G. Bar= 20 μm in A, B, D and E. Bar= 50 μm in C and F.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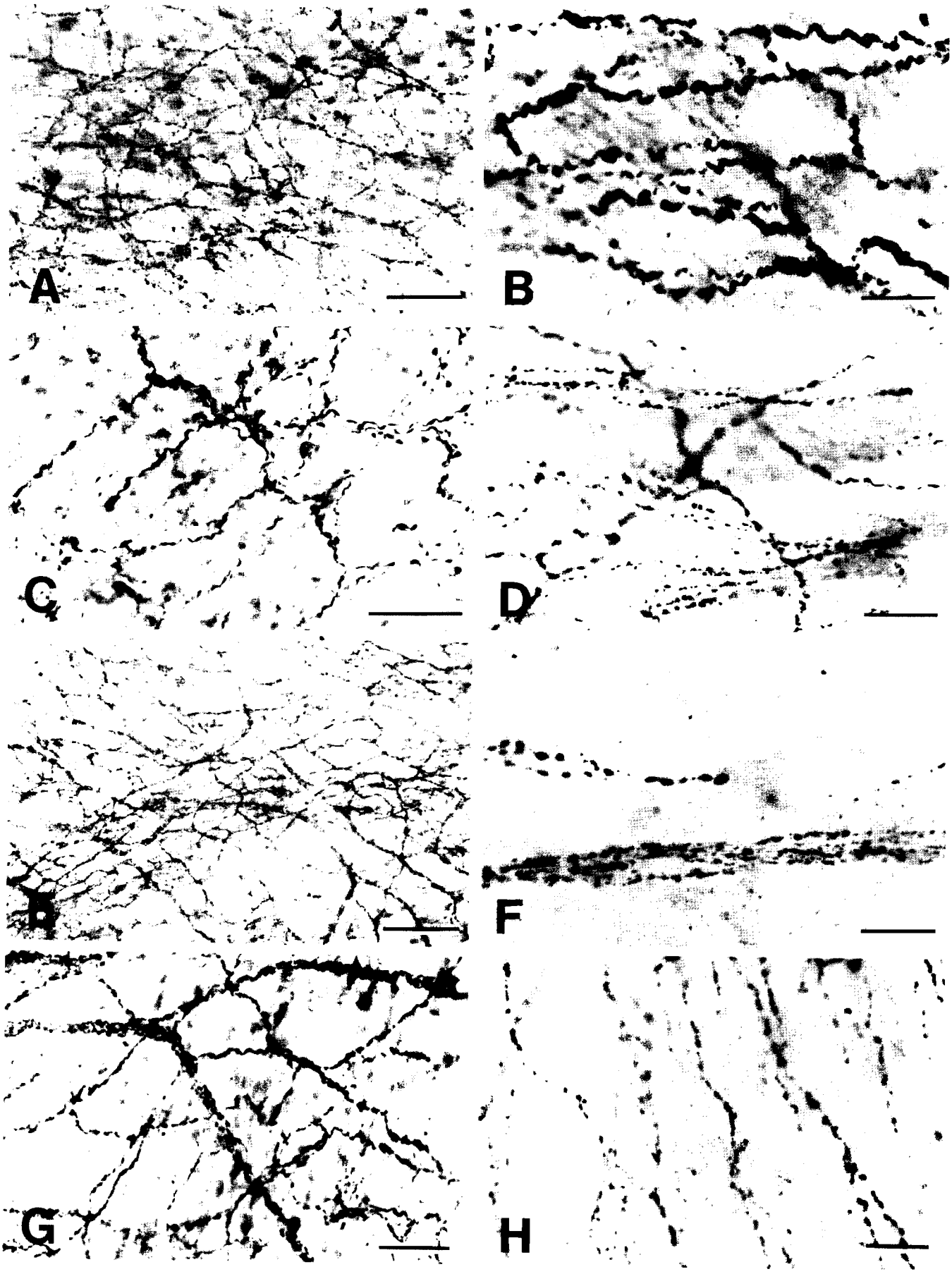
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Fig. 2. Distribution of NPY-immunoreactive nerve fibres in the tricuspid valve **(A-D)** and in the mitral valve **(E-H).** A dense, net-like plexus covers the anterior cusp of the tricuspid valve (A). Anastomosis and branching of the fibres in the anterior (B) and posterior (C) cusp of the tricuspid valve are shown in enlarged photographs. Fine varicose fibres run toward the free margin (right side of photograph) of the posterior cusp of the tricuspid valve (D). A dense, net-like plexus is seen in the body of the anterior cusp of the mitral valve (E). F and G show loosely-bound fibres in the body of the anterior cusp of the mitral valve. Fine varicose fibres are observed near the free margin of the anterior cusp of the mitral valve (H). Bar= 20 μm in B, F and H. Bar= 40 μm in C, D and G. Bar= 100 μm in A and E.

contractile properties, which could account for a controlled tonus. Such properties would be actively correlated with the cyclically changing forces that act on valves during diastole ans systole. Further physiological studies are needed to show that valvular NPY-IR nerve fibres might be able to participate in the control of tension in the cusp via interstitial cells that have contractile ability.

Some previous investigators have proposed that the valvular nerve apparatus might have a sensory function because the nerves are densely distributed within the cusps and have no apparent target elements (Williams, 1964; Lipp and Rodin, 1968; Anderson, 1971). Ferreira and Rossi (1974) found a well-developed neural apparatus in the human valves and they suggested that both the mitral and the tricuspid valves might have special sensory areas or reflexogenic zones. In contrast, ultrastructural studies have indicated that the valvular nerve elements include terminals that are filled with numerous mitochondria and are probably sensory (Hibbs and Ellison, 1973; De Biasi et al., 1984; Dixon, 1987; Tsumori et al., 1994). Thus, the afferent fibres might be included among the valvular nerve elements.

Kawano et al. (1989) confirmed the distribution of CGRP-IR nerve fibres in human mitral valves. In the present study, we have demonstrated the occurrence of CGRP-IR nerve fibres in every cusp of the rat valve. It is of interest that the ramifications of CGRP-IR nerve fibres seemed simpler than those of VIP- or NPY-IR nerve fibres in the rat valves. Most of CGRP-IR nerve fibres ran as single varicose fibres with few branches. Such features are very similar to those of the afferent sensory fibres in the atrioventricular valves of the cat and the rabbit (Voloschenko, 1965). CGRP immunoreactivity is widely distributed in sensory nerve fibres in the cardiovascular system (Wharton and Gulbenkian, 1987). Taking into account the vigorous mechanical deformation of the valves during systole and diastole, we can postulate a sensory function for the valvular CGRP-IR nerves. The distribution patterns of CGRP and SP immunoreactivity are very similar, and these two peptides are co-localized to a high degree within sensory neurons (Wharton and Gulbenkian, 1987). In the present study, however, SP-IR nerve fibres were not detected in the rat valves. The differences between results obtained in this and previous studies (Papka et al., 1981, 1984) might be due to species-species variations.

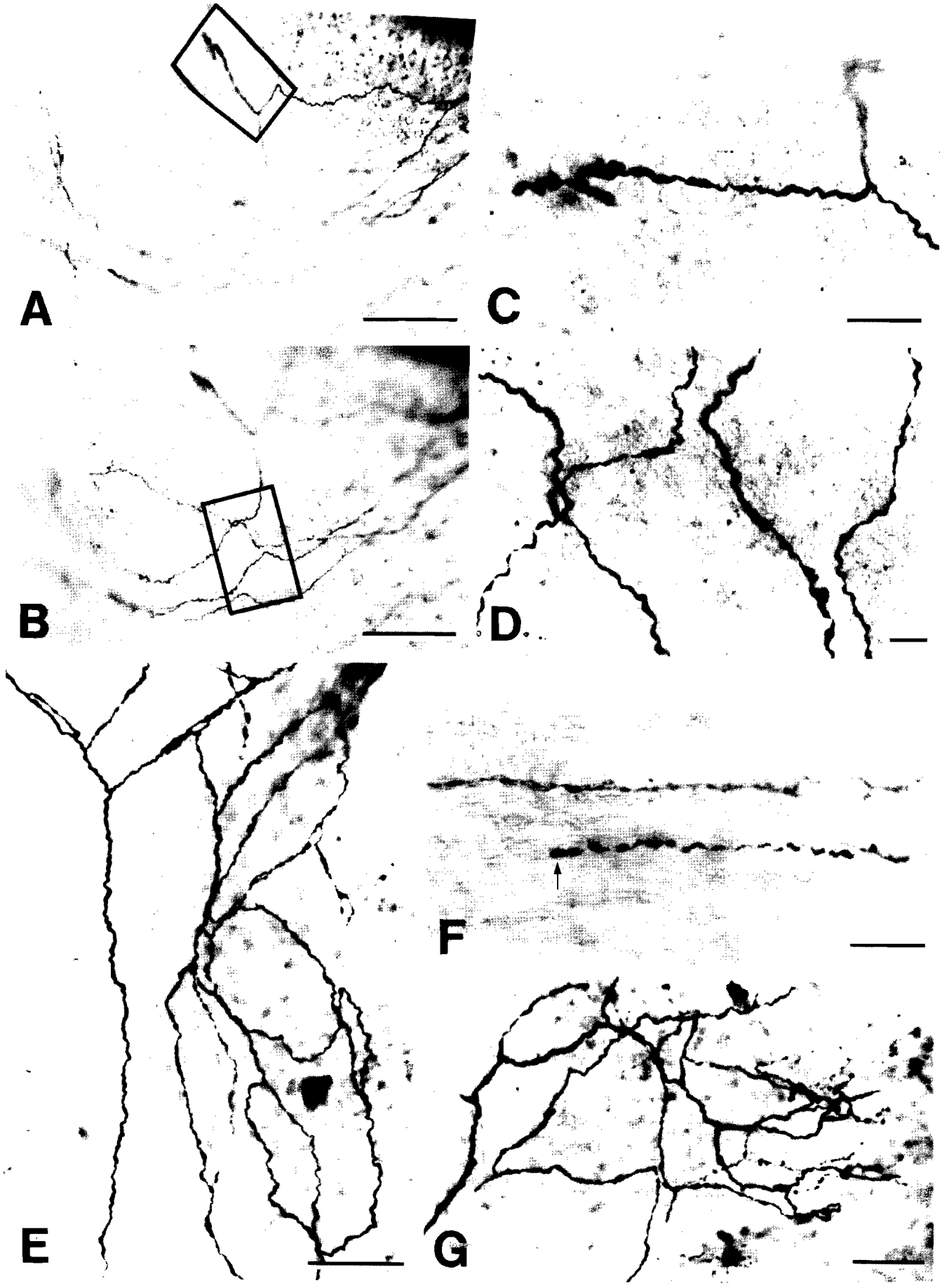
VIP-IR nerve fibres in several mammalian hearts have been detected for the most part in the atria, in the conduction system, in the coronary vessels and, to a lesser extent, in the atrioventricular valve (Weihe and Reinecke, 1981; Della et al., 1983; Weihe et al., 1984). It has been suggested that the main cardiac VIP-IR innervation is intrinsic and originates from postganglionic parasympathetic neurons, although possible extrinsic innervation from vagal or sympathetic nerves cannot be excluded (see Discussion in Weihe et al., 1984). The origin of VIP-IR fibers in the valve remains to be clarified and their target structures are still unknown (Weihe et al., 1984). Therefore, little information is available at present to suggest the possible role of VIP-IR nerves in valvular function.

VIP innervation was the densest in the anterior cusp of the mitral valve among the valve cusps. From the anatomical and physiological points of view, the anterior cusp of the mitral valve forms an important boundary of the left ventricular outflow tract (Lam et al., 1970). This might imply that the anterior cusp of the mitral valve is a possible candidate for a special sensory area among the cusps. Furthermore, a characteristic distribution of VIP-IR nerve fibres has been demonstrated in the cardiac glomus-like chemoreceptor areas in the vicinity of the origin of coronary arteries (Weihe et al., 1984). The basal attachment of the anterior cusp of the mitral valve is in direct continuity with the wall of the ascending aorta (Perloff and Roberts, 1972). Taken together, these data suggest that VIP-IR innervation in the anterior cusp of the mitral valve might be closely related to viscerosensory functions.

In the tricuspid valve, VIP-IR nerve fibres were distributed in the medial cusp conspicuously. The medial cusp of the tricuspid valve has the same embryological origin as the anterior cusp of the mitral valve, differing from the anterior or posterior cusp of the tricuspid valve (Van Mierop et al., 1963). The unique pattern of distribution of valvular VIP-IR nerve fibres might be related to their developmental origins.

In conclusion, the present study reveals the difference in distribution of peptidergic nerve fibres in the atrioventricular valves of the rat. The valvular nerves

Fig. 3. Distribution of CGRP-immunoreactive nerve fibres in the tricuspid valve **(A-D)** and in the mitral valve **(E-G).** A and B show the same field, with photographs taken on different focal planes, of the posterior cusp of the tricuspid valve. The left sides of these photographs are in the direction of the base of the cusp. C and D show enlarged views of the areas enclosed by rectangles in A and B, respectively. A rough mesh-like network is observed in the body of the anterior cusp of the mitral valve (E). F shows an enlarged view of some of the varicose fibres. An arrow shows a presumptive nerve ending. A plexus is also seen near the free margin (right side of photograph) of the anterior cusp of the mitral valve (G). Bar= 10 μm in D. Bar= 20 μm in C and F. Bar= 50 μm in E and G. Bar= 100 μm in A and B.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might contribute to regulation of the tension of cusps via contractile elements, or to transmission of sensing of local pressure or stretching during diastole and systole via afferent fibres. Pathological changes in valvular peptidergic innervation should be investigated in the future.

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