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Nerve fibres containing neuropeptide Y in the atrioventricular valves of Japanese monkey and rat; a light and electron microscopic study

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Summary. Dense distribution of varicose fibres containing neuropeptide Y-like immunoreactivity (NPY-LI) was found in the atrioventricular valves of the Japanese monkey, and moderately in the rat. The immunoelectron microscopy using immunogolds resulted in the localization of NPY-LI within the dense-cored vesicles which existed with the small clear vesicles in the unmyelinated axons near the endocardium. These NPY-LI-containing fibres may participate in regulation of vasomotor role or other functions of the atrioventricular valves.

Key words: Atrioventricular valves, Neuropeptide Y, Post-embedding immunogold method, Monkey, Rat

Introduction

In addition to the classical transmitters (acetylcholine and noradrenaline), neuropeptides and other putative neurotransmitters or modulators, including substance P (SP), vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide, 5-hydroxytryptamine, galanin and neuropeptide Y (NPY), have been revealed in nerves supplying the mammalian cardiovascular system where these nerves are prominent in the endocardium, myocardium and around coronary vessels (Williams, 1964; Della et al., 1983; Hassall and Burnstock, 1987; Wharton and Gulbenkian, 1987; Parsons et al., 1989; Wharton and Polak, 1990). Although the distribution of nerve fibres containing acetylcholinesterase, SP and VIP were found in the atrioventricular valves of several mammalians (Williams, 1964; Papka et al., 1981, 1984; Della et al., 1983), the similar descriptions concerning nerve fibres containing NPY-like immunoreactivity (NPY-LI), which appeared to be the most widespread populations in the mammalian cardiovascular system (Wharton and Gulbenkian, 1987), were scarcely reported in the valves. In the present study, the distribution of the nerve fibres with NPY-LI in the mitral and tricuspid valves was examined in the Japanese monkeys and rats. Furthermore, localization of NPY-LI was undertaken in the valves of Japanese monkey by immunoelectron microscopy.

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Materials and methods

Three Japanese monkeys (4-6 kg) and eight Wistar rats (250-300 g) were used. The monkeys were anaesthetized by an intramuscular injection of Ketalar (Sankyo Co. Ltd., Tokyo, 0.25 ml/kg) and sacrificed by bleeding via the severed femoral artery. The rats were anaesthetized with an overdose of ether. The hearts were removed and fixed immediately by immersion in 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.2) or Zamboni's solution overnight. The mitral and tricuspid valves were dissected out from the hearts and divided into several cusps. Whole mount techniques (Della et al., 1983) were employed for immunostaining, so as to preserve the intact structure. The cusps of valves were immunostained by the methods of avidin-biotin-peroxidase complex (ABC) (Hsu et al., 1981) using antiserum for NPY (Cambridge Research Biochemicals). The tissues were incubated for 48 hours in NPY antiserum (1:500) and 1-3 hours in biotinylated antirabbit IgG (DAKO, 1:100) and ABC (DAKO, 1:100) successively. Triton X-100 (0.2%) was included in the incubation medium. Following visualization of immunoreactivity by the diaminobenzidine reaction, the postintensification was performed by Gallyas' methods (Gallyas et al., 1982). Finally the whole mount specimens were transferred to glass slides coated with gelatin, dehydrated and mounted in Entellan (Merk).

Part of the material from the monkeys was prepared for immuneoelectron microscopy with the methods of post-embedding immunogold (Bendayan and

Zollinger, 1983). The tissues were fixed with a solution of 2% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate buffer followed by postfixation in 1% osmium tetroxide. Each valve was cut into proximal and distal portions and embedded in Epon. The ultrathin sections were cut across the whole thickness of the valves. The sections on the nickel grids were pretreated in saturated aqueous solution of sodium metaperiodate and hydrogen peroxide (3%) in turn and successively transferred to 1% bovine serum albumin (BSA) in PBS. The grids were incubated in the antiserum for NPY (1:400), and subsequently in goat anti-rabbit antisera labelled with 10 nm gold particles (1:50, Amersham) which were diluted in 1% BSA-PBS for 60 min at 30 °C. Washed with PBS after each step, the grids were counterstained with lead citrate and uranyl acetate. The control experiments were performed by means of substituting normal rabbit serum for the primary antiserum.

Results

A dense plexus of nerve fibres with varicosities containing NPY-LI was distributed in both mitral and tricuspid valves in Japanese monkey (Figs. 1A-1C). In the portion attached to the atrioventricular ring, many bundles and varicose fibres with NPY-LI were interwoven in several directions (Fig. 1A). Some bundles ran parallel to the ring. Delicate varicose fibres with NPY-LI formed an interlacing network in the middle region where the majority of them appeared perpendicular to the atrioventricular ring (Fig. 1B). Approaching the free margin of the valves, the density of the fibres and terminals slightly decreased. Some fibres travelled towards the chorda tendinae (Fig. 1C) and gradually disappeared at the route from the attached portion to the chorda tendinae. There was no difference in distribution of NPY-LI fibres between mitral and tricuspid valves. The distribution and configuration

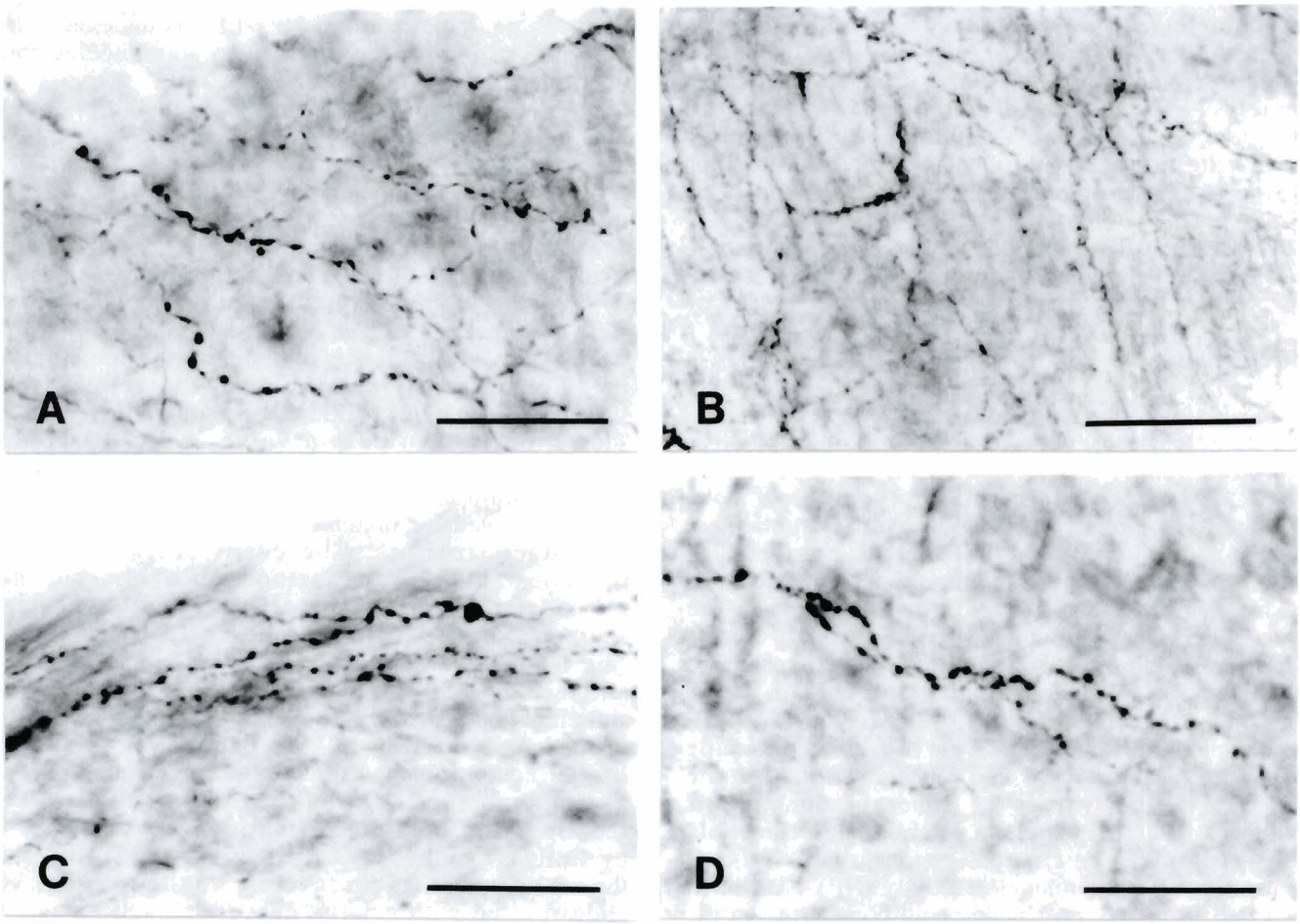

Fig. 1. Distribution of the fibres containing NPY-LI in the tricuspid valves of monkey **(A-C)** and rat (D). In A-C, the varicose fibres with NPY-LI are seen in the portion attached to the atrioventricular ring (A), in the middle region (B) and the area towards the chorda tendinae (C). Similar fibres are indicated in the attached portion of the tricuspid valve of rat **(D)**. The upper edge of A and B are parallel to the atrioventricular ring. Bar = 20 μm in A, C and D. Bar = 40 μm in B.

of the fibres with NPY-LI in the valves of the rat were generally similar to those of the monkey. However, these fibres were less dense in the rat than in the monkey (Fig. 1D).

Under the electron microscope, some bundles of unmyelinated fibres were encountered in the deep stroma of the valves, and many axon varicosities containing vesicles were found completely or incompletely ensheathed by the Schwann cell near the endocardium throughout both the mitral and tricuspid valves in the monkey (Fig. 2). In a few cases, distended terminals filled with vesicles were found in the stroma. Small clear vesicles were usually mixed with large dense-cored vesicles (50-100 nm) in the axons. Immunogold particles for NPY-LI were concentrated only within some dense-cored vesicles in many axon profiles (Fig. 2). Numerous interstitial cells, in accordance with the descriptions by Hibbs and Ellison (1973), were scattered within the stroma of the valves. Most of the axon terminals containing NPY-LI vesicles were seen between the interstitial cells and epithelial cells, and a few of them appeared close (2000-3000 Å) to the interstitial cells.

Discussion

The present results show a rich distribution of fibres with NPY-LI in the atrioventricular valves of Japanese monkey, which was apparently denser than in the rat. Ultrastructural localization of NPY-LI in the monkey valves has been observed for the first time in the present study. These results indicate that the atrioventricular valves are also innervated by nerves containing NPY-LI in addition to SP and VIP (Della et al., 1983; Papka et al., 1984).

Immunohistochemical studies have revealed that the distribution of nerve fibres with NPY-LI in the cardiovascular system is quite similar to that containing catecholamine synthesising enzymes (Wharton and Gulbenkian, 1987; Wharton and Polak, 1990), suggesting the sympathetic origin of NPY-LI fibres. This was confirmed by an observation that the removal of the stellate ganglia resulted in almost complete loss of NPY- and tyrosin-hydroxylase-immunoreactive nerve fibres in the guinea pig heart (Dalsgaard et al., 1986). Although most NPY-LI-containing nerve fibres in the heart seem to represent extrinsic sympathetic nerves, there is evidence that some intrinsic cardiac neurons may also contain NPY-LI (Hassall and Burnstock, 1984). Further

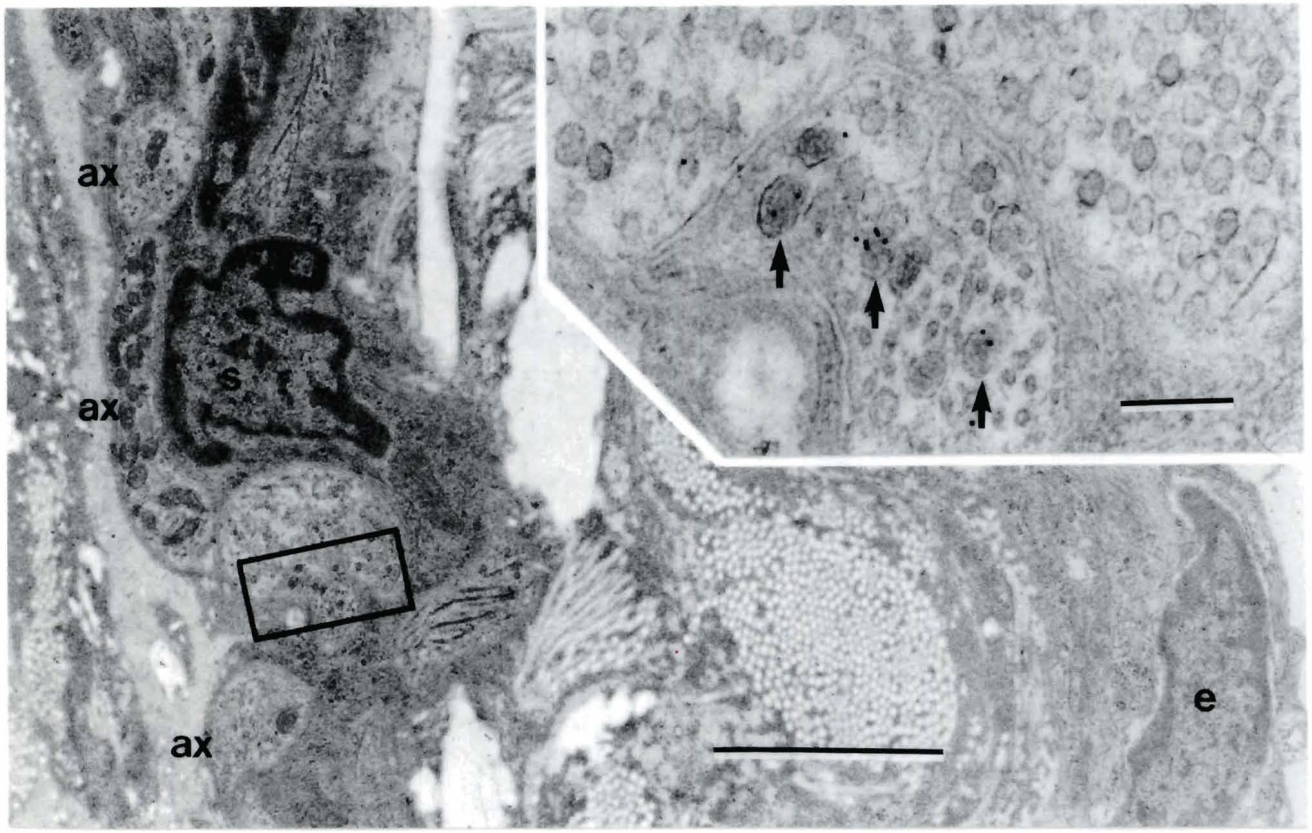

Fig. 2. Electron micrograph showing the axon profiles (ax) beneath the endothelium (e) of monkey mitral valve. An axon profile containing immunogolds for NPY-LI is enclosed by a rectangle and enlarged in an inset. It is clearly found that the gold particles are concentrated in the dense-cored vesicles (arrows) which exist with the clear small vesicles in the axon. S = Schwann cell. Bar = 2 μm. Bar = 0.2 μm in an inset.

examination is necessary to reveal the nerves with NPY-LI in the atrioventricular valves in order to study their origin and coexistence with catecholamine.

The large dense-cored vesicles in the unmyelinated axon varicosities contained NPY-LI in the cardiovascular system (Wharton and Gulbenkian, 1987). This is also demonstrated in the atrioventricular valves by the results in the present study. It is very likely that NPY released from the axon varicosities affects the endothelial cells or interstitial cell functions, because many NPY-LI axon varicosities were found near the endothelial cells and sometimes close to the interstitial cells. It is well known that NPY can influence sympathetic vascular control as a vasoconstricter by pre-junctional and post-junctional effects (Wharton and Gulbenkian, 1987). On the other hand, Williams (1964) proposed a role for extensive nerve plexuses in the valves as possibly sensory to measure the flow characteristics; namely the recording of blood movements across different portions of each valve cusp. More direct demonstration of target component of these axon terminals will help to understand the role of NPY-containing fibres in the atrioventricular valves.

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