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Light and electron microscopic demonstration of neuropeptide Y-like immunoreactive nerves in human cardiac muscle

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Summary. Neuropeptide Y (NPY)-like immunoreactive nerves were demonstrated in human cardiac muscle. The atrial specimens were obtained from open-heart surgery. The PAP method was applied for immunocytochemistry for light and electron microscopy. A dense, extensive network of NPY-like immunoreactive nerve fibres was seen between cardiac muscle cells and around blood vessels. In electron microscope PAP precipitates were localized in large dense-cored vesicles of 80–120 nm in size in separate nerve terminals or in the terminals situated in the nerve bundles. Close contacts were observed between NPY nerves and muscle cells and blood vessels.

The possible functional role of NPY innervation in the human heart is discussed.

Key words: Innervation of human heart – Neuropeptide Y nerves – Peptidergic innervation of human cardiac muscle

Introduction

Dual innervation of human cardiac muscle with parasympathetic fibres from the vagus nerves and sympathetic fibres from the sympathetic trunks, has been established both ultrastructurally (see e.g. Kyösola et al. 1976; Borchard 1978; Williams 1980) and cytochemically (Kyösola et al. 1976; Borchard 1978; Forssman et al. 1982). During recent years the peptidergic innervation of various organs has been intensively studied (see ref. e.g. Forssman et al. 1982). Consequently, nerves immunoreactive to neuropeptides such as substance P-, vasoactive intestinal peptide (VIP)-, and leu-enkephalin-like peptide have been demonstrated in human heart (Weihe et al. 1981; Rechardt et al. 1985). Recently Gu et al. (1983, 1984) demonstrated both in human and in mouse and rat hearts a dense network of nerve fibres exhibiting immunofluorescence for a new bioactive neuropeptide tyrosine (NPY).

In the present study, light and electron microscopic immunocytochemistry was applied to investigate the localization and characterization of NPY fibres and endings in human heart.

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Material and methods

Myocardial tissue specimens were excised from 8 patients undergoing open heart surgery. These patients belonged to a clinical series, where heart muscle was studied ultrastructurally before and after cardiopulmonary bypass. The findings will be correlated with later clinical status (to be published). Before starting the cardiopulmonary bypass myocardial specimens were excised from the right auricular appendage used for inserting the atrial cannula of the perfusion system, and after the perfusion from the right atrium (below the purse-string suture). Small blocks of tissue were immersed in a fixative containing 4% paraformaldehyde and 0.2% glutaraldehyde in phosphate buffer (0.1 M, pH 7.4) for six hours and washed overnight in the same buffer. The tissue pieces were then cut serially with a Vibratome at 20–40 micra. Pre-embedding modification (Pickel et al. 1979) of peroxidase-antiperoxidase method (Sternberger 1974) was carried out as follows. Prior to incubation with antibody to neuropeptide Y, the sections were incubated with a 0.25% solution of detergent Triton X-100 (Sigma Chemicals) in tris saline for 10 min and in 10% normal goat serum in tris saline for 30 min. After two washes in tris saline the sections were incubated for 12–16 h at +4° C with antiserum against NPY diluted 1:1,000 in tris saline containing 1% normal goat serum. The antiserum was purchased from Cambridge Research Biochemicals (Cambridge, England). The antiserum shows neglible crossreactivity to other neuropeptides (suppliers notice). After specific antiserum the sections were immersed in goat-anti-rabbit IgG (Sternberger-Meyer Co., Jarretsville, MD, USA) diluted 1:40 for 30 min and in rabbit peroxidase-

antiperoxidase complex (Dako, Copenhagen) diluted 1:40 for 30 min. Peroxidase reaction was visualized with 0.025% 3,3'-diaminobenzidine-HCl (Sigma Chemicals) and 0.01% hydrogen peroxidase in tris water for 5 min. The sections were postfixed for one hour in 2% aqueous solution of osmium tetroxide and flat embedded in Epon. The sections were photographed and desired areas were cut out of the sections and glued to the surface of previously polymerised epon blocks. Semithin and thin sections were cut for light- and electronmicroscopy. The sections were viewed and photographed without poststaining, i.e. without uranyl and lead staining.

The control sera included preimmune serum and antiserum preabsorbed with 50 µg of neuropeptide Y, substance-P, enkephalins and vasoactive intestinal polypeptide in 1 ml of antiserum diluted 1:1,000. (Only the addition of neuropeptide Y to the antiserum abolished the immunostaining.)

Results

NPY-like immunoreactive nerves or nerve bundles were found in abundance throughout the atrial specimens. The fibres were running between the cardiac muscle cells (Figs. 1–3). The blood vessels also had a rich NPY-like immunoreactive innervation (Fig. 1 and 3). The immunoreactive nerve networks were demonstrated in the 20–40 µ Vibratome sections (Figs. 2, 3) and in 2 µ thick Epon embedded sections, where an excellent preservation of the cardiac muscle cells with distinctly visible cross striations can be seen (Fig. 1).

The control sections with first antibody omitted and with the antiserum preabsorbed with 50 ng of neuropeptide Y did not yield any visible nerve fibres. The immunoreactive nerve staining was unchanged if the antisera were absorbed with substance P, enkephalins or with vasoactive intestinal polypeptide.

Compared with the excellent preservation observed in the light microscope the ultrastructure of the nerve terminals was found to be adequate only. With the fixative used and following the immunocytochemical procedures no small dense-cored vesicles were visualized in the nerve terminals. PAP precipitates were found in the separate nerve endings (Figs. 4 and 6) or in the nerve terminals in the nerve bundles (Figs. 5). The immunoreactive nerves were close to the cardiac muscle cells (Figs. 4 and 5) or close to the blood vessels. The PAP precipitates were localized in the large dense cored vesicles of 80–120 nm in size (Figs. 4 and 6).

Discussion

Recently a new polypeptide, NPY, which belongs to the pancreatic polypeptide family and contains 36 amino acids, was isolated and characterized, originally from porcine brain (Tatemoto 1982; Tatemoto et al. 1982). Co-localization of NPY and catecholamines was demonstrated both in the central nervous system (Hökfelt et al. 1983) and in the peripheral nervous system (Lundberg et al. 1982, 1983; Ekblad et al. 1984).

The dense networks of NPY-like immunoreactive nerve fibres were a not unexpected finding. In human cardiac muscle obtained from necropsys Gu et al. (1983) also demonstrated NPY innervation also present in mouse and rat hearts (Gu et al. 1984). The NPY innervation seems to be dense which is confirmed biochemically by the high concentrations of this peptide in cardiac tissue (Gu et al. 1984). These observations have led to the proposal that NPY could be a major cardiac neuropeptide.

Our light microscopic localizations are in accordance with the earlier findings (Gu et al. 1983, 1984) regarding the close contacts of nerves with the cardiac muscle cells and blood vessels. We further confirmed these localizations with electron microscopic demonstrations, where intimate contacts were seen between NPY-like immunoreactive nerve terminals containing PAP reaction products in the large dense-cored vesicles of 80–120 nm in size and myocardial cells and blood vessels. We have previously localized also substance P- and vasoactive intestinal polypeptide-like immunoreactions in the large dense-cored vesicles of the same size in human cardiac muscle (Rechardt et al. 1986). Whether these different neuropeptides are all localized in the same vesicles needs further double-labelling immunostudies *e.g.* with gold-particles, studies which are in progress in our laboratory. Co-localization of NPY and catecholamines has been reported by Gu et al. (1984), although they do not show any data on that.

There is evidence that NPY exerts a vasoconstrictory effect (Lundberg and Tatemoto 1982; Edvinsson et al. 1984; Ekblad et al. 1984), which is abolished by sympathectomy (Ekblad et al. 1984). So far the mechanism is not clear, either it is independent of catecholamines, additive to their effect, or NPY modulates the release of catecholamines. NPY also has an effect on the cardiac musculature. It induces positive inotropic and cronotropic effects in spontaneously beating guinea pig atria (Lundberg et al. 1984). Allen et al. (1983) did not observe any significant change in heart rate as reported by Lundberg et al. (1984): in their experiments they observed a reflexogenic bradycardia after systemic administration of NPY to guinea pigs, which they interpreted as a phenomenon due to inhibition of sympathetic tone (Lundberg and Tatemoto (1982).

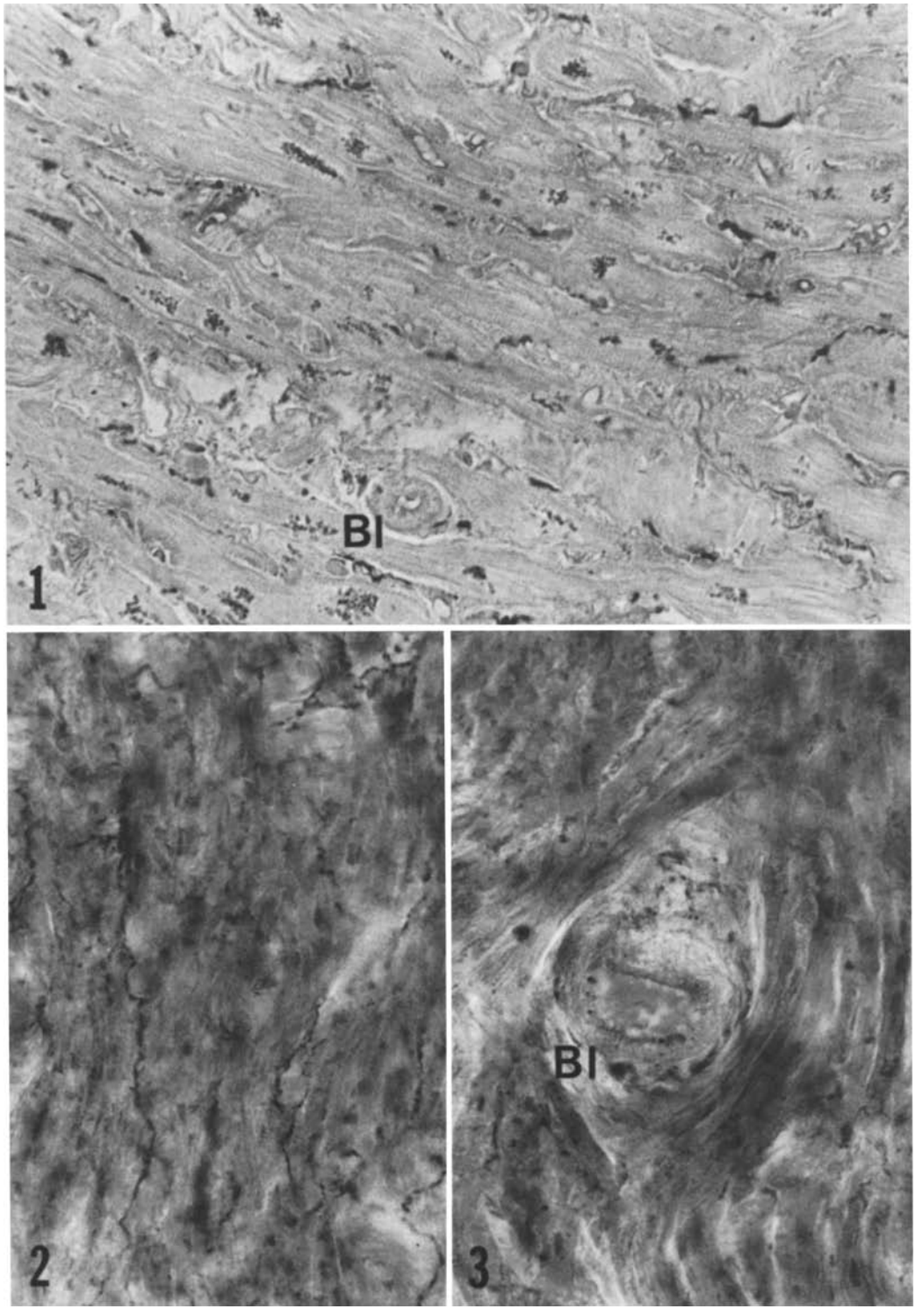

Fig. 1. Light microscopic view of human atrial cardiac muscle. A dense network of NPY-like immunoreactive nerves is observed between the cardiac muscle cells. PAP reaction seen in 2 micron thick Epon section. No counter staining. *Bl*=blood vessel. ×150

Fig. 2. NPY-like immunoreactive nerves are seen running as long fibres between the cardiac muscle cells. 30 micron thick Epon section. ×310

Fig. 3. A blood vessel is surrounded by NPY-like immunoreactive nerve fibres. 30 micron thick Epon section. *Bl*=blood vessel. ×500

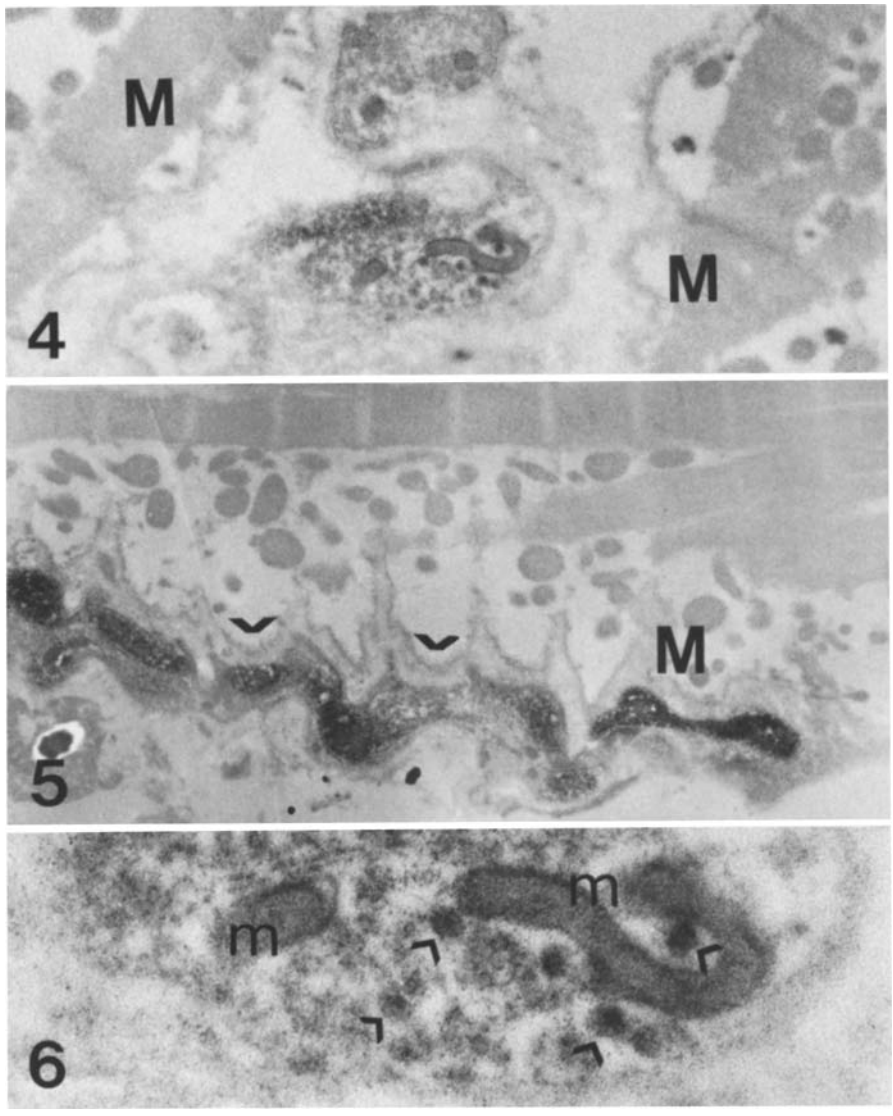

Fig. 4. Electron microscopic view of human atrial cardiac muscle cells. Between the muscle cells (*M*) a separate NPY-like immunoreactive nerve ending is localized. No poststaining. ×17500

Fig 5. A nerve bundle with several NPY-like immunoreactive nerve terminals is seen in close connection with the cardiac plasmalemma. *M* = cardiac muscle cell. ×10300

Fig. 6. Higher magnification of the nerve terminal seen in Fig. 4. Heavy PAP precipitates are seen in the large dense cored vesicles of about 110 nm size (*arrowheads*). (*m* = mitochondrion). ×52500

The mapping ot the NPY-like immunoreactive innervation and the preservation of these immunoreactive nerves after open-heart surgery are a new and valuable tool in the study of heart biopsies. By regulating the formulation and the concentration of the coronary perfusate during the surgery it is possible to influence coronary perfusion flow and ensure optimal preservation of the cardiac tissue during and after the procedure. Studies on the preservation of NPY nerves after open-heart surgery in connection with different coronary perfusates (cardioplegia) are in progress.

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