IMMUNOHISTOCHEMICAL LOCALIZATION OF ANP IN THE PULMONARY VEINS OF THE RAT

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The presence of atrial natriuretic peptide (ANP) in the striated myocytes of pulmonary veins in rat was studied by immunohistochemical methods. In general, the immunoreactivity of the pulmonary myocardium was less intense than found in the left atrium. In the inner circular layer of the pulmonary myocardium, some striated myocytes were moderately granular stained. The staining was mainly localized to the paranuclear areas, while some granules were also distributed throughout the sarcoplasm.

Cardiac muscle cells are present in the adventitia of the pulmonary venous wall of the rat and other rodentia (7-10, 12, 15, 18). The striated muscle coat of the pulmonary veins is continuous with the myocardium of the left atrium, and contributes to the major thickness of the wall in the extrapulmonary veins, whereas, it turn thinner in the intrapulmonary veins and finally disappears. The striated myocytes are structurally similar to those found in the atrium. Also, “atrial specific granules” (ASG) have been reported to be present in some of the myocytes of the extrapulmonary portion of the veins (14). These specific granules, including A-granules and B-granules (2), are morphologically similar to the ASG of the atria. In the atria, the specific granules have been shown to contain “atrial natriuretic peptide” (ANP) by immunocytochemical methods (3, 4, 16, 19, 23, 25). ANP has been demonstrated to play a role in the body fluid homeostasis by increasing the natriuresis and the diuresis as well as being capable of reducing the blood pressure (1, 5, 17, 22).

Based on the structural similarities between the ASG in the striated heart muscle cells of the pulmonary veins and the atria, this study has aimed at demonstrating the presence of ANP in the pulmonary myocardium by immunohistochemical methods.

MATERIALS AND METHODS

Tissue samples:

The heart of the Wistar rats was arrested by injection of ice-cold fixative into the right atrium. The fixative used was composed of 4% formaldehyde, 0.25 M sucrose and 0.01 M sodium phosphate buffer (pH 7.4). Thereafter the hearts were excised
together with the lungs. Still in a bath of fixative, the samples were collected from the following areas: 1) the sinus venarum cavarum of the left atrium, 2) the extrapulmonary veins, and 3) the pulmonary veins at the lung hilus. The samples were fixed at 4°C for 16 h, washed in buffered sucrose, and thereafter embedded in paraffin and cut into 3 μm sections.

**Antiserum:**

Solid phase synthesized ANP (fragment Arg101-Tyr126, a generous gift of Dr. N. Ling, The Stalk Institute) was conjugated to thyroglobin and the antiserum against the complex was raised in rabbits (24). The specificity criteria of the antiserum have been published elsewhere (19, 24). Swine antirabbit immunoglobulins and rabbit peroxidase-antiperoxidase complex were purchased from Dakopatts, Copenhagen.

**Immunohistochemistry:**

The unlabelled peroxidase-antiperoxidase complex method of Sternberger et al. (20) was used. The immunohistochemical staining was performed as described by
Rinne et al. (19). Briefly: the deparaffinized sections were incubated in 20% swine serum, anti-ANP serum (1/200, 1/400 and 1/800), swine antirabbit immunoglobulins (1/20), and PAP complex (1/100), with PBS-washes between incubations. The peroxidase label was visualized by the 3,3'-diaminobenzidine-H2O2-reaction. The specificity of the immunohistochemical staining was controlled by replacing the primary antiserum with normal rabbit serum.

RESULTS

The left atrium:
Almost every myocyte as shown in single sections is showing considerable immunoreactivity (Fig. 1). The specific staining reveals accumulation of granules which are mainly localized in close proximity to the nuclei. However, some granular staining are also seen in the subsarcolemmal areas.

The pulmonary veins:
The pulmonary myocardium contributes to the major thickness of the venous wall. Close to the left atrium the venous muscle coat comprises 5-6 concentric layers of myocytes, and when approaching the hilus it comprises 4-5 myocytes. The inner myocytes are circularly organized, while the outer myocytes are longitudinally organized. The striated muscle coat is well vascularized.

The inner circular layer of the pulmonary myocardium exhibits immunoreactivity, although not as much as the atrial myocardium (Fig. 2). Groups of granular stained myocytes are observed. At the same time, other areas of the pulmonary myocardium as seen in cross section of the veins, are almost free of specific staining. Further, with some enhancement of granularity in the paranuclear zone, the stained granules appear more evenly spread throughout the cytoplasm than found in the myocytes of the left atrium (Fig. 3). In the outer longitudinal layer only a few cells exhibit immunoreactivity.

DISCUSSION

The results of the present study demonstrate the presence of ANP in the cardiac muscle coat of the pulmonary veins. While almost every striated myocyte of the left atrium exhibited considerable immunoreactivity in the paranuclear and subsarcolemmal areas, only some of the myocytes as seen in single sections of the pulmonary veins were immunostained. In general, the latter myocytes displayed moderate granular staining, and, the staining appeared to be more evenly distributed in the cytoplasm than found in the left atrium. The present findings are comparable to previous studies regarding the presence of ASG in the pulmonary myocardium of the rat (14). In the latter study, both A-granules and B-granules were observed in the extrapulmonary veins, whereas, in the intrapulmonary veins ASG were only present at limited numbers after congestion of the left atrium and the pulmonary veins following left ventricular infarction.

ANP is secreted into the blood and is capable of inducing natriuresis and diuresis as well as being able to lower the blood pressure (1, 5, 17, 22). Studies have shown that in water-deprived animals there is a significant decrease of plasma ANP levels (11, 21) and a corresponding increase of plasma ANP in volume-loaded animals (13).
A similar increase of plasma ANP has been described in hamsters with congestive heart failure (6). Subsequently, it has been proposed that stretching of the striated myocytes following atrial distention in volume-loaded animals induces secretion of ANP (13, 17). Provided that the latter mechanism of regulation of the secretion of ANP is present in the pulmonary myocardium as well, this may explain the distinct localization of ANP to the inner striated myocytes of the pulmonary myocardium. These myocytes are circularly organized and are therefore particular vulnerable to stretch following the increased intraluminal pressure during expansion of blood volume.

The moderate immunoreactivity as well as the evenly distribution of granular staining in the cytoplasm of some of the striated myocytes in the extrapulmonary veins, indicate a lower production of ANP in these particular areas than in the left atrium. Nevertheless, its presence in the pulmonary myocardium may suggest that the pulmonary veins are participating to some extent in the regulation of the water and electrolyte balance as well as of the blood pressure.

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REFERENCES


