THE MORPHOLOGY AND LOCATION OF ATRIAL SPECIFIC GRANULES AND THE DEMONSTRATION OF ATRIAL NATRIURETIC FACTOR IN PORCINE, LAPINE AND BOVINE HEART BY IMMUNOELECTRONMICROSCOPY

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ABSTRACT


The atrial specific granules (ASGs) were studied in samples collected from the right and left auricles of conventionally slaughtered cows (10), pigs (16) and rabbits (8). In addition, the presence of atrial natriuretic factor (ANF) was detected by immunocytochemistry. Mature ASGs, characterized by the presence of highly osmiophilic and electron-dense material surrounded by a membrane, were present in all atrial myoendocrine cells and their diameters ranged from 100 to 470 nm in pigs, from 100 to 235 nm in cattle, and from 12.5 to 275 nm in rabbits. Immunoelectronmicroscopic studies revealed the presence of ANF in the ASGs of pigs and cattle, whereas anti-ANF polyclonal serum failed to detect any significant reaction in lapine ASGs. The ultrastructural features of the ASGs of pigs, cattle and rabbits described may be useful in comparing the morphological picture of several cardiac endocrine pathological conditions.

Keywords: atrial natriuretic factor, atrial specific granules, cattle, heart, immunoelectronmicroscopy, pig, rabbit.

INTRODUCTION

Mammalian, avian, amphibian and piscine atrial myocardium (Bencosme and Berger, 1971; Forssmann, 1988; Reinecke et al., 1988; Shao et al., 1989) contains a cell population characterized by the presence of electron-dense membrane-bound granules referred to as atrial specific granules (ASGs). Although both ATPase and nucleotide phosphatase activities, and chromogranins A and B have been detected in ASGs (Navaratnam, 1987; Steiner et al., 1990), bioassay analysis, radioimmunoassay and immunohistochemical studies in man and laboratory animals have previously shown that ASGs are the storage sites of bioactive polypeptides referred to as atrial natriuretic peptide (ANP) or atrial natriuretic factor (ANF), with potent natriuretic, diuretic and hypotensive properties (De Bold et al., 1981; Chapeau et al., 1985; Lang et al., 1985). In the atria and ventricles of rats, ANF is synthesized (prepro-ANF), stored (pro-ANF), and activated as a 28-residue peptide, known as ANF, by a membrane-bound enzyme localized in a microsomal fraction (Onwochei et al., 1987; Shields and Glembotski, 1987; Inagami and Imada, 1989).
In order to better characterize the ultrastructural morphology and the distribution of ASGs and to detect the presence of immunoreactive ANP in ASGs in normal heart, we studied tissue sections taken from the atrial myocardium of pigs, rabbits and cattle by transmission electron microscopy, before and after incubation with rabbit antiserum raised against alpha-human ANP (Hassall et al., 1988; Wharton et al., 1988).

MATERIALS AND METHODS

Tissue samples were taken from the right and left auricles of conventionally slaughtered animals (10 cows, 16 pigs and 8 rabbits) and fixed in 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer (pH 7.4). The tissue blocks were then post-fixed in 0.1% osmium tetroxide in sodium cacodylate buffer for 2 h, dehydrated in a series of graded ethanols, stained en bloc in a 3% solution of uranyl acetate in absolute alcohol, and embedded in a mixture of Epon-812 and Araldite. Thick sections (1 μm) were stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate, or processed for immunostaining. Briefly, for immunoelectron-microscopy, the sections were mounted on nickel grids, treated with 5% sodium metaperiodate solution for 10 min and then incubated in serum from a normal rabbit for 1 h. Incubation in the primary antiserum (1 : 200 dilution titre) (Cambridge Research Biochemicals, Cambridge, UK) was carried out overnight at 4°C. The grids were then covered with gold-conjugated anti-rabbit IgG (1 : 16 dilution titre) for 1 h, post-fixed in 2.5% glutaraldehyde for 10 min and stained with uranyl acetate and lead citrate.

RESULTS

In all the atrial myoendocrine cells, the typical location of the ASGs was the sarcoplasmic cone adjacent to the nuclear poles (Figure 1), although some granules were scattered among the mitochondria and myofibrillae and in close relationship to the sarcolemma and the T-tubule system (Figure 2). Mature granules were spherical or ovoid with a diameter of 100–470 nm (mean 266.26 ± 12.16 SEM) in pigs, 100–235 nm (168.43 ± 3.83) in cows and 125–275 nm (191.05 ± 4.12) in rabbits, and contained a homogeneous, osmiophilic and electron-dense matrix, which was often separated from the membrane by an electron-lucent halo. In any given section from all three species, the number of mature granules localized in the nuclear poles ranged from a few up to ten or more. Smaller progranules could also be observed within the cisternae of the Golgi area. Apart from the presence of ASGs, the morphology of the atrial myoendocrine cells did not differ from that of the ventricular myocytes. Ultrathin sections, immunolabelled with anti-ANP antibody, showed the presence of immunogold particles localized within the ASGs in porcine and bovine atrial myoendocrine cells (Figure 3), whereas in rabbits no noticeable labelling was detected.