MORPHOMETRY OF RIGHT VENTRICULAR PAPILLARY MUSCLE IN RAT DURING DEVELOPMENT AND RECOVERY OF HYPOXIA-INDUCED HYPERTENSION

Kuen-Shan Hung, Henry Pacheco, Dianna Lessin, Kerry Jordan, and Leone Mattioli
Departments of Anatomy and Pediatrics
University of Kansas Medical Center
Kansas City, KS 66103

ABSTRACT

Morphometric analyses of the right ventricular papillary muscle, as well as measurements of right ventricular pressure and weight, were carried out in the rat during the development and recovery of hypoxic pulmonary hypertension. Animals were divided into hypoxic and normobaric control groups. The hypoxic rats were placed in hypobaric chambers for 1, 2, and 3 wks; and after 3 wks exposure, subgroups of hypoxic rats were allowed to recover in normoxia for 1 to 9 wks. Hematocrit (HCT) and right ventricular systolic pressure (RVSP) were measured prior to sacrifice. The heart was perfused, and the right ventricle (RV) was separated from the left ventricle and septum (LV+S) and weighed. The papillary muscles were dissected and processed for ultrastructural morphometry. Results showed that HCT, RVSP, and RV weight increased in the rats during the hypoxic exposure and then gradually returned to control levels after 3 to 4 wks of normobaric recovery. The papillary muscle of the hypoxic rats showed increased volume density of interstitium, increased diameter and cross sectional area of the cardiac myocytes, reduced volume density of mitochondria, and reduced mitochondria to myofilament ratio. During normoxic recovery, these morphometric indices returned toward control values at various periods of time ranging from less than 3 wks to 9 wks. The results indicate that the adaptive ultrastructural changes of the papillary muscle in RV hypertrophy paralleled the RVSP changes, and also demonstrate the reversibility of these changes in ambient oxygen.

INTRODUCTION

It has been repeatedly shown in various experimental models that chronic hypoxia leads to the development of pulmonary arterial hypertension and right ventricular hypertrophy (RVH). Both the pulmonary arterial pressure and RV mass return to near normal levels upon removal of the hypoxic stimulus (Ressl et al., 1974; Herget et al., 1978; Kay, 1980; Kentera and Susic, 1980; Rabinovitch et al., 1981; Sobin et al., 1983; Kentera et al., 1985). However, the ultrastructural changes underlying the development and regression of hypoxia-induced RVH have received little
attention. Previously, morphometric studies by Herbener et al. (1973) have shown no changes in the volume density of mitochondria in the right ventricular myocytes of mice exposed to hypobaric hypoxia.

The purpose of this study is to correlate the ultrastructural changes of the RV papillary muscle with indices of hypoxic pulmonary hypertension (hematocrit and RV systolic pressure and weight) in rats during the progression and regression of hypoxic pulmonary hypertension.

METHODS

Male Sprague-Dawley rats (n = 93) with initial body weights (BW) of 150 to 175g were used. The hypoxic rats (n = 51) were placed in hypobaric chambers for 1, 2, and 3 wks; the controls (n = 42) were pair-fed in the same room in a normobaric environment (Hung et al., 1986). Subgroups of hypoxic rats (n = 32) were removed from the chambers after 3 wks of hypoxic exposure, and allowed to recover in normoxia for 1, 2, 3, 4, 5, 7 and 9 wks. Each hypobaric chamber has 0.25 m² capacity; and the chamber pressure was adjusted to approximately 370 torr. At the end of each exposure period, the animals were anesthetized with pentobarbital sodium (60 mg/kg) and weighed. Hematocrit (HCT) was measured from a tail blood sample. A 3.5 French catheter was placed in the right ventricle via the right jugular vein (Stinger et al., 1981), and the systolic pressure (RVSP) was measured by a Statham 23DB transducer and recorded on an EFM recorder while the animal was breathing spontaneously in ambient oxygen.

The chest was opened, and the heart was arrested in diastole by injecting 1 ml of 1 N KCl into the RV through the catheter and perfused with 2% glutaraldehyde in phosphate buffer, pH 7.2, under 100 cm H₂O pressure for two minutes. After further fixation in fresh 2% glutaraldehyde for at least 2 hours the RV was separated from the left ventricle and septum (LV+S), and weighed.

The papillary muscles from at least 3 animals from each of the following subgroups were obtained for quantitative ultrastructural morphometric analysis: 1, 2, and 3 wks in hypoxia, and their controls under 3 wks normoxia; 3 wks hypoxia followed by 3 wks normoxic recovery and their controls; 3 wks hypoxia followed by 7 wks recovery and their controls; and 3 wks hypoxia followed by 9 wks recovery and their controls. After perfusion fixation, two RV papillary muscles from each rat were removed and rinsed in 0.1M phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated in graded ethanols and embedded in Araldite.

The muscle was oriented for exact cross sections and viewed under a JEOL 100S transmission electron microscope. Low power micrographs (total magnification, 2,500X) were used to determine the volume densities of the myocytes, capillaries (including endothelium and lumen), and interstitial tissue using a point counting technique (Marino et al., 1986). The myocyte diameters and cross sectional areas were measured with a Bioquant Image Analysis System consisting of a Hipad digitizer interfaced with an Apple IIe microcomputer. Intermediate power micrographs (total magnification, 10,000X) were used to determine the volume densities of mitochondria and myofilaments by the same point counting technique. The mitochondria-to-myofilaments ratios were calculated by their volume densities.

Certain tissue sampling criteria were established to insure consistency regarding the areas photographed. A random grid opening was selected and micrographs were taken of the cells lying at each corner of the grid opening as well as two cells from the center of the grid. Only the cells cut in cross sections were used, and cells in the border region of the papillary muscle were disregarded.