Collagen Type Distribution in the Mammalian Left Ventricle During Growth and Aging*

I. Medugorac

Physiologisches Institut II der Universität Tübingen (Director: Prof. Dr. R. Jacob), Gmelinstr. 5, D-7400 Tübingen, Federal Republic of Germany

Summary. Left ventricular collagen of rats aged 1, 4, 8, and 24 months, as well as young and adult pigs and beef, was successively extracted with neutral salt and dilute acid solutions and limited pepsin digestion. The distribution of type I and III collagen molecules in pepsin-solubilized collagen was analyzed with sodium dodecyl sulphate polyacrylamide gel electrophoresis in the presence of 3.6 M urea, under nonreducing and reducing conditions.

Yields of dilute-acid-soluble collagen and neutral-salt-soluble collagen were extremely low (0.3–0.8%, and less than 0.3% of total starting collagen, respectively), indicating the presence of an extensive interchain and intermolecular cross-linking network in left ventricular tissue of young and mature mammals.

Comparison of protein patterns in electrophoresis gels indicated that myocardial collagen consists primarily of type I collagen molecules. The components of type III collagen also occurred in all investigated preparations, in varying and consistently lower proportions. The ratio of type III to type I collagen changed during postnatal growth and aging.

Key words: Left ventricular collagen – Collagen type distribution – Hydroxyproline – Growth – Aging

Numerous recent investigations have been devoted to the general understanding of the structure and biochemistry of the collagen molecule and collagen fibers. Structurally and genetically distinct collagen types have been shown to exist in different mammalian organs and within a given tissue. Different proportions of type I and type III collagen molecules have been identified in interstitial connective tissue of most mammalian organs. An age-related alteration in the distribution of these two collagen types has been discovered in human [4] and calf [1] skin. Despite rapid progress in the identification and chemical characterization of collagen molecules in various organs, analysis and characterization of heart collagen has

* Supported by a grant from the Deutsche Forschungsgemeinschaft
presumably been hampered because of its extreme insolubility, difficult separation from noncollagenous proteins and other components, and occurrence in relatively low amounts.

We investigated the quantity, solubility, and composition of collagen in i.m. left ventricular connective tissue of aging rats (1, 4, 8, and 24 months old) and young and adult beef and pigs.

Materials and Methods

Experimental Animals and Tissue Preparation

**Rats.** Male Wistar rats were used in the investigation. The 1-month-old animals weighed approximately 50 g, the 4-month-old approximately 295 g, the 8-month-old approximately 380 g, and the 24-month-old approximately 480 g. The experimental rats were obtained from Messrs. Ivanovas, Kiflegg (FRG).

**Beef and Pigs.** Young and adult beef were 3 and 18 months old and weighed approximately 120 and 600 kg, respectively; young and adult pigs were 2 and 7 months old and weighed approximately 25 and 120 kg, respectively. Left ventricles of young and adult beef and pigs were obtained immediately after slaughter from a local meat-packing plant.

Left ventricular muscle from the animals of various ages and species was dissected free of adhering fat, large vessels, visible connective tissue and atria. The free wall of the right ventricle was removed, and the weight of the left ventricle with septum was determined. Several left ventricles of each rat age-group were pooled, and left ventricular tissue was frozen and lyophilized [12].

Preparation of Soluble Collagen

Neutral-salt-soluble and acid-soluble collagen was prepared according to the method outlined by McClain et al. [10]. Solubilization of ventricular collagen with pepsin was performed as reported previously [13]. Estimation of collagen in all samples was based on hydroxyproline determination [12]. The amount of collagen was calculated assuming that collagen contained 13.4% hydroxyproline.

Sodium Dodecyl Sulphate Gel Electrophoresis and Densitometry

Sodium dodecyl sulphate gel electrophoresis (SDS-PAGE) was carried out essentially according to the method outlined by Hayashi and Nagai [7] using 0.4 M glycine buffer, pH 8.8, containing 3.6 M urea [13].

Distribution of type I and III collagen molecules was determined by densitometer tracing of the protein pattern in thoroughly destained gels at 550 μm [13].

Results

**Collagen Concentration Analysis**

Results based on hydroxyproline content indicated that left ventricular tissue of various mammals contains different amounts of collagen per mg dry tissue [12]. Collagen concentration increases during growth and aging. In the rat of 1, 4, 8, and 24 months of age, collagen accounts for 1.8, 2.1, 2.3, and 2.6% of the dry left ventricular tissue respectively.