MORPHOLOGICAL ANALYSIS OF SPECIALIZED CARDIOMYOCYTES OF THE CONDUCTING SYSTEM OF THE VAGOTOMIZED RAT HEART

I. A. Chervova and E. R. Pavlovich

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Light-optical and electron-microscopic investigations were made of the specialized conducting system of the rat heart at various times after vagotomy. The dynamics of the tissue and cellular changes are described. A mosaic pattern of cellular responses was found at all times after denervation in all parts of the conducting system and in all types of specialized cardiomyocytes.
KEY WORDS: conducting system of the heart; denervation.

The influence of the vagus nerves on morphological indices for the principal parts of the conducting system (CS) of the heart has not been studied. However, the results of physiological investigations indicate that the vagus nerves help to determine the structural characteristics of CS. Knowledge of the character of the tissue and cellular changes in CS of the mammalian heart after right-sided cervical vagotomy is essential for a global idea of the influence of the autonomic nervous system on the structural basis for the function of specialized myocardial cells responsible for correct and harmonious involvement of all the chambers of the heart in the contraction process. This paper describes the phenomenon of cellular changes in the sinoatrial (SAN) and atrioventricular nodes (AVN), and the bundle of His (AVB) at its main branches in the rat heart on the basis of analysis of the results of light-optical and electron-microscopic investigations at various times after vagotomy.

EXPERIMENTAL METHOD

Sexually mature noninbred male rats weighing 200-250 g were used. In 12 animals anesthetized with pentobarbital (0.05 mg/g body weight) the right vagus nerve was isolated in the neck and a segment of it 1 cm long was removed below the ganglion nodosum. In three animals the nerve was not divided. These rats served as the control. Material was taken from the animals 7, 15, and 30 days after the operation. The hearts of three animals (one at each time) were used for light-microscopy of serial sections, whereas the hearts from the remaining 12 animals were used for light microscopy of semithin sections and for electron microscopy. Material was fixed in 10% formalin and embedded in parafin wax. Sections 7 μ thick were stained by Van Gieferson's or Crossmon's methods [4]. For electron microscopy the hearts were fixed by perfusion with 2% glutaraldehyde and postfixed with 1% OsO_4. The subsequent stages of dehydration, embedding, and cutting and staining of sections were carried out in the usual manner. The material for examination was located under direct vision as described previously [2, 3], followed by histological verification of the tissue of the conducting system of the heart in semithin sections. The semithin sections were stained with 1% toluidine blue.

EXPERIMENTAL RESULTS

At all times after the operation vasodilatation was observed in the conducting myocardium, many connective-tissue cells appeared, and the nuclei of the myocytes and their cytoplasm were changed. The structural changes in the myocytes increased in intensity until 7-15 days after the operation, and their normal structure was subsequently restored by the 30th day after right-sided cervical vagotomy. Changes in the myocytes were expressed as swelling or shrinking of their nuclei and loss of clarity of the myofibrillary pattern. These changes were particularly marked in the working myocardium, surrounding the structures of the CS of the heart. On the whole the intensity of staining of the cardiomyocytes was reduced, and as a result of this, the conducting myocardium was more difficult to distinguish from the surrounding contractile

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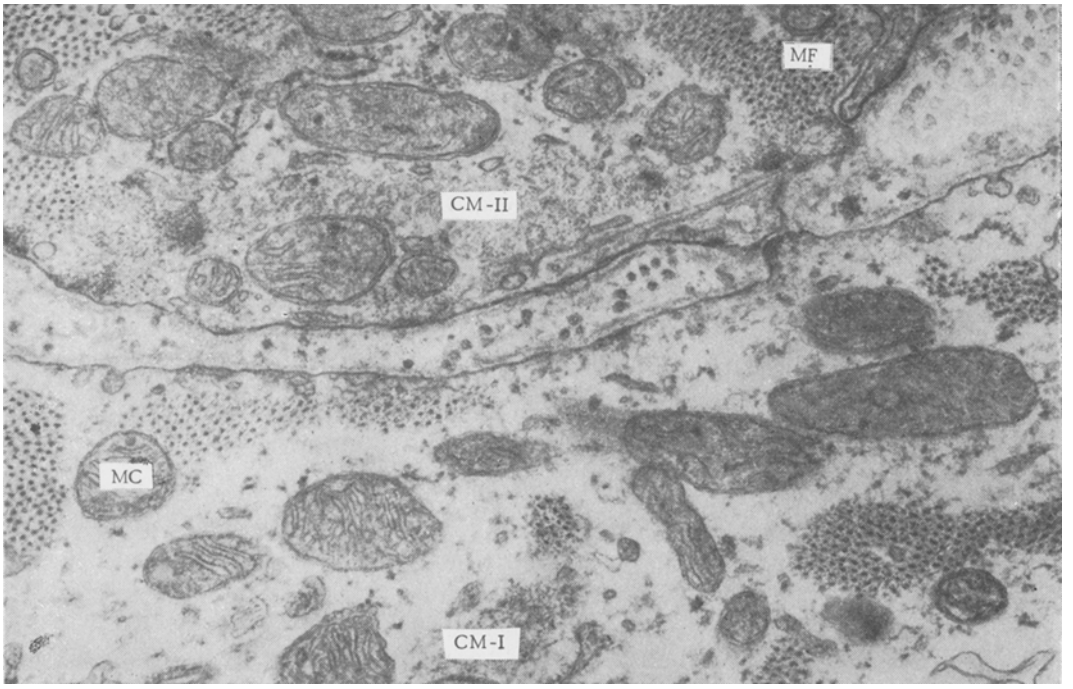

Fig. 1. Intact cardiomyocytes of types I (CM-I) and II (CM-II) 15 days after vagotomy. MC) Mitochondrion; MF) myofibril. Magnification 20,000 ×.

myocardium, although the differences did not disappear completely. For this reason it was possible to use the method of histological verification of the material of the CS of the rat heart in semithin sections, followed by trimming of the block around the target area for electron-microscopic study of changes taking place in the specialized myocardiocytes at different times after right-sided vagotomy.

Previously three different types of specialized myocytes were described in different parts of the CS of the intact rat heart; they were distinguished by their set of four ultrastructural features, such as the shape and size of the cell, the degree of prominence of the myofibrillary system, and the character of its organization [2, 3]. It was on the basis of this classification that the results of the present investigation were analyzed. The ultrastructural characteristics of the control material from the CS of the heart [7, 15, and 30 days) demonstrated very slight differences from material obtained from normal rats [2] but significant differences from material from the CS of the vagotomized rats. In the early stages (7 days) after the operation edema of the myocardium was present, edema fluid accumulated in the intercellular space, and many blood cells could be seen in the interstices. The shape of the cells and the character of the intracellular organelles were changed in the specialized cardiomyocytes of all types. Less marked changes were present in the specialized cardiomyocytes of type I [2] in SAN and AVB. Ultrastructural changes in the specialized type II cardiomyocytes [2] were manifested as an increase in their pinocytotic activity, dilatation of the tubules of the sarcoplasmic reticulum, and the appearance of dense bodies (in SAN, AVN, and AVB). The glycogen content was sharply reduced 7 days after vagotomy, as was particularly conspicuous in the type III cells in AVN, AVB, and the right branch of AVB. Under normal conditions these myocytes were rich in glycogen. In the type III cardiomyocytes the sarcoplasm was reduced in size and devoid of organelles; dilated membrane profiles and dense bodies appeared. By the 15th day after the operation the morphology of the myocytes in CS of the rat heart was heterogeneous: most cells had normal ultrastructural characteristics (Fig. 1) or were very slightly changed, and a minority of cells showed severe destructive changes (Fig. 2). The slight changes were expressed as widening of the intercristal spaces of the mitochondria and translucency of their matrix; the tubules of the sarcoplasmic reticulum were very slightly dilated. All three types of cells preserved the mutual arrangement of their contacts. Gross destructive changes were most characteristic of a few type I cells in AVB and type III cells in AVN and AVB (Fig. 2). In these myocytes total damage to the structures of the mitochondria was observed (to the extent of rupture of both membranes), the myofibrils were overcontracted, their myofilaments were indistinct, and the integrity of the sarcolemma was disturbed or its continuity was broken. However, at these same times cells with evidence of intensified structural metabolism were present. The number of distinctly outlined vesicles in the region of the Golgi complex was increased and zones of myofibril formation were

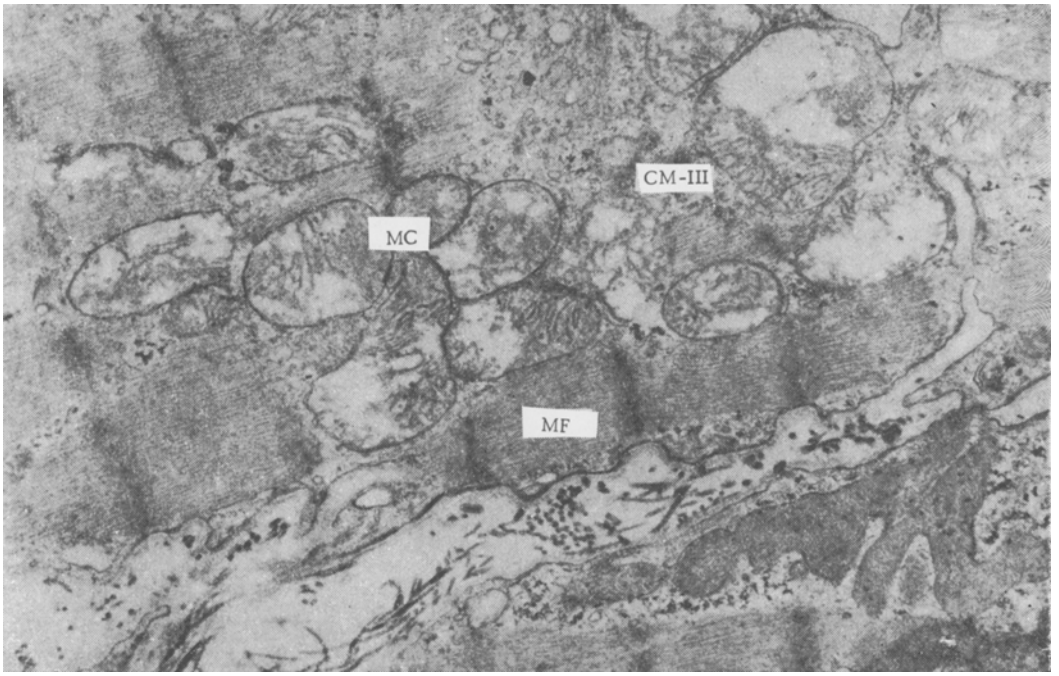

Fig. 2. Modified type III cardiomyocyte (CM-III) in bundle of His 15 days after vagotomy. Legend as in Fig. 1. Magnification 54,000 ×.

visible. In the type I cells the number of ribosomes was increased. It must be emphasized that the various cell types in the same region of CS of the heart varied in the intensity of their destructive and regenerative changes; this was also true of cells of the same type in different parts of CS. By the 30th day after the operation the number of cells involved in the reaction had increased. Individual myocytes of types I and III were irregular and branching in shape. The osmiophilia of the cardiomyocytes was altered, and some cells with strongly osmiophilic sarcoplasm appeared. The width of the space between contacting myocytes was changed. Changes in the cells were expressed as swelling of individual mitochondria and the appearance of myelin-like structures, and α-particles of glycogen, not characteristic of the myocytes of the intact organ. Signs of regeneration were found in some cardiomyocytes. On the whole, the changes in the specialized cardiomyocytes of CS of the rat heart formed a mosaic pattern, as reflected in variation of the intensity of destructive and reparative changes in different cell types within the same part of CS and for any one type of specialized myocyte in different parts of CS.

Nervous control over tissue nutrition is known not only to establish an adequate level of metabolism for each given moment, but also to maintain an appropriate structural organization of the cell; for that reason a disturbance of innervation causes changes in cellular ultrastructure [1]. Accordingly, right-sided cervical vagotomy evidently leads to an imbalance between the functional load in the structures of CS of the heart and nutritional provision for that load. Consequently, the renewal of the intracellular structures is disturbed, i.e., intracellular physiological regeneration is upset [1, 5]. Ultrastructural changes observed in the specialized cardiomyocytes of CS of the rat heart are evidently the result of disturbances in the metabolic system of the cell, which was exposed to the action of: a) disturbance of the nervous control of nutrition (a change in the hormonal-mediator background); b) disturbance of the circulation after vagotomy (changes in the supply of nutrients and oxygen); c) interstitial edema (changes in the ion-electrolyte balance of the cell, mechanical compression by edema fluid). The character of the ultrastructural changes in the conducting cells, and also in the working mycardium [5, 6], is in all probability nonspecific in character and composed of reversible and irreversible changes. The latter were visible on the 15th day after the operation but were not found earlier or later (possibly on account of death of the respective cells). The dynamics of the ultrastructural changes was thus expressed as predominance of edematous and destructive processes in the early period after the operation and a subsequent increase in the intensity of compensatory and regenerative processes. The character of the reactions of components of CS in the rat heart to division of the right vagus nerve was heterogeneous. This heterogeneity was expressed as differences in the intensity of destructive and regenerative reactions in different parts of CS (in SAN, ANV, AVB, and its main branches) for different cell types (specialized cardiomyocytes of types I, II, and III) and for the same cell type in different parts of CS.

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