Cardiac Hypertrophy in Spontaneously Hypertensive Rats

Ultrastructural Cytochemistry of $\beta$-Hydroxybutyrate Dehydrogenase

Kikuko IMAMURA,* Tadasu TAKATSU,* Chujiro KASHII,** and Keishiro KAWAMURA***

From the previous histochemical and biochemical observations about cardiac hypertrophy in spontaneously hypertensive rats (SHR), it was confirmed that the activity of $\beta$-hydroxybutyrate dehydrogenase ($\beta$-HBDH) in mitochondria of myocardium in SHR was increased prior to the development of cardiac hypertrophy. Besides, we also reported that ultrastructural stereology showed the decrease of the volume fraction of mitochondria in the hypertrophied myocardium of SHR. In the present study ultrastructural cytochemistry was applied to ascertain the fine structural localization of the $\beta$-HBDH activity and to investigate the correlation between biochemical and histochemical data and the structural changes of mitochondria in hypertrophied myocardium of SHR.

Materials and Methods:
Five, 9, and 15-week-old SHR and control animals of Wistar-Kyoto were anesthetized with ether. After thoracotomy the heart was fixed by 6.25% hydroxyadipaldehyde. The thick sections (40–60 μ) from the left ventricular papillary muscle were made with vibratome. The tissues were incubated for 30 min at 37°C in incubation medium according to the method of Ogawa (copper ferrocyanide method). For control study the substrate was omitted from the incubation medium. After incubation the tissues were osmicated in 1% OsO$_4$ at room temperature for 1 hour, dehydrated in graded ethanol and propylene oxide and embedded in epon. Ultrathin sections were cut, usually unstained, or stained with uranyl acetate and lead citrate and examined with electron microscope.

Results:
Electron-opaque copper ferrocyanide deposits marking the sites of the $\beta$-HBDH activity were observed in mitochondria in the myocardium of SHR and control rats. The reaction product was deposited in the outer compartment, the intracristal space and the cristal membrane of mitochondria. No deposit was seen in the mitochondrial matrix.

In control animals, numerous ovoid-formed mitochondria containing a great number of cristae were observed in the interspaces between the myofibrils in the myocardium. There were very few mitochondria with the altered structure. At the age of 5 weeks mitochondria containing the reaction product by $\beta$-HBDH were

---

From the Third Department of Internal Medicine,* Osaka Medical College, Takatsuki; Department of Internal Medicine,** Osaka Red Cross Hospital, Osaka; Third Department of Internal Medicine,*** Faculty of Medicine, Kyoto University, Kyoto.

581
scattered rarely in the myocardium. At the age of 9 and 15 weeks numerous mitochondria exhibited the reaction product in the outer compartment or within the intracristal spaces and the cristal membrane in addition to the outer compartment localization.

In SHR, on the other hand, at the age of 5 and 9 weeks few differences were seen in the number, distribution and structure of mitochondria as compared to controls. At the age of 5 weeks the reaction product by $\beta$-HBDH was deposited in slightly larger numbers of mitochondria than that in controls. The deposition was found mostly in the outer compartment of mitochondria. At the age of 9 weeks the majority of mitochondria exhibited heavy accumulations of the reaction product in the outer compartment, intracristal space and cristal membrane. At the age of 15 weeks mitochondria showed a wide variety of size, frequently with a predominance of the decreased size. In some areas of the myocardium there was marked accumulation of the mitochondria (mitochondriosis), which revealed intensive enzymatic activity. On the other hand, some mitochondria showed swelling and reduction in the number of cristae, and those mitochondria revealed low intensity of the reaction.

Discussion and Conclusion:

It was confirmed by the use of ultrastructural cytochemistry that the reaction product by $\beta$-HBDH was found in the outer compartment, the intracristal space and the cristal membrane of mitochondria. The heterogeneous staining pattern observed in mitochondria might indicate the existence of functional heterogeneity among mitochondria.

In addition, it might be suggested that the elevation of the $\beta$-HBDH activity in the myocardium of SHR results from the intensive enzymatic activity in the majority of mitochondria at the age of 9 weeks, while at the age of 15 weeks it is due to the markedly increased activity of the enzyme in mitochondria at the certain area. These observations provide the possibility that the functional heterogeneity of mitochondria exists not only within the same cell but also in neighboring cells in hypertrophied myocardium.