Ultrastructural Changes in Heart Muscle Associated with Anaphylaxis in the Guinea Pig

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SUZUKI, T. *Ultrastructural Changes in Heart Muscle Associated with Anaphylaxis in the Guinea Pig.* Tohoku J. exp. Med., 1972, 106 (2), 109-123 — The ultrastructural changes of heart muscle following anaphylactic shock induced by egg white injection have been studied with guinea pigs to elucidate the exact nature of the cardiac anaphylaxis. The main changes were observed in the contractile elements and they consisted of dissociation of myofibrils from intercalated discs, fragmentation of intercalated discs, lengthening of sarcomere with contraction of adjacent several sarcomeres, and fragmentation and disarrangement of myofilaments. The other changes were shrinkage or disruption of mitochondria, dilation of endoplasmic reticulum, appearance of numerous vacuoles between and inside the myofibrils, and slight increase of glycogen granules. In these changes of the heart muscle, those of the contractile elements and the mitochondria seemed to have resulted from the direct effects of anaphylactic reaction, and dilation of endoplasmic reticulum and appearance of numerous vacuoles were considered to be caused by anoxic effects of respiratory disturbance or reduction of coronary blood supply due to anaphylactic reaction. The increase of glycogen granules seemed to be caused by disturbance of oxidative enzymes in the mitochondria. These findings suggested that the effects of anaphylactic reaction on the heart muscle resulted from the direct toxic effects of anaphylactic reaction rather than the hypoxia or ischemia due to respiratory disturbance or reduction of coronary blood supply, and they seemed to support the conception that the heart should be considered to be a potential "shock organ" in the anaphylactic reaction. ——— anaphylactic shock; heart muscle; electron microscopy

The essential features of anaphylaxis have been demonstrated upon the isolated uterus from sensitized guinea pigs (Schultz 1910) and the isolated segments of the intestine from the animals of the same kind (Dale 1913). On the other hand, there is evidence that the heart is affected in the process of anaphylactic shock. Some authors (Auer 1911, Longcope 1915, Klinge 1930, Seegal *et al.* 1932) have reported morphological alterations such as degeneration of the myocardial cells with round cell infiltration and later scar formation, and others (Auer and Robinson 1913, Koenigsfeld and Oppenheimer 1922, Criepp 1931) have demonstrated disturbance of conduction and abnormalities of origin and spread of excitatory process in the heart during anaphylactic shock.

In 1913, Dale showed in his immunological study that the prime target in

anaphylactic reaction was the bronchiolar smooth muscle. Although cardiovascular disorder had been accepted to occur in anaphylactic shock, it was recognized that the circulatory collapse associated with anaphylaxis was not a primary symptom but was caused by asphyxia resulted from bronchospasm. However, it has not been clarified whether the effect of anaphylaxis to the heart is direct or secondary to asphyxia or to any substance which secondarily reacts upon the heart muscle.

It is said that the first direct proof of involvement of the heart itself as a primary target in anaphylactic shock was given by Cassaris-Demel in 1911, who showed that the isolated hearts of immunized rabbits and guinea pigs reacted specifically to the antigens with which the animals had been sensitized, and that the hearts were depressed following transient acceleration after adding small quantities of antigen into the perfusing fluid (Feigen and Prager 1969).

The study of anaphylaxis in the isolated hearts of young guinea pigs by Wilcox and Andrus (1938) showed that the response of the sensitized guinea pig heart to a specific antigen was characterized by an increase in the rate and amplitude of contraction, a delay in the atrioventricular conduction time, abnormalities in the origin and spread of excitation in the ventricles, and a severe reduction in the coronary flow. In this study, the authors considered that the functional changes of the heart in anaphylactic shock were the consequence of an impairment of myocardial oxygenation caused by decrease of the coronary flow.

Later, Penna *et al.* (1959) observed the anaphylactic reaction in the isolated atria of immunized guinea pigs and proved that the cardiac effects of anaphylactic reaction were exerted directly on the heart muscle, and were not dependent on changes in the coronary flow, since in the isolated atria preparation, the coronary circulation was not involved and antigen reached the atria by diffusion from outside.

The present work was carried out on guinea pigs in order to investigate the effects of anaphylactic reaction on the ultrastructure of the heart muscle and elucidate the exact nature of the cardiac anaphylaxis.

MATERIALS AND METHODS

Twenty guinea pigs were rendered sensitive to egg white by intramuscular injections of 0.2–0.5 ml with Freund's adjuvant three times a week for three weeks. Two or three weeks later, the toxic dose of egg white, 1.0–2.0 ml, was injected into the lateral vein of the ear. All of the animals showed severe symptoms of anaphylactic shock and died within several minutes after the injection. Continuous electrocardiographic tracings were made after the toxic dose injection. Immediately after the heart was exposed, small pieces of the papillary muscle of the left chamber were excised, fixed in cold 1% osmium tetroxide in barbiturate acetate buffer, dehydrated in a graded series of alcohol and embedded in epoxyresin. Sections were cut on a Hitachi UM-3 ultra microtome with glass knives and mounted on 150 mesh copper grids with collodion film and carbon coating. The thin sections were stained with lead citrate and uranyl acetate and examined with a Hitachi HS-7 electron microscope, and micrographs were taken at magnifications of 4,000 to 10,000, which were photographically enlarged to a desired size. The remaining tissue was fixed in 10% formalin solution, embedded in paraffin, sectioned and

stained with hematoxylin and eosin for light microscopy.

The other five guinea pigs were injected only Freund's adjuvant without egg white intramuscularly three times a week for three weeks. Two or three weeks later, 1.0–2.0 ml of egg white were injected in the lateral vein of the ear and they were killed by decapitation at intervals of several minutes. Their hearts were quickly excised and used as controls.

RESULTS

Gross observations

In highly sensitized guinea pigs, very marked panting respiration and rapid feeble heartbeats were seen after a short period of incubation, and then tonic and clonic convulsions set in, which were followed by death. Respiration ceased first, and the heart usually continued to beat for several minutes, then slowed down and stopped.

In electrocardiogram, sinus tachycardia at first, then bradycardia with or without sinus irregularity, prolongation of P-R interval, A-V dissociation even complete heart block, inversion of T wave and elevation or depression of S-T segments were noted in the earlier stages and terminally the heartbeats slowed down markedly and then stopped.

On opening the chest, the lungs were found collapsed well, but not fully. The heart was fairly dilated, especially the right auricle and ventricle. Hemorrhages were seen on auricular and ventricular surfaces. The endocardial surfaces of the ventricles usually showed subserous hemorrhages of variable extents. The lungs look faintly mottled, and the surfaces and borders showed numerous elevated areas which were due to emphysema. Occasionally slight hemorrhages were seen on the surfaces of the lungs.

Five control guinea pigs, which received 1.0–2.0 ml of egg white intravenously, did not show any symptoms resembling anaphylaxis.

Light microscopic observations

The hearts of most of the anaphylactic guinea pigs did not show any abnormal appearances except many subpericardial, subendocardial and intramural hemorrhages.

Electron microscopic observations

1) Control guinea pig. The heart muscle demonstrated that all the myocardial cell components were similar to the well-known classical appearances described by many investigators. The contractile elements and mitochondria were of normal appearance and there was no abnormal finding in other cell organelles of the heart muscle (Fig. 1).

2) Anaphylactic guinea pig. The examination of the hearts revealed specific cellular abnormalities differ from controls. The most general alterations were seen in the contractile elements. They consisted of dissociation of myofibrils from intercalated discs (Figs. 2 and 3), fragmentation of intercalated discs (Fig. 3), and

lengthening of the sarcomeres adjacent to the intercalated discs, which was constantly associated with a certain degree of contraction of the adjacent several sarcomeres (Fig. 4). In some of the heart muscle cells, fragmentation and disarrangement of myofilaments in the parts adjacent to the intercalated discs were noted (Fig. 5).

The majority of mitochondria appeared to be normal structurally and their number and distribution also normal. However, occasionally in some sections, they had shrunken appearance with closely arranged cristae and dense matrices (Figs. 2, 5 and 7), and in other sections, the majority of mitochondria were disrupted severely (Fig. 6). Endoplasmic reticulum was often observed to be dilated to various degree (Fig. 7). In some sections, large vacuoles with limiting membrane appeared between and inside the myofibrils (Fig. 5). The glycogen granules increased slightly in number, especially in the heart muscle cells with shrunken mitochondria (Figs. 2, 5 and 7).

In the capillaries surrounded by pericytes, the cytoplasmic surfaces of the endothelial cells facing the capillary lumina were irregular and very tortuous, and they appeared to be severely contracted (Fig. 8), while in other capillaries, the endothelial cells projected into the capillary lumina in various degree (Fig. 9). Interruption of capillary wall was noted in a few capillaries.

DISCUSSION

Previous studies of anaphylaxis had shown the guinea pig to be the most reliable and most sensitive test animal for immunologic aspects of anaphylaxis (Schultz 1910, Dale 1913). Therefore, the author used guinea pigs as experimental animals in the present work. The symptoms occurred shortly after the injection of egg white in toxic dose, were generally characteristic for asphyxia accompanied by a complex of concomitant pathologic signs.

The changes of electrocardiogram observed in the state of anaphylaxis were bradycardia, partial and complete block, inversion of T wave and elevation or depression of S-T segments. Terminally, the heartbeats slowed markedly down. The pathologic finding of the hearts of the experimental animals was only superficial and intramuscular hemorrhages of variable extent. These functional and morphological findings of the hearts seemed to be not specifically due to anaphylactic state of the heart but rather to the state of asphyxia induced by anaphylaxis. Therefore, from these findings, it was not defined whether the anaphylactic reaction exerted effects directly on the heart or not.

The ultramicroscopic observations of the heart muscle in the present study revealed that the main changes of the heart muscle were in the contractile elements and they consisted of dissociation of myofibrils from intercalated discs, fragmentation of intercalated discs, lengthening of sarcomeres with contraction of adjacent several sarcomeres, and fragmentation and disarrangement of myofilaments. The other findings were the shrunken mitochondria with close cristae and dense matrices, disruption of mitochondria, dilation of endoplasmic reticulum, appearance

of numerous vacuoles between and inside the myofibrils, and slight increase of glycogen granules in the heart muscle cells with the shrunken mitochondria.

All of these ultrastructural changes found in the anaphylactic heart muscle were not always attributable to direct effects of the anaphylactic reaction on the heart muscle, because in anaphylactic reaction the respiration was disturbed firstly which might contribute to anoxic anoxia, and the flow rate through the coronary vessel seemed to reduce strikingly (Andrus and Wilcox 1939), which might contribute to anemic anoxia. Therefore, in the electron microscopic observations of the anaphylactic heart muscle, the changes due to anoxia or ischemia were also expected to be observed.

In these ultrastructural findings, the most striking changes were those of the contractile elements and they suggested that the heart muscle cells contracted severely each other and very strong tension resulted at the uniting areas of the heart muscle cells, that is, the intercalated discs and the sarcomeres adjacent to them. Such changes of the contractile elements had not been reported in the anoxic or ischemic heart muscle, and they were considered to be caused by the anaphylactic reaction of the heart muscle. These findings suggested also that the anaphylactic reaction caused not only the smooth muscle but also the heart muscle to contract severely. This agrees with the constant increase of both frequency and amplitude of contraction observed by Penna *et al.* (1959) in the anaphylactic reaction of the isolated left atrium.

In the ultrastructural changes of the heart muscle except contractile elements, the shrunken mitochondria with closely arranged cristae and dense matrices have not been reported, and this finding was also considered to be caused by the toxic action resulted directly from the anaphylactic reaction. The disruption of mitochondria was reported in the heart muscle of rats in cyanide or carbon monoxide poisoning (Suzuki 1968, 1969). But these substances have no relation to the anaphylactic reaction. Therefore, the disruption of mitochondria noted in the anaphylactic heart muscle seemed also to be caused by direct toxic action resulted from the anaphylactic reaction. Since the increase of glycogen granules was seen mainly in the heart muscle cells with shrunken mitochondria, the oxidative enzymes in these mitochondria seemed to be severely disturbed. Dilation of the endoplasmic reticulum and appearance of numerous vacuoles were generally found in the heart muscle in anoxic or ischemic condition (Mölberty 1957, Caulfield and Klionsky 1959). These changes were considered to be caused by anoxic effects from respiratory disturbance and striking reduction of the coronary flow due to anaphylactic reaction. In the present work, anoxic or ischemic changes of the mitochondria such as swelling could not be found. This fact seemed to be due to shortness of duration in which the heart muscle was exposed to anoxic and ischemic condition, because the experimental animals became lethal within several minutes after anaphylactic reaction occurred.

Considering that the ultrastructural changes based on ischemia or anoxia were generally slight, most of functional cardiac changes observed in the present

work seemed to be attributable to the direct effects of anaphylactic reaction to the heart muscle rather than ischemia or anoxia of the heart muscle.

These ultrastructural changes of the heart muscle in the anaphylactic condition seemed to indicate clearly that the heart muscle itself participated in tissue sensitization and was capable to respond directly to anaphylaxis, and to support the conception that the heart should be considered to be a potential "shock organ" during the anaphylactic reaction (Penna *et al.* 1959). This fact may explain some cases of sudden death during anaphylactic shock in human beings.

In the electron microscopic observations of the capillary endothelial cells in the heart muscle, the irregular cytoplasmic expansions in the capillary lumina, severe contraction of capillary surrounded by pericytes and interruption of capillary wall were noted. In these findings, contraction of the capillary surrounded by pericytes seemed to be caused by contraction of pericytes due to anaphylactic reaction. The cytoplasmic expansion of the capillary endothelial cell in the capillary lumen has been observed in the capillary of the lung of rabbit injected with epinephrine to cause acute pulmonary edema (Kisch 1958). The cytoplasmic expansion observed in the present study was also considered to be caused by epinephrine released from the adrenal cortex in the state of anaphylactic shock. These capillary alterations suggested that striking reduction in the coronary blood supply to the heart muscle occurred in the anaphylactic state.

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Legend for Figs. 1-9

ss, mitochondrion	id, intercalated disc
cr, crista	f, fat droplet
sr, sarcoplasmic reticulum	g, glycogen granule
mf, myofibril	v, vacuole
z, Z band	ce, capillary endothelial cell
m, M band	cl, capillary lumen
s, sarcomere	p, pericyte
sl, sarcolemma	n, nucleus

Fig. 1. Longitudinal section of the control guinea pig heart muscle. All myocardial cell components were similar to the well-known appearances of those of the normal heart muscle cells. $\times 20,000$.

Fig. 2. Longitudinal section of the anaphylactic guinea pig heart muscle. Note dissociation of the myofibril from the intercalated disc ($\uparrow$). Mitochondria are shrunk-en prominently and have very closely arranged cristae and dense matrices. Slightly increased glycogen granules are seen. $\times 25,000$.

Fig. 3. Longitudinal section of the anaphylactic guinea pig heart muscle. Fragmentation of the intercalated disc ($\uparrow$) and dissociation of the myofibril from the intercalated disc are noted. From these findings, the intercalated disc appears like dissociating. $\times 18,000$.

Fig. 4. Longitudinal section of the anaphylactic guinea pig heart muscle. Lengthening of the sarcomeres adjacent to the intercalated discs and severe contraction of adjacent several sarcomeres. Dissociation of the myofibrils or fragmentation of the intercalated disc can not be seen. The mitochondria appear to be almost normal. $\times 18,000$.

Fig. 5. Longitudinal section of the anaphylactic guinea pig heart muscle. In the right myocardial cell of this figure, fragmentation and disarrangement of the myofilaments are seen in the large part adjacent to the intercalated disc. In both right and left cells there are several large vacuoles with limiting membranes. Mitochondria are shrunk and have very closely arranged cristae and dense matrices. Glycogen granules are increased slightly. $\times 10,000$.

Fig. 6. Longitudinal section of the anaphylactic guinea pig heart muscle. In many mitochondria seen between the myofibrils, disruption of external membranes and cristae is prominent. $\times 20,000$.

- Fig. 7. Longitudinal section of the anaphylactic guinea pig heart muscle. Dilation of the sarcoplasmic reticulum is prominent. Mitochondria have closely arranged cristae and dense matrices. Glycogen granules are increased slightly. $\times 11,000$.
- Fig. 8. Cross section of the capillary surrounded by pericytes in the anaphylactic guinea pig heart muscle. The cytoplasmic surface of the endothelial cell is irregular. The capillary lumen becomes very narrow and the capillary appears severely contracted. $\times 10,000$.
- Fig. 9. Cross section of the capillary in the anaphylactic guinea pig heart muscle. The endothelial cell projects a large cytoplasmic expansion into the capillary lumen ($\uparrow$). $\times 14,000$.