

Age-related Changes in Heart Tissue with Special Reference to ATPase and 5'-nucleotidase



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Abstract : The scanty distribution of 5'-Nucleotidase enzyme in sections of cardiac tissue from infant in comparison to young. It is more distinct in adult and proves an indication of the extent of replication process of adipocytes replacing senile and debilitating cardiac tissue. The enzyme activity is more marked in epicardium although the traces in endocardium and myocardium; also it indicates replacement of debilitating tissue through altered replication of adipocytes.

There is an increment of the adenosine triphosphatase (ATPase) activity with advancement of age but distribution becomes highly uneven indicating in non-uniform availability of the enzyme which can possibly explain the reduction in functional capacity due to possibly either alteration in the sequential synthesis of adenosine triphosphatase enzyme in the biochemical structural terms or differential capacity to synthesize enzyme on morphological structural terms.

Key words : Heart, Age, Enzymes, Cardiac tissue, 5'-Nucleotidase, Adenosine triphosphatase.

Introduction

Heart's structural/functional derailment is the single largest cause of death with advancement of age. Physiologists have found that the performance of many organs such as the heart, kidneys, brain, or lungs shows a gradual decline over the life span. Part of this decline is due to a loss of function mass of cells from these organs. Certain cellular enzymes may be less active and thus more time may be required to carry out chemical reactions. Ultimately the cell may die.

Contractile strength and prolonged work capacity in muscle are related to its structure and metabolism (Talesara and Arora, 1994). Multitude of senescent changes such as decrease in muscle working capacity, limitation of motor activity and weakening of muscle strength, strongly implicate certain structural and metabolic changes in muscle. Histochemical study of enzyme activity in old age has received little attention though it is of

considerable value in the investigation of muscle metabolism, strength and other structural changes within it, which may influence its electrical properties and contractile potential.

The present study has been undertaken to show age related changes in muscle fibre characteristics and enzyme level by correlative histochemical investigation in heart.

Material and Methods

Rat (*Rattus norvegicus*) bred in breeding centre of J. N. V. University duly licensed and registered for the purpose by expert committee of social justice, Govt. of India under prevention of animal cruelty act were subjected to study from day one of the birth. The rats were maintained under identical condition. All animals were given standard diet with free access to drinking water. New animals were obtained after breeding. Age matched healthy and disease free animals were used for the study purpose. Body weight was determined

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with the help of standard weighing machine. Investigations were performed on the rats bred in maintained condition at fix time of life cycle. Rats of different age groups *viz.* infant (1-3 months), young (5-6 months), adult (12 months & above) were used for study purpose. A single animal was taken in single day and chloroform was used for anaesthesia. Animal was sacrificed and heart muscles were excised free of blood vessels.

Heart was taken out, cut longitudinally into two pieces, and washed in 0.9% saline solution. After it, heart was kept on watch glass and blotted on filter paper. The heart tissue was kept in 10% neutral buffered formaline at 4°C in the freezer for 3 hours. After keeping in freezer, it was taken out and tissue was cut on a freezing microtome. Sections were obtained of 25 microns with the help of freezing microtome. Then sections were taken for staining purposes. Staining was done in the same day.

5'-nucleotidase enzyme of heart tissue was stained by Lead method (Wachstein & Meissel, 1957) as described by Bancroft *et al.* in histochemical techniques, page 248, W.H. Freeman & Co., San Francisco and London, 1975 and ATPase enzyme of heart tissue was stained by Lead method (Wachstein & Meissel, 1960) as described by Bancroft *et al.* in Histochemical Techniques, pages 248 and 250, respectively. W.H. Freeman & Co., San Francisco and London, 1975.

Results

5' -Nucleotidase

Microphotograph for the infant at 100x Fig. 1(a) shows a very scanty distribution of the enzyme 5'-nucleotidase in comparison to that of young {Fig.(1b)} while in adult the enzyme activity is more marked {Fig.1(c)}. Fortunately, in only one section field has been microphotographed at 100x fig.1(b), with reference to young which shows the epicardium and endocardial area as well. In this section the enzyme activity is conspicuous

along epicardium in the form of thin dark line while endocardium shows preponderance of enzyme activity, although the myocardium is also not devoid of enzyme activity.

The section of that cardiac tissue demonstrates lightened density of 5'-nucleotidase in the form of clumped deposit {fig1(c)}. There is marked presence of activity around sarcolemma also in fig 1(c).

ATP-ase

Comparing the microphotograph of 100x of the infant, young and adult {fig 2. (a),(b)and(c)} shows a scattered patchy distribution of ATP-ase. Interestingly a field of the section of Fig 2(b) cardiac tissue has been caught up in the view demonstrating branched tubular structure having total brown appearance with scattered or patches or bands of dark zone in the tubular frame work itself. This appearance is different from irregular patchy dark spots of cardiac tissue. This tubular structure appears to be nerve, which is known to have high ATPase activity.

Discussion

5'-nucleotidase is a hydrolase enzyme responsible for cleaving phosphate ion from the nucleotide converting the nucleotide to corresponding nucleoside. The substrate is not bioenergetic molecule but a component of the genetic molecule comprising of purines or pyrimidines. In cardiac tissue like an every other tissue presence of nucleotidase is expected for the simple reason that nucleotides of senile and degraded tissue has to be taken care off for reutilization of the self purines and pyrimidines as it is well known that purines and pyrimidines of the nonself origin are not incorporated in the self genetic material.

The endocardium, myocardium and epicardium have graded responsibility of replication with least replication in myocardium. In addition the connective tissue intervening the myocardium has fibrous component which is relatively less replicating in comparison to adipocytes. The principle

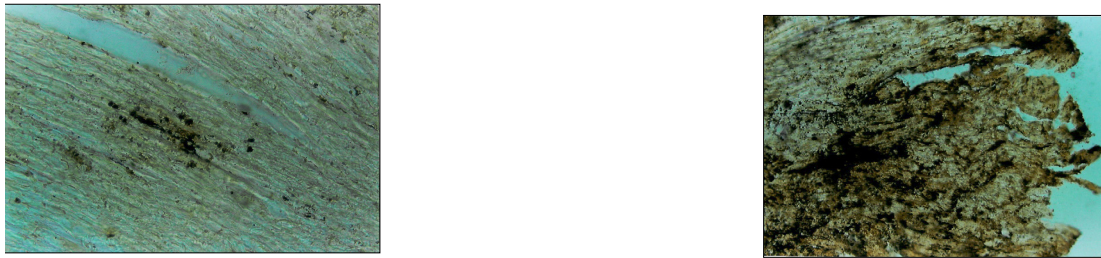


Fig 1. (a) Microphotograph shows a patchy distribution of light and heavy activity of 5'-Nucleotidase in myocardial area.(100x)(Age - Infant group)
(b) Microphotograph shows that the enzyme activity is conspicuous along the epicardium in the form of thin dark line while endocardium shows preponderance of enzyme activity.(100x)(Age - Young group)
(c) Microphotograph shows very high deposition of 5'-nucleotidase enzyme. Clumped distribution pattern of enzyme is seeing here.(100x)(Age - Adult group)

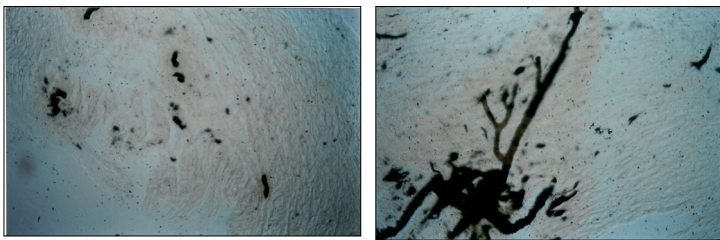


Fig 2. (a) Microphotograph shows very light minute deposition of ATP-ase enzyme in myocardial tissue. It is distributed as patchy pattern.(100x) (Age – Infant group)
(b) Microphotograph shows branched tubular appearance with scattered or patches or bands of dark deposit (Age – Young group)
(c) Microphotograph shows that high concentration of enzyme deposit and parallel dark margins of a tubular appearance (Age – Adult group)

underlying the demonstration of 5'-nucleotidase is cleavage of extraneous purines phosphate *i.e.* Adenosine monophosphate into adenosine and phosphate ion when combines with lead nitrate which forms the lead phosphates, later on it gets converted into lead sulphide and forms the black-brown deposits.

ATPase is also a phosphatase, which cleaves the phosphate group from Adenosine triphosphate (ATP) to yield Adenosine diphosphate (ADP). The phosphate group so released is precipitated as insoluble salt as lead phosphate, which is converted to brownish black pigmented deposit. Although Adenosine triphosphate is present in biological tissue as carrier of high energy phosphate group and is

dynamically kept in equilibrium by varying speed of Adenosine triphosphate synthesis from the metabolism of carbohydrate, lipids and proteins, the same when comes out from mitochondria is variable to attack by ATPase. Thus, it would be hardly available and demonstrable in significant quantities. Therefore, extraneous Adenosine triphosphate is used as a part of incubating medium.

If the basic energy yielding molecule, glucose is to be overlooked than the storage and energy production can be only assessed by the extent of availability of ubiquitous molecule, high energy containing molecule, high energy releasing molecule the Adenosine triphosphate and energy released is finally

dependent on conversion of their Adenosine triphosphate to Adenosine diphosphate and high energy carrier phosphate group. This reaction of conversion of Adenosine triphosphate to Adenosine diphosphate and phosphate group is kinetised and materialised only through enzyme ATPase.

The present study reveals an increment in the ATPase content of the cardiac tissue with advancement of age is in conformity with observations made by other workers (Talesara and Arora, 1994). This increment has been found to be significant in comparison to that of brain (Garg, Khanna and Sharma, 1992). Roubal and Tappel (1967) held this increment as a result of bodies countering mechanism to degradation of ATPase with increment in free radicals, which occurs with advancement of age. Talesara *et al.*, (1994) suggested a sequence changes or post synthetic modification of ATPase enzyme resulting into reduction in muscle contractile performance. Maciel *et al.*, (1990) observed that the level of calcium, ATPase and mRNA of sarcoplasmic reticulum were 60% lower in old rats as compared to those of young rats. Bhalla *et al.*, (1986) observed that no any changes occurs in myosin ATPase activity and myosin isoenzyme distribution in the moderately hypertrophied left ventricle of spontaneously hypertensive rats.

Our observations confirm that there is an increment of ATPase activity with advancement of age but distribution becomes highly uneven indicating in nonuniform availability of the enzyme which can possibly explain the reduction in functional capacity due possibly to either alteration in the sequential synthesis of ATPase enzyme in the biochemical structural terms or differential capacity to synthesize enzyme on morphological structural

terms. The distribution of Adenosine triphosphatase around the possible nerve in comparison to the surrounding myocardium only indicates that myocardium is more energetically dynamic in comparison to nerve fibre and utilizes the maximum Adenosine triphosphate molecules released from intermediary metabolism while the nerve manifesting high Adenosine triphosphatase activity indicates less consumption of Adenosine triphosphatase thus deposition.

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