Electronmicroscopic Study on Sodium Ion Distribution in Cardiac Ventricle Cells

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There are no detailed reports on the ion distribution in the heart muscle cells. This paper describes the histochemical method for the detection of sodium ion, the relation of autoradiography to its method, the influence of potassium ion to the sodium ion detection, and the sodium ion distribution in the heart ventricle muscle cells.

ON THE DISTRIBUTION of sodium ion in the heart muscle cells, there are some speculations from the standpoint of electrophysiology. Some studies for detection of sodium ion in the other tissue at the electronmicroscopic level have been reported since Komnick1, Zadunaisky2 reported on the location of sodium ion in the transverse tubules of the skeletal muscle.

The present report describes the histochemical method for the detection of sodium ion and the distribution of sodium in the ventricle muscle cells.

MATERIALS AND METHODS

Mice were sacrificed by cutting the head. The left heart ventricle was dissected free and minced into small pieces of about 1 mm³ in size in chilled (4°C) fixatives. These tissue were fixed for 2 hours at 4°C by one of the following three fixatives. (1) 2% aqueous solution of potassium pyroantimonate was prepared by boiling. It was then cooled to room temperature, and to it was added an equal volume of a 2% aqueous solution of osmium tetroxide, controlling pH at 7.2. (2) For controlling the osmotic pressure at 0.28 M and pH at 7.2, 0.1M potassium phosphate buffer2 was added to the above-mentioned fixative. (3) For the observation of the influence on the reaction of sodium ion and potassium pyroantimonate by potassium ion, (1)-fixative was controlled by adjusting the osmotic pressure to 0.3M and pH to 7.2 by adding potassium chloride.

The tissue blocks were dehydrated with graded ethanol and embedded routinely in Epon 812.

Ultrathin sections were stained with uranyl acetate and lead citrate. The specimens were examined with an electronmicroscope, HITACHI HS-75.

In order to know whether the precipitate of sodium pyroantimonate indicates the localization of sodium in the cells or not, electronmicroscopic autoradiography was performed by using 22Na in the following way. Two hours after intraperitoneal injection of 22NaCl at the rate of 10 μc/g body weight, fixation using (1)-fixative and embedding was performed routinely. Ultrathin sections were applied to grid meshes and over-coated with the nuclear research photosensitive photographic recording HITACHI HS-75. Following exposure for 4 weeks, the preparations were developed by MIZUHIRA-UCHIDA’s ultrafine grain development procedure3 and observed by electronmicroscope.

RESULTS

1) Distribution of sodium in ventricle muscle cells

Sodium ion reacted with potassium pyroanti-
Fig. 1. Precipitates of sodium pyroantimonate appear inside the transverse tubule (T), terminal cisternae (tc) and around the lipid droplets (L) and in a lesser amount in the myofilaments (mf). X 44,000

Fig. 2. The precipitates are not observed on the intercalated disc and the desmosome. X 44,000

Fig. 3. The precipitates in the nucleus (N) are larger than in the other regions. X 44,000

*Japanese Circulation Journal Vol. 33, June 1969*
monate producing a minute electron-dense precipitate of sodium pyroantimonate. In these ventricle muscle cells, the fine structure of the cell membrane, the mitochondria, the transverse tubule and the sarcoplasmic reticulum were kept intact. The reaction product and the structure were observed to be similar from the periphery to the center of blocks.

Small precipitates of sodium pyroantimonate were observed mainly in the intercellular spaces and inside the terminal cisternae and the nucleus of ventricle muscle cells. Likewise, they were observed around the lipid droplets and the Z band, inside the transverse tubule and in a lesser amount in the myofilaments (Fig.1). They were also observed scatteringly in the mitochondria but not on the intercalated disc and the desmosome (Fig.2). The precipitates are larger in the nucleus and minute in the T system (Fig.3).

2) Inhibition of precipitation by potassium ion

The precipitation seemed to be inhibited by mixing the potassium phosphate buffer with the (1)-fixative (Fig.4). But there were no changes in cellular structure. As a similar phenomenon was observed by adding potassium chloride to the fixative, potassium ion was thought to inhibit the production of the precipitate of sodium pyroantimonate (Fig.5).

3) Electronmicroscopic autoradiography of $^{22}\text{Na}$

Those precipitates of sodium pyroantimonate in the cell were overlapped by the developed grain caused by $^{22}\text{Na}$ in the electronmicroscopic autoradiography (Fig.6). This seemed to indicate that potassium pyroantimonate combined with sodium ion to produce sodium pyroantimonate. Since $^{22}\text{Na}$ lost during the fixation procedure was $3 \times 10^{-6}$ of the initially administered dose, it was thought that the precipitates of sodium pyroantimonate were hardly washed out during the process of dehydration.

**Discussion**

The first study on the detection of sodium ion
in the tissue at the electronmicroscopic level was reported by Komnick\(^1\) in the avian salt gland. It has been demonstrated\(^1,4\) that the antimonitic acid deposit is produced from potassium pyroantimonate and is mistaken for the true sodium pyroantimonate deposit when the pH of the fixative becomes acidic. Although Zadunaisky\(^2\) utilized a potassium phosphate buffer for the control of pH, the authors obtained the finding that the inhibition of precipitation was seen by using a potassium phosphate buffer. Since similar findings were observed by adding potassium chloride to the fixative, potassium ion was thought to inhibit the precipitation reaction of sodium ion and potassium pyroantimonate. By adding potassium pyroantimonate to the osmium tetroxide fixative, the authors were able to obtain stable results of producing a precipitate from the sodium ion without changing the cellular structure.

Since those precipitates of sodium pyroantimonate in the cells were overlapped by the developed grain caused by \(^{22}\)Na in the electronmicroscopic autoradiography, it was shown that the precipitates of sodium pyroantimonate demonstrated the localization of sodium ion in the cells. This is similar in finding in which the localization of vitamin B\(_1\) in the cells was shown by Mizuhira et al\(^5\).

In the ventricle muscle cells, sodium ion may be present in a much higher concentration of about ten times outside the cells than inside\(^6\). The intracellular distribution is not known yet in the heart muscle. However, it was reported on the skeletal muscle\(^2\) that the presence of the precipitate inside the transverse tubules and outside the sarcolemma was observed but not inside the sarcoplasmic reticulum. Since the fluid inside the transverse system has a high sodium content as in the extracellular space, Zadunaisky is inclined to think that the concentration of sodium in regions other than the transverse tubule and the basement membrane around the sarcolemma is too low to reveal any precipitate. In the heart muscle cells the precipitates were present in the intercellular space and inside the transverse tubule, the terminal cisternae and the nucleus. There may be no direct communication between the sarcoplasmic reticulum and the transverse system in skeletal muscle cells, but there may be in

ventricle muscle cells. Since the sodium ion is present in a high concentration in the terminal cisternae which is the calcium storage with a close relation to the concentration, the terminal cisternae is thought to be the site of the excitation-contraction coupling. However, the precipitates inside the terminal cisternae may be of calcium pyroantimonate which coexist with sodium pyroantimonate. As the precipitates are larger in the nucleus and minute in the transverse system, there may be a different meaning to the existence between the two. Spicer studied this problem recently on the rat trigeminal ganglia and mouse cervical lymph nodes and interpreted that the nuclear deposit can be the precipitate of pyroantimonate anion with Na cation salt linked to the nucleic acid. Sodium pyroantimonate was observed markedly in the intercellular space and outside the sarcolemma but not on the intercalated disc and the desmosome. As the intercalated discs demonstrate a low sodium concentration and as there is no difference in the sodium concentration between the two adjoined cells, this may be one of the certification that the ventricle muscle cells act as a functional syncytium.

**Summary**

1. For the detection of sodium ion, the mixture of osmium tetroxide and potassium pyroantimonate may be excellent for controlling the pH.
2. Potassium ion inhibits the production of the precipitate from the reaction of sodium ion and potassium pyroantimonate.
3. It is shown by the electronmicroscopic autoradiography using 22Na that the precipitate of sodium pyroantimonate demonstrates the localization of sodium ion in the cells.
4. In the ventricle muscle cells the distribution of sodium ion was observed markedly in the intercellular space and the nucleus and inside the terminal cisternae.
5. The excitation-contraction coupling and the functional syncytium were discussed from the point of distribution of sodium ion in the ventricle muscle cells.

**References**