Measurement of Na^+ Activity in Endolymph in Guinea Pig Cochlea with a Sodium Sensitive Glass Microelectrode

Takumi HAYASHI, Kotaro TAKE, Yozo ORITA and Moto MATSUMURA*

*Department of Otorhinolaryngology, *Department of Physiology, Kawasaki Medical School, Kurashiki 701-01, Japan*

Accepted for publication on February 9, 1994

ABSTRACT. Measurement of the Na^+ concentration of endolymph in guinea pig cochlea was investigated with a Na^+ sensitive double-barrelled ion electrode. The Na^+ sensitive exchanger, Fluka 71732, was poured into the tip of one of the double electrodes. The other electrode was filled with 0.5 M KCl solution. Measurement of the Na^+ diffusion potentials in the solutions containing different concentrations of Na^+ indicated that the potential was directly proportional to the logarithm of the Na^+ concentration with a slope of 37 to 45 mV per 10 times change in the Na^+ concentration at 22-24°C. Electrodes having smaller potential change than 35 mV were abandoned.

When a hypoxic condition was loaded on a guinea pig by stopping the respirator, EP declined rapidly and the Na^+ concentration increased by about 13 mV, which corresponded to an increase of 1.65 mM in the Na^+ concentration. The results indicate that our Na^+ electrodes were constructed well enough to measure the Na^+ activity in endolymph. The endolymphatic Na^+ concentration is considered to increase as a result of inhibition of Na^+-K^+ATPase activity, which is located in the basolateral membrane of the marginal cells of the stria vascularis.

Key words: Na^+ sensitive electrode — endolymph — endocochlear DC potential — hypoxia

In the composition of endolymph, the concentration of Na^+ is maintained at a low level, whereas that of K^+ is at high level.[1] The ionic environment is comparable to the composition of intracellular fluid. Blockade of Na^+-K^+ active transport by ouabain produces a decline in the endocochlear DC potential (EP) accompanied by an increase in $[Na^+]$.[2] Other physiological as well as histochemical investigations have revealed that the differences in the ion concentrations between endolymph and interstitial space are produced by Na^+-K^+ active transport on the basolateral side and non-selective cation channels on the apical side in marginal cells of stria vascularis.[3,4] Therefore measurement of $[Na^+]$ seems to be important for investigation of the nature of the DC potential. In the present paper, the principles of and preparation methods for our Na^+ sensitive glass microelectrode will be first described. Then a small change in $[Na^+]$ associated with hypoxic loading well be discussed.

林 琢巳，武浩太郎，折田洋造，松村幹郎

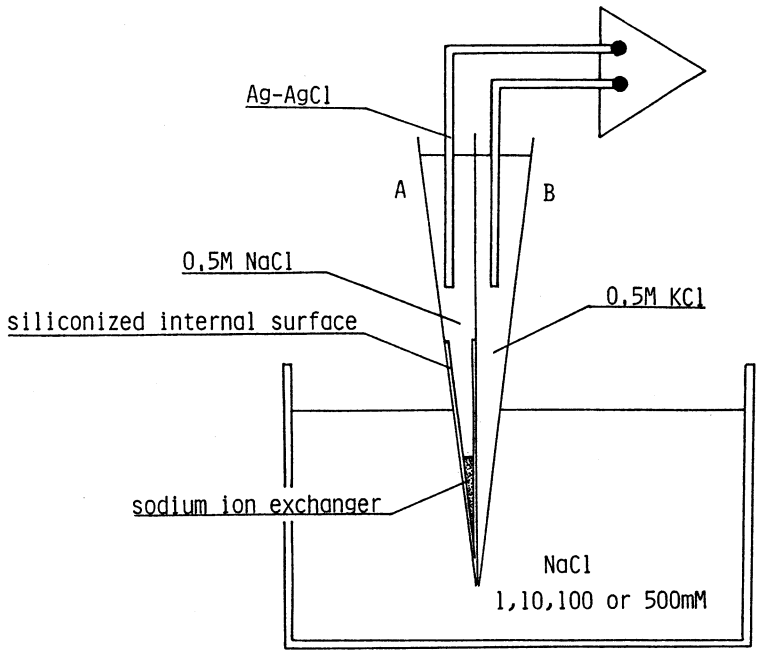

Fig. 1. Schematic diagram of the Na^+ sensitive double-barrelled glass microelectrode

A : Electrode to measure total potential of Na^+ diffusion potential and EP
B : Electrode to measure EP only A-B : Na^+ diffusion potential

PRINCIPLES

Na^+-sensitive ion exchanger is injected into the glass microelectrode. The scheme of the tip of the electrode is illustrated in Fig. 1. When the glass electrode is submerged in two solutions with different Na^+ activities, αA and αB, respectively, the potential difference, E_A-E_B, will be generated through the Na^+ ion exchanger. The activity of Na^+ is expressed as follows;

$$E_A - E_B = -\frac{RT}{F} \ln \frac{\alpha B}{\alpha A}$$

R, T and F represent the gas constant, absolute temperature and Faradays constant, respectively.

At body temparature, this is rewritten as follows;

$$E_A - E_B = -61 \log \frac{\alpha B}{\alpha A}.$$

At a room temperature of 20°C, the constant is -58 instead of -61.

Other electrode is filled with 0.5 M KCl and is used for the measurement of EP, thus, it is the EP electrode. Solutions for electrode do not contain Na^+. If they did, a leak of Na^+ from the tip of the EP electrode would disturb the measurement and result in an overestimation of the Na^+ concentration.

Generally, the exchanger should be selected so as to match physiological conditions. It bears high sensitivity to Na^+, essentially in the range between 1 and 10 mM; that is the cytoplasmic [Na^+] content of most cells. The selectivity for Na^+ is also of importance. Endolymph contains many cations, such as K^+, Mg^{2+} and Ca^{2+}, in addition to Na^+, anions, such as Cl^-, HCO_3^- and HPO_4^-, many amino acids, polypeptides and glucose, all of which can interfere with Na^+. The pH is also significantly influences the sensitivity of the exchanger. Therefore, the exchanger should be independent of these substance. Next, the electrode filled with exchanger should have high resistance. Usually, an electrode resistance is 10^{10} Ω or more.[5] So, an amplifier with high input resistance is required. Coincidentally, intensification of S/N ratio is necessary in order to avoid the electrical noise which is exaggerated with an increase in the electrode resistance.

The data detected with the ion electrode is not the molar concentration but the activity of the ion. The relationship between the activity of the ion, represented as d, and the molar concentration, represented as m, is expressed as follows;

$$d = r \cdot \mathrm{m}.$$

In a fluid with a low concentration, r is nearly equal to 1.

CONSTRUCTION OF A Na^+ SELECTIVE ELECTRODES

Among exc' ıgers for a Na^+ selective electrode, Fluka ETH 227 has been commonly em ɔyed.[4,6,7] In this study, Fluka 71732 was used as a Na^+ exchanger. The Na^+ sensitive electrodes were double-barrelled microelectrodes made from thickwalled filamented glass tube an outside diameter filamented of 1.0 mm (Narishige, Tokyo). The electrodes were soaked in 1 M HCl solution overnight to clean the inner surface. On the next day, the two tubes were put together, heated and pulled off, the tubes were twisted to each other with the rotation angle of 270°. One electrode was provided for measurement of Na^+ diffusion potential (Na^+ electrode) and the other for measurement of EP (EP electrode). Next, acetone was injected into the tip of the EP electrode to prevent siliconization, and the double-barrelled electrode was submerged in 20 ml trichloroethylene containing a drip of silicone (Shinetsu, Tokyo). Thus, only the tip of the exchanger electrode was siliconized successfully without siliconization of the tip of the EP electrode. Then, the electrode was dried at 200°C for 30 min on a hot plate to release the acetone. Subsequentry, the Na^+ selective exchanger, Fluka 71732, was injected into the Na^+ electrode, and was diffused to the tip by the capillary action existing in the internal cavity of the glass electrode. Then 0.5 M NaCl was injected into Na^+ electrode above Na^+ exchanger and 0.5 M KCl was injected into the EP electrode. Finally, they were stored in a refrigerator until used for measurement. With the double-barrelled electrode prepared as described, Na^+ activity was measured, *in vitro*, in Na^+ fluid of diverse concentration in which only NaCl without any other ions was involved. One finding regarding the relation between Na^+ concentrations and the Na^+ diffusion potential at respective concentrations is shown in Fig. 2. The change in the potential per 10 times change in the Na^+

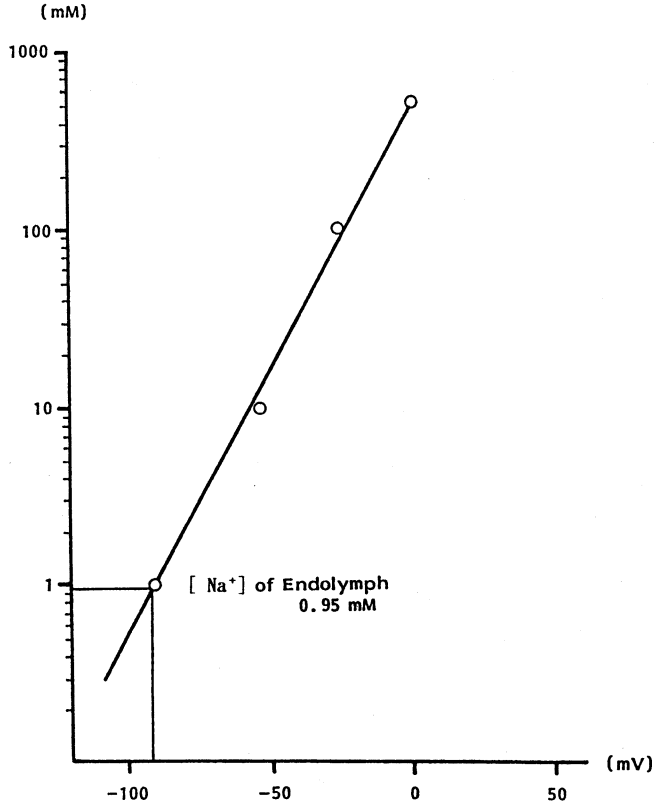

Fig. 2. Changes in Na^+ diffusion potential at diverse concentrations of Na^+

Na^+ diffusion potential was measured in solutions of 1, 10, 100 and 500 mM Na^+. The potential was first measured in the order of solution of 1 mM, 10, 100, 500 mM Na^+ solution. Then it was measured in the reverse order. Finally it was measured again in the order of the first trial. The average of three measurements in each Na^+ solution is indicated by circles. The straight line was drawn by hand. The Na^+ concentration in the endolymph of the guinea pig obtained with this electrode was estimated to be 0.95 mM from the value of the Na diffusion potential.

concentration was 42 mV, which was considerably smaller than the theoretical value of 58 mV. Some conceivable explanations are;

1. Essentially, the permeability of the Na^+ exchanger is not infinitely high.
2. The method of injection of the Na^+ exchanger is problematic.
3. There may have been a potential leak between the two electrodes across the glass wall.

More trials with many electrodes are necessary to enhance the sensitivity of the electrode.

Since the value which was measured from the Na^+ elecrtode was the sum of the sodium diffusion potential and EP in the experiment *in vivo*, the true potential for Na^+ activity was obtained by subtracting the value of EP from the measured value of Na^+ electrode.

RESULTS AND DISCUSSION

When the double-barrelled ion electrode was inserted into the scala media, a potential of +67 mV was obtained by the EP-electrode. When a hypoxic condition was loaded by stopping the respirator, EP declined immediately to −30 mV. The Na^+ diffusion potential was −91 mV at first, which corresponded to Na^+ activity of 0.95 mM, and it increased to −78 mV during hypoxic loading. The increase in the potential by 13 mV indicated a gain in lymphatic $[Na^+]$ which resulted in the rise from 0.95 to 2.60 mM, calculated from the calibration line in Fig. 2. Both changes were completely restored after recovery from hypoxia (Fig. 3). The rise in $[Na^+]$ started before a decay in

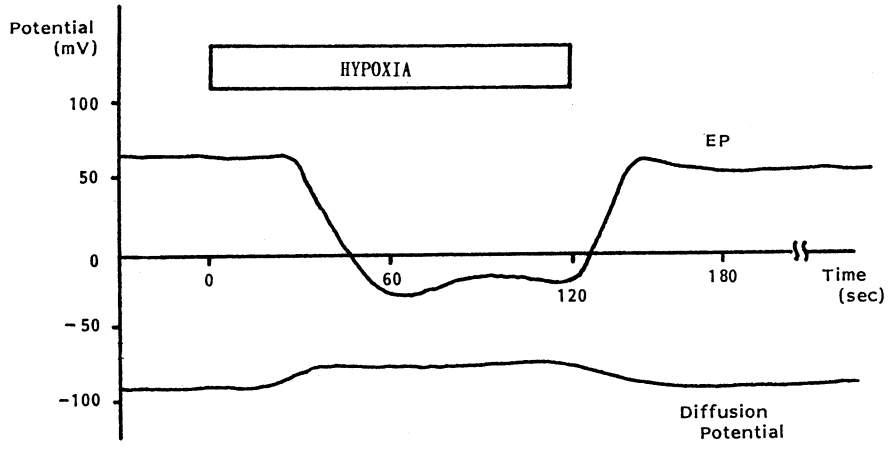

Fig. 3. Simultaneous recordings of the changes in EP and Na^+ diffusion potential during hypoxia

EP, but it was within 2 mM and reached a steady level although the EP decreased further. The change in $[Na^+]$ was very small, differing from the previous results of other authors.[8,9] The reason for this discrepancy has not yet been determined. In summary, it is suggested that endolymphatic $[Na^+]$ increased as a result of the increase in intracellular $[Na^+]$ of the marginal cells of the stria vascularis because Na^+-K^+pumps located in the basolateral membrane of those cells were inhibited due to hypoxia. The influence of ouabain or perilymphatic perfusion with Na^+ free solution on the endolymphatic $[Na^+]$ required further research.

ACKNOWLEDGMENT

This study was supported in part by a Reseach Project Grant (No. 3-606) from Kawasaki Medical School.

REFERENCES

1) Johnstone CG, Schmidt RS, Johnstone BM: Sodium and potassium in vertebrate cochlear endolymph as determined by flame microspectrophotometry. Comp Biochem Physiol **9**: 335-341, 1963

2) Marcus DC, Marcus NY, Thalmann R : Changes in cation of stria vascularis with ouabain and potassium-free perfusion. Hear Res **4** : 149-160, 1981
3) Offner FF, Dallos P, Cheatham MA : Positive endocochlear potential : Mechanism of production by marginal cells of stria vascularis. Hear Res **29** : 117-124, 1987
4) Ikeda K, Morizono T : Electrochemical profiles for monovalent ions in the stria vascularis : Cellular model of ion transport mechanisms. Hear Res **39** : 279-286, 1989
5) Fozzard HA, Chapman RA, Friedlander IR : Measurement of intracellular calcium ion activity with neutral exchanger ion sensitive microelectrodes. Cell Calcium **6** : 57-68, 1985
6) Boyett MR, Hart G, Levi AJ : Dissociation between force and intracellular sodium activity with strophanthidin in solated sheep Purkinje fibers. J Physiol **381** : 311-331 1986
7) Dagostino M, Lee CO : Neutral carrier Na^+ and Ca^{2+}-selective microelectrodes for intracellular application. Biophys J **40** : 199-207, 1982
8) Komune S, Wakizono S, Nakagawa T, Kimitsuki T, Hisashi K, Uemura T : Ototoxic effect of potassium canrenoate on the guinea pig cochlea. Acta Otolaryngol (Stockh) **111** : 719-727, 1991
9) Komune S, Nakagawa T, Hisashi K, Kimitsuki T, Uemura T : Movement of monovalent ions across the membrane of marginal cells of the stria vascularis in the guinea pig cochlea. ORL **55** : 61-67, 1993