POSSIBLE FUNCTIONING OF ACTIVE ION TRANSPORT MECHANISM IN THE MUCOUS EPITHELIAL CELLS OF NEWT STOMACH AT LOW TEMPERATURE

Yoshinobu Kanno, Yojiro Muneoka, and Toshiaki Yamami*

Department of Physiology and *Department of Crown and Bridge Prosthodontics, Hiroshima University School of Dentistry, Hiroshima, 734 Japan

Abstract The membrane potential of mucous epithelial cells of isolated newt stomach was —23 mV on the average at 7°C. The potential decreased when the temperature was lowered further, the normal external solution was replaced with K+-free solution, or ouabain or DNP was applied. The decreased membrane potential level of the cells in K+-free solution was not changed any more by adding ouabain to the solution. The membrane potential increased transiently beyond the control level when the stomach was returned to the normal solution after being exposed to isotonic NaCl solution with EGTA. The transient potential increase was blocked by ouabain. These results suggest that, even at such a low temperature as 7°C, the active ion transport mechanism(s) of mucous epithelial cells in the newt stomach is still capable of functioning to some extent.

It has been well demonstrated that H+ and Cl− are actively transported in the isolated amphibian stomach epithelium (Hogben, 1955; Heinz and Durbin, 1959; Forte et al., 1963). In addition to these ions, Na+ is also actively transported in the epithelium (Flemström, 1971; Flemström and Öbrink, 1972). The active Na+ transport in mammalian stomach epithelium has been also reported by several authors (Wright, 1962; Cummins and Vaughan, 1965; Kitahara, 1967; Kendall and Wright, 1967).

It is known that the mechanism of active Na+ transport is sensitive to temperature lowering: it is, in general, inhibited at low temperatures (Carpenter and Alving, 1968; Carpenter, 1970; Gorman and Marmor, 1970a, b). This may be also the case with the mechanism in the stomach epithelium. However, as the body temperature of amphibia varies with the environmental temperature, it
can be supposed that the active Na\(^+\) transport mechanism in the amphibian stomach epithelium might be rather resistant to temperature lowering as compared with that in the mammalian stomach epithelium. In fact, Tanaka and Teruya (1973) investigated the effect of temperature on the activity of (Na\(^+\), K\(^+\))-ATPase which is essentially involved in active Na\(^+\) transport mechanism and found that the (Na\(^+\), K\(^+\))-ATPase of bullfrog kidney is still active at 10°C, whereas the enzyme of bovine brain shows little activity at this temperature.

In the present study, the transmembrane potentials of stomach mucous epithelial cells of the newt, an amphibian, were measured and the effects of several factors which are known to influence active Na\(^+\) transport, such as metabolic inhibitors or K\(^+\)-free medium, were investigated under low temperature conditions. The results obtained indicate that the membrane potential is affected by the above factors even at a low temperature.

METHODS

Japanese newts \textit{(Triturus pyrrhogaster)} kept at a room temperature of about 20°C for more than 30 days were used throughout the study. The stomach was isolated, cut to expose its mucosal surface which was mounted, surface side up, in a small lucite chamber filled with physiological salt solution. After mounting the stomach, the experimental chamber was drained and the mucosal surface was washed several times by flushing with physiological salt solution and then the chamber was again drained and filled with the solution.

Membrane potential was measured in the conventional way using glass capillary microelectrodes filled with 3 M KCl (10–30 MΩ resistance). A microelectrode was inserted into a mucous epithelial cell under a binocular microscope and the membrane potential was fed into a Nihon Kohden Co. VC-7 oscilloscope through a Nihon Kohden Co. MZ-3B negative capacitance amplifier. In order to measure the effective membrane resistance and the electrical coupling ratio, two microelectrodes were inserted into a cell: one electrode (Es) served to pass a rectangular pulse of current \(10^{-7}\) Amp for 200 msec, inward) delivered by a constant-current device, and the other (Er), to record the resulting voltage change. The voltage change from the electrode Er was fed into the oscilloscope. After the voltage change \(V_i\) was recorded from the cell in which the electrodes Es and Er were inserted, the electrode Er was pulled out and inserted into an adjoining cell to record the voltage change \(V_{11}\) in it. Thus, the membrane potential changes in two contiguous cells during inward current flow were measured (Fig. 1). Effective membrane resistance was calculated by dividing the membrane voltage change \(V_i\) with the current flowed. The ratio of electrical coupling due to permeable junctional membrane between two contiguous cells was calculated by dividing \(V_i\) with \(V_{11}\) according to the methods of Loewenstein \textit{et al.} (1965).

The physiological salt solution had the following composition: 111.2 mM NaCl, 1.3 mM KCl and 1.4 mM CaCl\(_2\). Solutions with higher K\(^+\) concentrations

were obtained by replacing an equivalent amount of NaCl with KCl; solutions with lower K+ concentrations were obtained by replacing KCl with NaCl. In some experiments, isotonic NaCl solution containing 2 mM EGTA was used. All of the solutions were adjusted to pH 7.2 with 10 mM Tris-buffer.

The temperature of bathing medium was changed by using a Komatsu-Seisakusho Co. CTE-120 thermoregulator as shown in Fig. 1, and it was monitored by a thermistor placed near the recording site. Throughout the study the room temperature was maintained at about 20°C. Each experiment, except the experiment shown in Fig. 7, was repeated at least three times, and it was confirmed that all gave qualitatively the same results.

RESULTS

Effects of changes in the temperature of bathing medium

An average value of the membrane potential of mucous epithelial cells was $-34$ mV (inside negative) when the temperature of bathing medium was 20°C. When the temperature was changed from 20°C to 7°C, the membrane potential decreased to a steady level in 10–30 min. An average value of the potential at about 30 min after changing the temperature was $-23$ mV. The decreased membrane potential level was maintained almost constant at least for 3 hr when the temperature was maintained at 7°C. Further, the effective membrane resistance and the coupling ratio remained almost constant during the temperature change; average values at about 30 min after changing the temperature from 20°C to 7°C were 340 kΩ and 0.64, respectively (Fig. 2). For these reasons, the subsequent experiments were carried out after immersing the stomach in physiological salt
solution of 7°C for 30–60 min and confirming that the membrane potential had reached a steady level.

The membrane potential of mucous epithelial cells decreased further when the temperature of bathing medium was lowered from 7°C to 3°C (Fig. 3). In Fig. 3, the membrane potential at 7°C was −27 mV on the average and it decreased to −23 mV with a decrease in temperature to 3°C. The decrease in the membrane potential was reversible.

*Effect of ouabain*

Ouabain decreased the membrane potential of mucous epithelial cells. Figure

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**Fig. 2.** Time courses of the changes in membrane potential (M.P.), effective membrane resistance (M.R.) and electrical coupling ratio (C.R.) after changing the temperature of bathing medium from 20°C to 7°C. Each point shows the mean with the standard deviation (n=7, from one animal).

**Fig. 3.** Effect of lowering the temperature (◯) of bathing medium from 7°C to 3°C on membrane potential (●). Each membrane potential point shows the mean with the standard deviation (n=6–15, from one animal).
4 shows the effect of $10^{-5} \text{M}$ ouabain on the membrane potential. The average of the membrane potential in normal solution was $-22 \text{ mV}$ and it decreased to $-13 \text{ mV}$ about 30 min after applying ouabain. The decreased membrane potential did not completely recover after washing out the ouabain: it was $-17 \text{ mV}$ on the average about 30 min after washing out the drug.

**Effect of DNP (2,4-dinitrophenol)**

DNP also decreased the membrane potential. Figure 5 shows an effect of $10^{-4} \text{ M}$ DNP. The membrane potential in normal solution was $-20 \text{ mV}$ on the average. The potential decreased to $-15 \text{ mV}$ about 20 min after applying DNP and $-13 \text{ mV}$ about 40 min after it. The membrane potential completely recovered after washing out DNP.

**Effects of changes in external $K^+$ concentration**

Removal of external $K^+$ resulted in a decrease in membrane potential. Figure 6 shows an effect of the removal of external $K^+$. The membrane potential in normal solution was $-21 \text{ mV}$ on the average. After immersing the stomach in $K^+$-free solution, the membrane potential decreased gradually and reached an almost steady level in 30 min at which the potential was about $-15 \text{ mV}$. The membrane potential completely recovered 30 min after returning the preparation in normal solution.

Figure 7 shows a relationship between the membrane potential and external $K^+$ concentration, and the effect of $10^{-5} \text{ M}$ ouabain on the relationship. The figure indicates that the membrane potential decreased in the solutions of both higher $K^+$ concentrations and lower $K^+$ concentrations than normal one and that

![Fig. 4. Effect of $10^{-5} \text{ M}$ ouabain on membrane potential. Each point shows the mean with the standard deviation ($n=9-13$, from one animal).](image1)

![Fig. 5. Effect of $10^{-4} \text{ M}$ DNP on membrane potential. Each point shows the mean with the standard deviation ($n=6-18$, from one animal).](image2)
Fig. 6. Effect of the removal of external K⁺ on membrane potential. Each point shows the mean with the standard deviation (n=4–14, from one animal).

Fig. 7. Relationships between membrane potential and external K⁺ concentration in the presence (○) and in the absence (●) of 10⁻⁵ M ouabain. The external K⁺ concentration is represented relatively to the normal concentration (1.3 mM). On each K⁺ concentration, membrane potentials were measured first in the absence of ouabain, then the preparation was exposed to ouabain for 30 min and the membrane potentials in it were measured. Each point shows the mean with the standard deviation (n=16–32, from 3 animals). Each paired points were obtained from each of 3 animals.

the membrane potential in K⁺-free solution was not changed any more by applying ouabain.

Effect of exposure to isotonic NaCl solution containing EGTA

It is well known that an increased intracellular Na⁺ stimulates a Na⁺ pump (Kerkut and Thomas, 1965; Thomas, 1969). Yamaguchi (1975) observed that
the cell membrane of the anterior byssal retractor muscle of *Mytilus* is markedly hyperpolarized when the muscle is returned to the normal solution after being exposed to isotonic NaCl solution with EGTA. He attributed the hyperpolarization to an increase in intracellular Na+ and the consequent activation of an electrogenic Na+ pump. A similar method for increasing intracellular Na+ concentration was used in the present experiment. The resting membrane potentials of mucous epithelial cells in normal solution were measured, the preparation was equilibrated in K+-free solution for 20 min, then it was exposed to isotonic NaCl solution containing 2 mM EGTA for 10 min and then the preparation was restored to the K+-free solution for another 10 min. After this period, normal solution was readmitted. One of the experimental results is shown in Fig. 8. The membrane potential in normal solution before the treatments was -20 mV on the average, and it decreased to -10 mV during the treatments. Following the readmission of normal solution, the membrane potential increased transiently beyond the control level and then returned to it with time. The transient increase in membrane potential, as well as the hyperpolarization, was blocked by 10^-5 M ouabain (Fig. 9).

When the preparation was treated only with K+-free solution, the transient increase in membrane potential following readmission of normal solution was not observed as shown in Fig. 6 or was small, depending on the time of the treating period. When the time of the treating period was 60 min or less, the transient increase was not observed, and when the time was 120 min or more, a small transient increase was observed.

![Fig. 8. Transient hyperpolarization of the membrane after exposing mucous epithelial cells to isotonic NaCl solution with 2 mM EGTA (0 K+, 0 Ca²⁺ solution). The preparation was exposed to K⁺-free solution (0 K⁺ solution) for 20 min and then to 0 K⁺, 0 Ca²⁺ solution for 10 min. After this period, the preparation was restored to 0 K⁺ solution for another 10 min and then normal solution was readmitted. Each point shows the mean with the standard deviation (n=4-10, from one animal).](image-url)
Fig. 9. Block of the transient hyperpolarization of membrane by 10^-5 M ouabain. The preparation was exposed to K+-free solution (0 K+ solution) for 20 min and then to isotonic NaCl solution containing 2 mM EGTA (0 K+, 0 Ca^2+ solution) for 10 min. After this period, the preparation was restored to 0 K+ solution for another 20 min and then normal solution was readmitted. The ouabain was introduced 10 min before the readmission of normal solution. Each point shows the mean with the standard deviation (n=4-13, from one animal).

DISCUSSION

The resting membrane potential of squid giant axon is essentially independent of temperature from 3°C to 20°C (HODGKIN and KATZ, 1949). In addition, exposure of the axon to DNP (HODGKIN and KEYNES, 1955) or ouabain (CALDWELL and KEYNES, 1959) has little effect on the potential. In contrast, the resting membrane potential of the lobster axon becomes 5-8 mV more negative when the temperature of external solution is increased 10°C. This potential change exceeds that predicted by the temperature coefficient of the Nernst equation, and it is reduced by adding DNP or ouabain to the solution (SENFT, 1967). The membrane potential of Aplysia neurone is more sensitive to temperature change and to ouabain. It increases with increasing temperature by as much as 2 mV/°C (CARPENTER, 1967), and decreases in the presence of 10^-4 M ouabain by about 25 mV at 25°C (CARPENTER, 1970). This temperature dependence of membrane potential in the Aplysia neurone has been attributed to the electrogenic Na^+ pump (CARPENTER and ALVING, 1968). The membrane potential of giant neurone of Anisodoris, a marine mollusc, is also very sensitive to temperature change and to ouabain, and it is considered to be contributed by an electrogenic Na^+ pump (GORMAN and MARMOR, 1970a). The membrane potential of the neurone is little affected by ouabain once external K^+ is removed (GORMAN and MARMOR, 1970b).

In the present experiments, it was showed that the membrane potential of mucous epithelial cells in the newt stomach is, like the membrane potential of the
cells in which an electrogenic Na+ pump seems to operate, sensitive to temperature lowering, metabolic inhibitors and removal of K+ from the external solution even at 7°C. The membrane potential decreases without significant changes in effective membrane resistance and electrical coupling ratio when the temperature of bathing medium is lowered from 20°C to 7°C. Furthermore, the potential, which has reached a steady level at 7°C, decreases further when the temperature is lowered to 3°C. The membrane potential, which has reached a steady level at 7°C, is decreased by applying ouabain or DNP to the epithelial cells and by immersing the cells in K+-free solution. The decreased membrane potential in K+-free solution is not changed any more by applying ouabain. From these facts, it is conceivable that an active Na+ transport is involved in maintaining the membrane potential even at as low a temperature as 7°C. The active Na+ transport mechanism might have an electrogenic function. However, even if this is the case, the whole of potential decrease by a factor which inhibits the active Na+ transport mechanism may not be attributed directly to the inhibition of the electrogenic function: not only the inhibition of the electrogenic function but also the subsequent changes in transmembrane ion concentration gradients and/or ion permeabilities of cell membrane may be involved in the potential decrease.

When the stomach is returned to normal solution after being exposed to isotonic NaCl solution with EGTA, the membrane potential of the epithelial cells increases transiently beyond the control level. The transient increase in the membrane potential is not found in the solution containing ouabain. During the treatment with the NaCl-EGTA solution, the external Na+ would enter into the cell through the cell membrane, resulting in an increase in intracellular Na+ concentration and the increased Na+ would stimulate the active Na+ transport mechanism after returning the preparation in normal solution. Therefore, the transient increase in the membrane potential may be brought about by the action of stimulated electrogenic Na+ pump and the block of transient potential increase by ouabain may be related to the inhibition of electrogenic Na+ pump.

As discussed above, it is possible to consider that an active Na+ transport mechanism contributes to the maintenance of the membrane potential of mucous epithelial cells in the newt stomach even under low temperature conditions and that the decreases in the membrane potential observed in the present study were brought about by the inhibition of the Na+ transport. However, another possibility can be also considered. Since H+ and Cl− are also actively transported in the stomach epithelium (Hogben, 1955; Heinz and Durbin, 1959; Forte et al., 1963), the potential decreases might be brought about by the inhibition of H+ transport or Cl− transport or both. Though ouabain is generally recognized as an inhibitor of Na+ transport, it also inhibits the active transport of Cl− in frog stomach epithelium (Cooperstein, 1959). Durbin and Kasbekar (1965) reported that addition of DNP to the external solution inhibited the activity of H+ transport in the frog stomach. Davis et al. (1965) reported that the removal of K+ from the medium
bathing the frog stomach epithelium resulted in a decrease of the H⁺ secretory rate to zero. Recently, it was demonstrated that H⁺ is transported in exchange for K⁺ by (H⁺, K⁺)-ATPase (SACHS et al., 1976; SCHACKMANN et al., 1977). These facts support the above alternative possibility.

In the present study, however, the membrane potentials were not recorded from oxyntic cells, which are considered to be H⁺ secretory ones, but recorded from mucous epithelial cells. The mucosal surface of newt stomach consists of mucous epithelial cells and gastric pits. The mucous epithelial cells have numerous mucous granules and are considered mucous secreting cells (KANNO et al., 1971). So that it seems unlikely that an inhibition of H⁺ transport is involved in the membrane potential decreases observed in the present study. SCHACKMANN et al. (1977) and SACHS et al. (1976) suggested that the ATP-dependent exchange of H⁺ for K⁺ in mammalian stomach epithelium is electroneutral one. The transport of H⁺ in the bullfrog tadpole stomach is not manifest until late in the metamorphic development, concomitant with the development of the characteristic oxyntic cell, while the transport of Cl⁻ and Na⁺ are observed even at the early metamorphic stages which contain only one epithelial cell type, the columnar epithelial cell (Forte et al., 1969). The columnar epithelial cell is a mucous secreting cell and has morphological similarities to the surface mucous epithelial cell of stomach epithelium in the adult frog. Forte et al. (1969) also reported that, for the early tadpole, the transmucosal potential difference was dependent upon the presence of Na⁺ in the nutrient bathing solution and that the potential difference was sensitive to ouabain or to the complete omission of K⁺ from the nutrient solution. From the facts mentioned above, the potential decreases observed in the mucous epithelial cells of newt stomach in the present study seem to be brought about by the inhibition of Na⁺ transport rather than the inhibition of H⁺ transport or Cl⁻ transport or both, though the possibility that the inhibition of Cl⁻ transport is involved in the potential decreases still remains because the mucous epithelial cell of newt stomach might have a mechanism for Cl⁻ transport.

The present experimental results suggest that, even at such a low temperature as 7°C, the active ion transport mechanism(s) of mucous epithelial cells in the newt stomach is still capable of functioning to a certain degree. BOWLER and DUNCAN (1968) reported that the activity of rat brain microsomal (Na⁺, K⁺)-ATPase at low temperatures was considerably lower than that of the frog brain microsomal (Na⁺, K⁺)-ATPase. Similar difference in the temperature sensitivities of (Na⁺, K⁺)-ATPase preparations from homiothermic and poikilothermic vertebrates was also reported by TANAKA and TERUYA (1973) using bovine cerebral cortex and bullfrog kidney. They attributed the difference in temperature sensitivities to the difference in membrane lipid fluidities; the membrane lipids of frog are likely to maintain more fluidity at low temperatures than those of bovine. There are some reports that the fluidity of membrane lipids is intimately correlated to membrane enzyme activity (WILSON et al., 1970; LYONS and RAISON, 1970; SEELIG
and Hasselbach, 1971; Thompson and Parks, 1972). Using lamb kidney, Grisham and Barnett (1973) suggested that the membrane lipids must be fluid for the (Na\(^+\), K\(^+\))-ATPase to function.

Thus, it can be supposed that the stomach mucous epithelial cell of the newt, a poikilothermic vertebrate, can maintain its membrane lipid fluidity even at 7°C and the enzyme activities of the cell membrane are maintained to a certain degree for functioning of active ion transport mechanism at low temperatures. However, it is not possible to ascertain from the available evidence whether this is the case or not. There are reports suggesting that the active ion transport mechanism in the mammalian kidney can operate even at 0°C (Burg and Orloff, 1964; Robinson, 1965). Therefore, factors other than the changeability of membrane lipid fluidity might be also related to the operation of active ion transport mechanism in the mucous epithelial cells of newt stomach at low temperatures.

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ACTIVE TRANSPORT IN NEWT STOMACH CELLS

