Transcellular Ion Transport and Bioelectric Potential (37174)

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The temperature characteristic of the resting muscle potential (1) agrees with the classical concept of Nernst that this particular type of bioelectric potential results from simple diffusion of ions across the K-selective plasma membrane. This type of bioelectric potential has been considered to be only indirectly related to cellular metabolism. However, in some other tissue membranes the interplay between metabolic energy and the maintenance of the potential is so close (2–5) that metabolic energy sources must be an immediate source and therefore some biochemical "electrogenic" process may underlie the generation of this type of bioelectrical potential. However, there is no *a priori* reason why a combination of Nernstian and "electrogenic" potentials could not occur at the same time. As a matter of fact, Senft (6) has shown that in lobster axon at least a portion of the resting potential is different in its temperature coefficient from the Nernst-type diffusion potential. The purpose of this investigation is to understand the origin of the electrical potential of cellular systems where the transport of ions is not just across the plasma membrane but all the way across the cell. Furthermore, an attempt has been made to answer the following two questions: What is the effect of temperature on the rate of movement of the ions by an active transport process? Is the temperature coefficient of the flux of an actively transported ion different from that of a passively transported ion?

Materials and Methods. The abdominal skin of frog (*Rana pipiens*) and the urinary bladder of toad (*Bufo marinus*) were isolated from pithed animals and mounted flat on a nylon ring fitted with pins. The ring-mounted epithelial preparation was then placed in a chamber in which aerated Ringer's solution can continuously flow past both surfaces of the tissue preparation. The bathing solutions were prepared from analytical grade reagents and the Ringer's solution consisted of the following: 57.5 m*M* Na_2SO_4, 2.5 m*M* $KHCO_3$ or (KH_2PO_4 + K_2HPO_4), 1.5 m*M* $CaSO_4$ and 0.1% glucose. The slightly hypotonic sulfate Ringer's solution was the optimal artificial environment for the bladder epithelium for maintaining normal transport activity for 8 hr or more. With frog skin preparations, 55 m*M* sucrose was added to this sulfate Ringer's solution. Freshly prepared solution was oxygenated for a period of half an hour prior to its use. The pH of the solution was between 7.8 and 8.2.

The temperature of the tissue was measured with a thermister bridge, which had the miniature thermister in contact with the tissue. Quick changes in temperature of the tissue were achieved by switching the chamber inputs onto another set of reservoirs whose solutions were maintained at the desired temperature. The experiments were performed at temperatures between 5° and 25°. Temperatures above 30° irreversibly depressed the ion-transporting activity of the tissue, and the tissue deteriorated. Therefore, such higher temperatures were not used.

Total transepithelial electrical potential difference was measured by a voltage clamp technique (2). The accuracy of potential measurement was ± 0.05 mV. The net rate of active transport of sodium ions was determined from the magnitude of the short-circuit current.

Transcellular unidirectional fluxes of Na^+ and K^+ ions across the epithelial tissues were measured by using ^{22}Na and ^{42}K isotopes as the respective tracers. During the entire period of flux measurements the tissues were kept short-circuited by the volt-

age clamp apparatus. When the short-circuit current across the tissue became steady, the nonradioactive bathing solution at one side of the tissue was replaced with radioactive solution. For the conservation of isotope, this bathing solution was recirculated by an air-lift pump. In order to maintain the recirculating radioactive bathing solution at a desired temperature a miniature heat-exchanging capillary network was used in series with the input tubing and the heat exchanger was kept immersed in a water bath at the appropriate temperature. The rate of flow of the solution bathing the opposite surface of the tissue was adjusted to approximately 6 ml/min. At this flow rate the isotopic flushing half-time was about 3 sec. Drops of the washing solution were collected on a filter-paper tape moving at a constant speed. The tape was then immediately dried and wound on a reel. For easy localization of individual drops on the tape, the flushing solution was tinted with a small amount of Evans Blue dye. This dye was found to be inert to the transport parameters of the tissues. At the end of an experiment the filter paper tape was cut in strips. The strips were individually pressed into test tubes and the total radioactivity of ^{22}Na and ^{42}K counted in a well-type scintillation counter. A week later the same strips were counted again for the determination of radioactivity of ^{22}Na alone.

Results. The typical time course of transepithelial electrical potential difference following a temperature change is shown in Fig. 1. In this example, a bladder preparation was initially stabilized with cold Ringer's solution (6°) for a period of 20–30 min, which allowed both potential and short-circuit current to reach their respective steady states. The temperature of the tissue was then raised by flowing warmer solutions into the chamber. Concomitant with the increase in the tissue temperature (Graph A) there was an increase in the magnitude of the transepithelial electrical potential difference (Graph B). Within 2 min the tissue temperature as well as the potential difference reached their new steady states. The temperature coefficient of the electrical potential during this period (solid curve) was positive

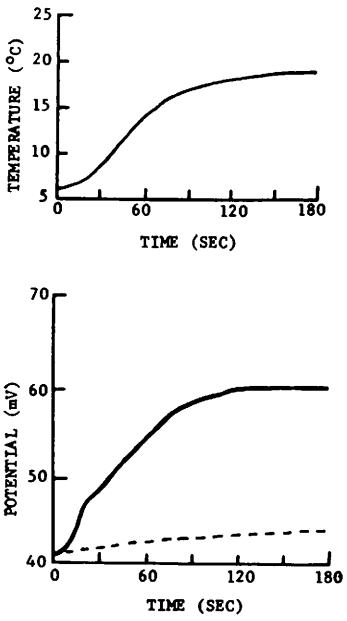

FIG. 1. Effect of temperature on electrical potential difference across a toad bladder. Abscissa: time; ordinates in A (top): tissue temperature (°) and in B (bottom): transepithelial potential difference (mV). At zero time the temperature of the bathing solutions was changed.

and much larger than that expected from the Nernst equation for membrane diffusion potential (dashed curve on Fig. B). The temperature coefficients of the transepithelial potential difference across frog skins were very similar to those of toad bladders. From a total number of sixteen experiments with toad bladder and eight experiments with frog skin, the average Q_{10} of the potential difference was found to be 1.51 with a standard error of $\pm$ 0.07. From a different group of experiments the Q_{10} of the rate of transcellular sodium ion transport was determined. Figure 2 represents the typical effects of temperature on the transepithelial fluxes of Na^+ and K^+ ions in the inward direction (*i.e.*, toward the internal bathing solution of frog skin or toward the serosal bathing solution of toad bladder). In this particular experiment, at time zero the temperature of the tissue was 6° and it reached its maximum value of 20° within 135 sec following the circulation of a warm bathing solution. The upper curve in Fig. 2 is drawn to fit (by eye) the data points on the inward flux of Na ions.

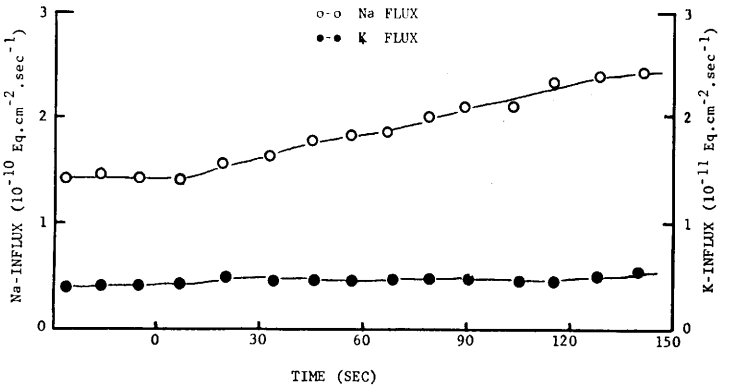

FIG. 2. Effects of temperature on inward fluxes of Na and K ions across a toad bladder preparation. Abscissa: time (sec); ordinates: influxes. Temperature of 6° at zero time rising to 20° at 135 sec and remaining stable thereafter. O—O: inward Na-flux, left-hand scale. ●—●: inward K-flux, right-hand scale.

The bottom curve represents the corresponding inward flux of K ions during the course of rise of the tissue temperature. Both the inward Na-flux and inward K-flux increased with temperature. However, the increase in the inward Na-flux was much larger than the corresponding increase in the K-flux. In a group of seven experiments, the average Q_{10} of the Na-influx was 1.46 ± 0.08, whereas that of the K-influx was 1.05 ± 0.01.

In the next group of experiments, isotopes were added to the serosal (or internal) bathing fluid and the transepithelial fluxes in the mucosal (or outward) direction monitored. Figure 3 is illustrative of six such experiments with toad bladder and two experiments with frog skin. Here both the sodium and potassium ion fluxes increased but slightly with temperature. It is interesting to note that the temperature. coefficient of sodium flux in the outward direction is much smaller than that in the inward direction.

Discussion. It is tempting to analyze the temperature coefficient of the potential in terms of the simple model of Koefoed-Johnsen and Ussing. In their model, which was proposed for frog skin only, the transepithelial potential difference was postulated to be the sum of (a) a sodium diffusion potential arising across the cell's outward facing membrane and (b) a potassium diffusion potential arising across the cell's inward facing membrane. In sulfate Ringer's solution the magnitude of the transepithelial potential difference, E_t, was proposed to be given by

$$E_t = E_{Na} + E_K = \frac{RT}{F}\left(\ln\frac{[Na]_o}{[Na]_c} + \ln\frac{[K]_c}{[K]_i}\right)$$

where the subscripts *o, i,* and *c* refer, respectively, to the external and internal bathing

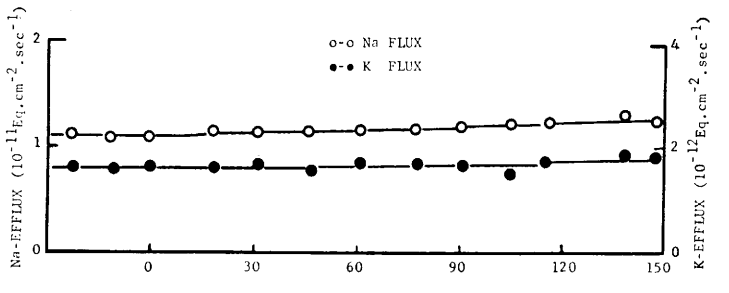

FIG. 3. Effects of temperature on outward fluxes of Na and K ions across a toad bladder preparation.

media and the intracellular medium. When studying the effect of temperature on transepithelial potential in the present experiments, $[Na]_o$ and $[K]_i$ were maintained constant. Furthermore, by using nonpenetrating sulphate ions as the major anions in the Ringer's solutions and making the potential measurement in the open-circuited condition, the rate of entry of any cation into the cell is certainly minimized. Therefore, during the short duration of time involved in raising the tissue temperature, $[Na]_c$ and $[K]_c$ cannot change to any significant extent. Thus, according to the membrane potential model of Koefoed-Johnsen and Ussing, due to a temperature increase of 10°, the transepithelial potential difference is expected to increase by 3–4% (*i.e.*, Q_{10} of 1.04). However, the observed increase in the potential difference is on the average 50% (*i.e.*, Q_{10} of 1.50). Alternatively, the Goldman–Hodgkin–Katz equation may be used so that the effects of temperature on the passive permeabilities of the cell membranes may be taken into consideration. In this regard the effect of temperature on the effluxes of Na and K ions would reflect the temperature effect on the passive permeabilities of the membranes to these two ions. As shown in Fig. 3, the magnitudes of the increase in the effluxes following an elevation of tissue temperature are quite small and therefore reflect only slight increases in the passive permeabilities. The magnitudes of the permeability increases are quite insufficient to account for the observed Q_{10} of the transepithelial electrical potential difference.

The large temperature coefficient of transepithelial potential observed in the transient phase persists even in the long-range steady state conditions. This latter finding is in agreement with data obtained by several other investigators (7, 8) from their long-term steady state measurements.

At this juncture the large Q_{10} of transepithelial potential may be examined from a different perspective. If this potential is produced by a biochemical mechanism, then it is conceivable that a chemically linked potential will have a large temperature coefficient. Since the Q_{10} of many biochemical reactions (9) is close to 2.0, it is likely that an electrogenic potential can have a Q_{10} of 1.51, as observed in the present experiments. Even though no biochemical analyses were undertaken in this investigation, the findings of other investigators along this line lend support to such contention. Following an early work of Dalton and Hendrix (10), Senft (6) carried out a rather extensive investigation on the effects of temperature on the resting potential in lobster axon. He observed that the Q_{10} of the resting potential was between 1.07 and 1.08 (the original data points are herein converted and expressed in terms of Q_{10}). When the lobster axons were perfused with solutions containing various inhibitors of ATP- synthesis, the magnitude of the resting potential decreased. Interestingly, Senft observed that the Q_{10} of potential in the inhibitor treated axons was between 1.03 and 1.04. In simultaneous biochemical analyses of inhibitor-treated axons the axonal ATP content decreased in conjunction with the decreased Q_{10} of the potential. In view of these findings, Senft concluded that the resting potential of lobster axon is composed of (a) a membrane potential and (b) a potential of some electrogenic biochemical mechanism. Similar conclusion was drawn by Gorman and Marmor (11) on the electrical potential of molluscan neurones. It appears that the contribution from an electrogenic process to the electrical potential of the present transcellularly transporting system is of a much higher degree than that in lobster axons or molluscan neurones.

The results of the experiments on the effects of temperature on ion fluxes indicate that more specifically the inward flux of Na ions is enhanced by elevated temperature. This specific directional effect of temperature lends strong support to the involvement of a carrier type mechanism in the transcellular transport of sodium ions, especially since a biochemical carrier molecule can have the type of specificity observed in the ion transporting systems. The effect of temperature on the passively diffusing K ions is very small. The Q_{10} for K-influx is 1.05 whereas that of K efflux is 1.03. On the other hand, the Q_{10} for Na-influx is 1.46 and that for Na efflux is 1.07.

In view of the above findings it is conclud-

ed that a portion of the generated transepithelial potential difference may indeed be due to a physical phenomenon of membrane diffusion. However, the major portion of this type of bioelectric potential is likely to be generated by an "electrogenic" transport mechanism. The transport mechanism is electrogenic in the sense that transport of sodium ion itself directly gives rise to the transcellular bioelectrical potential.

Summary. The transient effect of temperature on the electrical potential of *transcellularly* transporting epithelial cells of frog skin and toad bladder has been studied. The Q_{10} of the transepithelial potential difference is 1.51. This temperature effect is much larger than what can be expected if this bioelectric potential had been strictly due to passive diffusion of ions across permselective membranes. In the study of the transient effects on ion fluxes it has been observed that the effect of temperature is more pronounced on the inward flux of Na ion than on its outward flux. Na-influx increases with temperature, as does the short-circuit current. Both of these parameters increase instantly with the elevated tissue temperature. The inward and outward fluxes of K^+ are not significantly affected by temperature. Thus, it is concluded that the passively diffusing K ions are only slightly affected by temperature. On the other hand, the active transcellular transport of Na ions is very sensitive to temperature. It is proposed that a biochemical electrogenic mechanism is likely to underlie the process of transcellular transport of sodium ions and also the generation of the associated electrical potential.

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