Cooperative Critical Thermal Transition of Potassium Accumulation in Smooth Muscle

Abstract. *The steady-state levels of potassium and sodium of taenia coli of guinea pig are critically affected by varying temperature in the narrow range 12° to 17°C. For the accumulation of both cations the critical temperature, T_c, is 13.8°C in the presence of 5 millimolar external potassium. The value of T_c decreases to 10.0°C when the external potassium is raised to 10 millimolar. Since, at a fixed temperature, the potassium accumulation follows a cooperative mechanism, the results are compared with the quantitative predictions of this approach. The critical thermal transition behavior can be described in terms of the cooperative accumulation process.*

It is generally known that tissues lose potassium and gain sodium on cooling to 0° to 4°C. However, there is little information on whether the ionic contents are progressively or critically affected by temperature. Since a knowledge of the behavior of the system at different temperatures can give information about the mechanisms responsible for cation accumulation, studies are made of cellular potassium and sodium contents under steady-state conditions at various temperatures. Such recent studies have suggested that accumulation of these cations in smooth muscles follows a cooperative mechanism (*1*). For example, a plot of steady-state electrolyte contents against external potassium concentration is sigmoidal. The sigmoidal behavior has been compared with the predictions of a model in which it is assumed that potassium and sodium are adsorbed on sites distributed throughout the cytoplasm and that there is cooperative interaction between the nearest-neighbor sites (see below). The temperature studies reported here reveal further that the electrolyte distribution in smooth muscle cells undergoes abrupt readjustment around a critical temperature. This behavior is shown to be in quantitative agreement with predictions based on the cooperative mechanism.

The experiments were performed with taenia coli of guinea pig. Pieces about 2 cm in length were incubated in a slightly modified Krebs solution (*2*) for periods of 12 to 20 hours at temperatures between 1° and 36°C in constant temperature baths (± 0.1°C). These incubation periods were used to assure that the tissues were in a steady state (*3*). After incubation, the pieces were blotted on a wet filter paper and weighed. The potassium and sodium were extracted in 2-ml portions of 0.1*N* HCl. The concentrations of the extracts and of samples of the Krebs solution were determined by flame photometry.

The behavior of total steady-state tissue potassium and sodium contents reached at different temperatures in the presence of 5 m*M* potassium in the external solution is shown in Fig. 1A. The potassium content decreased by only 9 μmole/g when the temperature was changed from 36° to 17.5°C. An abrupt change in the electrolyte distribution took place, however, when the temperature was lowered from 17.5° to 12.5°C. In this narrow range of temperature, the tissue potassium content decreased from 75 to 17 μmole/g (Fig. 1A). Lowering the temperature below 12°C caused a further, although gradual, fall in the remaining potassium content. The behavior of the sodium content with temperature was inversely related to that of potassium. Decreasing the temperature from 36° to 17.5°C had little effect on the tissue sodium content. The sodium content rose abruptly in the temperature range 17.5° to 12.5°C (*4*) (Fig. 1A). Here, the critical temperature (T_c) is defined as the temperature at which the cellular cation content has fallen (or risen) to one-half its value at 36°C. On this basis, the value of T_c for the tissue potassium as well as the tissue sodium transition is found to be 13.8°C. We will next see if this transition can be predicted quantitatively on the basis of a model in which the behavior of potassium and sodium follows a cooperative mechanism.

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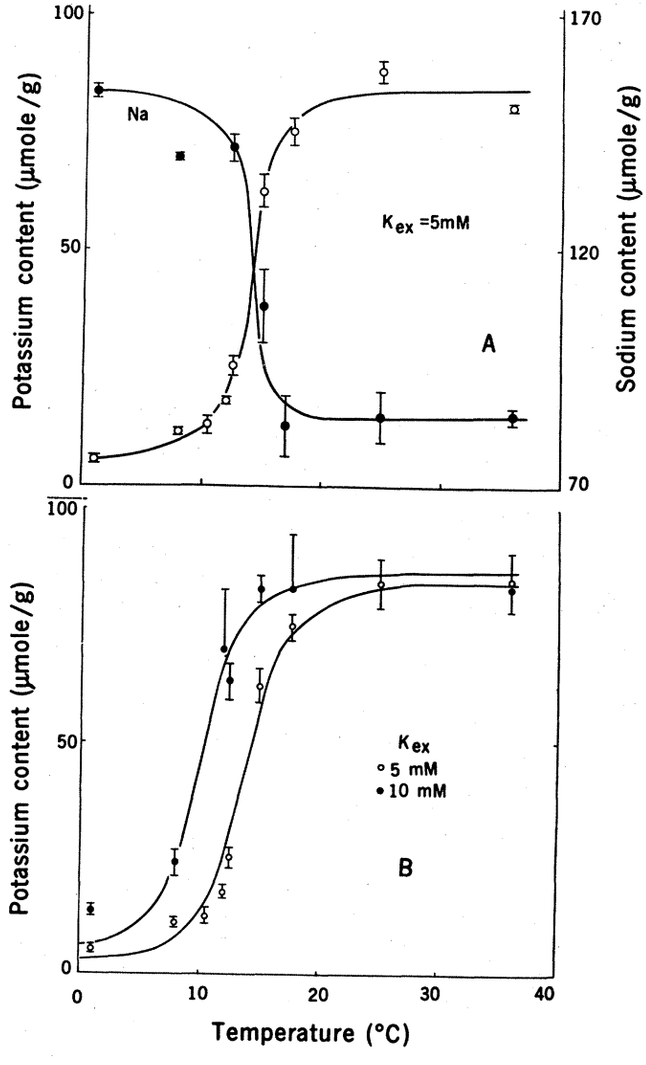

Fig. 1. (A) Effect of temperature on the steady-state potassium (open circles) and sodium (closed circles) contents of taenia coli. The high sodium content above 17°C is mainly due to the large extracellular space in smooth muscles (*17*). (B) Effect of K_{ex} on T_c for the thermal transition. The solid lines are theoretical curves derived by combining Eqs. 1 and 3, as described in the text. In the curves the potassium contents in the sorbitol space (2.15 μmole/g at 5 m*M* K_{ex} and 4.3 μmole/g at 10 m*M* K_{ex}) were added to the theoretically calculated values. The points in these figures are the means of 6 to 16 determinations in three different experiments.

derived a cooperative adsorption isotherm that explicitly relates the cellular potassium content to the external potassium concentration (*5*). The isotherm has been shown to describe adequately the equilibrium distribution of potassium and sodium in frog sartorius muscle (*6*) and in carotid artery of dog (*1*). This isotherm was derived by assuming that the cellular cations are selectively adsorbed onto negatively charged sites [for detailed discussions of the model see (*7*)]. The cooperative behavior is the result of nearest-neighbor interactions among the sites. Equation 1 shows the cooperative isotherm in which the cellular potassium is a function of the ratio K_{ex}/Na_{ex}

$$K_{ad} = \frac{F_T}{2}\left\{\frac{\xi - 1}{[(\xi-1)^2 + 4\xi n^{-2}]^{\frac{1}{2}}}\right\} \quad (1)$$

where F_T represents the amount of fixed negative sites (in micromoles per gram) which can interact cooperatively with cations; ξ is defined as $K^{00}{}_{Na\to K}$ (K_{ex}/Na_{ex}), where $K^{00}{}_{Na\to K}$ is the intrinsic equilibrium constant (selectivity ratio of potassium over sodium); K_{ex} and Na_{ex} are the external potassium and sodium concentrations; and n is a function of the energy of nearest-neighbor interactions. The quantity n is similar to Hill's parameter (*8*) and gives a measure of cooperation among sites. Equation 1 is formally similar to those previously derived for describing conformational changes of macromolecules (*9*) since all of them are based on the Ising model (*10*). When the potassium content approaches ½ F_T, Eq. 1 can be rearranged

$$\ln\left(\frac{K_{ad}}{F_T - K_{ad}}\right) = n \ln\frac{K_{ex}}{Na_{ex}} + n \ln K^{00}{}_{Na\to K} \quad (2)$$

If F_T and n are not significantly affected by temperature, the following relationship can be obtained by differentiating Eq. 2 with respect to the reciprocal of absolute temperature

$$\frac{\partial \ln[K_{ad}/(F_T - K_{ad})]}{\partial(1/T)} = n\frac{\partial \ln K^{00}{}_{Na\to K}}{\partial(1/T)} = -n\frac{\Delta H^{00}{}_{Na\to K}}{R} \quad (3)$$

where

$$-RT \cdot \ln K^{00}{}_{Na\to K} = \Delta F^{00} = \Delta H^{00} - T\Delta S^{00} \quad (4)$$

and ΔF^{00}, ΔH^{00}, and ΔS^{00} are changes in the intrinsic free energy, intrinsic enthalpy, and intrinsic entropy, respectively, for the exchange of potassium by sodium on adsorptive sites.

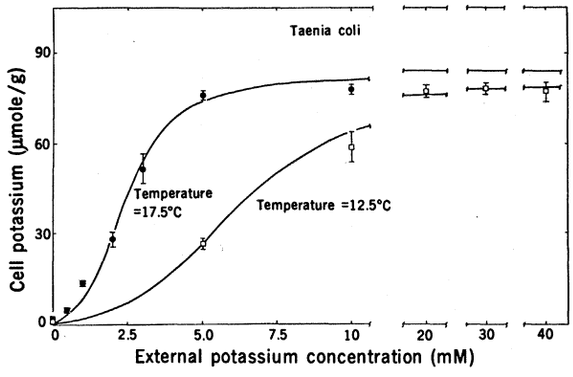

Fig. 2. Effect of K_{ex} on the cellular potassium content at 17.5°C (closed circles) and 12.5°C (open squares). The basic incubation medium was a physiological salt solution in which changes of the KCl concentration were compensated for by equivalent amounts of NaCl ($Na_{ex} + K_{ex} = 150$ m*M*). The cellular potassium content was calculated by subtracting from the total tissue potassium the potassium content in the sorbitol-^{14}C space [wet weight 0.430 ml/g (*17*)]. The points are the means of six to eight determinations; the error lines indicate the standard mean error. The solid lines are theoretically determined from Eq. 1. The only parameter significantly dependent on temperature is $K^{00}{}_{Na\to K}$.

The relationship (Eq. 3) emphasizes that for an adsorptive process there must be a critical thermal transition. Noncooperative mechanisms (when $n = 1$) also present thermal transitions. However, for a fixed ΔH^{00} value the transition for a cooperative process is steeper by a factor of n.

In order to construct a theoretical thermal transition curve with the use of Eqs. 1 and 3, it is necessary to determine experimentally the temperature dependence of F_T, $K^{00}{}_{Na\to K}$, and n. To do this, we examined the steady-state potassium content as a function of external potassium concentration at two different temperatures, 17.5° to 12.5°C. These temperatures were selected to focus observations around the transition process. Figure 2 shows that the cellular potassium content as a function of K_{ex} follows saturable sigmoid curves at both temperatures and can be fitted by Eq. 1. At 17.5°C, half saturation is reached when K_{ex} is 2.5 m*M*, but at the lower temperature half saturation takes place at a higher K_{ex} (7.5 m*M*). This implies that the selectivity ratio for potassium over sodium, $K^{00}{}_{Na\to K}$, has decreased. This effect of temperature on $K^{00}{}_{Na\to K}$ is similar to the known thermal behavior in *Escherichia coli* (*11*). The parameters used for the theoretical isotherms are as follows: at 17.5°C, 85 μmole/g (F_T), 59.6 ($K^{00}{}_{Na\to K}$), 3.0 (n); at 12.5°C, 80 μmole/g (F_T), 22.8 ($K^{00}{}_{Na\to K}$), 3.0 (n). The value of F_T at 17.5°C is not significantly different from that at 12.5°C. Similarly, the interaction parameter n remains constant at both temperatures. The only parameter that appears to be sensitive to temperature is $K^{00}{}_{Na\to K}$, as was assumed to obtain Eq. 3.

From a plot of the known values of $\ln K^{00}{}_{Na\to K}$ against $1/T$, we extrapolated values of $K^{00}{}_{Na\to K}$ for different temperatures. By introducing these intrinsic association constants in Eq. 1 together with experimental values of F_T and the interaction parameter n, we calculated cell potassium contents at different temperatures for 5 m*M* K_{ex}. Figure 1B shows that the calculated curve and the experimental data for the thermal transition of potassium contents are in good agreement.

The study with temperature offers yet another means of testing the applicability of the cooperative mechanism for the electrolyte accumulation process. A simple analytical expression relating T_c (the value of T at $K_{ad} = \frac{1}{2} F_T$) to the external potassium and sodium concentrations can be obtained from Eqs. 2 and 4 as follows

$$T_c = \frac{\Delta H^{00}/R}{\left(\ln\frac{K_{ex}}{Na_{ex}} + \frac{\Delta S^{00}}{R}\right)} \quad (5)$$

This equation reveals the dependence of T_c on the ratio K_{ex}/Na_{ex} and predicts that an increase in K_{ex} should result in a decreased value of T_c. Experiments were done to test this prediction at 10 m*M* K_{ex}. As shown in Fig. 1B, the curve relating tissue cell potassium to temperature for 10 m*M* K_{ex} is shifted to the left compared with the curve for 5 m*M* K_{ex}. In other words, when K_{ex} is high the cell maintains a high potassium content until a lower temperature is reached. The value of T_c is found to be about 10.0°C. Equation 4 predicts a value of 9.7°C for T_c when the known value of ΔH^{00} (30.9 kcal/mole) is inserted. These results establish that a cooperative mechanism is involved in the electrolyte accumulation processes in the smooth muscle.

Thermal transitions have been observed in many biological systems (*12–16*). Transition with temperature oc-

curs in the activity of Na,K–adenosine triphosphatase (*12*) and other enzymes (*13*), in the fluorescence of excitable nerves (*14*), and in calorimetric studies of lipids and water (*15*). In a few cases the thermal behavior is actually known to reflect the underlying cooperative conformational alterations.

The approach examined in the present report outlines a theoretical model that predicts the behavior of smooth muscle around the transition temperature. The model makes a quantitative prediction relating the cell electrolyte levels to temperature. A second prediction made by this model is that the transition temperature should be lowered by raising K_{ex}. Both of these predictions are quantitatively confirmed.

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2. The composition of the basic incubating medium is: NaCl, 121 m*M*; KCl, 5 m*M*; $NaHCO_3$, 22.5 m*M*; PO_4H_2Na, 1.2 m*M*; $MgCl_2$, 1.2 m*M*; $CaCl_2$, 2.5 m*M*; and glucose, 5.6 m*M*. The solution is gassed with a mixture of 95 percent O_2 and 5 percent CO_2.
3. The total potassium and sodium contents of taenia coli incubated at 36°C for periods of 13, 15, and 17 hours were (in μmole/g): 77.9 ± 2.9, 77.6 ± 2.3, and 79.7 ± 2.7 for potassium and 84.4 ± 3.1, 82.2 ± 3.4, and 84.0 ± 4.4 for sodium. The total potassium and sodium contents for another group of muscles incubated at 1.0°C for the same period of incubation were: 7.6 ± 1.5, 6.9 ± 0.7, and 7.8 ± 1.3 for potassium and 152.7 ± 2.0, 156.6 ± 4.7, and 156.5 ± 5.6 for sodium, respectively.
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Cooperative Thermal Effects on the Accumulation of Potassium and Sodium in Frog Muscle

Abstract. *Sodium-rich frog muscles are found to extrude sodium and reaccumulate potassium at 0°C. The uptake of potassium by these muscles is studied at three different temperatures as a function of external potassium concentration, K_{ex}. The steady-state potassium content of the tissue is related to K_{ex} by a sigmoidal cooperative curve at all temperatures. These results are compared with findings on a mammalian smooth muscle.*

Unlike many tissues when they are cooled, frog muscle is known to retain the bulk of its potassium both in vivo and in vitro (*1*). This is seen in Table 1, where the electrolyte contents are given for muscles stored at 0°C in normal Ringer solution (*2*). In 5 days the tissue electrolytes changed less than 10 percent compared with the fresh tissue values. In contrast, many mammalian smooth muscles lose more than 90 percent of cellular potassium during overnight incubation at 0°C (*3*). We now describe the electrolyte-accumulating properties of frog muscles which, as a result of preliminary treatment, contained high sodium and low potassium concentrations. The effects of temperature on these properties are compared with those reported for mammalian smooth muscle (*4*).

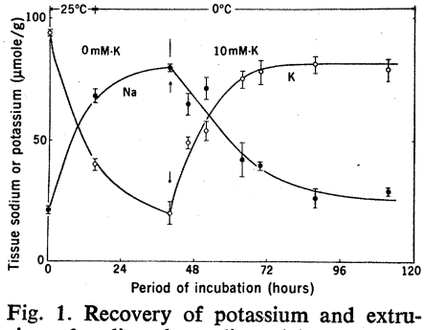

Fig. 1. Recovery of potassium and extrusion of sodium by sodium-rich muscles at 0°C. In these experiments the tissue potassium and sodium were extracted in 0.1*N* HCl overnight at room temperature. The ions were measured with a Beckman DU flame photometer.

Sodium-rich muscles in these experiments were obtained by incubating the tissues in potassium-free medium overnight at 25°C and then for 24 to 48 hours at 0°C (*5*). After this treatment, the total potassium content decreased to 10 to 30 μmole/g, and the sodium content increased to 90 to 110 μmole/g. For recovery, these muscles were transferred to a Ringer solution of known external potassium concentration, K_{ex}. The accumulation of potassium and extrusion of sodium was complete within 48 hours after the beginning of recovery at 0°C (Fig. 1) and within 20 hours at 25°C (not shown).

The ability of these tissues to reaccumulate potassium and extrude sodium against their gradients at 0°C in the presence of 10 m*M* external potassium was striking. This indicates that the potassium accumulation mechanism continues to operate at this temperature. However, potassium would be accumulated only up to 30 μmole/g when the potassium concentration of the recovery medium is 2.5 m*M*, the physiological concentration (Fig. 2A). Thus, it may be claimed on a qualitative basis that the temperature-dependent active transport mechanism is considerably slowed at 0°C. On the other hand, an alternative biophysical model was recently shown to quantitatively predict the effects of temperature on smooth muscle of the guinea pig taenia coli (*4*). The results presented below show that this model also applies to the effects of temperature on frog muscle.