Biochimica et Biophysica Acta, 736 (1983) 241–245
Elsevier

BBA Report

BBA 70135

THALLOUS ION PERMEATION THROUGH THE CATION-SELECTIVE CHANNEL OF THE SARCOPLASMIC RETICULUM

ANOMALOUS MOLE FRACTION DEPENDENCE

JAMES A. FOX

Neuroscience Program, Brain Research Institute, U.C.L.A., Los Angeles, CA 90024 (U.S.A.)

(Received April 19th, 1983)
(Revised manuscript received September 15th, 1983)

Key words: Sarcoplasmic reticulum; Cation channel; Anomalous mole fraction; Thallium

The thallous ion was found to permeate the cation-selective channel of rabbit sarcoplasmic reticulum and to block current through this channel when present in mixtures with other permeant ions. Channel conductance in pure thallium acetate saturates with increasing concentration, with a maximum limiting conductance of 60 pS. The conductance ratio G_K/G_{Tl} at 1 M is 3.7, while the permeability ratio is near 0.4 over the concentration range 0.01 to 1 M. Thallium blockade in mixtures can be described by the equation of Neher (Neher, E. (1975) Biochim. Biophys. Acta 401, 540–544).

The thallous ion exhibits anomalous behavior in many cation channels in that, while permeant, it blocks current when present in solutions containing other ions. It is more permeant than potassium in the potassium channel of squid [1] and muscle [2], in the anomalous rectifier channel of egg [3] and muscle [4], and as permeant in the gramicidin channel [5,6]. Gramicidin conductance goes through a minimum at low mole fractions of thallium in mixtures with sodium [7] or potassium [6]. Thallium ion interactions are of interest because they enable one to investigate ion specific interactions with channels.

The sarcoplasmic reticulum channel has been described in great detail through the work of Miller and coworkers [8–11]. It is a cation-selective channel most permeant to potassium which can be inserted into artificial bilayers. Single and multichannel currents may be studied with control of solution composition on both membrane faces under these conditions. The reported properties of this channel are consistent with a single ion pore model.

In this paper I report that thallous ion is permeant in this channel, and describe the conductance behavior in both single-salt and mixed-salt solutions. In symmetric 1 M thallium acetate solutions the single channel conductance is 56 pS. This value may be compared with the reported sarcoplasmic reticulum channel conductances at 1 molar salt of 214 pS for potassium and 72 pS for sodium [10]. When present in mixtures of permeant ions thallium blocks current through the channel. The zero-current potential under bi-ionic conditions (potassium and thallium) decreases somewhat, while the conductance ratio doubles, as activity increases.

Black lipid membranes were formed across a 0.3 mm hole in a polystyrene cup. Membranes were composed of PE/PC (9:1) (70 mM in decane). Rabbit sarcoplasmic reticulum vesicles were prepared by the method of Miller and Rosenberg [11], and stored in 0.4 M sucrose at −70°C until used. Membrane currents were monitored via potassium acetate agar bridges by a high-gain current to voltage converter with outputs to oscillo-

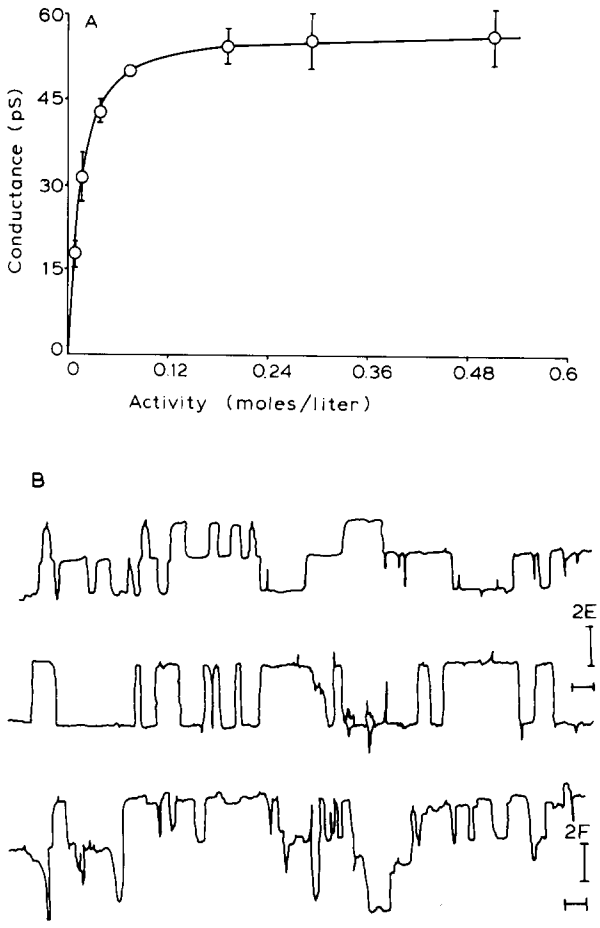


Fig. 1. (A) Channel conductance in thallium acetate. The conductance of the sarcoplasmic reticulum channel is shown as a function of thallium acetate activity. The activity at which the conductance is half maximal is 16 mM, which corresponds to a concentration of 18 mM. The maximal conductance G_{max} is 60 pS. Activity of 0.513 M corresponds to a thallium acetate concentration of 1 M. Activities are from Robinson and Harned [13]. Bars indicate mean conductance ± S.D. (absence of bars indicates that the deviation is less than 1 pS). (B) Single-channel records in pure thallium acetate. Top trace: 0.5 M concentration; vertical scale 100 pS conductance. Middle trace: 0.1 M concentration; vertical scale 50 pS. Bottom trace: 0.01 M concentration; vertical scale 25 pS. Horizontal scale shows 15 s for all three records.

scope and chart recorder. Acetate salts were used in all solutions (unless noted) to insure adequate thallium solubility, with 0.1 mM EDTA, 5 mM Mops and Tris buffers (to pH 7) also present. After membrane formation, 1 mM calcium acetate and 5–10 μg protein/ml vesicles were added to the cis compartment (trans side ground) with +40 mV applied. The solution was stirred until single-channel currents appeared, and then 1.5 mM EDTA was added to stop the insertion of channels. Occasionally, single channels appeared without addition of calcium to the solution; in these cases, no EDTA was added. Results were similar in both situations.

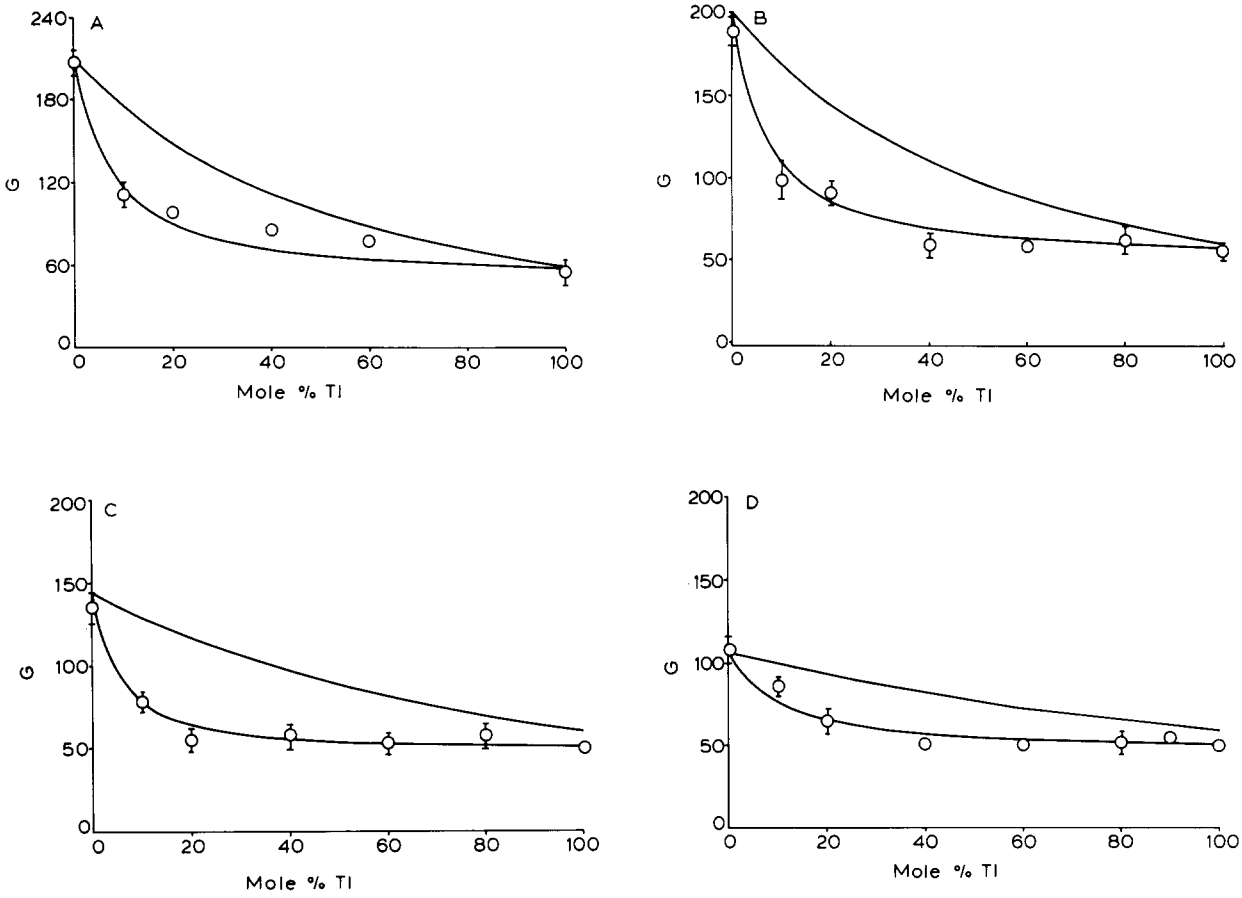
The conductance of the sarcoplasmic reticulum channel was determined as a function of concentration in symmetric solutions of thallium acetate. The results are shown in Fig. 1. The channel shows saturating behavior with a maximal conductance of 60 pS and half-maximal conductance at a thallium activity of 16 mM (corresponding to a concentration of 18 mM). This is much lower than the 240 pS maximal conductance for potassium, while similar to the value for sodium of 77 pS, and greater than the 7 pS G_{max} for lithium [10]. The K_m of 16 mM is similar to the 19 mM value for lithium, but is well below the value of 54 mM for potassium [10].

Using the values from the single-salt data, one can calculate the conductances for mixtures of ions predicted by the single-ion pore theory of Läuger [12]. In experiments with mixed solutions where the fractions of thallium and another cation are varied, while the total concentration is constant, clear deviations from this theory are observed. For example, in a 0.5 M (10% thallium/90% potassium) solution the sarcoplasmic reticulum channel conductance is 48% less than the value in pure 0.5 M potassium acetate. Only a 10% drop in channel conductance is expected from Läuger's theory in this case. These results are shown in Fig. 2.

The data from thallium-potassium mixtures and thallium-ammonium mixtures can be fit using Neher's equation for thallium blockade of sodium conductance in gramicidin [7]. His equation, as used to fit my data from the sarcoplasmic reticulum channel is:

$$G = \frac{G_{m\mathrm{Ion}}}{\left(1 + \frac{1 + K_b \cdot C_{Tl}}{K_{\mathrm{Ion}} \cdot C_{\mathrm{Ion}}}\right)} + \frac{G_{mTl}}{\left(1 + \frac{1}{K_{Tl} \cdot C_{Tl}}\right)} \qquad (1)$$

(where $G_{m\mathrm{Ion}}$ is the maximal conductance in single salt solutions of K or NH_4; G_{mTl} is that for Tl;



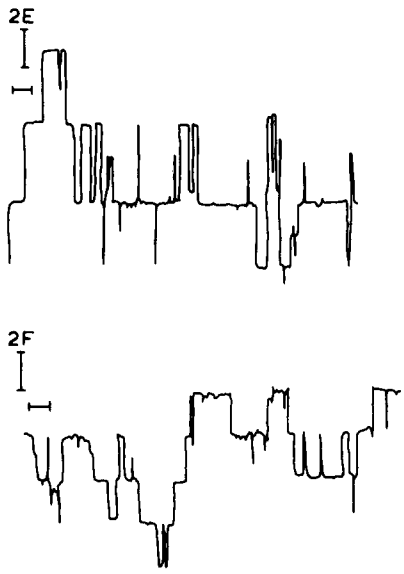


Fig. 2. sarcoplasmic reticulum channel conductance in mixed solutions. Anomalous dependence of conductance on mole fraction of thallium in mixtures with potassium (A–C) or ammonium (D) at constant total salt concentration (A: 1 M; B: 0.5 M; C: 0.1 M; D: 0.1 M). 0% indicates pure potassium or ammonium acetate; 100% indicates pure thallium acetate at the indicated concentrations. The upper curves show the conductance expected assuming simple exclusion proportional to the binding constant in the pure salt, derived from Läuger [7,12]. The lower curves show the conductance predicted by assuming a block by thallium, but not by the other permeant ion (Eqn. 1; Ref. 7). The lower curves were calculated using a blocking constant value for all three potassium concentrations of 380 M^{-1}; for ammonium the value was 250 M^{-1}. The symbols indicate mean conductance ± 1 S.D. (absence of error bars indicates a standard deviation smaller than the size of the symbol at the indicated scale). (E and F) Single-channel records from 0.5 M potassium acetate (E) and 0.45 M potassium acetate thallium acetate plus 0.05 M (F). The vertical scale for both records is 100 pS, the horizontal 15 s.

TABLE I

CONDUCTANCE AND PERMEABILITY RATIOS FOR POTASSIUM AND THALLIUM

The permeability ratios were determined under bi-ionic conditions, and calculated as

$$\frac{P_K}{P_{Tl}} = \left(\frac{a_{Tl}}{a_K}\right) e^{-V_0 F/RT}$$

where a_{Tl} and a_K are the activity coefficients for thallium and potassium at the experimental concentration, V_0 is the observed zero-current potential, and F, R, and T have their usual meanings.

Ion concentration (M)	G_K/G_{Tl}	P_K/P_{Tl}
0.01	1.672 ± 0.52	0.471 ± 0.03 ($n = 3$)
0.1	2.717 ± 0.23	0.409 ± 0.02 ($n = 3$)
0.5	3.431 ± 0.49	0.362 ± 0.01 ($n = 4$)
1.0	3.717 ± 0.46	0.303 ± 0.01 ($n = 4$)

C_{Ion} and C_{Tl} are the corresponding ion concentrations; K_{Ion} and K_{Tl} the binding constants for the indicated ions determined in single-salt solutions; and K_b the blocking constant which is fit empirically).

Data from potassium-thallium mixtures at all three concentrations can be fit by Eqn. 1 with a blocking constant value of 380 M^{-1}. The ammonium data is best fit with a value of 250 M^{-1}. These values may be compared to the value of 800 M^{-1} for the block of sodium by thallium in the gramicidin channel [7]. The lower curves in Fig. 2 illustrate these fits.

The upper curves in Fig. 2 illustrate the expected conductance in mixtures assuming that the presence of one ion in a pore simply excludes other ions with no other effect on conductance. These curves are from the equation for the channel conductance in a mixed solution with no blocking, as derived from Läuger [7,12]. The conductance data are clearly not fit by using this assumption, since this does not explain the large decreases in conductance at low mole percent of thallium seen with both potassium and ammonium mixtures. (The equation for these curves can be formed if, in Eqn. 1 above, the term K_b is replaced by the binding constant K_{Tl}, and the product $K_{Ion} \cdot C_{Ion}$ is added to the 1 in the numerator of the fraction in the denominator of the term on the right [7]).

Table I compares the conductance ratio G_K/G_{Tl} to the permeability ratio P_K/P_{Tl} as a function of concentration. The conductance ratio is simply the ratio of the conductances found in symmetric solutions of the pure salts, while the permeability ratio is found by measuring the zero-current potential under bi-ionic conditions at the indicated concentration. The permeability ratio P_K/P_{Tl} is near 0.4 at low activity, but as activity increases, it falls to 0.3 at a concentration of 1 M. (For all concentrations, the measured V_0 was 20 ± 1 mV, but activity corrections at high concentrations of thallium lead to this decrease in the calculated value). The permeability ratios for all pairs of ions tested by Coronado et al. [10] were constant with respect to ion concentration in this channel.

Blocking effects of thallium ion in mixed solutions have been observed previously in gramicidin channels [5–7], nerve membrane [1], muscle [2,4], and egg [3]. In gramicidin, the permeability ratio P_K/P_{Tl} is concentration dependent. Eisenman et al. [5] explain the 10-fold change observed in this ratio under asymmetric conditions (from 0.1 to 10 mM), and the predicted 2-fold change for the bi-ionic case, with a model which allows multiple occupancy of the gramicidin channel. My data for the sarcoplasmic reticulum channel do not show such large changes in the ratio P_K/P_{Tl}.

The conductance data in pure thallium acetate are consistent with a single-ion pore model of this channel. The blocking data in mixed solutions may be accounted for without invoking multiple occupancy of the channel if one postulates that thallium binding to an external site, not in the conduction path, alters the channel conductance to the two cations in solution. With this assumption, which has been used to account for similar effects of thallium in the inward rectifier channel [14], equations formally equivalent to Eqn. 1 above can be derived (personal communication from Dr. Sergio Ciani). Other possible models, which might be compatible with my data, would be to allow fluctuation of channel barriers between different conformational states, along the lines of model discussed by Läuger et al. [15], or to allow for multiple occupancy. This last suggestion is least attractive at this point, unless double occupancy is limited to channels which contain thallium and a

different cation, since the pure thallium data, and the behavior of this channel in all other salt solutions do not show evidence of multiple occupancy.

In summary, my observations of thallium ion permeation in the sarcoplasmic reticulum channel include anomalous blocking effects in mixtures of permeant ions, probably without multiple occupancy of the channel. This effect is also seen in the presence of sulfate ions, indicating that it is not due simply to altered membrane surface charge as a result of nonspecific thallium binding. Thus thallium ion appears to interact specifically with the sarcoplasmic reticulum channel to block current to other ions.

The author thanks Dr. Sergio Ciani for his helpful suggestions, and Dr. Christopher Miller and his associates for their technical assistance. This research was supported by a grant from the Muscular Dystrophy Association to the J.L.N.R.C. at U.C.L.A. and by U.S.P.H.S. grant GM07171. The author received an Integrative Biology Predoctoral Fellowship, USPHS GM07191.

References

1 Hagiwara, S., Eaton, D., Stuart, A. and Rosenthal N. (1972) J. Membrane Biol. 9, 373–389
2 Hille, B. (1973) J. Gen. Physiol. 61, 669–686
3 Hagiwara, S., Miyazaki, S., Krasne, S. and Ciani, S. (1977) J. Gen. Physiol. 70, 269–281
4 Gay, L. (1981) J. Physiol. (Lond.) 312, 39P
5 Eisenman, G., Sandblom, J. and Neher, E. (1977) in Metal-Ligand Interactions in Organic Chemistry and Biochemistry (Pullman, B. and Goldblum, N., eds.), part 2, pp. 1–36, Reidel, Dordrecht
6 Andersen, O. (1975) Int. Biophys. Congress, Copenhagen, 1975, p. 112, Abstr.
7 Neher, E. (1975) Biochim. Biophys. Acta 401, 540–544
8 Miller, C. (1978) J. Membrane Biol. 40, 1–23
9 Labarca, P., Coronado, R. and Miller, C. (1980) J. Gen. Physiol. 76, 397–424
10 Coronado, R., Rosenberg, R. and Miller, C. (1980) J. Gen. Physiol. 76, 425–446
11 Miller, C. and Rosenberg, R. (1979) Biochemistry 18, 1138–1145
12 Läuger, P. (1973) Biochim. Biophys. Acta 311, 423–441
13 Robinson, R. and Harned, H. (1941) Chem. Rev. 28, 419–477
14 Krasne, S., Ciani, S., Hagiwara, S. and Miyazaki, S. (1983) in The Physiology of Excitable Cells (Grinnell, A. and Moody, W., eds.), pp. 83–96, A.R. Liss, New York
15 Läuger, P., Stephan, W. and Frehland, E. (1980) Biochim. Biophys. Acta 602, 167–180