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TAURINE EFFECTS ON THE TRANSITION TEMPERATURE IN ARRHENIUS PLOTS OF ATP-DEPENDENT CALCIUM ION UPTAKE IN RAT RETINAL MEMBRANE PREPARATIONS

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Abstract—The transition temperatures calculated from Arrhenius plots of ATP-dependent calcium ion uptake in rat retinal membrane preparations differed depending upon the presence or absence of exogenous taurine. At a constant calcium ion concentration of 10 μM and in the absence of taurine the transition temperature was 17.9 ± 4.9°, whereas in the presence of 20 mM taurine the transition temperature was raised to 25.4 ± 0.8°. Arrhenius plots of maximum velocities of calcium ion uptake (calculated from six calcium ion concentrations which varied from 5 to 300 μM) also demonstrated a taurine effect on the transition temperatures (13.5° vs 25.5°). In addition, taurine lowered the apparent activation energy for calcium ion uptake.

The vertebrate retina contains high concentrations of taurine with values of 50 μmoles/g wet weight in the rat retina [1]. However, there is little information as to the physiological and molecular action of taurine in the retina. Taurine appears to be participating in at least two functions: (1) as a modifier of calcium fluxes, and (2) as a membrane stabilizer. Taurine has been demonstrated both to stimulate and to inhibit ATP-dependent calcium ion uptake in rat retinal membrane preparations depending upon the calcium ion concentration. At low calcium ion concentrations taurine stimulates calcium ion uptake [2–7], while at high calcium ion concentrations taurine is inhibitory [3, 8]. The suggestion that taurine might be a membrane stabilizer was first made by Huxtable and Bressler [9] and Gruener *et al.* [10] and is further supported by observations in the cat and the rat that taurine is necessary for photoreceptor structural integrity which can be disrupted by taurine depletion or illumination [11–13].

Previous observations from our laboratory indicated that taurine inhibits the phosphorylation of specific membrane proteins in the rat retina which possibly affect ATP-dependent calcium ion uptake [14]. The studies presented in this report further demonstrate that taurine affects Arrhenius profiles by changing the transition temperature of the discontinuity observed for the ATP-dependent calcium ion uptake and thus give additional support to the hypothesis that taurine alters retinal membrane proteins.

MATERIALS AND METHODS

Materials. Retinal tissue was obtained from adult Wistar rats (175–225 g) which were fed Purina rat chow and water *ad lib.*

Preparation of membrane homogenate. Retinal tissue was gently homogenized in 0.32 M sucrose (2°) and then centrifuged for 20 min at 16,000 *g*. The retinal membranes located in the pellet were then washed twice in bicarbonate buffer, pH 7.4 [2] ($NaHCO_3$, 50 mM; KCl, 50 mM; NaCl, 50 mM; KH_2PO_4, 1.2 mM; $MgCl_2$, 2 mM; ouabain, 50 μM), recentrifuged after each wash, and finally homogenized in a glass–glass homogenizer in the above bicarbonate buffer. Protein concentrations were determined by the method of Lowry *et al.* [15].

Calcium uptake assay. The incubation system contained the above bicarbonate buffer, $^{45}CaCl_2$, (0.5 μCi), $CaCl_2$ (10 μM or varied), ATP (1.2 mM or varied) and retinal preparation (≅0.25 mg protein). The reaction mixture minus the retinal preparation was preincubated for 4 min at various temperatures in plastic disposable tubes with the cap on. The retinal preparation was then added, and the system was incubated for an additional 3 min (no shaking). A linear range of temperatures from 9 to 39° was maintained in a copper bridge which contained 19 wells for the incubation tubes. One end of the bridge was placed in a water bath held at 48° while the other end was immersed in a −8° ice bath (containing NaCl). The reaction was terminated by adding 3 ml of ice-cold bicarbonate buffer to the incubation system and immediately filtering on a Millipore glass fiber filter (Whatman GF/B filter). The filter was washed three times with 3 ml of the above bicarbonate buffer and then counted for radioactivity with Aquasol scintillation fluid. The amount of $^{45}Ca^{2+}$ taken up by the retinal preparations was determined by subtracting the counts retained on the filter after a zero-time incubation with retinal preparation.

RESULTS

The ATP requirements for the calcium ion uptake at six different temperatures which ranged from 9 to 39° are demonstrated in Fig. 1. The optimal ATP

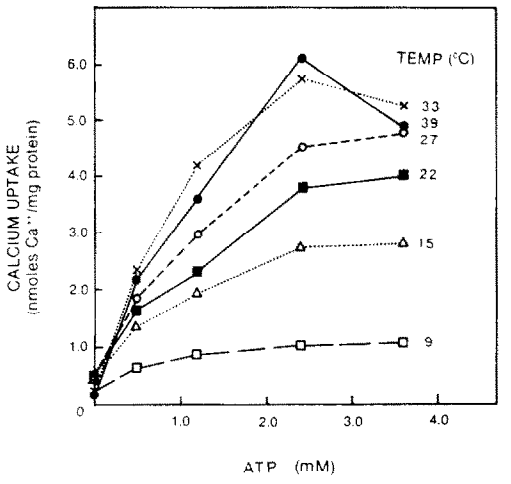

Fig. 1. Calcium ion uptake in rat retinal membrane preparations at various temperatures as a function of the ATP concentration. Details of the calcium ion uptake assay are presented in Materials and Methods. Calcium ion concentration was 10 μM.

concentration is 2.4 mM. However, in all subsequent experiments a concentration of 1.2 mM was used because taurine (20 mM) had a greater stimulatory effect at submaximal ATP concentrations. In the absence of exogenous ATP in the incubation mixture, a small amount of radioactive calcium was retained on the Whatman GF/B filters (Fig. 1). This retained radioactivity is probably due to calcium binding to retinal proteins or to calcium uptake because of endogenous ATP not removed during the preparation of the retinal membranes.

Figure 2 shows the uptake of calcium ion as a function of time. In all subsequent experiments an incubation period of 3 min was used.

Both the ATP-dependent and taurine-stimulated ATP-dependent calcium ion uptake were temperature dependent at a calcium ion concentration of 10 μM, as demonstrated in Fig. 3 (typical experiment). Arrhenius plots for the ATP-dependent calcium ion uptake in the presence and absence of 20 mM taurine were constructed from the data presented in Fig. 3 and are depicted in Fig. 4. The values of the transition temperatures and activation energies are presented in Table 1. The transition tem-

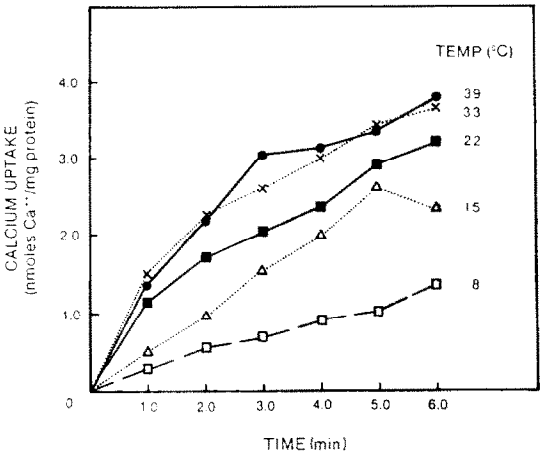

Fig. 2. Linearity of ATP-dependent calcium ion uptake as a function of time. Details of the calcium ion uptake assay are presented in Materials and Methods. Calcium ion concentration was 10 μM; ATP concentration was 1.2 mM.

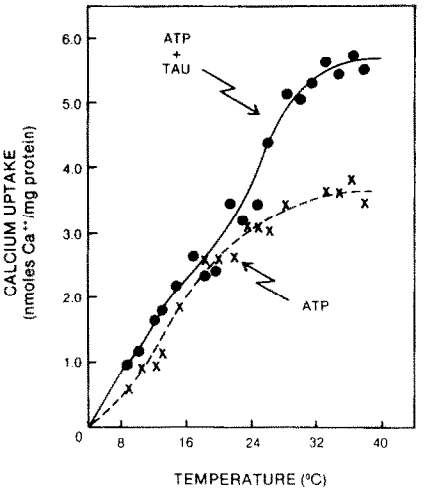

Fig. 3. Effect of temperature on ATP-dependent calcium ion uptake in the presence and absence of 20 mM taurine. Details of the calcium ion uptake assay are presented in Materials and Methods. Calcium ion concentration was 10 μM; ATP concentration was 1.2 mM.

Table 1. Transition temperatures and activation energies for ATP-dependent and taurine-stimulated ATP-dependent calcium ion uptake in rat retinal membrane preparations*

Condition	Transition temperature (°C)	Activation energy above transition temperature (kcal/mole)	Activation energy below transition temperature (kcal/mole)
ATP	17.9 ± 4.9†	5.96 ± 0.76†	22.5 ± 3.6
ATP + taurine	25.4 ± 0.8	3.41 ± 0.68	16.2 ± 1.2

* Values are means ± S.E.; N = 7.
† Significance was determined by Student's *t*-test, $P < 0.05$.

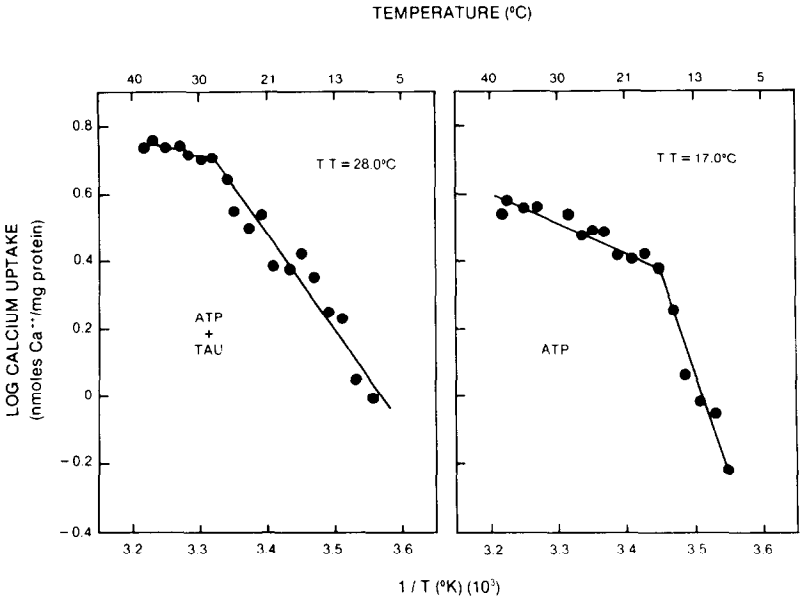

Fig. 4. Typical Arrhenius plots of the logarithm of the ATP-dependent calcium ion uptake velocity at a calcium ion concentration of 10 μM versus temperature. Details of the calcium ion uptake assay are presented in Materials and Methods. Calcium ion concentration was 10 μM; ATP concentration was 1.2 mM; taurine concentration was 20 mM. The data presented in this figure are from Fig. 3.

perature of the ATP-dependent calcium ion uptake in the absence of taurine was 17.9 ± 4.9°, whereas the addition of 20 mM taurine to the incubation system increased the transition temperature to 25.4 ± 0.8°.

The apparent activation energies obtained from Arrhenius plots are also given in Table 1. The activation energies of the ATP-dependent calcium ion uptake in the absence of taurine were approximately 6 and 23 kcal/mole at temperatures over and under 17.9° respectively. However, above the transition temperature (25.4°), taurine lowered the activation

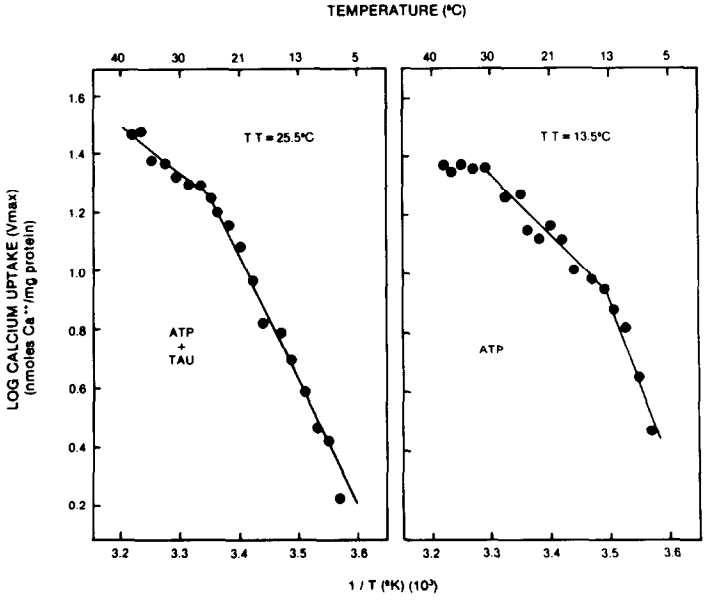

Fig. 5. Arrhenius plots of the logarithms of the ATP-dependent calcium ion uptake maximum velocity versus temperature. Details of the calcium ion uptake assay are presented in Materials and Methods. Velocities of the calcium ion uptake were measured at six calcium ion concentrations (5, 10, 25, 50, 125, and 300 μM) at each of the nineteen temperatures between 7 and 39°. The maximum velocities were calculated by linear regression analysis from plots of initial velocity versus initial velocity/calcium ion concentration. ATP concentration was 1.2 mM; taurine concentration was 20 mM.

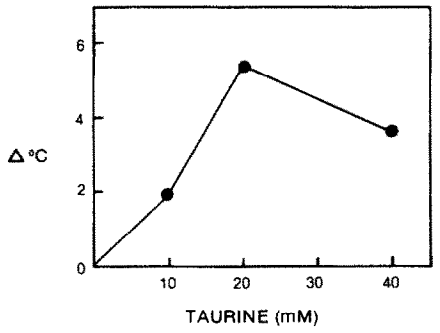

Fig. 6. Absolute change in the transition temperature versus taurine concentration calculated from Arrhenius plots of ATP-dependent calcium ion uptake. Details of the calcium ion uptake assay are presented in Materials and Methods. Calcium ion concentration was 10 μM; ATP concentration was 1.2 mM.

energy for calcium ion uptake (3.41 ± 0.68 vs 5.96 ± 0.76 kcal/mole) ($P < 0.05$). There was no significant difference in the activation energy below the transition temperature for the taurine-stimulated and non-taurine-stimulated ATP-dependent calcium ion uptake.

It has been reported for other biological systems that variations in temperature can alter the substrate-binding affinity and thus influence the behavior of Arrhenius plots by changing the activation energy and the transition temperature, and even produce an artifactual transition temperature, if activity is measured at a single substrate concentration [16, 17]. Thus, a series of maximum velocities for calcium ion uptake were measured at six calcium ion concentrations (5–300 μM) at nineteen temperatures between 7 and 39°. A plot of the maximum velocity (V_{max}) values as a function of temperature is shown in Fig. 5. The transition temperature for the ATP-dependent calcium ion uptake was calculated to be 13.5°, while a value of 25.5° was calculated for the taurine-stimulated ATP-dependent calcium ion uptake.

The increase in transition temperature due to the addition of exogenous taurine to the incubation system was dose dependent (Fig. 6). A maximal change in temperature of approximately 5.5° was observed at a taurine concentration of 20 mM.

DISCUSSION

In previous studies we have shown that calcium ion uptake has two transport systems, high and low affinity, with K_m values of 35 μM and 2 mM respectively [6]. In these present studies involving the effects of temperature on the ATP-dependent and taurine-stimulated ATP-dependent calcium ion uptake, we studied the effects of taurine on the high affinity uptake system: (1) by measuring the velocity at a constant calcium ion concentration (10 μM), and (2) by measuring the maximum velocity of the calcium ion uptake calculated from Eadie–Hofstee plots utilizing six calcium concentrations between 5 and 300 μM.

We have demonstrated that taurine produces definite differences in the overall effect of temperature on the ATP-dependent high affinity calcium ion uptake system. The addition of taurine to the incubation system for calcium ion uptake caused a marked increase in the transition temperature from 17.9 ± 4.9 to 25.4 ± 0.8° when uptake was measured at a low calcium ion concentration, that is, 10 μM. An increase in transition temperature from 13.5 to 25.5° was also observed when the high affinity maximum velocities for calcium ion uptake were measured at various temperatures.

A sudden change in the activation energy at a specific temperature for membrane-bound enzymes has been interpreted as an alteration in the crystalline-liquid crystalline phase transition of the membrane [18]. In particular, it has been demonstrated repeatedly that for Na^+/K^+ ATPase activity the occurrence of a transition temperature in the Arrhenius plots may be due to a lipid phase transition [19–21]. Thus, the elevation in the transition temperature due to the addition of taurine to the calcium ion uptake incubation system, as demonstrated in the Arrhenius plots of activity versus temperature, suggests that taurine induces a conformational change within the membrane.

However, there exists at least two possibilities concerning the nature of the conformational change: (1) taurine may cause a change within the structure of the membrane, such as affecting protein conformation by changes in the physical state of the membrane lipids, or (2) taurine may have a direct interaction on the carrier protein for the calcium ion uptake system. While the data presented in this paper do not directly address this question, we have reported recently [14] that taurine inhibits phosphorylation of retinal membrane proteins. Taurine and a close structural analogue, 2-aminoethylhydrogen sulfate, inhibited phosphate incorporation into rat retinal membrane preparations by 35–40%, whereas guanidinoethanesulfonic acid and isethionic acid had no effect. Autoradiograms of sodium dodecyl sulfate (SDS)-polyacrylamide gels indicated that, in the presence of 20 mM taurine, at least five bands of proteins with molecular weights ranging from 20,000 to 40,000 incorporated less quantities of radioactive phosphate than the control proteins.

Thus, there is direct evidence that taurine alters proteins within rat retinal membranes. However, in contrast to these results obtained for rat retinal membrane proteins, Pasantes-Morales [4] has not demonstrated a transition temperature in Arrhenius plots for calcium ion uptake in frog rod outer segment preparations. We offer no explanation for our differing results except that different species were used in the experiments.

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