METABOLIC COOPERATIVE CONTROL OF ELECTROLYTE LEVELS BY ADENOSINE TRIPHOSPHATE IN THE FROG MUSCLE

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ABSTRACT This study examines the effects of metabolic inhibitors on the content of cellular K, Na, and adenosine triphosphate (ATP). ATP and K are seen to fall in the inhibited tissues. The ATP content is correlated with the K content. The role of ATP is examined according to a recent biophysical approach. It is suggested that ATP may control the electrolyte levels by inducing conformational changes in the cytoplasmic proteins.

INTRODUCTION

There is now considerable support for the hypothesis (association-induction hypothesis, Ling, 1962) that the physical state of water and other solutes is different in muscle cells than in the external medium (Cope, 1970; Cope and Damadian, 1970; Czeisler et al., 1970; Hazlewood et al., 1969; Hinke, 1970; Jones and Karreman, 1969; Ling and Nagendank, 1970; Miller and Ling, 1970; Reisin et al., 1970; Rome, 1968). The altered physical state of water in the cell results mainly from the electrostatic polarizing influence exerted by the organized structure of cell proteins. This influence is exerted by polar groups on side chains as well as by the peptide amide bonds. The water is highly polarizable; furthermore, as a result of the alternating positive and negative spatial distribution of charges on the proteins, the polarization would be exerted in multilayers, thereby influencing the bulk of cell water (Ling, 1970 *a*).

The cells are known to have asymmetric properties. Whereas sodium is excluded from the cell water K, on the other hand, is accumulated to a much higher concentration in the cells than in the external medium. The exclusion of sodium and other solutes from the cells can be understood by considering properties of the more

tightly organized polarized water. The polarized water, by offering increased restriction to the rotational movement, can make it entropically unfavorable for the solutes to remain dissolved in the interstitial spaces.

The accumulation of potassium in the cells can be understood, according to the association-induction theory, on the basis of selective adsorption on fixed anionic sites of proteins (mainly on β and γ carboxyl groups of the aspartic and glutamic acid side chains). The theory assumes that the sites are distributed throughout the cytoplasm and that both K and Na compete for adsorption on the same sites. These sites, however, are selective for K over Na in the resting cell. The results of recent experiments have provided some support for these assumptions (Gulati et al., 1971; Ling and Bohr, 1970; Ling and Cope, 1969; Ling and Ochsenfeld, 1965).

In the association-induction hypothesis, the cell is considered to be in a state of metastable equilibrium. The control of electrolyte levels by external agents is an important part of this hypothesis. The controlling agents can act by inducing a shift in the equilibrium state of the cell.

This report describes experiments that illustrate an aspect of the biologic control that may be exerted through metabolism.

THEORY

In the frog muscle, cooperative adsorption of K in the presence of Na follows the Yang-Ling equation (Ling and Bohr, 1970):

$$K_{ad} = \frac{F_T}{2}\left[1 + \frac{\frac{[K]_{ex}}{[Na]_{ex}} \cdot K^{00}_{Na\to K} - 1}{\left\{\left(\frac{[K]_{ex}}{[Na]_{ex}} \cdot K^{00}_{Na\to K} - 1\right)^2 + 4\,\frac{[K]_{ex}}{[Na]_{ex}} \cdot K^{00}_{Na\to K} \cdot \exp\left(\gamma/RT\right)\right\}^{1/2}}\right], \quad (1)$$

where K_{ad} is the cell K in micromoles per gram, F_T is the maximum number of sites available for K adsorption in micromoles per gram, $K^{00}_{Na\to K}$ is the intrinsic equilibrium constant (selectivity ratio), and $-\gamma/2$ is the energy of interaction between nearest neighbors in kilocalories per mole.

The control of cooperative adsorption on proteins can be exerted via cardinal adsorbents. Let the sites be arranged in gangs of g sites; let each gang have one cardinal site. If ATP acts as a cardinal adsorbent, then, with the occupation of each cardinal site, let the controlled sites adsorb K; they otherwise adsorb Na. With these considerations in mind, we may write the equations:

$$F_T = g \cdot \mathrm{ATP}, \quad (2)$$

and

$$K_{ad} = \frac{g \cdot ATP}{2} \cdot \left[1 + \frac{\frac{[K]_{ex}}{[Na]_{ex}} \cdot K^{00}_{Na \to K} - 1}{\left\{ \left(\frac{[K]_{ex}}{[Na]_{ex}} \cdot K^{00}_{Na \to K} - 1 \right)^2 + 4 \frac{[K]_{ex}}{[Na]_{ex}} \cdot K^{00}_{Na \to K} \exp(\gamma/RT) \right\}^{1/2}} \right], \quad (3)$$

$$N_{ad} = F_{Tm} - K_{ad}, \quad (4)$$

where F_{Tm} is the value of F_T with normal ATP.

METHODS

Rana pipiens frogs were kept in a large stainless steel sink with water running continuously at 25°C. They were force-fed ground beef and cereal one or two times a week. Four pairs of muscles (semitendinosus, tibialis anticus longus, sartorius, and iliofibularis) were isolated from each frog. Four muscles, each from a different frog, were incubated in 100 ml of experimental solution in a 250 ml Erlenmeyer flask. Composition of the solution was (mM): Na, 104; K, 2.5; Mg, 1.2; Ca, 0.71; Cl, 99; HCO_3, 6.6; HPO_4, 1.2; H_2PO_4, 2.0; glucose, 24; and the inhibitor. The flasks were shaken at 150 rpm at room temperature for 8–48 hr. Antibiotics were routinely added in solution; for incubations longer than 24 hr at 25°C, sterile techniques were employed (see Ling and Bohr, 1969). At different intervals (usually every 1 or 2 hr) individual flasks were removed, transferred to a bath at 1°C, and shaken for an additional 2–4 hr. Incubation at this low temperature was carried out in order to assure that the tissue was in equilibrium (see Table I). The relationship between ATP and K did not change significantly between 3 and 12 hr at 1°C. This was taken to indicate that the tissue had equilibrated.

At the end of the incubation period, the tissues were blotted between eight layers of moist, pre-chilled filter paper, frozen in liquid nitrogen, and weighed on a torsion balance to the nearest 0.1 mg. The weighed tissue was then transferred to a cold tube (in liquid N_2) for ATP and electrolyte extraction.

TABLE I

EQUILIBRATION OF TISSUES AT 1°C AFTER TREATMENT WITH 0.01 mM *o*-IODOSOBENZOATE

Time of equilibration	ATP	K	$g = \frac{K}{ATP}$
hr	*μmoles/g*	*μmoles/g*	
3	1.4	35	25
	2.7	59	21.8
	1.6	36	22.5
	2.5	46	18.4
		Mean ± SE	21.9 ± 1.3
12	1.9	53	27.9
	2.6	61	22.7
	2.3	59	25.6
	1.8	32	17.8
		Mean ± SE	23.4 ± 2.2

ATP Extraction and Measurement

The procedure for the extraction of ATP from the tissue was adapted from Davies (Cain and Davies, 1962). Stainless steel centrifuge tubes and a pestle designed to fit the tubes were kept cold in liquid N_2. The weighed sample in each tube was crushed fine with the cold pestle. 3 ml of 0.3 M perchloric acid (PCA) was added, and the tube was transferred to a rack placed in iced water. The contents of the tube were then centrifuged at 0°C and 3000 rpm for 5 min. 0.2 ml of the supernatant was immediately used for the ATP determination. 2 ml of the remaining PCA extract was used for K^+ and Na^+ determinations. ATP was measured enzymatically using the Sigma Chemical Co. (St. Louis, Mo.) Kit No. 366UV.

Na^+ and K^+ Measurement

The electrolytes were measured on a Beckman DU Spectrophotometer with flame attachment (Beckman Instruments, Inc., Palo Alto, Calif.). The samples were prepared from the PCA extract by mixing 1 ml extract in 2 ml of diluent (containing 75 mM NaCl + 75 mM NaH_2PO_4 for K^+ determination, 75 mM KCl + 75 mM KH_2PO_4 for Na^+ determination). The presence of radiation buffers in the samples and in the standards eliminates both self-interference and radiation interference in the flame photometry (Ling, 1962, p. 201).

RESULTS AND DISCUSSION

The role of metabolism in the ion accumulation process was studied by measuring the effects of 10 different metabolic inhibitors (Table II). When the cell metabolic machinery is blocked with an inhibitor, the tissue ATP content is expected to fall. The model (equation 3) predicts that the decline in ATP content should be related to the amount of potassium in the cell. In fact, since $[K]_{ex}$ and $[Na]_{ex}$ are fixed, the prediction of the model is that ATP and K should be linearly related. This

TABLE II

CALCULATED VALUES FOR THE CONSTANTS IN CORRELATING EXPERIMENTAL ATP AND POTASSIUM CONTENTS:

$K = g \cdot ATP + c$

Poison	Concentration	Slope, g	Intercept, c	Correlation coefficient $r_{ATP,K}$
	mM			
o-Iodosobenzoate	0.01	24.0	0.1	0.91
Iodoacetate (IAA)	0.1	20.5	9.6	0.88
Chlorpromazine HCl (CPZ)	0.1	17.7	8.9	0.90
Dinitrophenol (DNP)	0.1	16.2	14.7	0.93
p-(Chloromecuri)-benzoate (PCMB)	0.5	23.7	3.1	0.81
Cyanide (NaCN)	1.0	15.1	18.5	0.93
Arsenite ($NaAsO_2$)	10.0	18.8	3.7	0.95
Azide (NaN_3)	10.0	19.1	4.4	0.96
Glyceraldehyde	10.0	21.9	−4.7	0.95
Malonic acid	20.0	19.7	4.0	0.97
	Mean ± SE	19.7 ± 0.9	6.2 ± 2.2	

is confirmed with *o*-iodosobenzoate at 0.01 mM (Fig. 1) and with iodoacetate (IAA) at 0.1 mM (Fig. 2 *a*). With IAA at 5 mM there is seen to be a deviation from the linear process (Fig. 2 *b*), suggesting that the findings in this report represent only an aspect of the control exerted by ATP.

A linear relationship between ATP and K was found with all the inhibitors examined at the concentrations indicated in Table II. The results are shown in Figs. 3 and 4. The line in each case is the least-square fit. The calculated values for the slopes and intercepts of these straight lines are summarized in Table II. The mean value of the intercepts is not significantly different from zero. An explicit relationship between ATP and K may therefore be written as:

$$K_{ad} = g \cdot ATP,$$

where g has a mean value of 20, as given by the slopes in Table II.

The tissue sodium contents were also measured in each case. The typical results are shown in Fig. 1 and follow the relationship in equation 4. It is seen that the (Na + K) contents are constant over the entire range of ATP values.

The above results establish the role of metabolism and ATP in the control of cell electrolyte levels. These results are explained quantitatively by assuming that ATP acts by binding to the controlling (or cardinal) sites. A simple cardinal site

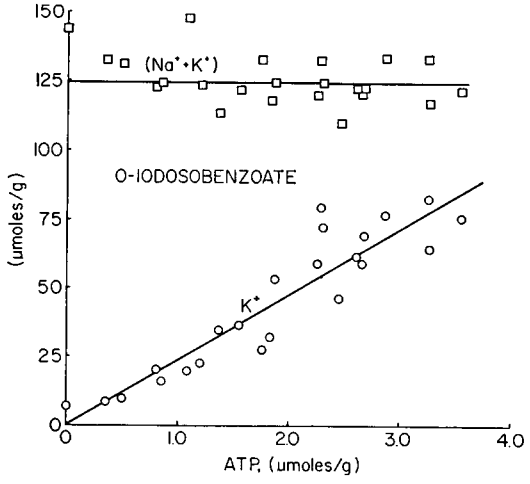

FIGURE 1

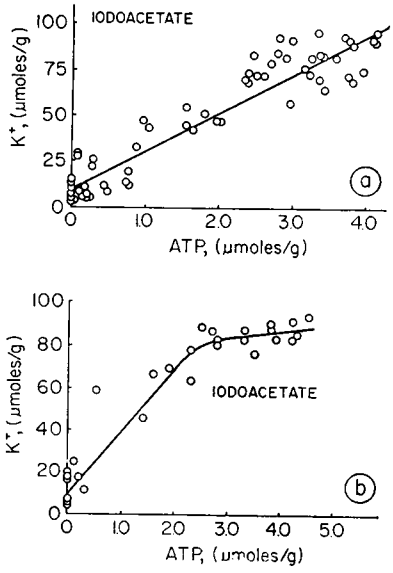

FIGURE 2

FIGURE 1 Relationships between tissue K, Na + K, and ATP contents in the presence of 0.01 mM *o*-iodosobenzoic acid. Each point represents one muscle. Na, K, and ATP were all measured on the same sample. The line is the least-square fit.

FIGURE 2 Relationships between tissue K and ATP contents in the presence of (*a*) 0.1 mM IAA and (*b*) 5 mM IAA. The straight line in *a* is the least-square fit. In *b* an arbitrary curve is drawn through the points.

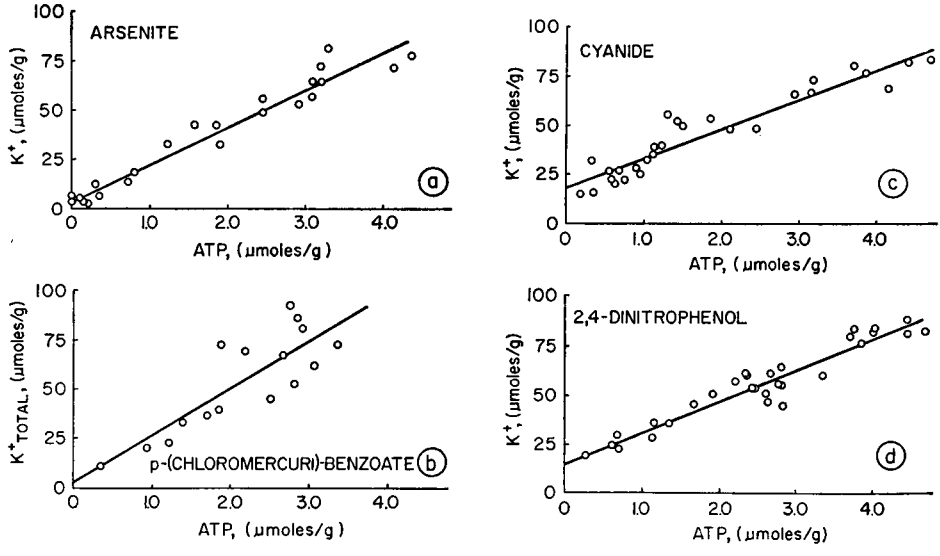

FIGURE 3 Relationships between K and ATP contents in the presence of (*a*) 10 mM arsenite, (*b*) 0.5 mM *p*-(chloromercuri)-benzoate, (*c*) 1.0 mM cyanide, and (*d*) 0.1 mM DNP. The least-square fit is indicated in each case.

model is able to describe these results. An integrated control of the cell function is known to involve an interaction among many cardinal sites. A quantitative description of this model has been presented elsewhere (Ling, 1970 *b*).

It is postulated in the association-induction hypothesis that a cardinal adsorbent controls the selectivity for adsorption of solutes on gangs of sites distributed throughout the cytoplasm. In the frog muscle, these sites are selective for K over Na with $K^{00}_{Na \to K} = 135$ (Ling and Bohr, 1970). The selectivity is controlled, in part, by the field strength of the sites (Ling, 1960; Eisenman, 1961; see also, Diamond and Wright, 1969). Gradual alteration in the strength of the negative sites induces progressive changes in the selectivity ratio for K over Na. Thus, the cardinal adsorbent can exert its effect on the selectivity by inducing alterations in the anionic field strength.

The uptake of K and Na by the sites follows a cooperative mechanism and the uptake of K can be completely described by equation 1 (Ling and Bohr, 1970; Jones, 1970) (see *Note added in proof*). The plot of K_{ad} as a function of $[K]_{ex}$ according to equation 1 results in a sigmoid-shaped curve which passes through the origin. As $[K]_{ex}$ is raised, K_{ad} increases gently at first, followed by a steep rise, and then approaches a saturation value ($= F_T$). The numerical value of the selectivity ratio is found from the expression:

$$K^{00}_{Na \to K} = \left\{ 1 \Big/ \frac{[K]_{ex}}{[Na]_{ex}} \right\}, \quad \text{at} \quad K_{ad} = \frac{F_T}{2}.$$

The value of $K^{00}_{Na \to K}$ determines the position of the curve. A decrease in the $K^{00}_{Na \to K}$ value, for instance, implies that a higher $[K]_{ex}$ is required to reach half-

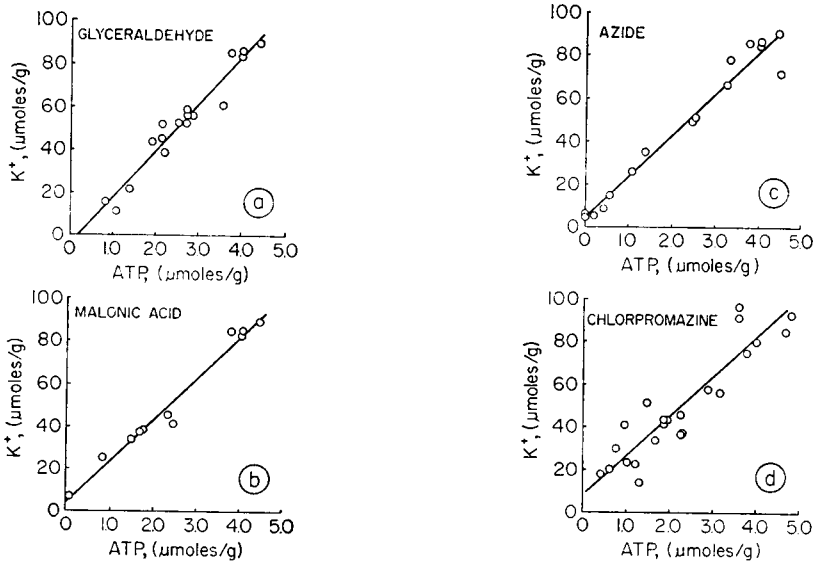

FIGURE 4 Relationship between K and ATP contents in the presence of (*a*) 10 mM glyceraldehyde, (*b*) 20 mM malonic acid, (*c*) 10 mM azide, and (*d*) 0.1 mM chlorpromazine. The line in each case is the least-square fit.

saturation. The sigmoidal plot obtained from equation 1, with the new lower value of $K^{00}_{Na \to K}$, will have shifted to the right.

The cardinal role of a controlling agent can thus be determined by studying its effect on the uptake of K in the presence of varying external potassium. With studies carried out in this manner, it was possible to show that calcium as well as ouabain may act as cardinal adsorbents in the control of the K accumulation process in vascular smooth muscle (Gulati, 1970; Gulati and Jones, 1971). Increasing concentrations of ouabain shifted the sigmoidal K-uptake curves towards the right. Increasing $[Ca]_{ex}$ levels, on the other hand, shifted these curves towards the left. It was shown that ouabain decreased and calcium increased $K^{00}_{Na \to K}$ values.

The action of ATP on the electrolyte accumulation process is also understood by assuming that ATP controls the selectivity of the adsorptive sites. The sites are assumed to be able to occur in two metastable conformational states. The field strength of the anionic sites is different in each conformation. When a cardinal site is occupied by ATP, the controlled gang of sites is in the state with higher selectivity for K over Na. Removal of ATP, by hydrolysis, may shift the system progressively towards the conformational state with lower selectivity for K over Na.

Note Added in Proof. In recent work on smooth muscle (guinea pig taeniae coli) in collaboration with Dr. I. L. Reisin, we have examined three predictions of equation 1. First, the distribution of K and Na shows a critical thermal transition as expected for *all* cooperative processes. The cellular electrolyte levels at temperatures around the transition were found to be in quantitative agreement with a prediction of equation 1. Second, thermal behavior of the smooth muscle was determined at $[K]_{ex}$ = 5 and 10 mM. The transition temperature (T_c) was lowered from 13.8°C at

$[K]_{ex}$ = 5 mM to 10.0°C at $[K]_{ex}$ = 10 mM. From equation 1, theoretically, T_c was predicted to be 13.4°C at 5 mM external potassium concentration and 9.7°C at 10 mM external potassium concentration. Third, from the expression determining $K^{00}_{Na \to K}$ it is seen that the sigmoidal potassium uptake curve as a function of $[K]_{ex}$ should be shifted to the left when $[Na]_{ex} + [K]_{ex}$ value is lowered. This was tested for $[Na]_{ex}$ = 150, 75, and 37.5 mM. Replacement for Na was made with osmotically equivalent sucrose. At all three Na levels, the $K^{00}_{Na \to K}$ value remained constant as expected and the sigmoidal relationship was shifted progressively to the left (I. L. Reisin, and J. Gulati, manuscript submitted for publication; see also J. Gulati et al. 1972. *Biophys. Soc. Annu. Meet. Abstr.* To be published.).

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