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Effects of External K Concentration on the Electrogenicity of the Insulin-Stimulated Na,K-Pump in Frog Skeletal Muscle

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Summary. Insulin hyperpolarized the membrane of frog skeletal muscle by stimulating the electrogenic Na,K-pump. At external K concentrations of 1, 2, 5 and 10 mM, both the insulin-induced hyperpolarization and the insulin-stimulated ouabain-sensitive Na efflux (an index of Na,K-pump activity) were observed. By increasing the external K concentration, the insulin-stimulated Na efflux increased, but the magnitude of the insulin-induced hyperpolarization decreased; i.e., although the activity of the insulin-stimulated Na,K-pump increased, on the contrary, the magnitude of the hyperpolarization decreased. To clarify the causes of this phenomenon, the specific membrane resistance was measured and found to decrease upon increasing the external K concentration.

One of the reasons for the decrease in magnitude of the hyperpolarization is the decrease in the specific membrane resistance. However, the decrease in magnitude of the hyperpolarization with a rise of the external K concentration, which increased the insulin-stimulated Na,K-pump activity, cannot be explained only by the decrease in the specific membrane resistance. It is suggested that the decrease in magnitude of the hyperpolarization is mainly caused by a decrease in the electrogenicity of the insulin-stimulated Na,K-pump upon an increase in the external K concentration. The conclusion of the present study is that the electrogenicity of the insulin-stimulated Na,K-pump in muscles is variable and decreases with increasing the external K concentration.

Key Words membrane potential · Na,K-pump · electrogenicity · membrane resistance · insulin · external K · skeletal muscle

Introduction

Insulin hyperpolarizes the membrane of rat skeletal muscles (Zierler, 1957, 1959). Moore and Rabovsky (1979) have reported that insulin induces a hyperpolarization of the membrane of frog skeletal muscle and that the hyperpolarization is caused by activating the electrogenic Na,K-pump without a significant change in the ratio of K^+ permeability to Na^+ permeability. Previous studies (Kitasato, Marunaka, Murayama & Nishio, 1980*a*; Marunaka & Kitasato, 1985) indicated that a rise of external K concentration increases the Na efflux mediated by the insulin-stimulated Na,K-pump, thus suggesting that the change in external K concentration has some effect on the magnitude of insulin-induced hyperpolarization. It was expected that the magnitude of insulin-induced hyperpolarization would increase with a rise of external K concentration, because a rise of external K concentration stimulates activity of the insulin-stimulated Na,K-pump (Kitasato et al., 1980*a*; Marunaka & Kitasato, 1985). In the present study, I investigated effects of external K concentration on the magnitude of insulin-induced hyperpolarization. Contrary to expectation, the magnitude of the hyperpolarization decreased upon increasing the external K concentration. While Na/K coupling of the Na,K-pump in erythrocytes is constant, as shown in the reports of Sen and Post (1964) and Post, Albright and Dayani (1967), various observations about coupling in muscles (Mullins & Noda, 1963; Beaugé, Sjodin & Ortiz, 1975; Beaugé & Sjodin, 1976; Lederer & Nelson, 1984) have suggested a variable coupling. The purpose of the present study was to estimate the Na/K coupling of the insulin-stimulated Na,K-pump in muscles at external K concentrations of 1, 2, 5 and 10 mM.

Materials and Methods

Solutions and Chemicals

The composition of Ringer's solutions used in the present study is summarized in Table 1. The pH was adjusted to 7.4 with Tris-HCl. The osmolarity was adjusted to 260 mOsm/liter with Tris-HCl and checked by the freezing point depression method. The concentration of insulin used was 500 mU/ml. Insulin was purchased from Sigma. $^{22}NaCl$ was purchased from New England Nuclear.

Muscles

Sartorius muscles from bullfrogs (*Rana catesbeiana*) were carefully dissected under a microscope. The wet weight of the muscle used was 200–300 mg; Table 2 shows the weight of some. The diameters of muscle fibers used were 30 to 50 μm; the diameter of 11 of those muscle fibers was 44.6 ± 2.8 μm (the mean ± SE).

Measurement of Water Content in the Intracellular Space and the Extracellular Space

Table 2 shows the wet weight (wet wt), the dry weight (dry wt) and the water content (*WC*) in the extracellular space (*ES*) of muscles, and the estimated value of the water content in the intracellular space (*IS*) of muscles. The wet weight and dry weight were measured by weighing before and after drying at 95°C for 3 hr, respectively. The dry weight of muscles was 20% of the wet weight. The water content in the extracellular space was estimated to be 25% of the wet weight by using ^{14}C-inulin. The water content in the intracellular space was estimated to be 55% of the wet weight, as follows:

The water content in *IS*
= (wet wt) − (dry wt) − (*WC* in *ES*)
= (1 − 0.2 − 0.25) × (wet wt)
= 0.55 × (wet wt)

Measurement of Membrane Potential

The membrane potential of muscles was measured by a glass microelectrode filled with 3 M KCl and with a resistance of 5–10 MΩ. The microelectrode was connected via a Ag-AgCl electrode to the input of a preamplifier (ME commercial, model ME-3211).

Estimation of the Rate Coefficient of ^{22}Na Efflux

The method of estimation of the rate coefficient of ^{22}Na efflux is described in the report of Kitasato, Sato, Murayama and Nishio (1980*c*). In short, sartorius muscles were soaked in 2.5 mM K-Ringer's solution containing ^{22}Na (0.1 mCi/mmol) for 3 hr at 4°C, then rinsed four times at intervals of 15 min with ^{22}Na-free 2.5 mM K-Ringer's solution for 60 min to wash out ^{22}Na from the extracellular space. The muscle was transferred from one tube containing the test solution to another. The radioactivity of ^{22}Na remaining in the muscle was determined by the "back add method" (Sjodin & Henderson, 1964). As adopted by Keynes (1965), the rate coefficient of ^{22}Na efflux was defined as the fraction of radioactivity remaining in the muscle. The radioactivity was counted by a gamma-counter (Packard, 5210).

Estimation of Intracellular Na Concentration

The method of estimation of intracellular Na concentration is similar to that reported by Keynes and Steinhardt (1968). The method is minutely described in a previous report (Kitasato et al., 1980*b*).

Estimation of Na Efflux

The Na efflux was estimated from the product of the rate coefficient of the ^{22}Na efflux and the intracellular Na concentration.

Measurement of Input Resistance and Estimation of Specific Membrane Resistance

The input resistance of the muscle was estimated by measuring the potential change (V') induced by passing the current (I') of ±2, ±4, ±6, ±8 and ±10 nA into the intracellular space of the muscle via the electrode. Namely, the magnitude of V' was plotted against that of I', and the slope (V'/I') was estimated. The slope constant was used as the magnitude of the input resistance.

Table 1. The composition of Ringer's solutions used

Ringer's solution	NaCl (mM)	KCl (mM)	$CaCl_2$ (mM)
1 mM K	110	1	2
2 mM K	110	2	2
5 mM K	110	5	2
10 mM K	110	10	2
2.5 mM K	110	2.5	2

Table 2. Estimation of water content in the intracellular space

	A	B	C	D	E
Muscle number	wet wt (mg)	dry wt (mg)	*WC* in *ES* (mg)	*WC* in *IS* (mg)	*D/A* (%)
1	281	53	70	158	56.2
2	279	56	67	156	55.9
3	215	45	58	112	52.1
4	220	43	59	118	53.6
5	295	58	68	169	57.3
6	289	58	70	161	55.7
Mean ± SE	263.2 ± 14.6	52.2 ± 2.7	65.3 ± 2.2	145.7 ± 9.9	55.1 ± 0.8

The relationship between input resistance ($\bar{R}$) and specific membrane resistance (R_m) is represented by

$$R_m = 8\pi^2 r^3 \bar{R}^2 / R_i \quad (1)$$

where r is a radius of muscle fiber and R_i is a specific resistance of myoplasma (Jenerick, 1953).

Estimation of Na/K Coupling of the Na,K-Pump

At steady state, the change in membrane potential caused by the Na,K-pump is expressed by

$$\Delta V_m = I_{passive} R_m \quad (2)$$

$$I_{passive} + F(J_{Na} - J_K) = 0 \quad (3)$$

where ΔV_m is the change in membrane potential caused by the Na,K-pump, R_m is the specific membrane resistance, $I_{passive}$ is the net passive current caused by ions transported by the Na,K-pump, J_{Na} is the Na efflux caused by the Na,K-pump, J_K is the K influx caused by the Na,K-pump, and F is Faraday's constant. From Eqs. (2) and (3), the following equation is deduced:

$$J_{Na}/J_K = \frac{1}{1 + \Delta V_m/(R_m F J_{Na})}. \quad (4)$$

Mullins and Noda (1963) have reported that the Na/K coupling is 3 under the normal condition. From their report, the Na/K coupling in 2 mM K-Ringer's solution was assumed to be 3. Using this, the Na/K coupling in 1, 5 and 10 mM K-Ringer's solutions was calculated.

Results

Effects of External K Concentration on Membrane Potential in the Presence and Absence of Insulin

Table 3 shows effects of external K concentration on membrane potential in the presence and absence of insulin. Preliminary experiments showed that insulin induced a change in membrane potential and the membrane potential attained a steady value within 40 min after addition of insulin. Moore and Rabovsky (1979) have also reported that the insulin-induced hyperpolarization reached its maximal value around 20 min after addition of insulin (250 mU/ml) and that for the following 30 min there was little change in the value of this hyperpolarization. To measure the maximal value of the insulin-induced hyperpolarization, the membrane potential was measured at 45 min after addition of insulin. The membrane potential in the presence of insulin shown in Table 3 is the value at that time. The membrane potentials at external K concentrations of 1, 2, 5 and 10 mM shown in Table 3 were those measured at 30 min after changing the external K concentration of 2.5 mM into the respective concentrations, because preliminary experiments showed that the membrane potential reached a steady value within 20 min after changing the concentration and that there was little change in the membrane potential for at least the following 20 min. At external K concentrations of 1, 2, 5 and 10 mM, insulin significantly hyperpolarized the membrane. The magnitude of the hyperpolarization decreased 1/4.9-fold upon increasing the external K concentration from 1 to 10 mM.

Effects of External K Concentration on the Rate Coefficient of ^{22}Na Efflux in the Presence and Absence of Insulin

The magnitude of insulin-induced increase in the rate coefficient of ^{22}Na efflux increased upon raising the external K concentration from 1 to 10 mM (Table 4). A previous report (Kitasato et al., 1980*d*) suggested that the insulin-induced increase in the rate coefficient of ^{22}Na efflux has reached its maximal value within 45 min after addition of insulin.

Table 3. Effects of external K concentration on the membrane potential (*MP*) in the presence and absence of insulin

	$[K]_o$ (mM)			
	1	2	5	10
MP (mV)				
Insulin	−108.9 ± 0.8(18)	−98.5 ± 0.3(14)	−80.9 ± 0.2(12)	−58.0 ± 0.4(32)
Control	−104.0 ± 1.0(18)	−94.5 ± 0.3(14)	−79.9 ± 0.4(12)	−57.0 ± 0.3(32)
Difference	−4.9	−4.0	−1.0	−1.0
	$P < 0.001$	$P < 0.001$	$P < 0.025$	$P < 0.05$

Each value is represented as the mean ± 1 SE of the mean (n), where n is the number of experiments. The membrane potential was measured at 22°C.

Therefore, to measure the maximal effect of insulin, the rate coefficient of ^{22}Na efflux was estimated around 45 min after its addition. The rate coefficient of ^{22}Na efflux at external K concentrations of 1, 2, 5 and 10 mM was estimated around 30 min after changing the external K concentration of 2.5 mM to the respective concentrations (Table 4).

Effects of External K Concentration on Intracellular Na Concentration in the Presence and Absence of Insulin

Table 5 shows the intracellular Na concentrations at external K concentrations of 1, 2, 5 and 10 mM in the presence and absence of insulin. The intracellular Na concentration in the presence of insulin is the value at 45 min after addition of insulin (Table 5). The intracellular Na concentrations at external K concentrations of 1, 2, 5 and 10 mM are the values obtained 30 min after changing the external K concentration of 2.5 mM to the respective concentrations. A rise of external K concentration had no significant effect on intracellular Na concentration irrespective of the presence of insulin. Insulin had no significant effect on the intracellular Na concentration.

Effects of External K Concentration on Insulin-Stimulated Na Efflux

Table 6 shows insulin-stimulated Na effluxes at external K concentrations of 1, 2, 5 and 10 mM. The insulin-stimulated Na efflux increased twofold upon raising the external K concentration from 1 to 10 mM.

Effects of External K Concentration on Membrane Resistance in the Presence and Absence of Insulin

With a rise of external K concentration, the magnitude of insulin-stimulated Na efflux increased, while the absolute value of insulin-induced hyperpolarization decreased. If electrogenicity of insulin-stimulated Na,K-pump does not change by increasing external K concentration, the data shown in

Table 4. Effects of external K concentration on the rate coefficient of ^{22}Na efflux in the presence and absence of insulin

	$[K]_o$ (mM)			
	1	2	5	10
Rate coefficient of ^{22}Na efflux (10^{-5}/sec)				
Insulin	12.4 ± 0.2	14.0 ± 0.3	18.8 ± 0.3	19.1 ± 0.4
Control	10.2 ± 0.2	11.2 ± 0.2	13.6 ± 0.4	13.8 ± 0.1
Difference	2.2	2.8	5.2	5.3
	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Each value is represented as the mean of 5 experiments ± 1 SE of the mean. The rate coefficient of the ^{22}Na efflux was measured at 22°C.

Table 5. Effects of external K concentration on intracellular Na concentration in the presence and absence of insulin

	$[K]_o$ (mM)			
	1	2	5	10
$[Na]_i$ (mmol/kg muscle water)				
Insulin	11.1 ± 1.1	10.2 ± 1.3	9.6 ± 0.9	9.0 ± 1.0
Control	11.5 ± 1.7	10.7 ± 1.0	9.9 ± 0.9	9.5 ± 1.1
	NS	NS	NS	NS

Each value is represented as the mean of 4 experiments ± 1 SE of the mean. The intracellular Na concentration was measured at 22°C. The intracellular Na concentration is not significantly affected by the external K concentration (1–10 mM), irrespective of the presence of insulin.

Tables 3 and 6 would be caused by a decrease in the specific membrane resistance. Since the magnitude of the insulin-stimulated Na efflux increased two-fold and the absolute value of the insulin-induced hyperpolarization decreased 1/4.9-fold upon increasing the external K concentration from 1 to 10 mM, measurements were made to see whether the specific membrane resistance decreased 1/9.8-fold [(1/4.9) × (1/2)].

To estimate the specific membrane resistance, the input resistance was measured. Table 7 shows effects of external K concentration on input resistance in the presence and absence of insulin. The input resistances at external K concentrations of 1, 2, 5 and 10 mM shown in Table 7 are the values obtained 30 min after changing the external K concentration of 2.5 mM to the respective concentrations. The input resistance in the presence of insulin shown in Table 7 is the value obtained 45 min after addition of insulin. Upon a rise of external K concentration, the magnitude of input resistance decreased irrespective of the presence or absence of insulin. Insulin had no significant effect on the input resistance at external K concentrations of 1, 2, 5 and 10 mM at 45 min after addition of insulin. Assuming that the specific resistance of myoplasma does not change upon increasing external K concentration (Jenerick, 1953), the relative specific membrane resistance was estimated from the input resistance.

Table 8 shows effects of external K concentration on the relative specific membrane resistance. It was calculated by using Eq. (1) with the data shown in Table 7. The specific membrane resistance decreased only about 1/1.8-fold upon increasing external K concentration from 1 to 10 mM. The magnitude of the decrease is much smaller than that (1/9.8-fold) expected on the presumption that the electrogenicity of the insulin-stimulated Na,K-pump does not change by increasing the external K concentration.

Effects of External K Concentration on Electrogenicity of the Insulin-Stimulated Na,K-Pump

Table 9 shows effects of external K concentration on electrogenicity of the insulin-stimulated Na,K-pump. The Na/K coupling at external K concentrations of 1, 5 and 10 mM is estimated, assuming that Na/K coupling at external K concentration of 2 mM is 3 (Mullins & Noda, 1963). Upon increasing external K concentration from 1 to 10 mM, the Na/K coupling approached 1; namely, the electrogenicity of the insulin-stimulated Na,K-pump decreased.

The observations in the present study suggest that the insulin-stimulated electrogenic Na,K-pump in frog skeletal muscles approaches an electroneutral pump upon increasing external K concentration from 1 to 10 mM, and that the decrease in magnitude of insulin-induced hyperpolarization upon raising external K concentration is caused not just by the decrease in the specific membrane resistance but mainly by the decrease in the electrogenicity of the insulin-stimulated Na,K-pump.

Table 6. Effects of external K concentration on Na efflux in the presence and absence of insulin

	$[K]_o$ (mM)			
	1	2	5	10
Na efflux (nmol/kg muscle water/sec)				
Insulin	1376	1428	1805	1719
Control	1173	1198	1346	1311
Difference	203	230	459	408

The Na efflux in the presence and absence of insulin was estimated by using the data shown in Tables 4 and 5.

Table 7. Effects of external K concentration on the input resistance in the presence and absence of insulin

	$[K]_o$ (mM)			
	1	2	5	10
Input resistance (MΩ)				
Insulin	2.9 ± 0.4(9)	2.6 ± 0.3(10)	2.3 ± 0.2(9)	2.2 ± 0.2(11)
Control	2.8 ± 0.4(9)	2.5 ± 0.4(9)	2.4 ± 0.2(9)	2.1 ± 0.4(9)
	NS	NS	NS	NS

Each value is represented as the mean ± 1 SE of the mean (n), where n is the number of experiments. The magnitude of the current passed into the intracellular space was ±2, ±4, ±6, ±8 and ±10 nA. The input resistance was measured at 22°C.

Table 8. Effects of external K concentration on the relative specific membrane resistance in the presence and absence of insulin

	$[K]_o$ (mM)			
	1	2	5	10
Relative specific membrane resistance				
Insulin	1.35	1.08	0.85	0.77
Control	1.25	1.00	0.92	0.71

Each value is the specific membrane resistance relative to that of 2 mM external K in the control. The relative specific membrane resistance was estimated by using Eq. (1) with the mean value of the input resistance shown in Table 7.

Table 9. Effects of external K concentration on Na/K coupling of the insulin-stimulated Na,K-pump

	$[K]_o$ (mM)			
	1	2	5	10
Na/K coupling	3.8	3	1.1	1.2

Assuming that Na/K coupling at the external K concentration of 2 mM is 3 (Mullins & Noda, 1963), each value is estimated by using Eq. (4) with the data shown in Tables 3, 6 and 8.

Discussion

Both insulin-stimulated Na efflux and insulin-induced hyperpolarization are sensitive to ouabain (Marunaka & Kitasato, 1985; Moore & Rabovsky, 1979). These observations suggest that insulin induces changes in Na efflux and membrane potential by activating the Na,K-pump.

Jenerick (1953) has reported effects of external K concentration on input resistance. The value of the input resistance reported by him is one-seventh of that in the present report. One of the reasons for this is the difference in the diameters of muscle fibers. However, the relative change in the input resistance upon changing the external K concentration reported by Jenerick is comparable to that shown here. Therefore, the difference of values for the input resistance has no effect on the calculation of the Na/K coupling.

In the present report, effects of external K concentration on Na/K coupling are studied. As a rise of external K concentration depolarizes the membrane, effects of membrane potential on Na/K coupling must be considered. Brinley and Mullins (1974) have reported that in squid axons the Na,K-pump activity is independent of the membrane potential. As shown by Beaugé et al. (1975) and Beaugé and Sjodin (1976), the activation of the Na,K-pump in frog skeletal muscles by a rise of external K concentration is independent of external K-induced change in the membrane potential. Lederer and Nelson (1984) have also reported that the Na transport mediated by the Na,K-pump is not voltage-dependent in barnacle muscles. These reports suggest that the stimulatory effect of increasing external K concentration on ouabain-sensitive Na efflux is not developed by depolarizing the membrane. Therefore, depolarization of the membrane induced by increasing the external K concentration may be disregarded in investigating effects of external K concentration on Na/K coupling.

A rise of external K concentration had no significant effect on intracellular Na concentration; however, the mean value of the intracellular Na concentration slightly decreased with a rise in external K concentration independent of the presence of insulin. Therefore, it follows that intracellular Na concentration has an effect on Na efflux. A decrease in intracellular Na concentration reduces Na efflux (Keynes & Swan, 1959; Mullins & Frumento, 1963; Kitasato et al., 1980*d*; Eisner, Lederer & Vaughan-Jones, 1981; Marunaka & Kitasato, 1985). In fact, within the range of intracellular Na concentration shown in the present report (about 9–12 mmol/kg muscle water), a change in Na efflux is observed (Kitasato et al., 1980*d*; Marunaka & Kitasato, 1985). The mean value of intracellular Na concentration decreases with a rise of external K concentration (Table 5). Therefore, under the condition where intracellular Na concentration at high external K concentration is the same as that at low external K concentration, Na efflux at high external K concentration would be larger than the observed value shown in Table 6. However, even if the observed value of Na efflux is underestimated and the underestimated value of Na efflux at high external K concentration is compensated for, Na/K coupling at only the high external K concentration approaches 1, and the conclusion reached in the present study is essentially the same.

In addition, effects of intracellular Na concentration on Na/K coupling must be argued. This is because Na/K coupling is dependent on intracellular Na concentration (Mullins & Brinley, 1969); i.e., Na/K coupling increases with a rise in intracellular Na concentration. The present study shows that the Na/K coupling at the low external K concentration is larger than that at the high external K concentration. This may be caused by increasing intracellular Na concentration, because at low external K concentration the mean value of intracellular Na concentration was slightly larger than that at high exter-

nal K concentration, although the intracellular Na concentration was not significantly affected by changing the external K concentration (1–10 mM).

I investigated whether the large value of Na/K coupling at low external K concentration was induced by the direct effect of lowering external K concentration or by its indirect effect through increasing the intracellular Na concentration; i.e., to remove effects on Na/K coupling of increasing intracellular Na concentration by lowering external K concentration, insulin-induced hyperpolarization at low external K concentration was measured under the condition that intracellular Na concentration was lower than that at high external K concentration. At the intracellular Na concentration (about 5–6 mmol/kg muscle water; Kitasato et al., 1980*b*), insulin hyperpolarized the membrane about 2 mV at an external K concentration of 1 mM (my preliminary observation). This shows that, even if the intracellular Na concentration is low (5–6 mmol/kg muscle water), the absolute value of the insulin-induced hyperpolarization at an external K concentration of 1 mM is larger by about twofold than that at high external K concentrations of 5 and 10 mM (Table 3) under the condition that the intracellular Na concentration is 9.0–9.6 mmol/kg muscle water (Table 5), which is higher than 5–6 mmol/kg muscle water. On the other hand, the value of the insulin-stimulated Na efflux at low external K (1 mM) and low intracellular Na (5–6 mmol/kg muscle water) concentrations is smaller by 1/2-fold or much more than that at high external K (5 and 10 mM) and normal intracellular Na (9–12 mmol/kg muscle water) concentrations (Marunaka & Kitasato, 1985).

Needless to say, these observations cannot be explained only by a change in the specific membrane resistance; namely, even under the condition that the intracellular Na concentration is low, Na/K coupling at low external K concentration (1 mM) is larger than that at high external K (5 and 10 mM) and normal intracellular Na concentrations. This suggests that lowering of the external K concentration itself directly increases Na/K coupling even if the lowering of external K concentration does not raise intracellular Na concentration. The large value of the Na/K coupling at low external K concentration (1 mM) may be caused partially by the large mean value of intracellular Na concentration; i.e., the slight change in the mean value of intracellular Na concentration may increase Na/K coupling. However, as mentioned above, Na/K coupling at the low external K concentration is larger than that at the high external K concentration even under the condition that the large mean value of intracellular Na concentration at low external K concentration is cancelled. Therefore, the conclusion of the present study is not essentially changed.

It is generally known that Na/K coupling of the Na,K-pump in erythrocytes is 3/2, as shown in the reports of Gardos (1964), Sen and Post (1964), Whittam and Ager (1965) and Post et al. (1967). Hodgkin and Keynes (1955) have reported the same observation in giant axons from *Sepia* and *Loligo*. On the other hand, according to the report of Sjodin and Beaugé (1967), Na/K coupling of the Na,K-pump is 2 in squid giant axon. In frog muscles, it has been reported that Na/K coupling is 3 (Mullins & Noda, 1963). Lederer and Nelson (1984) have reported that Na/K coupling is between 3/2 and slightly more than 2 in barnacle muscles. Mullins and Brinley (1969) have reported that at high intracellular Na concentration the Na/K coupling is 3 and at normal intracellular Na concentration, 3/2 in squid axons, which suggests that Na/K coupling is variable with a change in ion environment. In the present study, it is concluded that Na/K coupling of the insulin-stimulated Na,K-pump is variable and approaches 1 as the external K concentration is raised from 1 to 10 mM.

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