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Effects of choline on Na^+- and K^+-interactions with the Na^+/K^+-ATPase

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Choline chloride, 100 mM, stimulates Na^+/K^+-ATPase activity of a purified dog kidney enzyme preparation when Na^+ is suboptimal (9 mM Na^+ and 10 mM K^+) and inhibits when K^+ is suboptimal (90 mM Na^+ and 1 mM K^+), but has a negligible effect at optimal concentrations of both (90 mM Na^+ and 10 mM K^+). Stimulation occurs at low Na^+ to K^+ ratios, but not at those same ratios when the actual Na^+ concentration is high (90 mM). Stimulation decreases or disappears when incubation pH or temperature is increased or when Li^+ is substituted for K^+ or Rb^+. Choline$^+$ also reduces the K_m for MgATP at the low ratio of Na^+ to K^+ but not at the optimal ratio. In the absence of K^+, however, choline$^+$ does not stimulate at low Na^+ concentrations: either in the Na^+-ATPase reaction or in the E_1 to E_2P conformational transition. Together, these observations indicate that choline$^+$ accelerates the rate-limiting step in the Na^+/K^+-ATPase reaction cycle, K^+-deocclusion; consequently, optimal Na^+ concentrations reflect Na^+ accelerating that step also. Thus, the observed $K_{0.5}$ for Na^+ includes high-affinity activation of enzyme phosphorylation and low-affinity acceleration of K^+-deocclusion. Inhibition of Na^+/K^+-ATPase and K^+-nitrophenylphosphatase reactions by choline$^+$ increases as the K^+-concentration is decreased; the competition between choline$^+$ and K^+ may represent a similar antagonism between conformations selected by choline$^+$ and by K^+.

Introduction

The reaction sequence of the Na^+/K^+-ATPase includes Na^+-activated phosphorylation of the enzyme by ATP, K^+-activated dephosphorylation, and cycling between two families of enzyme conformation [1–3]:

$$E_1 \xrightarrow[+ATP]{Na^+} E_1P \rightarrow E_2P \xrightarrow[-P_i]{K^+} E_2 \rightarrow E_1$$

After the dephosphorylation step, K^+ is bound to E_2 in an 'occluded' form, such that it exchanges only slowly [4–6]. Deocclusion of K^+ is accelerated by ATP binding at low-affinity sites on E_2, but under usual assay conditions *in vitro* deocclusion is the rate-limiting step in the overall Na^+/K^+-ATPase cycle [4–7].

For transport and enzymatic studies on this enzyme it is often necessary to maintain ionic and osmotic strength constant, and thus when Na^+, K^+, or Mg^{2+} is being varied the problem arises of finding an 'inactive' cation, which neither stimulates nor inhibits, to vary reciprocally. Choline$^+$ is frequently chosen, despite reports of its Na^+-like effects [8,9], but we recently showed that choline$^+$ could either stimulate or inhibit Na^+/K^+-ATPase activity, depending on the concentrations of Na^+ and K^+ and their ratio [10].

Here we describe experiments designed to characterize the effects of choline$^+$ on the enzyme, with emphasis on the stimulation occurring with suboptimal Na^+ but optimal K^+. The results, from studies examining the various potential modes of interaction, indicate that choline$^+$ promotes K^+-deocclusion under those circumstances. The observations thus bear not only on the practical concerns for an 'inactive' cation but also on general issues of the reaction mechanism: as studied *in vitro*, a significant role for Na^+ – beyond activation

Abbreviations: CHES, 2-(*N*-cyclohexylamino)ethanesulfonic acid; Et_3N, triethylamine; Hepes, 4-(2-hydroxyethyl)-1-piperazinesulfonic acid; IAF, 5-iodoacetamidofluorescein; Mes, 2-(*N*-morpholino)ethanesulfonic acid; NMG, *N*-methylglucamine.

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of enzyme phosphorylation – is promoting K^+-deocclusion, a role in which choline$^+$ can substitute when Na^+ concentrations are suboptimal.

Methods and Materials

The enzyme preparation was obtained from the medullae of frozen canine kidneys [11].

Na^+/K^+-ATPase activity was assayed in terms of P_i production measured spectrophotometrically [12], routinely at 37 °C. The standard medium contained 20 mM Hepes-Et_3N (pH 7.5), 0.1 mM EGTA, 3 mM ATP (Et_3N salt), 3 mM $MgSO_4$, 90 mM NaCl, and 10 mM KCl. K^+-nitrophenylphosphatase activity was assayed in terms of nitrophenol production measured spectrophotometrically [13], at 37 °C. The standard medium was the same as that for the Na^+/K^+-ATPase reaction except that 3 mM nitrophenyl phosphate (Et_3N salt) was substituted for ATP and NaCl was omitted. Data presented are the means of four or more experiments each performed in duplicate or triplicate, and are listed ± S.E. where appropriate.

Na^+-ATPase activity was assayed in terms of P_i production measured fluorometrically with a linked enzyme assay [14], at 25 °C. The incubation media contained 25 mM imidazole-HCl (pH 7.0), 1 mM EDTA, 4 mM $MgCl_2$, 1 mM ATP, the concentrations of NaCl and choline chloride indicated, 45 μM 7-methylguanosine, and 0.1–0.3 units/ml nucleoside phosphorylase; the reaction was initiated by adding Na^+/K^+-ATPase enzyme and the fluorescence was recorded over time with a Perkin-Elmer MPF-66 spectrophotofluorimeter (excitation at 300 nm, emission at 410 nm).

Presteady-state kinetic measurements were made at 24 °C using enzyme labeled with IAF [7] and a Kinetics Instrument stopped flow fluorimeter, as previously described [10]. IAF-labeled enzyme in 25 mM imidazole-HCl (pH 7.0), 1 mM EDTA, 4 mM $MgCl_2$, and the concentrations of NaCl and choline chloride indicated, was rapidly mixed with an equal volume of the same buffer and salt composition (no enzyme) plus 2 mM ATP (final concentration 1 mM). Excitation was at 492 nm, emitted light was recorded after passage through a Corning 69 cut-off filter (528 nm), and the time course of the fluorescence change after mixing was measured; 6–8 traces were recorded and the rate constant for a single-exponential transition, k, was then calculated.

Frozen kidneys were obtained from Pel Freeze; ATP, CTP, nitrophenyl phosphate, oligomycin, Hepes, Mes, CHES, 7-methylguanosine, and calf spleen nucleoside phosphorylase from Sigma; IAF from Molecular Probes; and Me_2SO and choline chloride from Fisher (choline chloride solutions were made fresh daily).

Results and Discussion

Comparison of choline$^+$ with other cations

Adding choline chloride to Na^+/K^+-ATPase incubation media can either stimulate or inhibit activity, depending on the concentrations of Na^+ and K^+ and their ratio (Table I), as previously reported [10]. Other organic salts commonly used in buffers or to maintain osmotic or ionic strength can also affect enzymatic activity, but diversely: Et_3N-HCl and Hepes-Et_3N inhibit more than choline chloride when Na^+ is 90 mM and K^+ is 1 mM, whereas NMG-HCl and Tris-HCl inhibit less; on the other hand, none stimulates more than choline$^+$ when Na^+ is 9 mM and K^+ is 10 mM (Table I). The major aim of this paper is an examination of such stimulation occurring at suboptimal ratios of Na^+ to K^+, and thus we shall concentrate on the effects of choline$^+$.

A further comparison is pertinent. Both the inhibition by choline$^+$ at suboptimal K^+ and the stimulation at suboptimal Na^+ are similar to the effects of added Na^+ (Table II). Moreover, the effects of choline$^+$, although a quaternary amine, are directly opposite to those of NH_4^+. The ability of choline$^+$ to favor Na^+-induced enzyme conformations has previously been reported, in terms of both tryptic digestion patterns [8] and enzyme fluorescence changes [9].

Before considering the various mechanisms by which choline$^+$ might stimulate or inhibit, the concentration-dependence of these processes should be noted (Fig. 1). The choline$^+$ concentration for half-maximal stimulation, when Na^+ is 9 mM and K^+ is 10 mM, cannot be determined precisely because of the biphasic response; however, it is at least 30 mM. The inhibition at higher choline$^+$ concentrations is similar to that with 90 mM Na^+ and 1 mM K^+, where the half-maximal concentration is 0.3 M. Such inhibition cannot be due to ionic strength effects on the enzyme generally, for no inhibition occurs at these choline$^+$ concentrations when Na^+

TABLE I

Effects of organic cations on Na^+/K^+-ATPase activity as a function of [Na^+] and [K^+]

Enzyme was incubated at 37 °C in the standard medium modified to contain the concentrations of Na^+ and K^+ listed, in the absence and presence of the cations listed. Relative activities with 90:1 and 9:10 ratios of [Na^+] to [K^+] are, respectively, 0.42, 1.00, and 0.58 (in the absence of added organic cations).

[Na^+]/[K^+] (mM)	Percent stimulation or inhibition due to 100 mM:					
	Et_3N-HCl	Hepes-Et_3N	Imidazole-HCl	Choline chloride	NMG-HCl	Tris-HCl
90· 1	−60±7	−54±4	−26± 3	−29±5	+2±4	−2±6
90:10	−22±7	−8±4	+5±10	+3±4	+5±1	+6±7
9:10	+15±6	+14±7	+25± 7	+43±6	+26±3	+19±6

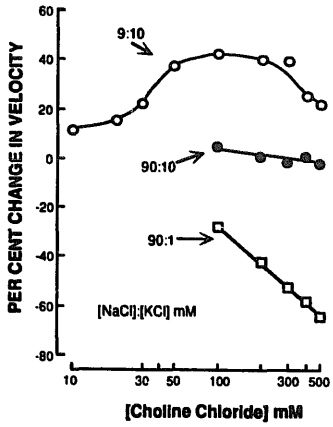

Fig. 1. Effects of choline$^+$ concentration on Na^+/K^+-ATPase activity. Enzyme was incubated in the standard medium modified to contain the concentrations of choline chloride indicated, and with 90 mM NaCl plus 10 mM KCl (●) or 9 mM NaCl plus 10 mM KCl (○) or 90 mM NaCl plus 1 mM KCl (□). The percent change due to adding choline$^+$ is plotted against the choline$^+$ concentration.

is 90 mM and K^+ is 10 mM. Thus, stimulation and inhibition seem each to reflect distinct consequences of Na^+- and K^+-dependent processes.

Choline$^+$ and the ratio of [Na$^+$] to [K$^+$]

Na^+ acts as a competitor to K^+ and K^+ as a competitor to Na^+ [15,16]. Thus, the observed stimulation by choline$^+$ might be due to relief of K^+-inhibition and the observed inhibition to increased Na^+-inhibition, in accord with the Na^+-like effects of choline$^+$ (Table II). With 9 mM Na^+, the effect of added choline$^+$ does range from inhibition when the K^+-concentration is 0.1 mM (a 90:1 molar ratio) to stimulation when the K^+-concentration is 10 mM (a 9:10 molar ratio) (Fig. 2A, open circles). There is a decrease in the stimulation at still higher K^+-concentrations, and this decrease parallels a fall in control ATPase activity (absence of choline$^+$) at high K^+ to Na^+ ratios (Fig. 2A, open squares).

With 90 mM Na^+, however, choline$^+$ does not stimulate at corresponding Na^+ to K^+ ratios (e.g., 90 mM Na^+ and 100 mM K^+: Fig. 2A, solid circles), yet there is a similar decrease in control ATPase activity at those higher K^+ to Na^+ ratios (Fig. 2A, solid squares). Although the inhibition by choline$^+$ does follow the [Na^+] to [K^+] ratio, the stimulation is a function also of the absolute concentrations of Na^+ (i.e., it is absent with 90 mM Na^+ at all ratios) and of K^+ (stimulation decreases or is absent above 30 mM K^+).

TABLE II

Effects of inorganic cations on Na^+/K^+-ATPase activity as a function of [Na^+] and [K^+]

Experiments were performed as in Table I, but in the absence and presence of the inorganic cations listed (as chloride salts); choline chloride is included for comparison.

[Na^+]/[K^+] (mM)	Percent stimulation or inhibition due to 200 mM:				
	K^+	NH_4^+	Li^+	Choline$^+$	Na^+
90: 1	+59±8	+80±8	+32±4	−42±6	−60±1
90:10	−35±1	−14±2	−25±2	+4±1	−10±2
9:10	−73±3	−63±4	−60±3	+44±3	+91±8

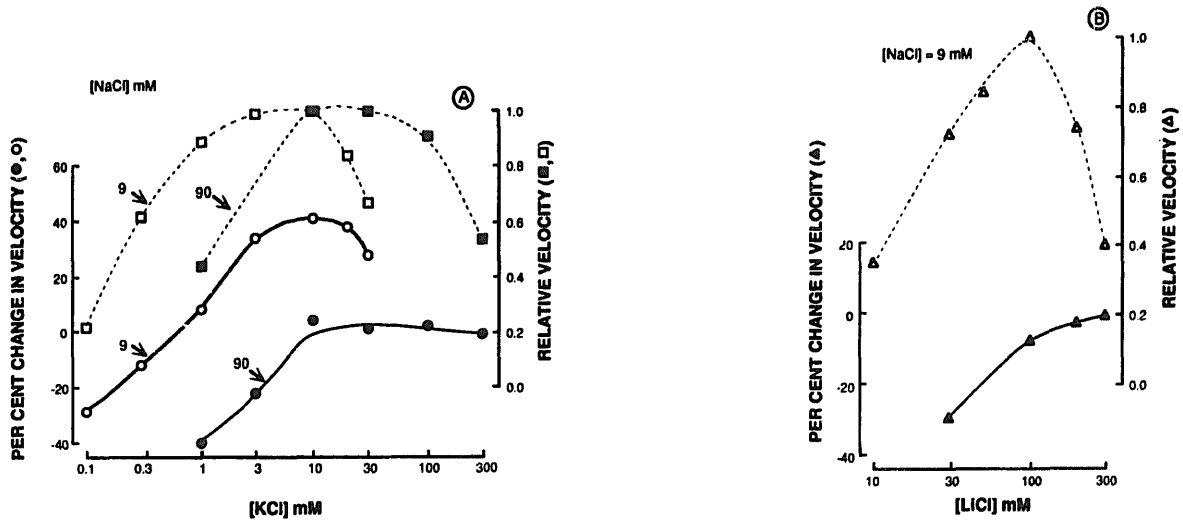

Fig. 2. Effects of Na^+ and K^+ concentrations on the response to choline$^+$ and on ATPase activity. In panel A are illustrated experiments like those in Fig. 1, in the absence and presence of 100 mM choline chloride, with the concentrations of KCl indicated, and either 90 mM NaCl (●, ■) or 9 mM NaCl (○, □). Against the lefthand ordinate are shown the changes in velocity due to adding choline$^+$, with 90 mM (●) or 9 mM (○) NaCl. Against the righthand ordinate are shown the Na^+/K^+-ATPase velocities in the absence of choline, with 90 mM (■) or 9 mM (□) NaCl, expressed relative to the velocity with 10 mM KCl defined as 1.0. In panel B are illustrated similar experiments but with LiCl substituted for KCl, all with 9 M NaCl. Against the lefthand axis are shown the changes in velocity due to adding choline$^+$ (▲), and against the righthand ordinate the Na^+/Li^+-ATPase velocity expressed relative to that with 100 mM LiCl (△).

TABLE III

Effects of pH and temperature on the response to choline $^+$

Enzyme was incubated at 37 or 20 °C in the standard medium modified to contain the concentrations of Na^+ and K^+ or Rb^+ listed, at either pH 6.5 (20 mM TAPS-Et_3N), 7.5 (20 mM Hepes-Et_3N), or 9.0 (20 mM CHES-Et_3N), and in the absence or presence of 100 mM choline$^+$.

$[Na^+]/[K^+]$ or $[Rb^+]$ (mM)	Percent stimulation or inhibition due to 100 mM choline$^+$			
	at 37 °C			at 20 °C
	pH 6.5	pH 7.5	pH 9.0	pH 7.5
90: 1 K^+	−20±3	−27±5	−33±7	+6±4
90:10 K^+	+12±3	+4±4	−1±4	+11±5
9:10 K^+	+47±7	+41±6	+11±5	+63±7
90:10 Rb^+	+11±4	+6±4	−3±5	-
9:10 Rb^+	+38±6	+58±4	+14±6	-

The nature of the interaction between choline$^+$ and K^+ is further illuminated when two K^+-like cations are substituted. When Li^+ is substituted for K^+, higher concentrations of Li^+ are required because of the lower affinity; nevertheless, choline$^+$ does not stimulate over the range 30 to 300 mM Li^+ in the presence of 9 mM Na^+, which includes suboptimal, optimal, and inhibitory concentrations for Na^+/Li^+-ATPase activity (Fig. 2B). By contrast, when 10 mM Rb^+ is substituted for K^+ in the presence of 9 mM Na^+ (Table III), choline$^+$ stimulates as with K^+ (the apparent affinity for Rb^+ is very close to that for K^+ [16] while the V_{max} at neutral pH is about half [6]). Thus, with a low concentration of Na^+, choline$^+$ stimulates when Rb^+ or K^+ are present but not when Li^+; the implications of this will be considered further, below, in terms of cation occlusion.

Effects of choline$^+$ on apparent affinity for Na^+

The stimulation by choline$^+$ with 9 mM but not 90 mM Na^+ (Tables I and II) represents a decrease in $K_{0.5}$ for Na^+ due to choline$^+$: from 7.5 mM in the absence of choline$^+$ to 3.4 mM with 100 mM choline$^+$ (data not shown). Raising the incubation pH decreases the $K_{0.5}$ for Na^+ [10], and at pH 9.0 choline$^+$ no longer stimulates with 9 mM Na^+ and either 10 mM K^+ or Rb^+ (Table III). Lowering the incubation temperature increases the $K_{0.5}$ for Na^+ [10], and 100 mM choline$^+$ stimulates with 9 mM Na^+ and 10 mM K^+ more at 20° than at 37 °C (Table III). The sensitivity of stimulation by choline$^+$ to pH and temperature is thus consistent with choline$^+$ antagonizing the effects of these changes on $K_{0.5}$ for Na^+. However, pH and temperature affect other enzymatic properties in addition to the $K_{0.5}$ for Na^+ (see below).

Effects of choline$^+$ on activation by Na^+ in the absence of K^+

Choline$^+$ cannot substitute for Na^+ in activating enzyme phosphorylation [17], but it might stimulate at low Na^+ concentrations by increasing the Na^+-affinity, as suggested by the preceding experiments. To determine whether choline$^+$ can stimulate at suboptimal levels of Na^+ in the absence of K^+ two processes were examined. (i) The overall rate of enzyme phosphorylation (the E_1 to E_1P step) and the subsequent conformational transition (to E_2P) can be followed by stopped-flow fluorescence changes in the IAF-labeled enzyme [7,10]. With a suboptimal Na^+ concentration, 5 mM, adding 150 mM choline$^+$ increases the rate of the E_1 to E_2P transition negligibly (Table IV). (ii) Steady-state measurements of Na^+-ATPase activity at suboptimal levels of Na^+ (measured in the absence of added K^+) also reveal no stimulation by choline$^+$ (Table IV). Thus, choline$^+$ does not appear to increase the affinity for Na^+ in these activities where K^+ is absent.

Effects of inhibitors of conformational transitions

As cited above, choline$^+$ does appear to favor Na^+-induced enzyme conformations in the absence of Na^+ and K^+. Two inhibitors of the Na^+/K^+-ATPase affect the transitions between E_1 and E_2 enzyme conformations: oligomycin favors E_1 conformations [18], at least in part by stabilizing the E_1(3Na) form [19]; and Me_2SO favors E_2 conformations [20–22]. However, adding either oligomycin or Me_2SO decreases or abolishes the stimulation by choline$^+$ in the presence of 9 mM Na^+ and 10 mM K^+ (Table V). Moreover, the inhibition by choline$^+$ in the presence of 90 mM Na^+ and 1 mM K^+ is increased when oligomycin or Me_2SO is added. Although the actions of these inhibitors are complex and undoubtedly include effects beyond the conformational transitions, these results with oligomycin and

TABLE IV

Effects of choline$^+$ in the absence of K^+

The rate constant for the fluorescence change from E_1 to E_2P was measured with a stopped-flow fluorimeter using IAF-labeled enzyme, with the concentrations of NaCl and choline chloride listed, as described under Methods. Na^+-ATPase activity was measured in the absence of KCl and the presence of the NaCl and choline chloride concentrations listed, using a coupled enzyme assay as described under Methods, and is expressed relative to the velocity with 155 mM Na^+ and no choline$^+$.

$[Na^+]/[choline^+]$ (mM)		Rate constant for fluorescence change (s^{-1})	Relative Na^+-ATPase activity
5	0	28.3±1.7	0.42±0.02
5	150	30.3±2.4	0.40±0.01
155	0	79.2±2.5	1.00
155	150	-	0.67±0.03
305	0	-	1.22±0.06

Me_2SO on stimulation by choline$^+$, together with the lack of stimulation by choline$^+$ on the E_1 to E_2P transition at suboptimal Na^+ (Table IV), argue against choline$^+$ acting primariiy through favoring Na^+-induced conformations.

Effects of choline$^+$ on K^+-sensitive steps

If choline$^+$ does not stimulate through promoting activation by Na^+, then it may through antagonizing inhibition by K^+. In favor of this interpretation are the observations that choline$^+$ does not stimulate when K^+ is absent (Table IV). Moreover, the slow step in the Na^+/K^+-ATPase is deocclusion of K^+ [7]. The deocclusion step is slower when Rb^+ is substituted for K^+ and faster when Li^+ is substituted [4,6]: correspondingly, choline$^+$ stimulates more with Rb^+ and not at all with Li^+. The deocclusion step is rate-limiting at lower pH values (< 8) and its rate increases as the pH is raised from 6.5 to 9.0 [6]: correspondingly, choline$^+$ stimulates more at pH 6.5 and little at pH 9.0. The deocclusion step is slower as the temperature is lowered [23,24]: correspondingly, choline$^+$ stimulates more at 20 °C than at 37 °C.

Forbush [6] found that the rate of deocclusion, at 20 °C, was increased about half by 100 mM Na^+ (and with a $K_{0.5}$ greater than 100 mM) whereas 100 mM choline$^+$ increased the rate even further. In the presence of ATP (which promotes K^+deocclusion [4–7]), however, Na^+ was about twice as effective as choline$^+$ and the $K_{0.5}$ was on the order of 50 mM [6]. At 0 °C, where the rate of deocclusion is slower still [23,24], Askari found, in the absence of ATP, a $K_{0.5}$ for Na^+ to promote deocclusion of 4–8 mM; choline$^+$ also promoted deocclusion, with a $K_{0.5}$ several-fold larger (Askari, A., personal communication (1991)). These observations demonstrate that Na^+ can act to accelerate the rate-limiting step in the Na^+/K^+-ATPase reaction through a site that at higher temperatures is of relatively low afinity, and that choline$^+$ can accelerate this step in the absence of (sufficient) Na^+.

K^+-deocclusion is accelerated by MgATP acting at low-affinity substrate sites [4–6]. If choline$^+$ stimulates by promoting deocclusion, then the stimulation should be more pronounced at lower ATP concentrations: choline$^+$ should decrease the K_m for MgATP at suboptimal Na^+ concentrations. With 9 mM Na^+ and 10 mM K^+ the K_m for Mg ATP decreases from 0.29 to 0.18 mM when 100 mM choline$^+$ is added; on the other hand, with 90 mM Na^+ and 10 mM K^+ the K_m for MgATP changes negligibly on adding 100 mM choline$^+$, from 0.23 to 0.20 mM (data not presented).

Effects of choline$^+$ on K^+-nitrophenylphosphatase activity

The effects of choline$^+$ in the absence as well as presence of Na^+ can be examined in terms of the K^+-phosphatase reaction that this enzyme also catalyzes. In this reaction, reflecting the terminal hydrolytic steps of the Na^+/K^+-ATPase catalytic sequence, K^+ activates through cytoplasmically-accessible sites [25], corresponding to those from which K^+ is released in the Na^+/K^+-ATPase cycle. Na^+ inhibits, consistent with competition toward K^+ for cytoplasmic sites, although in the presence of Na^+ and K^+ nitrophenyl phosphate hydrolysis may be effected through the entire Na^+/K^+-ATPase reaction sequence [21].

Choline$^+$ inhibits the K^+-nitrophenylphosphatase reaction, more at low than at high K^+ concentrations (Fig. 3; Table VI), in accord with earlier reports that choline$^+$ is a competitor toward K^+ in the phosphatase reaction [10,26] and in direct measurements of K^+ binding [27]. Even with 10 mM K^+, the near-optimal concentration for this reaction [13,16], choline$^+$ inhibits more than with the Na^+/K^+-ATPase at suboptimal (1 mM) K^+ (Fig. 1). However, adding Na^+ with either 0.6 mM K^+ (Fig. 3) or 0.3 mM K^+ (Table VI)

TABLE V

Effects of choline$^+$ in the absence and presence of Me_2SO or oligomycin

Experiments were performed as in Table I, in the absence and presence of 100 mM choline chloride, and also in the absence and presence of either 20% (v/v) Me_2SO or 10 μg/ml oligomycin. Enzymatic activities are listed relative to control values for that [Na^+] to [K^+] ratio in the absence of modifiers, defined as 1.0; in addition, the percent changes due to adding choline$^+$ are listed in parentheses.

[Na^+]/[K^+] (mM)	Relative activity (and % change due to choline$^+$)				
	choline$^+$	Me_2SO	Me_2SO plus choline$^+$	oligomycin	oligomycin plus choline$^+$
90: 1	0.74 ± 0.04 (−26%)	0.38 ± 0.02	0.23 ± 0.03 (−39%)	0.35 ± 0.03	0.19 ± 0.02 (−46%)
90:10	1.04 ± 0.02 (+4%)	0.44 ± 0.02	0.47 ± 0.02 (+7%)	0.24 ± 0.01	0.11 ± 0.03 (−54%)
9:10	1.45 ± 0.02 (+45%)	0.50 ± 0.02	0.61 ± 0.02 (+22%)	0.43 ± 0.03	0.38 ± 0.03 (−12%)

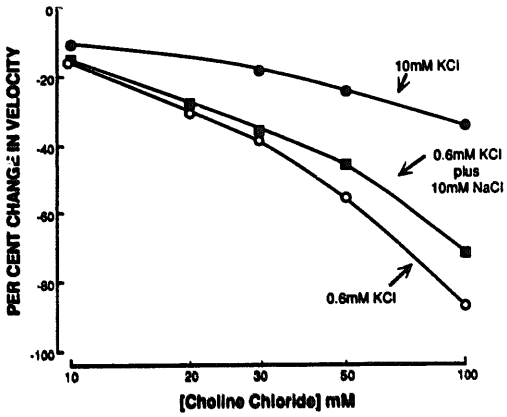

Fig. 3. Effect of choline$^+$ concentration of K^+-nitrophenylphosphatase activity. Enzyme was incubated in the standard medium modified to contain the concentrations of choline chloride indicated, and with either 10 mM KCl (●), 0.6 mM KCl (○), or 0.6 mM KCl plus 10 mM NaCl (■). The percent change due to adding choline$^+$ is plotted against the choline$^+$ concentration.

decreases inhibition by choline$^+$. Adding a nucleotide that phosphorylates the enzyme in the presence of Na^+, such as CTP, stimulates the phosphatase reaction at low K^+ concentrations by markedly decreasing the $K_{0.5}$ for K^+ [13]. With CTP plus Na^+, choline$^+$ inhibits still less. Taken together, these observations indicate that choline$^+$ inhibits the phosphatase reaction by reducing the affinity for K^+, an inhibition that is diminished when the affinity for K^+ is increased by CTP plus Na^+.

Me_2SO stimulates the K^+-phosphatase reaction, in part by increasing the affinity for K^+ [28], and Me_2SO decreases inhibition by choline$^+$, particularly at low K^+ concentrations (Table VI). Moreover, Me_2SO stimulates nitrophenylphosphatase activity in the presence of Na^+ plus K^+, probably by promoting hydrolysis over a Na^+/K^+-phosphatase pathway with an inherently higher affinity for K^+ [21]; with 0.3 mM K^+ and 10 mM Na^+ adding Me_2SO nearly abolishes inhibition by 30 mM choline$^+$ (Table VI).

If significant nitrophenylphosphate hydrolysis occurs over a pathway like that for the Na^+/K^+-ATPase reaction in the presence of Na^+ and low K^+ concentrations, then choline$^+$ should stimulate at certain Na^+ to K^+ ratios. However, despite examining a wide range of such ratios, in the absence and presence of CTP and of Me_2SO, no stimulation by choline$^+$ could be detected (data not presented). In any case, the inhibition of the K^+-phosphatase reaction by choline$^+$ is in accord with the inhibition of the Na^+/K^+-ATPase reaction at low K^+ to Na^+ ratios, indicating a common antagonism toward K^+.

Conclusions

Choline stimulates Na^+/K^+-ATPase activity at suboptimal Na^+ and optimal K^+ concentrations. Under these conditions, choline$^+$ appears to accelerate the rate limiting step in the cycle, K^+-deocclusion, for stimulation decreases when Li^+ replaces K^+ or Rb^+ and when incubation temperature or pH is increased: all changes that themselves promote cation-deocclusion [4,6,23,24]. Moreover, Na^+ and choline$^+$ have both been shown to promote deocclusion in direct studies (Ref. 6, and Askari, A., personal communication (1991)). MgATP also accelerates K^+-deocclusion [4–7], and when Na^+ is suboptimal, choline decreases the K_m for MgATP at the low-affinity sites that promote deocclusion. Together, these observations indicate that under usual experimental conditions, *in vitro*, a significant aspect of Na^+-activation involves acceleration of K^+-deocclusion. Thus, when Na^+ concentrations are suboptimal, choline$^+$ can substitute at that specific reaction step. Conventional evaluations of the

TABLE VI

Effects of choline$^+$ on K^+-nitrophenylphosphatase activity

Experiments were performed as in Fig. 3, in the absence and presence of 30 mM choline chloride, and with the K^+ concentrations and the additions to the standard medium (where K^+ is 10 mM) indicated; the Me_2SO concentration was 10% (v/v). Enzymatic activities are listed relative to control values in the standard medium, defined as 1.0; in addition, the percent changes due to adding choline$^+$ are listed in parentheses.

Changes to standard medium	Relative activity (and % change due to choline$^+$)			
	no choline$^+$ or Me^2SO	choline$^+$ (30 mM)	Me_2SO (10%)	Me_2SO plus choline$^+$
None	1.00	0.82±0.01 (−18%)	1.49±0.03	1.28±0.02 (−14%)
0.3 mM K^+	0.24±0.01	0.12±0.02 (−50%)	0.54±0.01	0.37±0.01 (−31%)
0.3 mM K^+ plus 10 mM Na^+	0.18±0.02	0.11±0.01 (−39%)	0.74±0.03	0.65±0.01 (−12%)
0.3 mM K^+ plus Na^+ and 0.3 mM CTP	0.70±0.03	0.58±0.02 (−17%)	0.98±0.04	0.84±0.02 (−14%)

$K_{0.5}$ for Na^+-activation of the Na^+/K^+-ATPase would therefore include a high-affinity component for activating enzyme phosphorylation and a low-affinity component for accelerating K^+-deocclusion. (This low-affinity component might also be inferred from the increased $K_{0.5}$ for Na^+ at low pH and low temperature [10], when antagonism toward K^+-occlusion is more significant.)

Choline$^+$ inhibits Na^+/K^+-ATPase and K^+-nitrophenylphophatase reactions, inversely with the K^+ concentration. Such competition may be indirect, reflecting K^+ and choline$^+$ favoring alternative enzyme forms [8,9], which may underlie as well the ability of choline$^+$ to promote deocclusion.

As a practical consideration, using choline$^+$ to maintain ionic strength in the ATPase reaction media is particularly hazardous in two situations (Fig. 2A): (a) when the Na^+ concentration is low but the K^+ concentration is optimal (10–30 mM), for choline can then stimulate; and (b) when the K^+ concentration is low and the Na^+ concentration is an order of magnitude or more greater, for choline can then inhibit. The sidedness of these actions of choline toward the enzyme was not examined in these experiments, however, and thus the potential problems for transport studies are uncertain.

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