

# Erythrocytes may contain a ouabain-insensitive $K^+$ -ATPase which plays a role in internal $K^+$ balance

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## Abstract

Erythrocytes are useful in evaluating  $K^+$  transport pathways involved in internal  $K^+$  balance. Several forms of  $H^+,K^+$ -ATPase have been described in nephron segments active in  $K^+$  transport. Furthermore, the activity of a ouabain-insensitive isoform of  $H^+,K^+$ -ATPase expressed in collecting duct cells may be modulated by acid-base status. Various assays were performed to determine if a ouabain-insensitive  $K^+$ -ATPase is present in rat erythrocytes and, if so, whether it plays a role in internal  $K^+$  balance. Kinetic studies demonstrated that maximal stimulation of enzyme activity was achieved with 2.5 mM  $K^+$  at pH 7.4. Subsequent experiments were performed on erythrocyte membranes collected from animals submitted to varying degrees of  $K^+$  homeostasis: control rats,  $K^+$ -depleted rats,  $K^+$ -loaded rats, and rats rendered hyperkalemic due to acute renal failure. As observed in the collecting duct cell studies, there was a significant decrease in the activity of ouabain-insensitive  $K^+$ -ATPase in the erythrocytes of both  $K^+$ -loaded and metabolically alkalotic  $K^+$ -depleted rats. However, this enzyme activity in erythrocyte membranes of rats with metabolic acidosis-related hyperkalemia was similar to that of control animals. This finding may be interpreted as resulting from two potentially modulating factors: the stimulating effect that metabolic acidosis has on  $K^+$ -ATPase and the counteracting effect that hyperkalemia and uremia have on metabolic acidosis. In summary, we present evidence of a ouabain-insensitive  $K^+$ -ATPase in erythrocytes, whose activity is modulated by acid-base status and  $K^+$  levels.

## Key words

- Erythrocyte
- $K^+$ -ATPase
- Potassium
- Acid-base status
- $H^+,K^+$ -ATPase
- Ouabain

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Internal  $K^+$  balance rapidly regulates plasma  $K^+$ . This runs in parallel to external  $K^+$  balance, in which the kidney and gastrointestinal tract play important regulatory roles. In the case of internal balance,  $K^+$  shifts between the extracellular and intracellular compartments via pathways such as channels, co-transport systems and pumps like  $Na^+,K^+$ -ATPase (1).

Although only about 7% of the body's potassium is stored in erythrocytes, these cells have been widely used in transport studies related to internal  $K^+$  balance. We have previously reported the presence of a ouabain-insensitive  $K^+$ -ATPase in inner medullary collecting duct cells of rats (2). We have also observed that  $K^+$  intake and acid-base balance modulated this enzyme activ-

ity. In addition, several reports have presented evidence of at least three  $H^+,K^+$ -ATPase isoforms in mammalian nephrons. Functional studies have indicated that this cation-transporting ATPase plays an important role in transepithelial  $K^+$  transport and may be modulated by alterations in acid-base balance (3,4). Although the presence of these  $H^+,K^+$ -ATPase isoforms is well documented in several organs such as kidneys, colon, gastric mucosa and vessels, their expression in erythrocytes has not been reported.

The aim of the present study was to determine if a ouabain-insensitive  $K^+$ -ATPase is present in erythrocytes that are active in internal  $K^+$  balance. Male Wistar rats were anesthetized with ether and 6 ml of blood was collected by heart puncture. Erythrocyte membranes were isolated and purified after several centrifugations at  $4^\circ C$  by the method of Post et al. (5). At the end of the procedure, the volume of the membrane suspension was adjusted to obtain a protein concentration of 0.5 to 1 mg/ml using the method of Lowry et al. (6). The ouabain-insensitive  $K^+$ -ATPase activity was immediately assayed in triplicate by measuring the inorganic phosphate (Pi) released from ATP labeled with  $\gamma$ - $[^{32}P]$ . The protocol used was similar to that described by us for fresh suspensions of rat inner medullary collecting duct cells (2). Briefly, 0.1 ml of purified erythrocyte membranes was incubated in 0.4 ml of medium for 1 h at  $37^\circ C$ . The incubation solution contained 100 mM NaCl, 3 mM  $MgCl_2$ , 5 mM  $NaN_3$ , 0.5 mM ethylene glycol-bis ( $\beta$ -aminoethyl ether)- $N,N,N',N'$ -tetraacetic acid (EGTA), 136 mM Tris base, 5 mM ATP (Sigma, St. Louis, MO, USA), and 1 mM ouabain (Sigma). It also contained trace amounts of  $\gamma$ - $[^{32}P]$ -ATP (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK) and 0.02% (w/v) deoxycholate. The reaction was stopped by cooling the samples on crushed ice and adding 1 ml of 10% activated charcoal suspension (w/v).

The tubes were centrifuged and  $^{32}P$  in the supernatant was counted with a liquid scintillation analyzer (Packard, Downers Grove, IL, USA). Blanks were determined in triplicate by assaying in the absence of membranes to determine non-enzymatic ATP hydrolysis.  $K^+$ -ATPase activity was calculated as the difference between the amount of  $^{32}P$  liberated in the presence of KCl and the amount liberated in its absence. Activity was reported as nM Pi/mg protein $^{-1}$  h $^{-1}$ . To assure that no P-type cation-transporting ATPases contaminated the assay, 15 mM sodium vanadate (Sigma) was added to a  $K^+$ -free incubation solution.

In a series of experiments, the effects of  $K^+$  concentration and incubation solution pH were determined in assays of erythrocyte membranes obtained from normal rats. As illustrated in Figure 1, a kinetic curve was obtained by varying  $K^+$  concentration in a pH 7.4 assay solution. Maximal stimulation was observed at 2.5 mM  $K^+$ , which was then used as the standard  $K^+$  concentration for incubation solutions used in subsequent experiments. The optimum pH for measuring the activity of ouabain-insensitive  $K^+$ -ATPase activity was determined by testing assays at various points between 6.8 and 7.8 on the pH scale. Maximal enzyme activity was observed at pH 7.2 and 7.4. A reduction in ouabain-insensitive  $K^+$ -ATPase activity resulted from either increases or decreases in incubation solution pH. Therefore, pH 7.4 was set as the standard for all subsequent experiments.

Following these kinetic studies, the activity of ouabain-insensitive  $K^+$ -ATPase was measured in erythrocyte membranes collected from animals submitted to varying degrees of  $K^+$  homeostasis. The animals were divided into four groups: 1) control; 2)  $K^+$ -depleted: rats fed a low  $K^+$  diet (14 mEq/kg) for two weeks; 3)  $K^+$ -loaded: rats fed a high  $K^+$  diet (700 mEq/kg) for two weeks, and 4) hyperkalemic: rats with acute renal failure induced by bilateral ureteral obstruction under ether anesthesia 24 h prior to the

experiments.

Table 1 summarizes data from animals submitted to different K<sup>+</sup> homeostasis protocols. Ouabain-insensitive K<sup>+</sup>-ATPase activity in erythrocyte membranes decreased significantly in rats submitted either to K<sup>+</sup> depletion or K<sup>+</sup> loading. In K<sup>+</sup>-depleted animals, metabolic alkalosis also developed. Erythrocyte membranes from hyperkalemic animals presented ouabain-insensitive K<sup>+</sup>-ATPase activity identical to that of controls. However, these hyperkalemic animals showed other metabolic disturbances associated with hyperkalemia, such as metabolic acidosis and uremia due to acute renal failure (Table 1).

The presence of a ouabain-insensitive K<sup>+</sup>-ATPase was first described in gastric mucosa. Later studies have well documented its expression in nephron segments (3,4). Because transport studies have demonstrated that the ouabain-insensitive ATPase exchanges K<sup>+</sup> and H<sup>+</sup>, this enzyme was named H<sup>+</sup>,K<sup>+</sup>-ATPase (7). In the present study, transport was not examined. However, erythrocyte membranes assayed with ouabain-insensitive K<sup>+</sup>-ATPase showed kinetic responses to variations in both the K<sup>+</sup> and pH levels of the incubation solutions.

Kidney studies have demonstrated that the activity of a ouabain-insensitive K<sup>+</sup>-ATPase in collecting duct cells was reduced when rats were fed a high K<sup>+</sup> diet (2,8). On the other hand, K<sup>+</sup> depletion stimulated this enzyme in the absence of acid-base disor-

ders. However, this enzyme activity was reduced in collecting duct cells if K<sup>+</sup>-depleted animals developed metabolic alkalosis (2,3). In agreement with the studies on kidney, the present study demonstrated a similar modulation of this enzyme activity in erythrocyte membranes. Reduced enzyme activity was demonstrated in K<sup>+</sup>-loaded animals and hypokalemic rats with associated metabolic alkalosis.

Other studies have also reported the effects of acid-base disturbances on H<sup>+</sup>,K<sup>+</sup>-ATPase activity in nephron segments (9,10). Rats submitted to acute and chronic respiratory alkalosis presented a decrease in renal H<sup>+</sup>,K<sup>+</sup>-ATPase activity. However, in chronic situations, both respiratory and metabolic acidosis stimulated this enzyme activity in the kidney (9,10).

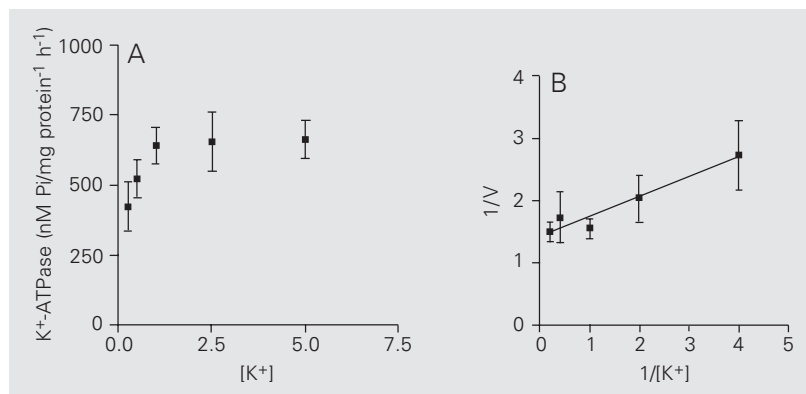


Figure 1. Effect of K<sup>+</sup> concentration on ouabain-insensitive K<sup>+</sup>-ATPase activity in control rat erythrocyte membranes. A, Kinetic curve where each point is the mean  $\pm$  SEM of six assays of membranes obtained from six animals. B, Lineweaver-Burk plots of these data.  $V_{max}$  = 700 nM Pi/mg protein<sup>-1</sup> h<sup>-1</sup> and  $K_m$  = 0.22 mM.

Table 1. Effect of K<sup>+</sup> depletion, K<sup>+</sup> loading and hyperkalemia by bilateral ureter obstruction (hyperkalemic) on body weight, plasma composition and K<sup>+</sup>-ATPase activity.

Group	N	Body weight (g)	Na <sup>+</sup> (mEq/l)	K <sup>+</sup> (mEq/l)	pH	HCO <sub>3</sub> <sup>-</sup> (mEq/l)	Urea (mg%)	Creatinine (mg%)	K <sup>+</sup> -ATPase (nM Pi/mg protein <sup>-1</sup> h <sup>-1</sup> )
Control	6	209 $\pm$ 11	133 $\pm$ 2	4.1 $\pm$ 0.2	7.39 $\pm$ 0.02	25 $\pm$ 2	45 $\pm$ 6	0.37 $\pm$ 0.04	538 $\pm$ 66
K <sup>+</sup> -depleted	7	229 $\pm$ 7	136 $\pm$ 1	2.2 $\pm$ 0.1*	7.51 $\pm$ 0.03*	33 $\pm$ 2*	40 $\pm$ 4	0.56 $\pm$ 0.04	266 $\pm$ 65*
K <sup>+</sup> -loaded	6	220 $\pm$ 21	135 $\pm$ 1	4.9 $\pm$ 0.2	7.37 $\pm$ 0.02	23 $\pm$ 2	33 $\pm$ 3	0.48 $\pm$ 0.03	291 $\pm$ 67*
Hyperkalemic	5	177 $\pm$ 6	136 $\pm$ 3	7.5 $\pm$ 0.6*	7.28 $\pm$ 0.04*	19 $\pm$ 2	252 $\pm$ 18*	2.2 $\pm$ 0.1*	537 $\pm$ 104

\*P < 0.05 compared to control (ANOVA followed by Dunnett's test).

In the present study, control rats and hyperkalemic rats with metabolic acidosis showed comparable levels of ouabain-insensitive  $K^+$ -ATPase activity in erythrocyte membranes. The inhibitory effect of hyperkalemia on this enzyme activity might counteract the stimulatory effect exerted by acidosis.

Another interesting finding concerns uremia. It is well known that uremia decreases other enzyme activities, such as  $Na^+,K^+$ -ATPase and  $Ca^{2+}$ -ATPase, which are present in erythrocytes (11,12). However, in the present study, values of a ouabain-insensitive  $K^+$ -ATPase in erythrocyte membranes of rats with acute renal failure showed activity similar to that of the control group. This observation suggests that the stimulatory ef-

fect exerted by metabolic acidosis may also counteract the inhibitory effect of uremia. On the basis of the present results, we suggest that metabolic acidosis prevents the reduction in the activity of the erythrocytic ATPase enzyme during the uremic state.

In summary, erythrocytes may contain a ouabain-insensitive  $K^+$ -ATPase whose activity plays a role in internal  $K^+$  balance and is modulated by changes in acid-base status.

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