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Studies on the mechanism of trimethoprim-induced hyperkalemia

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Studies on the mechanism of trimethoprim-induced hyperkalemia. We examined the effects of trimethoprim (TMP) on metabolic parameters and renal ATPases in rats after a 90 minute infusion (9.6 mg/hr/kg body wt, i.v.) and after 14 days (20 mg/kg body wt/day, i.p.). After one dose of TMP, plasma electrolytes, arterial pH and aldosterone levels were normal, but a natriuresis, bicarbonaturia, and decreased urinary potassium excretion occurred. Na-K-ATPase activity in microdissected segments from these animals was decreased by 36 ± 0.9% in proximal convoluted tubule (PCT) ($P < 0.005$); decreases of 50 ± 2.1% and 40 ± 1.1% were seen in cortical and medullary collecting tubules (CCT and MCT), respectively ($P < 0.005$). Na-K-ATPase activity was unaffected in medullary thick ascending limb (MTAL). H-ATPase (in PCT and collecting duct) and H-K-ATPase (in CCT and MCT) activities were not changed. Following chronic TMP administration, plasma potassium increased as compared to control (5.16 ± 0.05 mEq/liter vs. 3.97 ± 0.05 mEq/liter, $P < 0.05$), however, acid-base status and plasma aldosterone levels were normal. Na-K-ATPase activity was decreased by 45 ± 2.6% in PCT ($P < 0.005$), 73 ± 2.0% in CCT ($P < 0.001$), and 53 ± 2.5% in MCT ($P < 0.005$). Na-K-ATPase activity in MTAL and H-K-ATPase activity in CCT and MCT were unchanged. H-ATPase activity in PCT and MTAL was normal, but in the collecting tubule (CCT and MCT) it was decreased by approximately 25% ($P < 0.05$). TMP inhibited Na-K-ATPase activity in a dose-dependent fashion in PCT, CCT, and MCT when tubules from normal animals were incubated *in vitro* with the drug; TMP *in vitro* did not affect H-ATPase or H-K-ATPase activity. These results suggest that TMP-induced hyperkalemia may result from decreased urinary potassium excretion caused by inhibition of distal Na-K-ATPase in the face of intact H-K-ATPase activity.

The drug trimethoprim-sulfamethoxazole (Bactrim®) is widely used as an antimicrobial agent, being effective against both gram-positive and gram-negative organisms [1]. Renal disorders associated with its use include decreased creatinine secretion, interstitial nephritis, and hyponatremia [2, 3]. Recently, the drug has been advocated both for therapy and prophylaxis of *Pneumocystis carinii* pneumonia in patients with the acquired immunodeficiency syndrome [4, 5]. Clinical studies show that over 20% of the patients develop hyperkalemia when a regimen of 20 mg/kg/day is given for more than one week [6–8]. The renin-angiotensin-aldosterone axis appears to be "intact" in those few patients studied, and the hyperkalemia disappears when the drug is discontinued [6–9].

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Trimethoprim (TMP), one of the two active components of Bactrim®, is a pyrimidine derivative (pKa 7.3) [10] which, when infused into rats, causes a natriuresis and a fall in urinary potassium excretion [11]. In perfused rat-distal tubule, Velázquez et al [11] showed TMP decreased net potassium secretion and transepithelial voltage. In the frog skin, a model of the mammalian collecting duct, TMP decreases short circuit current and Na^{22} flux. Amiloride was found to competitively displace TMP with a K_d of 46×10^{-8} M (K_d of TMP is 53×10^{-5} M) [12]. Recent studies show that TMP also blocks an amiloride-sensitive sodium channel in A6 cell monolayers, a model of mammalian cortical collecting tubule principal cells [13].

The collecting tubule is the final site for regulation of urinary potassium excretion and urinary acidification [14, 15]. Potassium secretion at this site is modulated by Na-K-ATPase activity and reabsorption is mediated by H-K-ATPase activity [15, 16]. Inhibition of the former results in hyperkalemia and inhibition of the latter would result in kaliuresis. Proton transport in the collecting tubule is controlled by both the H-ATPase and the H-K-ATPase, enzymes found in the α-intercalated cells [15, 16]. As the effect of TMP on these renal transport enzymes is unknown, we examined acute and chronic administration of the drug to rats in microdissected permeabilized nephron fragments. We also studied the direct effect of the drug *in vitro*.

Methods

Male Sprague-Dawley rats weighing 100 to 200 g were used in this study. All animals had free access to the usual laboratory diet and were given tap water to drink *ad libitum*, unless otherwise noted.

In vivo studies

A. Acute study (single dose intravenous infusion). Animal preparation. Animals were first anesthetized with sodium pentobarbital and ketamine. The femoral vein was then catheterized with PE-50 tubing for continuous infusion; the femoral artery was cannulated for blood samples [17]. A solution containing NaCl (140 mEq/liter) and KCl (4 mEq/liter) (pH 7.4) was infused at 15 ml/hr/kg body wt and was the vehicle for TMP. The bladder was cannulated for urine collection to determine glomerular filtration rate (as measured using creatinine clearance) and electrolyte excretion [18]. A blood sample (0.5 ml) was obtained at the midpoint of each urine collection. One hour was allowed for equilibration before the beginning of the clearance collections. Three urine collections (30 min) were made as baseline data and three collections (30 min) were obtained during the experimental

period. All blood and urinary losses were replaced with the solution described above. The animals were divided into the following groups ($N = 6$ in each group):

Group 1: Control (C). The animals received the salt solution intravenously during the baseline and experimental periods.

Group 2: Trimethoprim (TMP). After the baseline period, animals received 0.64 g/liter TMP at a rate of 9.6 mg/hr/kg body wt throughout the experimental period. Previous work has shown that this dose results in a natriuresis and decreased urinary potassium excretion [11].

B. Chronic study (14 days intraperitoneal administration). Animal preparation. A twenty-four hour urine collection was performed on each animal as baseline after three days on a diet of standard composition (ICN Pharmaceuticals Inc., Costa Mesa, CA, USA) and 0.3% NaCl p.o. *ad libitum* to prevent volume contraction as we have described [18]. The animals were then divided into the following groups ($N = 6$ in each group):

Group 1: Control (C). The animals were treated daily ($\times$ 14 days) with the vehicle described below (0.75 ml, i.p.).

Group 2: Trimethoprim (TMP). The animals were treated daily with TMP (20 mg/kg/day, i.p.) for 14 days. TMP (16 mg/ml) was dissolved in 40% propylene glycol, and at the time of injection was diluted to 0.75 ml (total volume) with distilled H_2O for i.p. administration [10].

On the morning of the thirteenth day, the animals were again placed in metabolic cages for a 24-hour urine collection. On the day of the experiment, after receiving the final TMP dose, the animals were anesthetized and blood samples were obtained from the aorta for measurement of pH, pCO_2, creatinine, electrolytes, and aldosterone.

Arterial blood gas, urine and plasma electrolytes and creatinine were measured as we have published in the past [17, 18]. Potassium concentration in muscle, renal cortex, and renal medulla was measured by atomic absorption spectrophotometry; plasma aldosterone concentration was determined by radioimmunoassay (COAT-A-COUNT® DPC, Los Angeles, CA, USA) [18].

Tubules microdissection and enzymatic measurements

Tubules were microdissected as we have previously described in detail [18, 19]. In brief, the left renal artery was cannulated and the kidney was perfused for 15 minutes *in situ* at a rate of 0.7 ml/min with a balanced salt solution containing 400 U/ml collagenase; 4°C, pH 7.4. The kidney was then cut into 5 $\times$ 5x10 mm pyramids along the cortico-papillary axis and incubated in 3 ml of collagenase-albumin containing Hank's solution at 35°C for 12 minutes, pH 7.4. The tissues were continuously bubbled with compressed air (3 psi). After incubation, the pyramids were rinsed and immediately microdissected. Tubule segments were identified as proximal convoluted tubule (PCT), medullary thick ascending limb of Henle's loop (MTAL), cortical collecting tubule (CCT), and medullary collecting tubule (MCT) [18, 19].

To remove most of the extracellular potassium, nephron segments were incubated for 15 minutes at 37°C (pH 7.4) in potassium-free buffer. The nephron segments were then subjected to a two-step hypotonic-hypothermic shock and ATPase activities were determined after incubation of the segments with γ^{32}P-labeled ATP (37°C, 15 min, pH 7.4). H-ATPase (N-ethylmaleimide-inhibitable), H-K-ATPase (K-stimulated), and Na-K-ATPase (ouabain-inhibitable) were measured by the radiochemical method of Doucet and Marsy [20] as subsequently described by us [21]. All assays were performed at V_{max} conditions (12 mM ATP, pH 7.4 and 37°C) [21].

In vitro study

To further examine the direct effect of TMP on the three renal ATPases, additional *in vitro* studies were performed in a manner we have described for amiloride [19, 22]. Segments of PCT, CCT, and MCT were first microdissected from acclimated *normal* Sprague-Dawley rats. After the hypotonic-hypothermic shock, tubule segments were pre-incubated for 90 minutes *in vitro* in 1.2 μl of buffer with varying concentrations of TMP (0.01 to 10 mM). After the preincubation period, the solution was then replaced with one appropriate for the enzyme analysis to be performed (that is, H-ATPase, H-K-ATPase and Na-K-ATPase). ATP (12 mM) plus γ^{32}P-ATP was added and the reaction was allowed to proceed for 15 minutes at 37°C, pH 7.4.

Enzyme activity is expressed as pmol/mm tubule length/hour of ATP hydrolyzed. All samples were run in triplicate or quadruplicate and appropriate corrections were made for the blank and for the spontaneous hydrolysis of ATP [21].

Materials

All chemicals and reagents were obtained from the Sigma Chemical Co. (St. Louis, MO, USA) and were of highest purity. Radiolabeled ATP (high specific activity) was obtained from New England Nuclear (Boston, MA, USA). Trimethoprim was graciously provided by the Roche Laboratories (Nutley, NJ, USA).

Statistics

Statistical significance was assessed using the Student's *t*-test (paired or unpaired) or analysis of covariance, where appropriate, with P values of 0.05 or less being significant.

Results

Effects of trimethoprim infusion on metabolic parameters and renal function

Acute infusion. There was no significant difference in any of the blood values or in creatinine clearance after a single TMP infusion was given to rats (Table 1, upper panel). Acid-base status, plasma potassium (and other electrolytes) and aldosterone levels were similar to control. The slight, but significant, increase in urine sodium excretion seen in the experimental period of control animals was due to the volume expansion caused by the infusion of the salt solution use as the vehicle (Table 1, lower panel). Following the same protocol, however, TMP-treated rats had a greater and significant natriuresis ($P < 0.01$) and bicarbonaturia ($P < 0.01$), as well as a decrease in urinary potassium excretion ($P < 0.05$). While TMP infusion did not change urine flow rate or chloride excretion, urine pH increased ($P < 0.05$).

Chronic administration. There was no significant difference in body wt between the two groups of animals after 14 days, indicating normal growth throughout the study. In control animals, mean body wt was 104 $\pm$ 2 g on Day 0 and after 14 days, body wt was 177 $\pm$ 4 g; in the TMP-treated animals, body wt was 103 $\pm$ 2 g and 173 $\pm$ 3 g, respectively (NS). As shown in Table 2 (upper panel), TMP-treated animals had normal acid-base status, normal plasma sodium and chloride concentrations, but plasma potassium was significantly higher than control (5.16 $\pm$ 0.05 mEq/liter vs. 3.97 $\pm$ 0.05 mEq/liter, respectively, $P < 0.05$).

Table 1. Effect of a single infusion (9.6 mg/kg/hr for 90 min) of trimethoprim on metabolic parameters and renal function in rats

	Body wt *g*	Aldosterone *ng/dl*	pH	pCO_2 *mm Hg*	P_{HCO_3}	P_{Na}	P_K	P_{Cl}	C_{Cr} *ml/min/100 g*
					mEq/liter				
Control	170 ± 3	27 ± 2	7.39 ± 0.01	40 ± 1	23.3 ± 0.1	140 ± 1	3.98 ± 0.05	98 ± 1	0.81 ± 0.01
TMP	172 ± 4	25 ± 2	7.38 ± 0.01	40 ± 0.6	23.0 ± 0.6	138 ± 1	4.28 ± 0.05	99 ± 1	0.71 ± 0.02

Abbreviation is: C_{Cr}, creatinine clearance, $N = 6$ in each group; NS.

	Group	U_V	U_{pH}	$U_{HCO_3}V$	$U_{Na}V$	U_KV	$U_{Cl}V$
Control	Baseline	11.2 ± 0.31	6.60 ± 0.06	179 ± 8	1361 ± 39	589 ± 22	1804 ± 36
	Exptl	13.3 ± 0.34	6.58 ± 0.05	188 ± 6	1627 ± 69[a]	629 ± 17	1992 ± 41
TMP	Baseline	11.0 ± 0.39	6.55 ± 0.05	183 ± 7	1379 ± 49	595 ± 19	1857 ± 52
	Exptl	13.8 ± 0.34	7.02 ± 0.04[a]	387 ± 20[b]	2114 ± 48[b]	306 ± 15[a]	2007 ± 42

Abbreviations are: U_V, Urine flow rate (μl/min); $U_{HCO_3}V$, urine bicarbonate excretion (nmol/min); $U_{Na}V$, urine sodium excretion (nmol/min); U_KV, urine potassium excretion (nmol/min); $U_{Cl}V$, urine chloride excretion (nmol/min), Exptl, experimental period; $N = 6$ in each group.
[a] $P < 0.05$, [b] $P < 0.01$ vs. baseline of the same group and experimental of the control group

Table 2. Effect of chronic trimethoprim administration (20 mg/kg for 14 days) on metabolic parameters and renal function in rats

	Aldosterone *ng/dl*	pH	pCO_2 *mm Hg*	P_{HCO_3}	P_{Na}	P_K	P_{Cl}	C_{Cr} *ml/min/100 g*
				mEq/liter				
Control	28 ± 2	7.40 ± 0.01	41 ± 1	23.3 ± 0.04	141 ± 1	3.97 ± 0.05	98 ± 1	0.76 ± 0.02
TMP	30 ± 2	7.39 ± 0.01	42 ± 2	22.6 ± 0.05	139 ± 1	5.16 ± 0.05[a]	99 ± 2	0.66 ± 0.02

Abbreviation is: C_{Cr}, creatinine clearance. $N = 6$ in each group. [a] $P < 0.05$ vs. Control.

	Group	U_V	U_{pH}	$U_{HCO_3}V$	$U_{Na}V$	U_KV	$U_{Cl}V$
Control	Pre	9.6 ± 0.2	6.58 ± 0.07	271 ± 14	1633 ± 61	2469 ± 96	1678 ± 65
	Post	9.8 ± 0.2	6.60 ± 0.08	280 ± 14	1749 ± 62	2603 ± 163	1683 ± 63
TMP	Pre	9.8 ± 0.2	6.55 ± 0.07	268 ± 19	1647 ± 58	2537 ± 80	1728 ± 113
	Post	10.0 ± 0.2	7.00 ± 0.06[a]	656 ± 74[b]	1839 ± 54	1954 ± 40[a]	1705 ± 56

Abbreviations are: U_V, urine volume/24 hours (expressed/100 g body wt); $U_{HCO_3}V$, urine bicarbonate/24 hours; $U_{Na}V$, urine sodium/24 hours; U_KV, urine potassium/24 hours; $U_{Cl}V$, urine chloride/24 hours; Pre, pre-treatment; Post, post-treatment; urine electrolyte excretion is expressed as μEq/100 g body weight/24 hours. $N = 6$ in each group.
[a] $P < 0.05$, [b] $P < 0.01$ Pre vs. Post TMP.

Despite the hyperkalemia, however, plasma aldosterone was not different from the controls (30 ± 2 ng/dl vs. 28 ± 2 ng/dl, respectively, NS). Chronic TMP therapy resulted in bicarbonaturia ($P < 0.01$) and a fall in urinary potassium excretion ($P < 0.05$) (Table 2, lower panel). Urine flow and sodium chloride excretion were unchanged presumably reflecting a new steady state of salt balance after 14 days of TMP therapy.

Effect of trimethoprim on potassium stores

Analysis of muscle and renal tissue (cortex and medulla) revealed that potassium concentration was unaffected after TMP, either following a single infusion or after 14 days. Control values in muscle, renal cortex and medulla were 0.38 ± 0.01, 0.28 ± 0.02 and 0.30 ± 0.02 mEq/g dry wt, respectively; after 14 days of TMP the values were 0.36 ± 0.01, 0.26 ± 0.01 and 0.38 ± 0.02 mEq/g dry wt, respectively ($N = 8$ to 12 samples per organ per group; NS). (Statistically identical results were obtained for the acute infusion study, data not shown.)

In vivo effects of trimethoprim on renal ATPases

Acute infusion. Na-K-ATPase activity in microdissected segments from TMP-treated rats was decreased by 36 ± 0.9% in PCT ($P < 0.005$), 50 ± 2.1% in CCT ($P < 0.005$), and 40 ± 1.1% in MCT ($P < 0.005$) (Fig. 1). TMP infusion had no effect on Na-K-ATPase activity in MTAL. Neither H-K-ATPase (Fig. 2) nor H-ATPase (Fig. 3) activity was affected in TMP infusion-treated animals (H-K-ATPase activity was examined only in collecting tubule segments).

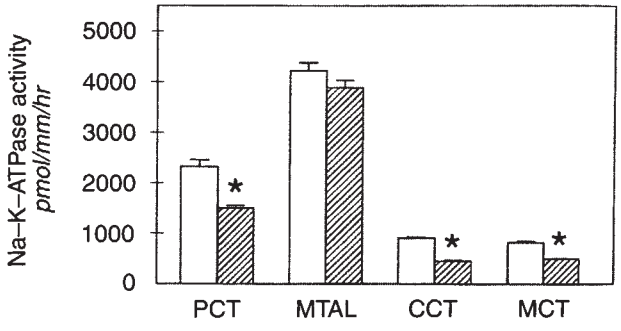

Fig. 1. *Effect of a single infusion of trimethoprim (shaded bars) on Na-K-ATPase activity in PCT, MTAL, CCT, MCT; N = 6 in each group; *P < 0.005 versus control animals.*

Chronic administration. The effect of chronic treatment with TMP on Na-K-ATPase activity is shown in Figure 4. Enzyme

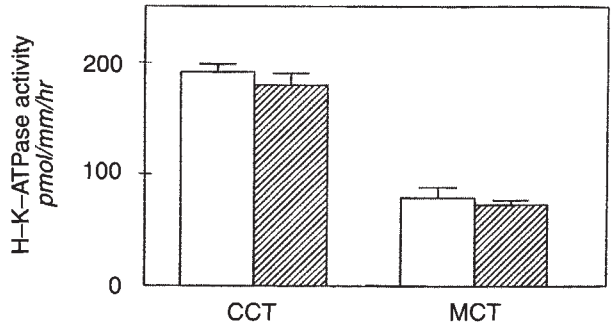

Fig. 2. *Effect of a single infusion of trimethoprim (shaded bars) on H-K-ATPase activity in CCT and MCT; N = 6 in each group (Control vs. TMP, NS).*

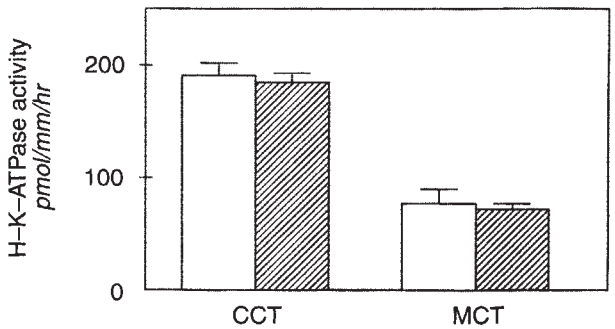

Fig. 5. *Effect of chronic (14 days) trimethoprim administration (shaded bars) on H-K-ATPase activity in CCT and MCT; N = 6 in each group (Control vs. TMP, NS).*

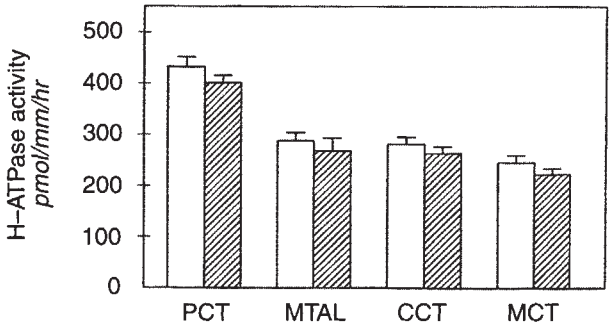

Fig. 3. *Effect of a single infusion of trimethoprim (shaded bars) on H-ATPase activity in PCT, MTAL, CCT, MCT; N = 6 in each group (Control vs. TMP, NS).*

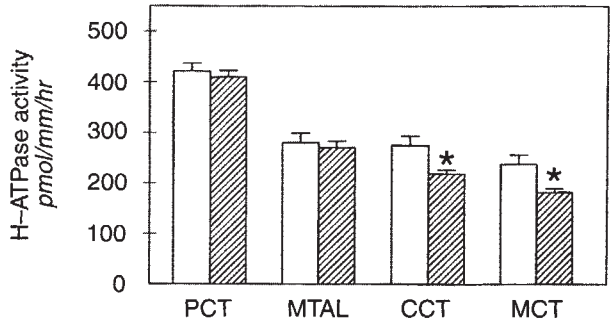

Fig. 6. *Effect of chronic (14 days) trimethoprim administration (shaded bars) on H-ATPase activity in PCT, MTAL, CCT, MCT; N = 6 in each group; *P < 0.05 versus control animals.*

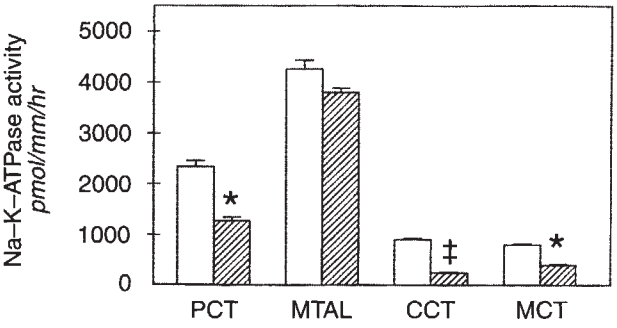

Fig. 4. *Effect of chronic (14 days) trimethoprim administration (shaded bars) on Na-K-ATPase activity in PCT, MTAL, CCT, MCT; N = 6 in each group; *P < 0.005; ‡P < 0.001 versus control animals.*

activity was decreased, as compared to control, by 45 ± 2.6% in PCT ($P < 0.005$), by 73 ± 2.0% in CCT ($P < 0.001$), and by 53 ± 2.5% in MCT ($P < 0.005$). Chronic drug treatment, again, did not affect Na-K-ATPase in MTAL. H-K-ATPase activity in CCT or in MCT was not different from their respective controls (Fig. 5). In contrast to the lack of effect of a single dose of TMP to alter H-ATPase activity in the collecting tubule (Fig. 5), chronic TMP treatment decreased the enzyme significantly; in CCT, it was decreased by 21 ± 1.3% ($P < 0.05$), and in MCT it fell by 24 ± 1.5% ($P < 0.05$) (Fig. 6). H-ATPase activity, however, did not change in either PCT or MTAL.

In vitro effects of trimethoprim on renal ATPase enzymes

To further examine the direct action of TMP on the three renal transport enzymes, we incubated the drug *in vitro* (0.01 to 10 mM) with tubule segments from normal animals (Table 3). Trimethoprim had a direct inhibitory effect on Na-K-ATPase in PCT, CCT, and MCT; it was without effect in MTAL. At 0.1 mM TMP, enzyme activity was decreased by 28 ± 1.1% in PCT ($P < 0.05$), 30 ± 1.1% in CCT ($P < 0.05$), and 27 ± 1.3% in MCT ($P < 0.05$). There was a further, and significant, decrease in Na-K-ATPase activity in each of the three segments when the concentration of TMP was increased to 1.0 mM. The percentage decrease, as compared to controls, was 53 ± 4.2% in PCT ($P < 0.01$), 54 ± 4.5% in CCT ($P < 0.01$), and 36 ± 1.7% in MCT ($P < 0.01$). The highest concentration (10 mM TMP) did not decrease Na-K-ATPase activity further.

The highest concentration of trimethoprim (10 mM) had no significant effect on H-K-ATPase or H-ATPase activity in any of the segments studied (Table 3). In additional studies ($N = 4$), the effect of 0.1 mM (100 μM) amiloride was examined. This agent, well known to inhibit apical Na channels directly, did not change H-K-ATPase or H-ATPase activity when it was incubated with collecting tubules microdissected from normal animals, confirming results reported previously [19, 22]. However, this concentration of amiloride (100 μM) decreased Na-K-ATPase in CCT from 905 ± 26 pmol/mm/hr to 463 ± 52 pmol/mm/hr ($P < 0.01$, $N = 4$).

Table 3. *In vitro* effect of trimethoprim (TMP) on renal ATPases in microdissected rat nephron segments

Enzyme	Group	PCT	MTAL	CCT	MCT
Na-K-ATPase	Control	2276 ± 134	4075 ± 160	903 ± 22	815 ± 12
	TMP (0.01 mM)	2127 ± 107	3987 ± 103	903 ± 22	802 ± 16
	TMP (0.1 mM)	1637 ± 67[a]	3968 ± 79	639 ± 24[a]	598 ± 29[a]
	TMP (1.0 mM)	1067 ± 85[b]	3942 ± 70	418 ± 35[b]	518 ± 25[b]
	TMP (10.0 mM)	923 ± 54[b]	3936 ± 64	382 ± 34[b]	510 ± 16[b]
H-K-ATPase	Control	ND	ND	184 ± 8	79 ± 7
	TMP (10 mM)	ND	ND	178 ± 14	75 ± 5
H-ATPase	Control	480 ± 11	275 ± 14	270 ± 15	233 ± 12
	TMP (10 mM)	414 ± 17	260 ± 11	264 ± 15	211 ± 12

Values are means ± SE expressed as pmol/mm/hr; $N = 6$ in each group. ND is not done (see **Methods** for details, 90 min pre-incubation with TMP).
[a] $P < 0.05$ vs. control [b] $P < 0.01$ vs. control and 0.1 mM TMP

Discussion

Trimethoprim is a widely used antimicrobial agent usually administered to humans as trimethoprim-sulfamethoxazole (Bactrim®). While the physico-chemistry and pharmacokinetics in humans and other animals have been reviewed in detail in the past [23–26], abnormalities in renal function and electrolyte homeostasis seem to be occurring more frequently. Some of the renal disorders associated with trimethoprim treatment include decreased creatinine secretion, interstitial nephritis, and hyponatremia [2, 3] (Sulfamethoxazole appears to be without effect). Recently, trimethoprim-induced hyperkalemia has been reported in patients with the acquired immunodeficiency syndrome (AIDS) who receive either high doses of the drug (20 mg/kg/day) or prolonged therapy (7 days or more) for *Pneumocystis carinii* pneumonia [4, 5]. The hyperkalemia appears to be reversible as it ameliorates after withdrawal of the agent [6–8]. The mechanism for the hyperkalemia is incompletely understood, but the renin-aldosterone axis is reported to be intact in most, but not all, cases [5, 9, 27]. Metabolic acidosis has also been seen in patients without AIDS who received long-term high-dose trimethoprim, although its incidence is far less than is the hyperkalemia.

The collecting tubule plays a major role in potassium secretion and sodium reabsorption, processes which depend, in part, on the basolateral Na-K-ATPase pump. Inhibition of the enzyme would affect the transport of both actions. Sodium wasting would occur, although its magnitude may not be substantial if the enzyme were intact in other parts of the nephron. Net potassium balance in collecting tubule results as a combination of secretion via K channels and luminal reabsorption via activity in the H-K-ATPase pump localized to α intercalated cells. Inhibition of Na-K ATPase would result in hyperkalemia as the gradient across as the basolateral membrane would be dissipated. If collecting duct H-K ATP remained intact, K absorption would continue, making the hyperkalemia more profound.

Velázquez et al [11] recently reported that an acute infusion of trimethoprim inhibited potassium secretion and enhanced sodium excretion in the *in vivo* perfused rat distal tubule. The decrease in potassium excretion was 40% and the change in sodium transport was twofold. The drug had no effect on urine flow rate or chloride excretion, findings confirmed in the present study. Velázquez et al [11] also showed a 50% decrease in transepithelial voltage when 0.1 mM trimethoprim was perfused. This dose is comparable to the one which we showed to significantly inhibit collecting tubule Na-K-ATPase activity *in vitro*.

Recent electrophysiologic measurements in the A6 cell line demonstrate that 1 mM trimethoprim directly inhibits the amiloride-sensitive Na^+ channel [28]. Apical, but not basolateral, administration affected the channels, suggesting that a direct interaction of the drug with the outer channel pore is required. Furthermore, the inhibitory effect of 1 mM trimethoprim on short-circuit current was rapidly reversed by the subsequent addition of amphotericin B (10^{-6} M) to the luminal bath [28]. The authors postulated that trimethoprim did not alter Na-K-ATPase or cellular metabolism since amphotericin B creates amiloride-*insensitive* ion channels allowing sodium to enter cells freely. They did not, however, measure Na-K-ATPase directly as we did in the present study. After either a single dose or chronic administration, trimethoprim was noted to inhibit Na-K-ATPase throughout the nephron, with the singular exception being the thick ascending limb. The pharmacologic agent also inhibited the enzyme when incubated *in vitro*. The discrepancy between electrophysiologic studies in an intact cell line in culture and our current biochemical studies in permeabilized cells are unknown. It appears that the A6 cell line is more sensitive (that is, current inhibition) to trimethoprim. Our data, however, do support the notion that trimethoprim's inhibition of short-circuit current is secondary to its direct effect on Na-K-ATPase activity. We say this because *in vitro* incubation of normal tubules with 0.1 mM TMP is associated with a significant fall in enzyme activity; a greater decrease occurs if the concentration is further increased (that is, to 1 mM TMP).

An additional explanation, however, is that trimethoprim may affect both apical sodium channels (or potassium) and basolateral Na-K-ATPase equally. Amiloride, a drug chemically similar to trimethoprim, results in hyperkalemia when given chronically. Amiloride also inhibits Na-K-ATPase when added to proximal tubules on collecting duct segments *in vitro* [22, 29].

The present study also shows that chronic trimethoprim administration causes hyperkalemia but acid-base status and plasma aldosterone levels remain normal. Bicarbonaturia and a fall in urinary potassium excretion were noted. Na-K-ATPase activity decreased in a pattern similar to that observed in the short term study, however, a more prominent fall was seen in the CCT. Again, H-K-ATPase activity remained unchanged, but collecting tubule H-ATPase activity was slightly decreased. Previous studies by our laboratory [18], as well as by Garg and Narang [30], clearly show that hyperkalemia inhibits H-K-ATPase activity. In the present study, however, we found H-K-ATPase activity to be normal in TMP-treated animals, despite the hyperkalemia. This was an unexpected finding as the drug was not found to have a direct effect on the enzyme when incubated *in vitro* (Table 3). To

us, this suggests that there must be offsetting events when the drug is administered chronically, however, precisely what those events are remain unknown at the present time.

Hyperkalemia is a well known potent stimulus for aldosterone release [18]. In our studies, however, plasma aldosterone was normal after chronic trimethoprim administration, despite a plasma potassium of 5.6 mEq/liter. Velázquez et al [11] reported in 7 of 30 patients with normal renal function receiving high dose trimethoprim (20 mg/kg/day) for a mean of five days, plasma potassium was 5.9 ± 0.9 mEq/liter. Plasma aldosterone levels in these patients was slightly elevated with a mean of 19.3 ± 9.5 ng/dl (laboratory control values were 8.1 to 15.5 ng/dl). In the face of hyperkalemia, we feel this is a suboptimal hormonal response, and combined with the result of our study, suggests that trimethoprim may indeed have a direct effect on the adrenal axis, possibly inhibiting aldosterone synthesis or release.

The kidney is known to avidly concentrate the drug [25], but it is not known whether the same occurs in the adrenal gland. If a crucial step in aldosterone biosynthesis were inhibited, the hormonal response to hyperkalemia would be altered. If Na-K-ATPase is required for aldosterone release, trimethoprim may inhibit its activity in the zona glomerulosa in a manner similar to that we observed in the kidney. These possibilities would explain "normal" plasma aldosterone levels in the face of hyperkalemia. Further studies are required to clarify this and to determine whether it is of clinical relevance in either normal or "at risk" humans.

Our experiments show, as has been reported in humans, that chronic trimethoprim results in bicarbonaturia, but plasma bicarbonate and pH are normal. Such findings suggest that the drug stimulates tissue buffer stores. While liver, bone or muscle are the likely candidates for the enhanced extra-renal buffering, there are no data in the literature examining this issue. Since trimethoprim seems to be similar in many ways to amiloride, we searched the literature for its effect on extra-renal buffering; we were unable to find any data on amiloride, either.

Finally, our study shows that trimethopim has no effect on Na-K ATPase in thick ascending limb, either *in vivo* or *in vitro*. While we have no ready explanation, it may be that there are different isoforms of the enzyme in this segment of the nephron as compared to other parts of the kidney. Further studies are required to verify whether this speculation is true.

In conclusion, acute trimethoprim infusion causes a decrease in urinary potassium excretion without hyperkalemia. This occurs in association with a fall in Na-K-ATPase activity in the proximal tubule and collecting duct. H-K-ATPase is normal. Long-term trimethoprim administration results in hyperkalemia as well as decreased potassium excretion. Na-K-ATPase activity was reduced in the proximal tubule, but a more prominent fall in the enzyme was seen in the cortical collecting tubule. After chronic administration there was a slight decrease in collecting tubule H-ATPase activity, possibly explaining the bicarbonaturia. *In vitro*, trimethoprim causes a direct inhibition of Na-K-ATPase activity in a dose-dependent fashion; the drug does not alter the H-ATPase or H-K-ATPase activity *in vitro*. Trimethoprim must also exert a direct effect on the adrenal axis as plasma aldosterone was within the normal range despite the rather marked hyperkalemia. Taken together, these data suggest that, from a functional standpoint, trimethoprim "is" amiloride, and should be used judiciously not only in the immunocompromised patient, but also in patients with renal insufficiency.

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