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Modulation of the secretion of potassium by accompanying anions in humans

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Modulation of the secretion of potassium by accompanying anions in humans. In animals, secretion of potassium (K) in the cortical collecting duct (CCD) is modulated by the properties of the accompanying anion. In humans, results are inconclusive as previous studies have not differentiated between a kaliuresis due to a rise in the concentration of K from one due to an increase in the volume of urine. Our purpose was to study the effects of chloride (Cl) and bicarbonate on the secretion of K in the CCD in humans using the transtubular K concentration gradient (TTKG), a semi-quantitative index of secretion of K in the terminal CCD. After control blood and urine samples were obtained, all subjects ingested 0.2 mg fludrocortisone to ensure that mineralocorticoids were not limiting the secretion of K. The anionic composition of the urine was varied using three protocols: Normal subjects ($N = 11$) ingested cystine and methionine to induce sulfaturia; nine subjects with a contracted ECF volume (to lower the concentration of Cl in the urine) were also studied during sulfaturia following the ingestion of cystine and methionine; 13 normovolemic subjects were studied during bicarbonaturia following the ingestion of acetazolamide. When the concentration of Cl in the urine was >15 mmol/liter, sulfate had no effect on the TTKG. With lower concentrations of Cl in the urine, the TTKG rose 1.5-fold. The TTKG rose 1.8-fold in the presence of bicarbonaturia despite concentrations of Cl in the urine that were >15 mmol/liter, suggesting that bicarbonate has additional effects on this K secretory process. At comparable concentrations of sulfate and bicarbonate in the urine, the TTKG was increased only with bicarbonaturia. We conclude that it is important to control for the effects of the accompanying anions when evaluating the role of the kidney in disorders of K homeostasis.

The purpose of this study was to evaluate the effect of an increase in delivery of anions other than chloride (Cl) to the distal nephron on the secretion of potassium (K) in humans. It is known that the rate of excretion of K in the urine increases following the administration of anions that are filtered by the kidney, but poorly reabsorbed [1]. It was proposed initially that this effect was mediated by an increase in luminal electronegativity [1, 2]. An alternative hypothesis is that a reduced delivery of Cl to the distal nephron per se augments the secretion of K [3–5].

Clinical tests to study the role of anions on the secretion of K include the induction of sulfaturia or bicarbonaturia. There are, however, no established criteria to evaluate these tests. For example, how high must the concentration of these anions be or how low must the concentration of Cl be in the urine to augment this secretion of K? What is the expected response if renal handling of K is completely normal? Further, previous reports have not distinguished between a kaliuresis due to an elevated concentration of K in the urine versus that due to a rise in the urine volume.

In this study, the transtubular K concentration gradient (TTKG) [6–8] and the rate of excretion of K were employed to assess the influence of non-reabsorbed anions on the K secretory process in the cortical distal nephron in man. The results are in keeping with animal studies [3–5], and indicate that the secretion of K increases with sulfaturia only when the concentration of Cl in the urine is <15 mmol/liter. Bicarbonaturia, however, leads to an increase in the TTKG even when the concentration of Cl in the urine is >15 mmol/liter, implying that bicarbonate has additional effects on the secretion of K.

Methods

Twenty-two healthy volunteers participated in the study. Since one aim was to develop a protocol for an out-patient setting, subjects consumed their usual diet, drank fluids ad libitum and performed their daily activities without restrictions. Informed consent was obtained in each case. Only urine samples with an osmolality equal to or greater than plasma were included in the analyses.

Group 1. Time course for the effect of fludrocortisone acetate (Fl)

Blood and urine samples were obtained from eight subjects prior to the ingestion of 0.2 mg Fl and urine samples were obtained hourly for twelve hours thereafter to provide data on the expected response of the TTKG due to Fl alone. To avoid mixing of urine samples with and without mineralocorticoid action, all subjects voided two hours after taking Fl and that urine was discarded. An additional blood sample was obtained five hours after ingesting Fl and the concentration of K in intervening samples was calculated by iteration.

Group 2. Effect of sulfaturia without ECF volume contraction (high concentration of Cl in the urine)

Blood and urine samples were obtained from eleven subjects as described above. Volunteers then ingested the precursors of

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sulfate, methionine (0.055 g/kg body wt) and cystine (0.055 g/kg body wt), a dose previously shown to increase sulfaturia [9]. A third blood sample was drawn one hour later, and urine samples were obtained at hourly intervals for three hours.

Group 3. Effect of sulfaturia during ECF volume contraction (low concentration of Cl in the urine)

The first stage of the protocol was designed to ensure that all subjects could elaborate a urine with a concentration of Cl which was <15 mmol/liter. This was achieved when nine subjects consumed a diet with no added salt for two days together with a natriuresis and chloruresis induced by the loop diuretic, furosemide, 40 mg orally at twelve hour intervals for three doses. No diuretics were taken within eighteen hours prior to study.

On the morning of the experiment, baseline blood and urine samples were obtained. Subjects were then given 0.2 mg Fl, and blood and urine samples obtained three hours later; subjects then ingested methionine and cystine as above. Urine samples were collected hourly, with a third blood sample drawn two hours after this amino acid load. Four to six hours later, when the excretion of K in the urine appeared maximal, 20 mg of furosemide was taken orally to increase delivery of Cl to the cortical distal nephron; urine samples were collected for the next four 30-minute intervals.

Group 4. Effect of bicarbonaturia

Four subjects had bicarbonaturia spontaneously during the Fl periods and six subjects were given 50 mmol of sodium bicarbonate to induce bicarbonaturia after the administration of Fl. Notwithstanding, bicarbonaturia was present in only three of these subjects; hence a more reliable protocol to induce bicarbonaturia was sought.

Blood and urine samples were collected from thirteen subjects before and after the administration of Fl as above. Each subject then ingested acetazolamide 250 mg p.o., and urine samples were collected hourly until the urine pH was >7. The next urine sample was taken for analysis. If the urine pH was <7 after two hours, a second dose of acetazolamide was administered.

Analytical methods

Electrolytes, osmolality, and creatinine in plasma and urine were measured as previously described [10]. The pH of the urine was measured by a pH meter (Radiometer, Copenhagen, Denmark). The concentration of sulfate in the urine was measured as described by Swaroop [11]. The concentration of aldosterone in the plasma was measured by radioimmunoassay (Coat-a-count Aldosterone, Diagnostic Product Corp., Los Angeles, California, USA).

Calculations

The TTKG is a semiquantitative index of the activity of the K secretory process in the terminal cortical collecting duct [6, 7]; it is calculated as follows, provided that the osmolality of the urine was equal to or higher than that of the plasma:

$$TTKG = (\{K\}_{urine}/(U/P)_{Osm})/\{K\}_{plasma}$$

Table 1. Time course for the kaliuretic actions of Fl in normal subjects

Time in hours	TTKG
0	6.3 ± 0.7
3	11.8 ± 0.5
4	12.1 ± 0.9
5	11.1 ± 3.5
6	10.8 ± 1.5
7	10.6 ± 1.0
8	9.7 ± 0.6
9	9.7 ± 0.3
10	9.5 ± 1.0
12+	4.2 ± 0.7

For details, see **Methods**. 0.2 mg of Fl was ingested at time 0 hr; urines were collected at hourly intervals and blood samples at time 0 hr and 5 hr. The TTKG was calculated only if the pH of the urine was < 6.5 and the osmolality > 285 mOsm/kg H_2O. Urines were discarded between 0 and 2 hours. Results are reported as mean ± SEM.

This provides a ratio of the estimated concentration of K in the luminal fluid of the terminal cortical collecting duct (that is, the concentration of K in the urine adjusted for water abstraction in the renal medulla) to that of K in the peritubular fluid in the renal cortex.

Statistics

The data are expressed as mean ± standard error. Statistical analyses were performed using analysis of variance (Anova, Statsview, Apple Macintosh). A P value of <0.05 was considered statistically significant.

Results

Group 1. Time course for the effect of fludrocortisone acetate

To ensure that changes in the TTKG were the result of modifications in the concentrations of anions in the urine and not due to actions of Fl alone, a time course for the effect of Fl was performed. The TTKG reached a peak of 12.1 at three hours and remained high for ten hours (N = 8; Table 1). Hence mineralocorticoid action should not have limited secretion of K in any protocol and a further rise in the TTKG could not be attributed to the actions of Fl alone.

Group 2. Effect of sulfaturia without ECF volume contraction (high concentration of Cl in the urine)

Before interventions, the concentrations of Na, K, and Cl in the urine were 127, 88, and 151 mmol/liter, respectively (N = 11, Table 2). The osmolality of the urine was 828 mOsm/kg H_2O and the TTKG was 7.1. The TTKG rose significantly three hours after taking Fl (11.0). Within two to three hours after these subjects ingested methionine and cystine, the concentration of sulfate in the urine rose almost threefold; this was accompanied by a significant fall in the concentration of Cl in the urine to 65 mmol/liter. The TTKG, however, did not change significantly (10.4). The rate of excretion of K in the urine increased somewhat with Fl, and did not change with sulfaturia. The difference in the concentrations of measured cations versus Cl in the urine (Na+K-Cl) increased significantly after the ingestion of the sulfate load, largely reflecting the sulfaturia. This difference in concentration can be adjusted for water abstraction in the medulla by dividing by the $(urine/plasma)_{Osm}$.

Table 2. Effect of sulfate on the excretion of K in normals

	Period		
	Control	Fl	Fl + SO_4
Plasma			
K *mmol/liter*	4.0 ± 0.1	3.9 ± 0.1	3.9 ± 0.1
Urine			
Na *mmol/liter*	127 ± 15	82 ± 11[a]	90 ± 14
K *mmol/liter*	88 ± 7	128 ± 14[a]	115 ± 12
K *μmol/min*	60 ± 4	84 ± 9[a]	87 ± 9[a]
Cl *mmol/liter*	151 ± 12	153 ± 18	65 ± 12[a,b]
SO_4 *mmol/liter*	15.9 ± 3.5	13.8 ± 2.8	46.1 ± 11.0[a,b]
Flow *ml/min*	0.7 ± 0.05	0.7 ± 0.2	0.9 ± 0.2
TTKG	7.1 ± 0.7	11.0 ± 1.4[a]	10.4 ± 0.9
$\frac{(Na + K\text{-}Cl)}{(U/P)_{Osm}}$ *mmol/liter*	24 ± 5	21 ± 3	50 ± 9[a,b]

Results (mean ± SEM) are reported for 11 normal subjects at three times during the study: before (control) and after Fl, and during sulfaturia (Fl ± SO_4). The TTKG was calculated in subjects with an osmolality of the urine > 290 mOsm/kg H_2O, and with a pH of the urine < 6.5.

[a] $P < 0.05$ as compared to the control period

[b] $P < 0.05$ as compared to the Fl period

This provides a rough estimate of the concentration of anions other than Cl in the terminal cortical collecting duct.

Group 3. Effect of sulfaturia during ECF volume contraction (low concentration of Cl in the urine)

After the period of salt restriction and diuretic therapy, subjects had laboratory evidence of ECF volume contraction ($N = 9$; Table 3). The concentration of Cl in the urine was 9 mmol/liter, the mean level of aldosterone in plasma was 1563 pmol/liter (56 ng/dl, normal range 111 to 860 pmol/liter (4 to 31 ng/dl)), and the TTKG was appropriately high (10.0). The mean concentration of Na in the urine was higher than expected during ECF volume contraction (19 mmol/liter); this reflected a high rate of excretion of organic anions (data not shown). Hypokalemia (2.9 and 3.4 mmol/liter) was present in two subjects.

The administration of Fl led to only small changes in the rates of excretion of electrolytes, perhaps because the level of aldosterone in the plasma was already high. The mean concentration of Na in the urine fell to 11 mmol/liter while that of Cl remained unchanged (10 mmol/liter). The rate of flow of urine and the excretion of K did not change significantly.

After the administration of cystine and methionine to induce sulfaturia, the concentration of Na in the urine was significantly higher (32 mmol/liter), and that of Cl fell to 4 mmol/liter. The TTKG and the rate of excretion of K were significantly higher (15.7 and 103 μmol/min, respectively). The rise in the rate of excretion of K reflected the increase in the concentration of K in the urine, and a small increase in the urine flow rate (which was not, however, statistically significant).

The delivery of Cl to the collecting duct was then increased by administering a small dose of furosemide; only the early time periods were examined (before there was a large increase in flow). The concentration of Cl in the urine increased significantly to 29 mmol/liter and the TTKG fell promptly to values comparable to the control period (Fig. 1). The rate of excretion of K fell significantly despite a small increase in urine flow after the administration of furosemide (0.6 ml/min pre-diuretic vs. 0.8 ml/min post-diuretic). Thus it is unlikely that the TTKG fell simply due to a diuretic-induced increase in distal nephron flow rate.

Effect of bicarbonaturia

In nine subjects with either spontaneous bicarbonaturia or with bicarbonaturia following the ingestion of 50 mmol of sodium bicarbonate, the TTKG rose to 19 and the rate of excretion of K rose after Fl, largely due to a rise in the concentration of K in the urine (urine flow 0.6 ml/min). Since bicarbonaturia was not induced in a reliable fashion, a protocol employing acetazolamide was undertaken (Table 4). Both means of inducing bicarbonaturia yielded identical values for the TTKG (19 ± 2 and 19 ± 1, respectively); since consistency is important, we focussed on the protocol with acetazolamide.

Group 4. Normal subjects given acetazolamide

After Fl, the concentration of Na in the urine decreased somewhat and the TTKG increased. After the administration of acetazolamide, the concentration of bicarbonate in the urine increased in all subjects ($N = 13$; Table 4). This is reflected by the difference in the concentration of measured cations versus Cl in the urine (Na+K-Cl). The difference, which only in part represents bicarbonate, rose from 56 to 182 mEq/liter. The urine pH of >7 suggests that much of this rise was due to bicarbonate. Adjusting for the urine/plasma osmolality, the concentration of bicarbonate (plus other anions in the cortical collecting duct) rose from 22 to 99 mEq/liter after administration of acetazolamide. The concentration of Na in the urine was unchanged, but that of Cl fell significantly to 41 mmol/liter. The TTKG was significantly higher when bicarbonaturia was present (19.0), despite an increase in the rate of urine flow. Both factors contributed to the very large increase in the rate of excretion of K (278 μmol/min).

Effect of delivery of Cl on the net secretion of K

To study the influence of the concentration of Cl on the TTKG, the data in subjects undergoing a sulfate diuresis (Groups 2 and 3) were separated into those with a concentration of Cl in the urine that was <5 mmol/liter, and those where it was >15 mmol/liter. When the concentration of Cl in the urine was lower, the TTKG was significantly higher (16.3 vs. 10.4, Table 5). The rate of excretion of sulfate was higher and the rate of flow of urine lower in the low Cl group (56 vs. 28 μmol/min, $P < 0.05$; 0.6 vs. 1.0 ml/min). Notwithstanding, even if samples with a rate of flow of urine that were >0.9 ml/min were excluded, there was still a higher TTKG when the concentration of Cl in the urine was <5 mmol/liter.

Comparison of effects of bicarbonate and sulfate on the net secretion of K

It is possible that both bicarbonate and sulfate increased the secretion of K by reducing the delivery of Cl to the distal nephron. If this were true, then these anions should increase the secretion of K to a comparable degree at similar concentrations of Cl in the cortical collecting duct. Table 6 shows the TTKG in subjects treated with Fl in Groups 2 and 4, in whom sulfaturia or bicarbonaturia led to varying concentrations of Cl in the

Table 3. Effect of sulfate on the excretion of K during ECF volume contraction

	Period			
	Control	Fl	Fl + So_4	post-Furos
Plasma				
K *mmol/liter*	3.9 ± 0.2	3.9 ± 0.1	3.8 ± 0.1	—
Aldo *pmol/liter*	1563 ± 275	—	—	—
Urine				
Na *mmol/liter*	19 ± 5	11 ± 5	32 ± 3[b]	37 ± 5[a,b]
K *mmol/liter*	102 ± 13	122 ± 24	174 ± 12[a,b]	86 ± 12[c]
K *μmol/min*	50 ± 17	45 ± 9	103 ± 11[a,b]	67 ± 9[c]
Cl *mmol/liter*	9 ± 1	10 ± 3	4 ± 1	29 ± 7[a,b,c]
SO_4 *mmol/liter*	29 ± 2.6	20 ± 2.5	93 ± 7.0[a,b]	52 ± 3.4[a,b,c]
Flow *ml/min*	0.5 ± 0.1	0.5 ± 0.2	0.6 ± 0.1	0.8 ± 0.1[a]
TTKG	10.0 ± 1.5	8.8 ± 1.0	15.7 ± 1.1[a,b]	10.8 ± 1.1[c]
$\frac{(Na + K\text{-}Cl)}{(U/P)_{Osm}}$ *mmol/liter*	42 ± 5.2	45 ± 8.3	70 ± 3.6[a,b]	45 ± 4.1[c]

For details, see legend to Table 2. Results (mean ± SEM) are reported for 9 subjects with ECF volume contraction, at 4 times during the study: before (control) and after Fl, and during sulfaturia, before (Fl + SO_4) and after 20 mg furosemide (post-Furos).

[a] $P < 0.05$ as compared to the control period

[b] $P < 0.05$ as compared to the Fl period

[c] $P < 0.05$ as compared to the Fl + SO_4 period

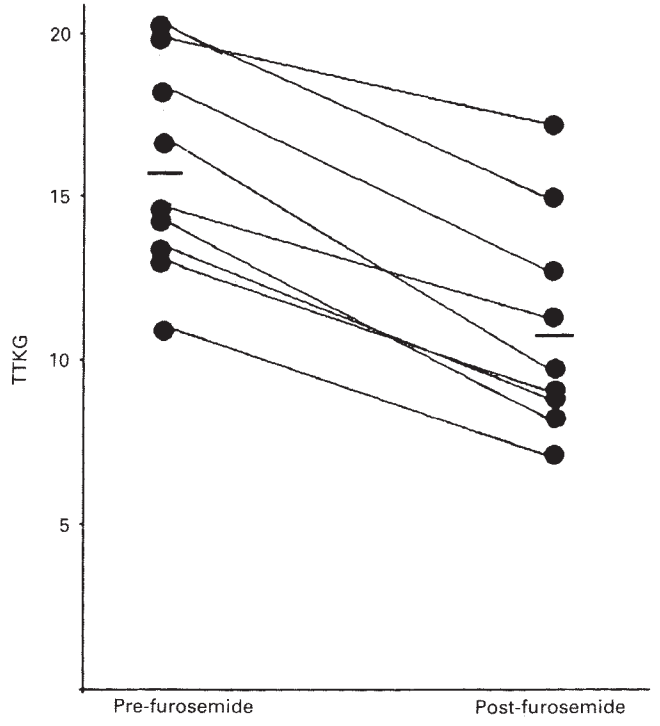

Fig. 1. *Effect of furosemide on the TTKG in subjects with ECF volume contraction during sulfaturia.* Following the ingestion of Fl and a sulfate load, the TTKG rose to 15.7 ± 1.1, coincident with a fall in the concentration of Cl in the urine to 4 ± 1 mmol/liter. After furosemide, the TTKG fell in all subjects (mean TTKG 10.8 ± 1.1); the concentration of Cl in the urine increased to 29 ± 7 mmol/liter.

Table 4. Effect of bicarbonaturia on the excretion of K in normals

	Period		
	Control	Fl	Fl + bicarbonaturia
Plasma			
K *mmol/liter*	4.2 ± 0.1	4.2 ± 0.1	4.0 ± 0.1[a,b]
Urine			
Na *mmol/liter*	111 ± 13	79 ± 13	87 ± 13
K *mmol/liter*	92 ± 11	142 ± 17[a]	136 ± 14[a]
K *μmol/min*	69 ± 10	108 ± 16	278 ± 25[a,b]
Cl *mmol/liter*	147 ± 17	161 ± 20	41 ± 5[a,b]
Flow *ml/min*	0.8 ± 0.1	1.1 ± 0.3	1.9 ± 0.1[a,b]
TTKG	8.0 ± 0.7	11.9 ± 1.1[a]	19.0 ± 1.3[a,b]
$\frac{(Na + K\text{-}Cl)}{(U/P)_{Osm}}$ *mmol/liter*	21 ± 3	22 ± 4	99 ± 2[a,b]

Results (mean ± SEM) are reported for 13 normal subjects at 3 times during the study: before (control) and after Fl, and during bicarbonaturia induced by acetazolamide. The TTKG was calculated in subjects with an osmolality of the urine > 290 mOsm/kg H_2O, and with a pH of the urine < 6.5 in the control and Fl groups; there was no restriction on the urine pH for the Fl + bicarbonaturia group.

[a] $P < 0.05$ as compared to control

[b] $P < 0.05$ as compared to Fl

urine. Although the mean concentration of Cl in the urine was lower in the bicarbonate compared to the sulfate group, this difference was no longer present when correction was made for abstraction of water in the renal medulla [$Cl/(U/P)_{Osm}$]. The TTKG was significantly higher in the subjects with bicarbonaturia versus those with sulfaturia (19.0 vs. 10.4). The urine flow rate was significantly higher in the bicarbonate group (who had been given the diuretic, acetazolamide); this effect alone should tend to lower the TTKG and suggests that bicarbonate has an even more powerful effect on the secretion of K than sulfate. Viewed another way, when the concentration of anions other than Cl in the terminal CCD were close to 50 mEq/liter, the presence of bicarbonate led to a significantly higher TTKG (15.7 vs. 10.4, Table 7). Hence bicarbonate seemed to act in a manner that differed from other non-resorbable anions.

Discussion

The aim of this study was to evaluate the relative importance of urinary anions on the rate of excretion of K in humans. A longer term objective was to apply these data to evaluate patients with hyperkalemia where a relative increase in the rate

Table 5. Effect of the concentration of Cl in the urine on the rate of excretion of K and on the TTKG during sulfaturia

	SO_4	
	Cl < 5 mmol/liter	Cl > 15 mmol/liter
Cl *mmol/liter*	4 ± 1	70 ± 12[a]
$Cl/(U/P)_{Osm}$ *mmol*	3 ± 2	29 ± 6[a]
TTKG	16.3 ± 1.3	10.4 ± 0.9[a]
K excretion *μmol/min*	106 ± 14	87 ± 9
Flow rate *ml/min*	0.6 ± 0.1	1.0 ± 0.3
SO_4 *μmol/min*	56 ± 10	28 ± 5[a]

The data of subjects undergoing a sulfate diuresis after Fl were separated into those with Cl concentrations in the urine < 5 mmol/l and > 15 mmol/liter. The TTKG was calculated in subjects with an osmolality of the urine > 290 mOsm/kg H_2O, and with a pH of the urine < 6.5. Results are reported as the mean ± SEM.

[a] $P < 0.05$

Table 6. Effect of the concentration of Cl in the urine on the rate of excretion of K and the TTKG during sulfaturia and bicarbonaturia

	SO_4	HCO_3
Cl *mmol/liter*	70 ± 12	43 ± 5[a]
$Cl/(U/P)_{Osm}$ *mmol*	29 ± 6	22 ± 1
TTKG	10.4 ± 0.9	19.0 ± 1.3[a]
K excretion *μmol/min*	87 ± 9	278 ± 25[a]
Flow rate *ml/min*	1.0 ± 0.3	1.9 ± 0.1[a]

The data of subjects with concentrations of Cl in the urine > 15 mmol/liter and undergoing a sulfate or a bicarbonate diuresis after Fl are compared. The TTKG was calculated in subjects with an osmolality of the urine > 290 mOsm/kg H_2O. Results are reported as the mean ± SEM.

[a] $P < 0.05$

Table 7. Effect of sulfaturia and bicarbonaturia on the TTKG at the same concentration of anions other than Cl in the CCD

	SO_4	HCO_3
Plasma		
K *mmol/liter*	3.9 ± 0.1	4.0 ± 0.1
Urine		
Na *mmol/liter*	90 ± 14	86 ± 20
K[*mmol/liter*	115 ± 12	149 ± 20
Cl *mmol/liter*	65 ± 12	112 ± 33
Flow *ml/min*	0.9 ± 0.2	1.2 ± 0.3
TTKG	10.4 ± 0.9	15.7 ± 1.1[a]
$\frac{(Na + K\text{-}Cl)}{(U/P)_{Osm}}$ *mmol/liter*	50 ± 9	57 ± 7

Data are derived from subjects who had a concentration of anions other than Cl in the terminal CCD that was close to 50 mEq/liter during a sulfate ($N = 11$) and bicarbonate ($N = 7$) diuresis while Fl was acting (concentrations of Cl in the urine were > 15 mmol/liter). The TTKG was calculated in subjects with an osmolality of the urine > 290 mOsm/kg H_2O. Results are reported as the mean ± SEM.

[a] $P < 0.05$

of reabsorption of Cl has been implicated in the pathophysiology [12]. The effects of altering the anionic composition of the urine on the secretion of K are well known in studies in animals. In contrast, this information is not available in humans. When the rate of excretion of K is evaluated in humans, two groups of factors are considered simultaneously, those that influence the concentration of K in the urine and those that influence the volume of urine ($K_{excretion} = \{K\}_{urine} \times$ urine flow rate). The advent of the TTKG has permitted the non-invasive evaluation of factors influencing the concentration of K in the terminal cortical collecting duct (CCD) in vivo [6–8]. This test is, in essence, a calculation which reflects, indirectly, the $(TF/P)_k$ in the CCD. Hence, the TTKG, urine volume and the rate of excretion of K should be examined together to gain insights into the underlying pathophysiology of disorders of excretion of K.

Effects of sulfate

The effects of sulfate on the excretion of K are similar in animals and humans. Velazquez, Wright and Good [4] showed that perfusion of the late distal convoluted tubule in rats with sulfate resulted in an augmented rate of secretion of K only if the concentration of Cl in the lumen was <10 mmol/liter. Further, they confirmed that this low concentration of Cl was responsible for an increased flux of K by finding a decrease in the rate of secretion of K when the delivery of Cl was increased, despite continued perfusion of sulfate.

The data in Tables 2 and 3 provide the information for humans. When sulfaturia was induced by consuming methionine and cystine in normal subjects, the concentration of sulfate rose and that of Cl fell in the urine. Nevertheless, the TTKG did not rise during this protocol because the concentration of Cl in the urine exceeded 15 mmol/liter in all specimens (Table 2). In contrast, sulfaturia did augment the TTKG 1.5-fold when the concentration of Cl in the terminal CCD was very low. This was achieved by contracting the ECF volume prior to the administration of methionine and cystine (Table 3). There was no correlation between the concentration of Na in the urine and the TTKG at this point in the protocol (data not shown). To confirm that this was a response to the low concentration of Cl in the urine, subjects were given 20 mg of furosemide in the final phase of the protocol and the next two to four urine samples were examined. This led to a prompt fall in the TTKG together with a decline in the rate of excretion of K (before the large diuresis occurred, Fig. 1, Table 3). These experiments suggest how low the concentration of Cl must be in the terminal CCD in order to imply that Cl modulates this secretion of K.[1]

[1] Over what range might the concentration of Cl vary in the terminal CCD in vivo? Estimating the osmolality and volume of filtrate delivered to the terminal CCD should help answer this question. First, the osmolality of fluid in the lumen of the terminal CCD should be the same as that in plasma, or close to 300 mOsm/kg H_2O, when ADH is acting. Second, the volume of urine delivered to the terminal CCD probably is close to 3 liter/day (2% of the GFR). Thus the maximum concentration of urea could be 150 mmol/liter (450 mmol excreted, volume is 3 liters) and that of electrolytes should also be 150 mOsm/liter (75 mmol Na + K, + 75 mEq of anions, the bulk of which are Cl + HCO_3). In the absence of profound bicarbonaturia, the concentration of Cl should be greater than 45 mmol/liter since the rate of excretion of phosphate, sulfate plus organic anions is 80 mEq/day (in 3 liters) or 27 mEq/liter [17]. Since the rate of secretion of K was only elevated once the concentration of Cl fell to <15 mmol/liter, it is difficult to imagine how variations in the concentration of Cl in the terminal CCD would, per se, normally promote or restrict the secretion of K.

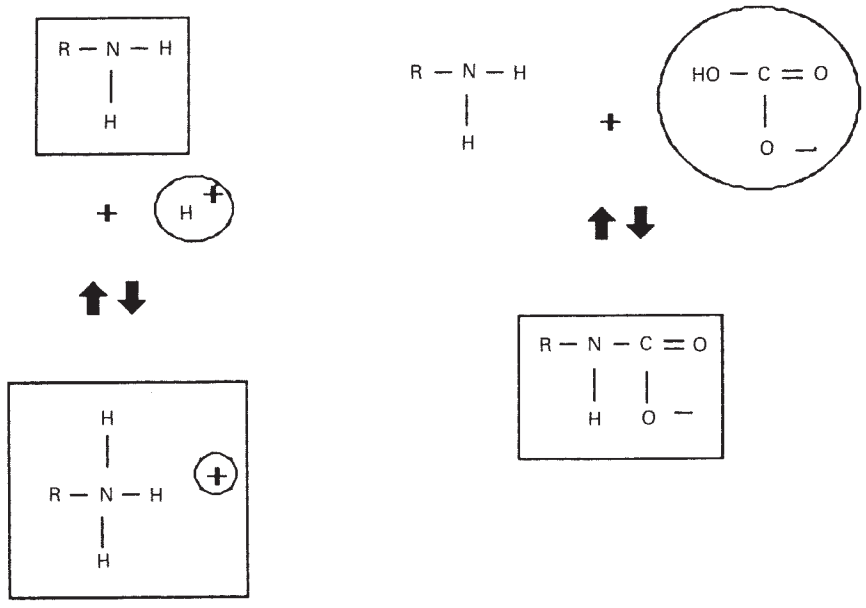

Fig. 2. *A proposed mechanism for the increase in the rate of secretion of K in the cortical collecting duct during bicarbonaturia, despite relatively high concentrations of Cl in the urine.* When the luminal pH is >7, the Cl channel in the luminal membrane of the cortical collecting duct should have a lower density of positive charges at its orifice (equation is driven to the right, $R\text{-}NH_3^+ \leftrightarrow R\text{-}NH_2 + H^+$). In the presence of bicarbonate, an anionic carbamino-compound is formed by carbamylating the uncharged amino group ($R\text{-}NH \cdot COO^-$). This anionic charge might repel the Cl anion in the lumen, diminishing the rate of reabsorption of Cl.

Effects of bicarbonaturia

Up to this point, we have suggested that the secretion of K may be indirectly related to the concentration of Cl in the terminal CCD, providing that this Cl concentration is <15 mmol/liter. Notwithstanding, when normals were treated with Fl plus either sodium bicarbonate or the carbonic anhydrase inhibitor, acetazolamide, there was a large increase in the TTKG. In these experiments, the concentration of Cl in the urine (and its estimated concentration in the terminal CCD) was much >15 mmol/liter (Table 4). Hence, it is difficult to attribute this kaliuretic response with bicarbonaturia only to a direct effect of the estimated concentration of Cl in the urine (Table 6). In addition, when the concentration of anions other than Cl in the terminal CCD was close to 50 mEq/liter and that of Cl was >15 mmol/liter, bicarbonaturia led to a higher TTKG (Table 7). This special effect of bicarbonate was also observed in micro-perfusion experiments [5, 13].

Changes in intraluminal pH might influence the secretion of K. A reduction in the luminal pH from 7.4 to 6.8 reduced secretion of K by 47% in the CCD of the rabbit [14]. Increasing the pH of the luminal fluid (without bicarbonate), however, had no effect on K flux in the superficial distal tubule of the rat [15]. One speculation (among many) that we favor is that bicarbonate itself might promote the secretion of K by inhibiting the transport of Cl through its specific channel, thus favoring electrogenic rather than electroneutral reabsorption of Na (Fig. 2).

Clinical implications of the so-called Cl shunt disorder

How might these results apply to patients with hyperkalemia due to a "Cl shunt"? Patients with chronic hyperkalemia almost always have an inadequate rate of excretion of K in the urine [16]. In a subgroup of these patients, the excretion of K rises if delivery of Cl to the distal nephron is markedly reduced—these patients are said to have a "Cl shunt" (recognizing, however, that the mechanism of the defect in Cl-modulated K excretion remains uncertain). In this report we evaluated, quantitatively, the response of excretion of K to modulation of the concentration of Cl in the terminal CCD in normal subjects. We hypothesize that if this protocol was satisfactorily carried out in a patient with a "Cl shunt", a similar pattern of excretion of K would be obtained.

The following points are very important for the correct interpretation of this diagnostic test:

(a) The concentration of Cl in the urine must be sufficiently low (<15 mmol/liter). This generally will entail pretreatment with diuretics and/or a diet low in sodium chloride. We also recommend ensuring avidity for Na throughout the test by the prior administration of mineralocorticoid.

(b) Stimulation of the excretion of K due to bicarbonaturia must not be present.

(c) The confidence in this diagnosis is increased if the excretion of K falls when Cl is again present in the luminal fluid of the distal nephron (following the administration of a loop diuretic). Care must be taken to obtain urine samples before a large diuresis occurs as the TTKG might decrease without a fall in the rate of excretion of K in this case.

(d) The TTKG, rather than simply the rate of excretion of K, should be used because the latter can be increased if administration of sulfate leads to a significant increase in the urine flow rate owing to the osmotic load. It is important to remember that the TTKG cannot be calculated in urine with an osmolality that is significantly hypo-osmolal to that of plasma.

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