The Effect of Diuretics on Extrarenal Potassium Tolerance

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A potassium loading study was performed in acutely nephrectomized rats to determine the extrarenal effects of diuretics on potassium tolerance. Four diuretics were evaluated: hydrochlorothiazide, furosemide, bumetanide, and spironolactone. Following an intravenous potassium load (0.17 mEq/100 g over one hour), plasma potassium concentration rose by 2.69 ± 0.26 to 3.67 ± 0.20 mEq/L in all groups. There was no difference in the observed increment in plasma potassium concentration between animals receiving diuretics and control animals. These results demonstrate that, at the doses used, diuretics do not impair extrarenal potassium disposal in the rat.

INTRODUCTION

It has become increasingly clear that extrarenal mechanisms play an important role in overall potassium homeostasis, particularly in acute potassium tolerance [1]. It has been shown in humans [2–4], in dogs [2], and in rats [5] that less than 50 percent of an acute exogenous K load is excreted by the kidney in the first four to six hours following administration. The greater part of the remainder of the K load is disposed of by extrarenal mechanisms, thus defending the extracellular fluid from life-threatening hyperkalemia. The major factors which regulate this extrarenal potassium disposal include acid-base balance, actions of hormones including insulin, catecholamines, mineralocorticoids, and glucocorticoids and drugs.

While diuretics are among the most commonly used pharmacologic agents in clinical medicine, there has been little emphasis on the role of diuretics in ion transport outside the kidney. It appears, however, that the actions of diuretics may not be limited to the renal epithelium. For example, furosemide has been shown to cause a prompt increase in venous capacitance which precedes its diuretic effect [6]. More recently, furosemide has been used in defining an ion cotransport mechanism which exists in several cell system such as the loop of Henle [7,8], as well as extrarenal cell systems such as avian [9,10] and human red blood cells [11–13]. This system, which transports Na, K, and Cl into the cell, is inhibited by furosemide. The ability of furosemide and other aminobenzoic acid derivatives, such as bumetanide, to act as inhibitors of the transport system appears to be directly correlated with their diuretic potency [9]. If this cotransport system has major significance in the whole animal, especially in muscle and liver cells where potassium is stored, then extrarenal potassium tolerance could be influenced by administration of loop diuretics.
Further evidence for an extrarenal effect of furosemide as well as thiazides is obvious from their use as antihypertensive agents. The blood-pressure lowering action of both of these drugs has been shown to persist even after their diuretic effect has ceased [14-16]. Data suggesting that thiazides may also have an effect on extrarenal potassium tolerance is found in a recent report demonstrating thiazide-induced correction of hyperkalemia without evidence for increased potassium losses [17].

Aldosterone has been shown to have an effect on extrarenal potassium disposal [1,21]. Mineralocorticoids appear to enhance K uptake by extrarenal tissues [18,19], and extrarenal K tolerance is diminished in their absence [20,21]. One might, therefore, expect then that mineralocorticoid inhibition with an agent such as spironolactone would impair extrarenal potassium tolerance.

We undertook this study to examine the effects of diuretics from three chemical families on extrarenal potassium disposal. The establishment of an extrarenal effect of diuretics on potassium transport would enhance our knowledge of the mechanism of action of these agents. Furthermore, the demonstration of an improvement in extrarenal potassium tolerance with certain diuretics would have important clinical implications in the treatment of hyperkalemia in patients with renal failure.

METHODS

In order to evaluate the effects of diuretics on extrarenal potassium tolerance, a potassium loading study was performed in male Sprague Dawley rats (Charles River, Boston, MA) following acute bilateral nephrectomy. Three classes of diuretics were evaluated: thiazides, loop diuretics (furosemide and bumetanide), and aldosterone antagonists. All rats weighed 225-265 grams and were divided into the following four groups:

Group I—Hydrochlorothiazide Group

Six rats received an intravenous dose of hydrochlorothiazide (5 mg/Kg) 30 minutes prior to the infusion of KCl (Fig. 1). The diuretic was dissolved in a 32 percent ethanol solution and delivered in a volume of 0.5 ml. Six rats receiving an equivalent volume of the ethanol solution alone, prior to KCl, served as controls for this group.

Group II—Furosemide Group

Six rats received an intravenous dose of furosemide (20 mg/Kg) 30 minutes prior to the infusion of KCl (Fig. 1). Six rats receiving an equivalent volume of the aqueous vehicle alone, prior to KCl, were studied as controls.

Group III—Bumetanide Group

Three rats received bumetanide (0.25 mg/Kg) intravenously 30 minutes prior to KCl infusion (Fig. 1). The bumetanide was dissolved in a 10 percent ethanol solution. Three rats receiving an intravenous dose of the ethanol vehicle alone served as controls.

Group IV—Spironolactone Group

Six rats received spironolactone (10 mg/Kg) subcutaneously 24-30 hours prior to study and again 210 minutes prior to KCl loading (Fig. 1). The spironolactone was dissolved in DMSO. Six rats receiving an equivalent volume of DMSO alone served as Group IV controls.
During each KCl loading study, a diuretic-treated rat was studied concomitantly with an untreated rat to control for time-related changes in each parameter examined.

In order to maintain similar potassium balance prior to study, all animals were maintained on 15 grams of Standard Purina Rat Chow (Na and K content equal to 2.5 and 4.0 mEq/day, respectively) for four to seven days before study. Each rat consumed the entire 15 grams of chow daily. All animals were allowed free access to water.

**Diuretic Dose**

In a separate study, the diuretic effectiveness of the doses used for hydrochlo-thiazide and furosemide were evaluated. Sprague Dawley rats (200–250 g) underwent a clearance study under Inactin anesthesia and following catheterization of blood vessels, urine was collected at thirty-minute intervals before and after administration of hydrochlo-thiazide (5 mg/kg) or furosemide (20 mg/kg). The dose of diuretic was considered effective if it resulted in an increase in both urine volume and sodium excretion.

The renal effect of bumetanide was not evaluated since it is ineffective as a diuretic in the rat [22]. Although it has been suggested that inhibition of the Na-K-Cl cotransport system correlates with naturetic potency [9], this fact has not yet been firmly established. We wished to evaluate the extrarenal effect of bumetanide since it is a more specific inhibitor of the cotransport mechanism than furosemide in avian erythrocytes [9]. As it is forty times more potent than furosemide on a molar basis [23], a dose of 0.25 mg/kg was chosen, which is approximately 2.5 times the intravenous dose effective at producing a diuresis on dogs [24]. Spironolactone was administered 24 hours and again approximately two hours before KCl loading, as previous data have shown that it takes eight to ten hours to achieve maximum aldosterone blockade in the rat and that binding at the receptor site may persist after plasma levels decline [25]. Effective blockade of aldosterone action in rat kidney with spironolactone occurs at a ratio of 400–800:1 spironolactone: aldosterone [26]. A dose of 10 mg/kg was chosen since this is two to seven times the diuretic dose used in man, and should block physiologic aldosterone production rates in the rat.

**Potassium Loading Study (Fig. 1)**

Rats were fasted the evening before study. On the morning of study, the animals were anesthetized with Inactin (Promonta, Hamburg, Germany), 12 mg/100 g body weight.
weight intraperitoneally. Body temperature was maintained between 37-38\(^\circ\) by means of a warming board. A tracheostomy was performed and an external jugular vein and carotid artery were cannulated to permit infusion of test substances and withdrawal of blood samples, respectively. Bilateral nephrectomy was then performed through a midline abdominal incision with care taken not to disturb the blood supply of the adrenal glands. After surgery, all animals received a 1 percent body weight bolus of 0.15 M NaCl over 20 minutes followed by a maintenance infusion of 0.15 M NaCl at 0.35 ml/100 g body weight/hour thereafter. A Harvard pump (Harvard Apparatus, Millis, MA) was used to deliver all infusions.

Following 60 minutes of equilibration (T = \(-105\) to T = \(-45\), Fig. 1), two carotid artery blood samples were obtained 15 minutes apart (T = \(-45\) and \(-30\)) for analysis of plasma Na and K concentrations prior to diuretic administration. Immediately following the second blood sample, an intravenous dose of a diuretic or its vehicle, in a volume of 0.5 ml, was given in Groups I-III. Group IV rats received the second dose of spironolactone or its vehicle at T = \(-210\) minutes. Two more carotid blood samples (P, and P\(_2\)) were obtained 15 minutes apart (T = \(-15\) and 0) for analysis of baseline plasma Na and K concentrations after diuretic administration and before KCl infusion. At T = 0 minutes, the saline infusion was replaced with an infusion of 0.5 M KCl administered at 0.35 ml/100 gm body weight/hour to deliver a K load of 0.17 mEq of potassium per 100 gm body weight over one hour. Four carotid blood samples (P\(_3\)-P\(_6\)) were obtained at 15-minute intervals during the KCl infusion for measurement of plasma sodium and potassium concentrations.

Blood pressure, measured with a mercury manometer attached to the carotid artery catheter, was taken at thirty-minute intervals before and after KCl loading. Hematocrit was measured on every blood sample as an approximate guide to changes in intravascular volume.

**Data Analysis**

Sodium and potassium concentrations were measured with a flame photometer (Instrumentation Laboratories, Lexington, MA) using an internal lithium standard. The rise in plasma potassium concentration at the end of each time interval during KCl loading was used as an index of potassium tolerance. This \(\Delta Pk\) was calculated by subtracting the baseline from the experimental plasma potassium concentration during each time period. Changes in plasma Na at these times as well as changes in hematocrit and blood pressure during each period were calculated and compared for all groups. Results of each experimental group were then compared with results from the concomitantly studied control group and analyzed by the Student \(t\)-test. All values are given as the mean \(\pm\) the standard error of the mean.

**RESULTS**

**Diuretic Dose**

The dose of thiazide chosen, 5 mg/kg, produced a three- to sixfold increase in urine flow rate and a 12- to 14-fold increase in sodium excretion ninety minutes after intravenous administration in two test rats. Similarly, furosemide, at 10 mg/kg, induced a three- to fourfold increase in flow rate and a greater than fourfold rise in sodium excretion 60 minutes after injection into two test rats, verifying the diuretic potency of the doses used.
Potassium Loading Study

Baseline plasma potassium values were similar to those obtained during equilibration in each group indicating that baseline plasma potassium concentration was unaltered by diuretic administration. Values for mean baseline plasma potassium concentration were similar between groups ranging from 4.57 ± 0.15 to 5.21 ± 0.13 mEq/L. Of note was the datum that the baseline plasma potassium level in animals receiving spironolactone (5.12 ± 0.11 mEq/L) was similar to the value obtained in the concomitantly studied control group (4.83 ± 0.19 mEq/L).

The effects of KCl loading on the rise in plasma potassium concentration (ΔPk) is shown in Fig. 2. The maximal ΔPk after one hour of KCl was similar in all groups and ranged between 2.69 ± 0.26 and 3.67 ± 0.20 mEq/L. Specifically, the increment in plasma potassium concentration in rats receiving diuretics was similar during each time period to the value obtained in the concomitantly studied control group.

Plasma sodium concentration ranged between 137 ± 3 to 143 ± 1 mEq/L during the baseline period. There were no significant differences in plasma sodium concentration between groups or within any given group during study. Mean blood pressures during equilibration was similar in all groups ranging from 100 ± 5 to 112 ± 6 mm Hg. Blood pressure remained stable throughout the study in all animals except for a slight, transient decrease in Group I rats soon after the administration of hydrochlorothiazide (decreased to 87 ± 9 mm Hg) or its ethanol vehicle (decreased to 94 ± 12 mm Hg). In these animals, blood pressure returned to baseline levels during K loading. Mean hematocrit values prior to KCl loading were similar in all groups (48 ± 1 to 52 ± 0.3 percent) and remained unchanged throughout the study.

![Graphs showing increments in plasma potassium concentration during 60 minutes of KCl infusion in Groups I-IV.](image)

FIG. 2. Increment in plasma potassium concentration during 60 minutes of KCl infusion in Groups I-IV. Each diuretic-treated rat was studied simultaneously with an untreated control rat from that group.
DISCUSSION

The purpose of this study was to examine the effects of diuretics on extrarenal potassium disposal. The results indicate that furosemide, bumetanide, hydrochlorothiazide, and spironolactone, at the doses employed, do not significantly alter the ability of the acutely nephrectomized rat to tolerate a potassium load. The increments in plasma potassium concentration following intravenous KCl administration were not significantly different in animals given diuretics as compared to controls given no diuretics.

The finding that neither furosemide nor bumetanide in the doses used influenced extrarenal potassium tolerance has important implications concerning mechanisms of extrarenal potassium disposal. Potassium can be transported into the cell via the classic Na-K ATPase pathway in which sodium is extruded from cells as potassium enters or via the newly described system of Na-K-Cl cotransport. In this latter pathway, which is stimulated by β-adrenergic agents, and inhibited by loop diuretics in the aminobenzoic acid family, sodium, potassium, and chloride are transported into cells by a cotransport mechanism [7,9,12,13,27–29]. While the system has been described in the loop of Henle [7,8] as well as in avian and human erythrocytes [9–13], its physiologic significance in other cells of the body is unknown. Liver and muscle are the most important organs of extrarenal potassium disposal during K loading. If the cotransport system was an important mechanism of potassium uptake in these organs, one might expect an impairment in potassium tolerance following blockade of this pathway with furosemide. As is evident in Fig. 2, potassium tolerance in furosemide-treated rats was similar to that of control animals. These results suggest that the cotransport mechanism of potassium uptake may not be a major regulator of in vivo extrarenal potassium homeostasis in the rat. Alternatively, it is possible that the concentration of free furosemide in the current experiment was too low to be effective. While the dose of furosemide used was effective in blocking sodium chloride absorption in the loop of Henle, concentrations of the unbound drug in this nephron segment should be considerably higher than circulating levels because the diuretic is secreted and concentrated in the tubular lumen [30]. Since furosemide is 95 percent protein-bound [31], it is possible that the amount of free circulating diuretic in the current experiment was less than the $10^{-5} - 10^{-6}$M concentration necessary to inhibit the cotransport mechanism [9]. Furthermore, circulating epinephrine levels are known to be extremely high in nephrectomized, K-loaded rats [21]. Failure to observe an effect of furosemide on extrarenal potassium tolerance in the present study may be attributable to maximal β-adrenergic stimulation of potassium uptake, not reversible with the doses of furosemide used.

It is less surprising that bumetanide failed to alter extrarenal potassium disposal. Our results support previous findings suggesting that the ability of diuretics, in the aminobenzoic acid class, to inhibit the cotransport mechanism is related to natriuretic potency [9]. Since bumetanide is not an effective diuretic in the rat [22], then it is likely that it has no extrarenal effect as results from the present study indicate. However, the current data do not exclude the possibility that an impairment in potassium tolerance may be seen at higher doses of the drug.

It has recently been suggested that the hypokalemia associated with thiazide administration may reflect redistribution of potassium rather than an absolute loss of total body potassium [32]. Furthermore, thiazides have been reported to correct hyperkalemia in certain patients with a concomitant increase in urinary potassium excretion [17], thus supporting the redistribution hypothesis. If the hypokalemic ef-
fect of thiazides were caused, at least in part, by cellular redistribution of potassium, one would expect that extrarenal potassium tolerance would be enhanced in the presence of this diuretic. In the current study, an improvement in extrarenal potassium tolerance was not observed in thiazide-treated animals. Although the thiazide dose chosen was effective in the distal nephron where the free drug is concentrated, it is again possible that circulating levels of the free drug were not sufficiently high to observe an effect on extrarenal potassium disposal. The extrarenal action of thiazides observed in other studies has been reported with long-term use of the diuretic. It is possible that chronic administration of thiazide is necessary before an effect on extrarenal potassium tolerance is observed.

Aldosterone administration can enhance extrarenal potassium disposal [18,19] and aldosterone insufficiency is associated with an impairment in extrarenal potassium tolerance [21]. It is therefore surprising that extrarenal potassium tolerance was unaffected by spironolactone administration in the current study. The dose chosen has been shown to block endogenous aldosterone action in rat colon [33] as well as the action of exogenously administered aldosterone in rat kidney [26]. It is possible, however, that this dose of spironolactone provided insufficient mineralocorticoid blockade for the elevated levels of aldosterone produced with potassium loading. It is also possible that the time course of spironolactone injections provided insufficient aldosterone blockade at the time of K loading. Since maximum spironolactone effect in the rat is observed eight to ten hours after administration of the drug and since the hormone may persist at the receptor sites after plasma levels decline [25], injection of the hormone 24 and two hours before KCl infusion was thought to produce adequate mineralocorticoid blockade. If, however, the biologic effect of the first spironolactone injection had declined before the effect of the second dose became maximal, then insufficient aldosterone inhibition would occur and potassium tolerance would remain normal. More prolonged administration of spironolactone was avoided in this study to avoid potassium retention prior to study.

In summary, the current results demonstrate that, within the limits of the experimental design of this study, diuretics do not appear to modify extrarenal potassium tolerance in the rat at the doses used. These data do not exclude the possibility that an extrarenal effect on potassium might be observed at higher doses of the drugs. However since the doses of diuretic chosen are similar to or higher than the doses employed to produce a diuresis in clinical settings, the evidence does suggest that diuretics would be ineffective in improving acute potassium tolerance in patients with significant renal failure.

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