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Effect of Sodium Bicarbonate Preloading on Ischemic Renal Failure

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Abstract. Rats pretreated with sodium bicarbonate were functionally protected from the damage of bilateral renal artery occlusion. The rise in serum creatinine (day 1 minus day 0) during the first 24 h after ischemia was 2.88 ± 0.28 mg% in the bicarbonate-loaded animals versus 3.90 ± 0.26 mg% in their matched controls ($p \leqq 0.01$). Pretreatment with acetazolamide produced a similar alkaline urine as the bicarbonate loading (pH 8.3 vs. 7.0 in controls) and a similar degree of protection (delta creatinine 2.85 ± 0.41 vs. 4.23 ± 0.26 mg%; $p \leqq 0.01$). A direct effect of sodium loading was excluded by comparing NH_4HCO_3 with $NaHCO_3$ loading and observing no difference in delta creatinine levels after ischemia (3.39 ± 0.69 vs. 3.20 ± 0.61 mg%). These data indicate that $NaHCO_3$ protects in this model of acute renal failure and further suggest that the mechanism of protection is not related to either systemic alkalosis or sodium loading.

Introduction

Bicarbonate loading has been shown to be beneficial in many forms of renal injury. It has been shown to be effective in the therapy of myoglobinuric renal failure [1] and has been used by one center as part of the resuscitation fluids in severe crush injuries [2]. Experimentally bicarbonate is protective in some [3, 4] but not all forms of toxic renal injury [5, 6]. A recent clinical trial has used bicarbonate in the initial therapy of renal failure regardless of etiology [7] and shown early promising results. Since clinically the etiology of renal failure is often multifactorial [8] and in many instances includes some degree of renal ischemia, we sought to determine the effects of bicarbonate preloading on the severity of renal impairment induced by renal artery clamping in the rat.

After observing better preservation of renal function with sodium bicarbonate loading we probed into the mechanism of this protection by inducing ischemia after carbonic anhydrase inhibition, a treatment which produces a similar alkaline urine without systemic bicarbonate loading. In addition, to exclude a direct effect of sodium loading per se, we compared sodium bicarbonate loading with ammonium bicarbonate loading.

Methods

Transient acute renal failure was induced in male Sprague-Dawley rats (153–318 g) by a period of bilateral renal artery occlusion. (In conducting the research described in this report, the investigator adhered to the 'Guide for the Care and Use of Laboratory Animals' as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.)

The decision of how long to occlude the renal arteries was made after a series of preliminary experiments in which 40- and 60-min occlusion times were examined (fig. 1). With 60 min of occlusion, serum creatinine rose substantially by the end of 24 h and showed little change over the next 2 days. However, during days 2 and 3, there was a 30% death rate from hyperkalemia. In order to avoid deaths from hyperkalemia, all subsequent experiments used a 50-min occlusion time, and the animals were examined at day 1. The increase in serum creatinine over the first 24 h after ischemia was used as a measure of renal damage.

The rats were divided into three treatment groups, each of which had its own control group: sodium bicarbonate loaded, Diamox treated, and ammonium bicarbonate loaded. The groups differed in their treatment prior to the renal artery occlusion. Bicarbonate-loaded animals had their drinking water replaced with 0.28 *M* $NaHCO_3$ 3 days prior to surgery. The day before surgery they were given a 10-cm^3 gavage of this same bicarbonate solution. The bicarbonate control group received normal drinking water during the 3 days prior to surgery. To control for the stress of handling, on the

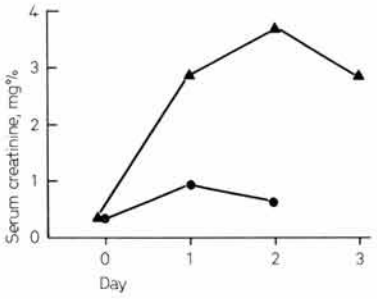

Fig. 1. Serum creatinine (autoanalyser). Rats underwent bilateral renal artery occlusion after collection of day 0 blood. ▲ = 60 min clamping; ● = 40 min clamping.

day before surgery they were gavaged with 0.1 cm^3 of tap water. The sodium bicarbonate loaded and the bicarbonate-loading control groups were matched and run concomitantly, starting 1 control with each bicarbonate-loaded animal. Diamox-treated animals were given normal drinking water throughout. For the 2 days prior to surgery and on the morning of surgery, the animals were given Diamox by gavage (0.5 cm^3 three times daily; 30 mg/kg in water). To control for the stress of handling, controls for this group received water by gavage (0.5 cm^3 three times daily) on the same schedule. Again controls were run concomitantly with the Diamox-treated animals. Ammonium bicarbonate loaded animals had their drinking water replaced with 0.28 *M* NH_4HCO_3 the 3 days prior to surgery and received a 10 cm^3 gavage of the same NH_4HCO_3 solution the day prior to surgery. The concomitant control for this group was a group of sodium bicarbonate loaded animals treated as above. In all groups of animals on the morning of surgery, urine was either collected in metabolic cages or obtained after induction of anesthesia through a small suprapubic incision. Urine pH (combination pH electrode, MI-414; Microelectrodes, Inc., Londonderry, N.H., USA) and osmolality (Wescor Vapor Pressure Osmometer) was measured.

On the day of surgery the rats were anesthetized with phenobarbital (35 mg/kg), and blood (1 cm^3) was obtained by cannulation of the superficial femoral vein for subsequent determination of serum creatinine (Beckman Creatinine Analyser 2). The kidneys were approached through small flank incisions, and loose ligatures were placed around the renal arteries proximal to the first bifurcation. After localizing both vessels, the left and right renal arteries were occluded in rapid succession using small metal clamps (Schwartz 1-in temporary clips, Roboz RS-5452). After 50 min the clamps were removed from the renal arteries, and the wounds were closed. The animals were observed during recovery from anesthesia, and thereafter all groups were allowed free access to food and regular water. One day after surgery the animals were again anesthetized with Nembutal, and blood was obtained from the abdominal aorta for analysis of serum creatinine.

In order to obtain more information on the condition of animals at the time of renal artery clamping, animals subjected to similar pretreatments were anesthetized at the time when the renal artery would normally have been clamped, and a large volume of blood was obtained for chemical analysis. Animals were divided into four treatment groups similar to those above. Sodium bicarbonate loaded animals, Diamox-treated animals, and ammonium bicarbonate loaded animals all received the same treatment as described above. Control animals received only regular water to drink and were given no gavage. All animals were placed in metabolic cages for 24 h prior to surgery, and urine was collected for measurement of volume, creatinine, sodium, and potassium (Beckman, Kline Flame). On the day that renal artery clamping would normally be performed, after the induction of anesthesia, 7 cm^3 of blood was obtained from the abdominal aorta for determination of arterial blood gas and pH (Instrument Laboratory System 1303, Allied Instrumentation Laboratory) and serum sodium, potassium, osmolality, and creatinine concentrations.

Results are shown as mean values ± SEM. In animals undergoing renal artery occlusion, statistical comparisons were made between the treatment group and its matched control group by a nonpaired Student t test. Comparisons between the four groups of animals not undergoing renal artery occlusion were made by one-way analysis of variance. Significance was accepted at the $p \leqq 0.05$ level.

Results

Table I depicts the mean values for urine pH, urine osmolality, and serum creatinine as measured immediately prior to surgery in all animals undergoing renal artery occlusion. The urine pH was significantly more alkaline in both the bicarbonate-loaded and the Diamox-treated animals when compared with their respective control groups. Sodium bicarbonate loading produced a more alkaline urine than ammonium bicarbonate loading. The urine osmolality was not significantly higher in the experimental groups than in their controls. Baseline serum creatinines were similar in all groups.

The average increase in serum creatinine over the 1st day after renal ischemia is shown for the 6 bicarbonate-loaded animals and their controls in the left panel of figure 2. Animals preloaded with bicarbonate had a significantly lower rise in their serum creatinine than did the controls (2.88 ± 0.28 vs. 3.98 ± 0.26 mg%; $p \leqq 0.01$).

Similarly, the average rise in serum creatinine over the 1st day for the 6 Diamox-treated animals and their controls is shown in the right panel of figure 2. Like the bicarbonate-loaded group, animals pretreated with Diamox also had a significantly lower increase in serum creatinine than their controls (2.85 ± 0.41 vs. 4.23 ± 0.26 mg%; $p \leqq 0.01$). No significant difference was detected in rise in serum creatinine between the ammonium bicarbonate and the sodium bicarbonate loaded animals (3.39 ± 0.69 vs. 3.20 ± 0.61 mg%, not shown).

Shown in table II are the average values for blood pH, calculated serum bicarbonate, and serum sodium at the time when the renal artery would normally have been clamped. Also shown is information calculated from the

Table I. Values prior to renal artery clamping

Treatment[1]	n	Urine pH	Urine osmolality mosm/l	Serum creatinine mg %
Diamox	6	8.3±0.1*	1,044±141	0.15±0.03
Control	6	6.8±0.2	1,189±306	0.25±0.03
$NaHCO_3$	6	8.3±0.3*	1,070±172	0.30±0.04
Control	6	7.2±0.3	775±175	0.32±0.02
$NaHCO_3$	9	8.2±0.2*	1,072±114	0.30±0.04
NH_4HCO_3	7	7.4±0.3	1,321±144	0.21±0.03

[1] Animals were orally loaded for 3 days.

*Significant difference between experimental group and matched control.

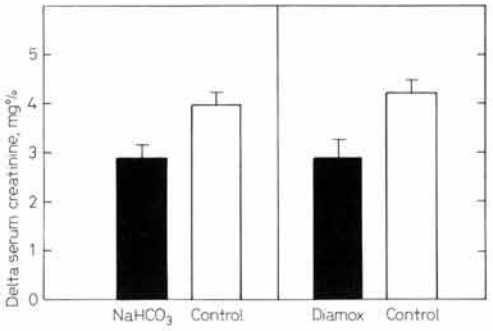

Fig. 2. Rise in serum creatinine (day 1 minus day 0) in rats 1 day after 50 min bilateral renal artery occlusion. Groups differed in their pretreatment before the clamp. Error bars indicate SEM. Controls differed significantly from experimental groups ($p \leq 0.01$).

Table II. Values after 3-day loading

Treatment	n	Blood pH	Serum bicarbonate mEq/l	Serum Na mEq/l	Urine volume cm^3/day	Na excretion mEq/day
Control	4	7.32±0.02	24.8±0.4	139.3±1.4	6.4±1.7	1,199± 180
Diamox	4	7.25±0.02*	23.8±1.9	144.3±1.0*	14.8±3.8	743± 37
$NaHCO_3$	4	7.40±0.01*	26.6±1.0	140.8±0.5	11.8±3.7	4,954±1,567*
NH_4HCO_3	5	7.36±0.01	26.3±0.8	139.6±1.3	18.4±1.3*	1,281± 98

*Significantly different from control.

24-hour urine collection ending at this time, including urine volume and sodium excretion. The Diamox-treated group had a significant systemic acidosis when compared to the control group. The bicarbonate-loaded group had a slight systemic alkalosis. Serum sodium was higher in the Diamox-treated group than in controls. There were no significant differences between the groups in calculated serum bicarbonate.

The average urine flow rate was high for the Diamox-treated and sodium bicarbonate loaded animals, but it did not differ significantly from the control animals. Ammonium bicarbonate loaded animals had significantly higher flow rates than controls. Consistent with the large sodium load given to these animals, the sodium bicarbonate loaded group had a higher sodium excretion rate than controls, while the urinary sodium excretion was not increased in the Diamox group. Not shown in table II are serum osmolality (mean 311 mosm/l), creatinine clearance (2.2 cm^3/min), serum potassium (4.0 mEq/l), and potassium excretion (2,227 mEq/day) which did not differ from controls.

Discussion

Sodium bicarbonate preloading led to better preservation of renal function at 24 h after an ischemic renal insult when compared to water-drinking controls. Since the effects of pretreating the animal may last for some period of time after the renal insult, the preservation of renal function may indicate either protection of the kidney from the initial injury or more rapid recovery during the early maintenance phase. In either case, this protection could be a consequence of any of the many physiologic changes induced by the bicarbonate loading: volume expansion secondary to concomitant sodium loading, natriuresis secondary to sodium loading, systemic alkalosis, alkalinization of the tubular fluid, or increased tubular flow rate. In order to examine which of these mechanisms were important, we induced a similar degree of tubular alkalinization with Diamox pretreatment and found a similar degree of protection. Both of these agents caused an increase in urine pH (table I), while Diamox produced systemic acidosis, and bicarbonate produced

alkalosis (table II). Sodium excretion was increased only in the sodium bicarbonate loaded animals. If the mechanism of protection is the same in both groups, it appears that neither systemic alkalosis nor increased sodium excretion is necessary for the beneficial effect. The results of clinical trials of bicarbonate loading in acute renal failure [7] have also led to the suggestion that systemic alkalosis is not an important protective mechanism, since only those patients that developed a bicarbonate diuresis benefited from the therapy irrespective of the degree of systemic alkalosis.

Serum sodium was higher in the present study in the Diamox-treated animals. This may reflect the changes in the acid-base status of the animal, increased water excretion, or it may be a consequence of loading the animals with the sodium salt of acetazolamide. To examine if sodium loading was important in the effect, we compared ammonium bicarbonate loading with sodium bicarbonate loading. There were no differences between these groups. We conclude that sodium loading is not critical for the effect. These results agree with previous studies on ischemic renal failure which have not shown any consistent protection from either preloading with sodium chloride or chronic Doca therapy [9–11].

In the present study, both the Diamox-treated animals and the sodium bicarbonate loaded animals had alkaline tubular fluid. Although there was no significant increase in urine flow rates with either agent, it is likely that both agents also increase end proximal tubular flow rates. By exclusion, one or both of these factors appear to be causally related to the protection from ischemic injury seen in the present study. The mechanism of protection is not known. Similar protection from ischemic injury has been seen by other agents that increase tubular flow rates such as Lasix and mannitol [12–14]. In other studies protection has correlated with a decreased number of tubular casts, and a direct washout phenomenon has been suggested [13, 14]. However, fewer casts may also indicate less cellular damage, and this dichotomy has not been resolved. Since outer medullary blood flow is compromised in the postischemic period [15–19], it is also possible that agents that increase tubular flow may supply some nutritive flow to the outer medulla and thereby limit cell death. Other methods will need to be applied to resolve these questions.

Bicarbonate has now been shown to be protective in a number of forms of renal failure [1, 3, 4], and it may have some general mode of action such as increase in tubular flow rate. However, it is possible that the mechanisms of protection are different in the other forms of renal failure. Unlike the findings in ischemic renal failure, volume expansion and chronic sodium loading have been shown to be protective in several of the toxic models of renal failure [10, 21, 23, 24]. In myoglobinuric renal failure the mechanism of action may be quite unique, since tubular alkalinization may prevent the formation of hematin which is a cellular toxin [25]. Further mechanistic studies of the protective effect of bicarbonate diuresis in ischemic renal failure seem warranted.

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