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0014-4754/88/050450-04$1.50 + 0.20/0

Alkalosis and renal excretion of ammonia by rat kidney

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Received 18 November 1987; accepted 18 February 1988

Summary. Upon sulfate administration, U_{pH} falls more in alkalotic rats than in controls. Alkalosis can lead to a reduction in $U_{NH3}V$ at highly acidic urine. The significance of this process is doubtful at U_{pH} ranging from about 6 to 7. At lower U_{pH} less NH_3 would be excreted, thereby less H^+ would be trapped in urine and some acid would be conserved.
Key words. Adaptation; diffusion trapping; sulfate infusion; P_{pH}; U_{pH}.

It has long been accepted that chronic acidosis results in an adaptive change so that at any urinary pH, more ammonia is excreted per unit time than in acute acidosis. From a search of the literature, one finds that this conclusion is based on only a single preliminary report [1] and one definitive paper [2]. Both of these used dogs as experimental animals. Much evidence exists to show that acidosis induces an increase in ammonia production by rat kidneys both in vivo and in vitro [3, 4]. In chronic acidosis no experiments seem to have been reported to show long-term adaptive increases in ammonia production and excretion as in dogs. In neither species do any data exist as to whether chronic alkalosis can produce suppression of ammonia excretion. We have evaluated changes in renal handling of ammonia in rats with chronic alkalosis in order to see if an adaptive change can be demonstrated under this condition.

Methods. Rats of the Wistar strain were raised in our animal facilities. They were studied when they had achieved a weight of greater than 300 g. Because sex differences have been reported in ammonia production [5, 6] only male rats were used. They were housed in individual cages and given standard laboratory chow ad libitum. Drinking solutions were different. Untreated rats were given free access to tap water. A second group of rats were given 75 mM $NaHCO_3$ to drink. Animals were maintained this way for 8–14 days.

On the day of study, animals were anesthetized with Inactin and placed on a heated table adjusted to maintain the rat's core temperature at 37.5–39.5 °C. A jugular vein was cannulated and an infusion of isotonic NaCl was started. Delivery of the infusate was 1 ml/h. A femoral artery was then catheterized and clotting prevented by filling the catheter with heparinized saline. The bladder was cannulated for collection of urine. Two hours after the start of the saline infusion, two 20-min control periods were run. Urine was collected under oil in preweighed glass vessels. At the midpoint, the arterial catheter was cleared and blood collected anaerobically in siliconized glass syringes. As soon as possible thereafter (usually less than 5 min after collection), urine and blood pH were measured using a Radiometer pH meter.

After the control periods, the infusate was changed to isomotic Na_2SO_4 (100 mM/l). Blood and urine were collected for an additional four periods. After completion of the six periods, urine was analyzed for ammonia concentration using the method of Bessman and Bessman [7]. Plasma was separated, and both plasma and urine were analyzed for Na and K by flame photometry. Differences between groups were compared using Student's t-test. The relationship between U_{pH} and U_{NH3} was calculated by regression analysis. All statistical calculation were made using the IBM program, 'Statpack'.

Results. Table 1 shows that giving rats a 75 mM bicarbonate solution to drink produces a mild alkalosis with the plasma pH increasing 0.05 pH units. In contrast P_{Na} and P_K showed no changes. U_{pH} increased markedly by close to 0.7 pH units. Changes in both P_{pH} and U_{pH} in experimental animals were significant.

After infusion of sulfate was started, P_{pH} was increased and U_{pH} decreased in progressive manner (table 2). There was little difference in the change in pH of plasma with sulfate infusion. The change in U_{pH} was greater in the alkalotic rats than in controls. In non-treated animals the pH of the final urine was 0.78 units less than in pre-sulfate infusion periods while in rats allowed to drink $NaHCO_3$, the drop was 1.57 units. The difference was 0.79 ± 0.22 pH units, a highly significant increment.

Figures 1 and 2 show the relationship between log $U_{NH3}V$ and U_{pH} for rats drinking tap water and bicarbonate solutions, respectively. Table 3 shows the least squares solution

Table 1. Preinfusion acid-base and plasma electrolyte values

Treatment	P_{pH}*	P_{Na}	P_K	U_{pH}*
None	7.37 ± 0.016 (14)	139.5 ± 4.3 (9)	6.11 ± 0.74 (9)	6.36 ± 0.12 (16)
HCO_3	7.42 ± 0.17 (15)	144.6 ± 2.9 (6)	5.81 ± 0.72 (6)	7.04 ± 0.16 (16)

All data are expressed as means ± SE; * $p < 0.05$ in comparison of the two groups. Numbers in parentheses indicate number of data in each mean. A total of 8 animals were used.

Table 2. Changes in plasma and urinary pH during continuous infusion of Na_2SO_4

Period	Untreated		Alkalotic	
	P_{pH}	U_{pH}	P_{pH}	U_{pH}
1	7.41 ± 0.032	5.86 ± 0.11	7.43 ± 0.016	6.21 ± 0.29
2	7.43 = 0.034	5.79 ± 0.081	7.45 ± 0.022	5.99 ± 0.20
3	7.44 ± 0.031	5.70 ± 0.084	7.48 ± 0.013	5.74 ± 0.11
4	7.44 ± 0.029	5.58 ± 0.083	7.51 ± 0.014	5.47 ± 0.068

All data are expressed as means ± SE.

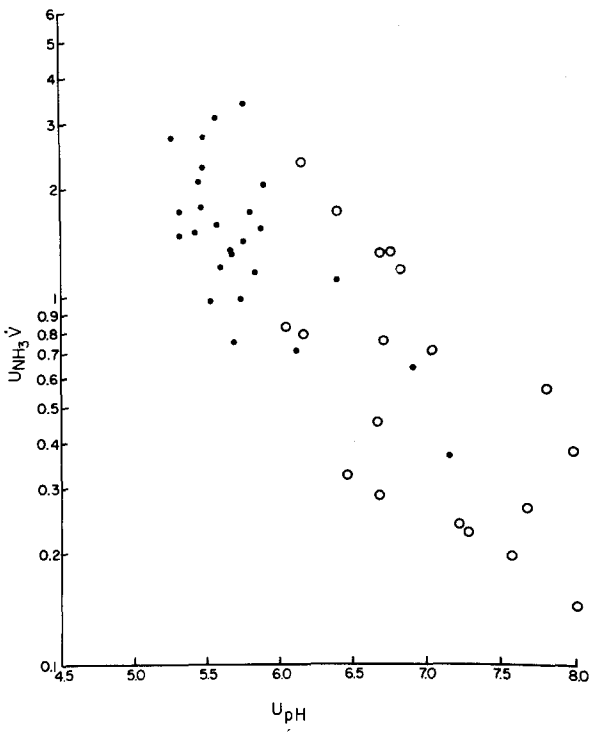

Figure 1. Ammonia excretion as a function of urinary pH in chronically alkalotic rats. $U_{NH3}V$ expressed as uM/min/kg b. wt. Open circles show data obtained under conditions of saline infusion while solid points show data obtained when the infusate was 100 mM Na_2SO_4.

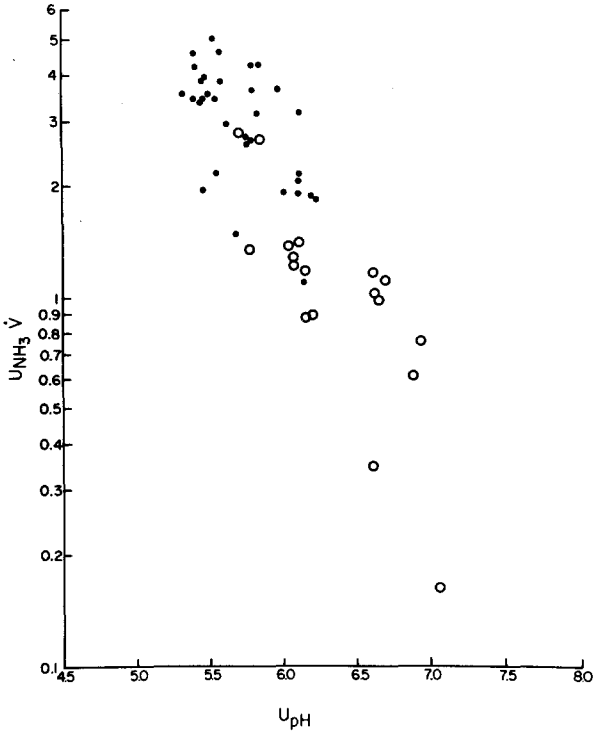

Figure 2. Ammonia excretion as a function of urinary pH in non-treated control rats. Dimensions and symbolism is the same as in fig. 1.

Table 3.

$U_{NH3}V = A + B\ (U_{pH})$			
	A	B ± S.E.	R
Non-treated	15.91	− 2.26 ± 0.25	0.787
Alkalotic	5.94	− 0.75 ± 0.11	0.727
LOG $U_{NH3}V = A + B\ (U_{pH})$			
	A	B ± S.E.	R
Non-treated	3.66	− 0.56 ± 0.05	0.825
Alkalotic	2.03	− 0.32 ± 0.04	0.756

for both a linear and semilogarithmic relationships. Although the fit is improved slightly by the dependence of log $U_{NH3}V$ on U_{pH}, it is not different enough to make it preferred by any objective criterion. When sulfate is infused into untreated rats, the slopes of the decrease in U_{NH3} as plasma pH increases is greater than in rats drinking 75 mM $NaHCO_3$ solution.

Discussion. Despite the rather minimal increase in plasma pH in our experimental animals, an effective alkalosis was produced as indicated by the increase in U_{pH} in the alkalotic animals. Whether the small change is the result of some compensation to the added bicarbonate, or a consequence of the experimental conditions we used would have to be established in further experiments. Although we did not measure fluid intake, it did seem from subjective observations that the rats drank the bicarbonate solution as well as other rats drank water.

By being a poorly absorbable anion at the level of the distal nephron, addition of sulfate to the tubular fluid forces an increase in secretion of K^+ and H^+ in exchange for reabsorbed Na^+ [8–10]. As a result plasma pH will increase since there is no evidence for increased production of acid for the forced increase in urinary excretion. It is not clear as to why there was a greater drop in U_{pH} in the alkalotic rats than in the controls. One could expect that the alkalosis would result in an increase in the amount of urinary bicarbonate ions capable of titrating secreted acid. Nor does it seem that the larger drop in U_{pH} can be explained by a decreased excretion of NH_3.

Another factor which could be involved in the response to alkalosis is changes in ionized calcium. Alkalosis could cause a decrease in Ca^{++} by increasing the number of negative groups capable of binding or chelating Ca^{++} both in plasma and in bone [11]. This change could trigger an increase in parathyroid hormone with a subsequent decrease in phos-

phate reabsorption. As in the case of bicarbonate, the end result would be an increase in tubular fluid buffering. Changes in cystosolic calcium could also cause changes in proximal secretory rates of H^+ [12]. As reviewed by Hulter [13], the issue of direct regulation of secretory activity needs additional work before a definitive mechanism can be used to explain these results.

The regression analysis of ammonia excretion as a function of pH shows that control rats have a greater slope and intercept than do chronic alkalotic rats. This result strongly suggests that in alkalosis an adaptive process is produced whereby the kidney excretes less ammonia than the more acidic control animals. This result is complimentary to that found in acidic dogs, wherein more NH_3 is excreted in acidotic animals at identical U_{pH} [1, 2]. Inspection of the data, however, makes it unlikely that this process is of great physiological significance. Over the midrange of U_{pH} (from about U_{pH} of 6 to 7) there is marked overlap of the data in both populations. The quantitative relationship is such that only at low U_{pH} (probably less than 5.5) can the reduced ammonia excretion be observed in alkalotic rats. It is at U_{pH} greater than 7.0 that alkalotic rats seem to have a greater $U_{NH_3}V$ than controls would have at these same U_{pH}.

In conclusion, evidence has been produced to indicate an adaptive decrease in excretion of ammonia in alkalosis. The physiological significance of the process may, however, be questionable.

Acknowledgments. This work was supported in part by a grant from NSF #PCM 8215938.

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0014-4754/88/050453-03$1.50 + 0.20/0

Postischemic ATP levels predict hepatic function 24 hours following ischemia in the rat

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Summary. Hepatic function was assessed by the aminopyrine breath test (ABT) in male Sprague Dawley rats 24 h after partial hepatic ischemia. ABT decreased progressively to 26.3 ($p < 0.05$) and 19.7% of dose ($p < 0.05$) after 90 and 120 min of ischemia, respectively. ABT at 24 h after injury was correlated to the concentration of ATP in the ischemic lobes 1 h after the onset of reperfusion ($r^2 = 0.971$) but not to ALT activity in plasma at 1 h ($r^2 = 0.391$). We conclude that postischemic ATP levels are a better index of subsequent hepatic function than ALT.
Key words. Liver ischemia; hepatic function; aminopyrine demethylation; ATP.

Ischemic damage to the liver is a major problem in clinical liver transplantation because it limits the time during which an explanted liver can be preserved without losing its viability [1]. Therefore, the development of protective agents that may prolong the ischemic interval which is still compatible with adequate function of the transplanted organ in the new host has high priority [2]. Putative protective agents are often judged by their effect on biochemical measurements shortly after a period of ischemia [3, 4]. However, the critical test of protective interventions is their effect on hepatic function following recovery from the operation. In order to screen protective agents it would be helpful to be able to predict functional recovery shortly after ischemia. In spite of improved knowledge regarding the pathogenesis of ischemic damage the critical events that mark the transition from reversible to irreversible loss of function in vivo are poorly defined. Many of the biochemical and morphological changes that can be observed immediately following a period of ischemia may be spontaneously reversible and may not predict hepatic function at a later point in time.

The aim of the present study was to correlate biochemical measurements made shortly after ischemia with hepatic function 24 h after injury in a rat model of partial hepatic ischemia, and to assess the predictive value of selected biochemical parameters for subsequent hepatic function.

Material and methods. Male Sprague-Dawley rats (Süddeutsche Versuchstierfarm Tuttlingen, FRG) weighing 250–300 g were kept in a climatized environment with a 12-h dark-light cycle and had free access to food and water until the morning of the study. The blood supply to the left lateral and part of the median lobe of the liver was interrupted by placing a surgical clip around the appropriate branches of the portal vein and the hepatic artery under brief ether anesthesia [5]. The clip was left in place for 45 to 120 min and was then removed under a second brief ether anesthesia. One hour after reperfusion a blood sample was obtained from the retro-orbital plexus under light ether anesthesia for the determination of alanine aminotransferase (ALT). At that time a portion of the liver was removed from some animals with a clamp cooled in liquid nitrogen for the determination of ATP. Sham-operated animals served as controls.

An aminopyrine breath test was performed 24 h after clamping the liver. (Dimethylamine-^{14}C)-aminopyrine (120 mCi/mmol, Amersham, Buckinghamshire GB) was dissolved in 0.9% NaCl and injected intravenously at a dose of 1 µCi/kg. The animals were then placed in a restraining cage that permits collection of exhaled CO_2 [6]. At the end of the study blood was obtained for determination of ALT, and in some animals the liver was removed for estimation of the extent of necrosis.