The following is an abstract of the article discussed in the subsequent letter:

Amlal, Hassané, Sulaiman Sheriff, and Manoover Kh Soleimani. Upregulation of collecting duct aquaporin-2 by metabolic acidosis: role of vasopressin. Am J Physiol Cell Physiol 286: C1019–C1030, 2004. First published December 24, 2003; 10.1152/ajpcell.00394.2003.—Metabolic acidosis is associated with alteration in fluid and electrolyte reabsorption in a number of nephron segments. However, the effects of metabolic acidosis on urine osmolality and aquaporin-2 (AQP-2) remain poorly understood. In these studies, we examined the effects of chronic metabolic acidosis on water handling by the kidney. Rats were placed in metabolic cages and subjected to water (control) or 280 mM NH₄Cl loading for 120 h to induce metabolic acidosis. The results indicated a significant increase in urine osmolality with no change in urine volume or urinary Na⁺ excretion in acid-loaded animals. This effect was independent of alteration in fluid intake or salt/Cl⁻ loading. Immunoblotting and Northern hybridization studies indicated that AQP-2 protein abundance and mRNA expression levels increased significantly along the collecting duct system of NH₄Cl- but not NaCl-loaded animals. RIA results indicated that metabolic acidosis was associated with a fourfold increase in circulating levels of vasopressin (AVP) and a significant increase in brain AVP mRNA expression levels. In conclusion, metabolic acidosis upregulates the expression levels of AQP-2 and increases urine osmolality, suggesting an adaptive increase in water reabsorption in the collecting duct. A concomitant increase in AVP synthesis and secretion likely plays an essential role in the adaptation of AQP-2 in metabolic acidosis.

Regulation of aquaporin-2 by metabolic acidosis

To the Editor: Amlal et al. (1) recently reported that an increase in vasopressin synthesis upregulated aquaporin-2 expression in the collecting duct in metabolic acidosis. They observed a fourfold increase in plasma levels of vasopressin in metabolic acidosis. They used 0.28 M NH₄Cl to induce metabolic acidosis. However, 0.28 M NH₄Cl is hypertonic, and rats become dehydrated in response. Therefore, a procedure used recently to induce metabolic acidosis was the addition of NH₄Cl to the food (2). We (3) reported that addition of NH₄Cl to the diet induced acidosis in rats without increasing their plasma levels of vasopressin. We (4) also reported that metabolic acidosis upregulated aquaporin-2 expression in the collecting duct but that urinary excretion of aquaporin-2 was decreased. Therefore, the results seem to be same. However, we think that Amlal et at. observed aquaporin-2 expression in dehydration, since their procedure increased plasma levels of vasopressin. According to their results, plasma osmolality and renin mRNA expression were the same as that in control rats. Amlal et al. could not reveal the reason for the increase in plasma levels of vasopressin. Although urine osmolality was increased in their acidosis group, plasma osmolality was not increased. In our method (4), urine volume was increased in metabolic acidosis together with decreased urine osmolality. We think that the possible reason for the increase in plasma levels of vasopressin is dehydration. We (3) reported that dehydration increased plasma levels of vasopressin threefold. The fourfold increase in plasma levels of vasopressin reported in the study by Amlal et al. is quite large. The data presented in the article could not explain the results. The authors should try the experiments using the proper method to induce metabolic acidosis to solve the above unanswered questions.

REFERENCES


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REPLY

To the Editor: The letter by Nonoguchi et al. questions the model of acidosis we used for our recent article (1). In this article, we demonstrated that metabolic acidosis, which was induced by the addition of 280 mM NH₄Cl in drinking water for 5 days, causes the upregulation of aquaporin-2 (AQP-2) and increases the synthesis and secretion of ADH (vasopressin). Nonoguchi et al. specifically state that 280 mM NH₄Cl added to the drinking water is hypertonic and causes dehydration. On the basis of this assumption, they conclude that the increased ADH levels in this experimental model are secondary to volume depletion and not acidosis. They further state that they used a different protocol to generate metabolic acidosis. In their protocol, metabolic acidosis was generated by addition of NH₄Cl in the diet (and not drinking water). They state that in their model, metabolic acidosis caused the upregulation of AQP-2 but did not affect blood ADH levels.

For the following five reasons, we believe that the conclusions by Nonoguchi et al. regarding our model of acidosis are not supported by facts and that their interpretation of our results is inaccurate.

1) To avoid any interference by the hypertonic drinking water (280 mM NH₄Cl added to the drinking water) with the results, we used a drinking solution with identical osmolality (280 mM NaCl) in another set of animals (see Fig. 3 in our article). In other words, the effect of NH₄Cl was compared with that of NaCl given at identical volume and osmolality. While we found no increase in the expression of kidney AQP-2 (see Fig. 9, A and B) and vasopressin preprohormone levels (data not shown) in rats subjected to 280 mM NaCl or water alone, we observed significant upregulation of AQP-2 (see Figs. 5–7) and increased plasma ADH levels (see Fig. 11) in rats given 280 mM NH₄Cl. These results clearly demonstrate that the stimulatory effect of NH₄Cl on plasma ADH and kidney AQP-2 expression levels are not secondary to dehydration.
tion or to the tonicity of the drinking solution but, rather, are due to acidosis.

2) Nonoguchi et al. make the erroneous assumption that since “0.28 M NH₄Cl is hypertonic,” then “rats become dehydrated.” In response, we should mention that hypertonic oral solutions (up to 310 mM salt or 2% saline) added to drinking water do not cause dehydration in either animals or humans and do not increase plasma ADH levels. Two well-designed studies (2, 3) in humans show that oral hypertonic saline either has no effect or may actually decrease plasma ADH levels. This is contrary to the assumption of Nonoguchi and colleagues. Furthermore, as we discussed above, the effect of 280 mM NH₄Cl was compared with that of 280 mM NaCl for the same duration, demonstrating that the effects of NH₄Cl on kidney AQP-2 and plasma ADH levels are clearly independent of the osmolality of the drinking water.

3) Serum osmolality and blood urea nitrogen (BUN) concentration did not change in rats given NH₄Cl added to their drinking water (see Table 1 in our article). These results clearly demonstrate that subjecting the rats to 280 mM NH₄Cl in drinking water for 5 days did not cause dehydration.

4) To exclude any remote possibility that NH₄Cl at 280 mM in the drinking water may cause volume depletion via unknown mechanisms, we examined the expression of kidney renin mRNA, which is a sensitive marker of volume depletion in rat. As shown in Fig. 12 in our article, while water deprivation, which is an established method of causing volume depletion, caused the upregulation of renin mRNA, rats given 280 NH₄Cl added to their drinking water for 5 days did not show any alteration in renin expression in their kidneys.

5) Finally, we wish to bring to the attention of Nonoguchi et al. that the stimulatory effect of acidosis on plasma ADH has also been demonstrated by other investigators. In studies by Wang et al. (4) and Wood et al. (5), induction of metabolic acidosis by intravenous administration of HCl increased plasma ADH levels in dogs and fetal sheep, respectively. These studies were cited in our article as Refs. 50 and 52.

Taking these results together, we believe that our experimental protocol of 280 mM NH₄Cl added to the drinking water for 5 days is an appropriate and reproducible model of metabolic acidosis and does not cause dehydration. We therefore conclude that increased blood ADH and kidney AQP-2 expression levels in the present studies (1) are clearly the result of metabolic acidosis and are not due to dehydration, as suggested by Nonoguchi et al.

REFERENCES


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