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Hippurate participates in the correction of metabolic acidosis

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Hippurate participates in the correction of metabolic acidosis. Hippurate (Hip), an endogenous conjugate, belongs to the group of uremic toxins. Hip stimulates P-independent glutaminase (PIG) localized at the proximal luminal membrane, desamidating glutamine with the formation of ammonia, a dominant and adaptive elimination product of H^+. This appears to be important because metabolic acidosis (MAC) does not stimulate PIG. Moreover, Hip inhibits ammonia production by P-dependent mitochondrial glutaminase (PDG) that is primarily stimulated by MAC. By this mechanism, it shifts the ammonia production from mitochondria to proximal tubular lumen. MAC stimulates Hip synthesis in the liver and kidney and increases Hip plasma concentration and even fractional excretion by the kidney, which creates an effective regulatory loop of ammoniagenesis. Thus, it appears that Hip by its participation in the correction of MAC possesses the modulatory function.

Hippurate (Hip) belongs to the uremic toxin family [1] and interferes with various physiological and metabolic processes (Table 1). One of its outstanding effects appears to be the participation in the correction of metabolic acidosis (MAC), developing as one of the key consequences of kidney function reduction and acceleration factor of kidney disease progression. MAC participates in mineral disbalance, induces negative nitrogen balance, modifies the balance of ureagenesis and glutamine (Gln) production, impairs enterocyte energetical balance and function, interferes with cellular immunity, inhibits growth hormone production with the resulting nephrogenic nanism in children, and modulates additional metabolic pathways [12, 13].

Hip stimulates ammoniagenesis [9–12], a dominant and adaptive mechanism of H^+ excretion. Glutaminase activity appears to be the key reaction of ammoniagenesis. Two isoenzymes, that is, mitochondrial P-dependent mitochondrial glutaminase (PDG) and luminal membrane Hip stimulated P-independent glutaminase (PIG), are limiting enzymes of ammonia production. The significance of PIG in ammonia production has been repeatedly discussed [9–11, 14]. Controversial views were caused by the absence of a suitable methodology for the simultaneous determination of PDG and PIG. This methodology exploiting the inorganic phosphate (Pi) dependence/independence of two isoenzymes has been recently published [15]. It allows the simultaneous determination of both isoenzyme activities in rat kidney cortex homogenates of acidified rats [13].

GLUTAMINASE ACTIVITIES DURING ACIDIFICATION AND/OR HIP STIMULATION

The basal PDG activity was twice as high as that of PIG (Fig. 1). Rat acidification by the administration of 1.5% NH_4Cl in drinking water for seven days doubled the PDG, while it only slightly stimulated PIG activity. Hip doubled PIG activity both at basal conditions and during acidification. On the other hand, Hip did not inhibit basal PDG activity, but it inhibited PDG activity markedly and significantly in acidified rats.

P-independent glutaminase stimulation by Hip was in good accordance with the previous indirect studies [9–11, 16], and the first direct evidence was with the direct determination of PIG activity. Its additive effect with MAC was physiologically reliable. The dominant localization of PIG, identical with γ-glutamyl transferase at the luminal membrane of proximal tubular cells, made it especially suitable for the ammonia production from Gln directly in the lumen of proximal tubule [17].

The small and insignificant inhibition of PDG activity by Hip at basal conditions, that during acidification showed marked and significant inhibition, was very surprising. It pointed to the shift of ammoniagenesis from mitochondria to the tubular lumen with the restriction of back ammonia diffusion to the blood stream.

HIP ACCUMULATION IN RENAL FAILURE

In healthy subjects, Hip is eliminated by the kidney very effectively, and its plasma concentration remains low until the clearance of endogenous creatinine decreases below 25 mL/min [18]. However, below this glomerular filtration rate, Hip serum concentration increases steeply, and at extreme situations, its concentration in-

Key words: metabolic acidosis, ammoniagenesis, P-independent glutaminase, proximal luminal membrane.

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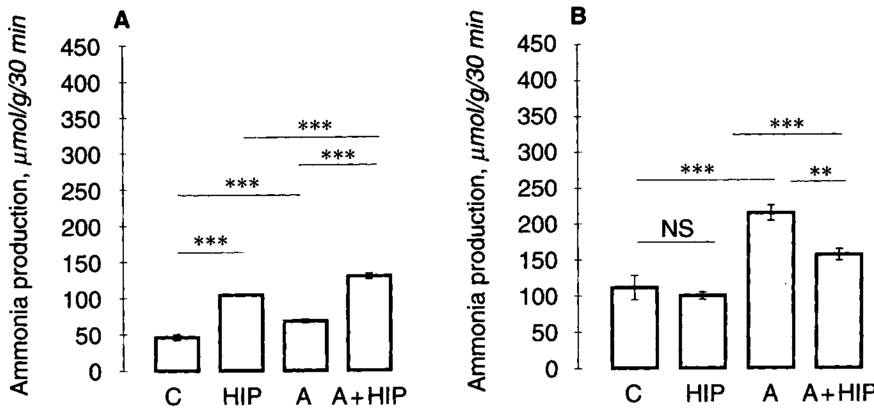

Fig. 1. Effect of Hip on P-independent glutaminase (PIG; *A*) and P-dependent mitochondrial glutaminase (PDG; *B*) activities in kidney homogenates at basal conditions and after acidification. Each bar represents the mean ± SEM. Abbreviations are: C, control; HIP, hippurate; A, acidification; NS, not significant. $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 1. Interference of hippurate with various physiological and metabolic reactions

Effect	Reference
Plasma protein binding	[2]
Organic anion excretion by the kidney	[3]
Indicator of middle molecules and uremic toxin accumulation	[4]
Inhibitor of glucose utilization in kidney and muscle	[5, 6]
Inhibitor of gluconeogenesis in the liver and kidney	[6]
Modulator of fatty acid metabolism	[7]
Inhibitor of tumor growth	[8]
Stimulator of ammoniagenesis	[9–12]

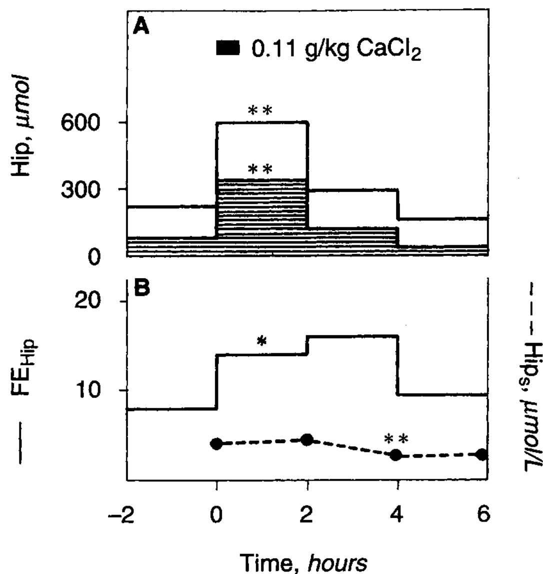

Fig. 2. Acidification test in healthy volunteers. (*A*) Hippurate (Hip) excretion (□) and synthesis (▤). Statistical significance is $^{*}P < 0.01$. (*B*) Hip serum concentration (Hip_s, dashed line) and fractional excretion of Hip (FE_{Hip}; solid line). Statistical significance, $^{*}P < 0.01$, $^{**}P < 0.05$.

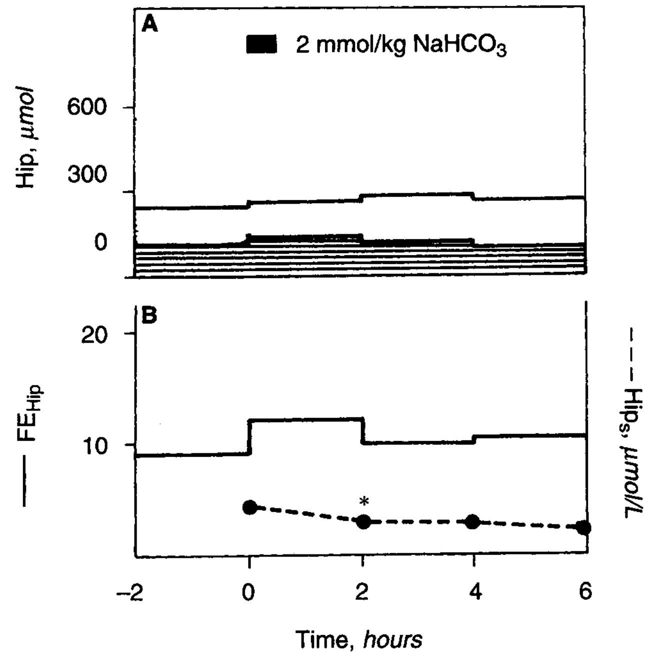

Fig. 3. Alkalization test in healthy volunteers. (*A*) Hip excretion (□) and synthesis (▤). (*B*) Hip serum concentration (Hip_s, dashed line) and fractional excretion of Hip (FE_{Hip}, solid line). Statistical significance, $^{*}P < 0.05$.

creases up to 100 times of normal values [19]. Hip could modulate ammoniagenesis at normal kidney function and a low plasma concentration because it is not only filtered in glomeruli, but completely secreted in the proximal tubule. Consequently, the luminal Hip concentration is high. On the other hand, in advanced renal failure and high plasma Hip concentration, the increased glomerular filtration load increases the Hip concentration in the lumen of proximal tubules. Thus, it is suggested that the Hip concentration in lumen depends on its synthesis, and that it participates in ammoniagenesis at any kidney function.

HIP AND ACID-BASE BALANCE

The Hip participation in acid-base balance would be physiologically reasonable in the case of MAC stimulation of Hip production. A human study found that acidi-

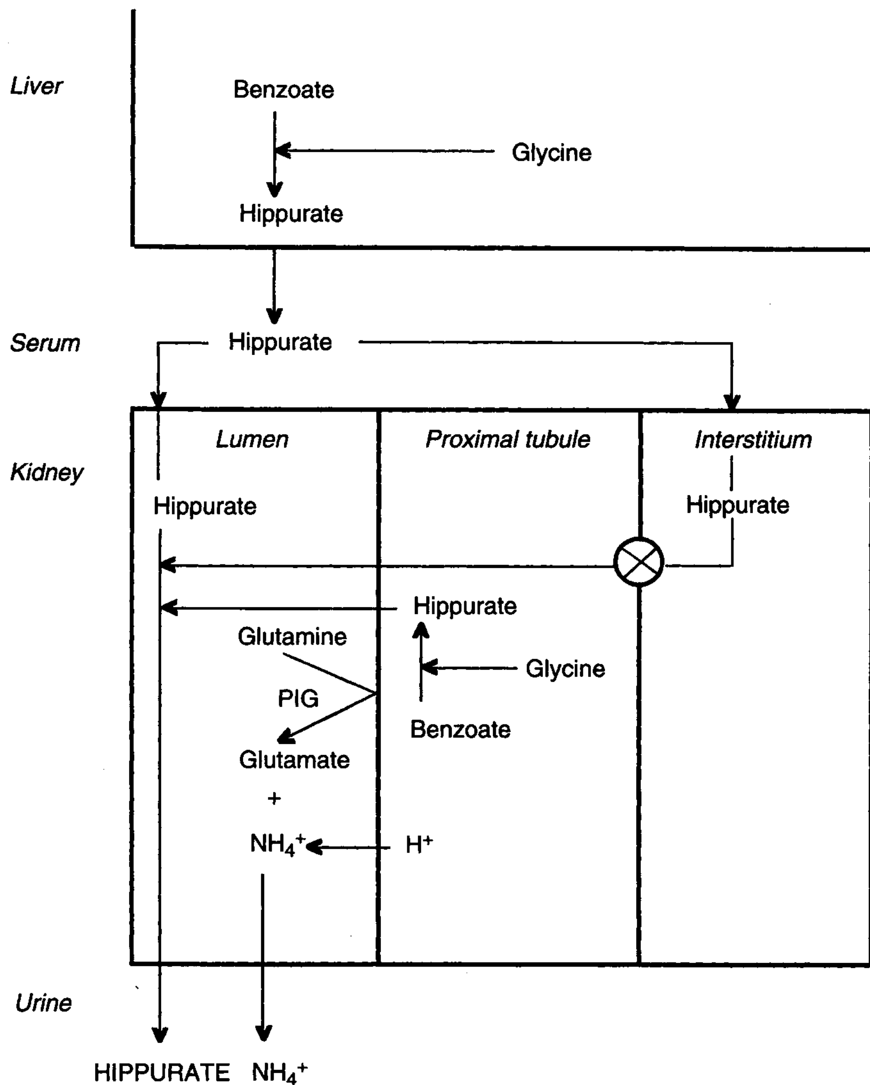

Fig. 4. Schematic representation of Hip synthesis in the liver and kidney and its effect on ammonia production.

fication increases both the liver and kidney Hip synthesis with the increased excretion and fractional excretion of Hip >5, which documents both the complete excretion and the Hip net synthesis in the kidney and the decreased plasma Hip levels (Fig. 2) [19]. On the other hand, alkalinization decreases both the Hip synthesis and excretion with no change in Hip fractional excretion (Fig. 3). We suggest the following mechanism of Hip action (Fig. 4): Benzoate, originating from various exogenous (food) and endogenous (metabolism) precursors, is conjugated with glycine to build Hip in the liver. It is released into the blood from where it is filtered in glomeruli and taken up from interstitium by the organic anion transport system [3]. Hip is synthesized even more actively in the kidney [20] and secreted directly into the primary urine. MAC stimulates Hip synthesis and its tubular secretion. Hip stimulates ammonia production by PIG, and consequently, both Hip and ammonia are excreted in increased amounts in urine. On the other hand, metabolic alkalosis inhibits both Hip synthesis and its excretion with no change in the fractional excretion of hippurate (FE_{Hip}), but sharply decreases plasma Hip concentration.

IS HIPPURATE AN ENDOGENOUS MODULATOR OR UREMIC TOXIN?

Hip is a "uremic toxin" with various biological effects (Table 1). Some of them, such as ammoniagenesis, are hardly seen as simply a consequence of Hip toxicity. Moreover, the direct stimulation of Hip synthesis and excretion by MAC, which is corrected by Hip directly stimulating PIG to produce ammonia intraluminally, draw the attention to the mediating nature of Hip. If true, other effects of Hip and even the effects of other "uremic toxins" synthesized in human body also should be re-evaluated.

In conclusion, Hip appears to be a physiological modulator of ammoniagenesis by stimulating PIG activity. Hip

synthesis is increased by MAC. The increased synthesis of Hip is reflected by an increased GFR_{Hip}, its tubular secretion, and increased PIG activity. We suggest that MAC, besides the stimulation of glucocorticoid production, also increases ammonia production by the stimulation of Hip production. The relationship between glucocorticoids and Hip remains to be elucidated.

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REFERENCES

1. Ringoir S: An update on uremic toxins. *Kidney Int* 52(Suppl 62):S2–S4, 1997
2. Gulyassy PF, Jarrard EA, Stanfel LA: Contributions of hippurate, indoxyl-sulfate, and O-hydroxyhippurate to impaired ligand binding by plasma in azotemic humans. *Biochem Pharmacol* 36: 4215–4220, 1987
3. Ullrich KJ, Rumrich G, Wieland T, Dekant W: Contraluminal para-aminohippurate (PAH) transport in the proximal tubule of the rat kidney. VI. Specificity: Amino acids, their N-methyl-, N-acetyl- and N-benzoylderivates: Glutathione- and cysteine conjugates, di- and oligopeptides. *Pflügers Arch* 415:42–50, 1989
4. Schoots AC, Dijkstra JB, Ringoir SM, Vanholder R, Cramers CA: Are the classical markers sufficient to describe uremic solute accumulation in dialyzed patients? Hippurates reconsidered. *Clin Chem* 34:1022–1029, 1988
5. Spustová V, Dzúrik R: The mechanism of hippurate inhibition of glucose utilization in rat diaphragm. *Biochem Clin Bohemoslov* 20:21–25, 1991
6. Spustová V, Dzúrik R: Effect of hippurate on glucose utilization in rat kidney cortex slices. *Renal Physiol Biochem* 14:42–47, 1991
7. Chorváthová V, Ondreička R, Spustová V, Červeň J, Dzúrik R: Effect of hippurate on tissue fatty acid metabolism. *Physiol Bohemoslov* 39:227–232, 1990
8. Spustová V, Oravec C: Antitumor effect of hippurate: An experimental study of various mouse tumor cells. *Neoplasma* 36:317–320, 1989
9. Welbourne TC, Dass PD: Role of hippurate in acidosis induced adaptation in renal γ-glutamyltransferase. *Life Sci* 30:793–802, 1982
10. Dass PD, Kurtz I: Renal ammonia and bicarbonate production in chronic renal failure. *Miner Electrolyte Metab* 16:308–314, 1990
11. Dass PD, Martin D: Adaptive ammoniagenesis in chronic renal failure. *Renal Physiol Biochem* 13:259–263, 1990
12. Spustová V, Krivošíková Z, Gavulová L, Gazdíková K, Dzúrik R: Nephrogenic metabolic acidosis. *Klin Biochem Metab* 26:170–175, 1997
13. Krivošíková Z, Spustová V, Dzúrik R: Participation of P-dependent and P-independent glutaminases in rat kidney ammoniagenesis and their modulation by metabolic acidosis, hippurate and insulin. *Physiol Res* 47:177–183, 1998
14. Silbernagl MS: Ammoniagenesis catalyzed by hippurate-activated γ-glutamyl-transferase in the lumen of the proximal tubule. *Pflügers Arch* 407(Suppl):S72–S79, 1986
15. Lardner AL, O'Donovan DJ: The hepatic and renal response to hydrochloric acid ingestion by the rat. *Contrib Nephrol* 110:170–175, 1994
16. Dass PD, Stuart BP, Holmes EW: Effect of benzoate and hippurate on the regulation of interorgan nitrogen flow: Therapeutic benefits. *Contrib Nephrol* 110:150–157, 1994
17. Tannen RL, Nissim I, Sahai A: Hormonal mediators of ammonia genesis: Mechanism of action of PGF_2 alpha and the implications for other hormones. *Kidney Int* 50:15–25, 1996
18. Spustová V, Geryková M, Gajdoš M, Dzúrik R: Effect of acidification and alkalization of the organism on hippurate excretion. *Bratisl Lek Listy* 89:377–380, 1988 (in Slovak)
19. Spustová V, Gajdoš M, Opatrný K Jr, Štefíková K, Dzúrik R: Serum hippurate and its excretion in conservatively treated and dialysed patients with chronic renal failure. *Physiol Res* 40:599–606, 1991
20. Spustová V, Geryková M, Dzúrik R: Hippurate synthesis by rat kidney cortex slices, in *Molecular Nephrology: Biochemical Aspects of Kidney Function*, edited by Kovačevič Z, Guder WG, Berlin-New York, Walter de Gruyter, 1987, pp 207–212