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The effect of urea synthesis on extracellular pH in isolated perfused rat liver

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In a non-recirculating system of isolated liver perfusion, stimulation of urea synthesis by NH_4Cl is followed by a decrease of effluent pH by up to 0.2 pH unit. This effect is not observed when urea synthesis is inhibited by amino-oxyacetate or norvaline. When the urea formed by the liver is immediately hydrolysed with urease before the effluent perfusate reaches the pH electrode, the urea-synthesis-induced acidification is no longer observed. This indicates that accompanying alterations in hepatic metabolism after stimulation of urea synthesis, such as increased energy provision and consumption, are not responsible for the extracellular acidification, but that the effect is due to the formation of urea itself. The acidification of the extracellular space after stimulation of urea synthesis by NH_4Cl is quantitatively explained by the consumption of 2 mol of HCO_3^-/mol of urea formed: 1 mol being incorporated into urea, the other being protonated to yield CO_2 and H_2O. The data match the theoretically predicted HCO_3^- consumption during ureogenesis and underline the role of hepatic urea synthesis for disposal of HCO_3^- by converting it into the excretable products CO_2 and urea.

INTRODUCTION

The maintenance of a physiological extracellular pH of 7.4 requires mechanisms to keep the extracellular HCO_3^-/CO_2 ratio constant. Because both compounds of this buffer system are continuously formed during the oxidation of ingested foodstuffs, it is necessary that HCO_3^- and CO_2 are eliminated from the organism at the same rate at which they are generated. The hydrolysis of proteins produces bipolar amino acids, yielding HCO_3^- and NH_4^+ in almost stoichiometric amounts, whereas CO_2 and H_2O are the only products of the complete oxidation of carbohydrates and fat and are excreted via the lungs and kidneys. In man, ingesting an average diet containing about 100 g of protein/day, about 1 mol of HCO_3^- and 1 mol of NH_4^+ are produced. In view of the limited volume of urine, such a high amount of HCO_3^- cannot be disposed of by the kidneys. In mammals, the major pathway for disposal of HCO_3^- is hepatic urea synthesis, which consumes HCO_3^- and NH_4^+ in stoichiometric amounts, yielding an excretable neutral product (Oliver & Bourke, 1975; Oliver *et al.*, 1977; Atkinson & Camien, 1982; Atkinson & Bourke, 1984; Häussinger *et al.*, 1984):

$$2\,HCO_3^- + 2\,NH_4^+ \rightarrow H_2N\text{–}\overset{\overset{O}{\|}}{C}\text{–}NH_2 + CO_2 + 3\,H_2O \quad (1)$$

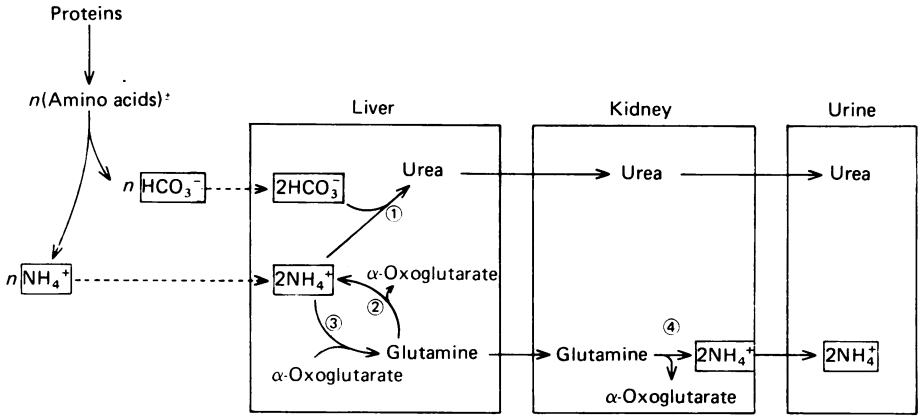

Fig. 1. NH_4^+ metabolism and regulation of HCO_3^- homoeostasis

NH_4^+ and HCO_3^- arise in almost stoichiometric amounts during protein breakdown. Whereas urea synthesis consumes HCO_3^-, HCO_3^- is spared when hepatic urea synthesis is switched off and NH_4^+ is excreted as such into the urine, with glutamine serving as non-toxic transport form of NH_4^+. In metabolic acidosis flux through reactions 1 and 2 (urea synthesis and glutaminase) is decreased, whereas flux through reactions 3 and 4 (glutamine synthetase and renal glutaminase) is increased, resulting in NH_4^+ disposal without concomitant HCO_3^- removal. When NH_4^+ ions are excreted as such into urine, there is no net production or consumption of 2-oxoglutarate in the organism.

In contrast, NH_4^+ fixation by glutamine synthesis does not affect HCO_3^- homoeostasis, and NH_4^+ may be excreted as such into urine after renal glutamine degradation (Fig. 1). Thus, in acidosis, HCO_3^- is spared when hepatic urea synthesis decreases (Atkinson & Camien, 1982; Atkinson & Bourke, 1984; Häussinger *et al.*, 1980, 1984), whereas waste nitrogen is increasingly excreted as NH_4^+ by the kidney, with glutamine being the non-toxic transport form between these tissues (Fig. 1). In this concept, the disposition of hepatic NH_4^+ either into urea or into glutamine synthesis will affect pH homoeostasis, whereas the renal step of NH_4^+ or urea excretion will not itself affect directly the extracellular pH in the organism. However, the co-ordinated action of both processes, hepatic HCO_3^- removal during urea synthesis and renal NH_4^+ excretion, is a prerequisite for an effective regulation of pH and HCO_3^- homoeostasis independent of the requirement for waste nitrogen disposal. This is met by a sensitive regulation of hepatic HCO_3^- consumption during urea synthesis (Oliver *et al.*, 1977; Häussinger *et al.*, 1975, 1983, 1984; Bean & Atkinson, 1984; Häussinger & Gerok, 1985) and of renal ammoniagenesis (for reviews see Tannen, 1978; Welbourne & Phromphetcharat, 1984; Tannen & Sastrasinh, 1984) by the extracellular pH and the absolute HCO_3^- and CO_2 concentrations.

Such a role of the liver in controlling the extracellular pH by regulating urea-cycle flux, however, has been questioned, and renal NH_4^+ excretion was considered to be the crucial step in the regulation of extracellular pH and generation of new HCO_3^- in the kidney (Halperin & Jungas, 1983; Welbourne & Phromphetcharat, 1984; Walser, 1986). The present study was undertaken to demonstrate experimentally the theoretically postulated effect of urea synthesis on extracellular pH (cf. eqn. 1).

MATERIALS AND METHODS

Liver perfusion

Livers of male Wistar rats (100–200 g body wt.), fed *ad libitum* on standard diet (Altromin), were perfused as described previously (Sies, 1978) in an open non-recirculating system. The perfusate was bicarbonate-buffered Krebs–Henseleit saline plus L-lactate (2.1 mM) and pyruvate (0.3 mM), both added as sodium salts. When ornithine was present, its concentration in the influent was 2 mM. NH_4Cl was added from neutralized stock solution by precision micro-pumps. The perfusion medium was gassed with O_2/CO_2 (19:1). The temperature was 37 °C. Perfusate flow was approx. 4 ml/g per min and was kept constant throughout each individual perfusion experiment.

Assays

The perfusate concentrations of NH_4^+ and urea were determined in enzymic optical tests based on the procedures given in Bergmeyer (1974). Effluent pH was monitored with a glass pH electrode (Fa. Ingold).

Materials

All enzymes, NADH and 2-oxoglutarate were from Boehringer (Mannheim, Germany). L-Lactic acid was from Roth (Karlsruhe, Germany). All other chemicals were from Merck (Darmstadt, Germany).

RESULTS AND DISCUSSION

Stimulation of urea synthesis in an open (non-recirculating) perfused rat liver system by addition of NH_4^+ ions was followed by a decrease in effluent pH. This acidification of effluent perfusate shows a time course similar to that for urea formation (Fig. 2). However, when urea synthesis from NH_4Cl is inhibited by amino-oxyacetate, the decrease in extracellular pH was no longer observed, indicating that not NH_4Cl itself, but its conversion into urea, caused the decrease in extracellular pH. As for control, amino-oxyacetate itself was without effect on the pH in the effluent (Fig. 2). Similarly, when urea synthesis was partly inhibited by norvaline (Marshall & Cohen, 1973), the decrease in effluent pH after NH_4Cl addition was largely diminished (results not shown).

When the urea synthesized by the liver was subsequently hydrolysed by addition of urease to the effluent perfusate, before it reached the pH electrode, stimulation of urea synthesis by NH_4Cl was without effect on pH (Fig. 3). This shows that the acidification of the extracellular space during urea synthesis is directly due to the formation of urea, whereas accompanying metabolic alterations after the addition of NH_4Cl, such as stimulation of pyruvate dehydrogenase flux or increased hepatic oxygen uptake (Häussinger *et al.*, 1975), do not contribute significantly to the acidification of the extracellular space. Similarly, when urea is added to the portal perfusate, its hydrolysis by urease addition leads to an alkalinization of the perfusate (Fig. 3). In the absence of added NH_4Cl or urea, urease infusion led only to a slight increase in effluent pH, owing to hydrolysis of the small amount of urea produced from endogenous proteins and amino acids.

From eqn. (1), per mol of urea formed by the liver, 2 mol of HCO_3^- is consumed and 1 mol of CO_2 is produced. Thus, when isolated rat liver is perfused with the bicarbonate-buffered Krebs–Henseleit medium ($[HCO_3^-] = 25$ mM; $[CO_2] = 1.2$ mM) in an open (non-recirculating) system, stimulation of urea synthesis by addition of NH_4Cl will theoretically result in a decrease of extracellular pH. From the urea concentration measured in effluent and the stoichiometry given in eqn. (1), the new pH of the effluent perfusate after stimulation of urea synthesis can be calculated as:

$$\mathrm{pH} = 6.1 + \log\left(\frac{25 - 2[\mathrm{urea}]}{1.2 + [\mathrm{urea}]}\right) \qquad (2)$$

(with [urea] = urea concentration in effluent) and the extent of acidification is calculated to be:

$$\Delta\mathrm{pH} = \log\left(\frac{25}{1.2}\right) - \log\left(\frac{25 - 2[\mathrm{urea}]}{1.2 + [\mathrm{urea}]}\right)$$

$$= 1.32 - \log\left(\frac{25 - 2[\mathrm{urea}]}{1.2 + [\mathrm{urea}]}\right) \qquad (3)$$

As shown in Fig. 2, the theoretically expected decrease in extracellular pH according to eqn. (2) closely matches the extent and time course of that directly measured with the pH electrode in the effluent. Agreement between calculated and measured changes in extracellular pH after urea synthesis is observed at different rates of urea production and is also maintained when urea synthesis is stimulated by ornithine (Fig. 4). The acidification of the extracellular space after stimulation of urea synthesis can

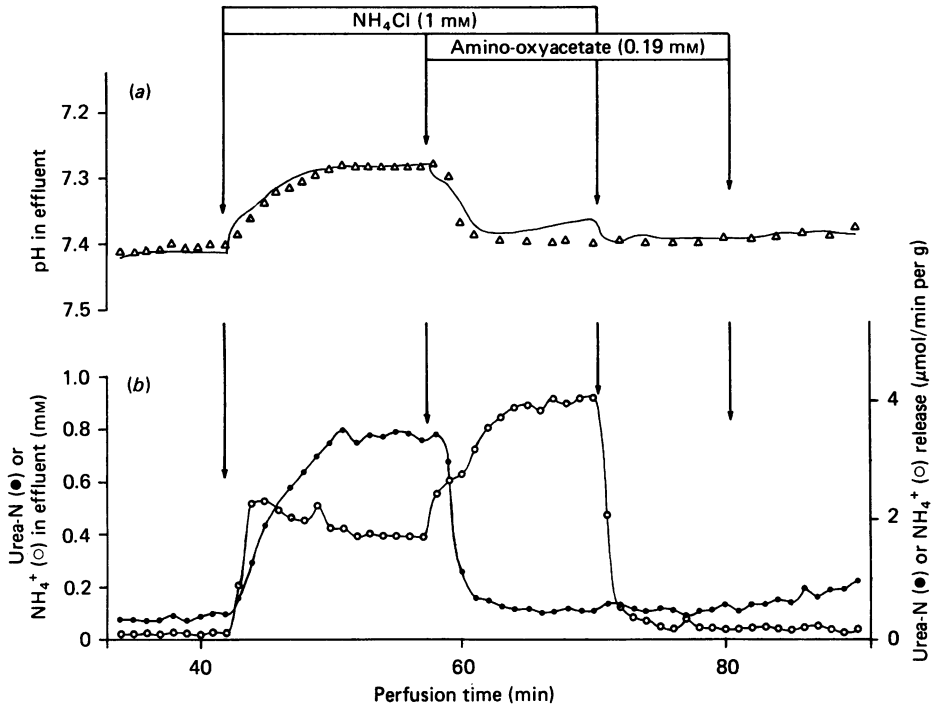

Fig. 2. Effect of stimulation of urea synthesis by NH_4Cl on perfusate pH

pH in the effluent perfusate was measured with the pH electrode (continuous line in *a*) or was calculated from the measured urea concentration in the effluent by eqn. (2) (△). NH_4Cl (1 mM) and amino-oxyacetate (0.19 mM) were added to the influent perfusate during the time periods indicated by the arrows.

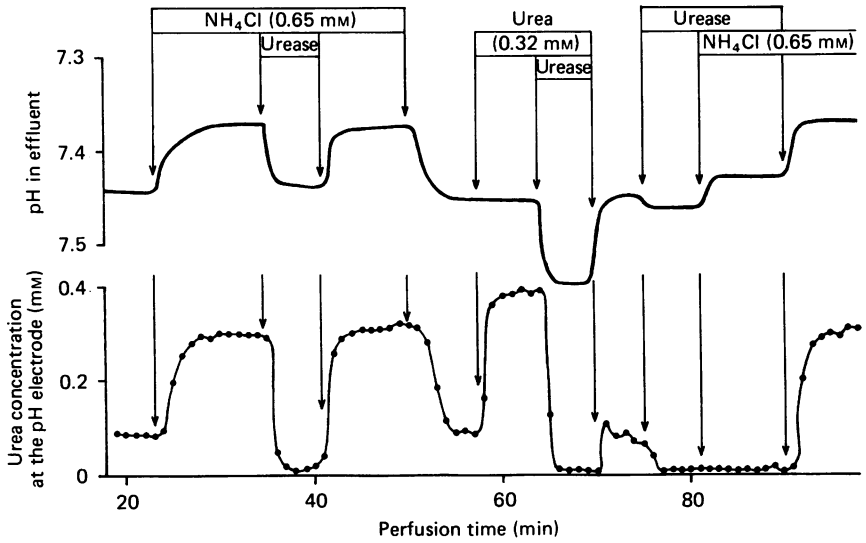

Fig. 3. Effect of urease on the urea-synthesis-induced acidification of the extracellular space

Urease (2.5 units/ml of perfusate) was added to the effluent perfusate, so that the urea synthesized by the liver was hydrolysed before the effluent perfusate reached the pH electrode. The concentration of urea was measured at the pH electrode. NH_4Cl (0.65 mM) and urea (0.32 mM) were added to influent perfusate during the time periods indicated by the arrows.

be reversed by infusion of $NaHCO_3$. Because urea synthesis not only consumes HCO_3^-, but also produces CO_2, the amount of $NaHCO_3$ required to restore the initial pH exceeds by far the amount of HCO_3^- consumed by urea synthesis (Table 1). These data demonstrate the importance of urea synthesis as a HCO_3^--utilizing pathway and prove experimentally the predicted effect of urea formation on extracellular pH (eqns. 1 and 2). Thus, in man, a daily excretion of about 30 g of urea is equivalent to the disposal of about 1 mol of HCO_3^-. The findings also support the view that a diminution of hepatic urea synthesis as a consequence of a decreased

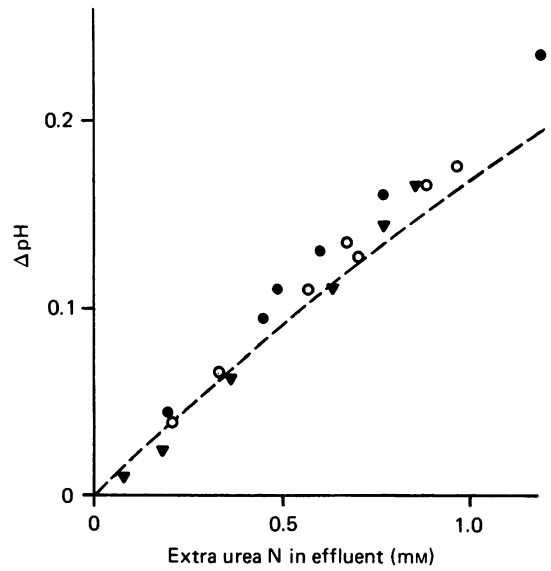

Fig. 4. Urea synthesis and extracellular acidification

Livers were perfused with different concentrations of NH_4Cl (0.05–3 mM) in the absence (○) or presence (●) of ornithine (2 mM) and after inhibition of glutamine synthetase with methionine sulphoximine (0.15 mM) (▼), and the urea-synthesis-induced acidification (ΔpH) was measured with the pH electrode during metabolic steady states. The broken line refers to a theoretically expected ΔpH, as calculated from eqn. (3) from the extra urea in the effluent perfusate.

Table 1. Effect of urea synthesis on pH in effluent perfusate

Urea synthesis was stimulated by addition of NH_4Cl (1 mM); further additions are indicated in the text. The pH in effluent perfusate was calculated from urea synthesis by eqn. (2) or was directly measured with a pH electrode. Data are given as means ± S.E.M. and are from six to nine different measurements during metabolic steady states. NH_4Cl, amino-oxyacetate (AOA) and $NaHCO_3$ were added to the influent perfusate.

Addition	pH		Urea production
	Measured	Calculated	(μmol/min per g)
Control	7.41	7.41	0.16 ± 0.01
NH_4Cl (1 mM)	7.28	7.29	1.70 ± 0.01
+ AOA (0.2 mM)	7.38	7.40	0.24 ± 0.01
+ $NaHCO_3$ (8.1 mM)	7.40	7.41	1.71 ± 0.01

extracellular pH (Oliver *et al.*, 1977; Häussinger *et al.*, 1984; Bean & Atkinson, 1984; Häussinger & Gerok, 1985) or a decrease in the absolute extracellular HCO_3^- and CO_2 concentrations (Häussinger *et al.*, 1975; Häussinger & Gerok, 1985) will lead to a decreased hepatic HCO_3^- consumption, in line with the proposed role of the liver in systemic pH regulation by adjusting the rate of HCO_3^- removal to the needs of acid–base homoeostasis. In this respect, it should be noted that HCO_3^- consumption by urea synthesis is irreversible and leads to the formation of an excretable metabolic end product. It has been argued that renal metabolism of 2-oxoglutarate via the oxidative or the gluconeogenic pathway derived from glutamine breakdown in acidosis will result in HCO_3^- formation or proton consumption by the kidney (Welbourne & Phromphetcharat, 1984; Walser, 1986). This, however, cannot affect HCO_3^- homoeostasis in the whole organism, because 2-oxoglutarate formation in the kidney is accompanied by an 2-oxoglutarate consumption in the glutamine-producing organs, such as liver and muscle (Fig. 1).

Fig. 2 also shows that addition or withdrawal of NH_4Cl to or from the extracellular space is nearly without effect on extracellular pH when urea synthesis is inhibited. This is simply explained by the comparatively high pK (9.3) of the NH_3/NH_4^+ system, and can be seen in rough analogy to renal NH_4Cl excretion. This important kidney function (see below) increases in a traditional sense the 'total body buffer base' (defined as the difference between non-metabolizable cations and non-metabolizable anions), but is without effect itself on extracellular pH (Atkinson & Camien, 1982; Atkinson & Bourke, 1984; Häussinger *et al.*, 1984). Because NH_4^+ and HCO_3^- arise in almost stoichiometric amounts during protein breakdown, renal excretion of NH_4^+ together with Cl^- or in exchange with Na^+ will leave behind HCO_3^-, which would otherwise be disposed of by hepatic urea synthesis. The question of whether the increased renal NH_4^+ excretion in acidosis is a consequence of a decreased hepatic NH_4^+ detoxication by urea synthesis or whether the decreased hepatic urea synthesis in acidosis results from an increased NH_4^+ removal by renal excretion is considered to be of little relevance, because it was shown in isolated liver and kidney preparations that the metabolism of both organs adapts independently of each other in a meaningful way to changes in pH. The complex regulation of hepatic urea synthesis (which is equivalent to HCO_3^- consumption) by extracellular pH and the absolute extracellular HCO_3^- and CO_2 concentrations (Häussinger *et al.*, 1975, 1984; Häussinger & Gerok, 1985), involving flux changes through the substrate-providing reactions catalysed by mitochondrial carbonic anhydrase (Häussinger & Gerok, 1985) and glutaminase (Häussinger *et al.*, 1980, 1983, 1984), is well designed to stabilize the extracellular pH near 7.4 and could also help to adjust the extracellular HCO_3^- concentration near 25 mM. The sophisticated structural and functional organization of the urea-cycle enzymes glutaminase and glutamine synthetase in the liver acinus (Häussinger, 1983; Häussinger & Gerok, 1984; Gaasbeek-Janzen *et al.*, 1984; Gebhardt & Mecke, 1983) guarantees the effective NH_4^+ detoxication by a compensatory increase in hepatic glutamine synthesis when HCO_3^- consumption by urea synthesis decreases in metabolic acidosis. Independent of these changes in hepatic metabolism, which directly counteract and compensate the pH derangement, the complex regulation of renal ammoniagenesis then removes surplus nitrogen. This contribution of the kidney prevents recirculation of free or amino-group-bound NH_4^+, whose accumulation might otherwise override the control of hepatic urea synthesis by extracellular pH, HCO_3^- and CO_2. Such a situation is observed in chronic renal insufficiency, when renal NH_4^+ excretion is impaired (Welbourne *et al.*, 1972; Tizianello *et al.*, 1980). Acidosis developing under these conditions can be explained as a consequence of an inadequate substrate

stimulation of HCO_3^--consuming urea synthesis, preventing hyperammonaemia for the price of hypobicarbonataemia.

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