# Renal ammoniagenesis in kidney slices from rats undergoing glycerol-induced acute tubular necrosis[1]

H.G. Preuss, R. Sundquist and S.J. Podlasek

*Department of Medicine and Pathology, Georgetown University Hospital, Washington (D.C. 20007, USA), 17 November 1981*

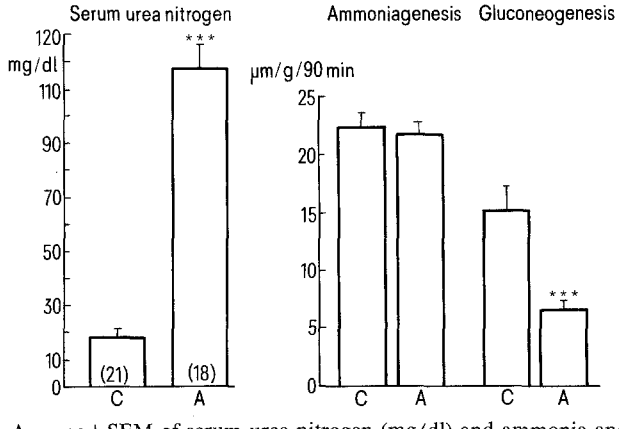
*Summary.* Compared to control, kidney slices from rats undergoing glycerol-induced ATN produce less ammonia from glutamate, but show no difference when glutamine is substrate. However, gluconeogenesis from glutamine, like glutamate, is decreased in acute tubular necrosis (ATN). We conclude that renal ammoniagenesis is influenced by ATN. Glycerol-induced ATN causes a relative increase in glutamine deamidation and a decrease in glutamate deamination.

In an early in vitro study[2], we reported decreased ammonia production by kidney slices from rats undergoing glycerol-induced acute tubular necrosis (ATN). In contrast, Westenfelder et al.[3], found depressed glucose reabsorption, PAH extraction, and bicarbonates reabsorption, but no decrease in ammonium excretion in their in vivo study concerning glycerol-induced ATN. What is the reason for discrepancy? To determine this, we performed expanded in vitro studies on ammoniagenesis by renal slices from rats undergoing glycerol ATN. Differently, we now found no significant changes in ammoniagenesis, but decreased gluconeogenesis was still present. The obvious difference between this and our previous investigation[2] was that glutamate was the ammonia precursor earlier, while glutamine was used in the present study.

*Methods.* Male Sprague-Dawley rats, weighing 250–350 g, were dehydrated for 24 h; 18 rats received a s.c. injection of 50% (V/V) glycerol-isotonic saline (1 ml/100 g b.wt) and 21 received an equal volume of isotonic saline. The rats were sacrificed 24 h later after blood was withdrawn from the lower aorta. Serum urea nitrogen was measured by standard procedures[4]. In vitro methodology and measurements of ammonia and glucose were as previously described[2,5]. Glutamine 0.6 mm was substrate. Statistics were assessed by Student's t-test using group analysis.

*Results and discussion.* The figure is a histogram depicting the serum urea nitrogen (SUN), slice ammonia and slice glucose production from control and rats undergoing glycerol ATN for 24 h. The average SUN of 21 control rats (C) was 18 mg/dl ± 2.6 (SEM). 24 h after injection of glycerol into dehydrate rats (A), the SUN rose to 120 mg/dl ± 9.8 (SEM). This was significantly different from control ($p < 0.001$).

The 2nd and 3rd set of bars depict the ability of slices removed from rats to produce ammonia and glucose when incubating in 0.6 mM glutamine. The gluconeogenic function following ATN (A) shows a significant decrease ($p < 0.001$) when compared to control (C). However, there was no difference in the ability of slices from A and C to produce ammonia in the presence of glutamine.




Average ± SEM of serum urea nitrogen (mg/dl) and ammonia and glucose production (μm/g/90 min). Number of rats studied in each group is shown in parenthesis. C = control; A = acute renal failure.

A substrate different from the original[2] was used in the present study. Glutamine, the natural precursor, forms ammonia from its amide and amino nitrogens, while glutamate forms ammonia principally from the amino group only[6]. The pathways through which ammonia is produced from the amide and amino nitrogens of glutamine are still under study, but most accept that phosphate dependent glutaminase (PDG) and glutamate dehydrogenase (GDH) are major routes[6,7].

The amino nitrogen of glutamate is deaminated via the glutamate dehydrogenase (GDH) pathway, which requires the coenzyme $NAD^+$. $NAD^+$ converts to NADH during deamination. Thus glutamate produces ammonia through oxidative processes, since NADH must be reoxidized to continue ammoniagenesis. Obviously, deamination of glutamate through the GDH pathway is somewhat limited with disruption of oxygen consumption[2,5]. Because ammonia produced from the glutamine after deamidation should continue through GDH to make more ammonia[8], one might expect ammoniagenesis from amino nitrogen to decrease even when glutamine is the starting substrate. It is likely that damaged mitochondria allow more glutamine to passively reach the sites of deamidation within mitochondria[6,7]. The combination of the increased deamidation of glutamine through PDG would be balanced by decreased ammonia formation through the GDH system from the glutamate formed after deamidation. Such a balance in events produces no change in overall ammoniagenesis in vivo and in vitro (fig.). The decrease in gluconeogenesis following ATN despite no significant change in ammoniagenesis is consistent with this conclusion. Accordingly, it is not surprising that Westenfelder et al.[5] found no significant changes in ammoniagenesis despite other cellular metabolic abnormalities being apparent. Both glutamine nitrogens are the natural precursors of in vivo ammoniagenesis[6,7]. Exogenous glutamate is in low concentration and penetrates mitochondria poorly in vivo[9]. Decreased glutamate deamination[2] in the face of increased glutamine deamidation accounts for no change in total ammoniagenesis in the present study and suggests mitochondrial disruption[2,5]. In further support, another in vitro model simulates our results. Anoxia produced in vitro leads to enhanced glutamine deamidation and decreased glutamate deamination[10].

1 Supported by a grant from the American Kidney Fund.

2 H.G. Preuss, Kidney int. *10*, S51 (1976).
3 C. Westenfelder, J. Arevalo, P.W. Crawford, P. Zerwer, R.L. Baranowski, F.M. Burch, W.R. Earnest, R.D. Coleman and N.A. Kurtzmann, Kidney int. *18*, 432 (1980).
4 C.J. Gentzkow, J. biol. Chem. *43*, 531 (1942).
5 H.G. Preuss, A. Tourkantonis, C.H. Hsu, P.S. Shim, P. Barzyk, F. Tio and G.E. Schreiner, Lab. Invest. *32*, 286 (1975).
6 R.F. Pitts, in: Handbook in Physiology, sect. 8, p. 455. American Physiology Society, Washington, D.C. 1973.
7 R.L. Tannen, Am. J. Physiol. *235*, F267 (1978).
8 H.G. Preuss and F.R. Weiss, Am. J. Physiol. *221*, 458 (1971).
9 R.F. Pitts, L.A. Pilkington and J.C.M. De Haas, J. clin. Invest. *44*, 731 (1965).
10 H.G. Preuss, O. Vavatsi-Manos, L. Vertuno and K. Baird, Proc. Soc. exp. Biol. Med. *146*, 803 (1974).