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Amino acid-mediated stimulation of renal phospholipid biosynthesis after acute tubular necrosis

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Amino acid-mediated stimulation of renal phospholipid biosynthesis after acute tubular necrosis. The mechanism by which amino acid infusion stimulates membrane phospholipid biosynthesis during renal regeneration after mercuric-chloride-induced acute tubular necrosis was studied in the rat. Amino acids can act directly on regenerating renal tissue to enhance net phospholipid synthesis because preincubation of cortical slices with amino acids induced an increase in [^{14}C]-choline incorporation into phospholipid without altering the rate of breakdown. This amino acid stimulation of phospholipid biosynthesis was studied further by measuring [^{14}C]-choline accumulation and its sequential conversion to phosphorylcholine, cytidine diphosphocholine (CDP-choline), and phosphatidylcholine via the Kennedy pathway in regenerating renal tissue. [^{14}C]-Choline accumulation was increased after amino acid infusion, compared to glucose infusion. There were also increments in the V_{max} of the choline kinase reaction, which converts entering [^{14}C]-choline into [^{14}C]-phosphorylcholine, and of the cholinephosphotransferase reaction in which [^{14}C]-CDP-choline is incorporated into [^{14}C]-phosphatidylcholine, whereas the apparent K_m of each reaction was unchanged. Thus, amino acids infused after tubular necrosis can act directly on regenerating renal cells to increase precursor availability and augment two reactions of the phospholipid biosynthetic pathway.

Stimulation par les acides aminés de la biosynthèse rénale de phospholipides après nécrose tubulaire aiguë. Le mécanisme par lequel la perfusion d'acides aminés stimule la biosynthèse de phospholipides membranaires au cours de la régénération rénale consécutive à la nécrose tubulaire aiguë déterminée par le chlorure mercurique a été étudié chez le rat. Les acides aminés peuvent agir directement sur le tissu rénal en régénération pour accroître la synthèse nette de phospholipides puisque la pré-incubation de tranches de cortex avec des acides aminés détermine une augmentation de l'incorporation de [^{14}C]-choline dans les phospholipides sans modification du catabolisme. Cette stimulation par les acides aminés de la synthèse des phospholipides a été étudiée par la mesure de l'accumulation de [^{14}C]-choline et de sa conversion séquentielle en phosphorylcholine, cytidine diphosphocholine (CDP-choline) et phosphatidylcholine, selon la voie de Kennedy, dans le tissu rénal en cours de régénération. L'accumulation de [^{14}C]-choline est augmentée après perfusion d'acides aminés, par comparaison avec celle de glucose. On observe aussi des augmentations des V_{max} de la réaction de la choline kinase, qui transforme la [^{14}C]-choline entrante en [^{14}C]-phosphorylcholine, et de la réaction de la cholinephosphotransferase, dans laquelle la [^{14}C]-CDP-choline est incorporée dans la [^{14}C]-phosphatidylcholine, alors que le K_m de ces réactions est inchangé. Ainsi les acides aminés perfusés après une nécrose tubulaire peuvent agir directement sur les cellules rénales en régénération pour augmenter la disponibilité en précurseur et accélérer deux réactions de la voie de biosynthèse des phospholipides.

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Amino acid infusions appear to alter favorably the course of acute renal failure in patients [1, 2] and animals [3]. A previous study from this laboratory indicates that infused amino acids decrease the level of renal functional insufficiency after mercuric chloride-induced acute tubular necrosis, and enhance phospholipid biosynthesis for new membrane formation in regenerating kidney cortical cells in the rat [3]. Phospholipid biosynthesis via the Kennedy pathway was studied using choline as a specific precursor of phosphatidylcholine, the major choline-containing phospholipid [4–8]. In this pathway, choline is phosphorylated to form phosphorylcholine, which reacts with cytidine triphosphate to form cytidine diphosphocholine (CDP-choline), the immediate precursor of phosphatidylcholine. To elucidate the mechanism of amino acid action on phosphatidylcholine synthesis, we examined the effect of amino acids on the steps of the Kennedy pathway in regenerating renal tissue. The results indicate that amino acids act directly on regenerating renal cells to increase precursor availability and to stimulate two reactions of the phospholipid biosynthetic pathway.

Methods

Male Sprague-Dawley rats (Sprague-Dawley Farms, Madison, Wisconsin), each weighing 175 to 275 g, were fed laboratory chow (Purina, Ralston-Purina Co., St. Louis, Missouri) and tap water ad lib. Mercuric chloride solution was injected into tail

veins of unanesthetized rats, temporarily immobilized in restraining cages, at a dose of 1.0 mg of mercury per kilogram to induce acute tubular necrosis [3, 9, 10]. Saline (1.0 ml/kg) was administered to control rats. Animals were then allowed free access to water but not food, and on the following day were either infused or offered chow. Animals chosen for infusion were anesthetized with ether, had PE-50 tubing introduced into the right jugular vein, and were then placed in restraining cages as previously described [3]. After a 2-hour equilibration period, during which 3 ml of normal saline was infused, the rats received an amino acid or glucose solution for 2 or 18 hr. The amino acid infusion contained 3.7 g of amino acids (nine essential and six nonessential)[1] as FreAmine II (McGaw Laboratories, Santa Ana, California) and 2.5 g of glucose in 50 ml of water (25.6 calories), whereas the glucose infusion had 6.25 g of glucose in 50 ml of water (25.0 calories). The infusions were delivered by a constant infusion pump (Harvard Apparatus Co., Millis, Massachusetts) at a rate of 3.2 ml/hr. Infusions were terminated by cutting the tubing, and the rats were then weighed and decapitated.

Studies of renal phospholipid metabolism were carried out 48 hours after mercury or saline injection. Kidneys were removed, decapsulated, and immediately placed in iced Krebs-Ringer bicarbonate medium. At least three sagittal slices of the inner cortical zone, which is necrotic in mercury-injected rats, were cut and trimmed as previously described [10]. One slice (22 to 40 mg) was placed in a 10-ml Erlenmeyer flask containing 2 ml of 20 μM [methyl-^{14}C]-choline chloride (specific activity, 30 mCi/mmole, 64 dpm/pmole) (New England Nuclear Corp., Boston, Massachusetts) in Krebs-Ringer bicarbonate medium, gassed for 30 sec with 95% oxygen and 5% carbon dioxide, incubated at 38° C for 30 min, and homogenized in 10% trichloroacetic acid. Radioactivity in the acid-insoluble and acid-soluble fractions was measured as previously described [11].

To determine if amino acids have a direct effect on renal phospholipid metabolism, we placed a cortical slice in a flask with 2 ml of choline-free Krebs-Ringer bicarbonate medium containing 12.75 mg of either amino acids (FreAmine II) or glucose, gassed, and preincubated at 38° C for 2 hr. The osmolality of each preincubation medium was about 325 mOsm/kg as measured with a Fiske osmometer (Fiske Associates, Inc., Uxbridge, Massachusetts). The slice was then rapidly dipped into each of two 100-ml beakers containing saline to displace surface preincubation medium, and blotted. Each slice was transferred to a fresh flask containing 2 ml of 20 μM [^{14}C]-choline in Krebs-Ringer bicarbonate medium, gassed, incubated for 30 min, and incorporation into the acid-insoluble and acid-soluble fractions was measured as described above.

More than 99.5% of the radioactivity in the acid-insoluble fractions obtained from regenerating and control tissue after treatment with amino acids or glucose in vivo or in vitro was found in lipid since this quantity of radioactivity was extracted by chloroform/methanol (2/1, volume/volume) and recovered with chloroform infranate after partition [12]. Thin-layer chromatography of the extracts [13, 14] indicated that the remainder was in lysophosphatidylcholine and sphingomyelin.

Phospholipid breakdown in regenerating renal tissue was compared during treatment with amino acids or glucose to evaluate amino acid enhancement of choline incorporation into phospholipid. Four rats were injected intravenously with mercuric chloride and on the following day intraperitoneally with 30 μCi of [^{14}C]-choline per 250 g body weight to prelabel renal phospholipid in vivo. Two days after mercury and one day after [^{14}C]-choline administration, cortical slices from these noninfused animals were cut and bisected to compare the breakdown of prelabeled phospholipid during preincubation with amino acids or glucose. One slice half was preincubated for 2 hours with amino acids and the other half with glucose. Then each slice half was incubated for 30 min in choline-free medium, and residual acid-insoluble radioactivity was measured. Differences between slice halves were assessed by paired anlysis. Four saline-injected noninfused rats were subjected to the same protocol to study the breakdown of prelabeled renal phospholipid during preincubation with amino acids or glucose. Previously, the breakdown of prelabeled renal phospholipid was assessed in mercury-injected rats infused with amino acids or glucose by incubating one cortical slice half in choline-free Krebs-Ringer bicarbonate medium for 30 min and immediately homogenizing the unincubated control half [3]. No detectable differences in acid-insoluble radioactivity were observed between incubated and unincubated halves. In the present

[1] Approximate quantity of amino acids infused (g/50 ml) was: L-histidine, 0.11; L-isoleucine, 0.27; L-leucine, 0.35; L-lysine HCl, 0.28; L-methionine, 0.20; L-phenylalanine, 0.22; L-threonine, 0.15; L-tryptophan, 0.06; L-valine, 0.25; L-alanine, 0.27; L-arginine, 0.14; L-cysteine, < 0.01; glycine, 0.77; L-proline, 0.43; L-serine, 0.23.

study, this protocol was used to estimate breakdown of prelabeled phospholipid in four saline-injected animals infused with amino acids or glucose, but it proved unsuitable because acid-insoluble radioactivity increased significantly during the incubation.

To study the mechanism of amino acid action on renal phospholipid biosynthesis, we compared the effect of amino acid and of glucose infusions for 18 hours on [^{14}C]-choline metabolism in regenerating tissue, at medium choline concentrations between 2 and 20 μM. Renal slices were incubated in 25-ml Erlenmeyer flasks containing 8 ml of Krebs-Ringer bicarbonate medium at various choline concentrations for 30 min. [^{14}C]-choline metabolites were measured by paper chromatography in perchloric acid extracts of the slices, and in 50% ethanol extracts of the medium [10]. Values for tissue [^{14}C]-choline and each [^{14}C]-metabolite were expressed as picomoles per milligram dry tissue weight per 30 min. The [^{14}C]-choline concentration in the extracellular fluid (ECF) was determined as the medium choline radioactivity after the incubation. The concentration of tissue [^{14}C]-choline attained in the slices was calculated as micromoles of [^{14}C]-choline per liter of total tissue water (μM). The concentration of [^{14}C]-phosphorylcholine and [^{14}C]-CDP-choline reached in the intracellular fluid (ICF) was estimated from the known choline specific activity and expressed as the number of micromoles formed per liter of ICF (μM). The ICF space was calculated as the difference between tissue water and extracellular fluid space, estimated from the distribution of [^{14}C]-inulin (New England Nuclear) [15], and was similar after amino acid and glucose infusion in mercury-injected rats (50.3 ± 0.7% of wet weight, N = 23.) Linear regression analysis was used to describe the relationship between tissue accumulation of each labeled compound and the concentration of its immediate [^{14}C]-precursor in cortical slices from amino acid or glucose-infused rats. Differences between slopes of these lines were evaluated by Student's t test [16] and Lineweaver-Burk analysis was used to calculate an apparent K_m and V_{max} where appropriate.

Results were expressed as means ± SEM. Data was compared by paired and unpaired Student's t test. P values less than 0.05 were accepted as significant.

Results

Amino acid treatment of regenerating renal tissue by infusion or preincubation for 2 hours increased the rate of [^{14}C]-choline incorporation into phospholipid in renal cortical slices compared to glucose (Fig. 1). Neither infusion for 18 hours, nor the presence of glucose in the preincubation medium were required to achieve these high rates of incorporation. An increase was also observed in renal tissue from saline-injected control animals following amino acid treatment in vivo and in vitro, although the absolute rates achieved were lower than they were in regenerating tissue (Fig. 2).

Regenerating tissue treated with amino acids or glucose in vitro (Table 1) had similar amounts of prelabeled phospholipid radioactivity. This absence of a detectable increase in phospholipid breakdown suggested that the increased rate of [^{14}C]-choline incorporation into phospholipid associated with amino acid treatment represented net phospholipid synthesis. Prelabeled phospholipid radioactivity in control tissue treated with amino acid in vitro was lower than it was after glucose treatment, which

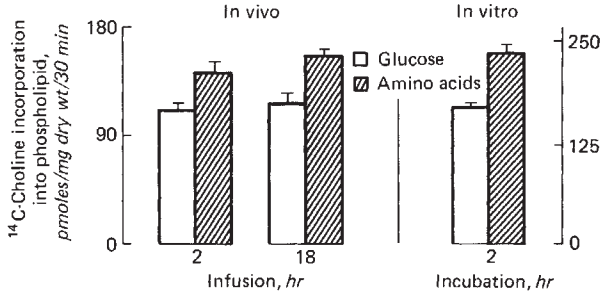

Fig. 1. *Effect of amino acids and glucose on incorporation of ^{14}C-choline into renal phospholipid 2 days after mercuric chloride injection.* Amino acid infusion for 2 and 18 hours or preincubation for 2 hours increased the rate of incorporation ($P < 0.005$). Values are means ± SEM for at least 16 determinations.

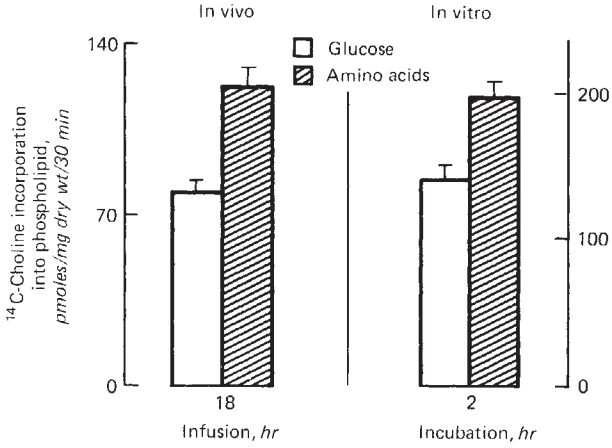

Fig. 2. *Effect of amino acids and glucose on incorporation of ^{14}C-choline into renal phospholipid in saline-injected control rats.* Amino acid infusion for 18 hours or preincubation for 2 hours increased the rate of incorporation ($P < 0.001$). Values are means ± SEM for at least nine determinations.

Table 1. Phospholipid radioactivity of cortical slice half, showing [^{14}C]-phospholipid breakdown in renal cortex 2 days after mercuric chloride injection and treatment with amino acids or glucose in vitro[a]

Injection	Preincubation with glucose *dpm/mg dry wt*	Preincubation with amino acids *dpm/mg dry wt*	*P*
Mercuric chloride	2531 ± 121	2392 ± 170	NS
Saline	1769 ± 127	1674 ± 110	< 0.01

[a] Animals were injected with mercuric chloride or saline i.v. and 1 day later with [^{14}C]-choline i.p. to prelabel renal phospholipid. Two days later the rats were killed and renal cortical slices cut and bisected. One slice half was preincubated for 2 hours in Krebs-Ringer bicarbonate medium containing glucose and then incubated for 30 min in glucose-free medium. The other half was preincubated for 2 hours with amino acids and for 30 min in amino acid-free medium. Radioactivity in the acid-insoluble fraction of each slice half was compared by paired analysis. Values are means ± SEM for eight determinations.

suggested that the observed increment in [^{14}C]-choline incorporation into phospholipid after amino acid treatment may not result in net phospholipid synthesis.

Tissue [^{14}C]-choline accumulation was 54% higher in mercury-injected animals after amino acid infusion than it was after glucose infusion (Fig. 3). The rate of [^{14}C]-phosphorylcholine formation was higher after amino acid infusion, when expressed as a function of the [^{14}C]-choline concentrations reached in the tissue. The V_{max} of this choline kinase reaction was 24% ($P < 0.02$) greater, whereas the apparent K_m was similar in the two tissues. In contrast, the rate of [^{14}C]-CDP-choline formation was lower ($P < 0.001$) after amino acid infusion, when expressed as a function of the intracellular [^{14}C]-phosphorylcholine concentrations attained during the incubation. [^{14}C]-phospholipid formation was faster after amino acid infusion at the [^{14}C]-CDP-choline concentrations reached in the tissue. The V_{max} of this cholinephosphotransferase reaction increased from 148 pmoles of [^{14}C]-CDP-choline incorporated per milligram dry weight per 30 min after glucose infusion to 188 pmoles of [^{14}C]-CDP-choline incorporated per milligram dry weight per 30 min after amino acid infusion ($P < 0.01$), whereas the apparent K_m was 0.8 μM [^{14}C]-CDP-choline in both tissues. Net formation of [^{14}C]-betaine and [^{14}C]-betaine aldehyde, the mitochondrial oxidative metabolites [17], detected in tissue and medium was similar for the two tissues.

Discussion

Increased renal cortical phospholipid biosynthesis takes place during recovery from mercuric chloride-induced acute tubular necrosis in the rat despite progressive azotemia, decreased food intake, and weight loss [10]. Increments in DNA, RNA, and protein biosynthesis, and in mitotic index also occur and precede the appearance of squamoid epithelial cells, which reline the denuded tubular basement membranes [18–20]. Amino acid infusion for 18 hours during the second day of the syndrome further augments renal membrane phospholipid biosynthesis and decreases the level of renal function insufficiency [3].

Phospholipid biosynthesis is required for new membrane and organelle formation in regenerating

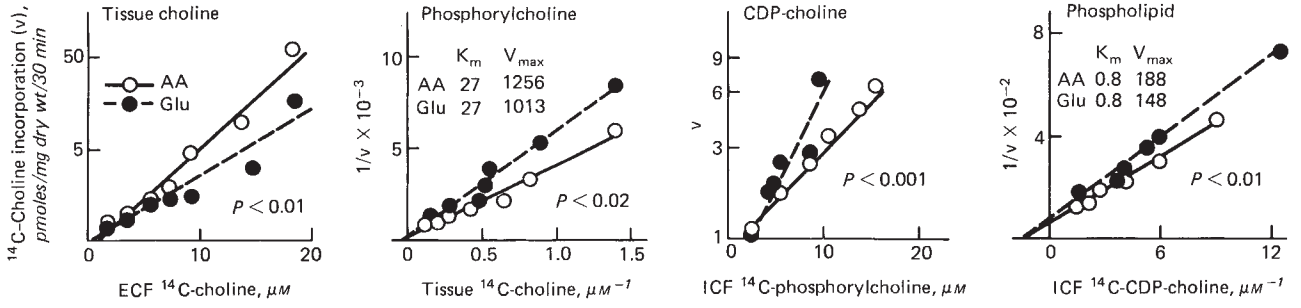

Fig. 3. *Effect of amino acid and glucose infusion on ^{14}C-choline uptake and incorporation into phosphorylcholine, CDP-choline, and phospholipid in renal cortical slices 2 days after mercuric chloride injection.* Slices were incubated in Krebs-Ringer bicarbonate medium containing [^{14}C]-choline at various concentrations for 30 min, and the formation of each labeled compound was measured as described in Methods section. Values for ^{14}C-choline reached in the slice after incubation were expressed as picomoles per milligram dry weight and as a concentration in total tissue water (micromolar). Values for rates of formation of phosphorylcholine, CDP-choline, and phospholipid at each medium choline concentration were expressed at the concentration of its immediate ^{14}C-precursor attained during incubation. Apparent K_m and V_{max} were calculated by Lineweaver-Burk analysis for ^{14}C-phosphorylcholine and ^{14}C-phospholipid formation. Each value is the mean of independent determinations on tissue from at least two rats. *P* values refer to differences in slopes of regression lines. The *r* value for each line was greater than 0.93. ECF is extracellular fluid; ICF, intracellular fluid; AA, amino acid-infused rats; Glu, glucose-infused rats.

renal cells. More than half the cellular membrane phospholipid is composed of three choline-containing phospholipids: phosphatidylcholine, lysophosphatidylcholine, and sphingomyelin [7]. In the present study [^{14}C]-choline was used as a precursor of phosphatidylcholine, the major membrane phospholipid, because 99% of the radioactivity found in renal lipid was in this phospholipid species. Since nearly all of the cellular phosphatidylcholine is located in membranes [21], measurements of choline incorporation into renal phosphatidylcholine can be used as an index of total cell phosphatidylcholine biosynthesis and membrane formation [10, 11, 14, 22-24].

The results of this study indicate that infused amino acids can act directly to stimulate net phospholipid synthesis in regenerating renal tissue because preincubation with amino acids resulted in increased [^{14}C]-choline incorporation into phospholipid without a detectable increase in breakdown. The absence of a glucose requirement for in vitro amino acid stimulation suggests that amino acid enhancement of phospholipid biosynthesis during renal regeneration is probably independent of the infused glucose.

As a consequence of amino acid infusion, the accumulation of [^{14}C]-choline in regenerating tissue was increased, whereas that of [^{14}C]-CDP-choline was decreased. In addition, the rate of formation of [^{14}C]-phosphorylcholine from choline (choline kinase reaction) and of [^{14}C]-phospholipid from CDP-choline (cholinephosphotransferase reaction) was enhanced. The accumulation of choline in renal tissue is the sum of its diffusion into the ECF space, net choline transport across the cell membrane, entry into the Kennedy pathway of phospholipid biosynthesis, and oxidation by the mitochondria. The increased accumulation of [^{14}C]-choline in regenerating tissue after amino acid treatment was associated with increased utilization of choline by the Kennedy pathway but not with alterations in ECF space or mitochondrial oxidation of choline. Therefore, amino acids appear to enhance net choline transport in regenerating cells. [^{14}C]-CDP-choline accumulation appears to be decreased after amino acid infusion when expressed as a function of the calculated [^{14}C]-phosphorylcholine precursor concentration. This difference in [^{14}C]-CDP-choline accumulation may be a consequence of the observed increment in V_{max} of CDP-choline: 1,2-diacylglycerol cholinephosphotransferase, a decrease in the dissociation of phosphatidylcholine into CDP-choline and 1,2-diacylglycerol, or a decrease in the tissue CDP-choline pool. The increments in the V_{max} of the choline kinase and cholinephosphotransferase reactions without a change in apparent K_m may be a consequence of increased availability of their coreactants (ATP, and 1,2-diacylglycerol, respectively), activation of existing enzyme molecules, or an increase in net enzyme synthesis.

The results of this study demonstrate that amino acid treatment can increase phospholipid precursor availability for utilization by the two augmented reactions of the biosynthetic pathway. This direct action of amino acids to increase phospholipid biosynthesis could thereby enhance new membrane and organelle formation in regenerating renal cells. Thus, amino acid infusion after acute tubular necrosis could act on renal and other tissues in patients with this syndrome to speed repair of damaged cells and the formation of new ones.

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