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Enhancement of Uridine Uptake in the Kidney Papilla by Urea (38074)

F. GARY TOBACK, PATRICIA D. SMITH, AND LEAH M. LOWENSTEIN
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Department of Medicine, Boston University School of Medicine, and Boston University Medical Center, Boston, Massachusetts 02118

Urea is distributed throughout the total body water in mammals at a concentration of 4 m*M*. However, in the mammalian kidney, the concentration of urea increases from 4 m*M* in the cortex to 700 m*M* in the papilla (1, 2). Thus the papilla functions in a unique physiological environment. Although urea is regarded as a metabolically inert end-product of protein metabolism, at high concentrations (80–700 m*M*) it diminishes protein synthesis in stimulated lymphocytes (3), O_2 uptake and ATP content of platelets (4), O_2 consumption by kidney cortex slices (5), and renal medullary and papillary (Na^+-K^+) ATPase activity (6). We report here that urea in the high concentrations normally observed only in the papilla *in vivo* stimulates uridine uptake by papillary cells, and that it may be required for optimal uridine transport in this tissue.

Methods. Renal cortical (18–36 mg) and papillary (13–25 mg) slices 0.4 mm thick were obtained from 110 male Sprague–Dawley rats (Charles River Breeding Laboratories, Inc.) weighing 120–160 g. The slices were placed into 25 ml Erlenmeyer flasks with 2 ml Krebs-Ringer–bicarbonate medium, pH 7.4, containing [2-^{14}C]uridine (spec act 55.8 mCi/mmole) at a concentration of 3 μM. Urea was added in various amounts to the Krebs-Ringer–bicarbonate medium, and the flasks gassed with 95% O_2–5% CO_2 and incubated for 1 hr at 37.5°C. The slices were then dipped quickly five times in 10 ml of 4 m*M* [^{12}C]uridine in saline to remove surface radioactivity. The slices were blotted, weighed, homogenized in 4 ml of iced 4 m*M* uridine in 10% trichloroacetic acid, and centrifuged at 1000*g* at 2°C for 15 min. The sediment was washed and both supernatants combined to give the acid-soluble fraction. The lipid was extracted from the acid-insoluble fraction. The remaining pellet, containing radioactive nucleic acid, and aliquots of the acid-soluble fraction were each dissolved in 10 ml Liquifluor (New England Nuclear) with 17% Bio-Solv (Beckman Instruments), and counted in a scintillation spectrometer using automatic quench correction and external standardization. The amount of [^{14}C]uridine taken up by the slice was calculated from the spec act of uridine in the medium and was expressed as pmoles/μliter intracellular fluid. The ratio of the concentration of [^{14}C]uridine and its metabolites in the intracellular fluid to the concentration of [^{14}C]uridine in the medium, termed the distribution ratio, is a measure of concentrative uridine uptake. A distribution ratio greater than one indicated net uptake of [^{14}C]uridine by the tissue. [^{14}C]inulin distribution and dry weights of cortical and papillary slices, measured as described previously (7), were used to obtain fluid spaces of the slices. The addition of increasing amounts of urea did not appreciably alter the intracellular or extracellular fluid spaces. This result was expected, since urea is a permeable solute.

Results. When papillary slices were incubated in media with increased urea concentrations, the [^{14}C]uridine distribution ratio increased 68%, from 3.27 ± 0.21 (SE) in urea-free medium to 5.50 ± 0.52 ($p < 0.002$) at 540 m*M* urea (Fig. 1). Thus, at

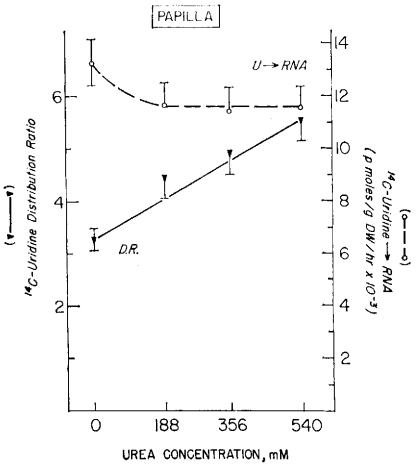

FIG. 1. Effect of urea on [^{14}C]uridine uptake and incorporation into RNA in renal papillary slices. The uptake of [^{14}C]uridine in the slices was measured and expressed as the distribution ratio (D.R.) (see text for explanation). The rate of [^{14}C]uridine incorporation into the acid-insoluble fraction (U → RNA) was expressed as pmoles [^{14}C]uridine incorporated into RNA/g dry wt/hr × 10^{-3}. Each value is the mean ± SE of at least five experiments.

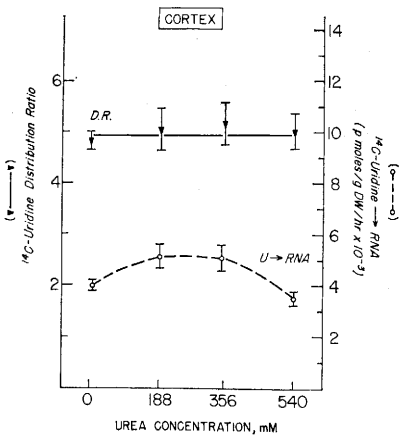

FIG. 2. Effect of urea on [^{14}C]uridine uptake and incorporation into RNA in renal cortical slices. See Fig. 1 for details.

concentrations found in the papilla *in vivo*, urea stimulated uridine uptake into kidney papillary slices. This increase in uridine uptake was not associated with a significant change in the rate of [^{14}C]uridine incorporation into RNA. In cortical slices there was no change in the [^{14}C]uridine distribution ratio or the rate of incorporation into RNA under these conditions (Fig. 2).

Concentrations of NaCl or sucrose up to 800 m*M* did not increase [^{14}C]uridine uptake in papillary or cortical slices; therefore, the increase in [^{14}C]uridine uptake into papillary slices observed in the media with urea was not the result of hypertonicity.

Discussion. The role of the renal cells in the uptake and metabolism of uridine has not yet been defined. These results suggest that both cortical and papillary cells can selectively accumulate uracil nucleotides. Since uridine occurs in the blood of animals (8, 9), and hence probably in the glomerular filtrate, renal tubular reabsorption of uridine may play a major role in uridine homeostasis.

Although urea at high concentrations has previously been considered either inert or damaging to cellular metabolism, we report here that urea at the high concentrations normally present in the renal papilla stimulates the uptake of uridine and may thereby conserve an important substrate for RNA synthesis and intermediary metabolism.

Summary. The urea concentration in the kidney papilla is normally 175 times higher than that in the kidney cortex and the rest of the body. The effect of the high urea concentration on the uptake of uridine was investigated in the rat kidney papilla and cortex. High concentrations of urea (540 m*M*) were found to increase [^{14}C]uridine uptake into papillary but not into cortical slices. Thus the urea concentration plays a role in regulating uridine uptake in the kidney papilla and may thereby conserve an important substrate for RNA synthesis and intermediary metabolism.

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