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Transport of Citrinin by Rat Renal Cortex

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Abstract. Citrinin, a secondary product of fungal metabolism, is nephrotoxic in the rat. Because citrinin is an organic anion, it might be expected to be transported by the renal organic anion transport system. Rat renal cortical slices were used to characterize the transport. ^{14}C-Citrinin uptake was enhanced by lactate and reduced by probenecid, a specific inhibitor of anion transport. Dinitrophenol is a metabolic inhibitor as well as competitive inhibitor of anion transport, and it also reduced citrinin transport. Organic cations did not alter citrinin accumulation by the slices. These data are consistent with the transport of citrinin by the renal organic anion secretory system.

Key words: Citrinin – Nephrotoxins – Fungal toxins – Anion transport – Renal slice transport

Introduction

Citrinin (Fig. 1) is a secondary product of fungal metabolism, e.g., of *Penicillium citrinum*. This substance, as well as other fungal products, has been shown to be nephrotoxic in the rat (Berndt and Hayes 1977) and in other species, e.g., the pig and guinea pig (e.g., Krogh 1976; Scott 1977; Elling and Moller 1973; Krogh 1972). In the rat, studies by Lockard et al. (1980) and Phillips et al. (1980) demonstrated that the nephrotoxic effect was exerted in the SI segment of the proximal tubule.

Citrinin, an organic anion, might be expected to be transported by the renal organic anion system of the proximal tubule known to transport *p*-aminohippurate (PAH). Citrinin excretion can be blocked by probenecid, as can its accumulation by renal cortical tissue (Berndt and Hayes 1982). Phillips et al.

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(1980) found citrinin : inulin clearance ratios of nearly three which suggests an important role of tubular secretion in the excretory process. The present study was undertaken to characterize further the renal secretory system for citrinin.

Materials and Methods

Male, Sprague-Dawley rat weighing from 200 to 250 g were used in these studies. Prior to use, the animals were housed in the central animal facilities of the medical center in rooms which provided a 12 h−12 h light-dark cycle and an ambient air temperature of 22−23° C.

Renal cortex slice accumulation of ^{14}C-citrinin was assessed using standard techniques as described elsewhere (Berndt and Hayes 1977). Slices (100−200 mg) were incubated in Kreb-Ringer phosphate buffer containing 1 mM Ca^{++}, and 5 mM K^{+} in addition to the other usual electrolytes. ^{14}C-citrinin was present at 5×10^{-5} M. Incubations were for 2 h unless stated otherwise. Uptake of citrinin was monitored by slice accumulation of radioactivity by liquid scintillation spectrometry. The data are presented as the S/M ratio (slice/medium ratio), i.e., the radioactivity per gram of tissue divided by the radioactivity per milliliter of bathing solution. In a few experiments tissue citrinin was determined by high performance liquid chromatography (HPLC) as reported previously (Phillips et al. 1979; Berndt and Hayes 1982). The quantity of citrinin in the renal tissue determined by HPLC agreed well with that calculated from the accumulation of radioactivity.

The ^{14}C-citrinin used in this study was prepared in this laboratory as reported previously (Phillips et al. 1979). The mold, *Penicillium citrinin* (NRRL 1982), was grown in 2% yeast extract, 5% sucrose liquid medium containing (1-^{14}C) acetate. The toxin was isolated and purified by the method of Davis et al. (1975). Authenticity and purity of the product were verified by HPLC and melting point.

The statistics used were either Student's *t* or Analysis of Variance followed by an appropriate ranking test, usually Student-Newman-Keuls.

Results

The accumulation of ^{14}C-citrinin at various times after initiation of incubation is presented in Fig. 2. In the presence of oxygen a steady state was achieved in approximately 2 h with S/M ratios near 30. In the absence of oxygen the steady state was achieved much earlier, i.e., approximately 30 min, with S/M ratios of the order of 5−8. Presumably the uptake in the presence of nitrogen was representative of nonspecific binding to tissue. Subtraction of these values from the uptake in oxygen yielded the time course of accumulation indicative of energy supported or active transport only. Although correction of the oxygen uptake values for tissue binding reduced the S/M ratios considerably, renal cortical slice uptake of the ^{14}C-citrinin was still high with steady state values of the order of 20.

The effects of substrates, inhibitors, and inorganic electrolytes on ^{14}C-citrinin uptake are presented in Table 1. Sodium lactate is well known to enhance the accumulation of organic anions by renal cortical tissue of the rat and this was observed with citrinin.

Various inorganic electrolytes are known to enhance the accumulation of organic anions by renal cortical tissue (Gerencser et al. 1973; Berndt and Beechwood 1965; Chung et al. 1970; Klahr et al. 1970). The magnitude of the

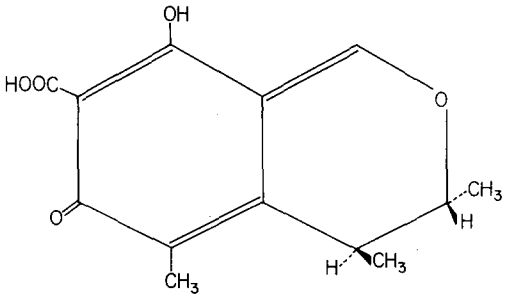

Fig. 1

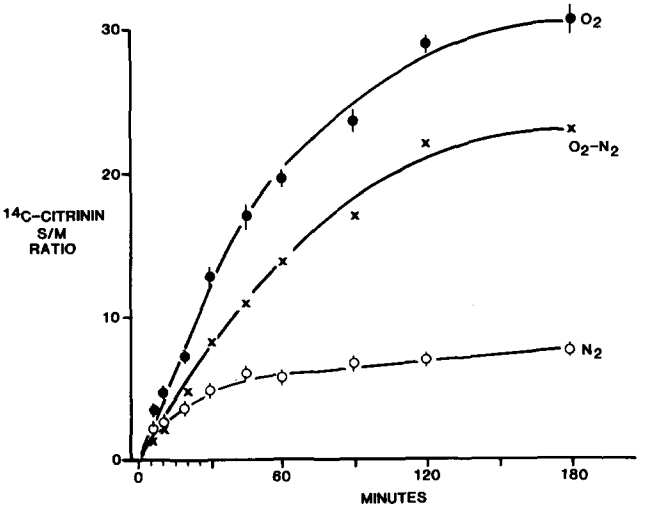

Fig. 2 Time course of ^{14}C-citrinin accumulation by rat renal cortex slices. Each point is the mean and bar the standard error for n = three to five experiments

Table 1. Effects of various substrates and inhibitors on ^{14}C-citrinin accumulation

		S/M ratio ± SE	p
Control	–	15.1 ± 1.2	–
Lactate	10^{-2} M	20.7 ± 1.1	< 0.05
Acetate	10^{-2} M	18.6 ± 0.6	n.s.
Glucose	10^{-2} M	16.8 ± 0.4	n.s.
Control	–	11.0 ± 0.4	–
DNP	10^{-5}	7.2 ± 0.3	< 0.05
	10^{-4}	4.2 ± 0.1	< 0.05
Probenecid	10^{-5}	7.4 ± 0.7	< 0.05
	10^{-4}	5.5 ± 0.1	< 0.05
PAH	10^{-4}	9.1 ± 0.2	< 0.05
	10^{-3}	8.7 ± 0.5	< 0.05
Control	–	14.5 ± 0.3	–
K^+	10 mM	16.8 ± 0.4	n.s.
	40 mM	19.5 ± 0.5	< 0.05
Ca^{2+}	0	10.1 ± 0.3	< 0.05

stimulatory effect of potassium varies considerably depending upon which organic anion is studied. Significantly enhanced uptake of citrinin was observed in the presence of 40 mM potassium. No significant stimulation was seen at potassium concentrations greater than 5, but lower than 40 mM. Removal of calcium from the bathing solution resulted in a significant depression of citrinin accumulation.

The ultimate characterization of organic anion transport rests with the effects of specific competitors and other inhibitors of transport. Neither tetraethylammonium (TEA) nor N-methylnicotinamide (NMN) affected the accumulation of ^{14}C-citrinin by rat renal cortex slices. Dinitrophenol (DNP) is both a competitive inhibitor of organic anion transport and a metabolic inhibitor (Berndt and Grote 1968). DNP reduced citrinin uptake. Probenecid, a specific competitor of organic anion transport, also reduced significantly the accumulation of ^{14}C-citrinin. In addition, the specific transport substrate, PAH, reduced the uptake of citrinin although the effect was not as marked as with the other competitors.

Discussion

Organic anion transport is known to occur in all three segments of the proximal tubule, although considerable quantitative variation exists from segment to segment and depending upon species (Tune et al. 1969; Roch-Ramel and Weiner 1980; Roch-Ramel et al. 1980). In the rat, however, organic anion transport in the convoluted segment of the proximal tubule is of considerable magnitude and it was in this nephron segment that citrinin produced necrosis. The effects of probenecid to block accumulation of citrinin by proximal tubular tissue (Berndt and Hayes 1982) is consistent with the importance of that transport in the production of nephrotoxicity.

The data presented in this study demonstrate that the transport characteristics for citrinin are similar to those for PAH, further documenting the likelihood of organic anion secretion as a mechanism for delivery of citrinin into the tubular fluid. Substrate stimulation, stimulation by inorganic cations, and high accumulation in the presence of oxygen all were noted with citrinin and are all transport characteristics noted with other organic anions. However, neither the stimulation of transport by lactate nor by potassium was as dramatic as noted with PAH (Gerencser et al. 1973; Chung et al. 1970). Nonetheless, this degree of variation has been observed with other anions, e.g., uric acid (Berndt and Beechwood 1965), whereas the effects of specific competitors of the organic anion transport system have been quite reproducible. Further, the poor stimulatory response may be in part relate to the relatively high non-specific binding of citrinin by the slices.

Both PAH itself and probenecid block the accumulation of citrinin, and neither compound has marked effects other than blockade of the organic anion transport system. DNP does block energy metabolism and by so doing can reduce transport processes. In addition, DNP can serve as a competitive inhibitor of organic anion transport as demonstrated in kinetic studies (Berndt

and Grote 1968). In particular the effects of PAH and probenecid, but also the action of DNP on citrinin accumulation are consistent with citrinin transport by the organic anion transport system.

The importance of the renal transport of citrinin in the production of nephrotoxicity has been demonstrated elsewhere (Berndt and Hayes 1982). The evidence presented here suggests that the transport mechanism whereby citrinin enters the renal tissue is the classical organic anion system associated with proximal tubular cell accumulation of PAH and its ultimate excretion. The transport data presented here and previously (Berndt and Hayes 1982) are important since they suggest that the citrinin must be present intracellularly to produce a disruption of renal function. In addition, these data indicate that a direct action of the toxin on renal cellular function is an underlying necessity for a toxic response. Whatever the ultimate pathogenesis of the resultant acute renal failure (altered renal blood flow, tubular obstruction, or proximal tubular leak), the initial disturbance appears to result from acute tubular necrosis of proximal tubular cells which is facilitated by transport of citrinin into those cells.

In summary, citrinin is transported by the same renal mechanism involved in the movement of many organic anions into the tubular fluid. This transport is consistent with the in vivo data of a large renal clearance of citrinin in the rat which can be demonstrated once plasma protein binding has been taken into account. Finally, the importance of renal transport in the production of a nephrotoxic response should be emphasized. Whether the intracellular action of citrinin is related to disruption of metabolism or some other important intracellular function (e.g., calcium transport or storage, Berndt and Hayes 1981) is not clear from these studies. It does seem clear, however, that damage to proximal tubular cells can result in the acute nephrotoxic response with acute renal failure.

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