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Interactions of Urea Transport and Synthesis in Hepatocytes of the Gulf Toadfish, *Opsanus beta*

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ABSTRACT. Although urea transport is receiving increased attention in mammalian systems, little is known about urea transport in fish tissues. Recently, we identified a phloretin-sensitive urea efflux pathway in hepatocytes of gulf toadfish (*Opsanus beta*), a ureogenic teleost. The present study sought to further examine this transport system and its potential interrelation with metabolic urea production. The transport inhibitors phloretin and 1-(3,4-dichlorophenyl)-2-thiourea (DCPTU) both had substantial inhibitory effects on hepatocyte urea production rates. These effects could be at least partially explained by inhibition of glutamate dehydrogenase (by phloretin) and argininosuccinate synthetase (by DCPTU) activities *in vitro*. Whereas phloretin substantially inhibited the efflux transport of urea, DCPTU did not. The metabolic effects of phloretin could be nearly eliminated by preparing hepatocytes in the absence of hyaluronidase, enabling its transport effects to be studied in isolation. In so doing we found that transporter inhibition did not lead to a substantial short-term buildup of intracellular urea concentration in hepatocytes. These results are discussed in the context of *in vitro* vs *in vivo* rates and pathways of urea transport or excretion. COMP BIOCHEM PHYSIOL 113B, 411–416, 1996.

KEY WORDS. Phloretin-sensitive urea transport, ureogenesis, hepatocytes, hyaluronidase, 1-(3,4-dichlorophenyl)-2-thiourea, *Opsanus beta*, gulf toadfish, ornithine-urea cycle

INTRODUCTION

Recent physiological observations in the mammalian kidney have rekindled interest in urea transport in vertebrates and have led to the view that urea is transported by facilitated diffusion and perhaps also by secondary active co-transport with Na^+ (1,2). In fact, urea transport by kidney tubules is inhibited by phloretin and a variety of competitive urea analogues (3,4), and recently, a specific transport protein has been cloned and characterized (5). An apparently active reabsorption of urea by the kidney of elasmobranchs also has been recognized for many years (6,7).

Furthermore, recent evidence suggests that urea carriers also occur in the vertebrate liver; phloretin sensitivity of urea efflux has been documented in hepatocytes of rats (8) and the gulf toadfish, *Opsanus beta* (9). The urea transport system in toadfish hepatocytes appears to be a simple carrier-facilitated diffusion without an active component or a secondarily active component (i.e., sodium requirement). The pathway also appears to have a high specificity for urea in that it is unaffected by moderate levels of potentially competitive substrates, including several urea analogues and glucose (9).

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The physiological significance of the toadfish hepatic urea transporter remains unclear. The simplest hypothesis would be that it ensures that urea produced in the liver exits rapidly to the extracellular space (i.e., more rapidly than by simple passive diffusion through the lipid bilayer). One potential advantage to such rapid efflux would be to avoid inhibition of hepatic metabolic enzymes (10), including those of the ornithine-urea cycle (O-UC), by intracellular urea buildup. Preliminary evidence suggests no urea feedback inhibition loop in toadfish hepatocytes (11), but we wished to more directly examine this possibility by testing whether transporter inhibition leads to a buildup of intracellular urea and subsequent metabolic inhibition. Therefore, our initial purpose for the present study was to determine whether the transport system had any linkage to, or regulatory effect on, hepatic urea synthesis. In the course of pursuing this initial goal, however, we discovered that urea synthesis is itself also directly inhibited by phloretin and that the degree of inhibition was affected by the conditions used to prepare hepatocytes. Another compound, 1-(3,4-dichlorophenyl)-2-thiourea (DCPTU), reported to be a highly specific and effective blocker of urea transport in other systems (12,13), was also found to be a significant inhibitor of urea synthesis in toadfish hepatocytes. Therefore, pursuit of the mechanisms of these inhibitor effects on ureogenesis became a second goal of this study. Our results

demonstrate that the effects of phloretin on transport and metabolism are independent and that the urea carrier and metabolic pathways do not appear to be strongly linked, at least in hepatocytes *in vitro*. The overall physiological role of the transporter is still unclear but may relate to proposed mechanisms for pulsatile whole-animal urea excretion from the head region in this species (14).

MATERIALS AND METHODS

Experimental Organisms

Sexually mature gulf toadfish, *O. beta* (Goode and Bean) (50–200 g), were captured by roller trawl by commercial shrimpers in Biscayne Bay, Florida, between January and May, 1994. Toadfish were held for <24 hr in an outdoor tank with running seawater at ambient seasonal conditions at the shrimpers' holding facility and then transferred to the laboratory where they were held in 45- or 80-l glass aquaria with a bed of beach sand and polyvinylchloride tubes for shelter and supplied with flowing aerated seawater (sand filtered and UV sterilised). Stocking densities were typically <5 g of fish l^{-1} of seawater. Temperature in these holding tanks was 22 ± 2°C. Fish were treated with a 2-hr static prophylactic dose of malachite green (final concentration 0.05 mg l^{-1}) in formalin (15 mg l^{-1}) (AquaVet, Hayward, CA, U.S.A.) on days 1 and 3 after transfer to the laboratory to prevent infection by a common ciliate (*Cryptocaryon irritans*) (15). Experiments were generally performed on days 6–10. Although gender was recorded when fish were killed, fish were randomly assigned to control or test groups without regard for gender. No significant differences between sexes was seen in any of our results.

Isolation of Hepatocytes and Transport/Metabolic Measurements

Toadfish hepatocytes were isolated by a collagenase/hyaluronidase perfusion method as described by Walsh (16), except that in some experiments hyaluronidase was omitted from the isolation procedure. Furthermore, glucose was omitted from the medium and substrates were 5 mmol l^{-1} $NaHCO_3$, 0.25 mmol l^{-1} aspartate, 1 mmol l^{-1} alanine and 1 mmol l^{-1} ornithine. Glutamine (5 mmol l^{-1}) was added when urea production rates were to be specifically measured. To measure urea production rates, 10–20 mg wet wt cells (measured gravimetrically) were added to 1 ml toadfish saline with the above substrates and incubated for up to 2 hr in stoppered 20-ml glass liquid scintillation vials on a rotary shaker as described previously (17). However, the radioactive assay for urea production was not used because subsequent to that study we found that urea can be measured directly by a chemical method with improved sensitivity (see below). At an appropriate time, cells were killed directly by the addition of 100 μl 70% perchloric acid (PCA). This mixture was centrifuged for 1 min at 13,000 ***g*** and 100 μl of supernatant was assayed for urea as detailed below. In most experiments, we also examined compartmentation of urea produced inside the cells vs released to the medium by rapidly centrifuging cells through oil into 75 μl of 7% PCA, as described below. In these experiments, cells and suspension medium were assayed separately.

Urea-N production rates were calculated from measurements of the difference in urea-N levels (μmol-N l^{-1}) between preparations "killed" at the start of the incubation (time 0) and at the experimental time (time t), factored by time (hr), cell weight (g) and incubation volume (l):

$$\text{Urea production rate} = \frac{([\text{urea-N}]_t - [\text{urea-N}]_0)\ \text{volume}}{(\text{time})\ (\text{cell weight})}.$$

In experiments where [urea-N] was measured separately in intracellular and extracellular compartments, the volume-weighted mean concentration was used in the calculation. In practice, at t_0, extracellular [urea-N] was negligible, intracellular [urea-N] was appreciable, and both were appreciable at later times.

Transport was measured in hepatocytes using ^{14}C labeled urea (2.04 GBq $mmol^{-1}$, Amersham, Arlington Hts., IL, U.S.A.) by methods previously applied to fish hepatocytes (9,16,18,19). Efflux experiments were performed by preloading concentrated suspensions of cells in high concentrations of radioactivity (typically 200 μl packed cells plus 10 μCi labeled substrate) for 30 min and then injecting small volumes of pre-labeled cells into a 100-fold larger volume of non-radioactive medium. Both loading and efflux media were toadfish salines plus all substrates except glutamine (to minimize metabolic contributions) plus 10 mmol l^{-1} cold urea.

Inhibitors were added both in the efflux medium only, or by pre-incubation of cells for 10 min after the 30-min loading period, before the flux measurement; no differences were observed in these two treatments. Typically, 12 μl cell suspension (containing up to 9 mg wet wt of cells) was injected into 1.2 ml saline without radioactive substrate, which was the top layer of three layers in a 1.8-ml microcentrifuge tube. This top layer had been layered onto a middle layer of 0.25–0.4 ml of 1-bromododecane oil, which in turn had been layered onto 50–75 μl of 70% PCA. After a timed interval up to 60 sec, the tube was centrifuged at 13,000 ***g*** to remove the cells to the acid layer, the cells being accompanied by minimal extracellular water (typically ≤ 10%) (16,19). Centrifugation was for 15 sec, although the cells are removed in the first 2–3 sec. Radioactivity was then quantified in the pellet by removing the supernatant and oil layer by aspiration, clipping the bottom 1 cm of the tube containing the acidified pellet with a dog pedicure tool and placing the bottom of the tube in Ecolume (ICN, Costa Mesa, CA, U.S.A.). Counts were made on a Beckman LS1801 liquid scintillation counter with an onboard quench correction program. The cpm remaining in the pellet at various timed intervals provided an inverse index of the urea efflux rate.

Experiments were conducted at 22.0 ± 0.5°C, except for one experimental series where temperature was varied between 7.5 and 35°C by pre-incubation of tubes with layered

solutions in a water bath, followed by injection of cells, followed by rapid transfer to the microcentrifuge.

Analytical Methods

Urea concentrations in cell pellets and supernatants were measured using the diacetyl monoxime method (20). As color development in the assay was reduced by up to 30% by the concentration of phloretin used, standards and unknowns always contained identical phloretin concentrations to samples being tested. ATP measurements were made with standard kits (No. 366-UV, Sigma Chemical Co., St Louis, MO, U.S.A.). Intracellular concentrations were expressed per l cell water using a measured water content of 65%.

For analysis of maximal enzyme activity, whole liver was homogenized on ice in three to four volumes homogenization buffer (20 mmol l^{-1} K_2HPO_4, 10 mmol l^{-1} HEPES, 0.5 mmol l^{-1} EDTA, 1 mmol l^{-1} dithiothreitol, 50% glycerol, adjusted with NaOH to pH 7.5 at 24°C) using a Brinkman polytron. Homogenates were spun at 8,000 *g* for 20 min at 4°C in a Jouan CR412 centrifuge. The supernatant or a 1:10 dilution was used directly for the assay at 24°C of alanine aminotransferase (AlaAT), aspartate aminotransferase (AspAT), glutamate dehydrogenase (GDH), glutamine synthetase (GNS), carbamoylphosphate synthetase (CPS), ornithine-citrulline transcarbamoylase (OTC), argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL) and arginase (ARG) by previously described methods (9,11,21).

All chemicals were reagent grade, and biochemicals were purchased from Sigma Chemical Company, except for DCPTU, which was purchased from Aldrich Chemical Company (Milwaukee, WI, U.S.A.). Treatment of cell suspensions or homogenates with inhibitors was done by dissolving the inhibitors at the appropriate high concentration in dimethyl sulfoxide (DMSO), such that 1 μl of this stock was added to 1-ml final volume of cell suspension or enzyme assay. Controls contained the same concentration of DMSO; however, DMSO itself had no effect on urea production rates. Statistical differences between treatments were determined using a Students' *t*-test (22) with each sample being a hepatocyte preparation from one fish.

RESULTS

Phloretin (250 μmol l^{-1}) did not lead to an enhanced accumulation of urea inside toadfish hepatocytes over a 2-hr incubation (Table 1). However, it did inhibit total urea synthesis substantially (~45%; Table 1). DCPTU (250 μmol l^{-1}) also inhibited total urea production rates to a comparable extent (Table 1). These apparent metabolic effects of phloretin and DCPTU could complicate the study of the interrelationship of metabolism and transport, but by serendipity we identified conditions under which phloretin's metabolic effects could be minimized.

Hyaluronidase was omitted from one preparation of hepatocytes, and virtually no metabolic effects of phloretin were seen. This observation was repeated to demonstrate significance; the percent inhibition was not significantly different from zero, in contrast to 47% inhibition when hyaluronidase was used (Table 2). Interestingly, control rates of urea production in hyaluronidase prepared cells were higher than in those prepared without hyaluronidase (Table 2).

The hepatocyte preparation method had no effect on inhibition of urea production rate by DCPTU (Table 2). Note however that the control rates were again lower when the hepatocytes were prepared without hyaluronidase. The lower control rates in the DCPTU series relative to the phloretin series probably reflected the fact that a different batch of toadfish, later in the year, was used.

The effect of phloretin inhibition on transport was assessed on a subset of hepatocytes prepared without hyaluronidase.

TABLE 1. Effects of phloretin and DCPTU on urea production rate (μmol urea N g^{-1} h^{-1}) and intracellular (i) and extracellular (e) concentrations (μmol urea-N l^{-1}) in gulf toadfish (*Opsanus beta*) hepatocytes prepared with hyaluronidase present

	Control		250 μmol l^{-1} Phloretin
Urea production Rate	24.84 ± 2.55		13.82 ± 3.13*
Initial [urea]i		1772 ± 203	
Final [urea]i	5999 ± 1456		3653 ± 702*
Final [urea]e	970 ± 130		570 ± 150*
Final [urea]i/[urea]e	5.83 ± 1.05		7.82 ± 1.77
	Control		**250 μmol l^{-1} DCPTU**
Urea production Rate	27.59 ± 5.72		17.36 ± 4.93*

Values are means ± SEM (n = 6–8 preparations per group).
*Indicates significantly different from control, $P < 0.05$.

TABLE 2. Effect of hepatocyte isolation method on urea production rate (μmol urea N g^{-1} h^{-1}) and on percent inhibition of urea production rate by phloretin and DCPTU (250 μmol l^{-1}) in gulf toadfish (*Opsanus beta*) hepatocytes

	+Hyaluronidase	−Hyaluronidase
Phloretin		
Control urea Production rate	27.02 ± 2.16 (11)	18.73 ± 3.64^{+} (7)
Phloretin urea Production rate	14.35 ± 1.03* (11)	16.88 ± 1.28 (7)
Percent inhibition	46.9 ± 7.2	9.9 ± 7.6^{+}
DCPTU		
Control urea Production rate	17.36 ± 5.32 (6)	11.46 ± 2.77^{+} (7)
DCPTU urea Production rate	10.69 ± 0.67* (6)	7.62 ± 0.72* (7)
Percent inhibition	38.4 ± 6.3	33.5 ± 9.4

Values are means ± SEM (*N*).
*Indicates significantly different from corresponding control, $P < 0.05$.
$^{+}$Indicates significantly different from +hyaluronidase treatment, $P < 0.05$.

Phloretin (250 μmol l^{-1}) continued to have a substantial effect on urea efflux rates (Table 3). DCPTU did not have an effect on efflux in hepatocytes prepared without hyaluronidase (Table 3), comparable with the phloretin inhibition of efflux that we had observed in an earlier study where hepatocytes were prepared with hyaluronidase (9).

Our initial studies with this system used 250 μmols l^{-1} phloretin (9). Because the effects of phloretin have been shown to be concentration dependent in red blood cells, with less specific effects noted at concentrations >300 μmol l^{-1} (23), we wished to confirm that the observed effects of phloretin on urea efflux could be duplicated using a lower phloretin concentration. Phloretin at 100 μmol l^{-1} decreased urea efflux (Table 3) as at 250 μmol l^{-1} phloretin (Table 3) (9).

A further way to determine whether cellular transport is likely due to a specific membrane-bound transporter is to examine the effects of temperature over a broad range. If there is a characteristic "break temperature" in an Arrhenius plot (log rate vs T^{-1} [°K]), it suggests that the rate of function of a membrane-bound transport protein markedly changes as the lipid bilayer goes through a transition. An Arrhenius plot for urea efflux rate is given in Fig. 1, and there is a break point at ~23.5°C.

TABLE 3. Effect of phloretin and DCPTU on urea efflux from gulf toadfish *Opsanus beta* hepatocytes prepared without hyaluronidase

	Urea Efflux (dpm mg^{-1} remaining after 15 s of efflux)
Control	11,738 ± 967
Phloretin (250 μmol l^{-1})	16,172 ± 1030*
DCPTU (250 μmol l^{-1})	12,174 ± 1053
Control	9,980 ± 1000
Phloretin (100 μmol l^{-1})	14,062 ± 995*

Values are means ± SEM (n = 4).
*Indicates significantly different from control, $P < 0.05$.

Once we had accumulated further evidence for the presence of a urea transporter and identified conditions (i.e., absence of hyaluronidase) under which its inhibition could be evaluated independently of phloretin effects on metabolism, we wished to determine whether transport inhibition would lead to intracellular accumulation of urea over a shorter time course. Facilitated diffusion might be expected to outstrip passive diffusion over a few minutes. However, inhibition of transport by phloretin (250 μmol l^{-1}) did not lead to intracellular urea accumulation at 1 and 5 min (Table 4).

Finally, we wished to identify potential mechanisms of the effect of phloretin and DCPTU on urea production. Neither compound had an effect on ATP contents of hepatocytes prepared with hyaluronidase ([ATP] in μmol g^{-1} wet tissue = 0.99 ± 0.12, control; 0.98 ± 0.10, 250 μmol l^{-1} phloretin; 1.06 ± 0.17 250, μmol l^{-1} DCPTU, values are means ± 1 SEM, n = 3) but did have substantial effects on enzymes of nitrogen metabolism. Phloretin substantially depressed the NADH-requiring GDH activity (Table 5), whereas DCPTU substantially depressed ASS activity (Table 6) *in vitro*. Also notable is the nearly significant effect of phloretin on AlaAT activity (Table 5, $P < 0.075$), as well as the fact that the linking enzyme in the AlaAT assay is the NADH-requiring lactate dehydrogenase.

DISCUSSION

This study demonstrates that the commonly used transport inhibitor, phloretin, as well as the more specific urea inhibitor DCPTU (12,13), can have substantial and direct effects on metabolic production of urea in toadfish hepatocytes (Tables 1 and 2). A substantial inhibition of NADH-linked GDH (Table 5), the mitochondrial enzyme responsible for ammonia production via deamination, as well as for supplying glutamate to the O-UC via glutamine synthetase, could be one cause

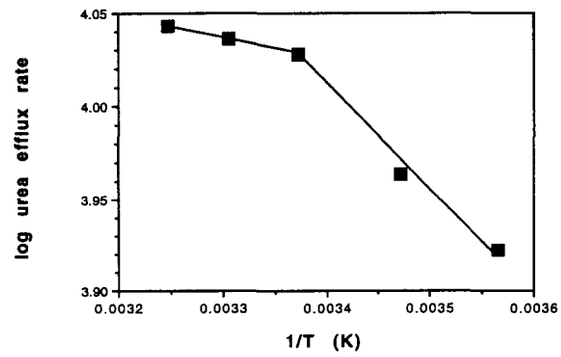

FIG. 1. Effect of temperature on urea efflux rate from gulf toadfish *Opsanus beta* hepatocytes expressed as an Arrhenius plot [log urea efflux rate (dpm min^{-1} mg^{-1}) vs T^{-1} ($°K^{-1}$)]. Points are means of duplicate determinations for T(°C) = 35.0, 29.5, 23.5, 15.0 and 7.5 moving from left to right on the x-axis.

TABLE 4. Short-term (1 and 5 min) effect of phloretin (250 μmol l^{-1}) on intracellular urea content (μmol l^{-1}) in gulf toadfish *Opsanus beta* hepatocytes

	Control	Phloretin
1 min	270 ± 50	240 ± 80
5 min	320 ± 30	260 ± 90

Values are means ± SEM (n = 3).

of the phloretin effect on metabolism. Phloretin had nearly significant effects on AlaAT (Table 5), whose assays use excess activities of linking dehydrogenases, and on toadfish lactate dehydrogenase (unpublished results) as well. Therefore, the metabolic effects of phloretin seen in this study could also result from more general effects on several dehydrogenase activities. DCPTU inhibition of urea production may result, at least in part from its effects on ASS, an O-UC enzyme (Table 6). Energy status of the cells, as indicated by ATP levels, was not affected by either drug.

The metabolic effects of phloretin and DCPTU could potentially impact interpretations of transport studies. However, we believe these effects do not invalidate the findings on urea transport by Walsh *et al.* (9) because of the way in which the studies were performed. Primarily, urea efflux rates were examined radioisotopically, and any depletion of intracellular urea by phloretin effects on metabolism would have increased intracellular urea specific activity, leading to a potential overestimate of efflux rates in phloretin treated cells relative to controls. In fact the opposite, inhibition of efflux, was observed in Walsh *et al.* (9) and the present study (Table 3), so we believe these potential complicating factors to be minimal. Furthermore, the 30-min loading period with 10 mmol l^{-1} urea would tend to swamp out any effects of production rate, given the much lower intracellular urea levels normally present *in vitro* (Table 4). In addition, the observations of efflux inhibition with a lower phloretin concentration (Table 3) where non-specific effects are less likely, as well as the demonstration of a characteristic temperature break point (Fig. 1),

TABLE 5. Effect of phloretin (250 μmol l^{-1}) on *in vitro* activities of hepatic O-UC and related enzymes in gulf toadfish *Opsanus beta*

Enzyme	Control	Phloretin
GDH	27.14 ± 1.29	4.31 ± 1.14*
AlaAT	41.80 ± 7.05	18.53 ± 5.98
AspAT	70.66 ± 8.65	54.29 ± 7.76
GNS	16.53 ± 0.98	16.52 ± 0.93
CPS	0.13 ± 0.06	0.12 ± 0.05
OTC	54.36 ± 5.54	52.86 ± 5.64
ASS	0.12 ± 0.01	0.13 ± 0.01
ASL	0.44 ± 0.02	0.37 ± 0.02
ARG	30.27 ± 7.09	26.29 ± 6.41

Values are means ± SEM (n = 3).
*Indicates significantly different from control, $P < 0.05$.

TABLE 6. Effect of DCPTU (250 μmol l^{-1}) on *in vitro* activities of hepatic O-UC and related enzymes in gulf toadfish *Opsanus beta*

Enzyme	Control	DCPTU
GDH	47.40 ± 7.25	51.71 ± 1.62
AlaAT	47.40 ± 5.38	54.94 ± 12.96
AspAT	75.40 ± 3.53	74.87 ± 6.35
GNS	23.15 ± 9.66	24.16 ± 10.30
CPS	0.38 ± 0.05	0.39 ± 0.05
OTC	79.02 ± 2.30	77.47 ± 3.74
ASS	0.09 ± 0.01	0.04 ± 0.01*
ASL	0.44 ± 0.03	0.44 ± 0.02
ARG	83.46 ± 2.71	82.07 ± 3.08

Values are means ± SEM (n = 3).
*Indicates significantly different from control, $P < 0.05$.

lend more confidence to the conclusion that a specific membrane transporter is responsible for urea transport in toadfish hepatocytes.

Nevertheless, the present findings that both phloretin and DCPTU inhibit urea synthesis in toadfish hapatocytes sounds a cautionary note for studies of urea transport with urea-producing tissues such as liver. If only the appearance of cold urea in the media had been measured in the present study, the results could have easily been misinterpreted as an inhibition of urea transport. In fact, for both drugs, this result was due to inhibited urea production rather than inhibited transport, and the two phenomena were experimentally dissociated. Thus, in hepatocytes prepared without the use of hyaluronidase, the phloretin effect on urea synthesis was virtually eliminated, whereas the transport inhibition remained. In contrast, DCPTU inhibited the urea production rate without altering transport rate. Clearly, only radioisotopic techniques can reliably detect effects on urea transport, and the possible effects of inhibited production rate on specific activity must be carefully considered.

DCPTU is a highly effective competitive inhibitor of facilitated urea transport in other systems; K_I values of 10 μmol l^{-1} in human red blood cells (13) and 32 μmol l^{-1} in frog urinary bladder (12) have been reported. It is curious that this drug did not inhibit urea transport in toadfish hepatocytes. Nevertheless, this result concurs with our earlier finding that other competitive urea analogues (acetamide, *N*-methylurea, thiourea) are without effect in this system (9), which suggests that the transporter in toadfish hepatocytes has an unusually high specificity for urea.

This study documented that the way in which hepatocytes are prepared can markedly effect metabolic rates and mode of action of inhibitors. Hyaluronidase aids in dispersing cells by its cleavage of hyaluronic acid, an important polysaccharide component of intercellular ground substance (24). Intuitively this would render cells more permeable, and this speculation is supported by our observations. Urea production rates were higher in hyaluronidase treated cells (Table 2), probably be-

cause exogenous substrates could more readily permeate the cell.

Our findings of different effects of phloretin with and without hyaluronidase treatment of cells enabled us to begin to test for linkage between the urea transporter and urea production. We reasoned that inhibition of the transporter would perhaps lead to a buildup of intracellular urea. This in fact was not observed (Table 4), suggesting that for hepatocytes in suspension, passive diffusion may be able to clear metabolically produced urea from the hepatocyte quickly enough to prevent buildup. Thus, the physiological impact of this transporter may only be important *in vivo* when the cell surface area exposed to extracellular fluid is more limited or when organ perfusion rate may limit the rate of simple diffusion. This speculation could perhaps be tested *in vitro* by examining urea transport in monolayer culture of toadfish hepatocytes (25) or with perifusion methods that have recently been applied to fish hepatocytes (26), both of which would restore substantial cell–cell contact. Using these experimental methods to establish the physiological significance of urea transport in hepatocytes may be an important line of investigation, because recently, we found that post-absorptive toadfish excrete nearly all their urea as a single burst per day from the head region (14). The mechanism of this excretory burst may be intimately linked to plasma urea concentrations, which in turn might depend on rapid equilibration of liver and plasma urea by the liver transporter.

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