

**EVIDENCE FOR FACILITATED DIFFUSION OF UREA IN THE GILLS  
OF THE FRESHWATER RAINBOW TROUT**

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**EXTENDED ABSTRACT ONLY- DO NOT CITE**

**Introduction**

Like most other freshwater teleosts, the rainbow trout (*Oncorhynchus mykiss*) excretes the majority of its nitrogenous wastes across the gills as ammonia, the form in which it is initially produced. However, research in the past decade has uncovered a handful of teleosts that expend energy to detoxify ammonia and excrete urea as their primary waste product. This is made possible by a fully functional ornithine-urea cycle (OUC) in internal tissues (especially the liver) and specialized facilitated diffusion urea transport mechanisms in the gill (UT-type transporters; Walsh et al. 2000, 2001). While adult rainbow trout do not have a full complement of OUC enzymes, they do maintain a surprisingly high level of circulating urea compared to endogenous ammonia concentrations (5–

50x more urea) and urea makes up about 10% of total nitrogen waste excretion. In addition, recent evidence suggests that, as in ureotelic fish, urea excretion is carrier-mediated in ammoniotelic organisms such as the rainbow trout (McDonald and Wood, 1998) and the plainfin midshipman (Walsh et al. 2001).

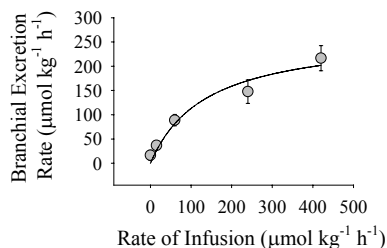
Therefore, preliminary evidence suggests the involvement of carrier protein in trout branchial urea excretion. The purpose of this study was to investigate the branchial urea transport mechanism hypothesized to be present in the rainbow trout through a combination of *in vivo* and *in vitro* techniques.

### Materials and Methods

*In vivo* studies were performed with fish that were surgically implanted with dorsal aortic catheters and allowed to recover for at least 24 hours. In Series *i*, fish were infused with consecutive isoosmotic solutions of urea balanced with NaCl at a rates of 0, 15, 60, 240 and 480  $\mu\text{mol kg}^{-1} \text{h}^{-1}$  in order to determine the branchial handling of urea in the face of exogenous urea loading. In Series *ii*, fish were injected with enough acetamide or thiourea, two urea analogues, so that analogue concentrations were equal to endogenous urea concentrations in order to determine if the gill preferentially transported urea. *In vitro* studies were performed by using the isolated basolateral membrane vesicle protocol as described by Perry and Flik (1988) where the basolateral membrane of the gill is isolated using differential centrifugation, and transport studies are performed using rapid filtration.

### Results and Discussion

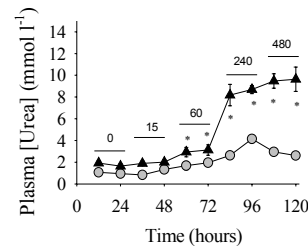
*In vivo*, the trout gill effectively cleared the plasma of excess urea during exogenous urea loading, suggesting a role for a facilitated diffusion UT-type transport mechanism for urea. However, saturation of this mechanism was suggested when branchial excretion rate could not keep up with infusion rate (Fig. 1). This apparent saturation resulted in an elevation in plasma urea concentrations, however, observations



**Fig. 1. Branchial excretion rate is not linearly proportional to infusion rate**

were complicated by the lethality of the elevated internal urea levels (Fig. 2). In

order to further investigate the saturation kinetics of branchial urea transport in the trout, the isolated basolateral membrane vesicle (BLMV) technique was employed (Perry and Flik, 1988). Basolateral membrane vesicles of the gill (BLMV) demonstrated urea uptake with a saturable component ( $K_m = 1.17 \text{ mmol l}^{-1}$ ;  $V_{max} = 0.42 \text{ } \mu\text{mol mg protein}^{-1} \text{ h}^{-1}$ ) at urea concentrations  $< 5 \text{ mmol l}^{-1}$ .



**Fig. 2. The reduced branchial excretion rate in proportion to infusion rate resulted in elevated plasma urea concentrations.**

Urea analogues, such as acetamide, thiourea and N-methylurea, are useful tools in identifying urea transport mechanisms as the specificity of a transporter leads it to preferentially transport urea over substances that are similar to it. In addition, analogues compete with urea in order to pass through the same transporter. Amongst teleosts where branchial UT mechanisms are present, a consistent pattern of urea and analogue handling is observed where acetamide clearance is 35-60% of urea clearance and thiourea clearance is only 16-19% (McDonald et al. 2000). This highly conserved pattern of differential urea and analogue handling was also observed in the rainbow trout where the ratio of analogue/urea branchial clearance was 48% for acetamide and only 22% for thiourea, strongly suggesting the presence of a UT-type diffusion mechanism. Using BLMV, the basolateral membrane alone was responsible for the differential handling of urea and acetamide, while urea and thiourea were handled similarly. In support of these findings, urea uptake by BLMV was significantly reduced by 73.2% in the presence of thiourea but was not affected by acetamide or N-methylurea.

The uptake of urea by BLMV was reduced by 62.5% when incubated with phloretin, a potent blocker of UT-type transport mechanisms giving strong evidence of carrier mediated diffusion of urea.

## Conclusions

Thus, branchial excretion of urea in adult rainbow trout appears to be mediated by a UT-type facilitated diffusion transport mechanism.

### **Acknowledgements**

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