GLYCINE STIMULATION OF ORGANIC ANION SECRETION IN TELEOST (*FUNDULUS HETEROCLITUS AND PSEUDOPLEURONECTES AMERICANUS*) RENAL TISSUES

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Renal proximal tubule cells are highly aerobic and are an important site of injury in models of acute renal failure caused by ischemia. Certain amino acids, e.g., glycine and alanine, are cytoprotective against hypoxic injury, and recent studies have implicated a receptor-gated anion channel in the mechanism of glycine protection against anoxia (Heyman et al., Kidney Intl. 42:41, 1992; Miller and Schnellmann, Tox. Let. 76:179, 1995). Here we examine interactions between glycine and organic anion transport in renal proximal tubules and proximal tubule cells from killifish (*Fundulus heteroclitus*) and winter flounder (*Pseudopleuronectes americanus*).

Isolated killifish renal proximal tubules and masses of flounder renal tissue were prepared as described previously (Miller and Pritchard, Am. J. Physiol. 267:R695, 1994). Fluorescein (FL) uptake by isolated renal tubules was measured using an inverted microscope equipped with epi-fluorescence optics, a video camera and Macintosh computer (Miller and Pritchard, op. cit.). Rates of FL efflux from flounder renal masses were determined by fluorometry of extracts of the masses and aliquots of the incubation media To determine unidirectional organic anion transport, flounder renal epithelial cells were isolated by cold trypsinization (Dickman and Renfro, Am. J. Physiol. 251:F424-F432, 1986), suspended in modified M-199 and plated to confluence on native rat tail collagen (Dickman and Renfro, Soc. Exp. Biol. Sem. Series 52:65-86, 1993). The floating collagen rafts were contracted from 35 mm to 17 mm after 12 days, and the cells forming the epithelial sheet had assumed their normal structure and function. Unidirectional ^{14}C-labelled *p*-aminohippuric acid (PAH) fluxes across these monolayers were determined in Ussing chambers under short-circuited conditions. In the presence of flounder saline (10 µM PAH) the flux ratio in control tissues was approximately 15 to 1 in the secretory direction. Net fluxes were calculated from the unidirectional fluxes in paired tissues

In initial experiments 5 mM glycine did indeed protect isolated proximal tubules from the effects of anoxia (30 min exposure to N_2 rather than air) when organic anion transport was the functional end-point (Fig. 1A). In addition, glycine seemed to increase cellular and luminal accumulation of FL, suggesting enhanced organic anion transport. Further examination of the latter finding in tubules kept under aerobic conditions showed that 2-10 mM glycine increased FL accumulation in both tissue compartments in a concentration-dependent manner (Fig. 1B). Significant glycine effects were first evident within 15-30 min of exposure and lasted for at least 60 min. Unidirectional fluxes of PAH in flounder proximal tubule cells (PTCs) are shown in Table 1. With 5 mM glycine present in both peritubular and luminal bathing medium, active PAH secretion was significantly stimulated to 133% of control transport rate. Glycine had no effect on reabsorptive (leak) flux.

At a minimum, this pattern of glycine-induced increases in net PAH secretion and cellular and luminal FL accumulation at steady state could be due to increased uptake or decreased efflux of organic anions at the basolateral membrane. Initial experiments in which the kinetics of efflux were determined in FL-loaded masses of flounder kidney were inconclusive, showing no

Table 1. Unidirectional and net fluxes of p-aminohippuric acid across flounder renal primary proximal tubule cultures in the presence and absence of glycine.

	^{14}C-PAH Flux (nmoles/cm^2/h)		
Treatment	Secretory	Reabsorptive	Net
Control	1.02 ± 0.242	0.07 ± 0.039	0.95 ± 0.281
5 mM Glycine	1.36 ± 0.349*	0.06 ± 0.026	1.31 ± 0.362*

Values are mean ± SEM of n = 4 preparations. ^{14}C-PAH and glycine were added at t = 0. Fluxes shown were determined at t = 90 min. *Significantly different form paired controls at P < 0.05.

significant effects of 5-10 mM glycine, although standard errors were quite high. For example, the half-time of efflux for controls averaged 22 ± 5 (n=19) min, that for masses exposed to 10 mM glycine, 33 ± 10 (n=8) min.

Little is known about mechanisms that control organic anion secretion in proximal tubule. Available evidence suggests that the basolateral membrane is one site of regulation (Halpin and Renfro, Am. J. Physiol., in press; Miller, unpublished data). The present results for teleost proximal tubules and cells in primary culture are consistent with glycine acting at the basolateral membrane to increase both organic anion uptake and transepithelial secretion. The relationship between this phenomenon and the glycine-gated anion channel suggested by the data of Miller and Schnellmann (*op. cit.*) remains to be determined. Supported by NSF IBN 9306619 (JLR). J.L. Renfro was supported by the Salsbury Cove Research Fund. Funding provided to L.M. from NSF ESI-9452682.

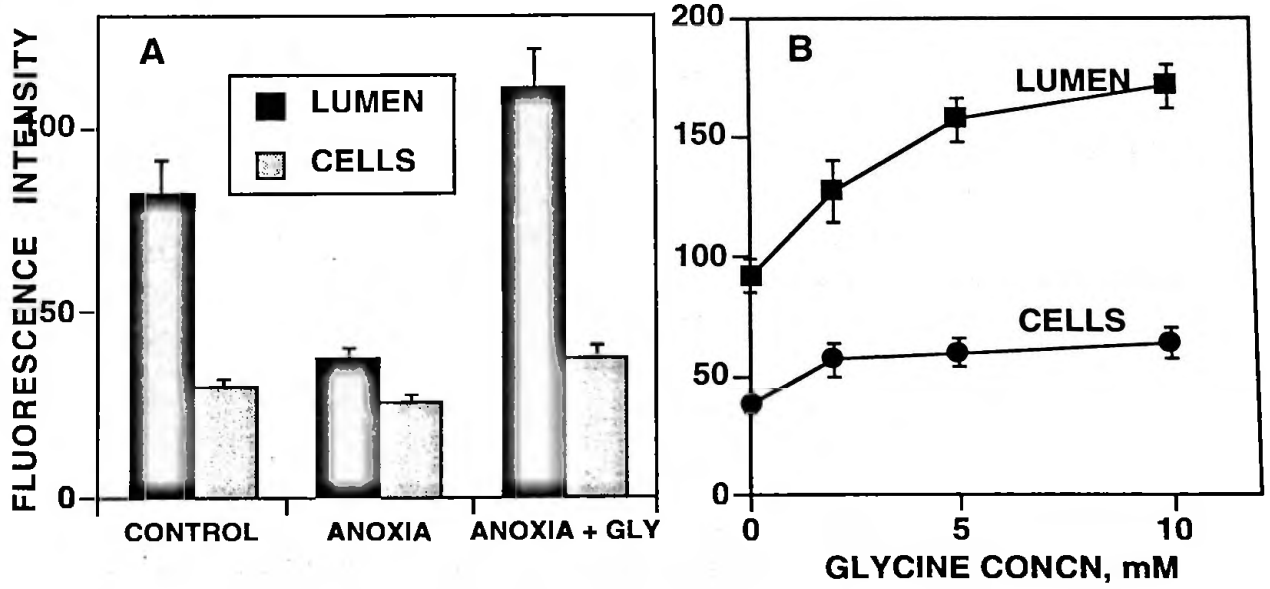

Figure 1. Glycine stimulation of fluorescein (FL) transport in killifish proximal tubules. (A) Tubules were preincubated 30 min in air (control), N_2 (anoxia) or N_2 with 5 mM glycine in the bath (anoxia + GLY). Tubules were then incubated for 60 min in medium with 1 µM FL under air. Anoxia significantly reduced cellular and luminal fluorescence, P<0.01. (B) Tubules were incubated for 60 min under air in media with 1 µM FL and 0-10 mM glycine. Glycine significantly increased cellular and luminal fluorescence (P<0.05 with 2 mM; P<0.01 with 5 and 10 mM).