STIMULATION OF RENAL ORGANIC ANION SECRETION BY GLYCINE AND STRYCHNINE

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Renal proximal tubule cells 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). We previously reported that millimolar concentrations of glycine protected killifish renal proximal tubules against hypoxic injury. Under aerobic conditions, glycine also increased organic anion secretion in isolated killifish (*Fundulus heteroclitus*) proximal tubules and in monolayers of winter flounder (*Pseudopleuronectes americanus*) proximal tubule cells (Miller et al., Bull. MDIBL, 36:73-74, 1997). The present report is concerned with the mechanism by which such transport is stimulated.

Isolated killifish renal proximal tubules, masses of flounder renal tissue and monolayers of flounder proximal tubule cells in primary culture (PTC) were prepared as described previously (Miller and Pritchard, Am. J. Physiol. 267:R695, 1994; Dickman and Renfro, Am. J. Physiol. 251:F424-F432, 1986). Two kinds of experiments were conducted with killifish tubules: 1) steady state fluorescein (FL) accumulation in the cells and lumens of isolated proximal tubules was measured using confocal microscopy and digital image analysis as described by Miller et al. (JPET 282:440-444, 1997); 2) initial rates of FL influx were measured using a microscope-based and computer-controlled microspectrofluorometer. Monolayers of flounder PTC mounted in Ussing chambers were used to measure efflux of ^{14}C-p-aminohippuric acid (PAH). PTC monolayers were equilibrated for 1 h with 10 μM PAH, washed twice with PAH-free medium and incubated in PAH free medium without (control) or with 5 mM glycine. All efflux media also contained 1 mM bromocresol green, a potent inhibitor of organic anion transport, to prevent reuptake of PAH.

Previous studies have shown that the drug, strychnine, like glycine, protects proximal tubules from hypoxic injury. Exposing killifish tubules to medium with 1-10 μM strychnine increased steady state cellular and luminal accumulation of FL by at least 50% (not shown). This is roughly the same amount of stimulation found with 5-10 mM glycine (Miller et al., op cit). When tubules were exposed to 10 mM glycine plus 10 μM strychnine, the effects were no greater than those of glycine or strychnine alone (Fig. 1A). Thus, glycine and strychnine appeared to stimulate transport through a common mechanism. It is unlikely that this stimulation is simply due to altered cellular metabolism, since strychnine has no known effect on metabolism in renal cells and since neither strychnine nor glycine affected active xenobiotic secretion mediated by the Na-independent organic anion transport system (not shown).

The observed increases in steady state accumulation of FL could have resulted from decreased efflux, increased uptake, or both. We previously measured FL efflux from preloaded flounder tubular masses and found no significant change (Miller et al., MDIBL Bulletin, 36:73-74, 1997). However, in those experiments variability was high, and small changes in efflux rate would not have been detected. To more closely examine this possibility, we measured the efflux of ^{3}H-PAH from flounder PTC monolayers and found that 5 mM glycine reduced 10 min basolateral efflux by 25 ± 9% (data from 6 pairs of control and glycine-treated PTC monolayers, $P<0.05$). We also measured initial (10 sec) rates of FL uptake into killifish tubules and found a 102 ± 38% increase in total influx with 5-10 mM glycine (data for 12 control and 12 glycine-treated tubules from 4 fish, $P<0.05$). Glycine caused no detectable change in nonmediated FL influx (measured in the

presence of 1 mM PAH), which was less than 5% of total influx . Together, these data indicate that the predominant effect of glycine on organic anion transport was to increase substrate influx into the tubule; to a smaller extent, glycine may also have decreased organic anion efflux.

Several laboratories have shown that renal organic anion secretion is under control of PKC. Activation of the kinase by phorbol ester or by diacyl glycerol reduces organic anion secretion and this reduction is prevented by protein kinase inhibitors. In the present experiments, we determined whether glycine or strychnine could also reverse the effects of PKC activators. Figures 1B and 1C shows that, in agreement with previous studies, PMA reduced both cellular and luminal accumulation of FL. These effects were reversed by 1 μM strychnine or 10 mM glycine; similar reversals were obtained when dioctylglycerol was used to reduce FL transport (not shown). Although these results are consistent with strychnine and glycine effects on organic anion transport being transduced through PKC, it is not yet clear which element of the signalling chain is the primary site of action of these compounds. Supported bv NSF IBN 9306619 (JLR). J.L. Renfro was supported by the Salsbury Cove Research Fund. NSF DBI 9531348

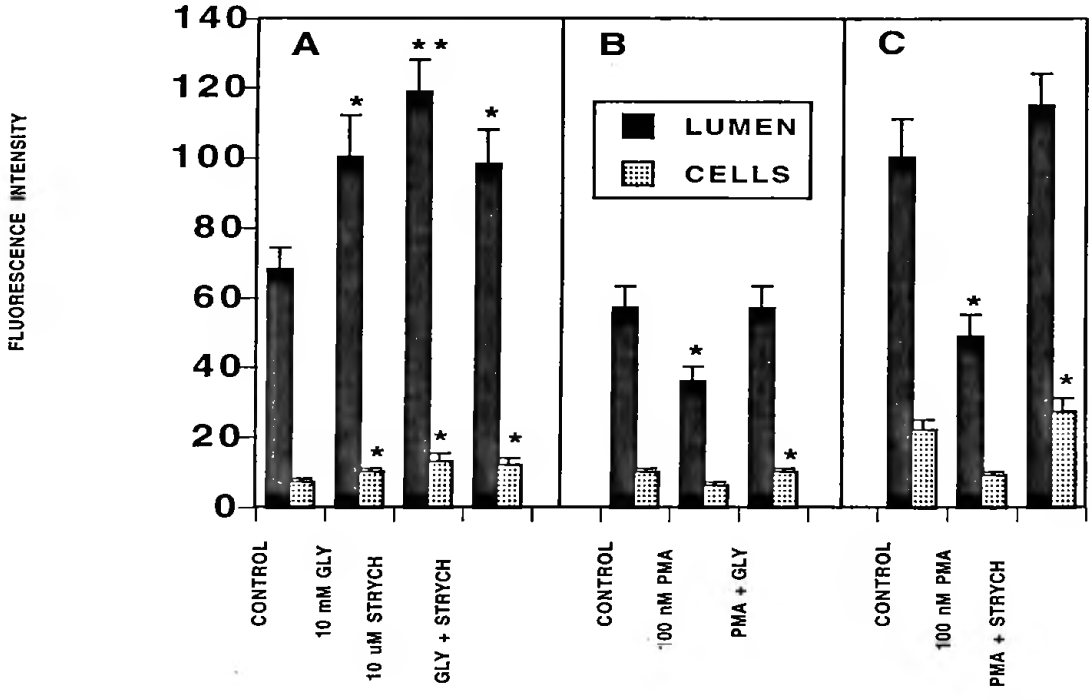

Figure 1. A) Effect of glycine (GLY), strychnine (STRYCH) or both on 1 μM fluorescein transport by killifish proximal tubules. B) Reversal of phorbol ester (PMA) effects by glycine. C) Reversal of phorbol ester effects by strychnine. Data given as mean values for 9-12 tubules; error bars indicate SE. Statistical comparisons: * $P<0.05$; ** $P<0.01$.