TISSUE INTERACTIONS DURING TAIL REGENERATION IN THE URODELE AMPHIBIAN, *Plethodon cinereus*

Charles E. Dinsmore, Department of Anatomy, Rush Medical College, Chicago, Illinois 60612

Limb regeneration in urodele amphibians is a well documented phenomenon and while investigations of urodele tail regeneration are relatively sparse, much is inferred from studies on the limb. Furthermore, most regeneration studies employ either aquatic larval ambystomids or the adult red-spotted newt, *Notophthalmus viridescens*, which is also aquatic. This choice of experimental organisms and the selection of the limb as the model system have created a paradoxical situation since a majority of urodele species are plethodontids and a majority of these have anatomical specialization for tail loss (Wake and Dresner, J. Morph. 122:265, 1967) and regeneration (Dinsmore, J. Exp. Zool., in press).

With these points in mind, experiments on one of the plethodontid species native to Mount Desert Island (*Plethodon cinereus*) have been designed to elucidate mechanisms associated with tail regeneration and either substantiate or refute ideas that tail regeneration is essentially the same process as limb regeneration.

Data obtained from initial experiments have demonstrated that, unlike limb muscle, myofibers in caudal myotomes do not normally, nor can they be experimentally induced to, participate in epimorphic regeneration in *Plethodon cinereus* although myofibers develop normally within the regenerate (Dinsmore, J. Exp. Zool., in press). The most likely source for blastema cells and, therefore, the tissues of the regenerate, is dedifferentiation and metaplastic transformation of the connective tissue cells in the well-developed dermis as well as from the intervertebral cartilage which remains associated with the stump following tail autotomy (Dinsmore, Am. Zool., 16:207, 1976).

Another parameter being examined is the inductive morphogenetic interactions between specific tissues during the initiation and elaboration of tail regeneration. Carlson (Devel. Biol., 39:263, 1974 and Devel. Biol. 47:269, 1975), has shown that by rotating a cuff of skin 180° around the long axis of the axolotl limb and subsequently amputating through the rotated skin, more than 80% of the regenerates are "multiple" or hypermorphic. Pilot studies on *Plethodon cinereus* show that in the tail, rotated skin has no comparable effect on the subsequent regeneration of the tail, control regenerates being identical with those arising from stumps with rotated skin.

The summary results of these investigations indicate clear-cut and significant differences between the morphogenetic interactions required during limb replacement and those occurring in tail regeneration.

Supported in part by NSF grants RR05764 and BMS 7503098 to the MDIBL.

EFFECT OF VARIOUS INHIBITORS ON SUGAR TRANSPORT IN TEASED RENAL TUBULES OF THE WINTER FLOUNDER *Pseudopleuronectes americanus*

George R. Dubyak and Arnost Kleinzeller, Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

The present report deals with the effects of several agents known to alter sugar transport in various cell types on the multiple sugar transport systems at the antiluminal face of flounder renal tubules. In this way it is hoped to obtain further information on the molecular properties of the transport proteins which are involved.

Cytochalasin B (CyB), para-hydroxy-mercuribenzoic acid (PMB), para-chloromercuribenzenesulfonic acid (PCMBS), and 1-fluoro-2,4,dinitrobenzene (FDNB) were incubated with teased proximal tubules from

flounder kidney and the effects of these inhibitors on the tissue uptake of D-glucose (Glc), 2-deoxy-D-glucose (2-dGlc), methyl-α-D-glucoside (α-meGlc), D-galactose (Gal), and 2-deoxy-D-galactose (2-dGal) were measured. Table 1 shows the percentage inhibitions of total (free plus phosphorylated forms) and free sugar uptake produced by these agents.

TABLE 1

Percentage Inhibitions By Various Agents on the Tissue Levels of Total and Free Sugar in Teased Tubules from the Kidney of the Winter Flounder

	Cyb (2.7 μM)	PMB (0.5 mM)	PCMBS (0.5 mM)	FDNB (2.0 mM)
D-Glucose				
Total	22%	11%	18%	
Free	0%	0%	0%	
2-deoxy-D-glucose				
Total	33%	6%	10%	65%
Free	0%	0%	0%	0%
methyl-α-D-glucoside				
Free		17%	31%	
D-galactose				
Total	40%	29%	34%	
Free	17%	0%	0%	
2-deoxy-D-galactose				
Total	32%	49%	50%	73%
Free	12%	30%	29%	32%

Groups of tissue (4-5) were incubated aerobically (air) at 15°C for 60 minutes without (controls) and with the respective inhibitors in saline with 0.5 mM sugar. Both the total sugar (free plus phosphorylated forms) and the free sugar accumulations were expressed as μmoles/g wet weight. In this table the % inhibitions (control = 0% inhibition) are given. In separate experiments the effects of the inhibitors (10 μM CyB, 0.1 mM PMB, and 0.1 mM PCMBS) on tissue water and ionic distribution were determined. (Data not given here.)

CyB has been implicated as a potent inhibitor of sugar transport in a number of cell types. In the teased tubule preparation, 2.7 μM CyB inhibited the uptake of all sugars to approximately the same degree as 0.25 mM phlorizin (Dubyak, Mullin, and Kleinzeller, Bull. MDIBL, 1975, in press). This finding is comparable with the relationship between the effects of both these inhibitors on sugar transport in adipocytes (Czech, J. Biol. Chem., 248:3636-3641, 1973). It should also be noted that in intestinal cells, CyB primarily inhibited sugar transport processes localized at the serosal cellular face (Kimmich, Intestinal Permeation. Proceedings, Fourth Workshop Conference Hoechst, Schloss Reisensburg, October 1975, in press). Our previous reports (Kleinzeller and McAvoy, Bull. MDIBL, 12: 62, 1972; and Dubyak, Kleinzeller, and Mullin, Bull. MDIBL, Vol. 15, provided data localizing sugar transport systems in the teased tubule preparation at the antiluminal face. The data in Table 1 show that CyB preponderantly inhibited the tissue levels of sugar phosphates. Since evidence exists from

other work (Czech, J. Biol. Chem., 248:3636-3641, 1973) that CyB acts on sugar transport at the membrane level and our studies show that CyB primarily affected the accumulation of sugar phosphate, it is reasonable to consider the possibility that sugar transport at the antiluminal face of renal cells involves a phosphorylating mechanism. The possibility that the inhibition of sugar uptake was related to an inhibition of cellular energy metabolism is unlikely in view of a lack of effect on tissue water and ionic distribution.

The sulfhydryl reagents PMB and PCMBS have been shown to be inhibitors of sugar transport in erythrocytes (Smith and Ellman, J. Memb. Biol., 12:177-188, 1973). In the teased tubule preparation both PMB and PCMBS inhibited the uptake of all sugars tested; PCMBS was consistently a more potent inhibitor than PMB. Like CyB, these inhibitors primarily affected the tissue levels of the respective sugar phosphates. A comparison of the inhibitory effects on the various model sugar transport systems showed that PMB and PCMBS were considerably less inhibitory on the Glc-2-dGlc system than on the Gal-2-dGal system; α-meGlc transport had an intermediate sensitivity to thg inhibitors. It would thus appear that the active center of the Glc-2-dGlc system has its reactive sulfhydryl groups in a less accessible position than either of the other sugar transport systems.

FDNB acted as a potent inhibitor of transport in both the Glc-2-dGlc and Gal-2-dGal systems; again it was the accumulation of sugar phosphate which was most affected. FDNB has been demonstrated to inhibit glucose transport in erythrocytes and, in addition, to enter the cells rapidly (Forsling, Remfry, and Widdas, J. Physiol., 194:535-543, 1968). Therefore it is possible to conceive two mechanisms of its inhibitory effect in renal cells (wherein a very high percentage of accumulated sugar is phosphorylated): first, the FDNB could bind to the cell membrane and so directly affect the translocation of the sugar; and second, the FDNB could act either as a respiratory inhibitor or uncoupler and so lead to ATP deletion which would subsequently inhibit the formation of sugar phosphate.

In summary, the various inhibitors used in these experiments affected sugar transport in flounder renal cells in a way consistent with the actions of phlorizin and phloretin: that is, by primarily affecting the accumulation of the phosphorylated form of the sugar.

This study was supported in part by USPHS Grant AM 12619. The assistance of Mr. Jonathan Goldstein is appreciated.

FAILURE OF HYPERTONIC SODIUM INJECTIONS OR EXTERNAL POTASSIUM TO INCREASE CHLORIDE EFFLUX ACROSS THE GILL IN *Anguilla rostrata*

Franklin H. Epstein, Patricio Silva, Sara Epstein and Jonathan Epstein, Department of Medicine and Thorndike Laboratory, Harvard Medical School and Beth Israel Hospital, Boston, Massachusetts

Active extrusion of chloride by the gill is the mechanism by which seawater teleosts excrete excess salt to maintain a constant internal environment. Chloride efflux in seawater-adapted fish has been reported to rise in response to two stimuli: (1) administration of hypertonic sodium chloride and (2) addition of potassium to a fresh water bathing medium.

The first question addressed in these experiments was whether chloride efflux across the gill responded immediately and automatically to induced hypernatremia. American eels (*Anguilla rostrata*) were adapted to seawater for six weeks. Serum Na averaged 160 mEq/L, serum Cl 140 mEq/L and gill Na-K-ATPase was at levels characteristic of salt-water-adapted fish (14-18 μMPi per mg protein per hr). Chloride efflux was measured over 30 minute periods by injecting 2-3 μCi of ^{36}Cl, allowing it to