**TRANSPORT PROPERTIES OF ANTERIOR CRUCIATE AND MEDIAL COLLATERAL LIGAMENT CELLS.**

**Introduction:** Significant differences in the healing of ACL and MCL injuries in vivo and in cell proliferation, chemotactic migration, integrin expression, and Ca ++ uptake have been demonstrated in vitro. Since these processes are intimately linked to cellular ion homeostasis, cell metabolism and uptake of precursors for collagen and proteoglycans, the present study was undertaken to define the role of key transport processes for sodium, glucose, proline, and sulfate in ACL/MCL cells. One of the major plasma membrane transport systems involved in maintaining homeostasis and asymmetrical distribution of Na+ and K+ across the plasma membrane is Na, K-ATPase. Therefore the activity of this transport system was investigated by two approaches: 1) by measuring ouabain-inhibitable Rb uptake by intact cells and 2) by determining the Na and K stimulated, ouabain-inhibitable Na, K-ATPase activity.

**Methods:** Cells were isolated from ACL & MCL obtained from canine knee joints, and cultured in M199 containing 10% Fetal Calf Serum at 37°C in a humidified 5% CO2 air atmosphere. Transport was determined by incubating cells with 14H-proline, 3H-sulfate or 3H-D-glucose in presence or absence of sodium (Na+), or with 3H in the presence or absence of ouabain (Ou), at 25°C and 4°C for 15 min. After incubation the cells were washed with phosphate buffered – saline (10mM PO4 - 0.15M NaCl, pH 7.4), dissolved in 2% SDS / 0.1N HCl and radioactivity of the samples was measured by liquid scintillation counting. The enzymatic activity of Na, K-ATPase was evaluated by measuring Na+ uptake into intact cells by incubating cell homogenates (obtained by repeated freezing and thawing of the cells) in the presence and absence of oubain at 37°C for 30 min.

**Results:** Both ACL and MCL cells at 25°C had a significant uptake of 3H-proline in the presence of sodium (Fig.1). The uptake was reduced by 80% at 4°C. The replacement of Na by N-methyl-D-glucamine (NMDG) did not alter Na uptake (n=4) while DIDS, an inhibitor of sulfate transport, was able to abolish sulfate uptake. Thus neither of the anion exchange pathways were involved in the transport of sulfate. The lower activity observed in the intact cell could be due to the low sodium concentration in the cell and the usual feedback on the pump exerted by the sodium gradient.

**Discussion:** The data demonstrated that both ACL and MCL possess a highly active sodium pump, a secondary active sodium-proline cotransport system, and sodium–independent transport system for D-glucose and sulfate. The rates of transport of these molecules in ACL and MCL cells were similar, supporting the hypothesis that under resting conditions these transport systems were not rate limiting for cell proliferation or migration. Whether these transport systems and the concomitant presence of transport are affected by application of mechanical load remains to be determined.

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