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RAPID COMMUNICATION

Regulation of Na^+, K^+-ATPase Density by the Extracellular Ionic and Osmotic Environment in Bovine Articular Chondrocytes

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Summary

The abundance of Na^+, K^+-ATPase in cartilage is controlled by the ionic composition of the extracellular environment of chondrocytes, and specifically depends on the local concentration of polyanionic matrix proteoglycans. In this study, it was found that the plasma membrane density of Na^+, K^+-ATPase in isolated chondrocytes is sensitive to both ionic and osmotic changes in the extracellular environment. The upregulation observed experimentally was similar in magnitude as measured by ^{3}H-ouabain binding, which indicates that chondrocytes respond adaptively to both ionic and osmotic stimuli. The precise mechanism for this novel mode of Na^+, K^+-ATPase regulation has yet to be elucidated. Physiological perturbation of the ionic and osmotic environment of chondrocytes may alter intracellular Na^+ concentration and this may be one of a number of stimuli responsible for alterations to the expression and plasma membrane abundance of Na^+, K^+-ATPase in the cells.

Key words

Na^+, K^+-ATPase • Upregulation • Chondrocyte • Articular cartilage

The Na^+, K^+ pump or Na^+, K^+-ATPase ((Na^+ + K^+)-stimulated adenosine triphosphatase; E.C. 3.6.1.37) is a Mg^{2+}-dependent pump responsible for maintaining the transmembrane gradients of Na^+ and K^+ in almost all animal cells (Skou 1998). Na^+, K^+-ATPase catalyses the active uptake of K^+ and extrusion of Na^+ at the expense of hydrolyzing ATP to ADP and P_i. The uphill transport establishes steep concentration gradients for Na^+ and K^+ which are harnessed by other membrane transport proteins for a variety of essential physiological functions including pH regulation and uptake of metabolites such as sugars and amino acids. The plasma membrane abundance of Na^+, K^+-ATPase in most cells is sensitive to the intracellular Na^+ concentration which in turn depends on cell permeability and ultimately, the extracellular Na^+ concentration (Wolitzky and Fambrough 1986, Brodie and Sampson 1989). In articular chondrocytes, the Na^+, K^+-ATPase is responsible for maintaining a low

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intracellular Na^+ : K^+ ratio, vital for the biosynthetic and secretory function of chondrocytes. The plasma membrane abundance of Na^+, K^+-ATPase in chondrocytes has been shown to be relatively high for these small cells (1.0 - 1.5 x 10^5 sites per cell; Mobasheri *et al.* 1997) and varies according to the local concentration of extracellular polyanionic glycosaminoglycans [GAG] and hence tissue Na^+ concentration (Mobasheri, 1998). Furthermore, multiple isoforms of the catalytic and regulatory subunits of Na^+, K^+-ATPase have been identified in bovine and human chondrocytes (Mobasheri *et al.* 1997, Trujillo *et al.* 1999). Although relatively little is known about the identity and mode of action of hormonal and chemical mediators involved in the regulation of Na^+, K^+-ATPase abundance in chondrocytes, it is understood that the extracellular physico-chemical environment is a key regulator of Na^+, K^+-ATPase expression and abundance in chondrocytes (Mobasheri 1999). In this study, the long-term effect of alterations in extracellular ionic and osmotic strength on the plasma membrane density of Na^+, K^+-ATPase was studied by quantitative ^{3}H-ouabain binding in isolated bovine chondrocytes.

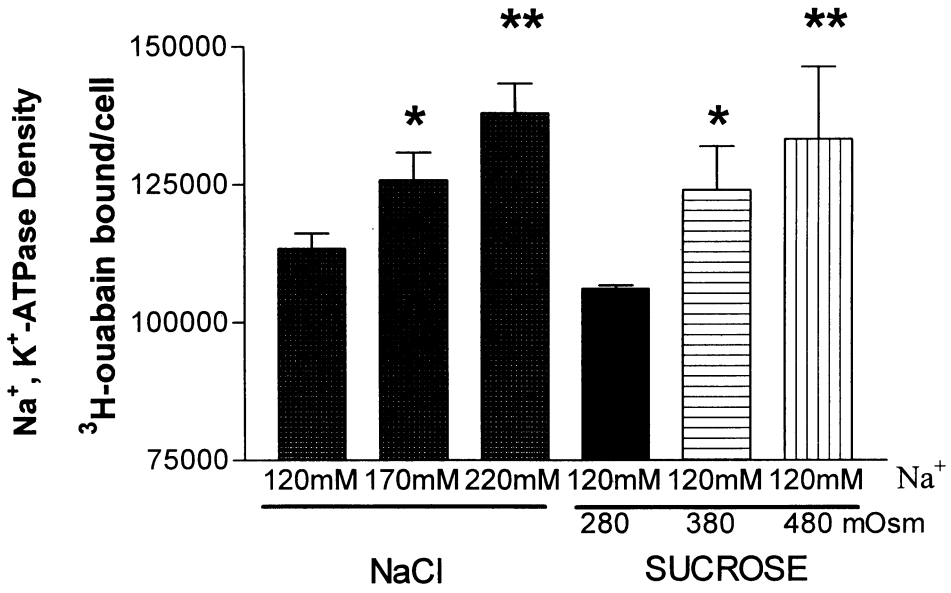

Fig. 1. *The effect of increased culture medium ionic strength and osmolarity on Na^+, K^+-ATPase density in isolated bovine chondrocytes. Increasing ionic strength by NaCl addition resulted in a significant upregulation of plasma membrane Na^+, K^+-ATPase sites. Increasing osmolarity (in 100 mOsm kg $^{-1}$ H_2O steps) by addition of sucrose (whilst keeping $[Na^+]_o$ constant at 120 mM) resulted in a very similar upregulation of slightly lower magnitude. Significant differences from chondrocytes isolated in isotonic medium are indicated by * $P<0.05$ and ** $P<0.005$ (Student's t-test) (mean ± standard error, n = 5 independent ^{3}H-ouabain binding experiments).*

Chondrocytes were isolated from bovine metacarpal-phalangeal joints as previously described in detail (Mobasheri *et al.* 1997). Briefly, type I collagenase from *Clostridium histolyticum* (0.8 mg . ml^{-1} or 100 collagen digestion units . ml^{-1}, for 18 h at 37 °C) was used to digest shavings of bovine metacarpal-phalangeal cartilage in Dulbecco's modified Eagle's medium (DMEM) in the presence of antibiotic/antimycotic solution (160 units penicillin, 0.16 mg streptomycin and 0.5 µg amphotericin B per ml, final DMEM concentrations). In experiments where the effect of medium osmolarity was under investigation (constant extracellular Na^+ concentration), the DMEM used was either isotonic (280 mOsm . kg $^{-1}$ H_2O) or hyperosmotic

(380 mOsm . kg $^{-1}$ H_2O and 480 mOsm . kg $^{-1}$ H_2O). The osmolarity of the medium was changed by sucrose addition to isotonic DMEM. In experiments where the effect of increased extracellular Na^+ concentration was under investigation, the Na^+ concentration in DMEM was altered by NaCl addition to give three different sodium concentrations (120, 170 and 220mM Na^+). At the end of the 18 h digestion period the resulting cell suspension was filtered through a 70 μm Nylon membrane. The chondrocytes were washed twice with fresh DMEM to remove collagenase. Cell counts and viability (which was normally above 95 %) was determined by counting cells on a hemocytometer and Zeiss light microscope using the vital dye trypan blue. Plasma membrane Na^+, K^+-ATPase density was measured by ^{3}H-ouabain binding according to the basic methodology described by Erdmann (1982) in a K^+-free artificial medium (to accelerate the rate of binding and to eliminate competition between K^+ and ^{3}H-ouabain) containing all the chemical components present in DMEM except amino acids and vitamins. Na^+, K^+-ATPase density was determined in this solution containing 4-8 kBq . ml^{-1} ^{3}H-ouabain. Non-specific ouabain binding was measured by the addition of excess non-radioactive ouabain (10^{-4} M unlabelled ouabain). Chondrocytes were then washed three times with ice-cold, non-radioactive K^+-free medium and the cells were lysed with 0.5 % Triton X-100 in deionized water. Half of the lysate was used for the determination of radioactivity by scintillation counting and the other half was used for DNA assays. Taking a value of 7.7 pg DNA per chondrocyte (Mobasheri 1998), these data yielded specific ouabain binding sites per chondrocyte or Na^+, K^+-ATPase density per chondrocyte.

Bovine chondrocytes isolated in isotonic medium containing 120 mM Na^+, 280 mOsm . kg $^{-1}$ H_2O) expressed between 1.06 x 10^5 (± 2828) and 1.13 x 10^5 (± 612) plasma membrane ouabain binding sites. Chondrocytes isolated in 170 mM Na^+ significantly upregulated plasma membrane ouabain binding sites over a period of 18 h (1.26 x 10^5 pumps per cell ± 5087). Chondrocytes isolated in DMEM containing 120 mM Na^+ and high sucrose (380 mOsm . kg $^{-1}$ H_2O) also upregulated plasma membrane ouabain binding sites expressing 1.24 x 10^5 (± 7867) sodium pump sites. Cells isolated in very high Na^+ (220 mM Na^+) and very high sucrose (480 mOsm . kg^{-1} H_2O) expressed significantly higher quantities of plasma membrane Na^+, K^+-ATPase (1.38 x 10^5 ± 5382 in 220 mM Na^+ and 1.30 x 10^5 ± 2828 in 480 mOsm . kg $^{-1}$ H_2O with constant Na^+). The results summarized in Figure 1 demonstrate that the increases in both extracellular Na^+ and osmolarity result in a similar and significant upregulation of plasma membrane Na^+, K^+-ATPase sites in isolated bovine articular chondrocytes.

Articular chondrocytes *in situ* routinely experience changes in extracellular Na^+ concentration during fluid loss under mechanical load. A physiological consequence of joint loading is expression of tissue fluid, increased extracellular Na^+ concentration and cartilage osmolarity. Such changes in extracellular Na^+ concentration will result in increased intracellular Na^+ concentration altering the Na^+ : K^+ ratio and the cell membrane potential. The Na^+, K^+-ATPase maintains the low Na^+ : K^+ ratio vital for the synthesis of extracellular matrix macromolecules (principally aggrecan and collagen II) in chondrocytes. Therefore, in response to ionic or osmotic stimuli the activity of Na^+, K^+-ATPase will acutely increase to match the homeostatic demands of the cell. Following long-term ionic or osmotic stimulation, the plasma membrane abundance and density of Na^+, K^+-ATPase is expected to change as has been observed in other cell systems. Indeed, recent studies using immunofluorescence confocal microscopy and ^{3}H-ouabain binding suggest that Na^+, K^+-ATPase upregulation induced by elevated ionic strength is the result of *de novo* Na^+, K^+-ATPase synthesis and targeting of newly synthesized Na^+, K^+-ATPase to the plasma membrane (Mobasheri 1999). The results presented here demonstrate, for the first time, that the abundance of Na^+, K^+-ATPase in isolated chondrocytes also appears to be sensitive to the osmotic composition of the extracellular environment, i.e. the incubation medium. However, the effect of simultaneously increasing osmolarity and ionic strength by addition of both NaCl and sucrose does not appear to be additive; there is no additional upregulation of Na^+, K^+-ATPase sites above the upregulation seen by increasing ionic strength or osmolarity alone (results not shown). Therefore, the ability to respond to changes in the ionic and osmotic environment is an important adaptive capability that will allow chondrocytes to respond to physiological changes that routinely occur in their unusually harsh ionic and osmotic extracellular environment *in vivo* by finely regulating the plasma membrane abundance of the Na^+, K^+-ATPase. Changes in the expression and turnover of the Na^+, K^+-ATPase may be required to maintain the optimal intracellular Na^+ : K^+ ratio for matrix turnover and may be achieved by transcriptional and translational regulation of Na^+, K^+-ATPase at the gene and protein levels respectively in articular cartilage (Mobasheri

1999). The possible role of other Na^+ transport systems such as epithelial sodium channels in the adaptation to increased ionic strength and osmolarity is unexplored. Also, the signaling mechanisms responsible for this adaptive homeostatic response mechanism remain to be elucidated.

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