Hydrostatic pressure modifies the membrane fluidity and desaturase gene expression in chondrocyte progenitor cells

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1. ABSTRACT

Membrane mechanical properties modulate the exchange of nutrients and metabolites as well as signal transduction. The cell membrane is a lipid bilayer containing cholesterol and proteins, whose fluidity is tightly regulated by cholesterol and lipid desaturases. Hydrostatic pressure affects the membrane fluidity of microorganisms but its effects on mammalian cells' mechanical properties are poorly understood. We here studied the effects of hydrostatic pressure on the membrane fluidity and the expression of four major desaturases in a mouse chondrogenic cell line. Hydrostatic pressure was found to decrease both membrane fluidity and the expression of the four desaturases, effects that were inhibited by cholesterol depletion. This study shows new effects of hydrostatic pressure on mammalian cell membranes and may help understand the mechano-sensing of hydrostatic pressure.

2. INTRODUCTION

The plasma membrane's lipid bilayer contains cholesterol and proteins, including numerous receptors and signaling molecules, and its physical properties, such as membrane fluidity, control many molecular events crucial to signal transduction.

Hydrostatic pressure (HP) has been shown to trigger homeoviscous adaptation, a process known to occur in deep sea organisms and during which the proportion of synthesized unsaturated fatty acids is boosted to increase membrane fluidity (1). In mammalian cells, membrane fluidity is controlled by two groups of fatty acid desaturases: the Δ9-desaturases, and the Δ5/Δ6-desaturases (2). Δ9-desaturases form monounsaturated fatty acids by introducing a cis-double bond at the Δ9 position of acyl chains in saturated lipids. In mice, four Δ9-desaturase genes (Scd1 to Scd4) have been identified, among which Scd1 and Scd2 are widely expressed. The Δ5- and Δ6-desaturases, respectively coded, in mice, by Fads1 and Fads2, are also widely expressed and are crucial to the synthesis of polyunsaturated fatty acids.

Previous studies on homeoviscous adaptation have been carried out on marine organisms but whether such adaptation also occurs in mammalian cells submitted to HP, such as chondrocytes, is still unclear. We therefore studied the effects of HP on membrane fluidity and desaturase gene expression in chondrogenic ATDC5 cells.

3. MATERIALS AND METHODS

Cell culture and pressurization

Mouse chondrogenic ATDC5 cells were purchased from the JCRB Cell Bank and cultured in DMEM/F12 (Sigma) supplemented with 5% of fetal bovine serum.

For cell imaging, we used a system consisting of a windowed culture chamber in which medium was injected with a high-pressure pump. The chamber consisted of 2 stainless steel plates with a round opening; the top plate opening was covered by a diamond window; the bottom plate opening was covered by a removable, 1-mm thick sapphire window (Edmund Optics) on which cells where cultured. A narrow space, sealed by an O-ring, was left between the two windows for the cells and medium, which was injected inside the chamber through flow channels. Before imaging, a sapphire window with cells was sandwiched between the two plates; the device was then placed on an inverted microscope, kept at 37°C on a thermally controlled Thermoplate (Tokai Hit), and the cells were observed through the window.

For PCR analyses, cells grown on fibronectin-coated glass slides were pressurized under constant HP for up to 24h using another system consisting of a pressure chamber placed inside a 37°C water bath and connected to a high-pressure cylinder. After culturing the cells on the glass slides placed in small Petri dishes for 2 days, the Petri dishes were sealed in polyolefin bags filled with culture medium and placed in the pressure chamber.

Live cell imaging

Membrane fluidity was assessed by staining the cells with the fluorescent probe laurdan (Molecular Probes) (3). For laurdan imaging, cells were cultured for 2 days on fibronectin-coated sapphire windows, stained for 30 min with laurdan and placed in the pressure chamber. Images acquired with a C7780 CCD digital color camera (Hamamatsu) were analyzed using AquaCosmos software. Laurdan was excited at 340 nm and the emitted light was collected by the blue and green channels of the CCD camera, respectively giving two intensity values I9 and I0, which were used to calculate the generalized polarization (GP) of laurdan using the formula GP = (I9-I0) / (I9+I0) (3).

Real-time PCR

cDNA was synthesized from total RNA using the ReverTra...
Ace qPCR RT Master Mix with gDNA Remover (Toyobo). Real-time PCR was carried out using Thunderbird SYBR qPCR Mix (Toyobo) using primers for Fads1, Fads2, Scd1, Scd2, Gapdh and Rpl13a; desaturase gene expression was normalized to the geometric mean of the reference genes Gapdh and Rpl13a.

4. RESULTS

Live imaging of pressurized ATDC5 cells stained with laurdan showed that under increasing HP, the average laurdan GP of the cells also increased (Fig. 1), indicating a shift from a more disordered state of the lipids in the bilayer to a more packed state; quantification of the change in GP as a function of HP showed a linear relationship between the two (Pearson’s r=0.779, p<0.01).

![Fig. 1: Change in laurdan GP in ATDC5 cells under HP.](image)

PCR analyses of pressurized ATDC5 cells showed that, compared to unpressurized cells, cells under 10 or 20 MPa saw the expression of all four desaturases (Fads1, Fads2, Scd1 and Scd2) significantly reduced after 24h (Fig. 2).

![Fig. 2: Modulation of desaturase gene expression in pressurized ATDC5 cells. Paired Student t-test: *p<0.05 and **p<0.01 compared to unpressurized cells.](image)

As Methyl-β-cyclodextrin (MβCD) removes cholesterol from the cell membrane, thus increasing its fluidity, we tested whether MβCD could reverse the effects of HP on desaturase gene expression by treating pressurized ATDC5 cells with MβCD (Sigma). MβCD significantly increased Scd1 expression in control and pressurized cells, HP decreased desaturase expression in MβCD-treated cells and the combined action of HP and MβCD tended to return desaturase expression to its basal level (Fig. 3).

![Fig. 3: Effects of cholesterol depletion on desaturase expression in pressurized cells. Paired Student t-test: *p<0.05; **p<0.01.](image)

5. DISCUSSION AND CONCLUSION

The main limitation of this study concerns the high HP applied to the cells (20 MPa). As GP imaging had only shown clear differences at pressures above 10 MPa, those pressures were chosen for subsequent experiments. In the human hip joint, peaks of up to 18 MPa have been recorded; 20 MPa is therefore a high pressure. High HP is known to decrease chondrocyte marker expression, promote inflammation and induce changes similar to osteoarthritis (4). The current findings should therefore be interpreted as reflecting a more pathological situation.

The fact that all four desaturases are simultaneously down-regulated suggests a concerted response to HP. However, the physiological significance of this down-regulation by HP may not be linked to homeoviscous adaptation but to the involvement of unsaturated lipids in cell proliferation or apoptosis. Indeed, Δ9 desaturase knockdown inhibits cell proliferation and promotes apoptosis (5), and polysaturated fatty acids produced by Δ5- and Δ6-desaturases participate in other essential cellular functions besides membrane fluidity regulation. This warrants further study.

In conclusion, this study shows that HP decreases membrane fluidity of ATDC5 cells and inhibits the expression of four important desaturases. HP is known to be important for cartilage differentiation. Therefore, understanding how chondrocytes respond to pressure may therefore be useful to better understand and ultimately treat osteoarthritis or create suitable growth conditions for tissue engineered cartilage.

6. REFERENCES

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