UNIAXIAL CYCLIC STRETCH INDUCES THE ACTIVATION OF TRANSCRIPTION FACTOR NF-KAPPA B IN HUMAN FIBROBLAST CELL.

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Introduction
Nuclear factor-kappa B (NF•B) is a transcription factor that plays a pivotal role in the regulation of a number of immune and inflammatory response genes and activation of various cellular and viral promoters. In the unstimulated cells, NF•B dimer in cytosol is present as an inactive form complexed with an inhibitory protein IκB. Upon cell stimulation with agents such as phorbol esters, TNF-α, IL-1, hydrogen peroxide, UV light and LPS, the NF•B dimer is dissociated from IκB and translocates to the nucleus. Mechanical stretch induces prostaglandin (PG) E2 which is considered to increase in cases of inflammation due to repetitive motion, such as tendonitis, bursitis and fasciitis in fibroblast cells and in skeletal muscle cells. It was reported that the level of the expression of COX-2 protein and the level of COX-2 mRNA in human lung fibroblasts increased in response to uni-axial cyclic stretch. Mechanotransduction pathway in human fibroblasts have not been clearly understood. However, stretch-activated (SA) channel is one of the candidates of mechanotransducer. It was suggested that COX-2 expression is up-regulated by uni-axial cyclic stretch via the activation of SA channel in human lung fibroblasts.

Recently it was reported that the human COX-2 promoter region contains transcription factors such as AP-1, CRE and NF•B, and the translocation of NF•B is induced by calcium signalling in B lymphocytes.

We examined here whether NF•B translocation into the nucleus and activation are induced by uni-axial cyclic stretch and investigated the involvement of SA channel in the NF•B translocation and activation in human fibroblasts.

Materials and Methods
Human lung fibroblasts (TIG-1) were transferred onto a fibronectin-coated silicon chamber and were subcultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum and 100 units/ml penicillin G sodium and 100 g/ml streptomycin sulfate. The silicon chamber attached to a stretching apparatus that was driven by a computer controlled stepping motor.

1. Immunostaining and Fluorescence Microscopy After the application of uni-axial sinusoidal stretch (120 % peak to peak, at 1 Hz), the cells were incubated with anti-RelA antibodies for 1 hour, and then were incubated with fluorophore-labeled antibodies for 30 minutes. The immunostaining was observed under an epifluorescence microscope.

2. Immunoblotting After the application of cyclic stretch, nuclei and cytoplasmic extracts were separated by 12.5 % SDS-PAGE. The proteins were transferred electrotherophoretically onto polyvinylidene fluoride membrane. The membrane were subsequently probed with anti-IκB or anti-RelA antibodies. The antibodies-antigen complexes were detected by using horseradish peroxidase-conjugated antibodies. Immunoreactivity was determined using the ECL chemiluminescence reaction (Amersham, Arlington Heights, IL, USA).

3. Luciferase assay After the transfection pNF•B-Luc plasmid that produces luciferase by activation of NF•B into the cells, cyclic stretch was applied. After the cells were incubated for 6 hours at 37 °C, 5% CO2, the light was measured by luminometer.

Results
1. Translocation of NF•B First we examined whether uni-axial cyclic stretch induces a translocation of NF•B into the nucleus as the translocation is reported to be an early event of the activation of NF•B. Immunostaining of NF•B subunit, RelA, demonstrated the translocation of NF•B into the nucleus in response to cyclic stretch. The time course of NF•B translocation into the nucleus during stretch was assayed by degradation of its cytosolic inhibitor IκB, or by accumulation of NF•B subunit, RelA, in the nucleus. The translocation of NF•B was detected as early as 2 minutes after the initiation of cyclic stretch, peaked at 4 min, and returned to the basal level within 10 min (Fig. 1). To investigate the involvement of SA channel in the regulation of NF•B activation, we examined the effects of the removal of extracellular Ca2+ and the application of Gd3+, SA channel blocker. When the cells were mechanically stretched in the absence of extracellular Ca2+, NF•B translocation was not detectable. The application of 20 M Gd3+ inhibited NF•B translocation.

2. Activation of NF•B Next, to investigate whether NF•B activates after translocation into nucleus, we assayed luciferase that the cells transfected pNF•B-Luc plasmids produced. The level of luciferase increased at 4 minutes after the initiation of cyclic stretch, peaked at 15 minutes (6.4 fold increase) and decreased gradually. But in the absence of extracellular Ca2+ and the application of 20 M Gd3+, the level of luciferase did not increase.

Discussion
Stretches is known to influence ionic fluxes in skeletal muscle cells, and to induce secretions of several endothelial cells. In human umbilical endothelial cells, an uni-axial stretch increased intra-cellular Ca2+ concentration, and this response was inhibited when the cells were in the absence of extracellular Ca2+ and the application of 20 M Gd3+, SA channel blocker. It was suggested that the increase in intracellular Ca2+ concentration arose from Ca2+ entry through SA channels in endothelial cells. In this study, when cells were stretched in the absence of extracellular Ca2+ and the application of 20 M Gd3+, the activation of NF•B was almost completely inhibited. Although we did not measure changes in the stretch induced intracellular Ca2+ concentration in TIG-1, these data strongly suggest that the activation of NF•B was regulated by stretch-induced release of Ca2+ from sarcoplasmic reticulum Ca2+ store.

Mechanical stretch induces PG E2 that is considered to increase in cases of inflammation due to repetitive motion, such as tendonitis, bursitis and fasciitis in fibroblast cells and in skeletal muscle cells. It is reported that COX-2 expression, which can be induced by inflammatory stimuli, hormones or mitogens, is up-regulated by uni-axial cyclic stretch via the activation of SA channel in human lung fibroblasts. The COX-2 expression is involved in the activation of NF•B deeply, because in fact the human COX-2 promoter region contains transcription factors NF•B. In other words, cyclic mechanical stretch increases intracellular Ca2+ concentration through SA channels and these signal activates NF•B. The activation of NF•B induces the expression of COX-2, the first rate-limiting enzyme in the synthesis of PG E2 in cases of inflammation.

In conclusion, uni-axial cyclic stretch up-regulated the activation of transcription factor NF•B via the activation of SA channel in human lung fibroblasts. NF•B is activated in cases of inflammation due to repetitive motion, such as tendonitis, bursitis and fasciitis.

Time (min.) 0 1 2 4 6 8 10 15 20 30 P.S.

RelA

Figure 1. The time course of NF•B translocation during stretch was assayed by degradation of IκB, or by accumulation of RelA. P.S.: positive control

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