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EFFECT OF LYSOPHOSPHATIDIC ACID ON C-JUN TERMINAL KINASE ACTIVATION BY MECHANICAL STRETCH IN HUMAN BLADDER SMOOTH MUSCLE CELLS.

Hypothesis / aims of study

Lysophosphatidic acid (LPA) is a bioactive lipid that influences a wide range of biological processes, including cell proliferation, migration, morphologic changes, and survival. However, a role for LPA in bladder pathology remains to be elucidated.

Bladder smooth muscle hyperplasia is a part of the usual compensatory response to bladder outlet obstruction (BOO) resulting often from benign prostatic hyperplasia (BPH). In the present study, we first evaluated whether LPA activates nuclear transcription factors and has a proliferative effect on human bladder smooth muscle cells(HBSMCs).

Since, mechanical stretch stress is considered the trigger inducing smooth muscle hyperplasia secondary to BOO, we used a in vitro model of mechanical stress, and investigated whether LPA is involved in stretch-activated signal transduction pathways, which leads to increased growth factor synthesis and cell proliferations in HBSMCs.

Study design, materials and methods

In all experiments, we used commercially established HBSMCs purchased from Lonza.

HBSMCs treated by LPA, and activity of MAPK was measured by western blotting method using anti-phosho JNK, p38, ERK antibody. Cell proliferation was measured using BrdU labelling and Detection kit (Roche). Cells were cultured in a 96-well microplate. BrdU was added to the culture medium and incorporated into freshly synthesized DNA. The absorbance of the sample was determined using a microplatereader.

After HBSMCs were treated by mechanical stretch, activity of MAPK was also measured. To simplify the analysis, we used uni-axial stretch device (STREX) which can stimulate adherent cells to a single direction stretch by controlled motor unit. Cells were stimulated by 15% elongated uni-axial stretch at 0.5Hz.

All values are expressed as means SEM. The data were statistically analysed by one-way ANOVA with the Dunnett test, and a probability value of $p<0.05$ was considered significant.

Results

1. LPA solely induced JNK, p38, ERK activation by dose-dependent manner, activation of JNK was most relevant among the 3 subsets of MAPK family members. This activity was suppressed by pre-incubation with LPA inhibitor Ki16425 (fig.1). The activities of JNK and ERK peaked at 15min, p38 peaked at 5min after incubation with 10^{-4}M LPA (fig.2).
   LPA induced cell proliferation by dose-dependent manner (fig.3), and the LPA-induced cell proliferation was suppressed by Ki16425.
2. Stretch (15% elongation,0.5Hz) stimulated HBSMCs were harvested at each time point and the activities of MAPK were measured. The activity of JNK peaked at 15 min after stretch about 3.7 fold. On the other hand, p38 and ERK did not appear to be appreciably activated by mechanical stretch (fig.4).
   This JNK activity was partially inhibited by pre-incubation with Ki16425 (fig.5).

Interpretation of results

To our knowledge, this is the first report measuring JNK activation and cell proliferation by LPA in HBSMCs. It was suggested that LPA induced cell proliferation through JNK activation.

Furthermore, JNK activation by mechanical stretch was partially suppressed by LPA inhibitor.

These results suggest that mechanical stretch may cause HBSMCs to release LPA, which then activates LPA receptor in autocrine fashion.

Concluding message

In this study, we showed that LPA induces JNK activation and cell proliferation in HBSMCs. Furthermore, it is demonstrated that LPA partially contributes to JNK activation by mechanical stretch, suggesting a potential involvement of LPA in bladder smooth muscle hyperplasia induced by BOO.

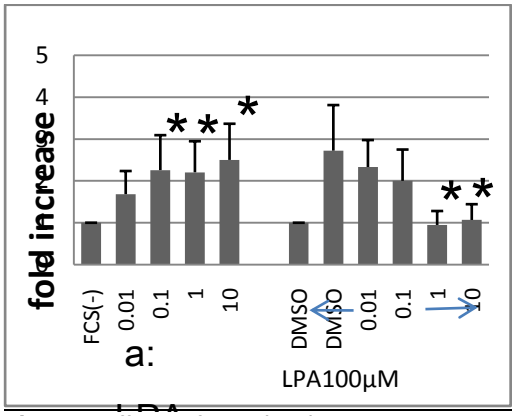


Fig 1. a: Effects of LPA (n=6)
Dose-dependent JNK activation in BSMCs The intensity of FCS(-) was set at 1.*p<0.05 vs. FCS(-).
b: JNK activity by pre-treatment Ki16425 (LPA1/3 inhibitor) (n=8)
*p<0.05 vs. LPA(+) pre-treatment DMSO

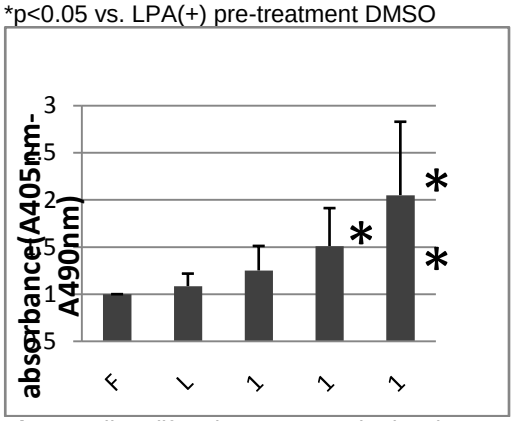
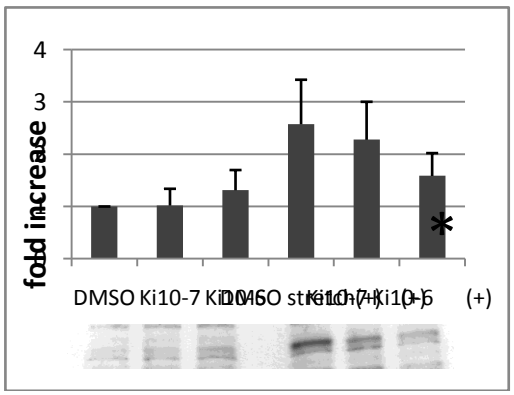


Fig 3. Cell proliferation ELIZA,BrdU (n=7)
Proliferation of BSMCs in response to LPA.
*p<0.05, **p<0.01 vs. FCS(-)



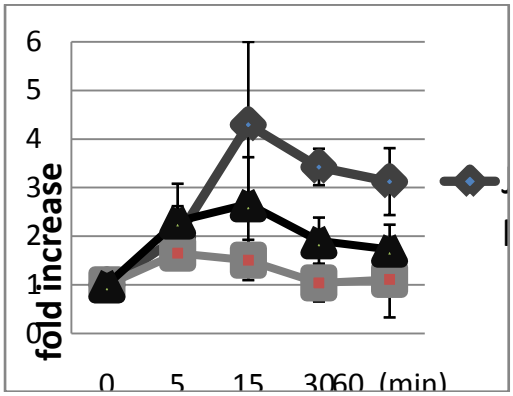


Fig 2. Time-dependent MAPK activation by LPA 10^{-4}M (n=4)

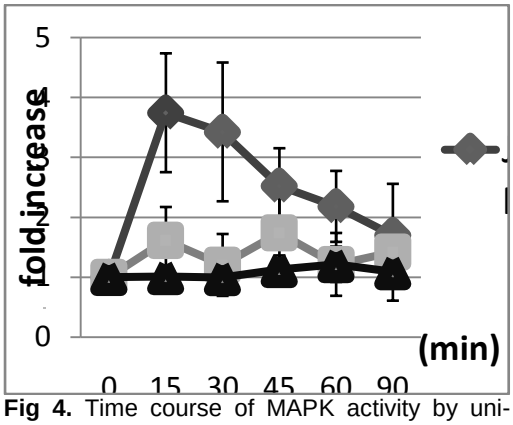


Fig 4. Time course of MAPK activity by uni-axial stretch (n=5)
BSMCs were exposed to uni-axial mechanical stretch
(15% elongation,0.5Hz) for period indicated.
JNK activity peaked at 15min.
The intensity at 0min for each kinase was set at 1.

Fig5. JNK activity by pre-treatment Ki16425 (n=6)
Activity of JNK was measured at 15min after stretch
*p<0.05 vs. stretch(+) pre-treatment DMSO

References

1. Am J Physiol Cell Physiol 281:C1165-C1172,2001
2. Biochem Biophys Res Commun 208:19-25,1995
3. Circ Res 88:925-932,2001

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What were the subjects in the study?	**NONE**