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MAPK FAMILY ACTIVATION IS ASSOCIATED WITH FREQUENT DETRUSOR CONTRACTIONS.

Hypothesis / aims of study

Bladder wall thickness has been noted to be increased in patients with overactive bladder (OAB). Due to a symptom of urinary frequency and detrusor overactivity, frequent detrusor contractions persist in this condition, which put mechanical stress on the bladder, resulting in hypertrophy of the detrusor muscle. This may explain the increase in bladder wall thickness. However, an early signal transduction mechanism that finally leads to detrusor hypertrophy has remained to be elucidated.

The mitogen-activated protein kinase (MAPK) family is signal transduction molecules that mediate external stimuli into intracellular signals that regulate cell growth. We hypothesized that MAPK is easily activated in the situations usually encountered, such as urinary frequency. In order to test this hypothesis, the *in vivo* study was undertaken to investigate whether increased voiding frequency activates MAPK family in the rat bladder. In addition, the *in vitro* study was carried out to evaluate the effects of frequency and duration of detrusor muscle contractions on MAPK activation.

Study design, materials and methods

In vivo study. 5 rats received the intraperitoneal administration of 20mg/kg furosemide (furosemide group) and 5 rats treated with vehicle were used as control (control group). Voiding frequency and urine output were monitored in the metabolic cage for 1 hour after the injections of furosemide or vehicle. Then, rats were sacrificed immediately and bladders were taken. The bladdrs were homogenized and the protein extracts were used to perform western blot for detecting total and phosphorylated MAPK family (extracellular signal-regulated kinase (ERK), c-jun NH_2-terminal kinase (JNK) and p38).

In vitro study. The bladder muscle strips removed from 5 rats were suspended in each 20ml organ bath containing krebs solution. Transmural nerve electrical field stimulation(EFS) was performed to induce contraction of detrusor muscle.(main interval : 60.0 s, cycles : 15, 30, or 45) Then, the bladder strips were immediately homogenized and the protein extracts were used to perform western blot for detecting total and phosphorylated MAPK family (ERK,JNK,p38).

Results

In vivo study. The rats in the furosemide group showed about 8.6 times voids by 1 hour after the injection of furosemide while number of voids was only 0.4 times in the control rats without diuresis (Table 1). In the bladders from the furosemide group, activation of JNK was the most relevant among the 3 subsets of MAPK family; a significant 4.7-fold increase was detected as compared with that in the non-diuresis control (Fig.1). Similarly, the activity of ERK was enhanced with a significant 1.6-fold increase. The significant activation of p38 was not detected.

In vitro study. EFS induced contractions of the bladder strip. In response to repetitive contractions, JNK activation was also prominent, and ERK activation was lower than JNK. P38 was not significantly activated. When duration of contraction was prolonged with a constant frequency of 60 times / h, the activities of JNK and ERK were also increased. (Fig.2)

Interpretation of results

The *in vivo* study shows that 8 to 9 times detrusor contractions for only 1 hour can activate JNK and ERK in the rat bladder. The *in vitro* study also suggests that both JNK and ERK activities will be increased more in the detrusor muscle that contracts with higher frequency and longer duration.

Concluding message

MAPK in the bladder may be very sensitive to increased bladder activity, which would provide a basis for the development of detrusor muscle hypertrophy that will manifest as a thickend bladder wall in patients with OAB.

	Number of micturition per hour	Urine output rate (mL/h)
Control (n=5)	0.4±0.2	0.5±0.3
Furosemide(n=5)	8.6±0.6	8.5±0.7

Table 1 Frequent urination is induced by administration of furosemide. Each value represent means ± SEM.

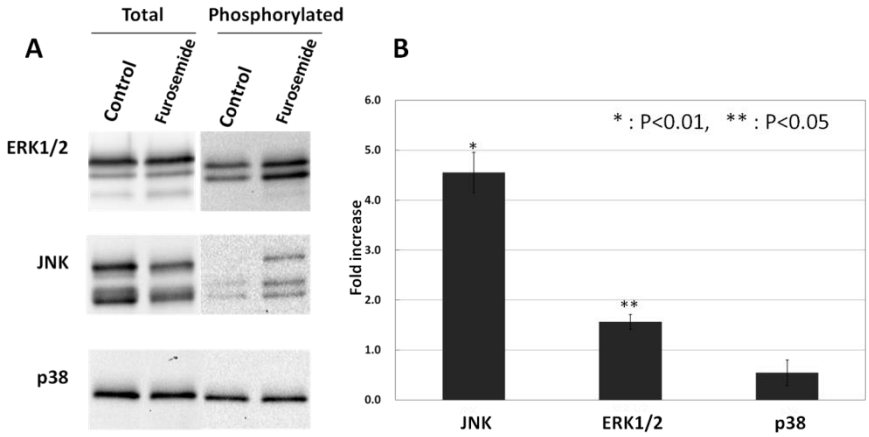

Fig. 1 Changes in MAPKs phosphorylation by frequent urination. (A) A example of blotting data, (B) The data is phosphorylated ratio in the furosemide group normalized by protein amount and intensity of Control group . Total and phosphorylated MAPK family is quantified by densitometry. Each value represent means ± SEM.

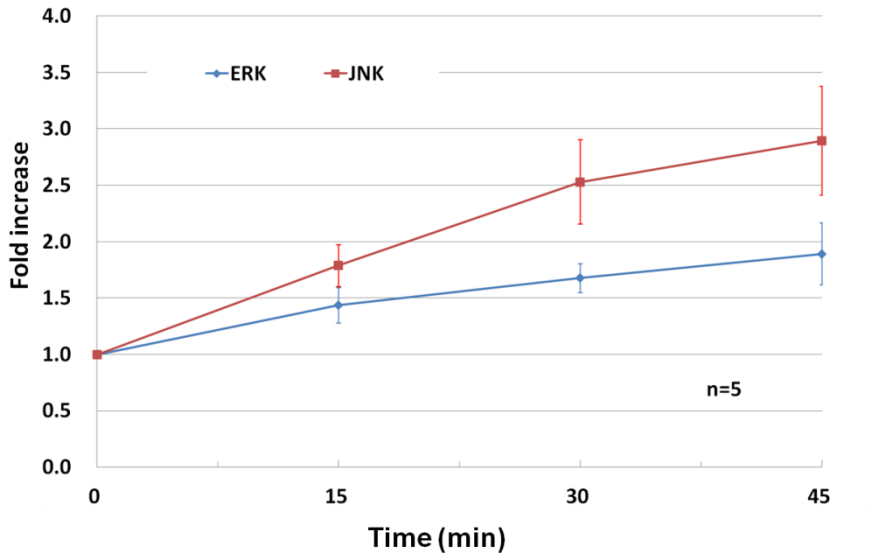

Fig. 2 MAPKs phosphorylation is facilitated by EFS induced frequent contraction.
Bladder muscle strips were performed EFS for indicated time with a constant frequency of 60 times / h. The data is phosphorylated ratio of each EFS performed that normalized by the protein amount and the intensity at each 0 min (EFS not performed). Total and phospho- rylated MAPK family is quantified by densitometry. Each value represent means ± SEM.

Disclosures
Funding: None **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** Nihon university