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# EFFECTS OF A NOVEL EP2 AND 3 RECEPTOR DUAL AGONIST (ONO-8055) ON BLADDER MUSCLE STRIPS FROM SHAM AND LUMBAR CANAL STENOSIS RATS AND MRNA EXPRESSION OF EP RECEPTORS IN THESE ANIMALS

Hypothesis / aims of study

Rat lumbar canal stenosis (LCS) model consistently demonstrates the objective characteristics of underactive bladder (UAB) / detrusor underactivity (DU), and seems useful to evaluate the effects of the drugs for UAB/DU (1, 2). A novel EP2 and EP3 receptor dual agonist (ONO-8055) contracts bladder smooth muscle and relaxes urethral smooth muscle in muscle strip studies from normal rats. Moreover, this agonist decreases residual urine rate in awake cystometry in LCS rats probably via a decrease in maximum cystometric capacity (MCC) as well as in intraurethral perfusion pressure (Pura). On the other hand, although this agonist decreases MCC and Pura in normal rats, these effects is demonstrated at the highest doses that are thirty times and ten times higher than the doses in LCS rats for MCC and Pura, respectively (3). Therefore, It is suggested that a pathway via EP2 and EP3 receptors does not have an important role in lower urinary tract function in normal rats and that this pathway would be more susceptible in LCS rats (3). In addition, it is conceivable that this agonist would affect bladder afferent system rather than detrusor itself (1, 2, 3).

In the present study, to further disclose the mechanisms of the effects of this agonist, we investigate the in vitro muscle contractile activity of this agonist and mRNA expression of EP receptors in the sham and LCS rats

Study design, materials and methods

1) In vitro functional study

The bladder from sham and LCS rats (female Jcl:Wistar rats, n=8 each) at ages 9 weeks were used. Bladder strips were prepared by cutting the bladder body into longitudinal strips approximately 10 x 3 mm. Prepared bladder strips were suspended in Magnus tubes. After it was confirmed that the KCl contractile response was maintained, physiological saline and ONO-8055 was cumulatively added from the lowest concentration.

2) mRNA relative quantification by quantitative PCR

The mRNA relative quantification was performed in the bladder and urethra (n=8 each) by quantitative PCR, using specific probes for EP1, EP2, EP3 and EP4 genes.

Responses to each concentration of ONO-8055 and mRNA expression of four EP receptors in the LCS groups were compared with those in the sham group, using ANOVA followed by student's t-test, and compared with pre-administration, usung Dunnet's test. p values $< 0.05$ were considered significant.

Results

1) In vitro functional study

Compared with saline, the muscle tension of both the sham and the LCS rats were significantly increased after adding the EP2 and EP2 dual agonist (Figure 1). Moreover, the response of muscle strips from LCS rats were statistically larger than from the sham rats at 1 and 10μmol/L of this agonist.

2) mRNA relative quantification by quantitative PCR

All the EP receptors (EP1 to EP4 receptor) were expressed in the lower urinary tract of both the sham and the LCS rats. Compared with the sham rats, the mRNA expression of four EP receptors in the lower urinary tract of the LCS rats did not show any statistically significant differences (Figure 2).

Interpretation of results

The EP2 and EP3 dual agonist effectively contracted the bladder muscle from the LCS rats as did in the sham rats. However, awake cystometry did not show the definitive evidence of augmenting bladder contractility in the LCS rats (2). Therefore, it is conceivable that this agonist might activate the afferent system before affecting the detrusor contractility, leading to voiding at lower bladder volume.

The present study showed that the different responsivites of the agonist on the sham and the LCS rats were not explained by mRNA expression of the EP receptors. The precise mechanism remained to be determined, however, the condition of the peripheral nervous system, that was intact in the sham rats but severy impaired in the LCS rats, might be associated with the different responsivities to the agonist.

Concluding message

A novel EP2 and EP3 dual agonist contracted the bladder muscle strips from both the sham and the LCS rats. The mRNA expression of EP receptors were not different between the sham and the LCS rats. Further studies, such as continuous awake cystometry, are planned to investigate the effects of this agonist on the bladder contractility in vivo.

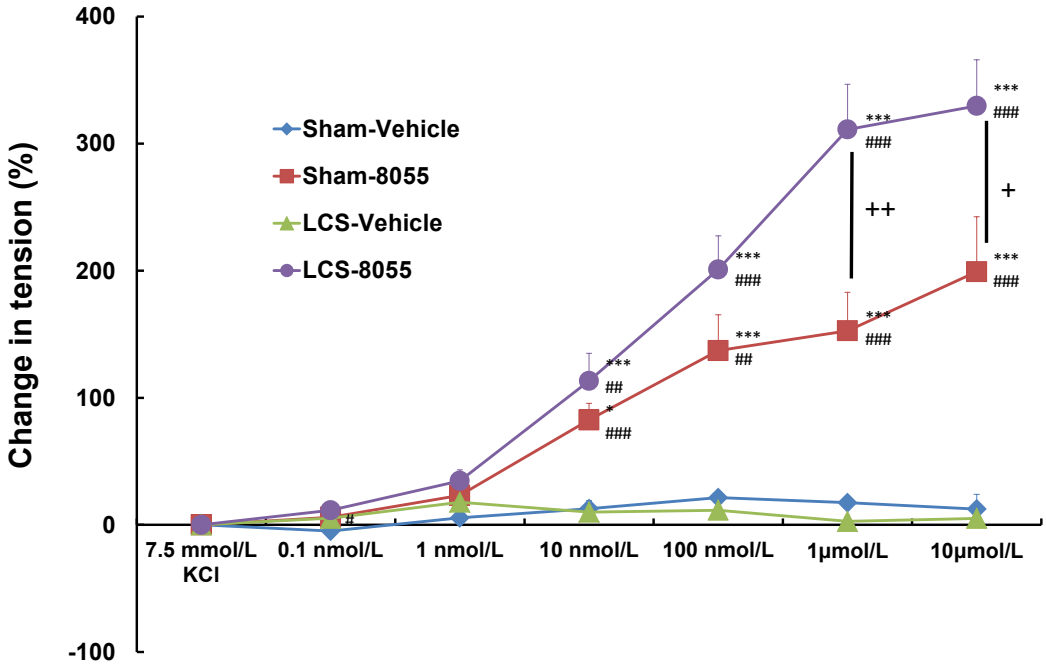


Figure 1. The results of in vitro functional study
*P＜0.05, ***P＜0.001 : vs. pre-administration (Dunnett's test), #P＜0.05, ##P＜0.01, ###P＜0.001 : vs. vehicle (student's t-test), +P＜0.05, ++P＜0.01 : Sham vs. LCS (student's t- test)
8055; EP2 and EP3 receptor dual agonist, LCS; lumbar canal stenosis

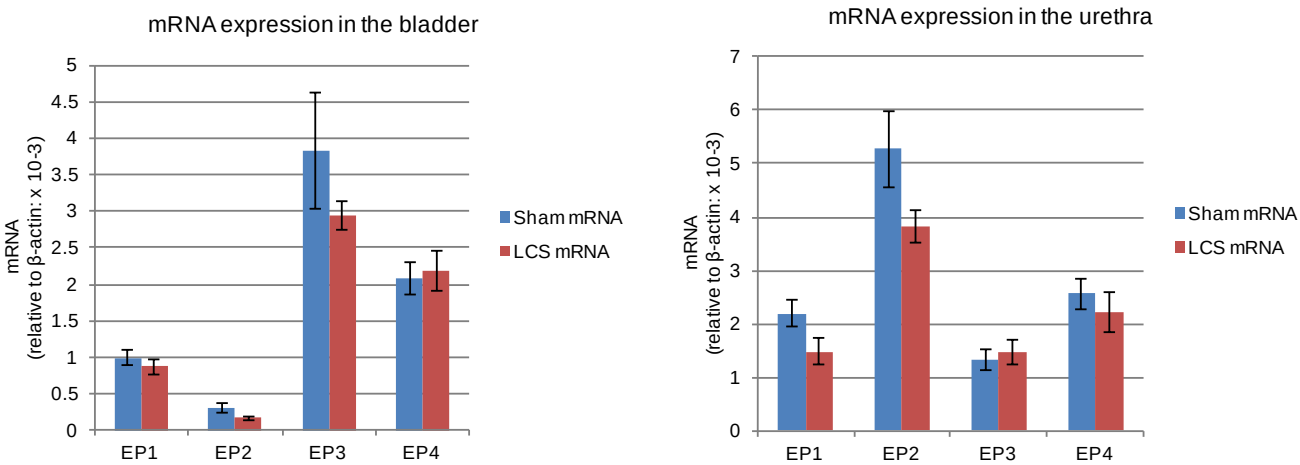


Figure 2. mRNA expression in the lower urinary tract of the sham and the lumbar canal stenosis rats
No significant differences in mRNA expression in the lower urinary tract between the sham and the lumbar canal stenosis rats are observed.
EP; prostaglandin $E_2$ receptor, LCS; lumbar canal stenosis

References
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3. ICS 2016 abstract 490

Disclosures
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