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ADRENERGIC α_1 ACTIVATION TO HEAL DAMAGED UROTHELIUM MIGHT CONTRIBUTE TO NORMAL MICTURITION REFLEX.

Hypothesis / aims of study

The dysfunction of urothelium has been thought to be related to the pathogenesis of interstitial cystitis(IC).Urothelium from IC patient exhibits common histological findings, including denudation or thinning of urothelium layer. The α_1 adrenergic receptor (α_1-AR) stimulation was reported to induce cell growth, proliferation and migration of vascular smooth muscle cell. We previously showed that urothelium expressed α_{1A} and α_{1D} –AR subtype. Our aim was to elucidate whether α_1-AR mediate the protective effects on disturbance of urothelium. We hypothesized that migration or proliferation of urothelium regulated by α_1-AR plays a role on the maintenance of urothelium function leading to normalize micturition reflex.

Study design, materials and methods

Cell Culture and wound healing assay: The bladders prepared from C57BL/6 mice (12-15 weeks) were incubated in MEM containing dispase for 1hr. The mucosa was gently scraped off and trypsinized to dissociate. Cells were plated onto collagen-coated glass coverslips in defined medium (HuMedia-KG2, Kurabo, Japan). The wound was made one day later when the cells formed a confluent. A sterile pipette tip was used to form scratch wound along the centre of the culture. The healing from the scratch was estimated to compare the wounded area immediately after scratching and 20 hours scratching. α_1 adrenergic agonist, phenylephrine was added to the culture 1 hour after scratching. α_1 adrenergic receptor non-selective antagonist, prazosin or α_{1A} -AR subtype selective antagonist, silodosin was added to the medium 20 min before the addition of phenylephrine. Phenylephrine and prazosin were purchased from Sigma. Silodosin was kindly gifted from Kissei Pharmaceuticals. **Immunocytotchemistry:** The cultures were rinsed with PBS and fixed in 4% paraformaldehyde in 0.1M phosphate buffer. After washing in TBS, samples were blocked in TBS contained 0.1% Triton X-100 and 3% bovine serum albumin. The slides were then incubated with rat anti-Ki67 antibody (DAKO). For visualization of primary antibody, samples were incubated with a donkey anti-rat-IgG secondary antibody conjugated with cy3. Then, alexa fluor-488 conjugated to phalloidin for staining F-actin and DAPI for nucleus were performed. Images were captured with confocal laser scanning microscope (LSM510meta, Zeiss). Width of lamellipodia was quantified with LSM image analyzer (Zeiss). Nucleus stained with DAPI was counted for nucleus density.

Results

Scratching of urothelium culture remained to form wounded edge (shown by dotted line in Fig.1A). Wound healing induced unidirectional and synchronous movement of cells, as a result wounded edges (dotted lines) moved close each other (Fig.1A) to close the wound. Wound healing assay showed that phenylephrine induced significant enhancement of healing of urothelium. This effect was blocked by the treatment of prazosin or silodosin prior to the addition of phenylephrine (Fig.1B). Cells located on wounded edge extended lamellipodia into open space (Fig.2A). Urothelium culture treated with phenylephrine significantly increased in number of lamellipodia along wounded edge and decreased the width of lamellipodia. These effects were cancelled by the pretreatment of silodosin (Fig.2D,E). The nucleus density was significantly increased in culture treated with phenylephrine, this effect was partially inhibited by silodosin (Fig.2F). Ki67 is a marker of cell proliferation, detected in nucleus. Positive signals of Ki67 was scare in control culture compared with that treated with phenylephrine (Fig.1A,B). Control culture and silodosin pretreated culture exhibited neally same level of positive signals of Ki67 (Fig2A,C).

Interpretation of results

The present results indicate that activation of α_1 -AR facilitates the healing from scratch wound and the acceleration is at least partially mediated by α_{1A} -AR subtype. The process might depend on cell proliferation and cell locomotive activity. The present data presents the novel idea that adrenergic α_1 receptor controls maintenance and repair of transitional cell layer in normal and abnormal condition and also proposes to explain the pathophysiology of damaged urothelium in IC.α_1 -AR activity of urothelium might play a protective role against abruption and /or denudation of urothelium related to the etiologic process of IC.

Concluding message

α_1-AR activity of urothelium might play a role on recovery of bladder function leading to normalization of micturition reflex.

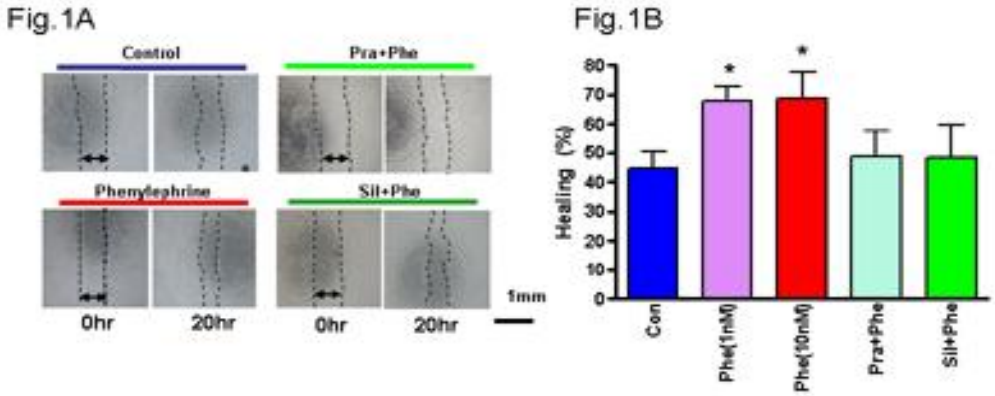

Fig.1: Typical example of healing from scratch was shown in Fig.1A.Wounded space after scratching was indicated by double arrow head lines. Dotted lines indicate wounded edge of urothelium culture. Healing was estimated in various conditions (Fig1B). Bars expressed mean +/- S.E.M.(n=4). The addition of reagents was described as materials and methods. Phenylephrine;1 nM, Pra+Phe: preteratment of 0.1 nM of prazocin and 1 nM of Phenylephrine, Sil+Phe; pre-treatment of 0.1 nM silodosin and 1 nM Phenylephrine. Phe(1nM); 1nM phenylephrine 1nM, Phe(10nM); 10 nM Phenylephrine. *<0.05

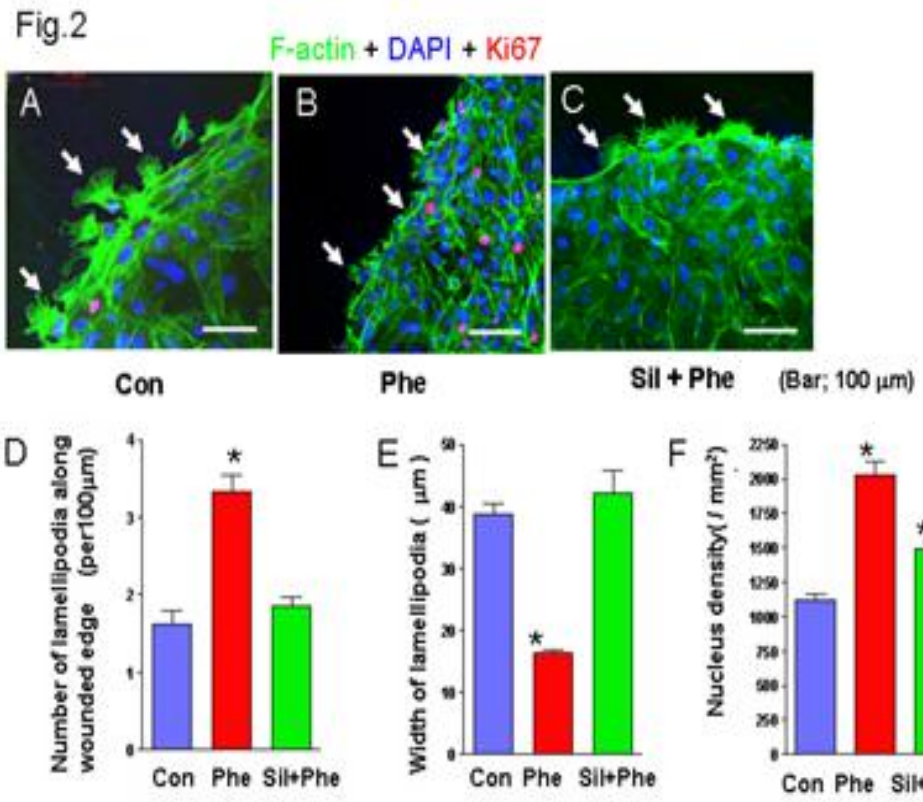

Fig.2: Confocal images of wounded edge was shown in Fig.2A,B,C. Note that the shape and number of lamellipodia was infuluenced by phenylephrine.The number (Fig.2D) and width (Fig.2E) of lamellipodia along the wounded edge was quantified. The nucleus density was shown in Fig.2F. Con; control culture, Phe; 1nM phenylephrine, Sil + Phe; 0.1 nM silodosin pretreatment before addition of 1 nM phenylephrine. *<0.001

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What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Animal Care Committee in Bioresearch-Education Centre, Akita University**