

## PHYSIOLOGICAL ROLE OF MINERALOCORTICOID RECEPTORS IN RAT BLADDER EPITHELIUM

### Hypothesis / aims of study

Previous studies have shown that mineralocorticoid receptors (MRs) are expressed in the epithelium of toad urinary bladder. On the other hand, to the best of our knowledge, no studies have shown whether similar MR expression occurs in the bladder epithelium of rats. In the present study, we examined the expression and the physiological role of MRs in rat urinary bladder.

### Study design, materials and methods

Male Sprague-Dawley rats (10–12 weeks old) were used for the in vivo and in vitro experiments. First, to examine MR expression, we performed RT-PCR and immunohistochemical analyses on urinary bladders from normal rats and sequenced the PCR products. Next, to examine the physiological function of MRs, we classified the rats into two groups: (1) normal and (2) fludrocortisone (FC). Rats in the FC group were orally treated with FC (6 mg/kg/day), a potent MR agonist, once daily for 3 days. After FC treatment, we performed cystometry; under anesthesia, the dome of the bladder was punctured, cannulated, and then filled with saline. Subsequently, the bladder was filled with 1 mM amiloride, a selective epithelial sodium channel (ENaC) inhibitor, in saline. Injection rate was 0.08 mL/min, and the intravesical pressure was recorded. The voiding intervals (VI) and the rate of changes of VI before and after infusion of amiloride were compared between the normal group and the FC group.

### Results

Expression of MR mRNA was observed in rat bladder. The base sequence of the PCR product was identical to the sequence reported before (Pietranera et al. 2012). In the immunofluorescence examination, MR proteins were clearly stained and localized at the bladder epithelium (Fig. 1). In the cystometry analysis, during infusion of saline, the VI of the FC group (n = 6) tended to be lower than that of the normal group (n = 4) ( $336.2 \pm 77.8$  s [FC group] vs.  $532.0 \pm 75.4$  s [normal group];  $P = 0.11$ ) (Fig. 2A, 2B[a]). Although the VI of the normal group (n = 4) before and after infusion of 1 mM amiloride remained unchanged ( $532.0 \pm 75.4$  s [saline] vs.  $535.0 \pm 95.1$  s [1 mM amiloride];  $P = 0.98$ ) (Fig. 2A[a], 2B), the values in the FC group (n = 6) tended to increase during infusion of 1 mM amiloride ( $336.2 \pm 77.8$  s [saline] vs.  $521.3 \pm 98.3$  s [1 mM amiloride];  $P = 0.17$ ) (Fig. 2A[b], 2B).

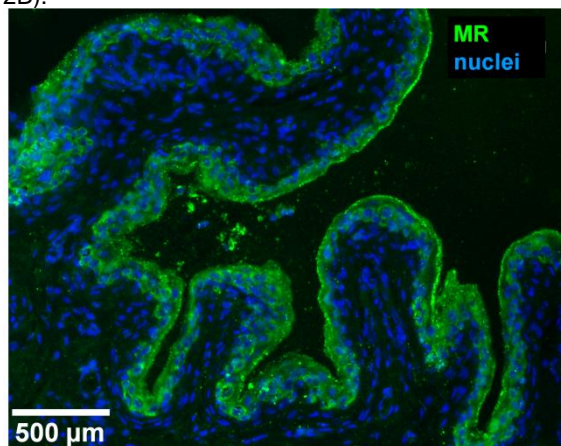
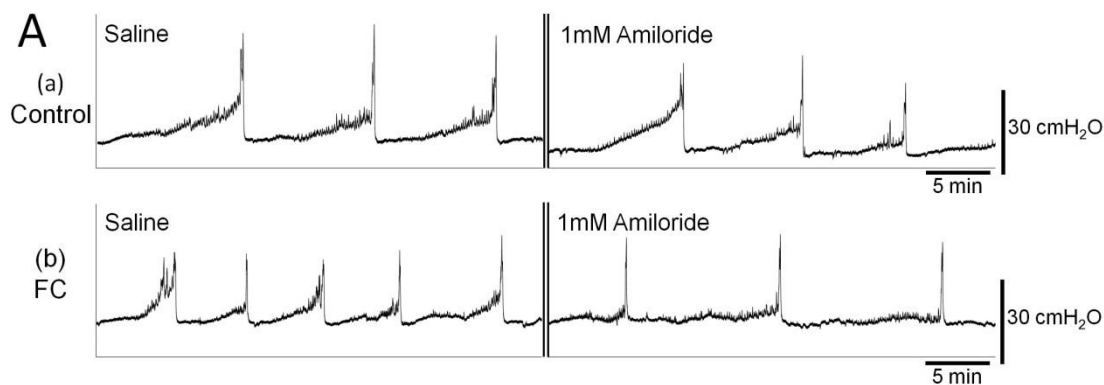


Fig. 1 Localization of MR in normal bladder  
Green, MR; Blue, nuclei



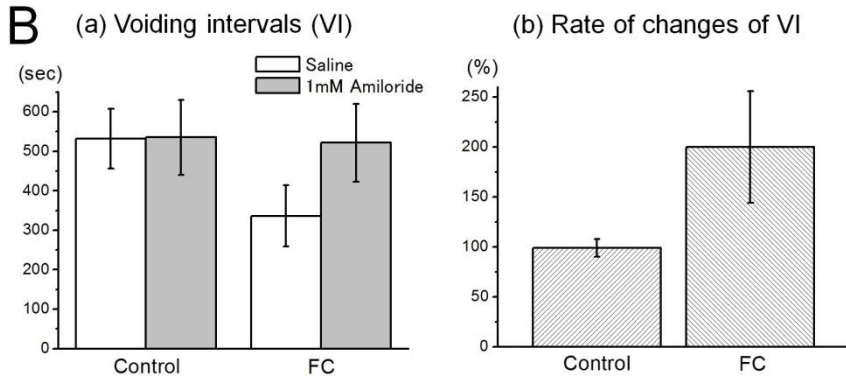


Fig. 2 The results of cystometry

A, representative charts for the normal group (a) and FC group (b); B, the voiding intervals (VI) (a) and the rate of changes of VI (b)

#### Interpretation of results

MR expression in rat bladder epithelium was similar to that reported in the toad urinary bladder. MR stimulation resulted in reduction of VI. In addition, the reduction was eliminated by amiloride. Therefore, in rat bladder epithelium, MR genomically regulates ENaC expression, as in other tissues (e.g., kidney and colon). In bladder epithelium, ENaC is reported as one of the mechanosensors for intravesical pressure. Therefore, our results suggested that activation of MR resulted in increase of both functional ENaC expression and sensitivity to intravesical pressure in rat bladder epithelium.

#### Concluding message

In the present study, we suggested a physiological function of MR in rat bladder epithelium. In some pathogeneses, aldosterone, a physiological MR agonist, may partly play a role in frequent urination, and MR antagonists or ENaC inhibitors may become effective therapeutic drugs for this condition.

#### Disclosures

**Funding:** None. **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** The ethics committee of Nagoya City University