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Full paper

Mineralocorticoid receptor stimulation induces urinary storage dysfunction via upregulation of epithelial sodium channel expression in the rat urinary bladder epithelium

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ABSTRACT

We aimed to evaluate mineralocorticoid receptor (MR) expression in rat bladder and the physiological role of the MR-epithelial sodium channel (ENaC) pathway in controlling bladder function in 10–12-week-old, male Sprague–Dawley rats. First, we examined the mRNA expression of MR and localization of MR and ENaC-α proteins in the urinary bladder. MR mRNA expression was observed in untreated-rat urinary bladders, and MR and ENaC-α proteins were localized in the epithelium. Next, rats were treated with vehicle (controls) or fludrocortisone (an MR agonist) for 3 days, and ENaC-α protein expression levels and bladder function were evaluated on day 4. ENaC-α protein expression was significantly higher in fludrocortisone-treated rats than in controls. In addition, cystometry was performed during intravesical infusion of saline and amiloride (an ENaC inhibitor). While intercontraction intervals (ICIs) during saline infusion were significantly shorter in the fludrocortisone group than in the controls, infusion of amiloride normalized the ICIs in the fludrocortisone group. However, no intra- or inter-group differences in maximum intravesical pressure were observed. Taken together, MR protein is localized in the rat urinary bladder epithelium, and may regulate ENaC expression and bladder afferent input. The MR-ENaC pathway may be a therapeutic target for ameliorating storage symptoms.

1. Introduction

Overactive bladder syndrome (OAB) exhibits urinary urgency and is typically accompanied by urinary frequency and nocturia (1,2). Anticholinergic drugs are currently the most common treatment for urinary incontinence and frequent urination in patients with OAB (1). However, adverse events are often associated with anticholinergic drug use, including dry mouth, constipation, and urination disorders, which can affect patient adherence (3,4). A selective β_3-adrenoceptor agonist, mirabegron, has recently been approved for the treatment of OAB in several countries (5). However, the treatment of some clinical cases has been difficult even by using this new drug. Therefore, a new therapeutic strategy for storage dysfunction (including OAB) is needed.

The pathology of bladder dysfunction involves altered bladder epithelial function (6), and a number of ion channels that are expressed at the bladder epithelium are reportedly involved in mechanosensation during bladder filling (e.g., TRPA1, TRPV4, or the epithelial sodium channel [ENaC]) (7,8). In the rat urinary bladder

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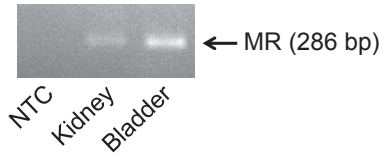

Fig. 1. Reverse transcriptase-polymerase chain reaction and agarose gel electrophoresis to analyze mineralocorticoid receptor expression in the rat urinary bladder. Mineralocorticoid receptor (MR) mRNA expression was observed in the rat urinary bladder. The kidney was used as the positive control, and the non-template control (NTC) was used as the negative control.

epithelium, ENaC is known to play a key role in releasing ATP, which is an important neurotransmitter for evoking detrusor contractions in response to mechanical stretch in the storage phase (9). Furthermore, among patients with bladder outlet obstruction and benign prostatic hyperplasia, bladder mRNA levels of the ENaC α, β, and γ subunits is significantly correlated with storage symptoms using the International Prostate Symptom Score (IPSS) (10). In addition, ENaC expression is regulated by mineralocorticoid receptors (MRs) in renal, colonic, and fetal lung alveolar type 2 epithelial cells (11,12); MRs are also expressed in the toad urinary bladder (13). Aldosterone-bound to MRs regulated the unidirectional transport of sodium from the mucosal phase to the serosal phase via the activation of amiloride-blockade sodium channels (13). However, to the best of our knowledge, no studies have shown that MRs are expressed in the bladder of rats or other mammals and the MR-ENaC pathway plays a physiological role in regulating bladder function. Therefore, the present study investigated whether MRs are expressed in the rat urinary bladder, and whether MR-ENaC signaling regulates bladder afferent nerve activity and bladder function.

2. Materials and methods

2.1. Animal and tissue preparation

We used 10–12-week-old male Sprague–Dawley rats (SLC Inc., Shizuoka, Japan) for all experiments. The rats were kept in a temperature- and humidity-controlled room, with a 12-h/12-h light/dark cycle and free access to laboratory chow and normal water. To prepare bladder tissue samples for the in vitro analyses, the rats were euthanized via excessive anesthesia, and their bladders were collected for reverse transcriptase-polymerase chain reaction (RT-PCR) and fluorescent immunohistochemistry analysis. All animal experiments were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals of the Science and International Affairs Bureau of the Japanese Ministry of Education, Culture, Sports, Science and Technology. The study design was reviewed and approved by the Ethics Committee of Nagoya City University.

2.2. RT-PCR

Total RNA was extracted from the whole kidney without the urothelium and the whole bladder by using the ISOGEN reagent (Nippon Gene, Tokyo, Japan), according to the manufacturer's recommended protocol. The RNA concentration and quality were measured via spectrophotometry at 260 nm and 280 nm. Then, 1 μg of total RNA was reverse transcribed using the ReverTra Ace-α-kit (Toyobo, Osaka, Japan) according to the manufacturer's recommended reaction protocol, with oligo $(dT)_{20}$ as the primer. The cDNA was mixed with AmpliTaq Gold DNA Polymerase in Buffer II and $MgCl_2$ (Applied Biosystems, Foster City, CA, USA), and the solution was combined with two primers that are specific for the MR gene: MR forward 5′-GTGGACAGTCCTTTCACTACCG-3′ and MR reverse 5′ -TGACACCCAGAAGCCTCATCTC-3′. The specificity of these primers was confirmed by a BLAST search of the rat genome database. The reaction mixtures were incubated at 95 °C for 10 min, which was followed by 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, and finally held at 72 °C for 7 min. Non-template control DNA was used as the negative control. The PCR products were subjected to electrophoresis on a 2% agarose gel, then stained with ethidium bromide and imaged under ultraviolet light. An automated DNA sequencer was used to confirm the DNA sequence of the purified PCR products, using the sequence of the Nr3c2 gene available in the online database.

2.3. Fluorescent immunohistochemistry

Extirpated urinary bladders and kidneys of untreated rats were fixed in 4% paraformaldehyde, which was replaced with 10%, 20%, and 30% sucrose solutions for >12 h, and then frozen in an optimal cutting temperature compound (Sakura Finetechnical Co., Ltd.,

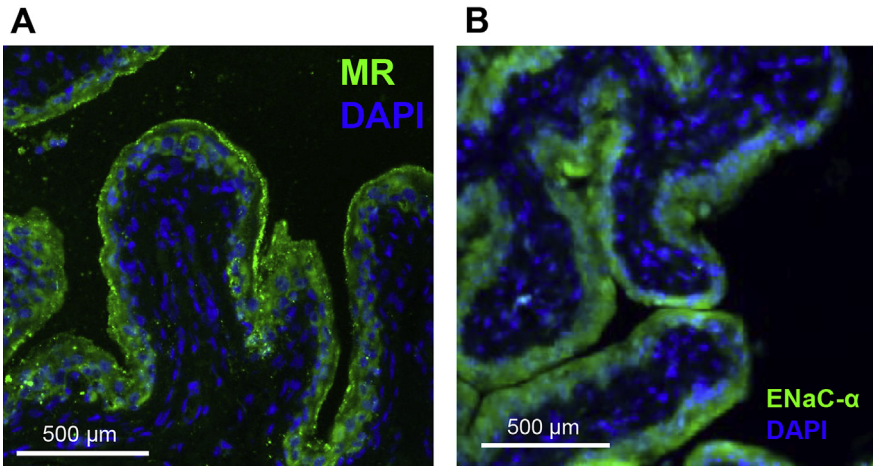

Fig. 2. Expression and localization of mineralocorticoid receptor protein and epithelial sodium channel-α protein in the rat urinary bladder. Blue staining indicates 4′,6-diamidino-2-phenylindole (DAPI)-positive nuclei. (A) Green staining indicates localization of the mineralocorticoid receptor (MR) protein at the rat urinary bladder epithelium. (B) Green staining indicates localization of the epithelial sodium channel-α (ENaC-α) protein at the rat urinary bladder epithelium. Scale bar = 500 μm.