59

Nishijima S[1], Sugaya K[1], Kadekawa K[1], Ashitomi K[1], Ueda T[2], Yamamoto H[3], Hattori T[4]
1. *Southern Knights' Laboratory,* ***2.*** *Institute for Animal Experiments, Faculty of Medicine, University of the Ryukyus.,* ***3.*** *Department of Biochemistry, Graduate School of Medicine, University of the Ryukyus.,* ***4.*** *Department of Medical Affairs, Asahi Kasei Pharma Corporation.*

ACTION OF NAFTOPIDIL ON SPINAL SEROTONERGIC NEUROTRANSMISSION FOR INHIBITION OF THE MICTURITION REFLEX IN RATS

Hypothesis / aims of study

Naftopidil, an α1D/A blocker, attenuates urethral outflow resistance in smooth muscle relaxation of the bladder neck and prostatic urethra, and is used to treat benign prostatic hyperplasia. In animal experiments, microinjection of naftopidil into the medial frontal lobe or intrathecal injection of naftopidil suppresses the micturition reflex.[1)] Oral administration of naftopidil reduces urinary ATP secretion and the plasma adrenaline level, and increases the plasma serotonin (5-HT) level.[2)] Thus, naftopidil suppresses the micturition reflex at the level of the frontal lobe, spinal cord, sympathetic nervous system, and bladder, in addition to relaxing the smooth muscles of the bladder neck and prostatic urethra. In the spinal cord, naftopidil mainly inhibits the afferent pathway of the micturition reflex by enhancing spinal inhibitory glycinergic and GABAergic neuronal activity. Additionally, naftopidil is also thought to inhibit the micturition reflex by affecting spinal descending neurotransmission, because naftopidil increases the plasma 5-HT level, intrathecal injection of 5-HT inhibits bladder contraction, and 5-HT differently modifies glutamate- and GABA- mediated effects, acting on distinct 5-HT receptor subtypes.[3)] In this study, therefore, we examined the action mechanism of naftopidil on spinal descending serotonergic neurotransmission for the micturition reflex.

Study design, materials and methods

Eighty-eight rats were studied in this experiment under urethane anesthesia (0.3 mg/kg intraperitoneally and 0.9 mg/kg subcutaneously). The abdomen was opened, the ureters were transected, and the distal ends were ligated. Then, a transurethral bladder catheter (PE-50, Clay-Adams, Parsippany, NJ, USA) was used to record the isovolumetric bladder pressure. After ligation of the catheter at the proximal urethra, the catheter was connected to a pressure transducer and an infusion pump through a three-way stopcock. A fine catheter (24 G, Clay-Adams) was inserted into the femoral vein for intravenous injection of naftopidil. For intrathecal injection of 5-HT receptor antagonists, a laminectomy was performed at L3, after which a polyethylene catheter (PE-10) was inserted into the subarachnoid space and advanced to the level of the sacral cord. When bladder activity was stabilized at 1 h after intravesical infusion of saline, the maximum bladder contraction pressure, the frequency of spontaneous bladder contractions, and the baseline intravesical pressure were measured to obtain an estimate of baseline bladder activity. In eight rats each (11 groups), 5-HT receptor antagonist, WAY100135 (a 5-HT1A receptor antagonist; 0.3-3 μg), GR55562 (a 5-HT1B receptor antagonist; 1-30 μg), BRL15572 (a 5-HT1D receptor antagonist; 1-30 μg), ketanserin (a 5-HT2A receptor antagonist; 0.1-1 μg), RS127445 (a 5-HT2B receptor antagonist; 1-10 nmol), SB242084 (a 5-HT2C receptor antagonist; 0.1-1 nmol), granisetron (a 5-HT3 receptor antagonist; 1-20 μg), GR113808 (a 5-HT4 receptor antagonist; 1-15 μg), SB399885 (a 5-HT6 receptor antagonist; 1-10 nmol), or SB269970 (a 5-HT7 receptor antagonist; 0.3-3 μg) dissolved in saline or 1% dimethyl sulfoxide was intrathecaly injected, or naftopidil (1 mg/kg) which dissolved in 0.1 M phosphate buffer was intravenously injected. After stable rhythmic bladder contraction was maintained, the maximum non-effective dose of each 5-HT antagonist and naftopidil were injected consecutively. Cystometric parameters were measured for 10-min intervals after drug injection and compared parameters between before and after, or between sole naftopidil and combined 5-HT receptor antagonist and naftopidil. Data were analysed by the paired or unpaired t-test, and $p < 0.05$ was considered to indicate statistical significance.

Results

Intravenous injection of naftopidil transiently abolished bladder contraction. Sole intrathecal injection of GR55562, SB269970 and granisetron significantly prolonged the interval between bladder contractions, and BRL15572 and RS127445 significantly shortened the interval between bladder contractions compared to the interval before intrathecal injection. Combined administration of the maximum non-effective dose of 5-HT receptor antagonists, except RS127445 and granisetron, and naftopidil significantly prolonged the interval between bladder contractions compared to the interval before administration. Meanwhile, the intervals between bladder contractions after combined administration of the maximum non-effective dose of BRL15572, ketanserin, RS127445, SB242084, or granisetron and naftopidil were significantly shorter than the duration of abolishment of bladder contractions after naftopidil was alone added.

Interpretation of results

In this study, according to the results of intrathecal injection of 5-HT receptor antagonists, 5-HT1B, 5-HT3, and 5-HT7 receptors were thought to promote the micturition reflex, and 5-HT1D and 5-HT2B receptors were considered to suppress the micturition reflex. When 5-HT2A and 5-HT2C receptor antagonists were intrathecally injected, no significant change was observed in the micturition reflex. However, the intervals between bladder contractions after combined administration of 5-HT2A or 5-HT2C antagonists at the spinal level and systemic naftopidil were shorter than the duration of abolishment of bladder contractions after naftopidil was alone added. These results suggest that 5-HT2A and 5-HT2C receptors also suppressed the micturition reflex caused by naftopidil.

Concluding message

In the spinal pathways controlling the micturition reflex, 5-HT1B, 5-HT1D, 5-HT2A, 5-HT2B, 5-HT2C, 5-HT3, and 5-HT7 receptors are involved in regulation of bladder activity. In our previous study, naftopidil increases the plasma 5-HT level and enhances spinal inhibitory glycinergic and GABAergic neuronal activity.[2)] Therefore, naftopidil may inhibit bladder contractions directly or

indirectly by an increase in the release or response of glycine and GABA in the spinal cord via 5-HT1D, 5-HT2A, 5-HT2B, 5-HT2C, and 5-HT3 receptors.

References
1. 1. Nishijima S, Sugaya K, Kadekawa K, et al. Effect of chemical stimulation of the medial frontal lobe on the micturition reflex in rats. J Urol 2012;.187:.1116-20.
2. 2. Sugaya K, Nishijima S, Kadekawa K, et al. Spinal mechanism of micturition reflex inhibition by naftopidil in rats. Life Sci 2014;116:106-11.
3. 3. Kadekawa K, Nishijima S, Sugaya K, et al. Mechanisms by which the serotonergic system inhibits micturition in rats. Life Sci 2009;85:592-6.

Disclosures
Funding: This study was supported by Asahi Kasei Pharma Corporation. **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rats **Ethics Committee:** The study protocol was approved by the President of the University of the Ryukyus based on the judgment of the Institutional Animal Care and Use Committee.