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EFFECTS OF RETIGABINE, A KV7 CHANNEL ACTIVATOR, ON NOCICEPTIVE BEHAVIOUR AND BLADDER OVERACTIVITY INDUCED BY INTRAVESICAL CHEMICAL IRRITATION IN CONCIOUS RATS

Hypothesis / aims of study
Voltage-gated K^+ 7 (Kv7) channels are known to reduce neural excitability and can inhibit nociceptive stimulation and transmission. Retigabine, a Kv7 selective activator, is a new class of antiepileptic drug used clinically and is an effective analgesic in animal models of chronic inflammatory and neuropathic pain (1). Kv7 channels are expressed in the rat urinary bladder, and its activator, retigabine, increases bladder capacity without concomitant reduction in blood pressure in anaesthetized rats with acetic acid–induced cystitis and increases bladder capacity in conscious rats with capsaicin-induced cystitis (2). These findings suggest that Kv7 channels may be a possible target for bladder pain syndrome. However, effects of Kv channels' inhibition on nociceptive behaviour induced by intravesical irritant instillation have not been investigated. We investigated effects of retigabine on nociceptive behaviour induced by intravesical instillation of resiniferatoxin (RTX) or acrolein, a main metabolite of cyclophosphamide, in conscious rats. In the experiments with intravesical acrolien, we also investigated the effects of retigabine on cystometric parameters.

Study design, materials and methods
Totally 24 female Sprague-Dawley rats were used. In the experiments with RTX-instillation, the experimental procedure was slightly modified from a previous study (3). Under general anesthesia with isoflurane, a catheter was inserted into the bladder through the urethra.
After recovery from anesthesia vehicle (saline) or retigabine (3 mg/kg) was injected intraperitoneally, and then RTX (3 μM, 0.3 ml) was instilled into the bladder and the urethral catheter was removed immediately after the instillation. The incidence of nociceptive behaviour (lower abdominal licking and freezing) was scored every 1-minute period for 15 minutes. In the experiments with acrolein-instillation, cystometry (CMG) measurements were carried out with constant intravesical instillation with saline at a rate of 6 ml/hour in conscious free-moving condition. Then saline or retigabine (1 mg/kg) was administrated intravenously (i.v.) and further CMG measurements were performed with acrolein (0.002%)-instillation at the same instillation rate. Nociceptive behaviour was scored and cystometric parameters measured for 60 minutes. The doses of retigabine used were chosen as un-affected doses on blood pressures based on our pilot study.

Results
RTX increased the licking at early-middle phases and the freezing at middle-later phases. In retigabine-administration group, this increased licking behaviour was significantly inhibited but freezing behaviour was not supressed significantly (Figure 1). Acrolein also increased the licking behaviour when voiding the urine (Figure 2). Moreover, acrolein significantly decreased the bladder capacity (Figure 3). Acrolein-induced increased licking behaviour was inhibited by the pretreatment with retigabine, but not saline (Figure 2), whereas neither saline nor retigabine changed the decreased bladder capacity induced by acrolein-instillation (Figure 3). The bladder weight was significantly increased after acrolein was instilled for 1 hour, and these values were not significantly different between saline- and retigabine-administration groups (sham group:122.4 ± 11.32 mg, saline group:225.5 ± 10.81 mg, retigabine group:227.5 ± 13.23 mg).

Interpretation of results
Licking behaviour induced by intravesical RTX-instillation is interpreted to occur through the mechanisms including an immediate response mediated by the activation of urethral afferents in the pudendal nerve and a late response evoked by the direct stimulation of C-fiber afferents in the bladder (3). Since retigabine inhibited more clearly the licking behaviour than freezing behaviour induced by both RTX and acrolein, it is likely that Kv7 channels play a role in nociceptive transduction in the urethra and bladder via the pudendal and pelvic nerves, respectively. The results with acrolein-instillation showed the increased bladder weight. This mechanical change, presumably reflecting edematous change in the bladder wall might contribute to the reduced bladder capacity, and retigabine could not attenuate this effect.

Concluding message
The present results demonstrate that the selective Kv7 activator, retigabine, can inhibit the licking behaviour induced by RTX and acrolein. These findings may give us a new insight into a possible role of Kv7 channels in the nociceptive transduction of the lower urinary tract.

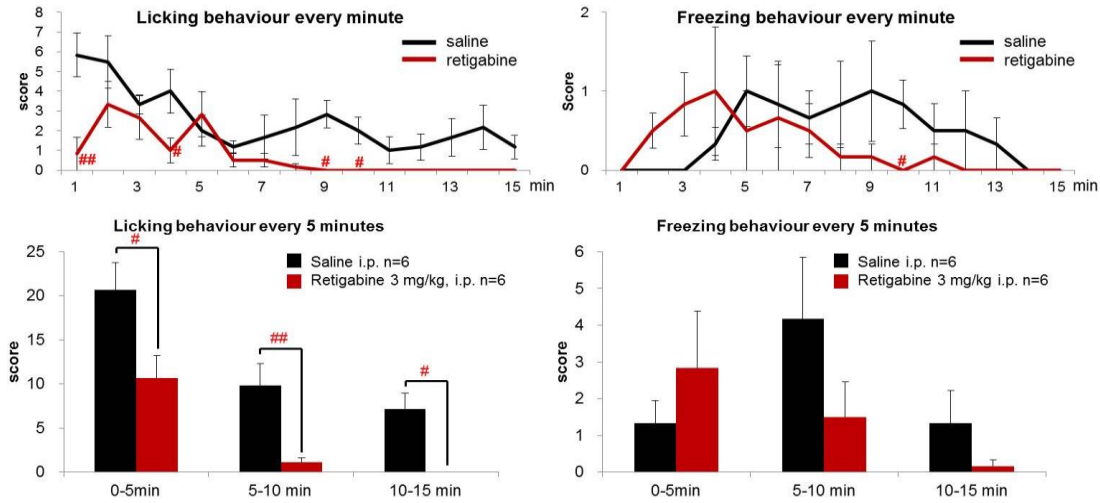

Figure 1. Effects of intravesical instillation of RTX on licking (left) and freezing (right) behaviour for every minute (upper) and totalled each 5-minutes period (lower) in saline- or retigabine-administrations. The values were expressed as mean ± SEM. $^{\#}P<0.05$, $^{\#\#}P<0.01$: significant differences between vehicle and retigabine groups (unpaired Student's *t*-test).

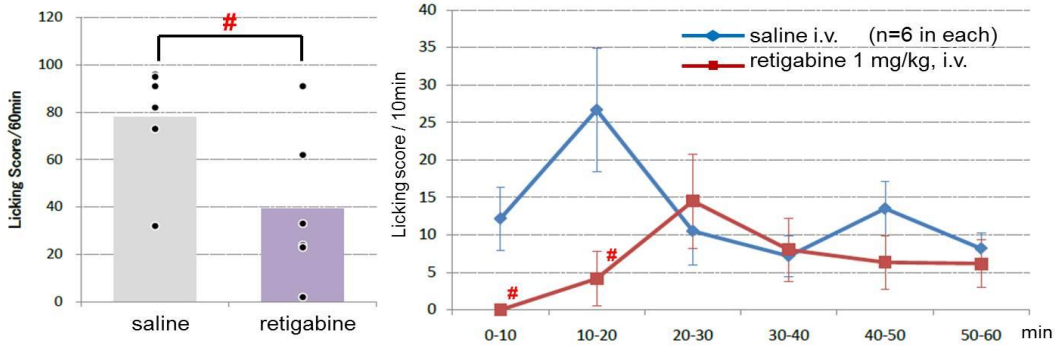

Figure 2. Effects of intravesical instillation of acrolein on licking behaviour for 60 minutes (left) and for each 10-minutes period (right) in saline- or retibabine-administrations. The values were expressed as mean ± SEM. $^{\#}P<0.05$: significant differences between vehicle and retigabine groups (unpaired Student's *t*-test).

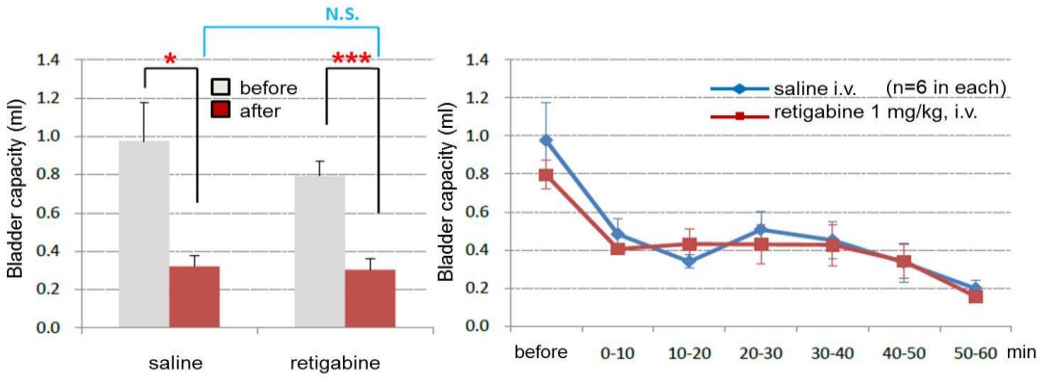

Figure 3. Effects of intravesical instillation of acrolein on bladder capacity for 60 minutes (left) and for each 10-minutes period (right) in saline- or retibabine-administrations. The values were expressed as mean ± SEM. $^{*}P<0.05$, $^{***}P<0.001$: significant differences before and after drug-administrations (paired Student's *t*-test).

References

1. Brown DA, Passmore GM. Neural KCNQ (Kv7) channels..Br J Pharmacol. 2009;156(8):1185-95.
2. Svalø J, Hansen HH, Rønn LC, et al. K(v) 7 Positive Modulators Reduce Detrusor Overactivity and Increase Bladder Capacity in Rats.Basic Clin Pharmacol Toxicol. 2011 Sep 6. doi: 10.1111/j.1742-7843.2011.00765.x.
3. Saitoh C, Chancellor MB, de Groat WC, et al. Effects of intravesical instillation of resiniferatoxin on bladder function and nociceptive behavior in freely moving, conscious rats. J Urol. 2008;179(1):359-64.

Disclosures

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