

## SPINAL GLYCINE TRANSPORTER-1 INHIBITION INFLUENCES THE MICTURITION REFLEX IN URETHANE-ANESTHETIZED RATS

### Hypothesis / aims of study

Glycine is an inhibitory neurotransmitter in the central nervous system, including neural pathways controlling the lower urinary tract (1), and higher concentrations of glycine are found in the spinal cord compared with supraspinal regions (2). So far, two types of glycine transporters (GlyTs), GlyT-1 and GlyT-2, have been cloned (3). However, it is not known whether GlyTs play an important role in the modulation of the micturition reflex. The aim of this study is to investigate the effects of a selective GlyT-1 inhibitor that can increase endogenous glycine concentration on the micturition reflex in urethane anesthetized rats.

### Study design, materials and methods

A total of 48 adult female Sprague-Dawley rats weighing 232-265 g were used in this study. Rats were anesthetized with isoflurane followed by urethane (1.2 g/kg, administered subcutaneously). Thereafter the abdomen was opened by a midline incision and a PE-60 polyethylene catheter was implanted into the bladder through the bladder dome. The catheter was connected via a three-way stopcock to a pressure transducer and a pump for continuous saline infusion. Continuous cystometrograms (CMG, 0.04ml/min) were performed in two groups of urethane-anesthetized rats. 24 rats were administered intrathecal ALX5407, a selective GlyT-1 inhibitor, via an PE-10 intrathecal catheters, which were implanted at Th11 via an incision in the dura under isoflurane anesthesia 3 days before the experiments. For the experiment, firstly saline was continuously infused for 2 hours to evaluate bladder activity during a control period. Then, ALX5407 (1, 3, 10 and 30 µg, n=6 per dose) was administered intrathecally to evaluate changes in bladder activity. The catheter was directed caudally into the spinal subarachnoid space and positioned at the level of the L6-S1 spinal cord. The volume of fluid in the catheter was kept constant at 6 µl. Single doses of drugs were then administered in a volume of 2 µl, followed by a 7 µl flush with saline. In the other group of 24 rats, ALX5407 (1, 3, 10 and 30 µg, n=6 per dose) was administered intracerebroventricularly. Using a stereotaxic micro-injector, a 30 gauge needle attached to a 10 µl Hamilton syringe was inserted into the lateral ventricle, and single doses of drugs were administered in a volume of 2 µl during 2 minutes. Cystometric parameters were recorded and compared before and after drug administration. All data values are expressed as the mean ± standard error. Statistical significance was determined with one-way ANOVA with p<0.05 considered to be statistically significant.

### Results

Intrathecal administration of ALX5407 at 1, 3, 10 and 30 µg increased intercontraction intervals at doses of 3 µg or higher in a dose-dependent fashion to 101.8 ± 5.7%, 122.1 ± 7.6%, 135.7 ± 7.8% and 156.9 ± 16.7% of the control value, respectively (at 3, 10 and 30 µg, p<0.01). These inhibitory effects were observed immediately after administration. Intrathecal administration of ALX5407 at 1, 3, 10 and 30 µg also increased threshold pressure at doses of 3 µg or higher in a dose-dependent fashion to 8.14 ± 0.77 cm H<sub>2</sub>O, 9.98 ± 0.79 cm H<sub>2</sub>O, 13.47 ± 0.91 cm H<sub>2</sub>O and 16.93 ± 1.83 cm H<sub>2</sub>O, respectively (at 3, 10 and 30 µg, p<0.01). However, when ALX5407 was administered intracerebroventricularly, there were no significant changes in intercontraction intervals, threshold pressure, maximum pressure or basal pressure or post void residual at any doses tested.

### Interpretation of results

In urethane-anesthetized rats, suppression of GlyT-1 by intrathecally administered ALX5407 has an inhibitory effect on the micturition reflex, as shown by the observed increases in intercontraction intervals and threshold pressure. The main function of ALX5407 seems to be mediated by modulation of afferent activity, rather than efferent or smooth muscle activity, because ALX5407 induced increases in intercontraction intervals and threshold pressure without affecting maximum pressure or basal pressure. Moreover, ALX5407 administered intracerebroventricularly in the present study failed to affect the micturition reflex. We postulate that the site of action may be the spinal cord.

### Concluding message

The results of our study indicate that GlyT-1 plays an important role in the modulation of micturition. Furthermore, these findings indicate that in urethane-anesthetized rats suppression of GlyT-1 can inhibit the micturition reflex at the spinal site. Thus, GlyT-1 could be a potential target for the treatment of bladder dysfunction such as overactive bladder.

### References

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<b>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</b>	Yes
<b>Name of ethics committee</b>	Tottori University Institutional Animal Care and Use Committee