

INHIBITION OF GLYCINE TRANSPORTER-2 (GLYT-2) IN THE SPINAL CORD AMELIORATES BLADDER OVERACTIVITY AND PAIN SENSATION IN RATS

Hypothesis / aims of study

Interstitial cystitis/bladder pain syndrome (IC/BPS) is one of the refractory diseases with unmet medical needs so that an innovative therapeutic strategy is in great demand. Spinal glycinergic neurons as well as GABAergic neurons are well known to be major inhibitory pathways against the nociceptive processing in peripheral inflammation and chronic pain situations [1]. Glycine is also well characterized to exert an inhibitory effect on the micturition reflex in the spinal cord level [2]. Therefore, enhancing glycinergic pathways in the spinal cord may be one of the strategic candidates for the treatment of overactive bladder (OAB) and IC/BPS. Since glycine transporters (GlyTs) play a pivotal role in the clearance of glycine in the synaptic terminals and/or glial cells in the CNS including the spinal cord [3], we hypothesized that GlyT inhibitors could ameliorate OAB and/or nociceptive processing in the organs relevant to the lower urinary tract. In this study, we investigated the effects of GlyT inhibitors on bladder activity in cystitis rats treated with cyclophosphamide (CYP) and on nociceptive behavior induced by intravesical application of resiniferatoxin (RTx). Also, we analyzed the expression levels of mRNA of GlyTs and glycine receptor (GlyR) subunits in sham or CYP-treated rats, using real-time reverse transcription PCR.

Study design, materials and methods

Female Sprague-Dawley rats (200-263gm) were used.

(1) Cyclophosphamide (CYP; 200 mg/kg, i.p) was given to rats 48hrs before cystometry. Continuous cystometry during saline infusion (0.04ml per min) was performed under urethane anesthesia (1.2 g/kg, s.c.). After stable cystometrograms (CMGs) were obtained, sacrocinine (a selective GlyT-1 inhibitor) and ALX-1393 (a selective GlyT-2 inhibitor) were administered intrathecally (at level of L6-S1 spinal cord), and then the changes of CMG parameters (intercontraction interval: ICI, maximum voiding pressure: MVP, basal pressure: BP, and threshold pressure: TP) were compared with the vehicle control groups. Also, in order to determine whether the effect was mediated by activation of GlyRs, strychnine (a GlyR antagonist; 10µg) was administered intrathecally after the administration of the GlyT inhibitor.

(2) In pain behavioral study, 3µM RTx (0.3ml for 1min) was injected into rat bladders using a urethral catheter and then, nociceptive behaviors, such as licking (lower abdominal licking) and freezing (motionless head turning) were scored every 5sec for 15min. The GlyT inhibitor was administered intrathecally 15min before application of RTx and the responses were compared with the vehicle control group.

(3) The spinal cord (a dorsal half containing the dorsal horn) at the level of L6-S1 from CYP-treated and sham rats, and the forebrain from sham rats were obtained. The expression levels of mRNA of GlyTs (GlyT1 and GlyT2) and GlyR subunits (alpha1, alpha2, alpha3, and beta) were quantified by real-time reverse transcription PCR. The expression levels were normalized to that of constitutive 18S ribosomal RNA.

Results

(1) CYP-treated rats showed a significant reduction in ICI compared to sham rats (345±29.7 vs 847±52.2 s). Sacrocinine (250 µg, i.t.) did not induce significant changes in any CMG parameters in either sham or cystitis rats though there was a tendency to increase ICI only in CYP-treated rats (p=0.0535). On the other hand, ALX-1393 at a dose of 10 µg (i.t) showed a significant increase in TP (137±10.4%) with a tendency of increases in ICI and TP in sham rats. In CYP-treated rats, ALX-1393 at a lower dose (3 µg, i.t.) clearly increased both ICI (144±10.5%) (p<0.05) and TP (119±4.48%) (p<0.01) compared to the predrug values. Moreover, ALX-1393 at a higher dose of 10 µg strongly suppressed the micturition reflex as shown by a greater increase in TP and/or appearance of overflow incontinence in CYP-treated rats. In the antagonist study, the inhibitory effect of ALX-1393 (3 µg, i.t) on ICI and TP were significantly reversed by the treatment with strychnine (p<0.05 and p<0.01, respectively).

(2) Freezing and licking behavior induced by RTx, which were known to be pelvic nerve- and pudendal nerve-dependent, respectively, were significantly suppressed by ALX-1393 at a dose of 10 µg (i.t.).

(3) In sham rats, GlyT2 mRNA was expressed much higher (23.0-fold) than GlyT1 mRNA in the dorsal spinal cord (L6-S1), whereas GlyT2 mRNA was expressed much less (13.9-fold) than GlyT1 mRNA in the forebrain. In CYP-treated rats, the expression levels of GlyT2 mRNAs in the dorsal spinal cord (L6-S1) were significantly reduced compared to those in sham rats (p<0.01). The significant reductions of mRNA levels of some of the GlyR subunits (alpha1 and beta) were also observed (p<0.05).

Interpretation of results

These results indicate that GlyT-2 plays a major role in the clearance of glycine in the spinal synaptic terminals and that the GlyT-2 inhibition leads to amelioration of bladder activity in CYP-treated rats and nociceptive behavior in RTx-treated rats. The reduction of GlyTs mRNA shown in CYP-treated rats might indicate a certain biological defense mechanism of glycinergic neurotransmission against excitatory synaptic transmissions in the cystitis condition.

Concluding message

These new findings suggest an important role of spinal glycinergic pathways in the suppression of bladder overactivity and pain sensation. Therefore, GlyT-2 inhibitors have a new therapeutic potential for the treatment of OAB and IC/PBS.

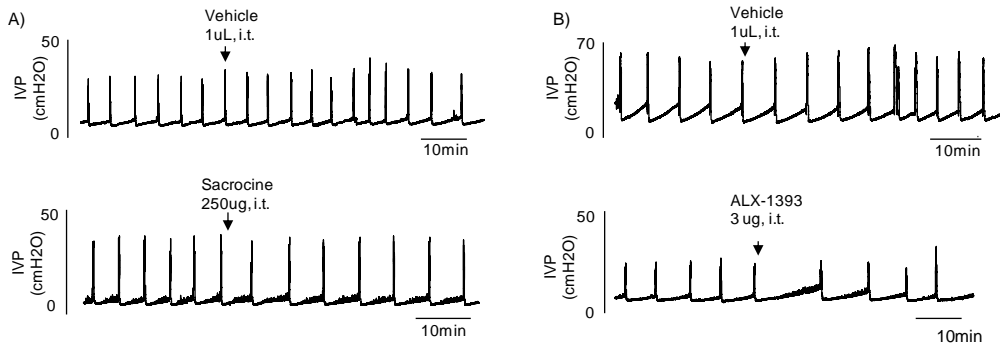


Figure 1. The effects of intrathecal administration of GlyT-1 inhibitor (A) and GlyT-2 inhibitor (B) on CMGs in rats treated with cyclophosphamide.

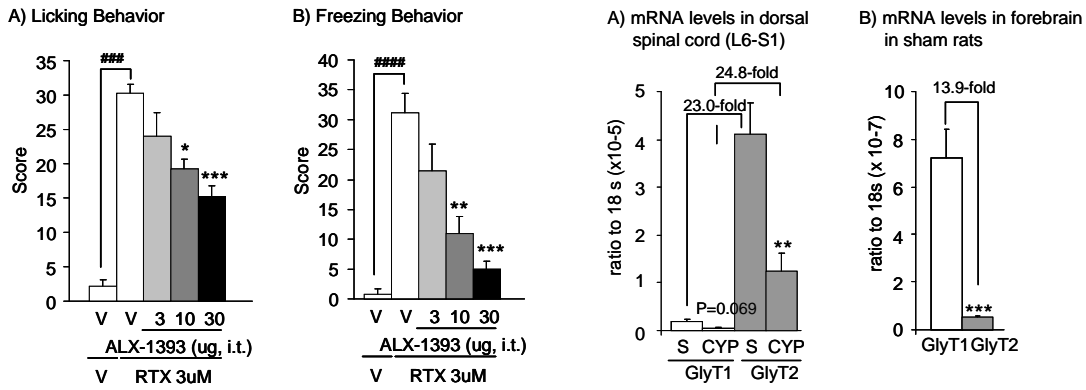


Figure 2 The effects of GlyT-2 inhibitor (i.t.) on nociceptive behaviors, licking (A) and freezing (B), in rats treated intravesically with RTX . N=5.

Figure 3 Relative expression levels of GlyTs mRNA in the dorsal spinal cord (L6-S1) in sham/CYP treated rats (A) and the forebrain in sham rats (B). N=5.

References

1. Cell Mol Life Sci 62: 2027-35, 2005.
2. Exp Neurol 183: 232-40, 2003.
3. Pharmacol Ther 123; 54-79, 2009.

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