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Oguchi T[1], Yokoyama H[1], Funahashi Y[1], Nishizawa O[2], Goins W F[1], Goss J R[1], Glorioso J C[1], Yoshimura N[1]
1. *University of Pittsburgh,* ***2.*** *Urology, Shinshu University*

EFFECT OF HERPES SIMPLEX VIRUS VECTOR-MEDIATED GLYCINE RECEPTOR GENE THERAPY ON BLADDER OVERACTIVITY AND NOCICEPTION

Hypothesis / aims of study

Bladder pain syndrome/interstitial cystitis (BPS/IC) is a serious disease whose main symptoms are bladder pain and frequent urination. Glycine is an inhibitory neurotransmitter which can affect bladder function [1]. We examined the effects of gene transfer of glycine receptors (GlyR) using replication-deficient herpes simplex virus (HSV) vectors [2] on bladder overactivity and pain behavior induced by intravesical application of resiniferatoxin (RTx) in rats.

Study design, materials and methods

Female Sprague-Dawley rats were used, and 20 µl of viral suspension of either GlyR (6.8×10^{10} pfu/ml) or control vector (E1G6) (7.1×10^{8} pfu/ml) was injected to the bladder wall under anesthesia. GlyR vectors also expressed Red Fluorescent Protein (RFP) as a marker protein. (1) The bladder and L6 dorsal root ganglia (DRG) were harvested for immunohistochemical analysis 2 weeks after viral injection. (2) Rats were divided into 4 groups, which were treated with control vector, GlyR, control vector with glycine, or GlyR with glycine. In the glycine administration groups, an osmotic pump (0.25 µl/h) filled with 200 µl of 0.9% glycine was placed in the abdominal cavity at the same time of viral injection to administer glycine continuously. Two weeks later, cystometry (n=7-10) was performed under urethane anethsesia. After 2 hours of saline infusion, 10nM RTx was infused to the bladder to induce bladder overactivity. Intercontraction intervals (ICI) were recorded, and the reduction rate of ICI was calculated. (3) Rats were divided into 2 groups; control virus and GlyR groups. In an awake condition, nociceptive behaviors such as lower abdominal licking (licking) and motionless head-turning (freezing) induced by 3 µM RTx intravesical administration (0.3ml for 1min) through a urethral catheter were scored every 5 sec for 15 min (n=7-8). Student t-test was used for statistic analysis. $P<0.05$ was considered as significant.

Results

(1) RFP positive cells were observed in both L6 DRG (Fig.1) and bladder of GlyR injected rats. (2) Virus (control or GlyR) injection without glycine administration did not alter the ICI reduction rate after RTx intravesical infusion. Both control virus and GlyR group given with glycine also showed a significant reduction in ICI, but GlyR virus-injected rats with 0.9% glycine showed a smaller reduction rate (67.8 ± 3.8% vs. 46.9 ± 5.9%, $p<0.05$) (Fig.2). (3) GlyR-treated rats showed a significant reduction in licking (45.4 ± 5.0 vs. 66.1 ±7.8, $p<0.05$) and freezing behavior (17.3 ± 4.1 vs. 34.3 ± 3.5, $p<0.01$) compared to E1G6 rats (Fig.3).

Figure.1

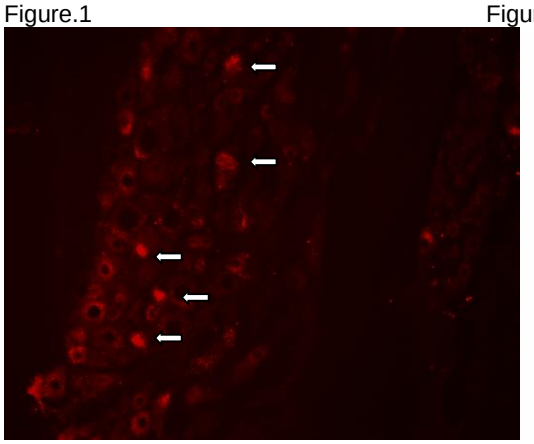
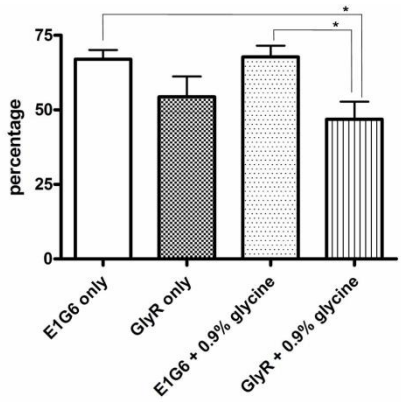


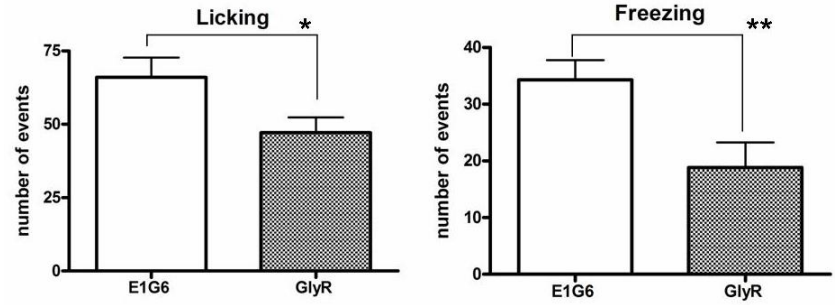
Figure.2



(Fig.1) Photomicrograph of a L6 DRG section from a HSV-GlyR-treated rat. RFP positive cells (arrows) were confirmed in the L6 DRG section (x100).

(Fig.2) ICI reduction percentage [(pre ICI- post ICI)/pre ICI x100] was significantly decreased in HSV-GlyR-treated rats with glycine application (*p<0.05).

Figure.3



(Fig. 3) Both licking and freezing behaviors were reduced in GlyR rats (*p<0.05, **p<0.01).

Interpretation of results

HSV-GlyR vectors injected into the rat bladder wall were transported to L6 DRG through bladder afferent pathways. Glycine administration in HSV-GlyR-treated rats, but not in control virus-treated rats, significantly suppressed RTx-induced bladder overactivity, indicating that activation of glycine receptor upregulated in bladder afferent pathways by exogenous glycine administration can reduce bladder overactivity induced by bladder nociceptive stimuli. In addition, bladder and urethral pain behavior was suppressed without glycine administration in HSV-GlyR-treated rats, suggesting that increased expression of glycine receptors in bladder afferent pathways by HSV-GlyR vector treatment can enhance endogenous glycinergic inhibitory mechanisms to suppress pain sensation.

Concluding message

The GlyR vector inoculation to the bladder wall intensifies the exogenous and endogenous glycine-mediated therapeutic effects on bladder overactivity and nociception, respectively, in rats with RTx-induced cystitis. Thus, HSV vector-mediated glycine receptor (GlyR) expression in bladder sensory pathways with glycine administration might be effective for treating BPS/IC symptoms such as bladder pain and frequent urination. In addition, systemic administration of inhibitory transmitters such as glycine in combination with local HSV gene therapy of their receptors could enhance the drug-receptor interaction in HSV-infected target organs and their afferent pathways, and avoid systemic side effects.

References

1. J Urol. 2005; 173: 314–317
2. Mol Ther. 2011: 19, 500-506

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Institutional Animal Care and Use Committee of University of Pittsburgh**