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Inhibitory effect of intrathecal glycine on the micturition reflex in normal and spinal cord injury rats

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Abstract

We examined the influence of lumbosacral glycinergic neurons on the spinobulbospinal and spinal micturition reflexes. Female rats were divided into intact rats, rats with acute injury to the lower thoracic spinal cord (SCI), and rats with chronic SCI. Under urethane anesthesia, isovolumetric cystometry was performed in each group before and after intrathecal (IT) injection of glycine or strychnine into the lumbosacral cord level. The glutamate and glycine levels of the lumbosacral cord were measured after injection of glycine or strychnine in intact and chronic SCI rats. Expression of strychnine-sensitive glycine receptor alpha-1 (GlyR α 1) mRNA in the lumbosacral cord was also assessed in both rats. In chronic SCI rats, the interval and amplitude of bladder contractions were shorter and smaller when compared with intact rats. IT glycine (0.1–100 μ g) prolonged the interval and decreased the amplitude of bladder contractions in both intact rats and chronic SCI rats. IT strychnine (0.01–10 μ g) elevated the baseline pressure in intact rats and induced bladder contraction in acute SCI rats. On amino acid analysis, IT glycine (0.01–100 μ g) decreased the glutamate level of the lumbosacral cord in intact rats, but not in chronic SCI rats. The glycine level of the lumbosacral cord was 54% lower in chronic SCI rats when compared with intact rats, while the GlyR α 1 mRNA level did not change after SCI. These results suggest that glycinergic neurons may have an important inhibitory effect on the spinobulbospinal and spinal micturition reflexes at the level of the lumbosacral cord.

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Keywords: Glutamate; Strychnine; Lumbosacral cord; Isovolumetric cystometry; Glycine receptor alpha-1 (GlyR α 1)

Introduction

In the central nervous system, some amino acids are known to be important neurotransmitters (Shapiro, 1997). For example, glutamate is a major excitatory neurotransmitter in both the upper and the lower central nervous system, and it facilitates the micturition reflex (Mayer and Westbrook, 1987). On the other hand, γ -aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system, and it inhibits the micturition reflex at the level of the lumbosacral cord (Igawa et al., 1993). Glycine is another important inhibitory neurotransmitter (Daly and

Aprison, 1983), and higher concentrations of glycine are found in the spinal cord than in supraspinal regions (Aprison et al., 1969; Elekes et al., 1986; Hall et al., 1976; Shaw and Heine, 1965). Simpson et al. (1995) have shown that intrathecal injection of glycine or a glycine agonist can reduce spasticity in rabbits with spinal cord ischemia, while injection of strychnine (a glycine receptor antagonist) increases muscle tone. They have also shown that intrathecal injection of glycine can reduce neuropathic pain in a rat model, while strychnine causes an increase in pain (Simpson et al., 1996). However, the influence of glycine on the micturition reflex has attracted little attention.

Micturition is mediated by the spinobulbospinal reflex pathway that passes through the pontine micturition center (Blok et al., 1995; Mallory et al., 1989; Noto et al., 1991). The peripheral afferent and efferent limbs of this reflex are travel via the pelvic nerves arising from the lumbosacral

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cord (de Groat et al., 1992). When the upper spinal cord is transected, a previously masked spinal micturition reflex gradually becomes manifest (de Groat, 1995; Yoshiyama et al., 1999). In our previous study, the micturition reflex disappeared immediately after transection of the thoracic cord in rats, and the glycine level in the lumbosacral cord showed a transient increase (Nishijima et al., 2001; Sugaya et al., 2000). However, the micturition reflex reappeared after 2 weeks and the glycine level of the lumbosacral cord decreased at this time. These findings suggested that glycine is likely to be involved in the mechanism of the micturition reflex. In order to clarify the influence of glycinergic neurons on the micturition reflex, and to explore the relationship between glutamatergic and glycinergic neurons, we examined the effects of intrathecal injection of glycine or strychnine on bladder activity and on the glutamate and glycine levels in the lumbosacral cord of rats with or without spinal cord injury (SCI). We also investigated the expression of strychnine-sensitive glycine receptor alpha-1 (GlyR α 1) mRNA in the lumbosacral cord.

Materials and methods

Animal model

One hundred and thirty female Sprague-Dawley rats weighing 250–300 g were used in this study. The rats were divided into the following three groups: (1) an intact group ($n = 80$), (2) an acute SCI group that was studied at 1 day after SCI ($n = 4$), and (3) a chronic SCI group that was studied at 4 weeks after SCI ($n = 46$). Rats in the SCI groups were anesthetized with 2% halothane and laminectomy was performed at the lower thoracic level. The dura was opened and the spinal cord was completely transected at T9 or T10 under direct vision. The animals were treated with an antibiotic (ampicillin: 100 mg/day intramuscularly for 2 days) to prevent infection. Postoperatively, the bladder was managed by expressing urine manually until 2 weeks after SCI. The protocol in this study was approved by Institutional Animal Care and Use Committee, University of the Ryukyus.

Intrathecal injection of glycine or strychnine during isovolumetric cystometry

Isovolumetric cystometry was performed in intact rats ($n = 14$), acute SCI rats ($n = 4$), and chronic SCI rats ($n = 8$). The rats were anesthetized by intraperitoneal and subcutaneous injection of urethane (1.2 g/kg for intact rats vs 0.6 g/kg for SCI rats), and a polyethylene catheter (PE-50) was inserted into the bladder through the urethra and the residual urine was removed. Then mineral oil (0.2 ml) was infused via the urethral catheter in order to prevent the absorption of intravesical fluid by the bladder wall (Sugaya et al., 1997). The urethra was ligated to the catheter near the external

urethral meatus to produce isovolumetric conditions in the bladder. After a lower abdominal incision was made, the ureters were transected at the level of the aortic bifurcation, and the distal ends were ligated. Laminectomy was performed at the level of the 3rd lumbar vertebra, and a catheter (PE-50) was inserted into the subarachnoid space to allow intrathecal injection. The tip of the catheter was advanced to the level of the sacral cord, with the length of the inserted catheter corresponding to the length of a microsyringe needle (10 μ l; Hamilton, Reno, NV). Bladder activity was monitored via the urethral catheter that was connected to a pressure transducer and a saline-infusion pump through a three-way stopcock. The bladder was filled with physiological saline (0.05 ml/min) to above the threshold volume, inducing rhythmic isovolumetric contractions. After the bladder contractions had become stable for over 30 min, glycine (0.001–100 μ g, $n = 5$ –7 per dose) or strychnine (0.001–10 μ g, $n = 5$ –7 per dose) was injected cumulatively every 15–30 min into the intrathecal catheter in intact rats, and the changes of bladder activity were recorded. In chronic SCI rats, glycine (0.001–100 μ g, $n = 5$ –6 per dose) was also injected intrathecally every 15–30 min. In 2 of the chronic SCI rats, strychnine (0.001–100 μ g) was additionally injected intrathecally every 15–30 min, in order to confirm whether changes of bladder contraction occurred like those seen in intact rats. In acute SCI rats, bladder contraction did not occur, so it was filled with physiological saline to the residual urine volume plus 0.5–1.5 ml and then intrathecal injection of strychnine (30–100 μ g, $n = 4$) was performed to confirm whether an increased glycine level in the lumbosacral cord could inhibit the micturition reflex. Glycine and strychnine were dissolved in physiological saline before injection at a volume of 5–10 μ l, and the intrathecal catheter was clamped for 1 min after each injection. The interval, amplitude, and duration of isovolumetric bladder contraction were evaluated, as well as the baseline pressure. These parameters were calculated and averaged for 15–30 min from the start of intrathecal injection of glycine or strychnine, and the results were compared with the control recordings obtained for 30 min before drug injection.

Measurement of glutamate and glycine levels in the lumbosacral cord after intrathecal injection of glycine or strychnine in intact and chronic SCI rats

Under urethane anesthesia, laminectomy was performed at the 3rd lumbar vertebra in intact rats ($n = 56$) and chronic SCI rats ($n = 28$). Intrathecal injection of glycine (0.001–100 μ g, $n = 4$ per dose) in intact and chronic SCI rats and intrathecal injection of strychnine (0.001–100 μ g, $n = 4$ per dose) in intact rats were performed. At 10 min after intrathecal injection, the rats were sacrificed, and the lumbosacral cord was removed immediately. The lumbosacral cords were also collected from intact rats ($n = 4$) and chronic SCI

rats ($n = 4$) under urethane anesthesia and they were used as controls.

Preparation of samples

The lumbosacral cord was separately homogenized in cold 0.5 M hydrochloric acid (1.0 ml/0.1 g tissue), and the homogenate was centrifuged at 15,000 rpm for 5 min. The supernatant was dechlorinated and was deproteinized by an Ultrafree C3 THK filter (Millipore, Bedford, MA). All samples were immediately stored -80°C prior to assay.

Amino acid determination by capillary electrophoresis

The amino acid levels in each sample were measured by a capillary electrophoresis system (Hewlett-Packard ^3DCE , Germany) (Nishijima et al., 2002; Soga and Ross, 1999) using a basic anion buffer. Samples were injected at 50 mbar for 4 s and were separated at a constant voltage of -30 kV at 30°C .

Quantification of GlyR $\alpha 1$ mRNA

Total RNA was extracted from the lumbosacral cords of intact ($n = 10$) and chronic SCI rats ($n = 10$) using a RNeasy Mini Kit (Qiagen, Germany). Total RNA ($0.2 \mu\text{g}$) was dissolved in ribonuclease-free water, mixed with 1X RT buffer, 1 mM dNTP, 1 μM oligo dT primer, 10 units of RNase inhibitor, and 1 unit of omniscript reverse transcriptase in a total volume of 20 μl , followed by incubation for 1 h at 37°C to synthesize complementary DNA (cDNA). Reverse transcription was terminated by heating for 5 min at 95°C , and the resulting cDNA was chilled on ice. The amount of GlyR $\alpha 1$ mRNA in each lumbosacral cord was quantified using a real-time polymerase chain reaction (PCR) SYBR Green assay (Dhar et al., 2001). Reactions were carried out in a 50- μl reaction volume containing 4 μl each of cDNA, 25 μl of 2X SYBR Green PCR master kit (PE Applied Biosystems, Japan), 1 μM of the forward (5'-CTGGCCTGCCCATGGACCTGA-3') and reverse (5'-CAGAAGGAGATCCAGGACAGGA-3') primers for the GlyR $\alpha 1$ gene (Sergeeva and Haas, 2001), and distilled water. To quantify PCR products and confirm the integrity of the RNA samples, a housekeeping gene (β -actin) was also amplified by the same procedure using forward (5'-TCATGAAGTGTGACGTTGCATCCGT-3') and reverse (5'-CCTAGAAGCTTTGCGGTGCACGATG-3') primers. Forty-five PCR cycles were performed in a GeneAmp 7700 thermocycler (Applied Biosystems, Japan), with each cycle consisting of denaturation for 15 s at 95°C and annealing and extension for 1 min at 60°C . After PCR, the data were analyzed using a GeneAmp detection system (Applied Biosystems, Japan) and GlyR $\alpha 1$ mRNA was quantified as a ratio to the amount of β -actin mRNA.

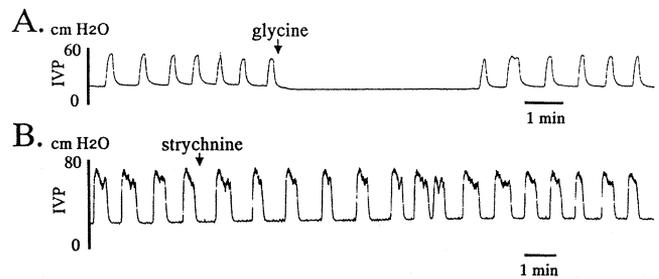


Fig. 1. Isovolumetric cystometry before and after intrathecal injection of 0.1 μg of glycine (A) and strychnine (B) in intact rats. (A) Isovolumetric bladder contractions ceased transiently after intrathecal injection of 0.1 μg of glycine. Eleven minutes later, bladder contractions reappeared without any change of the interval or amplitude. (B) After intrathecal injection of 0.1 μg of strychnine, the baseline pressure gradually increased and the amplitude gradually decreased. In this intact rat, the interval between bladder contractions was transiently prolonged, and the duration was shortened for several minutes after intrathecal injection of strychnine. However, the maximum bladder contraction pressure (baseline pressure plus amplitude) was unchanged. IVP, intravesical pressure.

Statistical analysis

Results are reported as the mean \pm standard error (SE). Student's t test for paired or unpaired data was used for statistical analysis, and significance was defined as $P < 0.05$.

Results

Changes of bladder activity after intrathecal injection of glycine in intact rats ($n = 7$)

The interval (1.97 ± 0.14 min), amplitude (30.4 ± 3.2 cm H_2O), and duration (1.23 ± 0.14 min) of bladder contraction and the baseline pressure (15.7 ± 1.5 cm H_2O) (controls) became stable at an intravesical volume of less than 0.6–1.0 ml from 1 h after the beginning of isovolumetric cystometry. Bladder contractions were transiently abolished immediately after the intrathecal injection of glycine (0.1–100 μg) [Fig. 1A]. The time until the reappearance of bladder contractions after intrathecal injection of glycine was 5.01 ± 1.49 min at a dose of 0.1 μg and 6.11 ± 2.01 min at 100 μg . The interval, amplitude, and duration of the reemerging bladder contractions were unchanged when the dose of glycine was very low (0.1 μg). However, after intrathecal injection of glycine at higher doses (1–100 μg), the interval, amplitude, and duration of the reemerging bladder contractions showed dose-dependent changes. The mean interval between contractions for 15–30 min after injection of glycine (0.1–100 μg) was significantly prolonged (8.63 ± 1.67 min, a 338% increase at 100 μg of glycine, $P = 0.0012$) [Fig. 2A]. Intrathecal injection of glycine (1–100 μg) also significantly decreased the ampli-

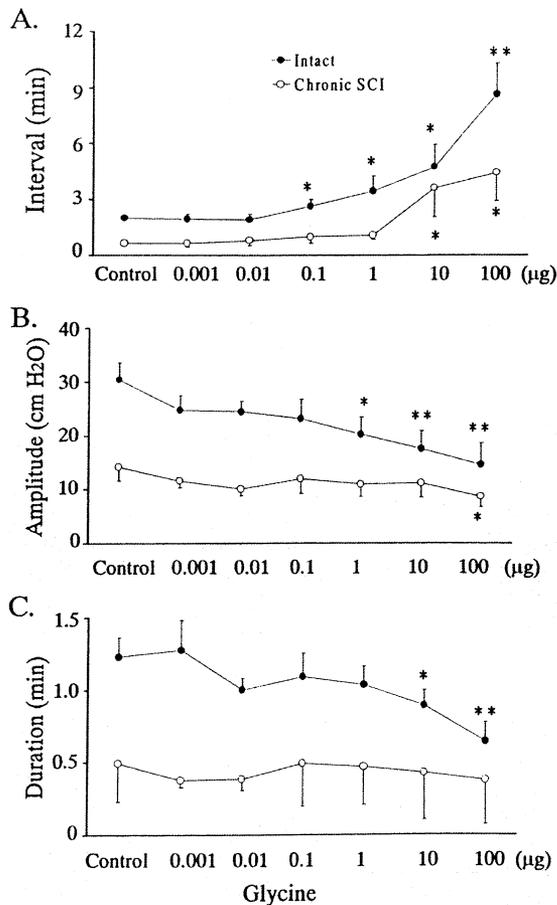


Fig. 2. Effect of intrathecal glycine (0.001–100 μg) on the interval, amplitude, and duration of isovolumetric bladder contractions compared with before injection (control) in intact ($n = 7$) and chronic SCI rats ($n = 6$). (A) The interval was significantly and dose dependently prolonged by intrathecal injection of glycine (0.1–100 μg) in intact rats (closed circles). In chronic SCI rats (open circles), the interval was significantly shorter (control) compared with that in intact rats, but it was prolonged after intrathecal injection of higher doses (10–100 μg) of glycine. (B) The amplitude was significantly and dose dependently decreased after intrathecal injection of glycine (1–100 μg) in intact rats (closed circles). In chronic SCI rats (open circles), the amplitude was significantly decreased (control) compared with that in intact rats. Intrathecal injection of the highest dose (100 μg) of glycine significantly decreased the amplitude of bladder contractions. (C) The duration was significantly shortened after intrathecal injection of glycine (10–100 μg) in intact rats (closed circles). In chronic SCI rats (open circles), the duration was significantly shortened (control) compared with that in intact rats, but it did not change after intrathecal injection of glycine. Values are the mean \pm SE. Significant differences from control in intact and chronic SCI rats are indicated by: * $P < 0.05$ and ** $P < 0.01$.

tude of bladder contractions (14.6 ± 4.0 cm H₂O, a 52% decrease at 100 μg of glycine, $P = 0.0035$) [Fig. 2B]. Furthermore, intrathecal injection of glycine at higher doses (10–100 μg) significantly decreased the duration of bladder contractions (0.64 ± 0.13 min, a 48% decrease at 100 μg of glycine, $P = 0.0025$) [Fig. 2C]. The changes of amplitude and duration persisted for 15–30 min until the next injection. The baseline pressure was not changed by intrathecal injection of glycine.

Changes of bladder activity after intrathecal injection of glycine in chronic SCI rats ($n = 6$)

In chronic SCI rats, the interval (0.63 ± 0.13 min), amplitude (14.2 ± 2.5 cm H₂O), and duration (0.49 ± 0.26 min) of bladder contraction were significantly ($P < 0.0001$, $P = 0.0009$, and $P = 0.0001$, respectively) reduced when compared with those in intact rats (Fig. 2), but the baseline pressure (12.3 ± 1.0 cm H₂O) was not different from that in intact rats. Bladder contraction was also transiently abolished immediately after the intrathecal injection of glycine at higher doses (10–100 μg), but contractions reappeared within 10–15 min. For 30 min after the intrathecal injection of glycine (10–100 μg), the mean interval between bladder contractions was significantly prolonged (4.40 ± 1.49 min, a 597% increase at 100 μg of glycine, $P = 0.0140$) [Fig. 2A]. Intrathecal injection of the highest dose of glycine (100 μg) also significantly decreased the amplitude of bladder contractions (8.6 ± 1.9 cm H₂O, a 39% decrease, $P = 0.0479$) [Fig. 2B]. However, the duration of contractions and the baseline pressure were not affected by intrathecal glycine (Fig. 2C).

Changes of bladder activity after intrathecal injection of strychnine in intact rats ($n = 7$)

Intrathecal injection of strychnine (0.01–10 μg) significantly elevated the baseline pressure (32.2 ± 4.2 cm H₂O, a 119% increase at 10 μg of strychnine, $P = 0.0025$) [Figs. 1B and 3A]. Along with elevation of the baseline pressure by intrathecal strychnine (0.01–10 μg), the amplitude of contractions was significantly decreased (16.5 ± 3.5 cm H₂O, a 60% decrease at 10 μg of strychnine, $P = 0.0009$) [Fig. 3B], so the maximum bladder contraction pressure (baseline pressure plus amplitude) did not change after intrathecal injection of strychnine. In some rats, the duration of the first bladder contraction was prolonged after intrathecal injection of strychnine, and the interval was shortened for a few minutes. However, the mean interval and duration of the contractions were not affected until a 10-μg dose of strychnine was injected. When strychnine was injected intrathecally at doses of 30–100 μg, bladder contractions disappeared and convulsions occurred.

Changes of bladder activity after intrathecal injection of strychnine in acute SCI rats ($n = 4$)

Flaccid paralysis and urinary retention were present from the time of SCI. Cystometry demonstrated the disappearance of bladder contraction. With an intravesical volume of 1.5–2.5 ml and a baseline pressure of 10.2–13.0 cm H₂O, however, intrathecal injection of strychnine (30–100 μg) induced bladder contractions with a small amplitude (10.1–12.3 cm H₂O) and short duration (0.71–0.82 min) [Fig. 4A]. The baseline pressure was not affected by intrathecal injection of strychnine (30–100 μg), but convulsions occurred.

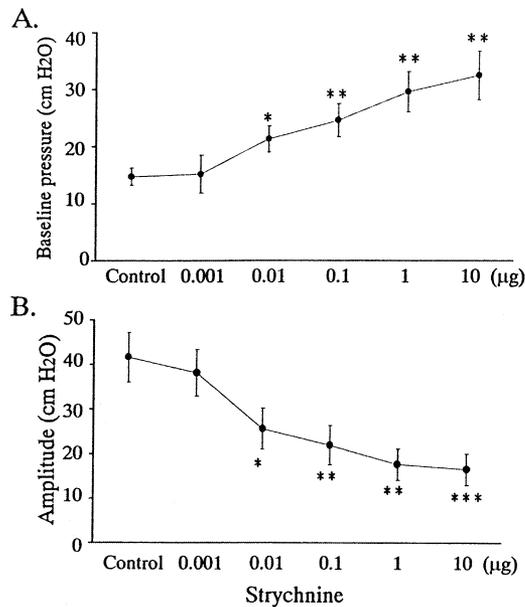


Fig. 3. Effect of intrathecal injection of strychnine (0.001–10 μg) on the baseline pressure and amplitude of isovolumetric bladder contractions compared with before injection (control) in intact rats ($n = 7$). (A) The baseline pressure was significantly and dose dependently elevated after intrathecal injection of strychnine (0.01–10 μg). (B) The amplitude was significantly and dose dependently decreased after intrathecal injection of strychnine (0.01–10 μg). Values are the means \pm SE. Significant differences from control are indicated by: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Changes of bladder activity after intrathecal injection of strychnine in chronic SCI rats ($n = 2$)

The interval, amplitude, and duration of bladder contraction were not altered by intrathecal injection of strychnine at low doses (0.001–10 μg), but the highest dose of strychnine (100 μg) elevated the baseline pressure (6.0–10.0 cm H₂O, a 33–67% increase) and induced convulsions. Bladder contractions disappeared about 10 min after intrathecal injection of 100 μg of strychnine. Under these conditions, additional intrathecal injection of 30 μg of glycine induced frequent bladder contractions (interval, 0.67–0.76 min; amplitude, 24.3–25.2 cm H₂O; duration, 0.52–0.75 min) [Fig. 4B].

Changes of the glutamate and glycine levels in the lumbosacral cord after intrathecal injection of glycine ($n = 28$) or strychnine ($n = 28$) in intact rats

In control rats, the glutamate and glycine levels of the lumbosacral cord were 4.48 ± 0.14 and 1.48 ± 0.13 μmol/g, respectively. Intrathecal injection of glycine (0.01–100 μg) caused a slight, but significant, decrease of the glutamate level (3.59 ± 0.15 μmol/g, a 20% decrease at 100 μg of glycine, $P = 0.0462$) in the lumbosacral cord (Fig. 5).

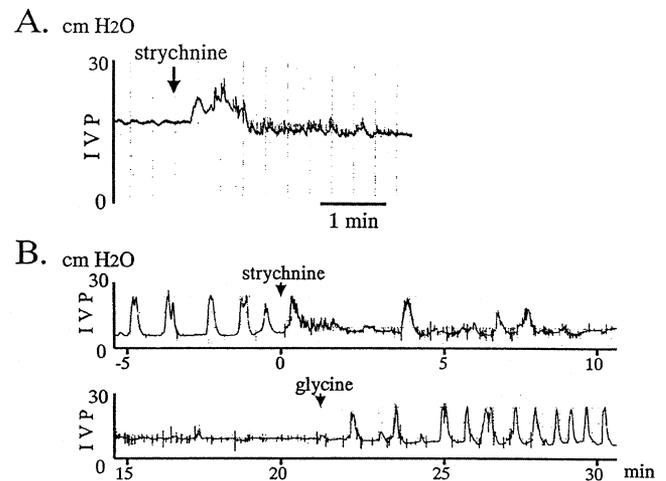


Fig. 4. Isovolumetric cystometry after intrathecal injection of strychnine in an acute SCI rat (A), and effect of intrathecal injection of glycine after intrathecal injection of strychnine in a chronic SCI rat (B). (A) A bladder contraction with a small amplitude (11.7 cm H₂O) and a short duration (0.77 min) appeared after intrathecal injection of strychnine (30 μg) in the acute SCI rat. (B) In the chronic SCI rat, the baseline pressure was elevated (6.0–10.0 cm H₂O) by intrathecal injection of strychnine (100 μg). The interval, amplitude, and duration of bladder contractions became unstable transiently, and bladder contractions disappeared about 10 min after intrathecal injection of strychnine. In this state, addition of intrathecal glycine (30 μg) evoked frequent bladder contractions. However, the amplitude (25.2 cm H₂O) was same as before intrathecal injection of strychnine. Frequent and small amplitude spikes on the cystometrograms (A and B) after intrathecal injection of strychnine were artifacts due to convulsions. IVP, intravesical pressure.

In contrast, intrathecal injection of strychnine (0.001–100 μg) did not influence the glutamate or glycine levels in the lumbosacral cord.

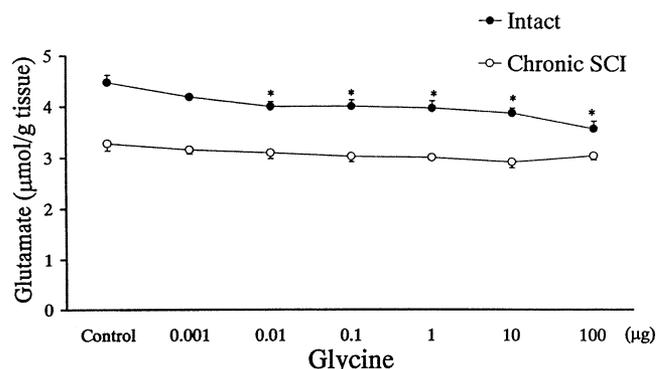


Fig. 5. Changes in the glutamate level in the lumbosacral cord of intact ($n = 28$) and chronic SCI rats ($n = 28$) after intrathecal injection of glycine (0.001–100 μg, $n = 4$ per dose) compared with control rats ($n = 4$). In intact rats (closed circles), the glutamate level was slightly, but significantly, decreased after intrathecal injection of glycine (0.01–100 μg). In chronic SCI rats (open circles), the glutamate level did not change after intrathecal injection of glycine. Values are the means \pm SE. Significant differences from control in intact and chronic SCI rats are indicated by: * $P < 0.05$.

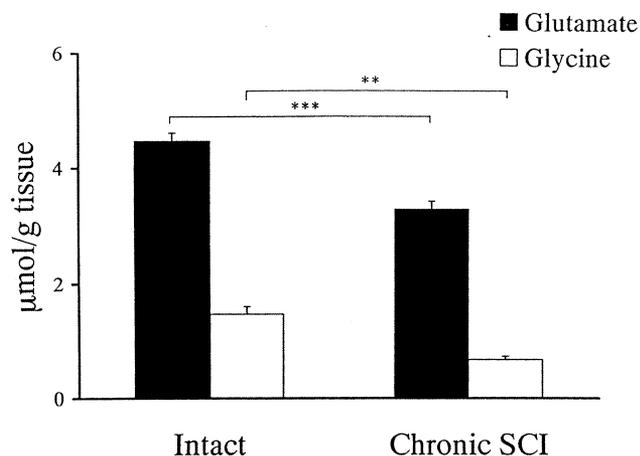


Fig. 6. Glutamate and glycine levels in the lumbo-sacral cord of intact ($n = 4$) and chronic SCI rats ($n = 4$). The glutamate level (closed bars) and glycine level (open bars) were significantly decreased in chronic SCI rats compared with intact rats. Values are the means \pm SE. Significant differences between intact and chronic SCI rats are indicated by: ** $P < 0.01$, and *** $P < 0.001$.

Changes of the glutamate and glycine levels in the lumbo-sacral cord after SCI and after intrathecal injection of glycine ($n = 28$) in chronic SCI rats

In chronic SCI rats, the glutamate ($3.29 \pm 0.14 \mu\text{mol/g}$) and glycine levels ($0.68 \pm 0.06 \mu\text{mol/g}$) of the lumbo-sacral cord were significantly ($P = 0.0002$ and $P = 0.0021$, respectively) lower (decreased by 27 and 54%, respectively) compared with those in intact rats (Fig. 6). The glutamate/glycine ratio (4.66 ± 0.32) was significantly ($P = 0.0141$) increased compared with that (3.14 ± 0.34) in intact rats. However, intrathecal injections of glycine (0.001 – $100 \mu\text{g}$) did not affect the glutamate level in the lumbo-sacral cord (Fig. 5).

Changes of the GlyR $\alpha 1$ mRNA level in the lumbo-sacral cord after SCI ($n = 20$) [Fig. 7]

In intact rats ($n = 10$), the β -actin mRNA and GlyR $\alpha 1$ mRNA levels of the lumbo-sacral cord were $0.36 \pm 0.09 \text{ ng/mg}$ and $1.66 \pm 0.20 \text{ pg/mg}$, respectively. The GlyR $\alpha 1$ mRNA/ β -actin mRNA ratio was 0.007 ± 0.003 . In chronic SCI rats ($n = 10$), the β -actin mRNA ($0.36 \pm 0.23 \text{ ng/mg}$) and GlyR $\alpha 1$ mRNA levels ($2.38 \pm 1.52 \text{ pg/mg}$) in the lumbo-sacral cord were not different from those in intact rats. The GlyR $\alpha 1$ mRNA/ β -actin mRNA ratio (0.009 ± 0.004) in chronic SCI rats was also not different from that in intact rats.

Discussion

It is already known that GABAergic mechanisms in the spinal cord inhibit the micturition reflex (Igawa et al., 1993).

The present study showed that a spinal glycinergic mechanism also caused inhibition of the micturition reflex. In intact rats, the intrathecal injection of glycine at lower and higher doses (0.1 – $100 \mu\text{g}$) transiently abolished bladder contraction, while higher doses of glycine decreased the amplitude (1 – $100 \mu\text{g}$) and duration of bladder contraction (10 – $100 \mu\text{g}$). These results suggest that a spinal glycinergic mechanism inhibits the spinal afferent and efferent limbs of the spinobulbospinal micturition reflex pathway, because the change in the interval of reemerging bladder contractions after the injection of glycine seems to be related to its effect on the afferent limb of the micturition reflex pathway, while the changes of amplitude and duration seem to be based on its effect on the efferent limb of the pathway (Sugaya et al., 2002). However, this glycinergic mechanism may predominantly inhibit the spinal afferent limb, because bladder contraction reappeared without any changes of the interval, amplitude, or duration after a very low dose of glycine ($0.1 \mu\text{g}$). On the other hand, intrathecal injection of strychnine caused the baseline pressure to increase. The baseline pressure normally shows no change when the nerves innervating the bladder are transected (Sugaya and de Groat, 1994a). If the efferent limb of the micturition reflex pathway only receives inhibitory inputs (including those from glycinergic neurons) during the collecting phase, then the blockade of glycinergic inputs by intrathecal injection of strychnine might not change the baseline pressure. However, Sugaya and de Groat (1994b) reported that the percentage change of the baseline intravesical pressure after transection of the dorsal or ventral lumbo-sacral spinal roots in rats depends upon bladder volume (0.35 – 1.8 ml), and that administration of hexamethonium (a ganglion blocker) after transection of these roots decreases baseline pressure. They concluded that the bladder is tonically excited or inhibited by a local reflex pathway and a parasympathetic pathway in the pelvic nerves, depending upon the intravesical volume. Therefore, this effect of strychnine suggests that the parasympathetic preganglionic neurons innervating the bladder may continuously receive both excitatory and inhibitory inputs during the collecting phase.

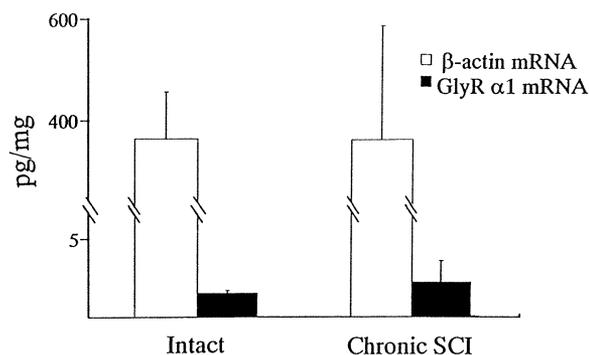


Fig. 7. β -actin and GlyR $\alpha 1$ mRNA levels in the lumbo-sacral cord of intact ($n = 10$) and chronic SCI rats ($n = 10$). The β -actin mRNA level (open bars) and GlyR $\alpha 1$ mRNA level (closed bars) did not change after SCI.

Fontana et al. (2001) reported that activation of glycine receptors inhibits the α -amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA)-evoked release of acetylcholine in cultured spinal cord motoneurons. Kerchner et al. (2001) found that dorsal horn inhibitory neurons express presynaptic kainate receptors, which stimulate glycine release by cultured spinal neurons. Therefore, there may be a close relationship between glutamatergic and glycinergic neurons in the spinal cord. In our previous study, intrathecal injection of glutamate caused an increase of the glycine level in the lumbosacral cord of intact rats, while intrathecal injection of MK-801 (a glutamate receptor antagonist) decreased both the glutamate and glycine levels in the lumbosacral cord of intact and acute SCI rats (Sugaya et al., 2000). These findings suggest that glutamatergic neurons have stimulatory projections to both glutamatergic and glycinergic neurons in the lumbosacral cord. In the present study, intrathecal injection of glycine decreased the glutamate level in the lumbosacral cord of intact rats. Although intrathecal injection of strychnine did not change the glutamate or glycine levels in the lumbosacral cord, these findings suggest that glycinergic neurons inhibit glutamatergic neurons to some extent. Therefore, glycinergic neurons may directly inhibit the spinal afferent and efferent limbs of the spinobulbosacral micturition reflex and/or may inhibit the glutamatergic neurons that facilitate these reflex limbs.

Spinal shock is a phenomenon that occurs after SCI and is characterized by flaccid paralysis and the loss of spinal cord reflexes below the level of injury. This phenomenon is not only induced by the initial spinal injury, but is also due to secondary damage caused by the excitatory amino acid cascade in the spinal cord (Butcher et al., 1987; Choi, 1985; Faden et al., 1989; Wahl et al., 1989). In humans, spinal shock typically persists for 1–2 weeks (Austin, 1985). Smith et al. (2002) showed that the glycine level of the spinal cord peaked at approximately 40 min after SCI in rats. In our previous study, the glycine level in the lumbosacral cord was also increased at 1 day after transection of the thoracic cord in rats (Nishijima et al., 2001; Sugaya et al., 2000). Simpson et al. (1993) have postulated that elevated glycine levels could mediate acute spinal shock after SCI. In the present study, cystometry demonstrated the disappearance of bladder contraction in acute SCI rats, but intrathecal injection of strychnine induced bladder contraction. Therefore, the delayed appearance of spinal micturition reflex activity after SCI, as well as the flaccid paralysis, might be mainly caused by an increase of glycinergic neuronal activity in the spinal cord. There could be at least two reasons for an acute increase of glycine in the lumbosacral cord after SCI. One possibility is the extinction of inhibitory projections to glycinergic neurons from the upper central nervous system, and the other possibility is the maintenance of stimulatory projections to glycinergic neurons from excitatory interneurons (such as glutamatergic neurons) in the lumbosacral cord, because our previous study showed that intrathecal injection of glutamate or MK-801 could respec-

tively increase or decrease the glycine level in the lumbosacral cord. (Sugaya et al., 2000).

In the chronic phase after SCI, various spinal reflexes become active. A potential spinal micturition reflex also subsequently becomes active and it evokes bladder contraction (de Groat, 1995; Yoshiyama et al., 1999). In the present study, bladder contractions with a shorter interval and smaller amplitude (detrusor hyperreflexia) were recorded in chronic SCI rats when compared with intact rats. In our previous study, the glycine level (but not the glutamate level) in the lumbosacral cord showed a reversible decrease at 2–8 weeks in rats with SCI (Nishijima et al., 2001). The ratio of excitatory amino acids to inhibitory amino acids is reported to be increased in the spastic state (Mertens et al., 2000). In the present study, both the glutamate and the glycine levels were decreased in the lumbosacral cord of chronic SCI rats, but the glutamate/glycine ratio was increased compared with that in intact rats. These findings suggest that both detrusor hyperreflexia and spinal hyperreflexia during the chronic phase of SCI are caused by a decrease of glycinergic neuronal activity in the spinal cord. Such a decrease of glycinergic activity may be induced by (1) decreased activity of glutamatergic neurons that facilitate glycine neurons (Sugaya et al., 2000), (2) a decrease in the number of glycinergic neurons in the spinal cord caused by death due to the excitatory amino acid cascade (Butcher et al., 1987; Choi, 1985; Faden et al., 1989; Wahl et al., 1989), or (3) changes of spinal neuronal mechanisms including the glycinergic system. However, the glutamate level of the lumbosacral cord did not show a significant decrease during the chronic phase of SCI in our previous study (Nishijima et al., 2001). Hereditary spastic mice have a 70–80% reduction of spinal cord glycine receptor levels (Monaghan, 1990), but the present study showed that the expression of strychnine-sensitive glycine receptor mRNA was not altered in the lumbosacral cord of chronic SCI rats. Therefore, the third possibility may be more likely.

In chronic SCI rats, a high dose of glycine prolonged the interval and decreased the amplitude of bladder contractions, suggesting that spinal glycinergic mechanisms inhibited both the afferent and the efferent limbs of the spinal micturition reflex. Intrathecal injection of a high dose of strychnine caused elevation of the baseline pressure in chronic SCI rats. Therefore, continuous glycinergic inhibition of the efferent limb of the spinal micturition reflex persisted to some extent after SCI. The weaker effects of glycine or strychnine on bladder activity when compared with those in intact rats may have been due to (1) decreased activity of other inhibitory mechanisms projecting from the upper central nervous system to the lumbosacral cord; (2) dysfunction of the glycinergic receptors expressed by glutamatergic neurons, because intrathecal injection of glycine did not influence the decreased glutamate level in lumbosacral cord of chronic SCI rats; and/or (3) increased activity of excitatory mechanisms (apart from glutamatergic mechanisms) in the lumbosacral cord.

Glycine acts on postsynaptic neuronal membranes via two receptor subtypes, a strychnine-sensitive chloride channel that produces membrane hyperpolarization and a much smaller population of strychnine-insensitive and *N*-methyl-D-aspartate-(NMDA) sensitive channels that mediate calcium flux and produce membrane depolarization (Chizhnikov et al., 1989; Larson and Beitz, 1988; McNamara and Dingledine, 1990; Patel et al., 1990). In the present study, after the suppression of isovolumetric rhythmic bladder contractions by intrathecal injection of strychnine in chronic SCI rats, the addition of glycine evoked rhythmic bladder contractions again. This finding suggests that exogenous glycine acted on the strychnine-insensitive and NMDA-sensitive subtype of glycine receptors to promote spinal micturition reflex activity. This glycinergic mechanism might influence the response of bladder activity to intrathecal glycine or strychnine or influence the glutamate and glycine levels in the lumbosacral cord, especially in SCI rats.

In summary, intrathecal injection of glycine prolonged the interval and decreased the amplitude of bladder contraction in intact and chronic SCI rats. Intrathecal injection of strychnine elevated the baseline pressure in intact rats and induced bladder contraction in acute SCI rats. In chronic SCI, although the expression of Gly α 1 mRNA in the lumbosacral cord was unchanged, glycinergic neuronal activity decreased and detrusor hyperreflexia appeared. Intrathecal injection of glycine decreased the glutamate level in the lumbosacral cord of intact rats. These results suggest that glycinergic neurons, as well as GABAergic neurons, may have a major inhibitory effect on the spinobulbosacral and spinal micturition reflexes at the level of the lumbosacral cord. The delayed appearance of spinal micturition reflex activity after SCI may be caused by an increase of glycinergic neuronal activity in the spinal cord, and detrusor hyperreflexia during the chronic phase of SCI may be largely related to a decrease of glycinergic neuronal activity in the spinal cord.

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