Intravenous Glycine Inhibits the Micturition Reflex in Normal and Spinal Cord Injury Rats

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ABSTRACT
We examined the influence of intravenous injection of glycine on the spinobulbospinal and spinal micturition reflexes in rats. Female rats were divided into an intact group and a chronic spinal cord injury (SCI) group with transection of the lower thoracic cord, and studied at 4 weeks after SCI. Under urethane anesthesia, isovolumetric cystometry was performed in each group before and after intravenous injection of glycine (0.01–100 mg/kg). Intravenous injection of glycine (0.1–100 mg/kg) transiently abolished bladder contractions after a delay of several minutes in both groups. In intact rats, intravenous injection of glycine prolonged the interval between bladder contractions, but did not change the amplitude of the contractions. On the other hand, intravenous glycine both prolonged the interval and decreased the amplitude of bladder contractions in SCI rats. However, intrathecal injection of low dose of strychnine (a selective glycine receptor antagonist; 0.001 μg) and following intravenous injection of glycine (0.1 mg/kg) did not affect the bladder contractions in intact rats. These results suggest that systemically administered glycine inhibits the afferent limb of the spinobulbospinal micturition reflex, and inhibits both the afferent and efferent limbs of the spinal micturition reflex at the lumbosacral cord level.

In the central nervous system, glutamate and aspartate are major excitatory amino acids, while glycine and gamma-aminobutyric acid (GABA) are the most abundant inhibitory amino acids (8). Some of these amino acids are considered to play an important role in micturition. In anesthetized normal rats that have an intact spinobulbospinal micturition reflex pathway, intrathecal or intravenous injection of glutamate receptor antagonists inhibits bladder activity (11). Intrathecal injection of glycine or GABA also inhibits bladder activity (4, 5). When the spinal cord is transected, the spinobulbospinal reflex pathway is blocked, and the previously masked spinal micturition reflex becomes active in the chronic phase (3). In chronic spinal cord injury (SCI) rats, however, intrathecal injection of glutamate receptor antagonists only has a weak effect (11, 12). On the other hand, intrathecal injection of glycine not only inhibits the spinobulbospinal micturition reflex but also the spinal micturition reflex (5). Our previous study showed that detrusor hyperreflexia developed in chronic SCI rats, and the glycine level decreased in the lumbosacral cord and serum, but the glutamate level in the lumbosacral cord did not change or only decreased slightly (5, 7). Therefore, intravenous injection of glycine might also inhibit both the spinobulbospinal micturition reflex and the spinal micturition reflex. In the present study, we examined the effects of intravenous injection of glycine on bladder activity in rats with or without SCI.
MATERIALS AND METHODS

Animals. Experiments were performed on 28 female Sprague-Dawley rats weighing 250–300 g. The rats were divided into an intact group (n = 18) and a SCI group (n = 10). Rats in the SCI group were anesthetized with 2% halothane, and the spinal cord was completely transected at the lower thoracic level (T9 or T10). Postoperatively, urine was manually expressed until 2 weeks after SCI, and the rats were tested at 4 weeks after the operation. The study protocol was approved by the Animal Care and Use Committee of the University of the Ryukyus.

Isovolumetric cystometry after intravenous injection of glycine in intact (n = 10) and SCI rats (n = 10). For cystometry, 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) connected to a pressure transducer was inserted into the bladder through the urethra. The urethra was ligated to the catheter near the external urethral meatus. The ureters were transected and the proximal cut ends were left open. A cannula was placed in the femoral vein for the intravenous administration of glycine. The bladder was filled with physiological saline (0.05 ml/min) to above the threshold volume, inducing rhythmic isovolumetric contractions. When the interval and amplitude of the bladder contractions had been stable for over 30 min, these parameters were measured as control values. The vehicle (physiological saline, 0.1 ml) was initially injected via the femoral vein to examine its influence on cystometry. Glycine (0.01–100 mg/kg, n = 10 per dose) was dissolved in physiological saline and was injected at 30-min intervals in the intact and SCI rats. The changes of bladder activity in response to glycine injection were recorded and compared with the control recordings.

Isovolumetric cystometry after intrathecal injection of strychnine and following intravenous injection of glycine in intact rats (n = 4). In order to confirm the effect of intravenous glycine on the micturition reflex in the lumbosacral cord, the effect of intravenous glycine after intrathecal injection of strychnine, a selective glycine receptor antagonist, on isovolumetric cystometry was investigated. Since intrathecal injection of strychnine (0.01–10 μg) affected isovolumetric cystometry in our previous study (5), 0.001 μg of strychnine was used intrathecally in this study. In intact rats, laminectomy was also performed at L3 and a catheter (PE-50) was inserted into the subarachnoid space through a small hole in the dura. The tip of the catheter was advanced to the level of the sacral cord, with the length of inserted catheter corresponding to that of a microsyringe needle (10 μl; Hamilton, Reno, Nevada) (5). The changes of bladder activity after intrathecal injection of strychnine (0.001 μg) and following intravenous injection of glycine (0.1–1 mg/kg) were recorded and compared with the control recordings before drug injections.

Isovolumetric cystometry by intravesical infusion of 1% glycine in intact rats (n = 4). Permeability of the bladder to water, electrolytes, or other substances has been investigated (1, 2), and the permeability occurs when intravesical volume is over 0.6 ml in rats (9). Therefore, in order to confirm the direct effect of glycine on the bladder smooth muscle activity, isovolumetric cystometry was performed by intravesical infusion of glycine in intact rats. Since 1% glycine in physiological saline corresponds to 40–50 mg/kg of intravenous glycine, we have decided to fill the bladder with 1% glycine to above the threshold volume, inducing rhythmic isovolumetric contractions. When the interval and amplitude of the bladder contractions had been stable for over 30 min, these parameters and bladder capacity were compared with those obtained from isovolumetric cystometry using physiological saline in intact rats.

Statistical analysis. Results are reported as the mean ± 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 intravenous injection of glycine in intact rats (n = 10) Intravenous injection of the vehicle did not influence either the interval between bladder contractions or the amplitude of contractions. The interval (2.06 ± 0.21 min) and amplitude (59.3 ± 6.39 cm H2O) of bladder contractions (controls) became stable at an intravesical volume of 0.95 ± 0.13 ml. Intravenous injection of glycine (0.1–100 mg/kg) abolished bladder contractions for 10–13 min after a delay of 2–3 min. Subsequently, bladder contractions reappeared, although the interval and amplitude did not recover completely. The interval was significantly (P < 0.05) prolonged (408–516%) after intravenous injection of glycine (0.1–100 mg/kg) compared with the
control value (Figs. 1A, 2A) However, the duration of disappearance of bladder contractions and the change of the contraction interval after administration of glycine were not dose-dependent. Intravenous injection of glycine (0.01–100 mg/kg) did not change the amplitude of bladder contractions (Figs. 1A, 2B).

Changes of bladder activity after intravenous injection of glycine in SCI rats (n = 10)
Intravenous injection of the vehicle did not influence either the interval between bladder contractions or the amplitude of contractions. Before the intravenous injection of glycine (control), the interval (1.10 ± 0.09 min) and amplitude (43.3 ± 1.84 cm H₂O) of bladder contractions were significantly (P < 0.01 and P < 0.05, respectively) reduced when compared with those in intact rats (Fig. 2). Intravenous injection of glycine (0.1–100 mg/kg) abolished bladder contractions for 8–10 min after a delay of 2–3 min, during which there was a gradual decrease of the amplitude. Bladder contractions subsequently reappeared, although the interval and amplitude did not recover completely. The interval was significantly (P < 0.05) prolonged (669–746%) compared with the control value after intravenous injection of glycine (0.1–100 mg/kg) (Figs. 1B, 2A). In addition, the amplitude was significantly (P < 0.05) decreased (27–40%) after intravenous injection of glycine (0.1–100 mg/kg) (Figs. 1B, 2B). However, the duration of disappearance of bladder contractions and the changes of the contraction interval and amplitude after administration of glycine were not dose-dependent.

Changes of bladder activity after intrathecal injection of strychnine and following intravenous injection of glycine in intact rats (n = 4)
The interval (1.43 ± 0.91 min) and amplitude (41.3 ± 4.50 cm H₂O) of bladder contractions were stable. Intrathecal injection of strychnine (0.001 µg) did not affect the interval and amplitude of bladder contractions, and following intravenous injection of glycine (0.1 mg/kg) also did not change these parameters. Additional intrathecal injection of strychnine (0.001 µg) did not change these parameters, but following intravenous injection of glycine (1 mg/kg) significantly (P < 0.01) prolonged the interval (3.05 ± 0.03 min) of bladder contractions. However, the amplitude (40.6 ± 4.10 cm H₂O) of bladder contractions was unchanged.

Changes of bladder activity by intravesical infusion of 1% glycine in intact rats (n = 4)
The interval (1.90 ± 0.06 min) and amplitude (56.0 ± 9.24 cm H₂O) of bladder contractions became stable at an intravesical volume of 1.00 ± 0.16 ml. These parameters were not different from those obtained from control values using physiological saline.

![Diagram](image)

**Fig. 1** Isovolumetric cystometry before and after intravenous injection of glycine in an intact rat (A) and a SCI rat (B). A: Isovolumetric bladder contractions were abolished by intravenous injection of 0.1 mg/kg of glycine after a delay of 2 minutes. Thirteen minutes later, bladder contractions reappeared without any change of the interval or amplitude. B: After intravenous injection of 0.1 mg/kg of glycine, the interval and amplitude of bladder contractions gradually decreased, and contractions almost disappeared after 4 minutes. Seven minutes later, bladder contractions reappeared. IVP; intravesical pressure.
DISCUSSION

In our previous study, intrathecal injection of glycine (0.1–100 μg) immediately (but transiently) abolished bladder contractions in both intact and SCI rats, and intrathecal injection of strychnine (0.01–10 μg) elevated the intravesical baseline pressure (5). In the present study, intravenous injection of glycine abolished bladder contractions for about 10 minutes after a delay of several minutes, prolonged the mean interval between isovolumetric bladder contractions in intact and SCI rats, and decreased the mean contraction amplitude in SCI rats. Intrathecal injection of low dose (0.001 μg) of strychnine and following intravenous injection of low dose (0.1 mg/kg) of glycine did not affect the bladder contractions, but additional intravenous injection of higher dose (1 mg/kg) of glycine prolonged the interval between bladder contractions in intact rats. These results suggest that intrathecal injection of low dose of strychnine at least block the effect of intravenous injection of low dose of glycine on the micturition reflex at the lumbar sacral cord level. On the other hand, isovolumetric bladder contractions by intravesical infusion of 1% glycine were not different from those using physiological saline, suggesting that the direct effect of glycine on the bladder smooth muscle activity is less or none. Therefore, intravenously injected glycine may cross the blood-brain barrier and affect the micturition reflex pathway in the lumbar sacral cord.

Intrathecal injection of glycine has been shown to prolong the interval and decreases the amplitude of bladder contractions in a dose-dependent fashion in intact rats (5). In the present study, intravenous injection of glycine prolonged the interval between
contractions independently of the dose, but it did not change the contraction amplitude in intact rats, suggesting that only part of an intravenous dose of glycine crosses the blood-brain barrier. Since changes of the contraction interval and amplitude are thought to be due to alterations of afferent and efferent activity in the micturition reflex pathway, respectively (10), intravenous injection of glycine may have blocked the afferent limb of the spinobulbospinal micturition reflex in the lumbosacral cord of intact rats. In SCI rats, however, intravenous injection of glycine prolonged the interval and decreased the amplitude of contractions independently of the dose. Intrathecal injection of higher doses of glycine also prolonged the contraction interval and decreased the amplitude without dose-dependence (5). Therefore, these results suggest that systemically administered glycine blocks the afferent and efferent limbs of the spinal micturition reflex in SCI rats, and that this reflex pathway is relatively simple and has fewer synapses compared with the spinobulbospinal micturition reflex pathway.

The glycine level is low in both the lumbosacral cord and serum of SCI rats compared with intact rats (5, 7). Clinically, the serum glycine level is also low in SCI patients and in patients who have benign prostatic hyperplasia and urinary frequency when compared with healthy controls (6). Therefore, an increase of the serum glycine level by administration of glycine may be able to improve micturition disorders like frequency or incontinence in these patients.

In conclusion, intravenous injection of glycine prolonged the interval between bladder contractions, but did not change the amplitude of the contractions in intact rats. On the other hand, intravenous glycine both prolonged the interval and decreased the amplitude of bladder contractions in SCI rats. However, intrathecal injection of low dose (0.001 µg) of strychnine and following intravenous injection of low dose (0.1 mg/kg) of glycine did not affect the bladder contractions. These results suggest that intravenous glycine inhibits the afferent limb of the spinobulbospinal micturition reflex, and inhibits both the afferent and efferent limbs of the spinal micturition reflex in the lumbosacral cord.

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