0022-5347/05/1731-0314/0
THE JOURNAL OF UROLOGY®

Vol. 173, 314–317, January 2005
Printed in U.S.A.
DOI: 10.1097/01.ju.0000141579.91638.a3

DIETARY GLYCINE INHIBITS BLADDER ACTIVITY IN NORMAL RATS AND RATS WITH SPINAL CORD INJURY

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ABSTRACT

Purpose: The influence of dietary glycine on bladder activity, and on glutamate and glycine levels in the serum and lumbosacral cord was examined in rats with or without spinal cord injury (SCI).

Materials and Methods: A total of 84 female rats were divided into an intact and an SCI group. Each group of rats was divided into 7 subgroups. Two intact and 2 SCI subgroups were fed a standard diet and the remaining subgroups were fed diets containing 0.1% to 3% glycine. After 4 weeks isovolumetric cystometry was performed with rats under urethane anesthesia. Following cystometry glutamate and glycine levels in the serum and lumbosacral cord were measured as well as the glycine receptor α1 mRNA level in the lumbosacral cord.

Results: Dietary glycine (1% to 3%) prolonged the interval between bladder contractions in intact rats but did not change the amplitude of contractions. On the other hand, dietary glycine (1% to 3%) prolonged the interval and decreased the amplitude of bladder contractions in SCI rats. The glycine levels in the serum and lumbosacral cord of SCI rats on the standard diet were respectively 43% and 45% lower than those in intact rats on the standard diet. Dietary glycine (1% to 3%) increased the serum glycine level in intact and SCI rats but the glycine receptor α1 mRNA level in the lumbosacral cord was unchanged.

Conclusions: Dietary glycine crosses the blood-brain barrier and inhibits the micturition reflex pathway in the lumbosacral cord but SCI and/or dietary glycine do not influence glycine receptor expression.

KEY WORDS: bladder; spinal cord injuries; glutamic acid; receptors, glycine; reflex

In the central nervous system glutamate and aspartate are major excitatory amino acids, while glycine and γ-aminobutyric acid (GABA) are the most abundant inhibitory amino acids.[1–3] GABA is known to have an important role in the inhibitory regulation of micturition via $GABA_A$ and $GABA_B$ receptors.[4] When the $GABA_B$ receptor agonist baclofen is administered intrathecally, intravenously or orally, it decreases urethral resistance and detrusor overactivity in animals and humans but this agent has not achieved wide acceptance clinically.[5–10] In our previous study rats with chronic spinal cord injury (SCI) showed frequent bladder contractions on cystometry and had lower glycine levels in the lumbosacral cord and serum compared with intact rats.[11,12] Intrathecal injection of glycine at the lumbosacral cord level inhibits the micturition reflex in normal and SCI rats.[11] Intravenous injection of glycine also inhibits the micturition reflex in normal and SCI rats but intrathecal injection of strychnine (a selective glycine receptor antagonist) and subsequent intravenous injection of glycine does not affect bladder contraction.[13] These results suggest that intravenous injection of glycine inhibits the micturition reflex at the lumbosacral cord level and glycine may be useful for the treatment of urinary frequency or overactive bladder. However, to our knowledge it is unknown whether dietary administration of glycine also inhibits the micturition reflex. Therefore, we performed the current study to examine the effect of dietary administration of glycine on bladder activity, glutamate and glycine levels in the serum and lumbosacral cord, and the expression of strychnine sensitive glycine receptor α1 (GlyR α1) mRNA in the lumbosacral cord of rats with or without SCI.

Submitted for publication May 6, 2004.
Supported by Grant-in-Aid for Scientific Research B-14370516 from the Japan Society for the Promotion of Science.
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MATERIALS AND METHODS

Animals. A total of 84 female Sprague-Dawley rats weighing 250 to 300 gm were divided into an intact group of 42 and a SCI group of 42). 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 the bladder was managed by expressing urine manually until 2 weeks after SCI. Each group of rats was divided into 7 subgroups of 6 each. A total of 12 intact rats and 12 immediately after SCI were fed a standard diet (Nihonkurea, Tokyo, Japan), while the other intact and SCI rats were fed diets containing 0.1% to 3% glycine. After 4 weeks the rats were weighed and anesthetized by intraperitoneal and subcutaneous injection of urethane (1.2 vs 0.6 gm/kg in intact vs SCI rats) before the experiments were performed.

Isovolumetric cystometry. A total of 30 intact and 30 SCI rats fed a standard diet (6 each) or 0.1% to 3% glycine diets (6 each) were used. After tail pinching a polyethylene catheter (PE-50, Clay-Adams, Parsippany, New Jersey) was inserted into the bladder through the urethra and residual urine volume was calculated. The urethra was ligated to the catheter near the external urethral meatus to produce isovolumetric conditions in the bladder. An abdominal incision was made, the ureters were transected and the distal ends were ligated. Bladder activity was monitored via the urethral catheter, which was connected to a pressure transducer and saline infusion pump. The bladder was filled with physiological saline (0.05 ml per minute) to above the threshold vol-

ume, inducing rhythmic isovolumetric contractions. After contractions were stable for more than 60 minutes the interval, amplitude and duration of isovolumetric bladder contractions were evaluated. These parameters were averaged for 30 minutes and results were compared among subgroups.

Amino acid determination. Following cystometry blood was withdrawn from the inferior vena cava in each rat, centrifuged for 10 minutes at 5,000 rpm to separate serum and deproteinized. The lumbosacral cord was also removed and homogenized in cold 0.5 M hydrochloric acid, after which the homogenate was dechlorinated and deproteinized. Amino acid levels in these samples were measured by capillary electrophoresis.[11]

Quantification of GlyR α1 mRNA. Lumbosacral cords were obtained from 12 intact and 12 SCI rats fed a standard (6 each) or 3% glycine (6 each) diet. Total RNA was extracted from the lumbosacral cords and cDNA was synthesized. GlyR α1 and β-actin mRNA levels in each lumbosacral cord specimen was quantified with a GeneAmp 7700 thermal cycler (Applied Biosystems, Foster City, California)[11] and the ratio of GlyR α1 to β-actin mRNA was compared.

Statistical analysis. Results are reported as the mean ± SE. Student's unpaired t test for data were used for statistical analysis with significance considered at $p < 0.05$.

RESULTS

Comparison of bladder activity between intact and SCI rats on standard or 0.1% to 3% glycine diets. Body weight and residual urine volume in intact rats on glycine diets were not different from those in rats on the standard diet (see table). Isovolumetric cystometry became stable at an intravesical volume of less than 0.6 to 1.0 ml in standard and glycine diet intact rats. Isovolumetric cystometry showed that the interval between bladder contractions was significantly prolonged in rats on a 1% and 3% glycine diet (3.19 ± 0.18 and 4.36 ± 0.31 minutes, respectively) compared with that in rats on a standard diet (1.89 ± 0.16 minutes, $p < 0.001$, figs. 1, *A* and 2, *A*). The amplitude, duration and baseline pressure of bladder contractions in rats on glycine diets were unchanged compared with those in rats on the standard diet (46.9 ± 2.90 cm H_2O, 1.23 ± 0.09 minutes and 8.02 ± 1.05 cm H_2O, respectively, figs. 1, *A* and 2, *B*).

Body weight in SCI rats on glycine diets was not different from that in rats on the standard diet but residual urine volume was significantly increased in rats fed the 3% glycine compared with that in rats on a standard diet (4.00 ± 0.63 vs 1.23 ± 0.41 ml, $p = 0.005$, see table). Isovolumetric cystometry became stable at an intravesical volume of 2.5 to 3.5 ml in standard and glycine diets SCI rats. Isovolumetric cystometry showed that the interval and amplitude of bladder contractions in SCI rats on a standard diet (0.56 ± 0.04 minutes and 34.5 ± 2.03 cm H_2O) were significantly decreased compared with those in intact rats on a standard diet ($p < 0.001$ and 0.004, respectively). The interval between bladder contractions was significantly prolonged by the 1% and 3% glycine diets (0.93 ± 0.01 and 0.89 ± 0.01 minutes, respectively) compared with the standard diet ($p < 0.001$, figs. 1, *B* and 2, *A*). The amplitude of bladder contractions was significantly decreased by the 1% and 3% glycine diets (23.2 ± 1.83 and 10.6 ± 0.78 cm H_2O) compared with the standard diet ($p = 0.002$ and < 0.001, respectively, figs. 1, *B* and 2, *B*). However, the duration and baseline pressure of bladder contractions in SCI rats on the glycine diet (0.63 ± 0.08 minutes

Body weight and residual urine volume after dietary administration of glycine

Group (diet)	Mean Wt ± SE (gm)	Mean Residual Urine ± SE (ml)
Intact:		
Control	267.8 ± 10.3	0.07 ± 0.05
0.1% Glycine	266.5 ± 5.8	0.13 ± 0.06
0.3% Glycine	243.8 ± 12.3	0.15 ± 0.08
1% Glycine	254.3 ± 7.1	0.19 ± 0.11
3% Glycine	252.7 ± 5.1	0.32 ± 0.11
SCI:		
Control	246.7 ± 7.0	1.20 ± 0.41
0.1% Glycine	243.5 ± 9.6	1.23 ± 0.40
0.3% Glycine	227.3 ± 7.5	1.33 ± 0.53
1% Glycine	274.5 ± 13.2	0.90 ± 0.05
3% Glycine	236.7 ± 1.4	4.00 ± 0.63*

* Standard (control) vs 3% glycine diet $p < 0.01$.

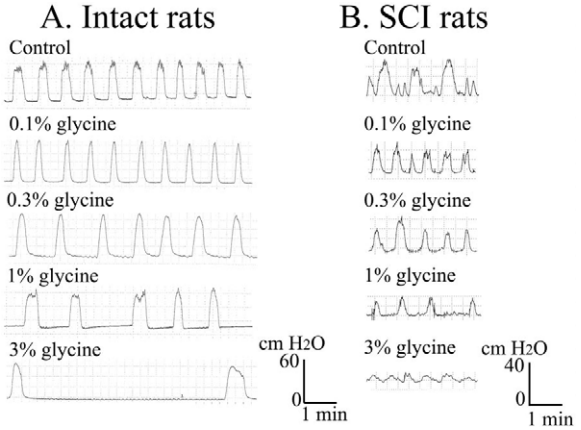

FIG. 1. Isovolumetric cystometry in standard diet (control) and 0.1% to 3% glycine diet groups. *A*, in intact rats interval between bladder contractions was gradually prolonged with increase in diet glycine but bladder contraction amplitude did not change. *B*, in SCI rats interval between isovolumetric bladder contractions was gradually prolonged and contraction amplitude was gradually decreased with increase in diet glycine.

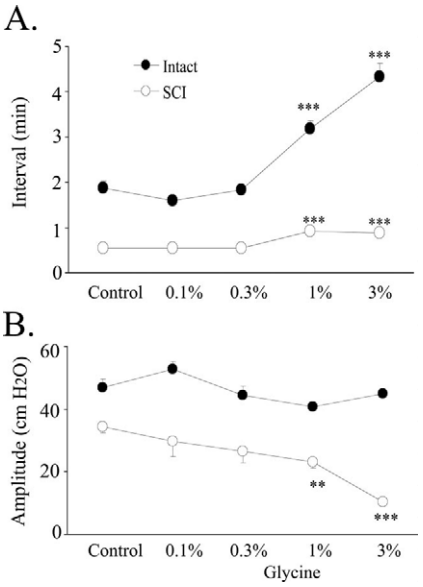

FIG. 2. Mean effect ± SE of dietary administration of 0.1% to 3% glycine on isovolumetric bladder contractions in intact and SCI rats. *A*, interval in intact and SCI rats on 1% to 3% glycine diets showed significant prolongation compared with interval in intact and SCI rats on standard diet (*Control*). In SCI rats on standard diet interval was significantly shorter than in intact rats on standard diet. Triple asterisks indicate significant difference ($p < 0.001$). *B*, amplitude did not change between intact rats on standard diet (*Control*) and 0.1% to 3% glycine diets. In SCI rats on 1% to 3% glycine diets amplitude was significantly decreased compared with that in SCI rats on standard diet. In SCI rats on standard diet amplitude was significantly lower than in intact rats on standard diet. There were 6 rats per subgroup. Double asterisks indicate significant difference ($p < 0.01$).

and 9.03 ± 0.53 cm H_2O, respectively) was similar to that in SCI rats on the standard diet.

Comparison of serum glutamate and glycine levels between intact and SCI rats fed standard or 0.1% to 3% glycine diets. In intact rats the glutamate level did not change after dietary administration of glycine. However, the glycine level was significantly increased in rats on the 3% glycine diet compared with that in rats on the standard diet (24.7 ± 1.42 vs 12.4 ± 1.06 mg/l, $p < 0.001$, fig. 3). The glutamate-to-glycine ratio, which is reported to be related to micturition reflex activity,[14] was significantly decreased by the 1% and 3% glycine diets (0.57 ± 0.04, $p = 0.048$ and 0.55 ± 0.05, $p = 0.044$, respectively) compared with the standard diet (0.87 ± 0.14).

In SCI rats the glutamate level also did not change after dietary administration of glycine. In SCI rats on standard diet the serum glycine level was significantly lower compared with that in intact rats on a standard diet (43% decrease, 7.02 ± 0.60 mg/l, $p < 0.001$, fig. 3). However, the glycine level was significantly increased by the 1% and 3% glycine diets compared with that in rats on a standard diet (10.3 ± 0.69 mg/l, $p = 0.010$ and 18.3 ± 0.81 mg/l, $p < 0.001$, respectively). The glutamate-to-glycine ratio was significantly decreased by the 3% glycine diet compared with that of rats on a standard diet (0.69 ± 0.09 vs 1.64 ± 0.64, $p = 0.015$).

Comparison of glutamate and glycine levels in the whole lumbosacral cord between intact and SCI rats with standard or 0.1% to 3% glycine diets. In SCI rats on the standard diet glutamate and glycine levels in the lumbosacral cord were significantly decreased (20% decrease, 3.41 ± 0.23 μmol/gm, $p = 0.013$ and 45% decrease, 0.71 ± 0.13 μmol/gm, $p = 0.007$) compared with those in intact rats on a standard diet (4.29 ± 0.18 and 1.29 ± 0.12 μmol/gm, respectively). The glutamate-to-glycine ratio in SCI rats on the standard diet was significantly increased compared with that in intact rats on the standard diet (10.2 ± 1.12 vs 6.89 ± 0.87, $p = 0.042$). However, glutamate and glycine levels as well as the glutamate-to-glycine ratio did not differ between intact rats or between SCI rats on standard vs glycine diets.

Comparison of the GlyR α1 mRNA level in the whole lumbosacral cord between intact and SCI rats on standard or 3% glycine diets. In intact rats on the standard diet β-actin mRNA and GlyR α1 mRNA levels in the lumbosacral cord were 0.43 ± 0.23 ng/ml and 0.36 ± 0.19 pg/ml, respectively. In intact rats on the 3% glycine diet β-actin mRNA and GlyR α1 mRNA levels were 0.82 ± 0.40 ng/ml and 0.39 ± 0.19 pg/ml, respectively, but the GlyR α1 mRNA-to-β-actin mRNA ratio in the lumbosacral cord did not differ from that in rats on the standard diet.

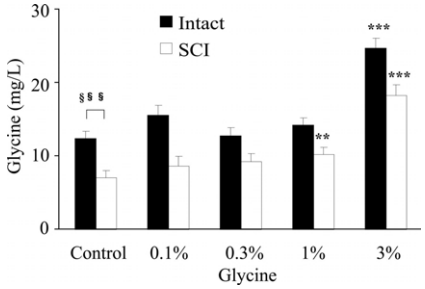

FIG. 3. Serum glycine in intact and SCI rats on standard (*Control*) or 0.1% to 3% glycine diets. Serum glycine in intact rats on 3% and SCI rats on 1% to 3% glycine diets was significantly higher than in intact and SCI rats on standard diet, respectively. In SCI rats on standard diet glycine was significantly lower than in intact rats on standard diet. There were 6 rats per subgroup. Values are shown as mean ± SE. Double asterisks indicate significantly different vs control ($p < 0.01$) Triple asterisks indicate significantly different vs control ($p < 0.001$). Triple curleys indicate significantly different between control intact vs SCI rats ($p < 0.001$).

In SCI rats on the standard diet β-actin mRNA and GlyR α1 mRNA levels in the lumbosacral cord were 0.85 ± 0.30 ng/ml and 0.17 ± 0.13 pg/ml, respectively, but the GlyR α1 mRNA-to-β-actin mRNA ratio in the lumbosacral cord did not differ from that in intact rats on the standard diet. In SCI rats on the 3% glycine diet β-actin mRNA and GlyR α1 mRNA levels were 2.25 ± 0.46 ng/ml and 0.64 ± 0.24 pg/ml, respectively, but the GlyR α1 mRNA-to-β-actin mRNA ratio in the lumbosacral cord did not differ from that in SCI rats on the standard diet.

DISCUSSION

In the current study dietary administration of 1% to 3% glycine prolonged the interval between bladder contractions in intact and SCI rats, and decreased the amplitude of bladder contractions in SCI rats. The serum glycine level was increased by the 3% glycine diet in intact rats or by the 1% to 3% glycine diets in SCI rats and the serum glutamate/glycine ratio was decreased by the in 1% to 3% glycine diets in intact rats or the 3% glycine diet in SCI rats compared with those in intact or SCI rats on the standard diet, respectively. However, there were no differences in glycine and GlyR α1 mRNA levels in the lumbosacral cord between intact or SCI rats on the standard and glycine diets. In our previous study intrathecal or intravenous injection of glycine inhibited the micturition reflex at the lumbosacral cord level in intact and SCI rats.[11,13] Therefore, the current results suggest that dietary glycine crosses the blood-brain barrier and inhibits the micturition reflex pathway in the lumbosacral cord.

In the chronic phase of SCI a potential spinal micturition reflex becomes active and detrusor hyperreflexia develops.[15] Glycine levels decrease in the lumbosacral cord and serum, while the glutamate-to-glycine ratio increases in the lumbosacral cord and serum.[11,12] In the current study the interval between bladder contractions was shorter in SCI than in intact rats and glycine was decreased in the serum and lumbosacral cord of SCI rats, while glutamate-to-glycine ratios in the serum and lumbosacral cord were increased compared with those in intact rats. These findings suggest that detrusor hyperreflexia during the chronic phase of SCI is caused by a decrease of glycinergic neuronal activity in the spinal cord. Dietary administration of 1% to 3% glycine increased the serum glycine level in SCI rats but did not change the glycine level in the lumbosacral cord. GlyR α1 mRNA level in the lumbosacral cord was also not changed by SCI and/or dietary administration of glycine. These results suggest that SCI and/or dietary glycine do not influence the level of glycine receptor expression in the lumbosacral cord.

Dietary administration of 1% to 3% glycine prolonged the interval and decreased the amplitude of bladder contractions in SCI rats. In intact rats dietary glycine also prolonged the interval but did not decrease the amplitude of bladder contractions. In our previous study intravenous injection of glycine also prolonged the interval between bladder contractions in intact and SCI rats, and decreased the amplitude of bladder contractions in SCI but not intact rats.[13] Since changes in the contraction interval and amplitude are thought to be due to alterations in afferent and efferent activity in the micturition reflex pathway, respectively,[16] these results suggest that dietary glycine blocks the afferent limb of the spinobulbospinal micturition reflex at the lumbosacral cord in intact rats and blocked the afferent and efferent limbs of the spinal micturition reflex in SCI rats. The results also suggest that this spinal micturition reflex pathway is relatively simple and it has fewer synapses than the spinobulbospinal micturition reflex pathway. Therefore, the inhibitory effect of dietary glycine on micturition may be more prominent in SCI rats, which may be the reason that residual urine volume was only increased in SCI rats after dietary administration of 3% glycine.

The serum glycine level is low in patients who have SCI or benign prostatic hyperplasia and urinary frequency compared with that in healthy controls.[17] In the current study dietary administration of glycine increased the serum glycine level and inhibited the micturition reflex. Therefore, dietary administration of glycine may be able to improve micturition disorders, such as urinary frequency or urge incontinence, in these patients.

CONCLUSIONS

Dietary glycine increased the serum glycine level and prolonged the interval between bladder contractions in intact rats. In SCI rats the baseline serum glycine level was 43% lower than in intact rats. Dietary glycine increased the serum glycine level, and prolonged the interval and decreased the amplitude of bladder contractions. However, the expression of GlyR α1 mRNA in the lumbosacral cord was unchanged by SCI and/or dietary glycine. These results suggest that dietary glycine crosses the blood-brain barrier and inhibits the micturition reflex pathway at the lumbosacral cord and SCI and/or dietary administration of glycine do not influence the level of glycine receptor expression in the lumbosacral cord.

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