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Strychnine-Sensitive and Strychnine-Insensitive Glycine Binding Sites in the Spinal Cord of the Wobbler Mouse

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Tomiyama, M., Kannari, K. and Matsunaga, M. *Strychnine-Sensitive and Strychnine-Insensitive Glycine Binding Sites in the Spinal Cord of the Wobbler Mouse.* Tohoku J. Exp. Med., 1997, **183** (1), 37-43 —— Using quantitative autoradiography, the strychnine-sensitive glycine site and strychnine-insensitive glycine site of the N-methyl-D-aspartate receptor were analyzed in the cervical segment of the spinal cord of the wobbler mouse, which is a purported model of human motor neuron diseases. Significantly increased density of the strychnine-sensitive site was found in the lamina II-inner (+17%) and laminae III & IV (+17%) of wobbler mice. The strychnine-insensitive site was also increased in lamina I & II-outer (+15%), lamina II-inner (+15%), laminae III & IV (+48%), laminae V-VIII (+43%) and lamina X (+26%) of wobbler mice. However, no significant differences were observed for the both sites in the ventral horn where motor neurons are located. These findings suggest that both inhibitory and excitatory-associated glycinergic dysfunctions are involved in the wobbler mouse motor neuron disease. ———— wobbler mouse; motor neuron disease; spinal cord; glycine receptors; NMDA receptors

The wobbler mouse is an autosomal recessive mutant whose mutation maps to chromosome 11 (Kaupman et al. 1992). Affected mice develop progressive muscular weakness, atrophy and contracture, predominantly in the distal, and later the proximal, forelimb muscles. Clinical signs appear at about 3 to 5 weeks of age. The anterior horn cells undergo vacuolar degeneration. For these characteristic manifestations, the wobbler mouse has been considered an animal model for human motor neuron diseases (Bradley 1984). Evidence indicative of glutamate dysfunctions has been described in the wobbler mouse, such as reduction of glutamate content (Krieger et al. 1991) and alterations of ionotropic glutamate receptors (Krieger et al. 1993a; Tomiyama et al. 1994) in the spinal cord. Although it has also been reported that glycine content is decreased in the

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spinal cord of the wobbler mouse (Krieger et al. 1991), its decrease has received little attention.

Glycine was once thought to be an exclusively inhibitory neurotransmitter mediated by strychnine-sensitive receptors (Aprison and Nadi 1978). However, it has been known that glycine also binds to the strychnine-insensitive site on N-methyl-D-aspartate (NMDA) receptor complex, a subtype of ionotropic glutamate receptors, and potentiates its excitatory transmission (Johnson and Ascher 1987). It might seem unlikely that glycine would have any significant influence on NMDA receptor-mediated neurotransmission in vivo (Johnson and Ascher 1987), since the endogenous glycine concentration is enough to saturate the NMDA glycine site. However, there is increasing evidence suggesting that NMDA receptors are not maximally potentiated by endogenous glycine, and that the glycine site is probably involved in excitatory transmission by the NMDA receptor in vivo (Kemp and Leeson 1993).

The aim of this study was to elucidate whether the decrease of glycine content in the wobbler mouse is related to any, or both, of the two glycine sites in the cervical segment of the spinal cord where motoneuronal degeneration is most evident.

Materials and Methods

Wobbler mice were obtained from our colony which is originally derived from breeding pairs provided by Prof. H. Mitsumoto, Department of Neurology, The Cleaveland Clinic Foundation. Wobbler mice (genotype, wr/wr; $n=7$; 4 male and 3 female; body weight, 18.1 ± 0.5 g [mean $\pm$ S.E.M.]) and gender-matched normal phenotype littermates (genotype, wr/+ or +/+; $n=7$; Body weight, 38.4 ± 1.2 g [mean $\pm$ S.E.M.]) were studied at 16 weeks of age. Cervical spinal cords were dissected from freshly decapitated mice and frozen on powdered dry ice. Tissue samples were stored at -70°C. For autoradiographic analysis, 20 μm cryostat sections at the cervical enlargement were thaw-mounted onto gelatin-coated glass slides.

Autoradiography for the strychnine-sensitive glycine site (SSGS) and the strychnine-insensitive glycine site (SIGS) was performed as previously described with [^{3}H]strychnine (23.0 Ci/mmol, New England Nuclear, Boston, MA, USA) (Zarbin et al. 1981) and [^{3}H]glycine (48.4 Ci/mmol, New England Nuclear) (McDonald et al. 1990), respectively. Sections were exposed to Amersham ^{3}H-Hyperfilm with tritium standard (Microscale, Amersham International PIC, Amersham, England) and exposed at 4°C for 6 weeks. The films were developed in D-19 (Kodak, Rochester, NY, USA). Analysis of autoradiographic images was performed using NIH Image (version 1.43) with Macintosh Quadra 900 computer and ARCUS scanner (AGFA, Leverkusen, Germany). Quadruplicate measurements were taken from six subregions of the spinal gray matter (laminae I & II-outer [IIo], lamina II-inner [IIi], laminae III & IV, the intermediate zone

[laminae V, VI and VII], lamina IX, and lamina X). Specific binding values for each assay were calculated by subtracting the nonspecific from the total binding values and averaging the quadruplicate subtracted values, and were expressed in fentomoles per milligram of protein. The significance of differences between the wobbler and control groups were assessed with t-test, $p<0.05$ being considered significant.

Results

In controls, SSGS and SIGS were mostly concentrated in the gray matter. The density of SSGS was high in the lamina IIi, laminae III & IV, intermediate zone and lamina X, moderate in the lamina IX, and low in the laminae I & IIo (Figs. 1A and 2A). On the other hand, SIGS was most abundant in the superficial laminae of the dorsal horn (laminae I & IIo and lamina IIi), moderately enriched around the central canal (lamina X), and less abundant in laminae III & IV, the intermediate zone and lamina IX (Figs. 1C and 2B). The distributional patterns

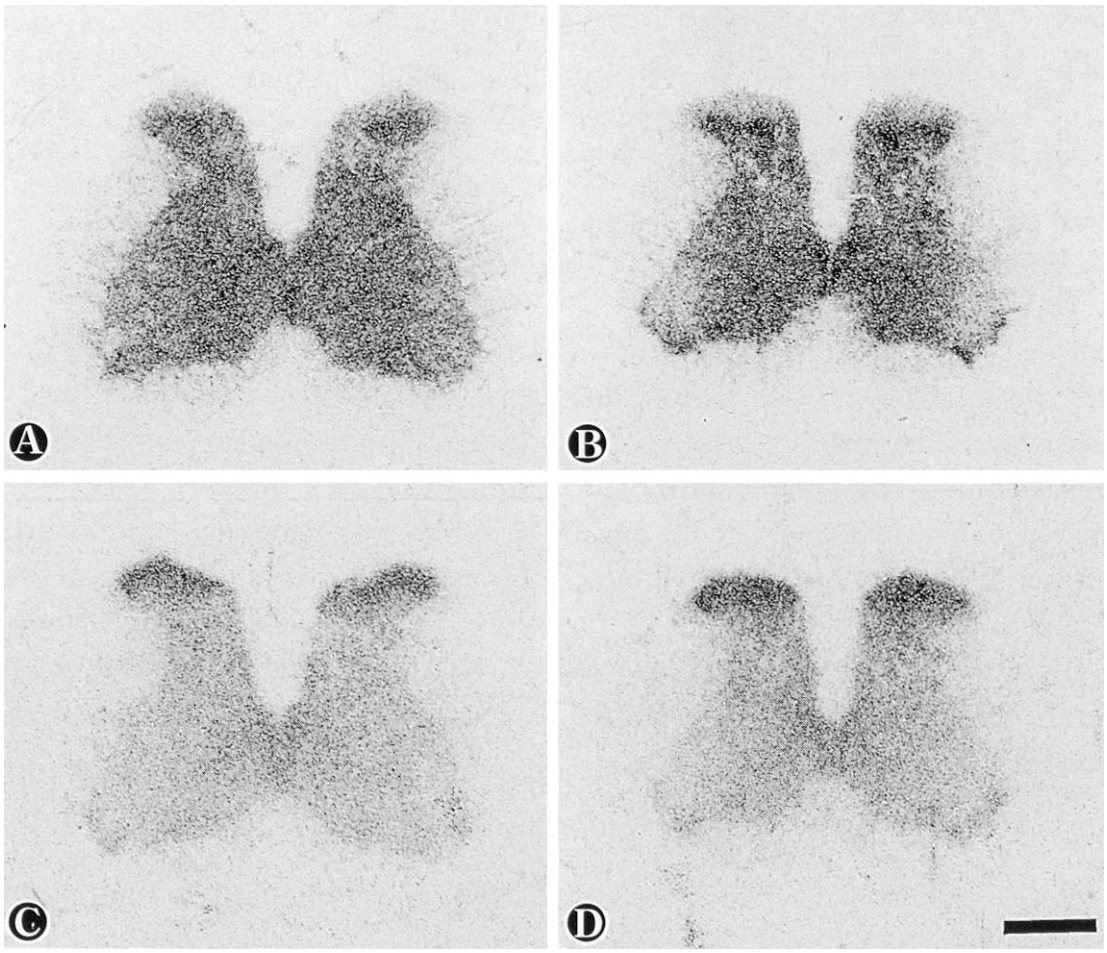

Fig. 1. Representative autoradiograms at the cervical enlargement of the strychnine-sensitive glycine binding site (A, control; C, wobbler) and the strychnine-insensitive glycine binding site (B, control; D, wobbler). The blacker the image, the greater the density of binding sites present. Scale bar, 1 mm.

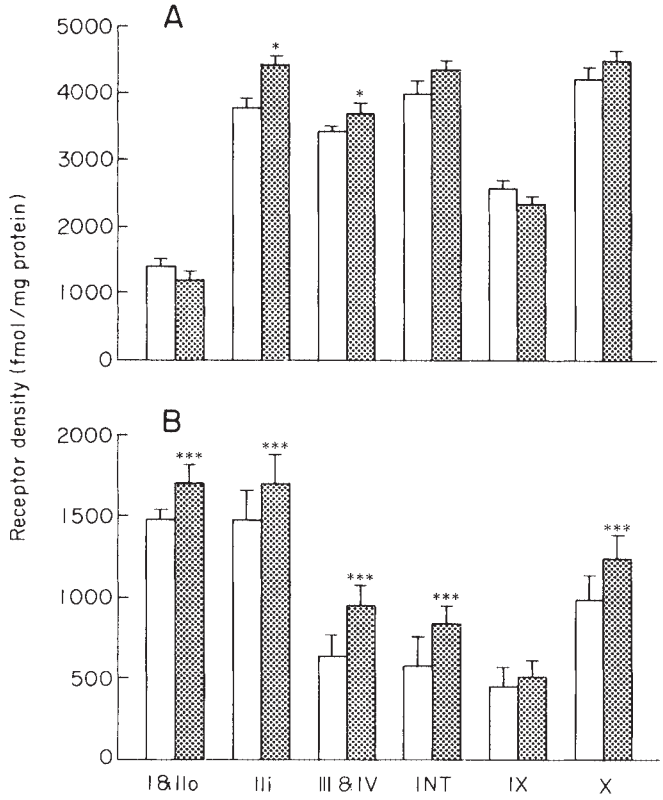

Fig. 2. Density of the strychnine-sensitive glycine binding site and the strychnine-insensitive glycine binding site is shown in A and B, respectively. Data are presented as the mean and S.E.M. in the laminae I and II-outer (I & IIo), lamina II-inner (IIi), laminae III and IV (III & IV), and intermediate zone (INT) corresponding to laminae V, VI, VII and VIII, lamina IX (IX), and lamina X (X). $^{*}p<0.05$, $^{***}p<0.001$.
□, control; ▩, wobbler

of the two binding sites in wobbler mice resembled those of controls (Figs. 1 and 2). However, the wobbler group had significantly higher density of SSGS in the lamina IIi (approximately +17%) and laminae III & IV (approx. +17%) when compared with controls (Figs. 1B and 2A). SIGS was increased significantly in laminae I & IIo (approx. +15%), lamina IIi (approx. +15%), laminae III & IV (approx. +48%), laminae V-VIII (approx. +43%) and lamina X (approx. +26%) in the wobbler group (Figs. 1D and 2B). This binding site did not show a significant alteration only in lamina IX of the wobbler mouse where motor neurons are located.

Discussion

The distribution of SSGS in the spinal cord of control mice was similar to that of rats (Zarbin et al. 1981). It is estimated that glycinergic neurons are interneurons within the spinal cord since the afferent and efferent fibers of the spinal cord do not contain glycine (van den Pol and Gorcs 1988). The finding that SSGS was increased in the wobbler group, taken together with the decrease of glycine content (Krieger et al. 1991), indicates abnormality of the glycinergic inhibitory

system in the wobbler mouse. Although motor neurons are lost in the wobbler mouse, SSGS was not altered in lamina IX. This may be caused by the remaining motor neurons expressing more SSGS or by the possible lower vulnerability of motor neurons rich in SSGS. The wobbler mouse has been considered as an animal model for human motor neuron diseases such as amyotrophic lateral sclerosis (ALS). Glycine content is decreased in the spinal cord from patients with ALS (Malessa et al. 1991) as in the wobbler mouse (Krieger et al. 1991). However, SSGS has been shown to be generally decreased in ALS spinal cords and significantly decreased in the ventral horn compared with controls (Hayashi et al. 1981; Whitehouse et al. 1983), whereas SSGS was increased in laminae IIi-IV of the wobbler group (Fig. 2A). This discrepancy suggests that the involvement of the inhibitory glycinergic system in the wobbler mouse differs from that of ALS.

The distribution of SIGS in the mouse spinal cord resembled that of the glutamate site of the NMDA receptor complex in mice (Tomiyama et al. 1994). The increase of SIGS observed in the wobbler group appears to correspond to the increment of the glutamate binding site in the wobbler spinal cord (Tomiyama et al. 1994), as the cloning of a functional NMDA receptor subunit has demonstrated that both the glutamate and glycine sites reside on the same subunit (NR1) (Moriyoshi et al. 1991). The elevation of the glutamate site and the glycine site of the NMDA receptor in the wobbler mouse, taken together with the decrease of glutamate content in the wobbler spinal cord (Krieger et al. 1991), suggests abnormality of the glutamatergic excitatory system associated with NMDA receptors. In the wobbler group, SIGS did not change in the lamina IX, while SIGS increased in all other subregions (Fig. 2B). Possibly, no alteration in the lamina IX reflects a balance between loss of binding sites due to motoneuronal death, with increased binding on the remaining neurons. On the other hand, it has been shown that NMDA receptors are decreased in both ventral horn and dorsal horn of the spinal cord from ALS patients (Allaoua et al. 1992; Krieger et al. 1993b; Shaw et al. 1994), although glutamate content is decreased in ALS spinal cords (Plaitakis et al. 1988; Malessa et al. 1991) like wobbler mice (Krieger et al. 1991). The present result supports that glutamate dysfunctions in the wobbler mouse may be different from those in ALS.

Three possible mechanisms can be postulated for the elevated binding sites, since increased number of neurons has not been shown in the spinal cord of the wobbler mouse (Bradley 1984). (1) The slow turnover of the receptor, (2) the change of binding affinity and (3) the up-regulation of the receptor. The reduction of glycine content in the spinal cord of the wobbler mouse (Krieger et al. 1991) may support the last possibility. However, the two sites are not necessarily increased in the same mechanism. The findings that both SSGS and SIGS were altered suggest that the reduction of glycine content in the spinal cord of the wobbler mouse may be associated with the both inhibitory and excitatory-associated glycinergic systems.

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