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The Influence of Glycine and Related Compounds on Spinal Cord Injury-Induced Spasticity

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Spasticity is a frequent and complex sequel to spinal cord injury. The neurochemical basis for the origin of spasticity is largely unknown. Glycine is among the most abundant neurotransmitters in the spinal cord. However, the role of glycine and related compounds in spasticity have received little attention. An ischemic spinal cord injury was created in rabbits, by an intraaortic balloon occlusion technique, which produced lower limb spasticity. A catheter was inserted into the cisterna magna and the spinal cord was bathed with 100 μM solutions of glycine, strychnine, D-serine, β-alanine, MK-801, or artificial CSF for 4 hours at a rate of 10 μl/min. H-reflexes were monitored before and during infusion by stimulating the posterior tibial nerve and recording from the plantar surface of the foot. Glycine, D-serine, and MK-801 depressed the H wave, strychnine produced a heightened H wave, and β-alanine caused no significant changes. These results indicate that glycine and related compounds may influence spasticity.

KEY WORDS: Intrathecal infusion; glycine; strychnine; NMDA; spasticity.

INTRODUCTION

Spasticity is generally defined as heightened stretch reflexes and excessive muscle tonus (1). It is a highly complex expression of neuronal activity following interruption of normal communication between the cerebrum and structures that are distal to the spinal cord. Segmental interneuron pools within the spinal cord play a key role as mediators of afferent inputs and descending motor control (2,3). Loss of this inhibitory interneuron activity contributes significantly to abnormal muscle behavior (4–7). Glycine is an abundant postsynaptic inhibitory amino acid neurotransmitter associated with segmental inhibitory interneuron pools, particularly those in proximity to alpha motor neurons (8–12). Since glycine is likely to be involved in the mechanisms responsible for the expression of spasticity, glycine administration as a single agent or in combination with other glycinergic compounds should influence the severity of this conditions. The primary hypothesis of the current study is that enhanced segmental glycine activity will reduce spasticity associated with neurological injury and disease. This is based on the assumption that a relationship exists between segmental glycine levels and and spasticity.

Current treatment for spasticity is quite limited and include some surgical procedures and a few pharmacological agents (13,14). Surgical procedures are not commonly used because they often produce new neurological deficits and the effects are not longlasting, and the few effective drugs have significant and often dangerous side effects (13,14). Additional drugs that may add to

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the limited armamentarium of spasticity treatment would be highly desirable. To produce such an agent, a clearer understanding of the underlying neurochemical mechanism responsible for spasticity is necessary. Many contemporary studies directed toward limiting or reversing the immediate consequences of neurologic injury are extremely valuable and very popular, however, treatment of the chronic debilitating sequela of such injuries deserves equal attention, including spasticity.

Although methods for patient management are limited, measurement of spasticity has often proven to be as difficult as treatment. The Ashworth Score (15), remains useful, but is subjective and lacks quantitative precision. Several types of electrophysiological recordings are generally accepted as providing quantitative measurements of spasticity (16,17). These include the raw or averaged electromyogram (EMG) and Hoffman or H-reflexes (18–21). Amplitude and latency measurements from these tests are used to gauge the severity of spasticity. New approaches to quantifying spasticity are being developed, such as the use of polyEMG activity evoked by transcranial motor cortex stimulation (22,23). However, H-reflex measurements have been shown to be the most reliable electrophysiologic measurement of spasticity (18–21). Ratios constructed from the amplitude of the H and M waves are frequently used to reflect the degree of muscle hypertonia are universally employed and were used in the current study to evaluate the influence of glycine and related compounds on spasticity (24–25).

EXPERIMENTAL PROCEDURE

Albino New Zealand male rabbits weighing between 3–5 kgs were used. Rabbits were anesthetized with methohexital sodium (Brevital 10 mg/kg IV) via an ear vein during the surgical procedures. The animals were ventilated with a volume-cycled rodent ventilator and maintained with 1-2% halothane (Fluothane) in 100% oxygen. Depth of anesthesia was judged during the surgical preparation by observing heart rate, blood pressure, depth of respiration and lid reflex. Following surgery, the animals were immobilized with pancuronium bromide (Pavulon 0.2 mg/kg IV). Body temperature was monitored and maintained at 38°C using a heating pad and lamp. The distal port of an arterial catheter, discussed below, was used to measure blood pressure and for blood sampling. Periodic measurement of blood gases and pH was performed and supplemental oxygen and/or lactated Ringer's solution was administered as needed.

The surgical preparation was performed using aseptic technique, including hair removal, betadine skin preparation, and the use of sterile drapes and instruments. A percutaneous cut-down was done in the left thigh through a lateral approach to transfemorally insert a sterile 4 French pediatric Swan-Ganz catheter. To produce ischemia, 0.6 cc of air was used to inflate the balloon just below the level of the renal arteries. A small vertex incision was made and sterile bipolar (4 mm Ag/AgCl cup) cortical stimulating electrodes were subcutaneously placed overlying the primary motor cortex for the left hindlimb. Two sterile stainless steel EMG needle recording electrodes, insulated except for the tips, were percutaneously inserted just lateral to the spinous process of the L5 and L6 vertebrae in contact with the lamina.

To insure that an ischemic injury to the spinal cord has occurred, corticomotor evoked potentials (CMEPs) were recorded from these paralaminar EMG electrodes. A detailed explanation of the technique has been previously published (15,26,27). CMEPs were optimally produced by transcranial anodal stimulation of the motor cortex at an intensity of 5-10 mA, for a duration of 1.0 msec, and at a frequency of 3 Hz using a Grass S-88 stimulator. The signals were recorded from the paralaminar EMG electrodes at L5 (active) and L6 (inactive). All signals were amplified, filtered (5-3000 Hz), and averaged (n=100) using a Metraco Inc. evoked potential system. Signals were digitized and stored on floppy discs (Apple Computer IIe) and paper with a digital printer. CMEPs were recorded before intraaortic balloon inflation, and every three minutes during ischemia. Records were also made immediately, 15, and 30 minutes after reperfusion.

After the ischemic session is complete the animals were awakened, extubated, and returned to their cages following full recovery from anesthesia. Approximately 30 minutes prior to extubation, atropine sulfate (0.1 mg/kg IM) was administered. Postoperative assessment of the animals was performed every eight hours, or more frequently if needed. The animal's overall condition and bladder function was carefully managed. Intravenous fluids were discontinued once oral intake was sufficient. Analgesics (butorphanol tartrate, Torbutrol 0.1 mg/kg SQ) were administered every 8 hours, as needed, and antibiotics (cephalothin sodium, Keflin 25 mg/kg IM) were administered every 8 hours. The majority of animals were paraparetic and a few were paraplegic. An animal was sacrificed if there was evidence of respiratory or any other distress. Daily assessments by H-reflex testing and Ashworth score determination were also done.

Each animal was restudied 72 hours after injury following final clinical assessment (Ashworth Score) and H-reflex records. At this point, the animal's neurological condition and degree of spasticity has stabilized as established by earlier studies (15,26,28). Introduction of anesthesia was performed in the same fashion as for the acute studies. Great care was undertaken to insure that the animals experienced no discomfort. After sufficient anesthesia, the surgical preparation consisted of replacing the femoral artery catheter. EMG electrodes were also placed into the muscle of the ipsilateral hindlimb. In addition, percutaneous insertion of a small caliber PE tube into the cisterna magna was performed, for drug infusion, and was secured to the skin. Each animal, remaining sufficiently anesthetized, was allowed to recover from the neuromuscular blockade. EMG recordings and H-reflex measurements were then made and the intrathecal drug infusions were begun. Infusion of either glycine, strychnine, D-serine, β-alanine, MK-801 (100 μM solutions in artificial CSF), or artificial CSF are performed continuously for 4 hours at a rate of 10 μl/min using a microsyringe driver (CMA/Microdialysis). The dose and duration of infusion were based on results from pilot data (29–32).

EMG records were obtained from surface disk and multiunit needle electrodes placed into the lateral muscle mass of the hindlimb. Raw EMG records were recorded before, during, and after continuous motor pathway stimulation and for 2 hours thereafter at 15 minute intervals. These records of 100 msec duration, sampled at a rate of 1/sec for 1 minute were averaged, and stored using an electromyographic recording system (TECA II). The number of spikes per second was tabulated from the raw EMG signals. Averaged EMG amplitude, and signal area was also analyzed. Using the same monitoring system, H-reflexes were recorded from the plantar surface of the foot using

surface disk electrodes in response to stimulation of the tibial nerve. M and H waves were measured in response to graded increments in stimulus intensity, from 0 mA to 20 mA. H/M curves were constructed based on peak waveform amplitudes. Signals were recorded before ischemic spinal cord injury, and then daily until the infusion protocol began. H-reflexes were then recorded just before and 4 hours after continuous infusion of the medication.

At the conclusion of the experiment the animals were transcardially perfused with heparinized saline and euthanized. The spinal cords were quickly removed and were placed in 10% buffered formalin for at least 24 hours prior to paraffin sectioning and hematoxylin-eosin and luxol fast blue staining for histopathological verification of the lesion. Data from experiments were analyzed using repeated measures analysis of variance (ANOVA). Significance was defined as $p < 0.05$.

RESULTS

Results from the present study indicate that the degree of EMG activity recorded from hindlimb musculature in the injured animal reflects the degree of spasticity. An example of these data is shown in Figure 1. The more complex and active the EMG record, the more spastic the animal. In addition, the amplitude and duration of the averaged EMG parallel the degree of spasticity. However, H-reflex records recorded from the plantar surface of the hind paw in response to tibial nerve stimulation were more sensitive to subtle changes in muscle hypertonia than either raw or averaged EMG records. An example of these data is shown in Fig. 2. Raw EMG data as well as averaged EMG signals was difficult to evaluate, lacked the quantitative precision of the H/M curve, and were not used in the final analysis of these data.

To produce the H/M curve, stimulus intensities were increased in a sequential fashion from 0 to 20 mA and the amplitudes of the elicited H and M waves were plotted as a ratio (H/M). The M waves, representing direct muscle stimulation, are not significantly altered in amplitude once the applied stimulus intensity is sufficient to produce a maximum contractile response. The H wave amplitudes, which reflect central excitability, are altered in proportion to the M wave depending upon the level of central excitability, or spasticity. Therefore, variation in the H/M ratio is produced reflecting heightened or suppressed segmental neuronal activity. Pharmacologically induced change in electrophysiologic measurements are presented in Fig. 3 and are plotted as H/M curves.

Glycine and D-serine depressed the H wave in spastic animals after 4 hours of infusion compared to artificial CSF. The primary effect of glycine was at low intensity tibial nerve stimulation, approximately 0.5–1.0 mA. However, D-serine, a powerful glycine analog, reduced the H/M ratio through a wide range of stimulus intensities (0.5–10 mA). Strychnine produced heightened H waves compared to artificial CSF infusion. The influence of strychnine on the H wave was most prominent using intensities ranging 0.5–4 mA. Infusion of an n-methyl-D-aspartate receptor blocker, MK-801, had no clear effect when low stimulus intensities were applied. However, at higher intensities the H/M ratios were reduced compared to infusion of artificial CSF. Likewise, another less powerful glycine analog, β-alanine, did not produce significant alteration in the H/M ratio. No changes in H wave amplitudes were seen by any infused compound when the highest intensities were used (above 10 mA), compared to artificial CSF.

DISCUSSION

Nearly 430,000 Americans sustain a serious head injury and approximately 20,000 more suffer from spinal cord injury each year (33). Many thousands more live with disabilities caused by cerebral palsy, stroke, multiple sclerosis, benign spine disease such as spondylosis, and degenerative disorders such as Parkinson's disease (33,34). Spasticity is a prominent feature associated with these neurological injuries and diseases. Spasticity is a highly complex expression of neuronal activity following interruption of normal communications between cerebrum and peripheral nerves (35–38). Several neurophysiologic mechanisms have been proposed to account for the increased muscle tone seen in spastic patients (39). Lack of descending inhibitory control of efferent output and/or exaggerated afferent input leading to an imbalance of inhibitory and excitatory activity, as originally proposed by Sherrington, is accepted as the general mechanism of spasticity (40).

Glycine is an abundant postsynaptic inhibitory amino acid neurotransmitter associated with these inhibitory interneuron pools (9–12,41,42). The role of glycine as an inhibitory neurotransmitter was initially proposed by Aprison in 1965, and since that time it has been established as the chief postsynaptic inhibitory neurotransmitter in the spinal cord (43,44–46). Autoradiographic and immunohistochemical studies show glycine to be closely associated with medial and, in particular, ventral interneuron pools (47–50). Glycine is liberated from cells corresponding in location to electrophysiologically defined Renshaw cells, and Ia and Ib interneurons (1,39,43,51,52). These segmental interneurons play a key role in mediating inhibitory control and govern the ultimate magnitude and quality of a motor response (2,3,40). As with γ-amino butyric acid (GABA), glycine

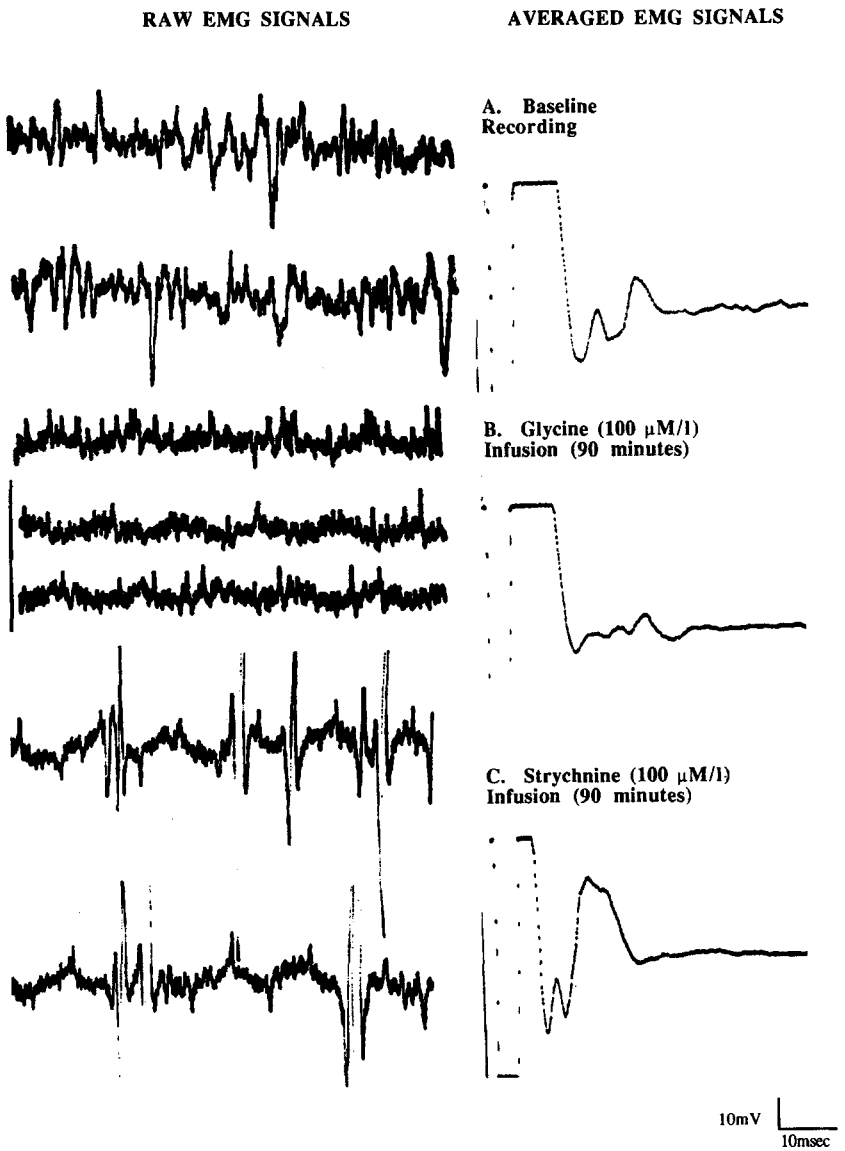

Fig. 1. Raw and averaged EMG recorded from the lateral muscle mass of the leg in response to noxious stimulation applied to the foot pad.

containing interneurons are also located within the dorsal horn (53,54) and may act in concert with GABA to modify peripheral afferent activity.

Results from earlier pilot studies, using microdialysis techniques, revealed that the segmental extracellular concentration of glycine was dramatically increased in response to spinal cord injury (55). In addition, sampling performed in 3 day post-ischemia animals revealed that injuries producing spasticity do not have a significant elevation in baseline glycine levels whereas animals sustaining severe, flaccid injuries had baseline glycine levels more than twice that of control pre-ischemia animals (30). These data indicate that glycine, in high concentration, may have played a role in suppressing residual motor activity. Also as part of the pilot investigation, glycine was found to be significantly elevated within 90 minutes of epidural spinal cord or peripheral afferent nerve stimulation (28), which are common methods used with modest success for treating patients with spasticity. These data indicate that glycine is released into the segmental extracellular space in response to stimulation of local segmental neural structures and, again, glycine may act to reduce spasticity.

The current study demonstrates that spasticity can be reduced by administration of glycine, or a closely related compound D-serine and by administration of the

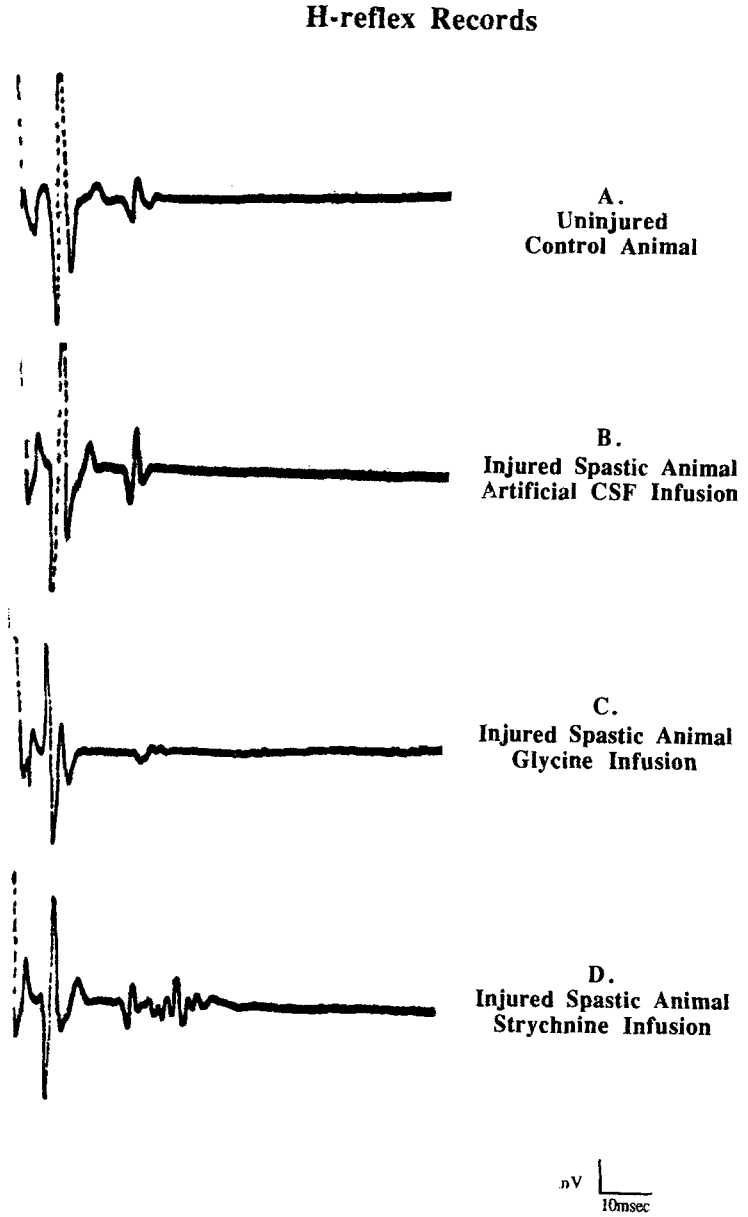

Fig. 2. H-reflexes recorded from the plantar surface of the hindlimb in response to tibial nerve stimulation.

NMDA channel antagonist, MK-801. Conversely, blockade of glycine mediated chloride channels with the selective antagonist strychnine amplified spasticity and, in addition, spasticity can be produced by nonspecific NMDA channel stimulation (56–59). Glycine acts on postsynaptic neuronal membranes through two receptor subtypes, a strychnine sensitive chloride channel (Gly 1) producing membrane hyperpolarization and a much smaller population of NMDA sensitive channels (Gly 2) mediating calcium flux and producing membrane depolarization (5,60–62). Both subtypes of glycine receptors are likely to be involved in the mechanisms responsible for spasticity. Gly 1 receptor activation may reduce muscle tone in response to low intensity afferent activation whereas NMDA receptor blockade may reduce muscle tone in response to high intensity afferent activation.

The dual role of glycine in the spinal cord as it pertains to spasticity has not been clearly deciphered using combinations of Gly 1 and Gly 2 agonists and antagonists (63–65). Determining the role of this particular neurotransmitter provides a unique opportunity to explore the relative contributions of two important glycine

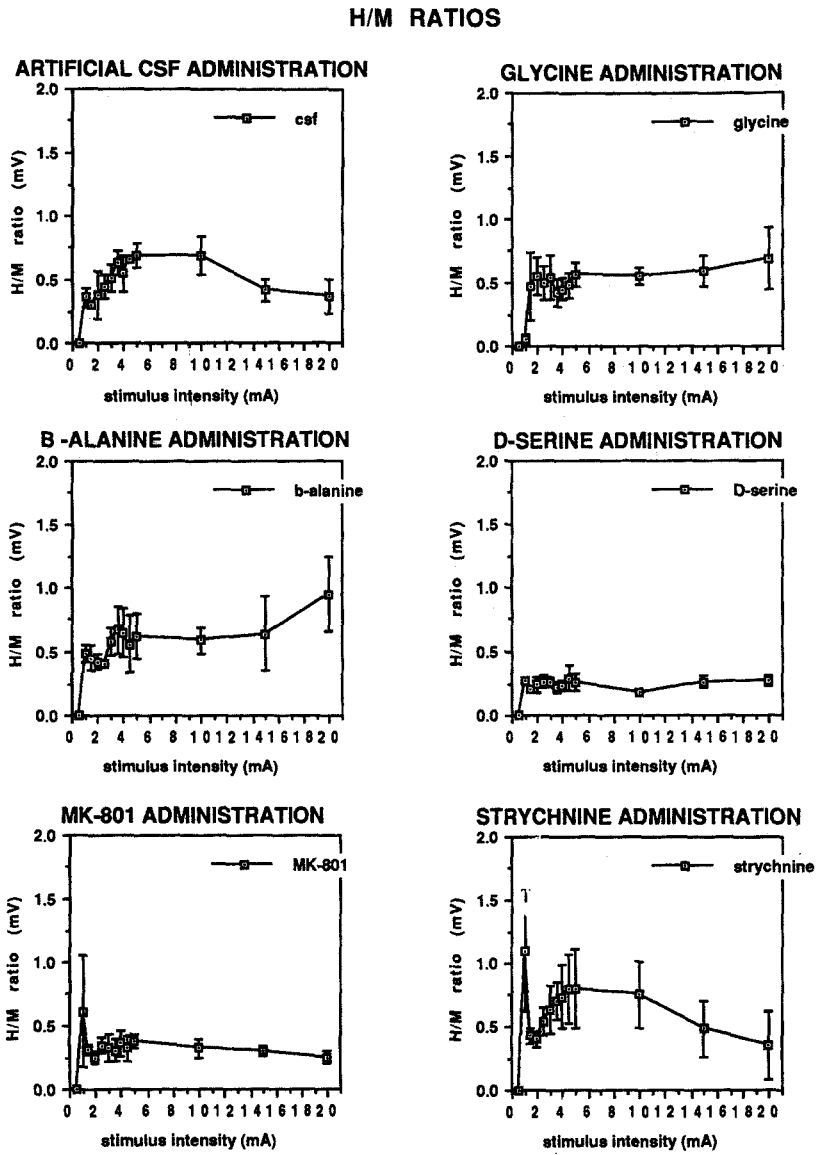

Fig. 3. H/M ratios calculated in response to increasing stimulus intensities. Data plotted as an H/M curve with standard error bars.

receptor subtypes to the production of spasticity (66–69). Clearly glycine is not the only neurotransmitter involved in the production of spasticity. Catecholamines, GABA, endogenous opiates, and possibly excitatory amino acids are likely to be involved (70–76). Glycine, however, because it has been established as the chief postsynaptic inhibitory neurotransmitter of the spinal cord and is closely associated with the modulation of segmental efferent activity within the ventral horn and afferent activity within the dorsal horn, may be a suitable compound to develop as a potential therapeutic agent for patients suffering with spasticity.

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