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Functional Changes of Spinal Sensory Neurons Following Gray Matter Degeneration

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=ABSTRACT=

Excitatory amino acids (EAA) are thought to play an important role in producing cell death associated with ischemic and traumatic spinal cord injury. The present study was carried out to determine if the response characteristics of spinal sensory neurons in segments adjacent to degeneration sites induced by EAA are altered following these morphological changes.

Intraspinal injections of quisqualic acid (QA) produced neuronal degeneration and spinal cavitation of gray matter. The severity of lesions was significantly attenuated by pretreatment with a non-NMDA antagonist NBQX. In extracellular single unit recordings, dorsal horn neurons in QA injected animal showed the increased mechanosensitivity, which included a shift to the left in the stimulus-response relationship, an increased background activity and an increase in the duration of after-discharge responses. Neuronal responses, especially the C-fiber response, to suprathreshold electrical stimulation of sciatic nerve also increased in most cases. These results suggest that altered functional states of neurons may be responsible for sensory abnormalities, e.g. allodynia and hyperalgesia, associated with syringomyolia and spinal cord injury.

Key Words: Spinal cord, Neurotoxicity, Excitatory amino acid, Allodynia

INTRODUCTION

It is well documented that the transmission of pain impulses to brain is integrated in the spinal gray matter under the influence of descending inhibitory signals from midbrain and pontomedullary nuclei (for reviews, Basbaum & Fields, 1984; Willis 1985; Fields, 1987). Lesions and diseases of the neuraxis involved in nociception are known to cause what is generally called "central pain syndrome (CPS)", which is characterized by severe, often excruciating, persistent dysesthetic pain not infrequently associated with bouts of lancinating pain and with sensory and somatomotor dysfunction(for reviews, Casey, 1991). Focusing on the spinal cord injury(SCI), it was clinically investigated that more than half of traumatic SCI patients complained of chronic pain, which developed within one year of injury, at or below the level of injury (for reviews, Davidoff & Roth, 1991). Also in animal studies, it has been well documented that many lesions of spinal cord are accompained with hyperalgesia or allodynia (Vierck et al, 1971; Davies et al, 1983; Hao et al, 1991b). Until now most experiments were conducted in a way where the subjects showed increased behavioral response to noxious stimulation after the lesion. On the other hand, alteration of electrophysiological responses of spinal sensory neurons resulting from SCI has not been extensively

studied, especially in cases of dorsal horn degeneration without involvement of spinal white matter, although altered dorsal horn processing following injury was suggested(Dubner, 1991).

Recently, several lines of evidence have been accumulated that suggest an important role of excitatory amino acid(EAA) in neuronal degeneration associated with ischemic and traumatic SCI (Faden et al, 1990; Panter et al, 1990; Simpson et al, 1990). EAAs are canidates for fast-excitatory neurotransmitters released at the primary afferent synapse(Aanonsen et al, 1987; Schneider & Perl, 1988; Jeftinija, 1989). Antagonists of EAA receptors such as MK-801(dizocilpine maleate) are known to reduce the severity of neurologic deficit associated with experimental SCI (Yum and Faden, 1990; Hao et al, 1991a), and block the development of hyperalgesia induced by spinal cord ischemia(Hao et al, 1991c). However, most of previous studies focused on the role of NMDA(N-methyl-D-aspartate) receptor and its antagonist.

The present study was carried out to investigate the effect of intraspinal injections of exogenous quisqualic acid (QA), an agonist of AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolpropionate) receptor and to evaluate the functional changes of spinal sensory neurons in region nearby gray matter lesion site.

METHODS

Animal preparation

Male Sprague-Dawley rats (250～350 g) were anesthetized with a mixture of ketamine, Acepromazine and Rhompum (0.5～0.7 ml/kg, s.c.). Following exposure of T_{11}-L_1 spinal segments via partial laminectomy, small holes were made in the dura and pia mater. Intraspinal injections of 60 mM or 120 mM QA were made using Hamilton microliter syringe attached to a micromanipulator (David-Kopf) into depth ranging 500～1200 μm below the surface of spinal cord. The total volume injected in each animal ranged from 0.3 to 0.8 μl. In 3 control animals, 0.5 μl of sterile normal saline was injected in the same way. Following injections a piece of Durafilm (Codman & Shurtleff Inc.) was positioned over the exposed cord, and the overlying muscles and skin were sutured.

Neuronal recording

Extracellular single-unit recordings were made from dorsal horn neurons in normal and QA injected animals anesthetized with urethane (1.25 g/kg). In QA group, recordings were performed 20～30 days after the QA injection. Tracheotomy and cannulations of external jugular vein and carotid artery were performed and the animals were artificially ventilated after an intravenous injection of pancuronium bromide. Arterial blood pressure and core temperature were monitored and maintained within physiological limits throughout the recording experiments. Following the holding of the animals on the stereotaxic apparatus, laminectomy was performed between T_{11} and L_2 vertebra. The dura was opened over this area and a mineral oil pool was formed around the spinal cord. In QA animals recordings were made 2.5 mm caudal to injection sites. In all animals recording sites were located in laminae II-VII of L_2-L_5 spinal segments. Spinal single cell units were recorded through carbon filament electrode (tip resistance, 1～4 MΩ). The recorded signal was amplified with AC differential amplifier (DAM 80, WPI), monitored on oscilloscope (5113, Tektronix), fed into window discriminator (WPI), and stored on a personal computer through a A/D interface (CED 1401 plus). The responses of neurons to mechanical stimulation of their cutaneous receptive field were characterized by applying graded mechanical stimuli, including calibrated von Frey filaments.

Histology

After completing electrophysiological study of the cell, electrolytic lesion was made (DC current of

100~200 μA, 20~30 sec duration) for histological identification of recording site. The animals were perfused transcardially with 1% solution of potassium ferrocyanide in 10% formalin. The spinal cord was removed and post-fixed for 3~5 days. Frozen sections were cut at 50 μm thickness and stained with cresyl-violet. Evaluation of the degree of lesion induced by QA injection was performed according to following criteria: grade 1, loss of less than 25% of gray matter as compared with control (un injected) side; grade 2, 25~50%; grade 3, 50~75%; grade 4, more than 75%. A degeneration score was calculated by summation of the grades of all sections of each animal.

Drug treatment

MK-801 (RBI) and NBQX (2,3-hydroxy-6-nitro-sulfamoyl-benzo(F)-quinoxaline, Novo-Nordisk) were used as antagonist of NMDA receptor and AMPA receptors, respectively. NBQX (30 mg/kg) and MK-801 (5 mg/kg) were administered intraperitoneally in a split-dose protocol with half given 20 min prior to and the rest 10 min after QA injection.

Statistics

Results are illustrated as mean ± S.E.M. Statistical comparisons were performed by using Student's t-test and Chi-square test. $P<0.05$ was used for significance.

RESULTS

Histologic study

All QA injected rats had areas of selective degeneration in the dorsal and central part of spinal gray matter. In 8/11 QA-injected rats, spinal cavity restricted in the gray matter were observed (see Fig. 1B), whereas the others showed a shrinkage of dorsal horn (Fig. 1A). No significant degenerative changes in the gray matter were observed in cresyl violet-stained sections of spinal cord of the control animals injected with sterile normal saline. The severity of lesion in QA-injected spinal cord extended in a graded fashion rostral and caudal from the epicenter of injction site which showed maximal cell loss. In general, the extent of degeneration was dependent on the depth, volume and concentration

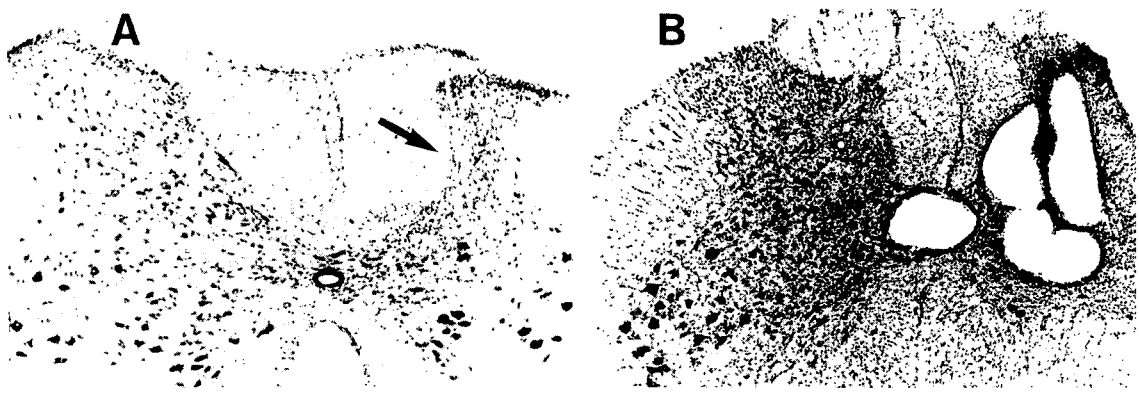

Fig. 1. Effects of intraspinal injections of quisqualic acid (QA) on neurons in spinal gray matter of the rat. A, unilateral neuronal degeneration following the injction of 0.4 ul of 60 mM QA into depth of 600 μm below the cord surface (26 day survival). Note the shrinkage (arrow marked area) of dorsal horn on the side of QA injection. B, neuronal degeneration and spinal cavitation following two injections of 0.3 ul of 120 mM QA into depths of 600 μm and 1200 μm (28 day survival).

of injected QA. The average rostrocaudal extent of gray matter loss in QA-injected animals was 1.61± 0.19 mm with degeneration score of 87.5±9.5, which was significantly reduced by pretreatments with NBQX (30 mg/kg). (Table 1).

Electrophysiologic study

Single unit recordings were made from 180 neurons, 89 of which were recorded in QA injected animals. No significant differences were found in the composition of both group neurons according to response profiles (Table 2) and laminar distribution of recording sites (Fig. 2). Most of neurons recorded in this study were wide dynamic range (WDR) cells located in dorsal horn of L_3-L_4. An example of typical response of WDR cells in control and QA group were shown in Fig. 3.

In QA-injected animals, WDR cells activity recorded in segments adjacent to degeneration area showed higher incidence and degree of spontaneous activity (Table 3). Neurons in QA group showed increased mechanosensitivity. In responses to a series of von Frey filaments applied to receptive fields, WDR cells in QA group revealed the lower threshold and the greater magnitude of responses

Table 1. Effect of MK-801 and NBQX on degeneration score and lesion length of gray matter induced by intraspinal QA injection

	Degeneration score	Lesion length (mm)
QA (120 mM, 0.5 μl) (n=8)	87.5±9.5	1.61±0.19
+ MK-801 (5 mg/kg) (n=5)	69.6±8.4	1.34±0.15
+ NBQX (30 mg/kg) (n=5)	36.8±6.9[1)]	0.88±0.16[2)]

1) and 2) represent significant difference at $p<0.01$ and $p<0.05$, respectively.

Table 2. Response profiles of control and QA group neurons

	Control	QA
WDR	51	59
LT	6	8
HT	7	8
Deep/tap	24	11
NR	3	3
	91	89

WDR, wide dynamic range; LT, low-threshold; HT, high threshold; NR, no receptive field found.

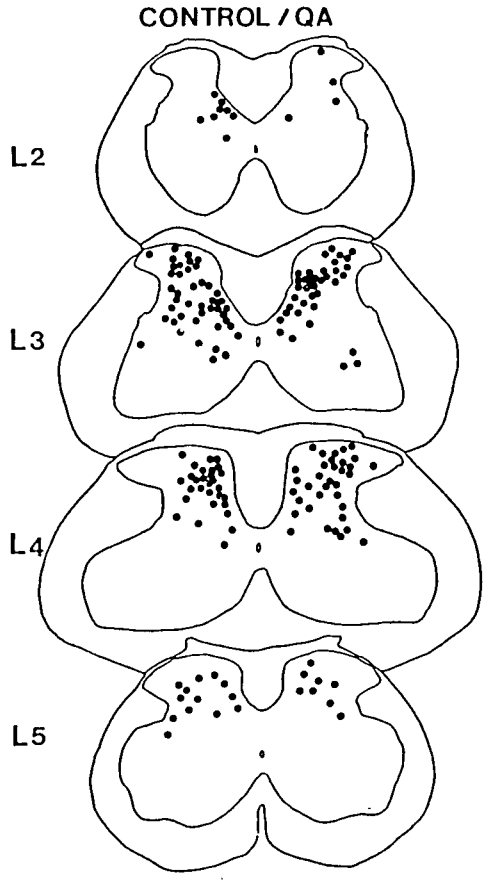

Fig. 2. Histological reconstruction of recording site of spinal sensory neurons. Control and QA group neurons were marked in the left and the right side of gray matter in each diagram, respectively.

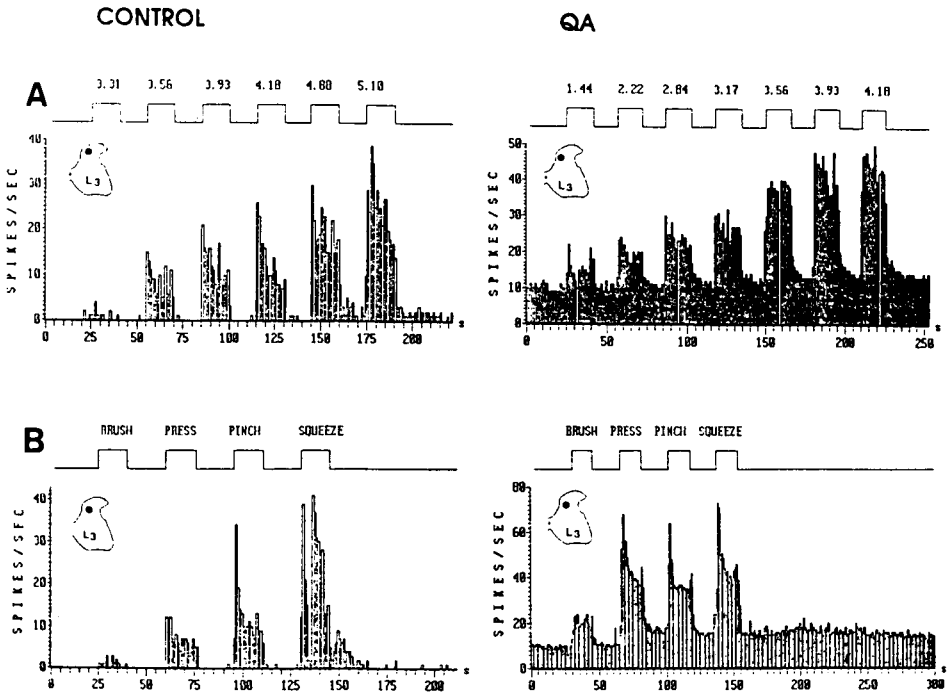

Fig. 3. Typical responses of spinal sensory neurons in control and QA injected animals to calibrated von Frey filaments (A) and natural stimuli (B) applied to the glabrous skin of the ipsilateral foot to intraspinal QA injection. Strength of stimulation is represent in log milligrams in (A).

Table 3. Incidence and degree of spontaneous activity for WDR cells in lamina I-VI

	Control	QA
Incidence of spontaneous activity	14/39	27/44*
Firing rate (spikes/sec)	12.2±2.02	18.7±1.85*

* represent significant difference at $p<0.05$.

(Fig. 4). Furthermore, in an analysis of after-discharge evoked by von Frey filaments at a supra-threshold (445 g) intensity, WDR neurons showed significantly long-lasting, afterdischarges (Fig. 5).

In the responsiveness to the electrical stimulation of sciatic nerve (5 mA, 1 msec), QA group cells showed marked increase in A- and C-fiber responses in comparison with those of control group cells (Fig. 6).

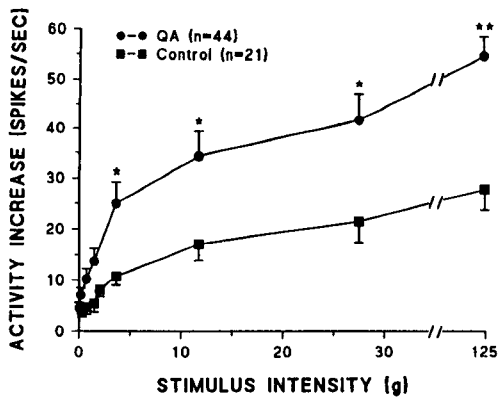

*Fig. 4. Summary of responses of wide dynamic range type neurons to receptive field stimulation with von Frey filaments. * and ** indicate significant difference at $P<0.05$ and $P<0.01$, respectively.*

DISCUSSION

Most of previous studies related to post-lesioned functional changes of spinal sensory neurons used photochemical ischemia (Hao et al, 1991b; Hao et al 1991c), compression (Holtz & Gerdin, 1991), contusion (Faden et al, 1990; Gale et al, 1985; Gómez-Pinilla et al, 1989; Wrathall et al, 1992), and hemisection (Sanner et al, 1994) for inducing spinal cord lesion. But all of these spinal lesions involve white matter as well as gray matter. Therefore, these experimental lesions are not adequate to investigation of selective loss of gray matter, which occurs sometimes as syringomylia-like degeneration. The present study provides direct evidence of functional changes of spinal sensory neurons which could be related with pain symptoms in syringomyelia patients.

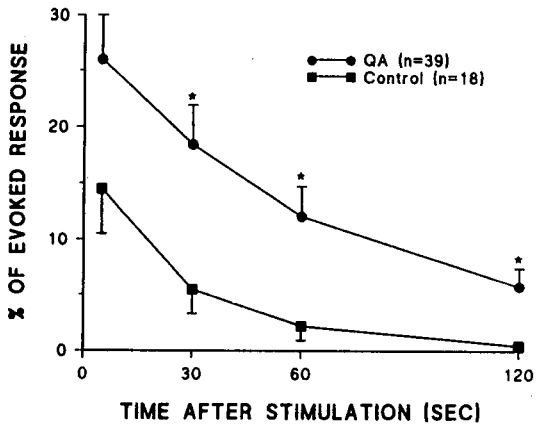

*Fig. 5. After discharges of spinal sensory neurons after 20 second stimulation with suprathreshold (445 g) stimuli in control and QA groups. * indicates significant difference at $p<0.05$.*

An important question in the present study is the effect of QA injection on the morphological and electrophysiological changes of gray matter neurons. Considering that in control saline-injected animals no degenerative findings such as cell loss and cavity formation were observed, it could be confirmed that these changes resulted from intraspinal QA reaction. Consistent with previous report (Yezierski et al,

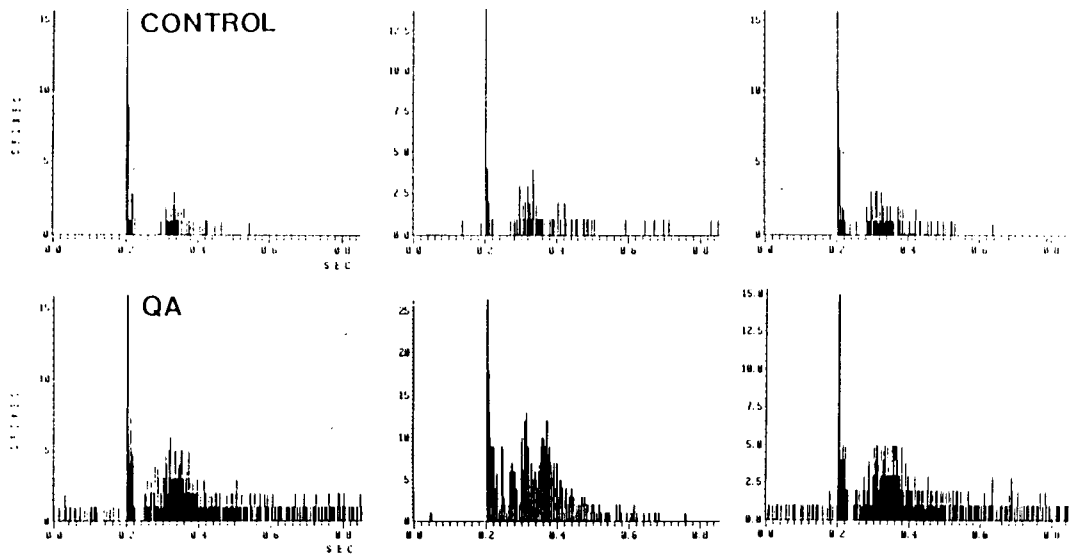

Fig. 6. Peristimulus histogram of dorsal horn neurons activated by electrical stimulation of sciatic nerve (5 mA, 1 msec, 0.5 Hz). Bin width in 2.5 msec, fifteen sweeps in 30 seconds.

1993), it was clear that lesional cavity in the gray matter was seperated from the central canal, although in some cases dilatation of the central canal was observed. This finding suggests that cavity formed in this study is similar to the noncommunicating type of syringomyelia and that lesional cavity results from segmental cell degeneration due to QA effect. In 3/11 QA-injected animals, shrinkage of dorsal horn without cavity formation was observed. Although in all of these 3 rats the dose of QA injected was relatively smaller (60 mM, 0.3 ~0.4 μl) than those of other animals (120 mM, 0.5 ~0.6 μl), it could not be confirmed that dose difference was the crucial factor in developing cavities. Other factors such as injection depth, volume, survival period, and spinal blood flow need to be considered when enough cases are accumulated. Although the pathophysiological mechanism for cavity formation following QA injection is not clear, this study provides further evidence supporting that non-NMDA(AMPA) receptor is involved in cell death in spinal gray matter.

There are at least three subtypes of EAA receptors in the spinal cord, NMDA receptors and two non-NMDA receptors selectively activated by QA and kainate (Watkins & Evans, 1981; Arancio & MacDermott, 1991; Stewart et al, 1991). With the development of pharmacological tools, QA receptor was later renamed as AMPA receptor (Bettler & Mulle, 1995). EAA levels in spinal cord are rapidly affected by traumatic injury (Panter et al, 1990; Liu et al, 1991). By 15 min after SCI, extracellular EAA concentrations can increase to levels that would be toxic to neurons. Thus EAA receptors could mediate some of the secondary pathological processes that occur after SCI. Focusing on the AMPA receptor, application of AMPA agonist to cultured spinal neuron (Stewart et al, 1991) and to spinal cord intrathecally (Nakamura et al, 1994; Brambilla et al, 1996) induced acute degeneration. Consistent with these reports, intraspinal injection of QA in the present study induced gray matter degeneration, which was significantly attenuated by pretreatments with NBQX, selective antagonist of AMPA receptor. These results suggest that activation of AMPA receptor might be at least partly involved in the pathophysiological mechanism in developing degenerative changes in rat spinal cord.

For electrophysiological investigation, we recorded the responses of dorsal horn cells below the level of the lesion, considering that pain symptoms usually develop below the level of SCI. During recording experiment, the boundary of the lesion site could be easily estimated because there was electrophysiologically silent area quite possibly due to neurodegeneration. Most of neurons in segments adjacent lesion site showed changes in neuronal excitability. Results related to these changes included higher tendency of spontaneous acitivty, lower mechanical threshold, increased sensitivity to stimuli and long-lasting after- discharge. These functional changes do not seem to result from direct activation of AMPA receptors by injected QA, considering that there were enough intervals more than 20 days between QA injection and response assessment. On the contrary, it may be reasonable to understand these functional alteration as secondary events following histopathologic changes. It was possible that loss of inhibitory interneurons could be induced through degeneration processes and that secondary changes such as increased concentration of neurotransmitter of nociceptive information could develop in surrounding area. Recent study on the increased distribution of substance P in the dorsal horn below the level of syringmyelia (Milhorat et al, 1996) supports this possibility.

Increased excitability of WDR neurons in QA injected animals much resemble allodynia-like changes after ischemic spinal cord injury in which lesion involves white matter as well as gray matter (Hao et al, 1991b). Therefore, it is estimated that spinal lesion does not have to involve axonal degeneration to induce secondary pain. Although we did not test the thermal hypersensitivity, the increased C-fiber response to electrical stimulation of sciatic nerve and increased firing rate accompained with pro-

longed afterdischarge evoked by noxious mechanical (445 g von Frey filament and squeeze) stimuli strongly suggested that WDR neurons in QA-lesioned animal might have hyperalgesia-like characteristics together with allodynic features.

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