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Short Communication

Aβ and Aδ but not C-fibres are involved in stroke related pain and allodynia: an experimental study in mice

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Abstract

Objectives Cerebral ischaemia is a leading cause of death and disability, including severe complications such as memory disturbance, palsy, and spasticity. Central post-stroke pain (CPSP) is a complication of cerebral ischaemia, and is characterized clinically by spontaneous pain and attacks of allodynia and dysaesthesia. However, the detailed mechanisms of CPSP are not well established. Herein, we have examined alterations of the current stimulus threshold of primary afferent neurons or the nociceptive threshold against mechanical stimuli in mice receiving left middle cerebral artery occlusion (MCAO).

Methods Alterations of current stimulus threshold and the development of mechanical allodynia in hind paws were measured after MCAO using a Neurometer and the von Frey filament test, respectively.

Key findings Development of cerebral infarction was clearly observed on day 1 and day 3 after MCAO. For the estimation of current stimulus threshold measured by the Neurometer, the sensitivity of Aδ and Aβ fibres (at 2000 and 250 Hz stimulation, respectively) was significantly increased on day 3 after MCAO, while that of C fibres (at 5 Hz stimulation) was unaltered. In addition, the paw withdrawal threshold of the left hind paw as measured by the von Frey filament test was significantly decreased on day 1 and day 3 after MCAO when compared with day 0, while that in the right hind paw was not different.

Conclusions The data suggested the development of bilateral hyperaesthesia in this model. Further, mechanical allodynia developed in the ipsilateral side to the MCAO. Potentially, myelinated A fibre-specific hypersensitization after stroke may have contributed to these symptoms.

Keywords central post-stroke pain; cerebral ischaemia; current stimulus threshold; mechanical allodynia; primary afferent neuron

Introduction

Focal cerebral ischaemia (stroke) is a major cause of death and disability including paralysis and memory disturbance.[1] Pain, characterized as spontaneous pain and evoked pain, is a refractory complication of stroke that markedly decreases the quality of life for patients.[2] Central post-stroke pain (CPSP) is observed in some patients with a low responsiveness to opioids or non-steroidal anti-inflammatory drugs. Further, clinically approved adjuvant analgesics such as antidepressants and anticonvulsants are only partially or noneffective depending upon the individual, while nonpharmacotherapies such as surgical operation or stimulation therapy do not completely prevent damage.[2,3] Although development of improved therapeutics is required, the detailed mechanisms of CPSP are largely unknown. As studies in animal models of ischaemia do not describe development of CPSP under ischaemic stress, in this study we have examined CPSP in middle cerebral artery occlusion (MCAO) mice, a popular model in experimental stroke research that causes prominent ischaemic damage.[4–6] As the development of neuronal hyperexcitability or sensory abnormality is involved in neuropathic pain syndrome, we focused on post-stroke alterations of sensitivity of primary sensory neurons including A and C fibres, and alterations of the pain threshold against mechanical stimuli.[7]

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Materials and Methods

Animals

Experiments were performed on male ddY mice (5-weeks-old) obtained from Japan SLC (Osaka, Japan). Animals were housed at 23–24°C with a 12 h light–dark cycle (lights on 0800 h to 2000 h). Food and water were freely available. This study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, adopted by the Japanese Pharmacological Society. In addition, all experiments were approved by the Ethical Committee for Animals of Kobe Gakuin University (approval number: A 060601-10).

Middle cerebral artery occlusion mouse model

Transient focal cerebral ischaemia was induced by MCAO as described previously.[8] Briefly, under 2% isoflurane anaesthesia (Abbott Japan, Osaka, Japan), a 8-0 nylon monofilament (Shirakawa, Fukushima, Japan) coated with silicon resin (Provil novo Medium; Heraeus Kulzer, Hanau, Germany) was inserted through a small incision into the left common carotid artery (occlusion) for 2 h. Sham-operated mice were subjected to the procedure without MCAO. Relative cerebral blood flow was measured by laser Doppler flowmetry (TBF-LN1; Unique Medical, Osaka, Japan) to assess the extent of the vascular occlusion and reperfusion, as described previously.[8]

Measurement of infarct volume

After brains were removed, coronal sections were cut 2-mm thick and incubated in saline containing 2% 2,3,5-triphenyltetrazolium chloride (Sigma, MO, USA) at 37°C for 10 min. After being fixed with 4% paraformaldehyde (Sigma), infarct areas were measured using image analysis software (Image J; NIH, MD, USA) and Adobe Photoshop Elements 5.0 (Adobe Systems Incorporated, Tokyo, Japan). The infarct volume was calculated based on infarct area and intensity.

Electrical stimulation-induced paw withdrawal test

The assessment of current stimulus threshold in response to transcutaneous constant electrical stimulation was conducted using the Neurometer (Primetech Co., Tokyo, Japan).[9,10] Electrodes were lightly touched to the plantar surface and instep of mice, and three sine-wave pulses (at 2000, 250 or 5 Hz) were applied. The minimum intensity (μA) at which each mouse withdrew its paw (behaviour such as stretching their plantar surface or briefly trembling their paw immediately after stimulation) was defined as the threshold.

Mechanical allodynia (von Frey test)

The assessment of paw withdrawal threshold (PWT) in response to mechanical stimulation was performed using von Frey filaments (North Coast Medical, Inc., CA, USA).[11,12] At least 30 min before the test, mice were placed in individual plastic boxes on a raised metal mesh. The plantar surface of the paw was stimulated with each filament (with a range between 0.07 and 2 g). For each filament, the stimulus was repeated five times with an interval of 1–2 s between each stimulus. The PWT was determined as the lower force that evoked a withdrawal response to three of the five stimuli.

Statistical analysis

The data for the current perception threshold were analysed using one-way analysis of variance followed by Scheffe's test. Data are presented as means ± SEM. The data for PWT in response to mechanical stimulation were analysed using a Steel-Dwass test of post-hoc nonparametric multiple comparison tests. Data are presented as medians (25th–75th percentile). The differences were regarded as statistically significant when the *P*-value was less than 0.05.

Results

The infarct volume gradually enlarged from day 1 (131.2 ± 48.0 (infarct area × mm) (× 10000)) to day 3 (197.9 ± 42.6 (infarct area × mm) (× 10000)) after MCAO (Figure 1a and 1b).

In the right hind paw, the current stimulus threshold at 5 Hz (i.e. C fibre stimuli) in the MCAO group determined on day 3 after MCAO was similar to that before MCAO; similar results were obtained in the sham group. By contrast, the current stimulus thresholds at 250 Hz (i.e. Aδ fibre stimuli) and at 2000 Hz (i.e. Aβ fibre stimuli) were decreased significantly compared with those before MCAO (Figure 2a); there were no changes in the sham group. In the left hind paw, the current stimulus threshold was significantly decreased on day 3 after MCAO at 250 and 2000 Hz stimuli compared with those before MCAO (Figure 2b); no changes were observed at 5 Hz.

On day 1 and day 3 after MCAO, the PWT of the right hind paw against mechanical stimuli did not change compared with that before MCAO (Figure 3a). However, in the left hind paw, the PWT in the MCAO group significantly decreased compared with that before MCAO (Figure 3b). There were no changes in PWT against mechanical stimuli between pre and post MCAO in the sham group (Figure 3a and 3b).

Discussion

Although there are many reports about the neuronal damage in focal cerebral ischaemic model mice, very few papers focus on the CPSP.[4,5] Recently, Wasserman and Koeberle[13] reported the development of CPSP in the haemorrhagic rat model, however, no one had shown it in the typical ischaemic model mice i.e. MCAO model mice.[8] In this study we determined post-stroke alterations of current stimulus threshold in hind paws that showed changes in responsiveness to primary afferent neurons. The current stimulus threshold was measured using the Neurometer, a clinical device for measuring perception and pain thresholds.[14] In addition, this device has been verified to provide selective stimulation for three subsets of nerve fibres of different diameters in humans and animals.[9,10,15,16] The pulses at 2000, 250 or 5 Hz primarily stimulate large myelinated Aβ, small myelinated Aδ or small unmyelinated C fibres, respectively.[17–21] In this study, the current stimulus thresholds in myelinated Aβ or Aδ fibres, but not in C fibres, were significantly decreased by cerebral stoke, suggesting that A fibre-mediated sensory responses to external

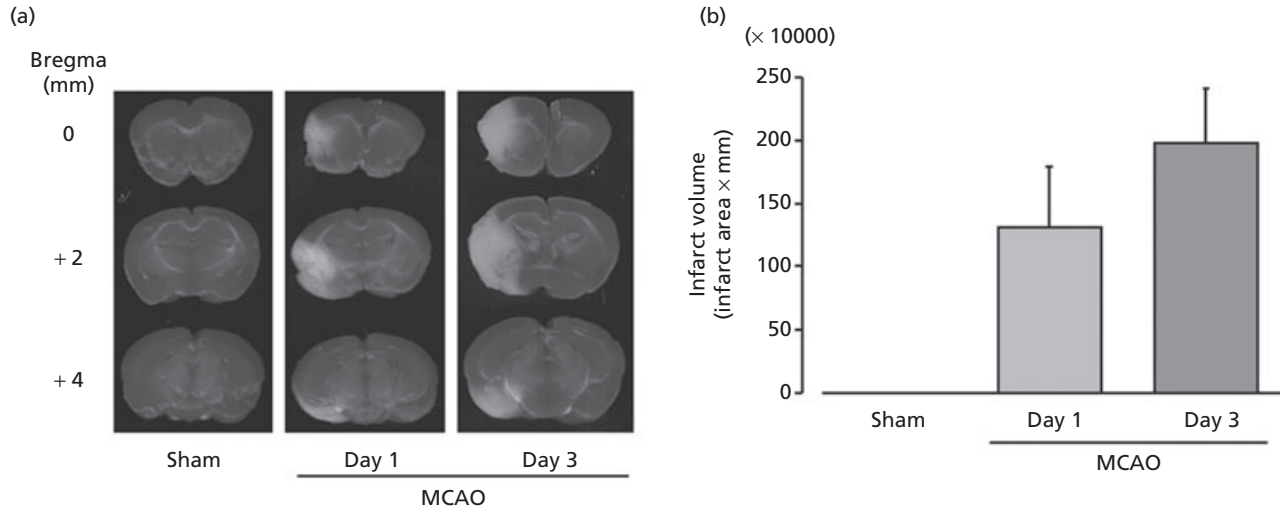

Figure 1 Development of neuronal damage after cerebral stroke in mice. (a) Typical image of 2,3,5-triphenyltetrazolium chloride- staining at 0, +2, +4 mm coronal sections from bregma on day 1 and day 3 after middle cerebral artery occlusion (MCAO). (b) Quantitative analysis of infarct volume. Date are mean ± SEM ($n = 5–12$).

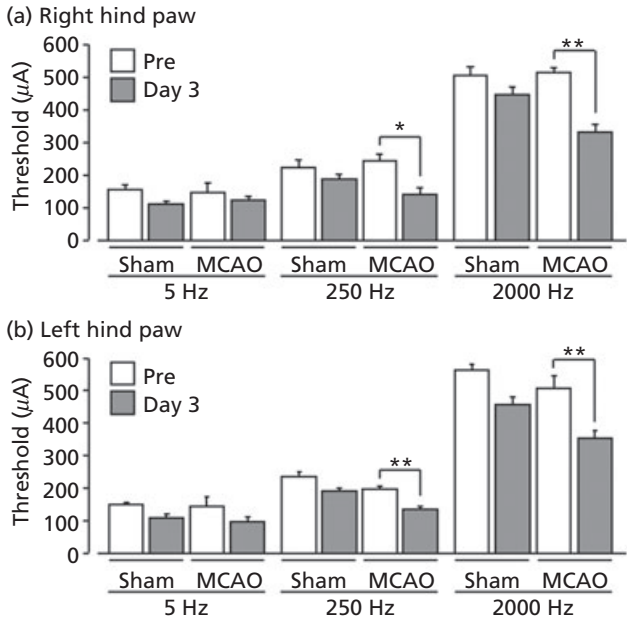

Figure 2 Determination of current stimulus threshold using an electrical stimulation after cerebral stroke in mice. The change in current stimulus threshold was measured with the Neurometer at 5, 250 and 2000 Hz on day 3 after middle cerebral artery occlusion (MCAO). 'Pre' indicates measurement before MCAO. Data are mean ± SEM (sham: $n = 6–8$; MCAO: $n = 4–8$). $^{**}P < 0.01$, $^{*}P < 0.05$, Scheffe's test.

stimuli may be enhanced. These data were supported by previous observations in the partial sciatic nerve ligation model (model of peripheral neuropathic pain) where hypersensitization developed only in myelinated A fibres, while the responses in C fibres were blunted.[10]

Interestingly, in this study an A fibre-mediated hyperaesthesia developed in the bilateral hind paws, despite induction of cerebral infarction only in the left hemisphere. As previously reported, neuronal activation may occur in both the ipsilateral and contralateral hemispheres after cerebral stroke.[22] Furthermore, our findings that both the ipsilateral and contra lateral sides show altered hyperalgesia suggested an involvement of a circulatory component in the post-stoke allodynia. In addition, some circulatory components including inflammatory cytokines such as interleukin-1β and tumour necrosis factor-α or chemokines have been reported to be involved in the development of neuropathic pain.[23–25] Therefore, it was possible that some circulatory components including inflammatory cytokines or chemokines may have been involved in the mechanisms of development of hypersensitization of the Aβ and Aδ fibres. These phenomena may have contributed to the bilateral hyperaesthesia in the bilateral hind paws in this study. A hypersensitization of primary nociceptive or non-nociceptive afferent A fibres is known to cause abnormal sensory phenomena, including tactile allodynia (the perception of normally innocuous stimuli, touching and brushing), hyperalgesia (a heightened response to painful stimuli) and spontaneous pain.[26] In this study, allodynia to mechanical stimuli developed in the left hind paw on day 3 after MCAO, suggesting that mechanisms such as demyelination or induction of the calcium channel $\alpha 2\delta$-1 subunit (Ca$\alpha 2\delta$-1) or the sodium channel 1.3 subunit ($Na_v1.3$) in myelinated A fibres may have been involved in the specific hyperexcitability.[27,28] Further studies are required to clarify the involvement of these molecules in post-stroke allodynia.

In contrast, the PWT against mechanical stimuli in the right hind paw did not decrease after MCAO. Although clinical studies suggested that the sensory symptoms of CPSP were usually contralateral to the stroke, there was some evidence to suggest that CPSP could occur on both ipsilateral and contralateral sides.[2] Further, since the signs and symptoms of CPSP varies with individuals, further studies are required to determine and characterize pain-like symptoms using other stimuli including heat or cold stimuli.[2,13]

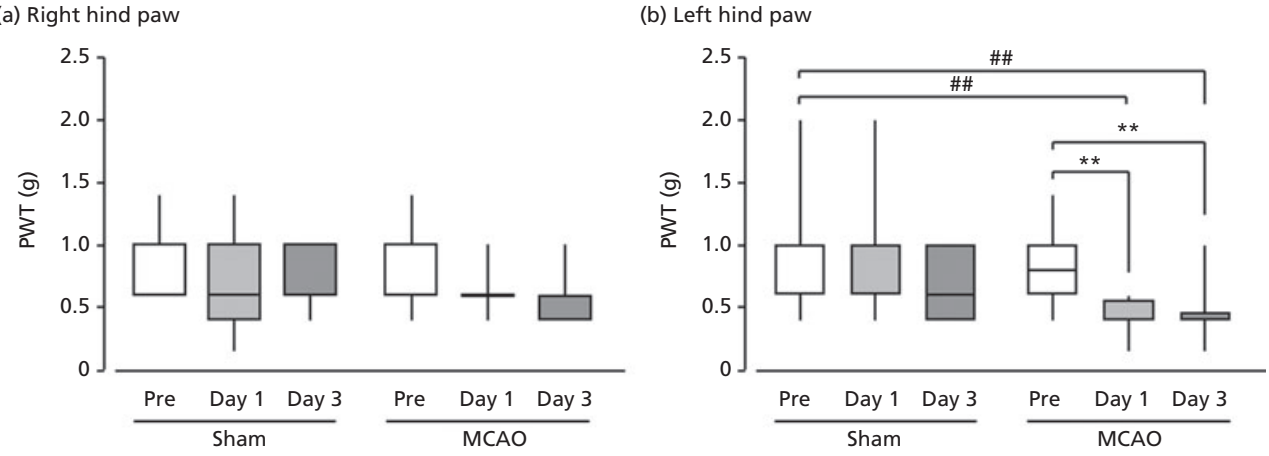

Figure 3 Determination of development of mechanical allodynia after cerebral stroke in mice. The time-dependent change of pain threshold after middle cerebral artery occlusion (MCAO). Paw withdrawal threshold, PWT. 'Pre' indicates measurement before MCAO. Data are shown as boxes containing the values between the 25th and 75th percentile, the line across the boxes represents the medians, and the whiskers extend to the highest and lowest values (sham: $n = 13–28$; MCAO: $n = 10–22$). $^{**}P < 0.01$, $^{\#\#}P < 0.01$ Steel-Dwass test of post-hoc nonparametric multiple comparison tests.

In general, neuropathic pain can involve peripheral sensitization, hypersensitization of primary sensory neurons in the peripheral nervous system, and central sensitization, a hypersensitization of nociceptive neurons in the spinal cord and brain.[29,30] Potential mechanisms that may contribute to central sensitization include increased N-methyl-D-aspartic acid (NMDA) and α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor-mediated excitatory synaptic transmission in the dorsal horn; a decrease in γ-aminobutyric acid (GABA) and glycine receptor-mediated inhibitory synaptic transmission; an increase in descending facilitation;[31–33] it is possible these mechanism may be involved in the development of post-stroke allodynia observed in this study. It is reported that the increase of synaptic glutamate or excess influx of calcium ion into neuronal cells following NMDA-receptor activation significantly exacerbated the development of infarction.[34,35] As described above, similar molecules are known to be involved in the mechanism of development of pain.[25,36] Thus, some drugs such as NMDA-receptor antagonists and calcium channel blockers would be effective in inhibiting the development of infarction as well as development of post-stroke allodynia.

Clinically, the appearance of CPSP is considered to result from damage to the thalamus, although damage to the suprathalamic brain regions is involved also.[7,37] Multiple brain regions were damaged in our ischaemic model, and neuronal damage/infarction was detected in the cerebral cortex, thalamus and amygdale (sites involved in the regulation of pain). We suggest that functional alterations in these brain regions may play a role in the development of hypersensitization of primary afferent neurons (especially A fibres) or of the ipsilateral allodynia in this model.

Conclusions

We have demonstrated the development of hypersensitization of the primary afferent neurons specifically with myelinated Aβ or Aδ fibres and the development of ipsilateral mechanical allodynia following transient cerebral ischaemia in mice. These data provide further understanding of the mechanisms of CPSP and may help in the development of novel therapeutic drugs/strategies for the treatment of CPSP.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Shuaib A *et al*. NXY-059 for the treatment of acute ischemic stroke. *N Engl J Med* 2007; 357: 562–571.
2. Kumar B *et al*. Central poststroke pain: a review of pathophysiology and treatment. *Anesth Analg* 2009; 108: 1645–1657.
3. Pickering AE *et al*. Analgesia in conjunction with normalisation of thermal sensation following deep brain stimulation for central post-stroke pain. *Pain* 2009; 147: 299–304.
4. Dirnagl U *et al*. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci* 1999; 22: 391–397.
5. Murphy TH, Corbett D. Plasticity during stroke recovery: from synapse to behaviour. *Nat Rev Neurosci* 2009; 10: 861–872.
6. Yiming L *et al*. Neuroprotective effects of breviscapine against apoptosis induced by transient focal cerebral ischaemia in rats. *J Pharm Pharmacol* 2008; 60: 349–355.
7. Kim JS. Post-stroke pain. *Expert Rev Neurother* 2009; 9: 711–721.
8. Harada S *et al*. Preventive effect of Morinda citrifolia fruit juice on neuronal damage induced by focal ischemia. *Biol Pharm Bull* 2009; 32: 405–409.
9. Kiso T *et al*. Neurometer measurement of current stimulus threshold in rats. *J Pharmacol Exp Ther* 2001; 297: 352–356.

10. Matsumoto M *et al*. Pharmacological switch in Abeta-fiber stimulation-induced spinal transmission in mice with partial sciatic nerve injury. *Mol Pain* 2008; 4: 25.
11. Zhao FY *et al*. GW406381, a novel COX-2 inhibitor, attenuates spontaneous ectopic discharge in sural nerves of rats following chronic constriction injury. *Pain* 2007; 128: 78–87.
12. Marcil J *et al*. Antinociceptive effects of tetrodotoxin (TTX) in rodents. *Br J Anaesth* 2006; 96: 761–768.
13. Wasserman JK, Koeberle PD. Development and characterization of a hemorrhagic rat model of central post-stroke pain. *Neuroscience* 2009; 161: 173–183.
14. Katims JJ *et al*. New screening device for assessment of peripheral neuropathy. *J Occup Med* 1986; 28: 1219–1221.
15. Tseng CH. Abnormal current perception thresholds measured by neurometer among residents in blackfoot disease-hyperendemic villages in Taiwan. *Toxicol Lett* 2003; 146: 27–36.
16. Koga K *et al*. Selective activation of primary afferent fibers evaluated by sine-wave electrical stimulation. *Mol Pain* 2005; 1: 13.
17. Masson EA *et al*. Current perception thresholds: a new, quick, and reproducible method for the assessment of peripheral neuropathy in diabetes mellitus. *Diabetologia* 1989; 32: 724–728.
18. Masson EA, Boulton AJ. The Neurometer: validation and comparison with conventional tests for diabetic neuropathy. *Diabet Med* 1991; 8: Spec No S63–S66.
19. Pitei DL *et al*. The value of the Neurometer in assessing diabetic neuropathy by measurement of the current perception threshold. *Diabet Med* 1994; 11: 872–876.
20. Liu S *et al*. Quantitative assessment of differential sensory nerve block after lidocaine spinal anesthesia. *Anesthesiology* 1995; 82: 60–63.
21. Tay B *et al*. Quantitative assessment of differential sensory blockade after lumbar epidural lidocaine. *Anesth Analg* 1997; 84: 1071–1075.
22. Takatsuru Y *et al*. Neuronal circuit remodeling in the contralateral cortical hemisphere during functional recovery from cerebral infarction. *J Neurosci* 2009; 29: 10081–10086.
23. Schäfers M *et al*. Tumor necrosis factor-alpha induces mechanical allodynia after spinal nerve ligation by activation of p38 MAPK in primary sensory neurons. *J Neurosci* 2003; 23: 2517–2521.
24. Kawasaki Y *et al*. Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. *Nat Med* 2008; 14: 331–336.
25. Kiguchi N *et al*. Macrophage inflammatory protein-1alpha mediates the development of neuropathic pain following peripheral nerve injury through interleukin-1beta up-regulation. *Pain* 2010; 149: 305–315.
26. Matsumoto M *et al*. Inhibition of paclitaxel-induced A-fiber hypersensitization by gabapentin. *J Pharmacol Exp Ther* 2006; 318: 735–740.
27. Inoue M *et al*. Initiation of neuropathic pain requires lysophosphatidic acid receptor signaling. *Nat Med* 2004; 10: 712–718.
28. Waxman SG *et al*. Type III sodium channel mRNA is expressed in embryonic but not adult spinal sensory neurons, and is reexpressed following axotomy. *J Neurophysiol* 1994; 72: 466–470.
29. Julius D, Basbaum AI. Molecular mechanisms of nociception. *Nature* 2001; 413: 203–210.
30. Gao YJ, Ji RR. Chemokines, neuronal-glial interactions, and central processing of neuropathic pain. *Pharmacol Ther* 2010; 126: 56–68.
31. Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms, and management. *Lancet* 1999; 353: 1959–1964.
32. Porreca F *et al*. Chronic pain and medullary descending facilitation. *Trends Neurosci* 2002; 25: 319–325.
33. Coull JA *et al*. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 2005; 438: 1017–1021.
34. Nakayama R *et al*. Effects of dantrolene on extracellular glutamate concentration and neuronal death in the rat hippocampal CA1 region subjected to transient ischemia. *Anesthesiology* 2002; 96: 705–710.
35. Kim YK *et al*. The effects of gabapentin pretreatment on brain injury induced by focal cerebral ischemia/reperfusion in the rat. *Korean J Anesthesiol* 2010; 58: 184–190.
36. Roh DH *et al*. Intrathecal clonidine suppresses phosphorylation of the N-methyl-D-aspartate receptor NR1 subunit in spinal dorsal horn neurons of rats with neuropathic pain. *Anesth Analg* 2008; 107: 693–700.
37. Yamamoto T *et al*. Pharmacological classification of central post-stroke pain: comparison with the results of chronic motor cortex stimulation therapy. *Pain* 1997; 72: 5–12.