European Journal of Neuroscience, Vol. 27, pp. 2373–2379, 2008 doi:10.1111/j.1460-9568.2008.06205.x

The role of RNA editing of the serotonin 2C receptor in a rat model of oro-facial neuropathic pain

Aya Nakae,[1] Kunihiro Nakai,[2] Tatsuya Tanaka,[3] Saotoshi Hagihira,[1] Masahiko Shibata,[4] Koichi Ueda[2] and Takashi Masimo[1]

[1]Department of Anesthesiology and Intensive Care Medicine, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita City, Osaka, Japan 565-0871

[2]Department of Plastic and Reconstructive Surgery, Osaka Medical College, Takatsuki, Osaka, Japan

[3]Center for Medical Research and Education and

[4]Department of Pain Medicine, Graduate School of Medicine, Osaka University, Suita City, Osaka, Japan

Keywords: antidepressant, mechanical allodynia, mRNA editing, neuropathic pain model, serotonin 2C receptor

Abstract

We examined whether infraorbital nerve injury affected the RNA editing efficiency of the serotonin (5HT) 2C receptor in the cervical spinal cord, in association with increased pain thresholds, and whether a 5HT reuptake inhibitor (fluvoxamine; Depromel®, Meiji Seika, Tokyo, Japan) altered this editing. Accordingly, we injured rats with an infraorbital nerve loose ligation and examined the pain thresholds, mRNA and mRNA editing of the 5HT2C receptor. We evaluated changes in mRNA editing and 5HT2C mRNA expression using cloning along with sequence analysis and quantitative reverse transcription-polymerase chain reaction to compare samples taken at post-injury day 28 from spinal cord sites, including the trigeminal nucleus caudalis, in naive, sham and injured rats (groups of each type had also received fluvoxamine). 5HT2C receptor expression was maintained post-injury. The RNA editing efficiency was statistically significantly lower at molecular sites A and B in ipsilateral spinal cord samples from injured rats than in bilateral samples from naive and sham rats, and in contralateral samples from injured rats. After injury, the proportional presence of two receptor isoforms changed, i.e. statistically significantly less VNV and significantly more INV and ISV. The proportions reverted after fluvoxamine administration. The post-injury change might be evidence of a functional adaptation mechanism that increases the expression of 5HT2C mRNA isoforms that encode receptors that are more sensitive to 5HT. This would activate the brainstem–spinal descending 5HT systems and, in effect, suppress nociceptive signals from primary afferent neurons to the spinal trigeminal nucleus caudalis.

Introduction

The serotonin (5HT) 2C receptor is a G-protein-coupled receptor whose pre-mRNA is a substrate for base modification that, via hydrolytic deamination of adenosines, yields inosines (Burns *et al.*, 1997). Five adenosines (present at editing sites A–E; Fig. 1), which are located within a sequence encoding the putative second intracellular domain of the 5HT2C receptor, can be converted to inosines. Fully edited transcripts and partially edited transcripts that include editing of at least site E or of sites E and C differ from non-edited receptors in their reduced ability to activate G-protein (Gurevich *et al.*, 2002a, b). This is apparent in their decreased agonist-independent constitutive receptor activity, which results in decreased agonist affinity and potency (Niswender *et al.*, 1999; Wang *et al.*, 2000). *In-vitro* studies have revealed differences in the biochemical and pharmacological properties of several isoforms, including affinity for 5HT, G-protein coupling and responses to atypical antipsychotics (Fitzgerald *et al.*, 1999; Herrick-Davis *et al.*, 1999; Niswender *et al.*, 1999, 2001; Wang *et al.*, 2000; Berg *et al.*, 2001; Price & Sanders-Bush, 2000; Price *et al.*, 2001; Marison *et al.*, 2004; McGrew *et al.*, 2004; Tohda *et al.*, 2006). *In-vivo* studies have shown that most of the 32 possible mRNA variants are produced in the human and rodent brain (Burns *et al.*, 1997; Niswender *et al.*, 1999). In rodents, changes in editing patterns have been induced by exposure to anxiety, including the forced swim test and learned helplessness, and by drugs, such as fluoxetine (Gurevich *et al.*, 2002b; Yang *et al.*, 2004; Englander *et al.*, 2005; Iwamoto *et al.*, 2005). In a previous investigation we found that spinal-contusion-injury rats exhibited 5HT2C mRNA editing, possible evidence that modulation of 5HT2C receptor mRNA editing could be an adaptive mechanism that functions in response to nociceptive stimuli (Nakae *et al.*, 2007). Most of the changes in editing patterns induced by disease, genetic background, stress and pharmacological treatment are subtle and the corresponding diversity produced by these changes at the protein level has not yet been studied. Even so, cumulative data suggest that 5HT2C receptor mRNA editing is biologically relevant.

The selective 5HT reuptake inhibitor affects 5HT2C receptor in response to increased synaptic 5HT (Honda *et al.*, 2006). Widely distributed in the spinal cord, 5HT2C receptors are reported, from results of pain threshold testing, to mediate antinociception (Obata *et al.*, 2004). The descending 5HT pathway serves to inhibit the input of noxious stimuli. Dysfunction along the pathway can produce hypersensitivity to pain and, furthermore, lower the pain threshold (Stahl & Briley, 2004). Dysfunction of the serotonergic pathways may be regarded as a common neurological abnormality involved in the

Correspondence: Dr Aya Nakae, as above.
E-mail: anakae@anes.med.osaka-u.ac.jp

Received 31 January 2008, revised 11 March 2008, accepted 13 March 2008

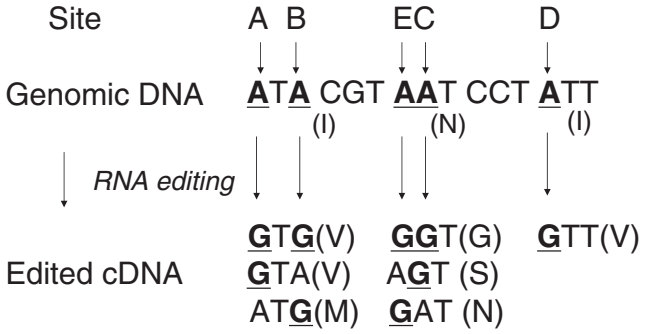

FIG. 1. Genomic context: 5HT2C receptor gene transcripts around RNA editing sites and amino acid changes associated with RNA editing of the 5HT2C receptor. Relationships between RNA editing and isoforms are shown.

etiology of pain disorders (Basbaum & Field, 1984; Stahl & Briley, 2004). Our hypothesis is that the reduced RNA editing should increase the number of constitutively active receptors and increase the responses to 5HT, both of which would be a compensatory response to pain. We speculated that infraorbital nerve (ION) loose-ligation-injured rats, which demonstrate pain-related behavior, would show allodynia reduced by 5HT2C mRNA editing efficiency and that this editing would be increased by fluvoxamine in the spinal cord, possibly by making more 5HT. To test this hypothesis, we created a rat model and performed pharmacological manipulations to increase 5HT using the selective 5HT reuptake inhibitor fluvoxamine.

Materials and methods

All surgical and experimental protocols in this study were reviewed and approved by the Institutional Animal Care and Use Committee of Osaka Medical College and carried out according to the National Institutes of Health guidelines. Animals were treated according to the Guidelines of the International Association for the Study of Pain (Zimmermann, 1983). In particular, the duration of the experiments was kept as short as possible and the number of animals (total $n = 45$) used was minimized.

Experimental animals

Each group consisted of five male Sprague-Dawley rats (body weight 170–230 g at time of surgery). With free access to chow and water, rats were housed four to a cage at 22 ± 2 °C in a 12 h light/dark cycle (illumination 08:00–20:00 h). Before surgery, the animals were allowed at least 1 week for habituation to the housing facilities.

Surgical procedures

Under direct control and viewed through an operation microscope, we applied unilateral chronic-constriction injuries to the right ION, essentially according to the method described by Vos *et al.* (1994). In brief, the animals were anesthetized with sodium pentobarbital (Nembutal, 60 mg/kg i.p.) and a midline scalp incision was made, exposing the skull and nasal bone. The edge of the orbit, formed by the maxillary, frontal, lacrimal and zygomatic bones, was dissected free. The ION was dissected free from its most rostral location in the orbital cavity, just caudal to the infraorbital foramen. Two nylon (5-0) ligatures (2 mm apart) were loosely tied around the ION. To obtain the desired degree of constriction, a criterion formulated by Bennett & Xie (1988) was applied, i.e. the ligature reduced the diameter of the nerve by a just noticeable amount and retarded, but did not interrupt, epineural circulation through the superficial vasculature. The scalp incision was closed in layers using nylon sutures (5-0). Sham operations were performed in the same way but the nylon ligatures were more loosely tied around the ION (2 mm apart). After surgery, the rats were allowed to recover in a warmed cage in which water and chow were easily accessible.

Behavioral testing

Behavioral tests were carried out 3 days before and 7, 14, 21 and 28 days after surgery. All experiments were carried out in a quiet room generally between 09:00 and 16:00 h. In sequence, increasingly resilient von Frey filaments were applied to determine pain hypersensitivity to mechanical stimulation. To observe behavioral responses to the mechanical stimulation, each rat was placed alone in a plastic cage ($25 \times 40 \times 18$ cm) with bedding. After 30 min accommodation, von Frey filaments (bending force 0.16, 0.4, 0.6, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10.0 and 15.0 g) were applied from above to the ION territory, near the center of the vibrissal pad, on the hairy skin surrounding the mystacial vibrissae. Each von Frey filament was applied five times to the same region at approximately 1 s intervals. Head withdrawal or touching or scratching of the facial regions upon application of a von Frey filament was recorded as a positive pain response. The response threshold was defined as the lowest-force filament application that prompted at least three positive responses in five trials. If an animal made no pain response to any of the tested filaments, 15 g was ascribed as the nominal threshold.

Drug treatment

From post-injury day 14, fluvoxamine (Meiji Seika, Tokyo, Japan) dissolved in saline was administered intraperitoneally to some animals at a dose of 30 mg/kg/day for 14 days. We observed no evidence that the drug treatment affected the general wellbeing of the animals.

Total RNA extraction, reverse transcription, nucleotide sequencing and real-time polymerase chain reaction

The animals were killed by decapitation on post-injury day 28. Two separate cervical spinal cord tissue samples (ipsilateral and contralateral) were then taken from each animal and saved in RNAlater solution (Ambion, Inc., Austin, TX, USA) for later RNA extraction. Following the method described in the manufacturer's protocol, total RNA was extracted with Rneasy Lipid Tissue Mini Kits (Qiagen, Tokyo, Japan). Total RNA (10 μg) was used for first-strand cDNA synthesis by Thermoscript reverse transcriptase and random hexamer (Invitrogen, Gaithersburg, MD, USA). Quantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed, according to the manufacturer's protocol, with an ABI PRISM 7900HT (Applied Biosystems, Foster City, CA, USA) using TaqMan probes supplied by Applied Biosystems. All reactions were run in duplicate. Results were then normalized to those obtained for amplifications of the same cDNA samples using glyceralaldehyde-3-phosphate-dehydrogenase (GAPDH), which acts as an internal control, and averaged for each treatment group.

To estimate the RNA editing efficiency, rat 5HT2C receptor amplification was performed using RT-PCR. The primers used in the polymerase chain reaction were ATCATGGCAGTAAGCATGGAGA-AGA and ATTGATATTGCCCAAACGATGGCA. Each sample under-

went cDNA synthesis and RT-PCR analysis. A portion of polymerase chain reaction product was cloned into the pCR2.1 vector (Invitrogen, Tokyo, Japan) and the reaction mixture was used for *Escherichia coli* transformation. Single bacterial transformations, planted on 150 mm dishes, yielded > 300 recombinant colonies from which 80 or more were randomly picked for plasmid DNA extraction and nucleotide sequencing using an ABI3700 genetic analyser (Applied Biosystems). Data from at least 50 colonies from each sample were analysed.

Data were compared by one-way ANOVA and statistical differences were resolved post-hoc using the Tukey-Kramer multiple-comparison test ($P < 0.05$).

Results

Behavioral responses to ION loose ligation and fluvoxamine administration

On post-injury day 14, ION loose ligation correlated statistically significantly with reflex withdrawal to punctuate mechanical stimulation. An ANOVA with Dunn's all-pairwise multiple comparison demonstrated that the pain thresholds at the ION territory of ION loose-ligation-injured rats were statistically significantly lower than the pain thresholds of sham and naive animals.

On post-injury day 28, injured animals that had received fluvoxamine for 2 weeks showed statistically significantly higher pain thresholds than injured animals that had not received the drug (Fig. 2). The fluvoxamine treatment did not affect motor performance.

Expression levels of 5HT2C receptor mRNA in the spinal cord

We evaluated the levels of GAPDH, as an internal control, when evaluating 5HT2C receptor gene expression levels. The measured presence of 5HT2C receptor mRNA was almost equal in all samples (Fig. 3). The results indicate that neither the surgery nor the drug treatment was associated with changes in the level of expression of 5HT2C mRNA.

5HT2C mRNA editing in the spinal cords of ION-injured rats

We first compared the basal spinal cord (including spinal nucleus caudalis) 5HT2C mRNA editing pattern in naive, sham and model

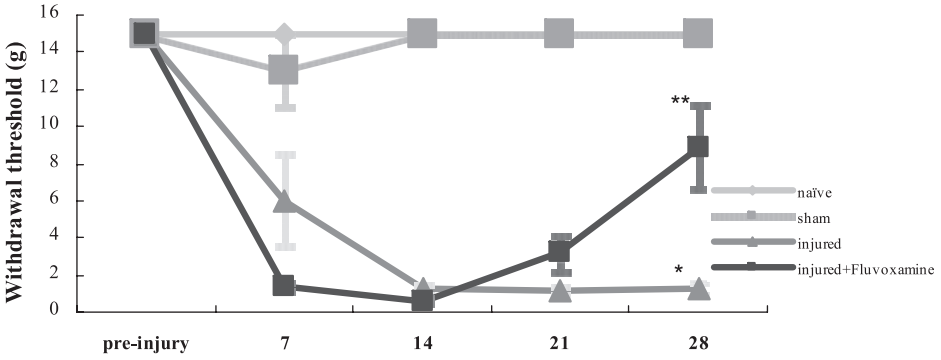

FIG. 2. Time course of change in tolerance to threshold bending force during von Frey filament stimulation. All data points represent mean ± SEM. Statistical significance of differences between groups was determined by an ANOVA with Dunn's all-pairwise multiple comparisons. (*$P < 0.05$, compared with the naïve group; **$P < 0.05$, compared with the injured group).

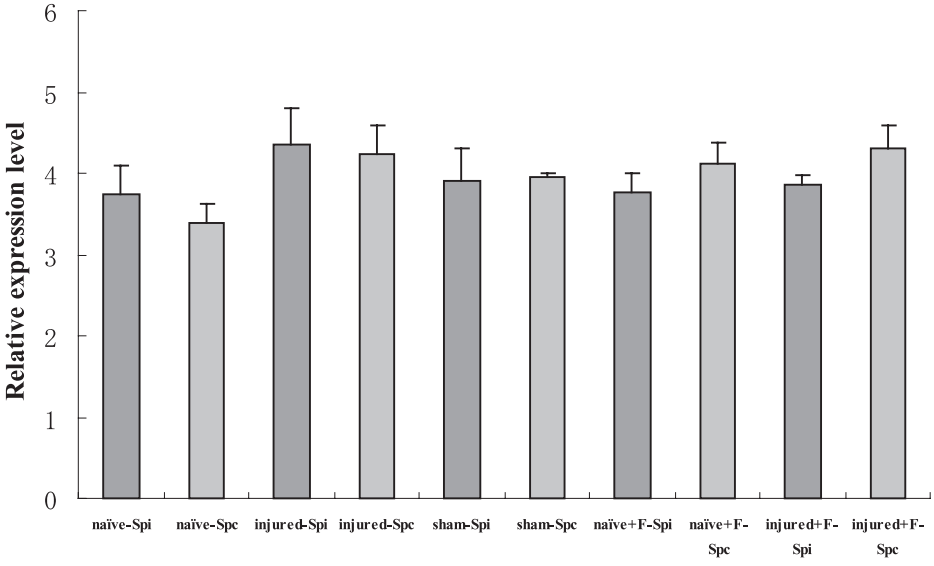

FIG. 3. Different 5HT2C receptor expression in spinal cord samples from the sites ipsilateral and contralateral to the site of injury. Data were compared by one-way ANOVA and statistical differences were resolved post-hoc using the Tukey-Kramer multiple-comparison test ($P < 0.05$). Spi, ipsilateral spinal cord site; Spc, contralateral spinal cord site; F, fluvoxamine treated.

rats. There are five RNA editing sites (sites A–E) at the 3′-end of exon 5 in the 5HT2C receptor. RT-PCR products, including those derived from these edited sites, were used for cloning along with sequencing analysis. To ensure reliable estimation of RNA editing efficiency, at least 80 colonies were sequenced per sample and at least 50 clones were analysed (naive rats, $n = 4$; injured rats, $n = 4$; sham rats, $n = 5$; fluvoxamine-administered naive rats, $n = 5$; fluvoxamine-administered injured rats, $n = 5$). As shown in Fig. 4a, compared with all other samples except sham-ipsilateral samples, statistically significantly lower RNA editing efficiency was detected at the A editing sites in ipsilateral samples from injured rats. In samples from injured rats, compared with naive-ipsilateral, sham-contralateral, injured-contralateral, fluvoxamine-administered naive-bilateral and fluvoxamine-administered injured-ipsilateral samples, statistically significantly lower RNA editing efficiency was also detected at ipsilateral B sites. When comparing results for sites C, D and E, no significant differences were found for samples from naive, sham and injured rats (Fig. 4b).

Given the differences that we found in RNA editing in samples from injured, sham and naive rats, to investigate the functional consequences of the RNA editing changes, we then compared the distribution of five major isoforms (Fig. 5). In ipsilateral-site samples from injured rats, the presence of the INV and ISV isoforms was statistically significantly greater than in bilateral naive and sham samples and contralateral-injured samples (Fig. 5a).

5HT2C mRNA editing in the spinal cord of fluvoxamine-treated or untreated injured rats

In the next series of experiments, we examined the effects of fluvoxamine, a selective 5HT reuptake inhibitor, on mechanical allodynia and the sites where fluvoamine acts. In naive rats, chronic fluvoxamine treatment produced no significant change. In ipsilateral samples from injured rats that did not receive fluvoxamine, the presence of INI, INV and ISV isoforms was statistically significantly greater and that of VNV isoforms was significantly less than in samples from the right side of fluvoxamine-free and fluvoxamine-treated naive rats, and from the ipsilateral sites of fluvoxamine-treated injured rats (Fig. 5b). The 5HT2C mRNA editing pattern in samples from the ipsilateral sites of fluvoxamine-treated injured rats resembled that found for contralateral sites. In other words, fluvoxamine administration suppressed lateral changes resulting from injury.

In summary, in the ipsilateral site of injured rats, statistically significantly lower RNA editing efficiency was detected at sites A and B. Administration of fluvoxamine to injured rats increased the

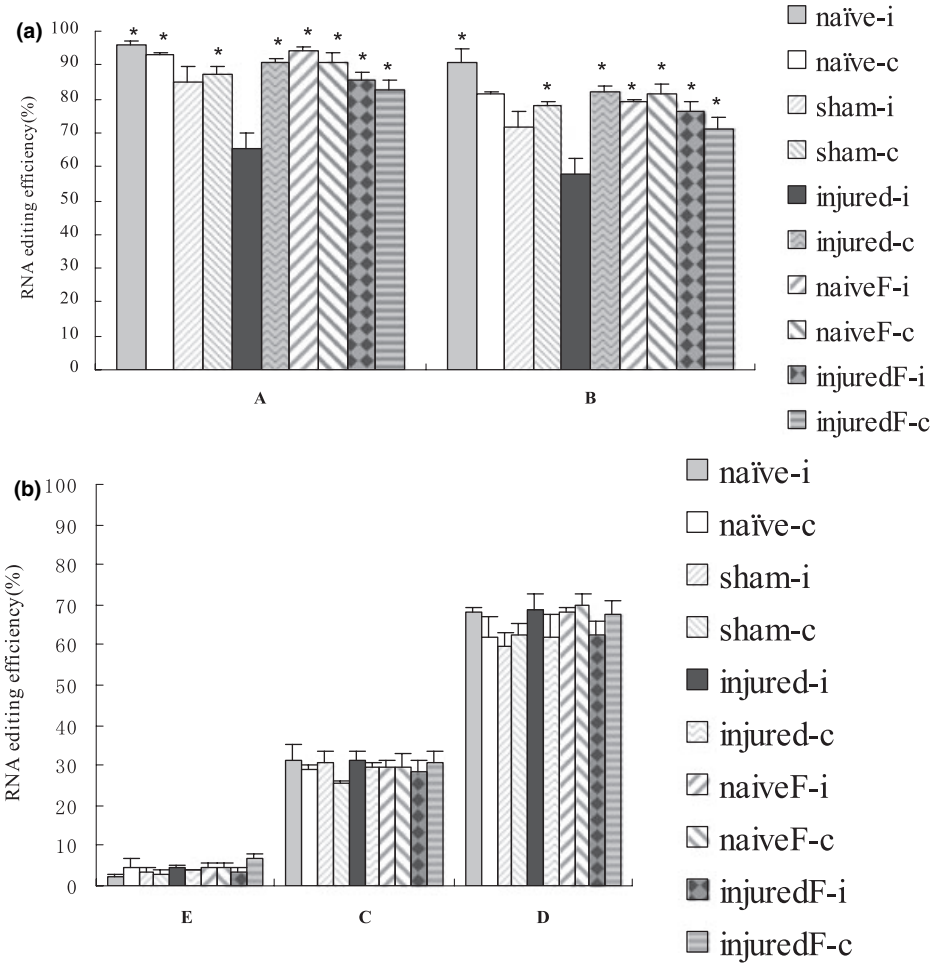

FIG. 4. Different editing efficiency of 5HT2C receptors at different RNA sites. RNA editing efficiency at each site. Sequencing of at least 80 colonies per sample (naive, $n = 3$; sham, $n = 5$; injured, $n = 5$; fluvoxamine-treated naive, $n = 5$; fluvoxamine-treated injured $n = 5$) was performed on 5HT2C receptor cDNA derived from single bacterial colonies. All of the more than 5000 sequenced clones contained the 5HT2C receptor gene. Data were compared by one-way ANOVA and statistical differences were resolved post-hoc using the Tukey-Kramer multiple-comparison test (*$P < 0.05$). (a) Site A and B, (b) site C, D and E. i, ipsilateral c, contralateral; F, fluvoxamine administered

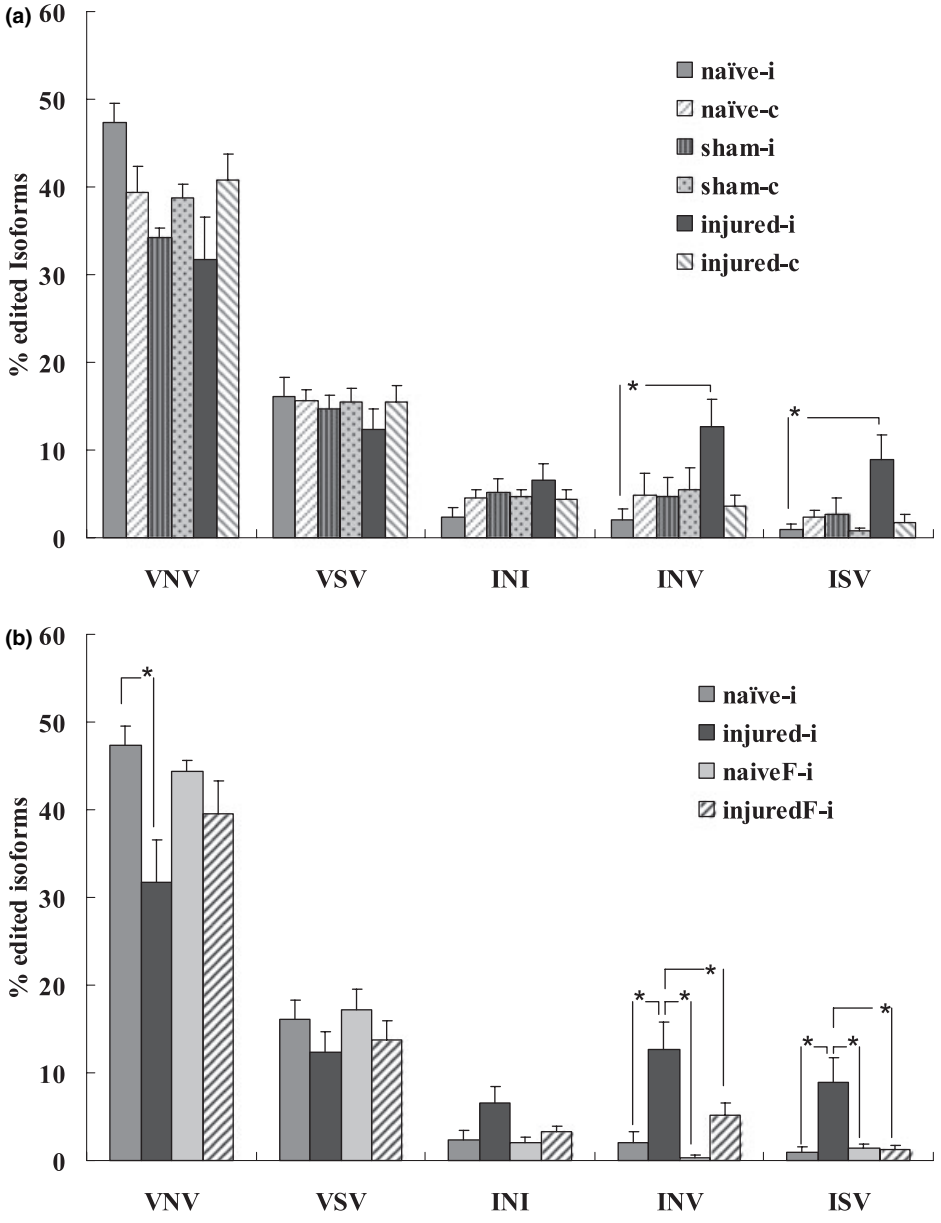

FIG. 5. Proportions of the major 5HT2C receptor isoforms present in spinal cord. (a) Comparison between naive, sham and injured bilateral samples. (b) Comparison between ipsilateral-naive, injured, fluvoxamine-treated-naive and fluvoxamine-treated-injured samples. Data were compared by one-way ANOVA and statistical differences were resolved post-hoc using the Tukey-Kramer multiple-comparison test (*$P < 0.05$). i, ipsilateral; c, contralateral; F, fluvoxamine adminstered.

pain threshold (compared with that in injured rats that did not receive fluvoxamine); it also ameliorated the symptoms of nerve injury and attenuated lateral differences. Using the same techniques that provided the results for Fig. 3, we also used quantitative RT-PCR to evaluate whether or not treatment with fluvoxamine alters the cytoplasmic expression levels of 5HT2C mRNA. No difference was found between rats that received and did not receive fluvoxamine.

Discussion

Rats injured by ION loose ligation clearly exhibited greater pain sensitivity, on post-injury day 28, as attested by lower thresholds to mechanical stimulation than uninjured rats. Injured rats that had received fluvoxamine (30 mg/kg i.p.) for 14 days (Fig. 2) tended to show less sensitivity to pain. For depressive patients, the onset of the selective 5HT reuptake inhibitor effect is slow, taking from 2 weeks to 1 month. Thus, it is interesting that we also found the onset of action of fluvoxamine on pain thresholds to take 2 weeks. The overall level of 5HT2C receptor mRNA expression remained similar in all samples. A study of the antiallodynic effect of selective 5HT reuptake inhibitor found that this antiallodynic effect, rather than resulting from a changed number of 5HT receptors, is attributable to changes in the synaptic concentration of 5HT (Gurevich *et al.*, 2002a). Moreover, as it has been found that RNA editing of 5HT2C receptor mRNA is influenced by antidepressants, the 5HT2C receptor has emerged as a promising target for antidepressants (Englander *et al.*, 2005). Even so, at the ipsilateral site, ION-loose-ligated rats showed statistically significantly less RNA editing than all of the other samples except sham-ipsilateral at A sites, and naive-right (ipsilateral), sham-ipsilat-

eral and fluvoxamine-treated-injured contralateral samples at B sites. Characterization of the isoforms revealed that injured rats had proportionally more of the INV and ISV isoforms at the ipsilateral spinal cord sample sites than naive and sham control rats (Fig. 5a), statistically significantly more of the INI, INV and ISV isoforms, and significantly less of the VNV isoform, in ipsilateral samples than in samples from naive-ipsilateral and fluvoxamine-treated naive and injured rats. RNA editing produces receptor isoforms with varying degrees of basal activity. An *in-vitro* study has shown that the INI isoform exhibits great basal activity, agonist affinity and potency, and that ISV and INV are slightly less active than INI. By contrast, the most statistically significant decreases in basal activity were observed with VNV and VSV isoforms (Herrick-Davis *et al.*, 1999). The results of the present study might support the conjecture that these responses to ION loose-ligation injury and the resultant increase in the synaptic concentration of 5HT could be an adaptive response to the low baseline levels of spinal cord 5HT. In the face of changing synaptic input, these responses occur to maintain receptor activation within an acceptable range for information processing together with low baseline 5HT2C mRNA editing. Our previous study (Nakae *et al.*, 2007) and these results were considered to be similar at the point that isoforms of greater basal activity were increased and less active isoforms were decreased, and the present study using ION loose-ligation rats showed a larger robust change. The larger change of 5HT2C editing might affect the pain-related behavior.

It has been reported that stimulation of spinal 5HT2C receptors by their selective agonists produces antiallodynic effects in rats with nerve injury (Obata *et al.*, 2004), which suggests that fluvoxamine might ameliorate tactile allodynia via spinal 5HT2C receptors by acting on the receptors or 5HT neurons. However, the report of Obata *et al.* (2004) suggests that pain relief by the 5HT2 agonist is an indirect action through facilitation of adrenergic activation of alpha2 adrenergic receptors, which inhibit transmission. Our results might be considered to have a direct relationship between direct 5HT2C receptor activation and pain reduction. Reports of investigations into changes in 5HT levels in mice with neuropathic pain have shown that 5HT levels in the raphe magnus nucleus decrease in sciatic-nerve-ligated mice (Sounvoravong *et al.*, 2004) and that the 5HT concentration in the lumbar spinal cord decreases in mice with chronic-constriction sciatic nerve injury (Vogel *et al.*, 2003). Thus, the antiallodynic effect of fluvoxamine might be attributable to an increased extracellular concentration of 5HT, which had decreased subsequent to nerve injury. However, 5HT itself induces hypersensitivity via 5HT3 receptors in the spinal dorsal horn in spinal nerve ligation (Rahman *et al.*, 2006). From our results, the effect of fluvoxamine and 5HT was considered to attenuate the impact of chronic-constriction injury of ION.

A relationship has been reported between 5HT2C receptor RNA editing in the brain and 5HT depletion (Gurevich *et al.*, 2002a). In conditions of 5HT depletion, 5HT2C receptor RNA editing decreases relatively more at sites C and E and there is increased expression of 5HT2C mRNA-encoding receptor isoforms with higher sensitivity to 5HT. Meanwhile, the 5HT2C receptor partial agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) increases the editing frequency at site E, resulting in the mRNA encoding of receptors that are less responsive to 5HT. We found that nerve injury caused by ION loose ligation alters 5HT2C receptor mRNA editing and, either as a result of this or because of other dysfunction of the ION in the spinal cord, the injury produced hyperalgesia or even lowered the pain threshold in response to normally non-noxious stimuli. In other words, 5HT2C receptor mRNA editing is liable to change in response to nerve injury or other conditions. Moreover, the changes in 5HT2C mRNA editing detected in fluvoxamine-treated rats may be attributable to an increased level of 5HT induced by this drug. For better elucidation, more studies of the spinal cord in a neuropathic pain model with and without 5HT-depleted rats are required.

Previous studies that characterized the functional properties of recombinant non-edited, partially edited and fully edited isoforms expressed singly in transfected cells have shown that the editing combinations A (B) CD [VSV] and A (B) ECD [VGV] differ from non-edited [INI] and other partially edited isoforms in their reduced ability to activate G-protein in response to agonist stimulation (Burns *et al.*, 1997; Fitzgerald *et al.*, 1999; Niswender *et al.*, 1999). However, editing at sites E and C, because it often results in the A (B) EC-edited isoform, is sufficient to achieve maximum down-regulation of receptor activity. We found, however, that the proportional production of isoforms was not altered in the same way in all groups. A previous study has also shown that receptor basal activity and 5HT-stimulated ^{3}H-IP production are inversely related (Herrick-Davis *et al.*, 1999). In situations where 5HT is depleted, the basal activities of receptors resulting from INV and ISV isoforms are greater and those from VNV are less. The present data suggest therefore that there is an overall increase in 5HT2C receptor basal activity in the ipsilateral spinal cord of ION loose-ligation-injured rats.

It is evident from the results shown in Fig. 5 that various differently edited 5HT2C receptor isoforms are expressed in the spinal cord; however, the net outcome of the activation of such mixed populations of receptors *in vivo* is not known. It is also clear that editing of 5HT2C mRNA does not lead to the kind of drastic changes that would be reflected in increased EC_{50} concentrations of full or partial 5HT2C receptor agonists. This lack of marked systemic alteration supports a recent hypothesis that this editing, like the majority of A-to-I site-specific editing of neuronal transcripts that have thus far been identified, enables 'fine-tuning' of the activity of distinct physiological process (Seeburg, 2000). However, differences in 5HT2C receptor activity resulting from the kind of altered editing described in the current study would predictably become most apparent when serotonergic neurotransmission is reduced. Thus, although the *in-vivo* significance of the altered editing described here is still unclear, and although the actual synaptic concentration of 5HT in the spinal cord is not known, the present data suggest that decreased expression of the AB-edited isoform, which occurs together with a increased expression of the AB non-edited or A-site non-edited mRNA, has functional consequences when serotonergic neurotransmission is compromised.

This, in turn, suggests that the post-transcriptional regulation of gene expression played a role in modulating the expression of distinct neurotransmitter receptors in the spinal cords of our neuropathic-pain-model rats.

Our finding is based on the analysis of total RNA from spinal cord including not only sensory but also motoneurons. Harvey *et al.* (2006) reported that increased 5HT2C receptor activity increases motoneuronal excitability in normal and injured rats. Thus, if all 5HT2C receptors change with nerve injury, this should lead to increased motoneuron excitability.

Post-injury 5HT2C receptor expression was maintained in a neuropathic pain model involving rats injured by loosely ligating the ION. The RNA editing efficiency was statistically significantly lower at molecular sites A and B in ipsilateral spinal cord (including trigeminal nucleus caudalis) samples from injured rats than in bilateral samples from naive and sham rats, and contralateral samples from injured rats. After injury, the proportional presence of two receptor isoforms changed, i.e. INV and ISV statistically significantly increased (Fig. 5a). In injured rats, we found statistically significantly more of the INV and ISV isoforms than in samples from naïve-right (ipsilateral) and fluvoxamine-treated naive and injured rats, and

significantly less of the VNV isoform in ipsilateral samples than in ipsilateral samples from naive rats. These changes are dissimilar to those from brain samples of depression suicides and spinal cord samples from spinal cord injury models. The current findings might suggest that modulation of 5HT2C receptor mRNA editing could be an adaptive mechanism that functions in response to serotonergic dysfunction due to 5HT depletion or surgical stress. Further study is required to clarify the clinical significance of 5HT2C receptor RNA editing in the spinal cord.

Acknowledgements

This work was supported by a Grant-in-Aid (no. 19591791) from the Ministry of Education, Culture, Sports, Science and Technology, Japan. We are grateful to Meiji Seika (Tokyo, Japan) for supplying the fluvoxamine malate. The authors thank Keiko Miyake for excellent technical assistance.

Abbreviations

5HT, serotonin; GAPDH, glyceralaldehyde-3-phosphate-dehydrogenase; ION, infraorbital nerve; RT-PCR, reverse transcription-polymerase chain reaction.

References

Basbaum, A.I. & Field, H.L. (1984) Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. *Annu. Rev. Neurosci.*, **7**, 309–338.

Bennett, G.J. & Xie, Y.K. (1988) A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain*, **33**, 87–107.

Berg, K.A., Cropper, J.D., Niswender, C.M., Sander-Bush, E., Emeson, R.B. & Clarke, W.P. (2001) RNA-editing of the 5-HT(2C) receptor alters agonist-receptor-effector coupling specificity. *Br. J. Pharmacol.*, **134**, 386–392.

Burns, C., Chu, H., Rueter, S.M., Hutchinson, L.K., Canton, H., Sanders-Bush, E. & Emeson, R.B. (1997) Regulation of serotonin-2C receptor G-protein coupling by RNA editing. *Nature*, **387**, 303–308.

Englander, M.T., Dulawa, S.C., Bhansali, P. & Schmauss, C. (2005) How stress and fluoxetine modulate serotonin 2C receptor pre-mRNA editing. *J. Neurosci.*, **25**, 648–651.

Fitzgerald, L.W., Iyer, G., Conklin, D.S., Krause, C.M., Marshall, A., Patterson, J.P., Tran, D.P., Jonak, G.J. & Hartig, P.R. (1999) Messenger RNA editing of the human serotonin 5HT2C receptor. *Neuropsychopharmacology*, **21**, 82S–90S.

Gurevich, I., Englander, M.T., Adlersberg, M., Siegal, N.B. & Schmauss, C. (2002a) Modulation of serotonin 2C receptor editing by sustained changes in serotonergic neurotransmission. *J. Neurosci.*, **22**, 10 529–10 532.

Gurevich, I., Tamir, H., Arango, V., Dwork, A.J., Mann, J.J. & Schmauss, C. (2002b) Altered editing of serotonin 2C receptor pre-mRNA in the prefrontal cortex of depressed suicide victims. *Neuron*, **34**, 349–356.

Harvey, P.J., Li, X. & Bennett, D.J. (2006) Endogenous monoamine receptor activation is essential for enabling persistent sodium currents and repetitive firing in rat spinal motoneurons. *J. Neurophysiol.*, **96**, 1171–1186.

Herrick-Davis, K., Grinde, E. & Niswender, C.M. (1999) Serotonin 5-HT2C receptor RNA editing alters receptor basal activity: implications for seronergic signal transduction. *J. Neurochem.*, **73**, 1711–1717.

Honda, M., Uchida, K., Tanabe, M. & Ono, H. (2006) Fluvoxamine, a serotonin reuptake inhibitor, exerts its antiallodynic effects on neuropathic pain in mice via 5-HT2A/2C receptors. *Neuropharmacology*, **51**, 866–872.

Iwamoto, K., Nakatani, N., Bundo, M., Yoshikawa, T. & Kato, T. (2005) Altered RNA editing of serotonin 2C receptor in a rat model of depression. *Neurosci. Res.*, **53**, 69–76.

Marison, S., Weiner, D.M. & Caron, M.G. (2004) RNA editing induces variation in desensitization and trafficking of 5-hydroxytryptamine 2c receptor isoforms. *J. Biol. Chem.*, **279**, 2945–2954.

McGrew, L., Price, R.D., Hackler, E., Chang, M.S. & Sanders-Bush, E. (2004) RNA editing of the human serotonin 5-HT2C receptor disrupts transactivation of the small G-protein RhoA. *Mol. Pharmacol.*, **65**, 252–256.

Nakae, A., Nakai, K., Tanaka, T., Takashina, M., Hagihira, S., Shibata, M., Ueda, K. & Mashimo, T. (2007) Serotonin2C receptor mRNA editing in neuropathic pain model. *Neurosci. Res.* October 23 (Epub ahead of print).

Niswender, C., Copeland, S.C., Herrik-Davis, K., Emeson, R.B. & Sanders-Bush, E. (1999) RNA editing of the human serotonin 5-hydroxytryptamine 2C receptor silences constitutive activity. *J. Biol. Chem.*, **274**, 9472–9478.

Niswender, C.M., Herrick-Davis, K., Dilley, G.E., Meltzer, H.Y., Overholser, J.C., Stockmeier, C.A., Emeson, R.B. & Sanders-Bush, E. (2001) RNA editing of the human serotonin HTR2C receptor: alterations in suicide and implications for serotonergic pharmacotherapy. *Neuropsychopharmacology*, **24**, 478–491.

Obata, H., Saito, S., Sakurazawa, S., Sasaki, M., Usui, T. & Goto, F. (2004) Antiallodynic effects of intrathecally administered 5-HT (2C) receptor agonists in rats with nerve injury. *Pain*, **108**, 163–169.

Price, R.D. & Sander-Bush, E. (2000) RNA editing of human serotonin 5-HT (2C) receptor delays agonist-stimulated calcium release. *Mol. Pharmacol.*, **58**, 859–862.

Price, R.D., Weiner, D.M., Chang, M.S. & Sanders-Bush, E. (2001) RNA editing of the human serotonin 5-HT2C receptor alters receptor-mediated activation of G13 protein. *J. Biol. Chem.*, **176**, 44 663–44 668.

Rahman, W., Suzuki, R., Webber, M., Hunt, S.P. & Dickenson, A.H. (2006) Depletion of endogenous spinal 5-HT attenuates the behavioural hypersensitivity to mechanical and cooling stimuli induced by spinal nerve ligation. *Pain*, **123**, 264–274.

Seeburg, P.H. (2000) RNA helicase participates in the editing game. *Neuron*, **25**, 261–263.

Sounvoravong, S., Nakashima, M.N., Wada, M. & Nakashima, K. (2004) Decrease in serotonin concentration in raphe magnus nucleus and attenuation of morphine analgesia in two mice of neuropathic pain. *Eur. J. Pharmacol.*, **484**, 217–223.

Stahl, S. & Briley, M. (2004) Understanding pain in depression. *Hum. Psychopharmacol.*, **1**, S9–S13.

Tohda, M., Nomura, M. & Nomura, Y. (2006) Molecular pathopharmacology of 5-HT2C receptors and the RNA editing in the brain. *J. Pharmacol. Sci.*, **100**, 427–432.

Vogel, C., Mossner, R., Gerlach, M., Heinemann, T., Murphy, D., Riederer, P., Lesch, K.P. & Sommer, C. (2003) Absence of thermal hyperalgesia in serotonin transporter-deficient mice. *J. Neurosci.*, **23**, 708–715.

Vos, B.P., Strassman, A.M. & Maciewicz, R.J. (1994) Behavioral evidence of trigeminal neuropathic pain following chronic constriction injury to the rat's infraorbital nerve. *J. Neurosci.*, **14**, 2708–2723.

Wang, Q., O'Brien, P., Chen, C.-X., Cho, D.-S.C., Murray, J.M. & Nishikura, K. (2000) Altered G protein-coupling functions of RNA editing isoform and splicing variant serotonin 2C receptors. *J. Neurochem.*, **74**, 1290–1300.

Yang, W., Wang, Q., Kanes, S.J., Murray, J.M. & Nishikura, K. (2004) Altered RNA editing of serotonin HTR2C receptor induced by interferon: implications for depression associated with cytokine therapy. *Mol. Brain Res.*, **124**, 70–78.

Zimmermann, M. (1983) Ethical guidelines for investigations of experimental pain in conscious animals. *Pain*, **16**, 109–110.