Published in final edited form as:

Neuroreport. 2008 July 16; 19(11): 1151–1154. doi:10.1097/WNR.0b013e3283086781.

Glutamine uptake contributes to central sensitization in the medullary dorsal horn

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Abstract

Mustard oil application to tooth pulp produces central sensitization in rat medullary dorsal horn (MDH) nociceptive neurons, which has been implicated in persistent pain mechanisms. We found that superfusion onto MDH of methylaminoisobutyric acid, a competitive inhibitor of the neuronal system A transporter for presynaptic uptake of glutamine (a glutamate precursor released from astroglia), significantly depressed development of mustard oil-induced central sensitization in rat MDH nociceptive neurons. This finding indicates that the system A transporter is required for the expression of central sensitization and confirms the important roles of astroglia, glutamine and presynaptic modulation of glutamate release in the development of central sensitization.

Keywords

astroglia; central sensitization; glutamine; medullary dorsal horn; methylaminoisobutyric acid; mustard oil; pulp inflammation; system A transporter

Introduction

Central sensitization as reflected by increases in nociceptive neuronal mechanoreceptive field size, responses to noxious stimuli, and a decrease in activation threshold is a crucial mechanism underlying the increased excitability of central nociceptive pathways after peripheral tissue injury and inflammation, and has been implicated in the development and maintenance of persistent pain [1–3]. Application of the small-fiber excitant and inflammatory irritant mustard oil to the rat tooth pulp produces central sensitization in nociceptive neurons of the caudal portion of the trigeminal spinal nucleus (the medullary dorsal horn, MDH) that is glutamatergic receptor-dependent and modulated by glial cell mechanisms [4–6]. The astroglial glutamate–glutamine shuttle between neurons and astroglia provides an important means for replenishing the increased amounts of glutamate used for neurotransmission under conditions of increased activity. On the basis of our recent findings that the specific inhibition of the astroglial tricarboxylic acid cycle by fluoroacetate [6], and of astroglial glutamine synthetase by methionine sulfoximine [5] significantly attenuates central sensitization in MDH; we speculated that blockade of uptake by the glutamatergic terminals of glutamine released from

astroglia would also attenuate sensitization in MDH. Recent work has shown that the system A transporter (SAT) mediates glutamine uptake by neurons and that methylaminoisobutyric acid (MeAIB) can competitively inhibit the SAT [7–11]. In this study, we provide evidence that superfusion onto MDH of MeAIB significantly depressed development of sensitization induced in MDH nociceptive neurons by application of mustard oil to the rat tooth pulp.

Methods

Detailed descriptions of most of the methods have been earlier reported [4–6], so only a brief outline follows.

Animal preparation

Male adult rats were anesthetized by intraperitoneal α -chloralose (50 mg/kg)/urethane (1 g/kg). The right maxillary first molar pulp was exposed and covered with a saline-soaked cotton pellet, and the dorsal surface of the caudal medulla was surgically exposed. The rat then received a continuous intravenous infusion of a mixture of 70% urethane solution (0.2 g/ml) and 30% pancuronium solution (1 mg/ml) at a rate of 0.4–0.5 ml/h, and was artificially ventilated throughout the whole experimental period. Heart rate, percentage expired CO₂, and rectal temperature were continuously monitored. All surgeries and procedures were approved by the University of Toronto Animal Care Committee in accordance with the regulations of the Ontario Animal Research Act (Canada).

Recording and stimulation procedures

The activity of single neurons was recorded by a tungsten microelectrode (5–15 M Ω) in histologically verified sites in MDH (lateral: 1.4–2.0 mm; posterior: 1.5–2.0 mm referred to the obex). Responses to stimulation of the orofacial region were amplified and displayed on oscilloscopes and also led to an analog-to-digital converter connected to a computer. Data were analyzed off-line with Spike 2 software (Cambridge Electronic Design, Science Park, Milton Road, Cambridge, UK).

Mechanical (brush, pressure, and pinch) and noxious thermal (radiant heat, 51–53°C) stimuli were applied to classify nociceptive-specific neurons in the deep laminae of the MDH [4–6]. A nociceptive-specific neuron's spontaneous activity was determined over an initial 1-min recording period, and its cutaneous orofacial mechanoreceptive field was determined with nonserrated forceps. Its activation threshold to a mechanical stimulus applied to its receptive field was assessed by force-monitoring forceps or an electronic von Frey monofilament (Stoelting Co., Wood Dale, Illinois, USA), and its responses to graded pressure or pinch were determined (25, 50, 75, 100, and sometimes 200 g, applied in ascending order, each for 5 s at an interval of >45 s). The pressure-evoked or pinch-evoked responses were assessed by summing the number of spikes evoked by each of these graded stimuli.

Experimental paradigm

After baseline values of neuronal properties were obtained, either MeAIB (0.5 mM; freshly dissolved in phosphate-buffered saline (PBS) at pH 7.4; Sigma-Aldrich, St Louis, Missouri, USA) or PBS (as control) was continuously superfused (i.t.) over the exposed ipsilateral medulla (at a rate of 0.6 ml/h). At 5 and 15 min after superfusion began, two assessments of neuronal properties were carried out. At 30 min, mustard oil (0.2 μ l; allyl isothiocyanate, 95%; Aldrich Chemical Co., Milwaukee, Wisconsin, USA) was applied to the exposed pulp, which was promptly sealed with CAVIT (ESPE, Seefeld/Oberbayern, Germany). Three minutes after mustard oil application, neuronal properties were again assessed at 10-min intervals over the next 50 min. Thus, two groups (MeAIB/mustard oil and PBS/mustard oil) of animals were studied. The selection of the dose (0.5 mM) of MeAIB superfused in this study was based on

preliminary experiments in seven neurons, which revealed that 5 mM but not 0.05 mM MeAIB superfusion produced marked increases in spontaneous activity, receptive field size, responses to noxious stimuli, decreases in activation threshold, and appearance of novel receptive fields sensitive to touch and also that 0.05 mM MeAIB did not affect the central sensitization induced by subsequent mustard oil application to the tooth pulp.

Statistical analyses

Statistical analyses were based on normalized data (in percentage). Differences between baseline values and values at different time-points with post-hoc mustard oil, MeAIB, or PBS application in each group were treated by repeated measures (RM) analysis of variance (ANOVA) or ANOVA on ranks, after Dunnett's test. Differences between MeAIB/mustard oil and PBS/mustard oil groups were treated by two-way ANOVA with post-hoc Bonferroni *t*-test. The level of significance was set at a $P < 0.05$. All values are presented as mean $\pm$ SEM.

Results

Sixteen functionally identified nociceptive-specific neurons in the deep laminae of the MDH were studied. Examples of effects of MeAIB or PBS on mustard oil-induced changes are shown in Fig. 1.

Orofacial receptive field size

In the PBS/mustard oil group, all eight neurons had at baseline an ipsilateral pinch/pressure receptive field (mean: size $\pm$ SEM: 1.3 $\pm$ 0.3 cm²) involving both trigeminal maxillary and ophthalmic divisions. Although superfusion of PBS did not significantly affect receptive field size, mustard oil application to the pulp produced a significant, long-lasting increase in size throughout the 50-min observation period ($P < 0.001$, RM ANOVA on ranks), with its peak (273 $\pm$ 41% of control) at 18 min (see Fig. 2a). In addition, a novel receptive field sensitive to touch appeared for 10–30 min in three of the eight neurons (Fig. 2b). After superfusion of MeAIB, mustard oil application to the pulp, however, produced only a small increase in the receptive field size (173 $\pm$ 24% of control at 18 min; $n=8$) throughout the 50-min observation period ($P < 0.001$, RM ANOVA on ranks; Fig. 2a), and no novel receptive field sensitive to touch was apparent in any of the eight neurons tested (Fig. 2b). The differences in touch receptive field and pinch receptive field size between the PBS/mustard oil and MeAIB/mustard oil groups was significant ($P < 0.002$ and $P < 0.001$, respectively, two-way ANOVA). No significant effect of MeAIB on baseline pinch receptive field size was observed ($P > 0.05$, RM ANOVA).

Activation threshold

In the PBS/mustard oil group, the eight neurons had at baseline a mean threshold of 94 $\pm$ 30 g that was unchanged after PBS superfusion but was significantly decreased to 55 $\pm$ 8% of control at 18 min after mustard oil application ($P < 0.001$, RM ANOVA; Fig. 2c). In the MeAIB/mustard oil group, however, mustard oil application, did not affect the threshold (82–118% of control; $P=0.2$, RM ANOVA, $n=8$; Fig. 2c). The differences between these two groups were significant ($P < 0.001$, two-way ANOVA). The mean baseline threshold (104 $\pm$ 27 g) was not significantly affected by MeAIB superfusion.

Responses to graded pinch/pressure stimuli

In the PBS/mustard oil group, the eight neurons had a mean baseline response of 72 $\pm$ 25 spikes, which was unchanged after PBS superfusion but significantly increased to 330 $\pm$ 100% of control at 18–28 min after mustard oil application ($P=0.001$, RM ANOVA; Fig. 2d). In the MeAIB/mustard oil group, mustard oil application after MeAIB superfusion failed to produce

a significant increase in response ($P=0.18$, RM ANOVA, $n=8$; Fig. 2d). The differences in responses after mustard oil application between these two groups were significant ($P < 0.001$, two-way ANOVA). The mean baseline response of 80 ± 27 spikes was not significantly changed after MeAIB superfusion.

Discussion

This study has demonstrated that continuous i.t. superfusion of MeAIB can significantly attenuate mustard oil-induced central sensitization as characterized by increases in pinch receptive field size, responses to graded noxious stimuli and decreases in mechanical activation threshold of MDH nociceptive neurons. MeAIB is a Na^+ -dependent, competitive blocker of SAT (including SAT-1 and SAT-2) [8–11]. In cloned transporter-transfected cell studies, MeAIB saturates transport by SAT-2 with a K_m of 1.6 mM, which is substantially higher than the K_m of 0.15–0.5 mM reported for SAT-1 [11–13]. As the dose of MeAIB used in this study was 0.5 mM, it is likely that the small increase in neuronal receptive field size observed in the MeAIB/mustard oil group (see Fig. 2a) may have resulted because of incomplete blockade of SAT (i.e. only blockade of SAT-1 but not SAT-2). In in-vivo cortical and thalamic dialysis studies, the glutamine concentration in the extracellular fluid (around 385 μM) is elevated from 1.5-fold to 1.8-fold upon perfusion of 50–250 mM MeAIB [14] and therefore it might be expected that the glutamine in the extracellular fluid in this study would be little if any higher than normal levels. Both in-vitro studies and in-vivo studies have suggested that SAT is involved in the modulation of synaptic glutamate and gamma-aminobutyric acid or GABA release [10,14–16]. In conclusion, the present findings provide the first demonstration that selective blockade of neuronal uptake of glutamine by MeAIB can significantly attenuate the development of MDH central sensitization that has been shown earlier to be glutamate dependent [4]. These findings further confirm the important roles of astroglia, glutamine, and presynaptic modulation of glutamate release in the development of central sensitization.

Acknowledgments

The authors acknowledge Ms S. Carter for her technical assistance. This study was supported by NIH Grant DE-04786 to B.J.S. and CIHR Grant MOP-82831 to J.O.D.

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Fig. 1.

Examples showing mustard oil-induced central sensitization in nociceptive-specific neurons during continuous i.t. superfusion of PBS or MeAIB over medulla. Neuronal pinch receptive field (upper panel), activation threshold (middle panel) and responses to pinch/pressure stimuli (lower panel) are shown at baseline, 18 min after MeAIB or PBS superfusion (i.e. before mustard oil application), 18 and 48 min after mustard oil application for each nociceptive-specific neuron. Note that the mustard oil induced changes only in the neuron illustrated from the PBS/mustard oil group. The vertical cut-off line defines the activation thresholds of a given neuron, for which neuronal discharges (dots) are displayed in instantaneous frequency distributions (in Hz). The responses to pinch/pressure stimuli are also shown in instantaneous frequency distributions during the 5-s stimulation period. Black areas in the diagrams indicate each neuron's receptive field. Abbreviations: MeAIB, methylaminoisobutyric acid; MO, mustard oil; PBS, phosphate-buffered saline; RF, mechanoreceptive field.

Fig. 2.

Time courses of mustard oil-induced central sensitization in nociceptive-specific neurons during continuous i.t. superfusion of PBS or MeAIB over medulla. (a) Neuronal pinch/pressure receptive field. (b) Tactile receptive field. (c) Mechanical activation threshold. (d) Responses to graded mechanical stimuli. Mean $\pm$ SEM values in percentage are shown in a, c, and d; b shows actual receptive field size values. Each group included eight nociceptive-specific neurons. * $P < 0.05$ compared with the baseline within the group (RM ANOVA with post-hoc Dunnett's test); # $P < 0.05$ compared with PBS group at the different time-points tested (two-way ANOVA with post-hoc Bonferroni t -test). MO, mustard oil; RF, mechanoreceptive field; RM ANOVA, repeated measures analysis of variance.