Published in final edited form as:

Neuroreport. 2010 March 10; 21(4): 313–317. doi:10.1097/WNR.0b013e32833774bf.

Role of spinal p38 α and β MAPK in inflammatory hyperalgesia and spinal COX-2 expression

Bethany L. Fitzsimmons¹, Michela Zattoni^{1, #}, Camilla I. Svensson[^], Joanne Steinauer, Xiao-Ying Hua, and Tony L. Yaksh

Department of Anesthesiology, University of California - San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0818, USA

Abstract

Pharmacological studies indicate that spinal p38 MAPK plays a role in the development of hyperalgesia. We investigated whether either the spinal isoform p38 α or p38 β is involved in peripheral inflammation-evoked pain state and increased expression of spinal COX-2. Using intrathecal antisense oligonucleotides, we show that hyperalgesia is prevented by downregulation of p38 β but not p38 α , while increases in spinal COX-2 protein expression at eight hours is mediated by both p38 α and β isoforms. These data suggest that early activation of spinal p38 β isoform may affect acute facilitatory processing, and both p38 β and α isoforms mediate temporally delayed upregulation of spinal COX-2.

Keywords

Spinal cord; Inflammation; Pain; Mitogen-activated protein kinase; Cyclooxygenase

Introduction

Previous studies have demonstrated that intrathecal (IT) delivery of inhibitors of p38 mitogen-activated protein kinase (p38) attenuate hyperalgesia including that produced by intraplantar carrageenan or IT substance P (SP) [1]. Importantly, IT p38 inhibitors, at analgesic doses, also block spinal PGE₂ release and prevent delayed increases otherwise observed in spinal COX-2 expression [1–3]. There are, however, at least four different p38 isoforms (α , β , γ and δ) expressed in adult mammalian CNS [4]. Although the p38 isoforms share certain structural and enzymatic properties, only p38 α and p38 β are sensitive to the conventional p38 inhibitors (SB203580, SD-282 [5]) known to alter pain processing. As p38 α and p38 β are both expressed in spinal dorsal horn [3], the question arises as to which, if not both, isoform is relevant to these two actions. In the present study we examined the effects of IT treatment with antisense (AS) oligonucleotides, targeted at the respective p38 α and p38 β isoform, on the thermal hyperalgesia produced by intraplantar carrageenan and IT SP-induced delayed spinal COX-2 expression in rats.

Corresponding author: Tony L. Yaksh, Department of Anesthesiology, University of California - San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0818, USA Tel: 619-543-3597; Fax: 619-543-6070; tyaksh@ucsd.edu.

¹B.L.F. and M.Z. contributed equally to this manuscript.

[#]Current address: University Hospital Zurich, Department of Neurosurgery Frauenklinikstrasse 10, 8091 Zurich, Switzerland.

[^]Current address: Department of Physiology and Pharmacology, Karolinska Institutet, 171 77 Stockholm, Sweden.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

2. Materials and methods

Animals and intrathecal drug delivery

These experiments were carried out according to protocols approved by the Institutional Animal Care Committee of University of California, San Diego. Male Holzman Sprague-Dawley rats (300–350 g) were prepared with lumbar IT catheters (PE-5, 8.5 cm) [6]. Intrathecal oligonucleotides were administered, starting 5–6 days after surgery.

Three oligonucleotides (ISIS Pharmaceuticals Inc., Carlsbad, CA and Altair Therapeutics Inc., San Diego, CA) were employed. ISIS 101757 (AGGTGCTCAGGACTCCATTT) is an AS oligonucleotide that hybridizes to rat p38 α mRNA (α -AS) beginning at position 1081, while ISIS 107211 (GTATGTCCTCCTCGCGTGGA) is a p38 β -AS oligonucleotide that hybridizes with the target beginning at position 439 of p38 β mRNA. ISIS 141923 (CCTTCCCTGAAGGTTCTCC) is a missense (MS) control. Both antisense(s) and missense compounds are synthetic 20-mer oligonucleotides, with a full phosphorothioate backbone and 2'-O-methoxyethyl modifications at positions of the ribose at nucleobases 1–5 and 16–20. Oligonucleotides were dissolved in artificial cerebrospinal fluid (ACSF) and administered IT (30 μ g in 10 μ l ACSF) once daily for 5 days. Behavioral experiments and spinal tissue collection were performed on day six.

Assessment of thermal nociception

Local inflammation was produced by unilateral injection of carrageenan (lambda, Sigma, St. Louis, MO, 100 μ l, 2% in saline) into the plantar surface of the left hind paw during brief isoflurane anesthesia (2–4%). To assess thermally evoked paw withdrawal response, a Hargreaves type device was employed [7]. Basal paw withdrawal latencies were assessed at time = –30 min and carrageenan injected intraplantarly at time = 0 min. Withdrawal latencies were assessed periodically. Response data were presented as response latency vs time and statistical analysis was undertaken using the hyperalgesic index (% change from pre-carrageenan baseline $\times$ min).

Western blot analysis

To verify the effect of IT AS on target protein expression, p38 α and β were measured in cords after 5-day IT AS/MS treatment. COX-1 and -2 expression was examined in spinal cords of rats harvested 8 hours after IT bolus SP (60 nmol) day six after 5-day AS or MS treatment. Lumbar spinal cords were homogenized by sonication in extraction buffer (50 mM Tris buffer, containing 0.5% Triton X-100, 150 mM NaCl, 1 mM EDTA, Protease inhibitor cocktail (P-8340, Sigma 1:100), and phosphatase inhibitor cocktail I and II (Sigma 1:100)). Samples were subjected to 4–12% Bis-Tris gel electrophoresis (Invitrogen, Carlsbad, CA) and transferred to nitrocellulose membranes (Invitrogen). After 1 hour incubation in 5% low-fat milk membranes were probed with antibodies overnight at 4°C followed by horseradish peroxidase-conjugated secondary antibody for 1 hour at ambient temperature. The signal was detected by chemiluminescent reagents (SuperSignal, Pierce, Rockford, IL). Nitrocellulose membranes were stripped with Re-Blot Western blot recycling kit (Chemicon, Temecula, CA) and incubated with different antibodies. Antibodies used in this study included: p38 α (Cell Signaling, Beverly, MA, 1:2000), p38 β (Zymed, San Francisco, CA, 1:1000), phospho-p38 (Cell Signaling, 1:1000), total p38 (Cell Signaling, 1:1000), COX-1 (Cayman, Ann Arbor, MI, 1:1000), COX-2 (Cayman, 1:1000) and β -actin (Sigma, 1:50,000). Intensity of immunoreactive bands was quantified using ImageQuant software (Molecular Dynamics, Sunnyvale, CA) and was normalized relative to β -actin.

Statistics

For measurements of protein levels by Western blotting and formalin test, four to seven animals were included per group. Groups were compared with one-way ANOVA and Bonferroni *post hoc* test, or student's *t*-tests with a criterion of $p < 0.05$ for significance.

Results

Down-regulation of p38 β , but not p38 α , attenuates carrageenan-induced thermal hyperalgesia

Five days of IT treatment with α -AS (30 μ g) or β -AS (30 μ g) oligonucleotides suppressed spinal p38 α and p38 β protein expression as compared with respective protein in the control MS-treated animals, i.e., 45 % reduction of p38 α protein, and 70 % reduction of p38 β protein ($p < 0.05$ vs MS group) (Figure 1).

Baseline latencies to thermal stimulation assessed before injection of carrageenan, were 8.9 ± 0.3 sec for the MS group, a value that is not different from the baseline of intrathecally catheterized and naive animals (data not shown). Treatment with neither α -AS nor β -AS altered thermal baseline latencies (α -AS, 8.7 ± 0.3 sec; and β -AS, 9.3 ± 0.3 sec, $N=5-6$ per group, $p > 0.05$ vs. MS). In the MS group, paw withdrawal latency decreased to 2.4 ± 0.4 sec at 120 min after carrageenan injection which is comparable to results observed in IT catheterized rats treated with intrathecal saline (data not shown). The carrageenan-induced thermal hyperalgesia was unabated in IT MS and IT α -AS treated animals (Figure 2A). In contrast, IT β -AS treatment produced a significant reduction in the thermal hyperalgesia ($p < 0.05$, Figure 2B). The thermal withdrawal latency in the contralateral (non-hyperalgesic) paw was unaltered by AS treatment.

SP-induced spinal COX-2 expression is reversed by knock-down of either p38 α or p38 β protein

Eight hours after IT SP (60 nmol), COX-2 protein expression in spinal cord was increased while COX-1 protein levels remained the same (Figure 3). Unlike the effect of IT β -AS treatment in preventing hyperalgesia, up-regulation of COX-2 protein in spinal cord was reversed by IT treatment with either β -AS or α -AS (Figure 3). None of the AS treatment affected COX-1 protein expression (Figure 3). In addition, the SP-induced upregulation of COX-2 expression was prevented by IT pre-treatment with p38 α/β inhibitor SB203580 (30 μ g, 30 min prior to SP, data not shown).

Discussion

The present study demonstrated that IT p38 α or p38 β antisense oligonucleotide treatment suppressed expression of the respective isoform in spinal cord. Reduction in the level of spinal p38 β , but not p38 α , prevented development of hyperalgesia following peripheral inflammation. Though the previous studies with p38 inhibitors indicate that p38 is involved in inflammation-induced hyperalgesia, the present work has further extended the previous finding to reveal that it is the p38 β , not p38 α isoform, which contributes to spinal pain processing initiated by an inflammatory stimulus. In the case of upregulation of spinal COX-2 protein expression evoked by activation of the spinal neurokinin 1 (NK1) receptor [1,8], the observation that knockdown of either isoform is able to suppress induction of COX-2 protein production suggests that both p38 α and p38 β isoforms participate in transcriptional and/or translational regulation of spinal COX-2. As it is known that COX-2 plays a pivotal role in maintenance of spinal sensitization, we hypothesize that p38 α/β -mediated upregulation of spinal COX-2 is one of underlying mechanisms of persistent inflammation-associated hyperalgesia.

The unique effect of p38 β in mediating the onset of hyperalgesia is an intriguing phenomenon. The present finding with anti-hyperalgesic effect of p38 β AS on carrageenan inflammatory model extended the previously reported work showing that specific knockdown of spinal p38 β results in blockade of facilitated pain states which focused on acute models including formalin-evoked flinching (1 hour) and IT SP-induced thermal hyperalgesia (< 0.5 hour) [3]. In parallel, with these acute effects, an increase in phosphorylation of spinal p38 (P-p38) is seen as early as 5 minutes after IT SP or intraplantar formalin [1,3], and this phosphorylation is prevented when spinal p38 β , but not p38 α , is down-regulated [3]. One important characteristic of p38 β is the selective cellular location of this enzyme in spinal cord. Immunohistochemical studies revealed that p38 β is present only in microglia [3]. Our previous work has shown that carrageenan-induced increases in spinal P-p38 occurred exclusively in microglia. Treatment with minocycline, an inhibitor of microglia activation, at the IT dose that blocked carrageenan-induced hyperalgesia, also attenuated the increased P-p38 in microglia [9]. The current finding of the anti-hyperalgesic effect of p38 β knockdown provides evidence for the hypothesis that p38 β in microglia responds rapidly to afferent signals generated by peripheral inflammation, and that the p38 β -mediated signal cascade is involved in the initial processing of spinal nociception.

It is widely accepted that COX-2 plays a critical role in the facilitation of spinal pain processing (see [10]). Although COX-2 is constitutively expressed in spinal cord, further upregulation of spinal COX-2 (both mRNA and protein) has been observed in a number of experimental models of hyperalgesia [11–14]. It is demonstrated that SP, the major neurotransmitter in primary afferents, is able to regulate COX-2 activity and expression via activation of NK1 receptors [1,8]. As shown by our previous work [1] spinal NK1 receptor activation-evoked synthesis of COX-2 protein is an acute event which can be prevented by IT pre-treatment, but not post-treatment, with p38 inhibitors. This indicates that the role of p38 in the triggering of spinal COX-2 expression is immediate and transient. p38 regulation of spinal COX-2 likely occurs at the level of COX-2 mRNA transcription or stability via phosphorylation of transcription factors or indirectly through other kinases [15]. Indeed, p38 is known to activate transcription factors such as CREB and ATF-2, and bind to the CRE element in the promoter region of the COX-2 gene [16]. Together with other kinases, p38 regulates RNA stability of COX-2 mRNA by stimulating binding of mRNA-stabilizing factor HuR to AREs at the 3'-UTR [17]. Our finding that antisense inhibition of either isoform partially prevents up-regulation of COX-2 protein in spinal cord following NK1 activation suggests that both p38 α and p38 β isoforms may participate in the regulation of spinal COX-2 protein expression. p38 α is present in dorsal spinal cord and distinct from p38 β , its location is largely neuronal [3]. Spinal COX-2 is reportedly present in neurons, microglia, radial glia and endothelial cells (see [10]). Thus, it is possible that the two isoforms may participate in individual cell types. Synergy between these two isoforms may also exist, since it is known that activation of p38 in microglia promotes release of pro-nociceptive mediators, such as cytokines [18], which further stimulate neurons and reinforce COX-2 expression.

Knockout of p38 α or p38 β genes has been reported. p38 α deficiency was found to result in embryonic lethality [19]. Mice with p38 β deficiency have been generated by two groups [4, 20]. Although both groups reported that inflammatory responses appear normal in p38 β deficient mice, one group [20] did observe, in agreement with this report, that inflammation-induced hyperalgesia was attenuated. It appears that permanent elimination of one of the p38 isoforms may be associated with compensation of function and/or expression of other isoforms, including other MAPKs (e.g. JNK, ERK) [4,21]. Accordingly, these findings achieved though the acute and specific knockdown of the two isoforms with IT antisense, are the first tangible evidence suggesting that spinal p38 α and β play distinct roles in acute spinal nociceptive processing and the delayed changes of protein expression induced by peripheral inflammation in the adult animals.

Conclusion

The present study reveals the intriguing organization that acute activation of spinal p38 MAPK can lead to constitutive effects leading to an immediate hyperalgesia, and that the same isoform can participate in the initiation of long term consequences such as the upregulation of an important pro-algesic enzyme. Blocking this linkage preemptively can not only alter early changes in pain processing but diminish the long-term consequences otherwise associated with acute activation of the spinal systems which process nociceptive information.

Acknowledgments

This work was supported by NIH NS 16541 and NIH DA02110. The oligonucleotides are gift from ISIS Pharmaceuticals, Inc. Carlsbad CA and Altair Therapeutics, Inc. San Diego, CA.

REFERENCES

1. Svensson CI, Marsala M, Westerlund A, et al. Activation of p38 mitogen-activated protein kinase in spinal microglia is a critical link in inflammation-induced spinal pain processing. *J Neurochem* 2003;86(6):1534–1544. [PubMed: 12950462]
2. Svensson CI, Hua XY, Protter AA, Powell HC, Yaksh TL. Spinal p38 MAP kinase is necessary for NMDA-induced spinal PGE(2) release and thermal hyperalgesia. *Neuroreport* 2003;14(8):1153–1157. [PubMed: 12821799]
3. Svensson CI, Fitzsimmons B, Azizi S, Powell HC, Hua XY, Yaksh TL. Spinal p38beta isoform mediates tissue injury-induced hyperalgesia and spinal sensitization. *J Neurochem* 2005;92(6):1508–1520. [PubMed: 15748168]
4. Beardmore VA, Hinton HJ, Eftychi C, et al. Generation and characterization of p38beta (MAPK11) gene-targeted mice. *Mol Cell Biol* 2005;25(23):10454–10464. [PubMed: 16287858]
5. Kumar S, McDonnell PC, Gum RJ, Hand AT, Lee JC, Young PR. Novel homologues of CSBP/p38 MAP kinase: activation, substrate specificity and sensitivity to inhibition by pyridinyl imidazoles. *Biochem Biophys Res Commun* 1997;235(3):533–538. [PubMed: 9207191]
6. Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. *Physiol Behav* 1976;17(6):1031–1036. [PubMed: 14677603]
7. Dirig DM, Salami A, Rathbun ML, Ozaki GT, Yaksh TL. Characterization of variables defining hindpaw withdrawal latency evoked by radiant thermal stimuli. *J Neurosci Methods* 1997;76(2):183–191. [PubMed: 9350970]
8. Hua XY, Chen P, Marsala M, Yaksh TL. Intrathecal substance P-induced thermal hyperalgesia and spinal release of prostaglandin E2 and amino acids. *Neuroscience* 1999;89(2):525–534. [PubMed: 10077333]
9. Hua XY, Svensson CI, Matsui T, Fitzsimmons B, Yaksh TL, Webb M. Intrathecal minocycline attenuates peripheral inflammation-induced hyperalgesia by inhibiting p38 MAPK in spinal microglia. *Eur J Neurosci* 2005;22(10):2431–2440. [PubMed: 16307586]
10. Svensson CI, Yaksh TL. The spinal phospholipase-cyclooxygenase-prostanoid cascade in nociceptive processing. *Annu Rev Pharmacol Toxicol* 2002;42:553–583. [PubMed: 11807183]
11. Hay CH, Trevethick MA, Wheeldon A, Bowers JS, de Belleruche JS. The potential role of spinal cord cyclooxygenase-2 in the development of Freund's complete adjuvant-induced changes in hyperalgesia and allodynia. *Neuroscience* 1997;78(3):843–850. [PubMed: 9153662]
12. Dirig DM, Yaksh TL. In vitro prostanoid release from spinal cord following peripheral inflammation: effects of substance P, NMDA and capsaicin. *Br J Pharmacol* 1999;126(6):1333–1340. [PubMed: 10217526]
13. Samad TA, Moore KA, Sapirstein A, et al. Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. *Nature* 2001;410(6827):471–475. [PubMed: 11260714]

14. Guhring H, Gorig M, Ates M, et al. Suppressed injury-induced rise in spinal prostaglandin E₂ production and reduced early thermal hyperalgesia in iNOS-deficient mice. *J Neurosci* 2000;20(17): 6714–6720. [PubMed: 10964977]
15. Shi Y, Gaestel M. In the cellular garden of forking paths: how p38 MAPKs signal for downstream assistance. *Biol Chem* 2002;383(10):1519–1536. [PubMed: 12452429]
16. Schroer K, Zhu Y, Saunders MA, et al. Obligatory role of cyclic adenosine monophosphate response element in cyclooxygenase-2 promoter induction and feedback regulation by inflammatory mediators. *Circulation* 2002;105(23):2760–2765. [PubMed: 12057991]
17. Subbaramaiah K, Marmo TP, Dixon DA, Dannenberg AJ. Regulation of cyclooxygenase-2 mRNA stability by taxanes: evidence for involvement of p38, MAPKAPK-2, and HuR. *J Biol Chem* 2003;278(39):37637–37647. [PubMed: 12826679]
18. Fiebich BL, Mueksch B, Boehringer M, Hull M. Interleukin-1 β induces cyclooxygenase-2 and prostaglandin E₂ synthesis in human neuroblastoma cells: involvement of p38 mitogen-activated protein kinase and nuclear factor-kappaB. *J Neurochem* 2000;75(5):2020–2028. [PubMed: 11032891]
19. Mudgett JS, Ding J, Guh-Siesel L, et al. Essential role for p38 α mitogen-activated protein kinase in placental angiogenesis. *Proc Natl Acad Sci U S A* 2000;97(19):10454–10459. [PubMed: 10973481]
20. Martin, WJ.; Murphy, BA.; Parson, JN.; Cupo, SH.; Zaller, DM.; O'Keefe, SJ. Society for Neuroscience. 2005. p38 β contributes to injury-induced nociceptive, but not inflammatory, responses in mice.
21. Kim C, Sano Y, Todorova K, et al. The kinase p38 α serves cell type-specific inflammatory functions in skin injury and coordinates pro- and anti-inflammatory gene expression. *Nat Immunol* 2008;9(9):1019–1027. [PubMed: 18677317]

Figure 1. Expression of p38α and p38β protein in rat spinal cord tissues was assayed by Western blotting. The protein expression in p38α-AS or p38β-AS treated group is compared with that in MS-treated animals. N=5–8 per group. * $p < 0.05$ vs. MS group.

Figure 2.

Effects of IT treatment with MS, p38 α -AS, p38 β -AS on thermal hyperalgesia evoked by injection of carrageenan (100 μ l, 2%, s.c.) into unilateral hind paw. Paw withdrawal thresholds are expressed in seconds (s) to thermal stimulation in each paw plotted against time (left panel). The antihyperalgesic effects are presented as a hyperalgesic index (area under the curve calculated from 0 to 240 min after carrageenan paw injection) in which the percentage reduction from baseline response is plotted against time (right panel). * $p < 0.05$, $N=5-6$, student's t -test.

Figure 3.

(A) Representative western-blots showing levels of COX-1, COX-2 and β -actin in lumbar spinal cord of rats 8 hours after IT SP and 5-day IT treatment of saline, MS, p38 α -AS and p38 β -AS (30 μ g). (B-C) Spinal COX-2 (B) and COX-1 (C) protein expression as percentage change from levels assessed in spinal cords of control rats (IT injection of MS and saline). * $p < 0.05$, 1-way ANOVA, N=3–6.