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Forum Minireview

ATP- and Adenosine-Mediated Signaling in the Central Nervous System: Chronic Pain and Microglia: Involvement of the ATP Receptor $P2X_4$

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Abstract. We have been studying the role of ATP receptors in pain and already reported that activation of $P2X_{2/3}$ heteromeric channel/receptor in primary sensory neurons causes acutely tactile allodynia, one hallmark of neuropathic pain. We report here that tactile allodynia under the chronic pain state requires an activation of the $P2X_4$ ionotropic ATP receptor and p38 mitogen-activated protein kinase (MAPK) in spinal cord microglia. Two weeks after L5 spinal nerve injury, rats displayed a marked mechanical allodynia. In the rats, activated microglia were detected in the injured side of the dorsal horn and the level of the dually-phosphorylated active form of p38MAPK (phospho-p38MAPK) in these microglia was increased. Moreover, intraspinal administration of a p38MAPK inhibitor, SB203580, suppressed the allodynia. We also found that the expression level of $P2X_4$ was increased strikingly in spinal cord microgila after nerve injury and that pharmacological blockade or inhibition of the expression of $P2X_4$ reversed the allodynia. Taken together, our results demonstrate that activation of $P2X_4$ or p38MAPK in spinal cord microglia is necessary for tactile allodynia after nerve injury.

Keywords: ATP receptor, $P2X_4$, microglia, p38 mitogen-activated protein kinase, allodynia

Introduction

Injury of primary sensory neurons produces long-lasting abnormal hypersensitivity to normally innocuous stimuli, a phenomenon known as tactile allodynia (1, 2). Tactile allodynia is the most troublesome of the neuropathic pain syndromes in humans and the mechanisms by which nerve injury develops tactile allodynia have remained unknown (3).

The present article introduces our recent study (4, 5) revealing crucial roles of two molecules, expression and activation of which are highly restricted in microglia in the spinal cord, in neuropathic pain: p38 mitogen-activated protein kinase (p38MAPK) and $P2X_4$ receptor, a subtype of ionotropic ATP receptors.

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p38MAPK was activated in spinal hyperactive microglia after nerve injury

Animals with spinal nerve injury displayed tactile allodynia. Paw withdrawal threshold (PWT) (ipsilateral side) to mechanical stimulation significantly decreased at 7 and 14 days. At day 7 and 14, the OX42 labelling was greater in the dorsal horn ipsilateral to the nerve injury. OX42-positive cells were more numerous and displayed hypertrophic morphology in the dorsal horn on the side of the nerve injury as compared with the contralateral side (4). To examine whether p38MAPK is activated in the spinal cord in rats that have developed tactile allodynia, we performed Western blot analysis using an antibody targeting the phosphorylated p38MAPK (phospho-p38MAPK). The band intensity of phospho-p38MAPK protein in the ipsilateral spinal cord dramatically increased 7 and 14 days after nerve injury compared with that in naive rat. Furthermore, we observed strong phospho-p38MAPK immunofluorescence

in the injury side of L5 dorsal spinal cord sections at 7 and 14 days after nerve injury (5). The bilateral difference in phospho-p38MAPK levels parallel with the emergence of the tactile allodynia. These results indicate that the p38MAPK is activated in the dorsal horn ipsilateral to the nerve injury, which may correlate with the nerve injury-induced tactile allodynia. We carried out double immunolabeling for phospho-p38MAPK and for cell type-specific markers to identify the type of cells and found that cells showing phospho-p38MAPK immunofluorescence was double labeled with OX42 but not with neuronal nuclei (NeuN) or glial fibrillary acidic protein (GFAP), indicating that activation of p38MAPK in the dorsal horn is highly restricted to microglia (5). OX42 recognizes the complement receptor type 3 (CR3), expression of which is greatly increased in hyperactive versus resting microglia after nerve injury (4 – 6). These results indicate that nerve injury induced a switch from the resting to the hyperactive phenotype in the population of microglia in the dorsal horn. We found that a marked phosphorylation of p38MAPK is observed in individual microglia in the ipsilateral dorsal horn (3.7-fold as compared with the contralateral side), particularly in hyperactive microglia that dramatically expressed OX42 (4). Therefore, we conclude that in the dorsal horn following nerve injury, hyperactive microglia are the cell type that activates p38MAPK and that the level of p38MAPK phosphorylation is dramatically increased in individual microglia.

p38MAPK activation in the spinal microglia caused development and maintenance of tactile allodynia

We examined whether intrathecal treatment with a potent inhibitor of p38MAPK, SB203580, alters the maintenance and development of tactile allodynia following nerve injury. Catheterized rats were treated with SB203580 (3 nmol/10 μL, n = 13) once at day 7 of the nerve injury. SB203580 treated-rats displayed a marked increase in PWT following nerve injury. When the rats were treated with SB203580 (30 nmol/10 μL, n = 9) once a day during 14 days from 0 day of the nerve injury, SB203580-treated rats showed only a slight decrease in PWT. These results suggest that inhibiting spinal p38MAPK activation in microglia by intrathecal treatment with inhibitor for p38MAPK suppresses the maintenance and development of tactile allodynia following spinal nerve injury.

The role of $P2X_4$ receptor in the tactile allodynia

We tested for the involvement of P2X receptors in the tactile allodynia by using ATP receptor blockers and found that tactile allodynia was reversed by the intrathecal administration of 2',3'-*O*-(2,4,6-trinitrophenyl) adenosine 5'-triphosphate (TNP-ATP) (30 nmol), an antagonist of P2X subtypes $P2X_{1-4}$, on day 7. Intrathecal administration of pyridoxial-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS), an antagonist of P2X subtypes $P2X_{1,2,3,5,7}$, but not of $P2X_4$, had no effect on either testing day. We observed no alteration in motor behaviour following TNP-ATP administration. These results together indicate that TNP-ATP caused a dose-dependent, reversible inhibition of allodynia on the nerve-injured side without a non-specific effect on motor or sensory functioning. At these intrathecal doses, PPADS is known to suppress nociceptive behaviors caused by intrathecal injection of the $P2X_{1,3}$ agonist α,β-methylene ATP. The lack of effect of PPADS on PWT together with the increase by TNP-ATP indicates that tactile allodynia caused by L5 nerve injury depends upon spinal P2X receptors that are sensitive to TNP-ATP and insensitive to PPADS. The pharmacological profile of these P2Xs is consistent with that of the $P2X_4$ subtype.

We found that $P2X_4$ protein in the ipsilateral spinal cord dramatically increased after L5 nerve injury. The increase in $P2X_4$ was detected as early as day 1 and the highest level was observed on day 14. The time-course of the change in $P2X_4$ level in the spinal cord and the bilateral difference in $P2X_4$ levels match the emergence of the tactile allodynia. We performed immunofluorescence on sections of the L5 spinal dorsal horn to examine the distribution of $P2X_4$. In the spinal cord ipsilateral to the nerve injury, we observed strong, punctate $P2X_4$ immunofluorescence in the dorsal horn on day 14. Furthermore, we found that cells showing $P2X_4$ immunofluorescence were not double-labelled for NeuN or GFAP. Almost all of the $P2X_4$-positive cells were double-labelled with OX42, indicating that $P2X_4$s were expressed in microglia.

Next we examined whether tactile allodynia following nerve injury is critically dependent upon functional $P2X_4$ in hyperactive microglia in the dorsal horn using an antisense oligodeoxynucleotide (ODN) targeting $P2X_4$. The nerve injury-induced allodynia was significantly suppressed in animals treated with $P2X_4$ antisense ODN as compared with that in animals treated with mismatch ODN (4). We also found that the level of $P2X_4$ protein in homogenates from the spinal cord of antisense ODN-treated rats was $32.0 \pm 4.8\%$ less than that of mismatch ODN-treated rats (4). These results indicate that intrathecal treatment with $P2X_4$ antisense ODN suppressed both the tactile allodynia and the increase in $P2X_4$ expression following nerve injury.

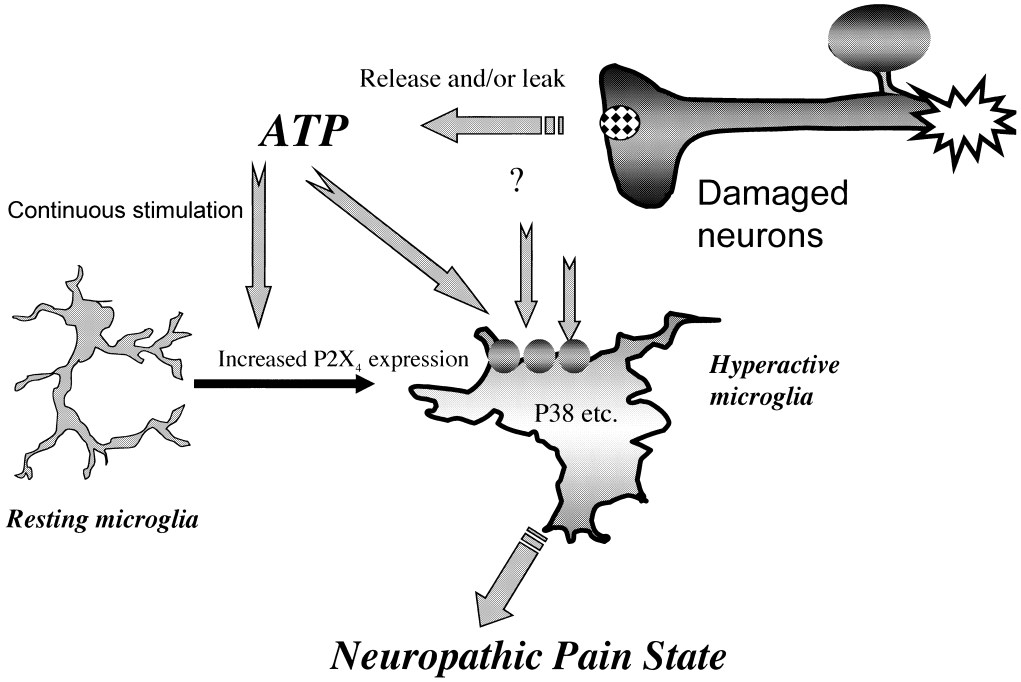

Fig. 1. Hypothesis: neuropathic pain after nerve injury. Tactile allodynia following nerve injury is critically dependent upon functional $P2X_4$ receptors in hyperactive microglia in the dorsal horn. ATP, which might be released or leaked from damaged neurons or astrocytes, stimulates resting microglia to be converted to hyperactive microglia. Hyperactive microglia increases the expression of $P2X_4$ receptors and p38-phosphorelation, resulting in tactile allodynia following nerve injury.

Conclusion

In the present article, we demonstrate that microglia in the spinal dorsal horn are converted to the hyperactive phenotype as a consequence of peripheral nerve injury and have dramatically expressed $P2X_4$ and activated p38MAPK. Also we suggested that the activation of p38MAPK and $P2X_4$ in spinal cord microglia are essential for allodynia after nerve injury (Fig. 1). The allodynia was reversed rapidly by pharmacological blockade of p38MAPK activation or inhibiting the expression of $P2X_4$ receptors, implying that nerve injury-induced pain hypersensitivity depends upon ongoing signaling via $P2X_4$ and/or p38MAPK, likely activated by ATP that may be released from primary sensory terminals (7 – 9), dorsal horn neurons (7, 10, 11), or dorsal horn astrocytes (12). Inhibition of $P2X_4$ expression, inhibiting the function of these receptors and/or p38MAPK in spinal microglia can be novel therapeutic approaches for treating tactile allodynia caused by nerve damage.

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