Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Involvement of phosphatidylinositol-3 kinase/Akt/mammalian target of rapamycin/peroxisome proliferator-activated receptor γ pathway for induction and maintenance of neuropathic pain

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ARTICLE INFO

Article history:
Received 16 February 2018
Accepted 19 March 2018
Available online xxx

Keywords:
N-type voltage-dependent Ca^{2+} channel
PI3K
Akt
mTOR
PPARγ
Neuropathic pain

ABSTRACT

Peripheral nerve injury induces neuropathic pain, which is characterized by the tactile allodynia and thermal hyperalgesia. N-type voltage-dependent Ca^{2+} channel (VDCC) plays pivotal roles in the development of neuropathic pain, since mice lacking $Ca_v2.2$, the pore-forming subunit of N-type VDCC, show greatly reduced symptoms of both tactile allodynia and thermal hyperalgesia. Our study on gene expression profiles of the wild-type and N-type VDCC knockout (KO) spinal cord and several pain-related brain regions after spinal nerve ligation (SNL) injury revealed altered expression of genes encoding catalytic subunits of phosphatidylinositol-3 kinase (PI3K). PI3K/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) signaling is considered to be very important for cancer development and drugs targeting the molecules in this pathway have been tested in oncology trials. In the present study, we have tested whether the changes in expression of molecules in this pathway in mice having spinal nerve injury are causally related to neuropathic pain. Our results suggest that spinal nerve injury induces activation of N-type VDCC and the following Ca^{2+} entry through this channel may change the expression of genes encoding PI3K catalytic subunits (p110α and p110γ), Akt, retinoid X receptor α (RXRα) and RXRγ. Furthermore, the blockers of the molecules in this pathway are found to be effective in reducing neuropathic pain both at the spinal and at the supraspinal levels. Thus, the activation of PI3K/Akt/mTOR/peroxisome proliferator activated receptor gamma (PPARγ) pathway would be a hallmark of the induction and maintenance of neuropathic pain.

1. Introduction

Voltage-dependent Ca^{2+} channels (VDCCs) play critical roles in the control of various cellular activities including muscle contraction and neurotransmitter release [1]. VDCCs are classified into several types (L, N, P, Q, R and T types) according to their physiological and pharmacological properties and all are composed of several subunits (α_1, α_2/δ, β, and γ). Of these subunits, α_1 is the essential pore-forming subunit and the others are auxiliary subunits [2,3]. By using a genetic strategy, we have previously demonstrated that mice lacking N-type VDCC show markedly reduced symptoms of neuropathic pain-related behavior induced by spinal nerve injury [4]. This suggests the critical role of N-type VDCC in the development of neuropathic pain, an intractable pain which is characterized mainly by two pathophysiological signatures, thermal hyperalgesia and mechanical allodynia [5,6]. We have also shown that the activation of N-type VDCC in excitable neurons contribute to thermal hyperalgesia and the activation of N-type VDCC in non-excitable microglia contribute to mechanical allodynia [7]. Although several lines of studies demonstrated that blockade of this channel is effective against neuropathic pain in rodents [8,9], it has been reported that clinical application of a direct N-type VDCC blocker has a limitation due to its serious side effects [10]. Nonetheless, gabapentin/pregabalin, which interacts with α_2/δ subunit of VDCC (speculated to be acting through the blockade of N-type VDCC function indirectly), is proven to be effective in blocking neuropathic pain without serious side effects and now used clinically [11].

PI3K plays important roles in many cellular functions and PI3K

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https://doi.org/10.1016/j.bbrc.2018.03.139
0006-291X/

signaling pathways can be potential targets for the treatment of various human diseases [12]. p110α, encoded by *Pik3ca* gene, is one of the catalytic subunits of Class IA PI3K, known to be expressed ubiquitously and activated downstream of receptor tyrosine kinases. p110γ, encoded by *Pik3cg* gene, is the catalytic subunit of class IB PI3K, known to be enriched in immune cells including microglia and activated mainly downstream of G protein coupled receptors. Results of cDNA microarray analysis of the spinal cord and several pain-related brain regions from wild-type and N-type VDCC knockout (KO) mice, two weeks after the spinal nerve injury or sham operation, indicated the differential changes in expression of *Pik3ca* and *Pik3cg* in both wild-type and the neuropathic pain-resistant N-type VDCC KO mice. Furthermore, some of the signaling molecules downstream of PI3K were also found to change their expression in the spinal cord. In the present study, we have tested whether these changes of expression are merely a coincidence associated with nerve injury operation or indicate the pathological mechanism leading to neuropathic pain.

2. Materials and methods

2.1. Animal experiments

All the animal experiments were approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University (Permission No. A2017-052C and its previous versions). Pain-related experiments were performed according to the ethical guidelines for investigations of experimental pain in conscious animals published by the International Association for the Study of Pain [13].

2.2. cDNA microarray analysis

cDNA microarray analysis was carried out in essentially the same way as described previously [14,15]. Two weeks after spinal nerve injury or sham operation of C57BL/6J and N-type VDCC KO mice [4], 5th and 6th lumbar level (L5/6) spinal cords, L5/6 DRG, cortex, mesencephalon, medulla oblongata and thalamus were dissected and collected for RNA preparation. cDNA microarray analysis was performed using the CodeLink™ UniSet Mouse 10K I (Amersham Biosciences, NJ, USA) and Atlas™ Glass Microarrays Mouse 1.0 and Mouse 3.8I (Clontech Laboratories, Inc. CA, USA) following the protocols provided by the manufacturers. Four data sets (wild type-SNL, wild type-sham, KO-SNL, KO-sham) were compared using the CodeLink™ System Software or Atlas Iris software.

2.3. Spinal nerve ligation (SNL)

Mouse spinal nerves (L5and L6) on the right side were ligated with fine silk thread according to the procedure described previously, with those on the left side kept intact for control [16,17].

2.4. Intrathecal and intracerebroventricular injections

Intrathecal (*i.t.*) injection was given in a volume of 5 μL by percutaneous puncture through an intervertebral space at the level of L5 or L6 vertebra according to the previously reported procedure [18] using a 25-μL Hamilton microsyringe with a 30-gauge needle. Mice were not anaesthetized during the *i.t.* injection.

Intracerebroventricular (*i.c.v.*) administration was performed as described previously [19,20]. In brief, mice were anaesthetized with ether and a 27-gauge needle attached to a microsyringe was inserted into the lateral ventricle. The volume for *i.c.v.* injection was 5 μL per mouse.

2.5. Agents

PI3K inhibitor LY294002, Akt inhibitor triciribine, GSK3β inhibitor SB216763, mTOR inhibitor rapamycin, and PPARα/γ inhibitor GW9662 were each first dissolved in dimethyl sulfoxide, diluted with physiological saline, and then administered intrathecally or intracerebroventricularly. LY294002 was from Calbiochem, triciribine was from BIOMOL International, SB216763 was from Tocris and rapamycin and GW9662 were from Sigma-Aldrich.

2.6. Pain-related behavioral tests

Mice were individually housed under temperature- and light-controlled environments (23 ± 1 °C, light and dark cycle of 12 h:12 h with the light on at 8:00 a.m.). Behavioral tests were performed 2–4 weeks after the SNL operation during the light phase in sound-proof rooms in essentially the same way as previously described [16]. Tactile allodynia was evaluated by determining the threshold to withdraw hindpaw from increasing mechanical stimuli. The threshold for paw withdrawal was determined by Dynamic Plantar Aesthesiometer (Ugo Basile, Italy), with the cut-off force 5 g. Thermal hyperalgesia was evaluated by determining the latency to withdraw a hindpaw from a local heat stimulus. The withdrawal latency was determined by Paw Thermal Stimulator (UCSD, San Diego), with the cut-off time 20.5 s.

2.7. Statistical analysis

Data are presented as mean ± s.e.m. One-way analysis of variance and post hoc Dunnett's tests were used to evaluate statistical significance. *P* value less than 0.05 was considered statistically significant.

3. Results

3.1. cDNA microarray analysis

Using the cDNA microarray techniques, we found that the expression of *Pik3cg* encoding p110γ in the mesencephalon was increased by 1.4-fold in the wild-type mice that developed neuropathic pain compared to sham operated wild-type mice but was decreased by 1.6-fold in the SNL-operated neuropathic pain-resistant N-type VDCC KO mice compared to sham operated N-type VDCC KO mice. On the contrary, the expression of *Pik3ca* encoding p110α in the mesencephalon and cortex was decreased by 1.7 and 1.6-fold, respectively, in the wild-type mice that developed neuropathic pain (vs sham operated wild-type mice) but was increased by 2.1 and 1.1-fold, respectively, in the SNL-operated neuropathic pain-resistant-N-type VDCC KO mice (vs sham operated N-type VDCC KO mice). We also found that the expression of *Pik3ca* in the spinal cord, medulla oblongata and thalamus was increased by 1.6, 2.5 and 1.1-fold, respectively, in the wild-type mice that developed neuropathic pain (vs sham operated control) but was decreased by 2.1, 1.3 and 1.6-fold, respectively, in the SNL-operated neuropathic pain-resistant N-type VDCC KO mice (vs sham operated N-type VDCC KO mice). We also found that gene expression of Akt, a signaling molecule downstream of PI3K, in the spinal cord was increased by 2.2-fold in the wild-type mice that developed neuropathic pain (vs sham operated control) but was decreased by 2.5-fold in the SNL-operated neuropathic pain-resistant N-type VDCC KO mice (vs sham operated control). Expression of the gene coding for mTOR, one of the signaling molecules downstream of Akt, was not changed but gene expression of RXRα and RXRγ in the spinal cord, signaling molecules downstream of mTOR functioning as a heterodimeric counterpart

Please cite this article in press as: D. Kondo, et al., Involvement of phosphatidylinositol-3 kinase/Akt/mammalian target of rapamycin/peroxisome proliferator-activated receptor γ pathway for induction and maintenance of neuropathic pain, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.139