Up-Regulation of Akt and Nav1.8 in BmK I-Induced Pain

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Dear Editor,

As the key contributor to the rising phase of action potentials in dorsal root ganglion (DRG) neurons, voltage-gated sodium channels (VGSCs) are important in physiological and pathological pain conditions. For instance, abnormal expression of VGSCs in DRG neurons is the main cause of the induction and maintenance of chronic inflammatory pain. Previously, we have reported that functional up-regulation of a peripheral VGSC isoform Nav1.8 is critical in pain behaviors induced by BmK I, a sodium channel activator purified from the venom of *Buthus martensi Karsch* (BmK) [1]. It is well known that protein kinase B/Akt participates in multiple cellular processes including protein synthesis, cell survival, proliferation, and metabolism. Moreover, it has been demonstrated that the Akt pathway plays an indispensable role in the hyperexcitability of peripheral sensory neurons and

therefore contributes to the generation and maintenance of pathological pain [2, 3]. The activation of Akt in DRG neurons has been reported in multiple inflammatory pain models including the carrageenan, formalin, and complete Freund's adjuvant (CFA) models [4–6]. However, whether and how the Akt pathway participates in BmK I-induced inflammatory pain is unknown.

In the present study, Western blot experiments revealed that the protein expression levels of both phosphorylated Akt (p-Akt) and Nav1.8 were increased in the ipsilateral DRG neurons both at 2 h (from 100.00% ± 0.98% to 129.71% ± 1.98%, $n = 3$, $P < 0.001$; from 100.00% ± 4.72% to 168.54% ± 9.73%, $n = 3$, $P < 0.05$) and at 8 h (from 100.00% ± 0.98% to 167.56% ± 4.50%; from 100.00% ± 4.72% to 249.69% ± 31.30%, $n = 3$, $P < 0.001$) after intraplantar injection of 0.05 mg/kg BmK I (Fig. 1A, B, D). The expression levels of p-Akt and Nav1.8 returned to baseline at 24 h post injection (Fig. 1A, B, D). The protein expression level of Nav1.8 was also increased at 2 h (from 100.00% ± 1.62% to 164.15% ± 8.58%, $n = 3$, $P < 0.001$) and 8 h (from 100.00% ± 1.62% to 137.23% ± 1.15%, $n = 3$, $P < 0.001$) in the contralateral DRG neurons (Fig. 1A, C, E). However, no significant change in p-Akt was detected in the contralateral DRGs at any time point (Fig. 1A, C, E). Unlike p-Akt, the protein expression levels of unphosphorylated Akt in DRGs remained unchanged on both the ipsilateral and contralateral sides after BmK I injection (Fig. 1A, F, G). Furthermore, immunohistochemistry experiments revealed that the percentage co-localization of Nav1.8 with calcitonin gene-related peptide (CGRP) neurons increased significantly at 2 h and 8 h in the ipsilateral L4–L5 DRG neurons, while the percentage co-localization of p-Akt and CGRP neurons was increased only at 2 h (Fig. 1J). As to the contralateral side, the percentage of either Nav1.8 or p-Akt co-localized

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Fig. 1 Expression patterns of Akt, p-Akt, and Nav1.8 in DRGs after BmK I injection. **A** Representative Western blots of Akt, p-Akt, and Nav1.8 immunoreactivity at different time points following intraplantar injection of BmK I. **B–G** Quantification of band intensity for the ipsilateral (**B, D, F**) and contralateral (**C, E, G**) sides. The fold change for the density of the Akt, p-Akt, and Nav1.8 bands was calculated after normalization to control. **H, I** Changes in the mRNA

with CGRP neurons was increased at 2 h and 8 h in L4–L5 DRGs (Fig. 1J). In the ipsilateral DRGs, $\sim 33.3\% \pm 1.78\%$ and $35.0\% \pm 3.49\%$ of p-Akt-labeled neurons were positive for Nav1.8 at 2 h and 8 h after saline injection (Fig. 2A, C). In the contralateral DRGs, the percentage co-localization of p-Akt and Nav1.8 at 2 h was $40.9\% \pm 4.04\%$ and at 8 h was $41.7\% \pm 2.43\%$ (Fig. 2B, D). After BmK I injection, the percentage of neurons double-labeled for p-Akt and Nav1.8 was increased from $33.3\% \pm 1.78\%$ to $42.50\% \pm 1.25\%$ at 2 h and from $35.0\% \pm 3.49\%$ to $46.51\% \pm 0.97\%$ at 8 h post injection in the ipsilateral DRGs (Fig. 2A, C). However, on the contralateral side, there was no significant difference in p-Akt and Nav1.8 double staining between BmK I and saline groups at either time point (Fig. 2B, D).

expression levels of Nav1.8. **J** Co-localization of Nav1.8 and Akt with CGRP neurons. **K, L** Ipsilateral (**K**) and contralateral (**L**) quantification of the co-localization in Nav1.8-positive cells. **M, N** Ipsilateral (**M**) and contralateral (**N**) quantification of the co-localization in pAkt-positive cells. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ ($n = 3$) compared with control groups. All data are presented as mean $\pm$ SEM.

We further examined the effect of pre-treatment with Akt IV, an inhibitor of Akt phosphorylation, on the expression of p-Akt and Nav1.8. We found that $5 \mu\text{g}/10 \mu\text{L}$ Akt IV, administered 30 min before BmK I injection, prevented the BmK I-induced up-regulation of the co-localization of CGRP neurons with either p-Akt or Nav1.8 on both the ipsilateral and contralateral DRGs (Fig. 1K–N). Moreover, the increase of Nav1.8 mRNA at 2 h and 8 h in the ipsilateral DRG neurons was prevented by Akt IV. Interestingly, the decrease of Nav1.8 mRNA at 8 h in the contralateral DRG neurons was also partially prevented by Akt IV (Fig. 1H, I). These results suggest that the BmK I-induced changes in the contralateral DRG neurons are triggered by the sensitization of ipsilateral DRG neurons. Therefore, the effects of Akt IV on the expression of

Fig. 2 Co-localization of p-Akt with Nav1.8 and the suppression of BmK I-induced pain behaviors by Akt IV. **A** Representative labeling for p-Akt and Nav1.8 in ipsilateral DRG neurons, and their merged images. **B** Representative labeling for p-Akt and Nav1.8 in contralateral DRG neurons, and their merged images. **C** Quantification of the percentage of neurons double-labeled for p-Akt and Nav1.8 in ipsilateral DRGs at 2 h and 8 h after saline or BmK I injection. **D** Quantification of the percentage of neurons double-labeled for p-Akt and Nav1.8 in contralateral DRGs at 2 h and 8 h after saline or BmK I injection. **E** Time-course of suppression of paw flinches/5 min by Akt IV.

Nav1.8 mRNA in the contralateral DRG neurons might be caused by the effects of Akt IV on the ipsilateral DRG neurons.

To test whether the increased Akt expression contributed to the pain behaviors induced by BmK I, Akt IV (1 µg/10 µL, 5 µg/10 µL, and 10 µg/10 µL, intrathecal) was administered 30 min before ipsilateral intraplantar injection of BmK I. We found that 5 µg and 10 µg Akt IV suppressed spontaneous pain behaviors including the number of spontaneous paw flinches, the total number of paw flinches, the total duration of lifting/biting, and the number of paroxysmal pain-like behaviors during the first 2 h after injection (Fig. 2E–L). Previously, we have reported that ipsilateral injection of BmK I induces both bilateral mechanical allodynia (or mirror mechanical allodynia) and unilateral (ipsilateral) thermal hyperalgesia during the induction phase of pain responses [7]. In the present study,

F Suppression of the total number of rat paw flinches in 2 h by Akt IV. **G** Total duration of lifting/biting behaviors in 2 h. **H** Suppression of the number of paroxysmal pain-like behaviors by Akt IV. **I–J** Suppressive effects of pre-treatment with intrathecal Akt IV on the ipsilateral (**I**) and contralateral (**J**) mechanical hypersensitivity. **K–L** Suppressive effects of pre-treatment with intrathecal Akt IV on the ipsilateral (**K**) and contralateral (**L**) mechanical hypersensitivity. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared with control groups ($n = 3$). All data are presented as mean $\pm$ SEM.

we found that pretreatment with Akt IV partially prevented the mirror mechanical allodynia and unilateral thermal hyperalgesia in a dose-dependent manner at 4 h and 8 h after BmK I injection (Fig. 2I–K). The results indicated that the activation of Akt is indispensable during the induction and maintenance phases of BmK I-induced pain. Notably, the expression of p-Akt in the contralateral DRGs was unaltered, while the contralateral mechanical hypersensitivity was inhibited by Akt IV. These results suggested that the BmK I-induced contralateral pain hypersensitivity might rely on the ipsilateral pain sensitization induced by BmK I. Inhibition of the Akt signal by Akt IV on the BmK I-injected side might reduce the transmission of pain signals into the CNS and might attenuate the contralateral pain responses.

In conclusion, Akt is activated along with Nav1.8 in peripheral DRG neurons by BmK I. Akt might regulate

BmK I-induced pain responses *via* modulation of Nav1.8 expression. Both Akt and Nav1.8 might be indispensable for the induction, development, and maintenance of BmK I-induced pain responses.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there are no competing interest.

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