ERK, synaptic plasticity and acid-induced muscle pain

Ming-Yuan Min,1,2,* Hsiu-Wen Yang,3 Chen-Tung Yen,1 Chien-Chang Chen,4 Chih-Cheng Chen5 and Sin-Jhong Cheng1

1Institute of Zoology and 2Department of Life Science; College of Life Science; National Taiwan University; Taipei; 3Department of Biomedical Sciences; Chung-Shan Medical University; Taichung; 4Institute of Biomedical Sciences; Academia Sinica, Taipei, Taiwan

Key words: amygdala, central sensitization, chronic pain, LTP, parabrachial nucleus, paraventricular thalamic nucleus anterior, T-channel

Abbreviations: ACC, anterior cingulate cortex; AIMP, acid-induced muscle pain; CeAC, capsular central amygdala; ERK, extracellular signal-regulated kinase; LTP, long-term potentiation; PBA, parabrachio-amygdaloid; PVA, paraventricular thalamic nucleus anterior

Chronic pain is characterized by post-injury pain hypersensitivity. Current evidence suggests that it might result from altered neuronal excitability and/or synaptic functions in pain-related pathways and brain areas, an effect known as central sensitization. Increased activity of extracellular signal-regulated kinase (ERK) has been well-demonstrated in the dorsal horn of the spinal cord in chronic pain animal models. Recently, increased ERK activity has also been identified in two supraspinal areas, the central amygdala and the paraventricular thalamic nucleus anterior. Our recent work on the capsular central amygdala has shown that this increased ERK activity can enhance synaptic transmission, which might account for central sensitization and behavior hypersensitivity in animals receiving noxious stimuli.

Physiological pain serves as an early-warning protective system attributive to individuals to avoid noxious stimuli or harmful contact, or creates a circumstance to disfavor movement and physical contact to assist injured bodies to repair. On the other hand, non-protective-related pathological pain results from abnormality and dysfunction of the nervous system and consequently creates clinical pain syndromes with the greatest unmet need.1 Chronic pain is among the most disabling and costly afflictions. The American Chronic Pain Association estimates that one in three Americans (~50 million) suffers from some type of chronic pain.2 Chronic pain is often accompanied by altered synaptic functions (usually hyperexcitability) of nociceptive pathways in the central nervous system, an effect known as central sensitization,3 in which the sensory system is rendered into a dysfunctional hyperalgesia state in which distinction of low-intensity stimuli and noxious stimuli is lost.

Central sensitization occurs not only in the dorsal horn of the spinal cord, but also in the thalamus, amygdala and anterior cingulate cortex (ACC) in inflammatory, neuropathic and chemically-induced chronic pain.4 Being a part of the limbic system, the amygdala plays a key role in emotionality, including the emotional evaluation of sensory stimuli, such as pain and emotional learning and memory5-8 and has been the subject of intensive investigation. The capsular central amygdala (CeAC) consists of many nociceptive neurons and is defined as the “nociceptive amygdala.”9-10 It receives large numbers of nociceptive-related fibers from the parabrachial nucleus (PB), a pontine structure that relays nociceptive information from spinofugal tracts to various forebrain areas, via the parabrachio-amygdaloid (PBA) pathway.10,11 In neuropathic and arthritic pain models, synaptic transmission of the PBA pathway on CeAC neurons (PBA-CeAC synapses) is enhanced.5,12,13 Nevertheless, the underlying cellular mechanism remains unclear.

Synaptic Plasticity and Chronic Pain

Long-term potentiation (LTP),14 is a prolonged increase in response to the same stimulus after a tetanizing stimulation train. LTP can be induced at the synapses of nociceptive inputs on dorsal horn neurons in the spinal cord15 and in the ACC,16,17 in which most neurons respond to both noxious and non-noxious stimuli, after peripheral injury. The occurrence of LTP in these nociceptive-related pathways and areas is considered to be a part of the cellular mechanism underlying the development of central sensitization in chronic pain.18 At some cortical synapses, induction of LTP requires activation of extracellular signal-regulated kinase (ERK) of mitogen-activated protein kinase (MAPKs) downstream of the activation of the N-methyl-D aspartate glutamate receptor (NMDAR).19-22 Interestingly, a biochemical hallmark of chronic pain is an increased level of phosphorylated (p)ERK and/or other MAPKs in the dorsal horn23-26 and the CeAC27 in formalin-induced pain and other chronic models. Direct injection of an ERK blocker or activator into the CeAC respectively increased or reduced nociceptive behavior in normal animals or those with formalin-induced or acid-induced pain, showing that activation of ERK in the CeAC directly causes central sensitization.27-29 Accordingly, it has been proposed that LTP and central sensitization may share common cellular mechanisms.18 In a
and mice with acid-induced muscle pain (AIMP), we provided a recent study of PBA-CeAC synaptic transmission in normal mice and found that the application of PDA also caused ERK-dependent postsynaptic enhancement of PBA-CeAC synaptic transmission, which might occur through the upregulation of non-NMDAR functions at synaptic sites.37,38 We also reported NMDAR-PKC-ERK-dependent LTP of PBA-CeAC synaptic transmission after the application of tetanizing stimulation (Fig. 1C). As PDA application to slices directly induces LTP and the induction of further LTP by tetanizing stimulation is occluded when slices are bathed in PDA, the postsynaptic enhancement by PDA and LTP by tetanizing stimulation might share common cellular mechanisms. Interestingly, PKC-ERK-dependent postsynaptic potentiation was not only observed when ERK was activated by bath-applied PDA, but also in animals with AIMP. Moreover, the postsynaptic enhancement by PDA and LTP by tetanizing stimulation seen in slices from normal mice was occluded in slices from AIMP mice. Based on these results, we propose that, in AIMP mice, an excessive nociceptive signal triggered by acidic saline injection into the gastrocnemius muscle activates ERK in the CeA, thereby enhancing PBA-CeAC synaptic transmission (Fig. 1).

**T-channel and PVA**

Although the observation of increased pERK levels in the central amygdala in AIMP mice is consistent with previous studies using other models, we found substantial differences between AIMP and other chronic pain models. First, mechanical hypersensitivity has been shown to be functionally lateralized to the right amygdala in intraplantar formalin and arthritic models,39-41 however, this phenomenon was not found in the AIMP model, as we constantly observed increased pERK in both sides of the central amygdala after acid injection.28,29 Second, in addition to the central amygdala, a profound increase in the number of neurons showing pERK immunoreactivity was found in the paraventricular thalamic nucleus anterior (PVA) in AIMP mice.28 The PVA belongs to the midline thalamic nuclei and is believed to serve as an important relay in the transfer of visceral/arousal and circadian information to parts of the limbic system, thereby priming them to a state of readiness for behavior responding.42,43 Recently, accumulating evidence has emerged to support a role of PVA in the modulation of nociception. PVA neurons receive nociceptive information indirectly from the PB44,45 and from regions that are important in generating pain perception, such as the ACC and central amygdala;46-50 they have been shown to respond to innocuous somatic and/or noxious stimuli51,52 with a widely receptive field. Pharmacological inactivation of ERK in PVA blocks acid-induced chronic hyperalgesia in mice.28

**Synaptic Plasticity in AIMP**

The AIMP model,30 first described and developed by Sluka et al. (2001), is generally accepted as an animal model for chronic musculoskeletal pain syndrome, such as fibromyalgia and myofascial pain syndromes. In this model, animals receive a single injection of acidic saline into one side of the gastrocnemius muscle and develop transient mechanical hyperalgesia in both hind paws. The hyperalgesia declines in 24 h but can become long-lasting for weeks if a second dose of acidic saline is given in 5 days. AIMP involves the activation of acid-sensing ion channel 3 in muscle nociceptors and requires central sensitization.31

Consistent with studies of other models, we found an increased pERK level in both sides of the central amygdala in an AIMP model.28,29 To investigate whether such elevated pERK in CeAC is related to a change in the efficacy of PBA-CeAC synapses and behavioral hypersensitivity in AIMP, we examined the effect of an ERK activator, phorbol 12,13-diacetate (PDA), on the function of PBA-CeAC synapses. In addition to the well-known facilitating effect on glutamate release at many cortical synapses (Fig. 1A and B) through PKC-dependent upregulation of voltage-gated calcium channels located at axonal terminals,32-34 and/or modulation of proteins involved in exocytosis,35,36 we found that the application of PDA also caused ERK-dependent postsynaptic enhancement of PBA-CeAC synaptic transmission, which might occur through the upregulation of non-NMDAR functions at synaptic sites.37,38 We also reported NMDAR-PKC-ERK-dependent LTP of PBA-CeAC synaptic transmission after the application of tetanizing stimulation (Fig. 1C). As PDA application to slices directly induces LTP and the induction of further LTP by tetanizing stimulation is occluded when slices are bathed in PDA, the postsynaptic enhancement by PDA and LTP by tetanizing stimulation might share common cellular mechanisms. Interestingly, PKC-ERK-dependent postsynaptic potentiation was not only observed when ERK was activated by bath-applied PDA, but also in animals with AIMP. Moreover, the postsynaptic enhancement by PDA and LTP by tetanizing stimulation seen in slices from normal mice was occluded in slices from AIMP mice. Based on these results, we propose that, in AIMP mice, an excessive nociceptive signal triggered by acidic saline injection into the gastrocnemius muscle activates ERK in the CeA, thereby enhancing PBA-CeAC synaptic transmission (Fig. 1).

![Figure 1. Schematic diagram summarizing PBA-CeAC synaptic transmission in normal and AIMP mice.](image-url)
hypersensitivity and elevation of the pERK level in the PVA in AIMP mice. The T-channels are involved in the regulation of many thalamic neuron functions, including the switching of firing modes between continuous or burst. Recently, we have shown that T-channels might also contribute to the synaptic transmission of corticothalamic (CT) inputs on neurons in the ventrobasal nucleus, as synaptic efficacy of CT inputs could undergo a firing-mode-dependent plastic change. In addition, a growing amount of evidence has suggested a role of the T-channel in pain. Taking these lines of evidences together, it is obvious that further exploration of T-channel function in synaptic plasticity and neuronal excitability in the PVA, a newly-identified pain-related area, should provide new insight into the cellular mechanisms underlying the development of central sensitization at the supraspinal level.

In conclusion, plastic changes in synaptic transmission between pain-related brain areas have been shown to contribute to central sensitization and behavior hypersensitivity and ERK activation appears to play an important role in linking noxious stimuli to such functional changes. Mechanisms by which ERK are activated by noxious signals may vary among different pain-related brain areas. While NMDAR and/or group 1 metabotropic glutamate receptor activation are involved in the central amygdala, PVA thalamic neurons involve T-channels.

References

1. Woolf CJ. What is this thing called pain? J Clin Invest 2010; 120:3742-4.
2. Gold MS, Gebhart GF. Nociceptor sensitization in pain pathogenesis. Nat Med 2010; 16:1248-57.
3. Woolf CJ. Evidence for a central component of post-injury pain hypersensitivity. Nature 1983; 306:686-8.
4. Lautermieler A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. J Pain 2009; 10:895-926.
5. Davis M. Anatomical and physiologic substrates of emotion in an animal model. J Clin Neuropsychi 1998; 20:378-87.
6. LeDoux JE. Emotion circuits in the brain. Annu Rev Neurosci 2000; 23:155-84.
7. Zald DH. The human amygdala and the emotional evaluation of sensory stimuli. Brain Res Rev 2003; 41:188-123.
8. Neugebauer V, Li W, Bird GC, Han JS. The amygdala and persistent pain. Neuroscientist 2000; 10:221-34.
9. Bernard JF, Huang GF, Benson JM. Nuclei centralis of the amygdala and the globus pallidus ventralis: electrophysiological evidence for an involvement in pain processing. J Neurophysiol 1992; 68:551-69.
10. Bernard JF, Bester H, Benson JM. Involvement of the spino-parabrachial -amygdaloid and -hippocamal pathways in the autonomic and affective emotional aspects of pain. Prog Brain Res 1996; 107:243-55.
11. Gauriau G, Casteilla L, Collingridge GL. A synaptic model of the hippocampal formation. Nature 1993; 361:31-9.
12. Chao MV, Nestler EJ. Gene expression and neuronal plasticity. Neuron 2000; 23:11-4.
13. Chen S, Squire LR. Hemispheric lateralization of a molecular signal for pain modulation in the amygdala. Mol Pain 2009; 5:42-4.
14. Ji G, Neugebauer V. Hemispheric lateralization of pain processing by amygdala neurons. J Neurophysiol 2009; 102:2253-64.
15. Kolber BJ, Montana MC, Carrasquillo Y, Xu J, Heinemann SF, Muglia LJ, et al. Activation of metabotropic glutamate receptor 5 in the amygdala modulates pain-like behavior. J Neurosci 2010; 30:8203-13.
16. Mon M, Weiss RF, Moore RY. Efferent projections of the paraventricular thalamic nucleus in the rat. J Comp Neurol 1995; 359:221-38.
17. Vertes RP, Hoover WB. Projections of the paraventricular and parathalamic nuclei of the dorsal midline thalamus in the rat. J Comp Neurol 2008; 508:212-37.
18. Bester H, Bourguin L, Villanueva L, Benson JM, Bernard JF. Differential projections to the intralaminar and gustatory thalamus from the parabrachial area: a PHA-I study in the rat. J Comp Neurol 1999; 405:421-9.
19. Krout KE, Loewy AD. Parabrachial nucleus projections to the midline and intralaminar thalamic nucleus of the rat. J Comp Neurol 2000; 428:475-94.
20. Chen S, Su HS. Afferent connections of the thalamic paraventricular and paratenal nucleus in the rat—a retrograde tracing study with somotophoric application of Fluoro-Gold. Brain Res 1990; 522:1-6.
21. Krout KE, Behler RE, Loewy AD. Brainstem projections to the midline and intralaminar thalamic nuclei of the rat. J Comp Neurol 2002; 448:53-101.
22. Novak CM, Harris JA, Smale L, Nunez AA. Paraventricular thalamic nucleus projections to the paraventricular thalamic nucleus in nocturnal rats (Rattus norvegicus) and diurnal nile grass rats (Arvicanthis nilineus). Brain Res 2000; 874:147-57.
23. Orkand K, Rugiero DA, Nakamura Y. Adrenergic innervation of forebrain neurons that project to the paraventricular thalamic nucleus in the rat. Brain Res 1995; 697:17-26.
50. Peschanski M, Besson JM. Diencephalic connections of the raphe nuclei of the rat brainstem: an anatomical study with reference to the somatosensory system. J Comp Neurol 1984; 224:509-34.

51. Dostrovsky JO, Guillbaud G. Nociceptive response in medial thalamus of the normal and the arthritic rat. Pain 1990; 40:93-104.

52. Reyes-Vazquez C, Prieto-Gomez B, Dafny N. Noxious and non-noxious responses in the medial thalamus of the rat. Neurology 1989; 11:177-80.

53. Bal T, McCormick DA. Mechanisms of oscillatory activity in guinea-pig nucleus reticularis thalami in vitro: a mammalian pacemaker. J Physiol 1993; 468:669-91.

54. Sherman SM. Tonic and burst firing: dual modes of thalamocortical relay. Trends Neurosci 2001; 24:122-6.

55. Hsu CL, Yang HW, Yen CT, Min MY. Comparison of synaptic transmission and plasticity between sensory and cortical synapses on relay neurons in the ventrobasal nucleus of the rat thalamus. J Physiol 2010; 588:4347-63.

56. Altier C, Zamponi GW. Targeting Ca2+ channels to treat pain: T-type versus N-type. Trends Pharmacol Sci 22004; 5:465-70.

57. Cheng JK, Lin CS, Chen CC, Yang JR, Chiou LC. Effects of intrathecal injection of T-type calcium channel blockers in the rat formalin test. Behav Pharmacol 2002; 13:1-8.

58. Choi S, Na HS, Kim J, Lee J, Lee S, Kim D, et al. Attenuated pain responses in mice lacking Ca(V)3.2 T-type channels. Genes Brain Behav 2007; 6:425-31.

59. Jagodic MM, Pathirathna S, Nelson MT, Mancuso S, Jokovic PM, Rosenberg ER, et al. Cell-specific alterations of T-type calcium current in painful diabetic neuropathy enhance excitability of sensory neurons. J Neurosci 2007; 27:3305-16.

60. Todorovic SM, Jevtic-Todorovic V, Meyenburg A, Mennerick S, Perez-Reyes E, Romano C, et al. Redox modulation of T-type calcium channels in rat peripheral nociceptors. Neuron 2001; 31:75-85.

61. Todorovic SM, Meyenburg A, Jevtic-Todorovic V. Mechanical and thermal antinociception in rats following systemic administration of mibefradil, a T-type calcium channel blocker. Brain Res 2002; 951:336-40.