Enhanced adenosine-mediated inhibition of excitatory transmission in the central amygdala receiving neuropathic pain

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Introduction: Long-lasting chronic pain is often accompanied with strong negative emotion, which in turn increases the subjective sensation of pain, leads to psychological problems including depression and neurosis, and inevitably lowers the quality of life of the patients. The complication of such pain-related emotional changes is that such negative affection is not always correlated with the severity or duration of the objective tissue damages: in many clinical cases, it might appear regardless of remaining tissue damages. Effective therapies for such aberrant central perception resulting from sustained pain are of primary demand for both the patients and clinicians [1]. The ascending pathway from the dorsal horn to the laterocapsular part of the central amygdala (CeA) via nucleus parabrachialis (PB) plays the pivotal role in the expression of negative emotion resulting from nociceptive information [2]. We have previously reported that excitatory synaptic transmission between the PB afferents and central amygdala (CeA) neurons become potentiated and consolidated in the rat model of the neuropathic pain [3]. We examined whether this potentiation is affected with adenosine, which is known to modulate synaptic transmission in the central nervous system and attenuate chronic pain sensation [4].

Materials and Methods: Young Wistar rats (P21-24) were anesthetized and neuropathic pain models were made. In “ligation group”, the left L5 spinal nerve was isolated and tightly ligated and in “sham group”, the same manipulations except the nerve ligation were made [5]. To quantify tactile sensitivity of the hind paw, we estimated paw withdrawal threshold to normally innocuous stimuli. Mechanical stimuli were applied with von Frey filaments. Transverse brain slices from ligation and sham groups were prepared on the sixth or seventh day after operation. The slice was perfused with artificial cerebrospinal fluid (ACSF). To isolate excitatory synaptic inputs, picrotoxin (100 μM) and strychnine (1 μM) were dissolved in ACSF and bath-applied. CeA neurons were identified visually with an infrared differential interference contrast microscopy (IR-DIC). Stimulating electrode was placed on the afferent fibers from the PB under visual guidance and stimulation-evoked excitatory postsynaptic currents (eEPSCs) were recorded from the CeA neurons in whole-cell configuration. Adenosine (100 μM; dissolved in ACSF) was locally applied to the recorded neurons with a help of electromagnetic valves for 60 s in the absence and presence of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; an antagonist against adenosine A1 receptors; 1 μM for longer than 10 min).

Results: Unilateral spinal nerve ligation established side-specific tactile allodynia. The synaptic transmission in the central amygdala was potentiated in the animals with long-lasting chronic neuropathic pain; this potentiation occurred predominantly in the PB-CeA synapses contralateral to the peripheral neuropathic pain (Figure 1). Adenosine significantly decreased the amplitude of EPSC evoked by PB afferents in both sham and ligation groups. However, the potency of adenosine at 100 μM was significantly larger in CeA neurons contralateral to ligation than other groups. In the presence of adenosine, the EPSC amplitudes were no more significantly different between two sides. DPCPX (1 μM) abolished the inhibitory effect of adenosine both in ligation and sham groups and in the ipsilateral and contralateral CeA (Figure 2).

Discussion: The present results imply that CeA neurons contralateral to the side with neuropathic pain are more easily excited by smaller synaptic inputs from parabrachial pathway. This means that even a small below-threshold input, which is not necessarily painful, from the parabrachial

nucleus can make the CeA neurons generate action potentials and subsequently evoke emotional responses mediated by CeA in the neuropathic animals. Activation of adenosine A1 receptors exerted inhibitory effects selectively on the side receiving afferents from neuropathic pain; i.e., it selectively attenuated the excitatory transmission that had been potentiated upon establishment of the neuropathic pain. These results suggest that purinergic receptors in the CeA might be a potent target of the drug therapies against unpleasant emotional responses in the patients suffering from persistent subjective hyperalgesia without tissue damages.

Figure 1. Summary of the normalized eEPSC amplitude at 400 μA current stimulation in the ligation and sham groups. Mean ± SEM. **P < 0.01 compared to the left CeA in the ligation group, and to bilateral CeA in the sham groups (ANOVA). NS, not significantly different between groups (ANOVA).

Figure 2. Summarized results of the effect of adenosine (ADO) 100 μM and DPCPX 1 μM. Mean ± SEM. * P < 0.05; ** P < 0.01 (Mann-Whitney U-test), * * P < 0.05; ** * P = 0.01 (paired-T test), respectively.