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Short Communication

Involvement of $GABA_B$ receptor in the antihypersensitive effect in anterior cingulate cortex of partial sciatic nerve ligation model

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ABSTRACT

The role of the $GABA_B$ receptor in the anterior cingulate cortex (ACC) of neuropathic pain is unclear. Injection of a $GABA_B$ receptor antagonist CGP35348 into the ACC induced mechanical hypersensitivity in normal rats. Activation of the $GABA_B$ receptor injected by a $GABA_B$ receptor agonist baclofen into the ACC attenuated mechanical hypersensitivity in partial sciatic nerve ligation (PSNL) rats. Co-microinjection of CGP35348 with a muscarinic M1 receptor agonist McN-A-343 into the ACC significantly inhibited McN-A-343-induced antihypersensitivity in PSNL rats. These results suggest that the $GABA_B$ receptor in the ACC contributes to mechanical hypersensitivity and is involved in muscarinic M_1 receptor-mediated antihypersensitivity.

Neuropathic pain is difficult to manage, resulting in a chronic condition at the peripheral and central levels.[1] Recently, it has been reported that the anterior cingulate cortex (ACC) is an important area for pain perception at central nervous system (CNS).[2,3] LaGraize et al. reported that activation of γ-aminobutyric acid type A ($GABA_A$), but not $GABA_B$ receptors, attenuated the escape/avoidance behavior induced by a noxious stimulus in the ACC of nerve-ligated animals.[4] In contrast, Masocha W. reported that levels of some $GABA_A$ receptor subunits and $GABA_{B2}$ receptor mRNAs increased in the ACC of paclitaxel-induced neuropathic pain mice.[5] However, the role of the $GABA_B$ receptor in the ACC of neuropathic pain remains unknown. In addition, our last report showed that activation of muscarinic M_1 receptors in the ACC produced antinociceptive effects via $GABA_A$ receptors in response to mechanical stimulation.[6] Muscarinic M_1 receptors distribute cortical area and are associated with many important brain functions, including the regulation of nociception and memory.[7,8] In the ACC, it has been reported that the muscarinic receptors participate in nociception-related memory acquisition.[9] However, it has also been unclarified whether $GABA_B$ receptor participates in antihypersensitive effect caused by muscarinic M_1 receptor in the ACC in the chronic pain model. We demonstrate here that $GABA_B$ receptor in the ACC plays an important role for mechanical hypersensitivity and is involved in a muscarinic M_1 receptor agonist McN-A-343-induced antihypersensitive effect in the ACC using animal model of chronic pain by partial sciatic nerve ligation (PSNL).

The experimental procedures were based on the Guidelines of the Committee for Animal Care and Use of Fukuoka University, and all efforts were made to minimize the number of animals used and their suffering. Male Wistar rats (250–300 g) were purchased from Kyudo (Kumamoto, Japan). The rats were anesthetized with an intraperitoneal injection of medetomidine (0.4 mg/kg), midazolam (2.0 mg/kg), and butorphanol (5.0 mg/kg). The sciatic nerve on the left side was tightly ligated with a surgical suture around approximately 1/3–1/2 of the diameter according to our previous study.[6] Microinjection of drugs was undertaken via a guide cannula placed into the right ACC.[6] Paw withdrawal thresholds (PWT) to

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pressure stimulation were assessed using the Dynamic Plantar Aesthesiometer (Ugo Basile, Italy).[6]

Western blots were performed with 50 μg of total protein as previously described.[10] Anti-GABABR1 and anti-GABABR2, anti-GAT-1, anti-decarboxylase 65/67 (GAD65/67) and anti-β-actin were purchased from Abcam (Cambridge, USA), Millipore (Billerica, USA), Enzo Life Sciences (New York, USA) and Sigma−Aldrich (St. Louis, USA), respectively.

Data are expressed as means ± SEM. Statistical analyses were done with StatView. Results of time course experiments were analyzed for significance with repeated measures analysis of variance (ANOVA) with post hoc tests, Dunnett's test (comparisons to pre-injection 0 min) or Tukey's test. The differences among the groups were analyzed by two-way repeated measures ANOVA, followed by the Tukey−Kramer test for multiple comparisons or Student's *t* test for two comparisons. A value of $P < 0.05$ was taken to indicate a statistically significant difference.

The GABAergic system is important for the regulation of nociceptive behavior in CNS. In the spinal cord, the hypersensitivity to mechanical stimulation was regulated by not only the $GABA_A$ receptor but also the $GABA_B$ receptor.[11] In the ACC, it is also reported that activation of $GABA_A$ but not $GABA_B$ receptors contributes to pain processing.[4] Recently, a report was shown that the level of $GABA_{B2}$ receptor mRNA in the ACC increased in paclitaxel-treated mice.[5] We first investigated the participation of the $GABA_B$ receptor in the ACC of normal rats in nociception (Fig. 1). Microinjection of a $GABA_B$ receptor antagonist CGP35348 into the right side of the ACC induced the hypersensitivity to the mechanical stimulation of the left hind paw. This result suggests that the $GABA_B$ receptors in the ACC contribute to mechanical hypersensitive behavior.

We next confirmed whether the activation of the $GABA_B$ receptor in the ACC caused an antihypersensitive effect in PSNL rats using a $GABA_B$ agonist baclofen (Fig. 2A). Microinjection of baclofen into the right side on the ACC blocked the hypersensitivity elicited by mechanical stimulation to the ligation side of the hind paw in a dose dependent manner. These results indicated that activation of the $GABA_B$ receptor in the ACC produced an antihypersensitive effect in the PSNL model.

We further investigated whether PSNL influenced on the amount of the GABAergic system-related protein expression in the ACC using western blot analysis (Fig. 2B). GABA is removed from the synaptic cleft by GABA transporter (GAT), which is subdivided into four subtypes (GAT-1~4).[12] GAT-1 is most abundant in the brain.[13] Glutamate decarboxylase 65 and 67 (GAD65/67) synthesizes GABA from glutamate.[14] The $GABA_B$ receptor is subdivided into $GABA_{B1}$ and $GABA_{B2}$ receptors.[15] Therefore, we measured the expressions of the $GABA_{B1}$ receptor, $GABA_{B2}$ receptor, GAT-1 and GAD65/67 in the left and right side of the ACC in PSNL rats. The results showed that all proteins were unchanged compared with naïve rats. Therefore, activation of $GABA_B$ receptor by agonist administration attenuated the mechanical hypersensitivity after PSNL, but did not show any changes in $GABA_B$ receptor expression in the PSNL model rats.

In addition, we recently confirmed that muscarinic M_1 receptor-mediated antihypersensitivity was blocked by the injection of the $GABA_A$ receptor antagonist into the ACC in PSNL rats (submitted manuscript). We thus tested in this experiment whether $GABA_B$ receptor was involved in McN-A-343-induced antihypersensitive effect (Fig. 3). The microinjection of McN-A-343 (189 pmol) into the right side on the ACC attenuated the hypersensitivity in response to ipsilateral mechanical stimuli to the ligation side of the hind paw at day 7 after PSNL rats. Co-microinjection of CGP35348 (100 pmol or 150 pmol) with McN-A-343 significantly inhibited McN-A-343-induced antihypersensitive effect. In addition, injection of 150 pmol CGP35348 alone did not change the PWT in PSNL rats. These results suggest that activation of muscarinic M_1 receptor in the ACC can attenuate nerve injury-induced hypersensitivity, and the signaling followed by muscarinic M_1 receptor may be mediated by $GABA_B$ receptor in addition to $GABA_A$ receptor.

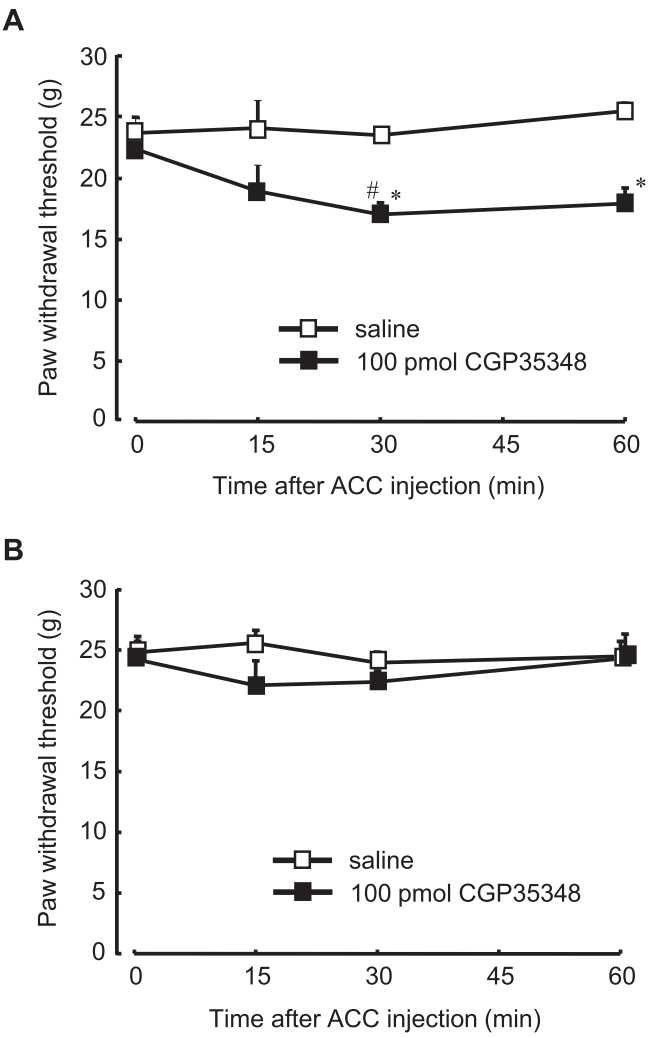

Fig. 1. Effect of $GABA_B$ receptor in the ACC on mechanical threshold in normal rats. (A) The microinjection of saline or $GABA_B$ receptors antagonist 100 pmol CGP35348 into the right ACC of normal rats decreased PWT to the mechanical stimulation of the left hind paw (two-way repeated measures of ANOVA, $F_{1,9} = 11.405$, $P = 0.0082$: saline, n = 7; 100 pmol CGP35348, n = 4). (B) The microinjection of saline or 100 pmol CGP35348 into the ACC of normal rats did not significantly change PWT to the mechanical stimulation of the right hind paw (two-way repeated measures of ANOVA, $F_{1,9} = 1.492$, $P = 0.2529$: saline, n = 7; 100 pmol CGP35348, n = 4). All values are means ± S.E.M. $^{\#}P < 0.05$ vs 0 min, $^{*}P < 0.05$ vs saline.

In conclusion, we showed that the $GABA_B$ receptor in the ACC participates in antihypersensitive effects. Furthermore, we found that nerve injury-caused mechanical hypersensitivity is inhibited by activation of muscarinic M_1 receptor through in part $GABA_B$ receptor in the ACC. These findings suggest that activation of muscarinic M_1 receptor which is located on GABAergic neurons cause the release of GABA and modulate of functional $GABA_B$ receptor in ACC. It is highly possible that activation of muscarinic M_1 receptor in the ACC is applicable to therapy for nerve injury-induced mechanical hypersensitivity.

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