Short Communication

Roles of β - and α_2 -Adrenoceptors Within the Central Nucleus of the Amygdala in the Visceral Pain–Induced Aversion in RatsSatoshi Deyama^{1,2}, Azusa Takishita², Sachi Tanimoto², Soichiro Ide¹, Takayuki Nakagawa², Masamichi Satoh³, and Masabumi Minami^{1,*}¹Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan²Department of Molecular Pharmacology, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto 606-8501, Japan³Department of Pharmacy, Yasuda Women's University, Hiroshima 731-0153, Japan

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Abstract. We investigated the roles of β - and α_2 -adrenoceptors within the central nucleus of the amygdala (CeA) in the negative affective and sensory components of visceral pain in rats. We observed a dose-dependent reduction of intraperitoneal acetic acid-induced conditioned place aversion by bilateral injections of timolol, a β -adrenoceptor antagonist, or clonidine, an α_2 -adrenoceptor agonist, without reducing writhing behaviors. These data suggest a pivotal role of intra-CeA adrenoceptors in the negative affective, but not sensory, component of visceral pain.

Keywords: amygdala, emotion, visceral pain

Pain consists of sensory and negative affective components. The neural systems involved in the sensory component of pain have been studied widely, while we poorly understand those underlying the negative affective component of pain. Recently, some behavioral studies using a conditioned place aversion (CPA) test revealed that several brain areas, such as the anterior cingulate cortex (1, 2), amygdala (3), and bed nucleus of the stria terminalis (BNST) (4, 5), mediate the negative affective component of pain. These reports show that sensory and negative affective components of pain can be separately evaluated. We previously demonstrated that bilateral lesions of the central nucleus of the amygdala (CeA) attenuated intraperitoneal (i.p.) acetic acid-induced CPA (A-CPA) without reducing nociceptive behaviors (6). In addition, i.p. injection of acetic acid induced *c-fos* mRNA expression, a marker of neuronal activation, highly within the CeA (7). Moreover, an electrophysiological study revealed that the CeA neurons undergo neuroplastic changes by persistent visceral pain (8). However, the neurochemicals involved in the signal transduction for visceral pain–induced aversive responses within the CeA

have not been fully identified.

The CeA is highly innervated by noradrenergic and adrenergic afferents (9), and the ascending noradrenergic systems to the CeA are involved in modulating negative affective response to stress (10, 11). Our previous behavioral study demonstrated that blockade of β -adrenoceptors in the CeA attenuates morphine withdrawal-induced CPA (12), suggesting that enhanced β -adrenergic transmission within the CeA plays an important role in the negative affective states. In addition, because a local administration of clonidine, an α_2 -adrenoceptor agonist, into the BNST is reported to suppress the noradrenaline release and emotional behavior in the odor-induced freezing model (13), it is possible that α_2 -adrenoceptors within the CeA could modulate pain-induced aversion. Thus, the aim of this study was to investigate the roles of β - and α_2 -adrenoceptors within the CeA in visceral pain–induced aversion.

Male Sprague-Dawley rats (180 – 230 g; Japan SLC, Hamamatsu) were used. The animals were kept at a constant ambient temperature ($24 \pm 1^\circ\text{C}$) under a 12-h light/dark cycle with free access to food and water. All experiments were performed with the approval of the Institutional Animal Care and Use Committee at Hokkaido University and Kyoto University. Clonidine and timolol (Sigma, St. Louis, MO, USA) were freshly dis-

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solved in phosphate-buffered saline on the day of injection.

Under thiamylal sodium anesthesia (50 mg/kg, i.p.), each rat was bilaterally implanted with 25-gauge guide cannulae above the CeA (1.8-mm posterior, 4.2-mm lateral, 3.0-mm ventral to the bregma) (14). After surgery, the rats were returned to their home cages and allowed to recover for 5–7 days before the following procedures. Injection cannulae (33 gauge) connected to a Hamilton microsyringe via PE-8 tubing were inserted to 8.0 mm below the skull surface when attached to the guide cannulae. Drugs were injected into the bilateral CeA 10 min before i.p. injection of acetic acid in a volume of 1 μ l per side over 2 min by a microinfusion pump. After the injection, injection cannulae were left in place for additional 2 min to prevent backflow of the drugs.

To evaluate the negative affective component of pain, the CPA test was conducted as previously described (3–6). We used a shuttle box (KN-80; Natsume Seisakusho, Tokyo) composed of two equal-sized compartments (30 $\times$ 30 $\times$ 30 cm) with distinct tactile and visual cues (one compartment with a smooth floor was black and the other with a textured floor was white). On day 1 and day 2 (preconditioning session), the rats freely explored the two compartments for 900 s, and the time spent in each compartment was automatically measured. We designated the compartment in which the rat spent a longer time (>450 s) on day 2 as the pain-paired compartment for each animal. On day 3, conditioning was performed as follows: the rats were given an i.p. injection of saline (1 ml) as a control non-noxious stimulus and then immediately confined in the non-pain-paired compartment for 1 h (conducted between 09:00 and 12:00). After 3–5 h, the rats were given an i.p. injection of 2% acetic acid (1 ml) as a noxious stimulus and then confined in the pain-paired compartment for 1 h (conducted between 14:00 and 18:00). On day 4, in the test session, each rat was allowed to freely explore the two compartments for 900 s, and the time spent in each compartment was recorded. CPA scores were calculated by subtracting the time spent in the pain-paired compartment on day 4 from that on day 2. To analyze the sensory component of pain, i.p. acetic acid-induced nociceptive writhing behavior was examined in a blind manner as previously described (4, 6). The writhing test was conducted between 9:00 and 18:00 with groups of rats different from those used in the CPA test. The number of writhing behaviors was counted for each 5-min period over 60 min. After the behavioral experiments, histological analyses were performed. The data of the rats having incorrect microinjection sites were excluded in the following analyses. Data are expressed as means $\pm$ S.E.M. Data were analyzed by the *t*-test, one-way ANOVA followed by a Newman-Keuls *post hoc*

test, or two-way ANOVA for repeated measurements, as indicated in the text. Differences with $P < 0.05$ were considered significant.

In the intra-CeA vehicle-injected rats, the time spent in the pain-paired compartment in the test session was 386 ± 44.3 s, which was significantly shorter than that in the preconditioning session (558 ± 36.3 s, $P < 0.05$, Student's *t*-test). In the intra-CeA timolol-injected rats, there was no significant difference in the time spent in the pain-paired compartment between preconditioning and test sessions (1 nmol: 556 ± 30.3 s in the preconditioning session and 489 ± 46.4 s in the test session, $P > 0.05$; 10 nmol: 547 ± 26.8 and 541 ± 38.6 s, $P > 0.05$; Student's *t*-test). CPA scores showed the dose-dependent inhibition of A-CPA by intra-CeA injection of timolol (Fig. 1A). One-way ANOVA demonstrated a significant difference among groups ($F_{2,20} = 3.76$, $P < 0.05$). The

Fig. 1. Effects of intra-CeA timolol on negative affective and sensory components of pain. A) Intra-CeA injection of timolol dose-dependently attenuated the A-CPA. The columns show the CPA scores of the intra-CeA vehicle ($n = 6$)–injected rats or timolol (1 nmol, $n = 8$; 10 nmol, $n = 9$)–injected rats. B) Intra-CeA timolol injection did not affect the acetic acid-induced writhing behavior (vehicle, $n = 4$; 1 nmol, $n = 5$; 10 nmol, $n = 5$). Data are expressed as means $\pm$ S.E.M. * $P < 0.05$, compared to the vehicle-injected rats (Newman-Keuls *post hoc* test).

Newman-Keuls *post hoc* test showed that timolol at a dose of 10 nmol/side (5.78 ± 26.1 s; $P < 0.05$), but not 1 nmol/side (67.3 ± 37.3 s; $P > 0.05$), significantly attenuated A-CPA compared with the vehicle-injected group (173 ± 68.1 s). In the rats injected with i.p. saline instead of i.p. acetic acid, intra-CeA timolol (10 nmol) did not change the time spent in the pain-paired compartment between preconditioning (548 ± 46.4 s) and test (576 ± 75.1 s) sessions ($n = 5$, $P > 0.05$; Student's *t*-test), indicating that intra-CeA injection of timolol *per se* has no rewarding or aversive effect. Intra-CeA timolol did not affect writhing behaviors (treatment, $F_{2,11} = 1.64$, $P > 0.05$; treatment $\times$ time, $F_{22,121} = 0.68$, $P > 0.05$), indicating no effect on the sensory component of pain (Fig. 1B).

In the intra-CeA vehicle-injected rats, the time spent in

Fig. 2. Effects of intra-CeA clonidine on negative affective and sensory components of pain. A) Intra-CeA injection of clonidine dose-dependently attenuated the A-CPA. The columns show the CPA scores of the intra-CeA vehicle ($n = 7$)-injected rats or clonidine (1 nmol, $n = 7$; 10 nmol, $n = 6$)-injected rats. B) Intra-CeA clonidine injection did not affect the acetic acid-induced writhing behavior (vehicle, $n = 4$; 1 nmol, $n = 4$; 10 nmol, $n = 5$). Data are expressed as means $\pm$ S.E.M. * $P < 0.05$, compared to the vehicle-injected rats (Newman-Keuls *post hoc* test).

the pain-paired compartment in the test session was 430 ± 32.9 s, which was significantly shorter than that in the preconditioning session (526 ± 14.8 s, $P < 0.05$, Student's *t*-test). In the intra-CeA clonidine-injected rats, there was no significant difference in the time spent in the pain-paired compartment between preconditioning and test sessions (1 nmol: 552 ± 30.2 s in the preconditioning session and 517 ± 40.0 s in the test session, $P > 0.05$; 10 nmol: 527 ± 21.0 and 534 ± 35.4 s, $P > 0.05$; Student's *t*-test). CPA scores showed the dose-dependent inhibition of A-CPA by intra-CeA injection of clonidine (Fig. 2A). One-way ANOVA demonstrated a significant difference among groups ($F_{2,17} = 3.79$, $P < 0.05$). The Newman-Keuls *post hoc* test showed that clonidine at a dose of 10 nmol/side (-7.17 ± 29.4 s, $P < 0.05$), but not 1 nmol/side (34.9 ± 15.4 s, $P > 0.05$), significantly attenuated A-CPA compared with the vehicle-injected group (95.4 ± 31.7 s). In the rats injected with i.p. saline instead of i.p. acetic acid, intra-CeA clonidine (10 nmol) did not change the time spent in the pain-paired compartment between preconditioning (535 ± 22.2 s) and test (533 ± 16.9 s) sessions ($n = 7$, $P > 0.05$; Student's *t*-test), indicating that intra-CeA injection of clonidine *per se* has no rewarding or aversive effect. Intra-CeA clonidine did not affect writhing behaviors (treatment, $F_{2,10} = 0.55$, $P > 0.05$; treatment $\times$ time, $F_{22,110} = 0.78$, $P > 0.05$), indicating no effect on the sensory component of pain (Fig. 2B).

In the present study, we demonstrated that intra-CeA injections of the β -adrenoceptor antagonist timolol and α_2 -adrenoceptor agonist clonidine suppressed visceral pain-induced aversive responses. These findings suggest a pivotal role of intra-CeA adrenoceptors in the regulation of pain-induced negative affective states. Recently, Kolber et al. reported that activation of metabotropic glutamate receptor 5 (mGluR5) in the CeA modulates pain-like behavior (15). Similar to the present study, the acute response to the inflammatory stimulus was not affected by conditional deletion of mGluR5 in the CeA. On the other hand, formalin-induced hypersensitivity was suppressed by the conditional deletion of mGluR5 and intra-CeA injection of the MEK inhibitor U0126. The effects of intra-CeA timolol and clonidine on inflammation-induced hypersensitivity should be examined in the future studies.

As chronic pain is frequently associated with psychological and emotional dysfunction, studies of the neural circuits and the molecular mechanisms involved in the negative affective component of pain may have considerable clinical importance in the treatment of chronic pain.

References

- 1 Johansen JP, Fields HL, Manning BH. The affective component of pain in rodents: direct evidence for a contribution of the anterior cingulate cortex. *Proc Natl Acad Sci U S A*. 2001;98:8077–8082.
- 2 Johansen JP, Fields HL. Glutamatergic activation of anterior cingulate cortex produces an aversive teaching signal. *Nat Neurosci*. 2004;7:398–403.
- 3 Deyama S, Yamamoto J, Machida T, Tanimoto S, Nakagawa T, Kaneko S, et al. Inhibition of glutamatergic transmission by morphine in the basolateral amygdaloid nucleus reduces pain-induced aversion. *Neurosci Res*. 2007;59:199–204.
- 4 Deyama S, Nakagawa T, Kaneko S, Uehara T, Mimami M. Involvement of the bed nucleus of the stria terminalis in the negative affective component of visceral and somatic pain in rats. *Behav Brain Res*. 2007;176:367–371.
- 5 Deyama S, Katayama T, Ohno A, Nakagawa T, Kaneko S, Yamaguchi T, et al. Activation of the β -adrenoceptor-protein kinase A signaling pathway within the ventral bed nucleus of the stria terminalis mediates the negative affective component of pain in rats. *J Neurosci*. 2008;28:7728–7736.
- 6 Tanimoto S, Nakagawa T, Yamauchi Y, Minami M, Satoh M. Differential contributions of the basolateral and central nuclei of the amygdala in the negative affective component of chemical somatic and visceral pains in rats. *Eur J Neurosci*. 2003;18:2343–2350.
- 7 Nakagawa T, Katsuya A, Tanimoto S, Yamamoto J, Yamauchi Y, Minami M, et al. Differential patterns of c-fos mRNA expression in the amygdaloid nuclei induced by chemical somatic and visceral noxious stimuli in rats. *Neurosci Lett*. 2003;344:197–200.
- 8 Han JS, Neugebauer V. Synaptic plasticity in the amygdala in a visceral pain model in rats. *Neurosci Lett*. 2004;361:254–257.
- 9 Asan E. The catecholaminergic innervation of the rat amygdala. *Adv Anat Embryol Cell Biol*. 1998;142:1–118.
- 10 Williams CL, Men D, Clayton EC, Gold PE. Norepinephrine release in the amygdala after systemic injection of epinephrine or escapable footshock: contribution of the nucleus of the solitary tract. *Behav Neurosci*. 1998;112:1414–1422.
- 11 Khoshbouei H, Cecchi M, Dove S, Javors M, Morilak DA. Behavioral reactivity to stress: amplification of stress-induced noradrenergic activation elicits a galanin-mediated anxiolytic effect in central amygdala. *Pharmacol Biochem Behav*. 2002;71:407–417.
- 12 Watanabe T, Nakagawa T, Yamamoto R, Maeda A, Minami M, Satoh M. Involvement of noradrenergic system within the central nucleus of the amygdala in naloxone-precipitated morphine withdrawal-induced conditioned place aversion in rats. *Psychopharmacology (Berl)*. 2003;170:80–88.
- 13 Fendt M, Siegl S, Steinige-Brach B. Noradrenaline transmission within the ventral bed nucleus of the stria terminalis is critical for fear behavior induced by trimethylthiazoline, a component of fox odor. *J Neurosci*. 2005;25:5998–6004.
- 14 Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 4th ed. San Diego: Academic Press; 1998.
- 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;30:8203–8213.