Inhibition of noradrenaline release by clonidine in the ventral bed nucleus of the stria terminalis attenuates pain-induced aversion in rats

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ABSTRACT

Pain is an unpleasant sensory and emotional experience. The neural systems underlying the sensory component of pain have been studied extensively, but we are only beginning to understand those underlying the affective component of pain. Previously, we showed the pivotal role of noradrenergic transmission in the ventral part of the bed nucleus of the stria terminalis (vBNST) in the negative affective component of pain using a conditioned place paradigm. In this study, we examined the effect of local administration of clonidine, an α_2-adrenoceptor agonist, into the vBNST on noradrenaline release and on conditioned place aversion (CPA) induced by an intraplantar formalin injection in male Sprague-Dawley rats. In vivo microdialysis showed that the formalin-induced increase in the extracellular noradrenaline level within the vBNST was significantly suppressed by clonidine (100 μM) perfusion through a microdialysis probe. Bilateral intra-vBNST injections of clonidine (1 and 10 nmol/side) dose-dependently attenuated formalin-induced CPA without reducing nociceptive behaviors. These results suggest that clonidine inhibits noradrenaline release by acting on α_2-adrenoceptors located in the vBNST and thereby attenuates pain-induced aversion. α_2-adrenoceptors in the vBNST play a pivotal role in the regulation of negative affective, but not the sensory, component of pain.

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1. Introduction

Pain is a complex experience consisting of sensory and negative affective components. Although neuronal pathways and brain regions involved in the sensory component of pain have been studied extensively, neuronal systems underlying the affective component of pain remain to be elucidated. Recently, behavioral studies using a conditioned place aversion (CPA) test showed the neural substrates involved in the negative affective component of pain. Johansen et al. (2001) reported that excitotoxic lesions of the anterior cingulate cortex (ACC) suppress intraplantar formalin-induced CPA (F-CPA) without reducing formalin-induced nociceptive behaviors. We showed that the central (CeA) and basolateral (BLA) amygdaloid nuclei are involved differently in F-CPA and intraperitoneal acetic acid-induced CPA (A-CPA) (Tanimoto et al., 2003). Additionally, we reported the involvement of the bed nucleus of the stria terminalis (BNST) in the affective component of pain (Deyama et al., 2007a).

The BNST, particularly the ventral part (vBNST), is densely innervated by noradrenergic fibers arising mainly from medullary A1/A2 cell groups (Forray et al., 2000; Woulfe et al., 1990). Noradrenergic transmission within this brain area is critical for mediating negative emotions such as anxiety, fear, and aversion (Cecchi et al., 2002; Delfs et al., 2000; Fendt et al., 2005; Schweimer et al., 2005). Recently, we demonstrated the pivotal role of enhanced noradrenergic transmission within the vBNST in somatic and visceral pain-induced aversion in rats (Deyama et al., 2008, 2009). α_2-adrenoceptors are known to play regulatory roles in the sensory component of pain. Specifically, α_2-adrenoceptor agonists, such as clonidine, produce antinociception at the spinal level (Reddy et al., 1980; Yaksh et al., 1995) at least in part through the inhibitory effect on glutamate release from the primary afferent terminal (Ueda et al., 1995). However, the roles of α_2-adrenoceptors in the affective component of pain remain unclear. Therefore, in this study, we investigated the effects of local administrations of clonidine into the vBNST on intraplantar formalin-induced noradrenaline release and on F-CPA.

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2. Methods

2.1. Animals

Male Sprague-Dawley rats (200–240 g; Japan SLC, Hamamatsu, Japan) were used. The rats were housed four per cage. Following implantation surgery of the guide cannulae, the rats were individually housed in cages. The rats were maintained at a constant ambient temperature (22 ± 1 °C) under a 12-h light/dark cycle with food and water available *ad libitum*. All experiments were approved by the institutional animal care and use committee of Hokkaido University and conformed to the guidelines of the International Association for the Study of Pain.

2.2. In vivo microdialysis

Thirty-two rats were divided into two groups; a control group ($n = 16$) and a clonidine-perfused group ($n = 16$). Each rat was unilaterally implanted with a microdialysis guide cannula (o.d. 0.5 mm, AG-7; Eicom, Kyoto, Japan) 1.0 mm above the left vBNST (−0.3 mm rostral, 1.6 mm lateral, 6.7 mm ventral to the bregma) (Paxinos and Watoson, 1998) under sodium pentobarbital anesthesia (50 mg/kg, i.p.). Following surgery, the rats were housed individually in cages for a recovery period of 1–2 days. Microdialysis experiments were then performed in freely moving rats. A microdialysis probe (dialysis membrane: length 1.0 mm, o.d. 0.22 mm, A-I-7-01; Eicom) was inserted through the guide cannula and continuously perfused with Ringer's solution (Na^+ 147 mM, K^+ 4 mM, Ca^{2+} 2.3 mM, Cl^- 155.6 mM) at a constant flow rate of 1 μl/min. The tip of the microdialysis probe protruded 1.0 mm from the tip of the guide cannula to reach the vBNST. The rats were placed in a Plexiglas chamber (width × length × height: 30 cm × 30 cm × 35 cm). After a stabilization period (>2 h), fourteen 15-min dialyzate fractions were collected. The first three samples served as baseline samples. After collecting three additional samples, each rat was given an intraplantar injection of 2% formalin (100 μl). In the clonidine-perfused group, the perfusion solution was switched from normal Ringer's solution to Ringer's solution containing 100 μM clonidine (Sigma, St Louis, MO, USA) immediately after collection of the last baseline sample. As Fendt et al. (2005) reported that clonidine perfusion at a concentration of 100 μM suppresses the intra-vBNST noradrenaline release induced by trimethylthiazoline (TMT), a component of fox odor, in rats, we selected this concentration to examine the effects of clonidine.

Each dialyzate sample was separated in a liquid chromatography column (Eicompak CAX, 2.0 mm i.d. × 200 mm; Eicom) at 35 °C with 0.1 M ammonium acetate buffer (pH 6.0) containing 0.05 M sodium sulfate, 50 mg/l ethylene diamine tetraacetic acid, and 30% methanol at a constant flow rate of 0.25 ml/min. Noradrenaline content in the dialyzates was measured with an electrochemical detector (HTEC-500; Eicom) with a working electrode set at +450 mV versus an Ag/AgCl reference electrode. Chromatogram peaks were analyzed using the PowerChrom data-recording system.

Histological analyses were performed on coronal brain sections following the *in vivo* microdialysis experiments. Briefly, rats were decapitated and the brain was rapidly removed and frozen in powdered dry ice. Coronal sections (50 μm) were prepared on a cryostat, thaw-mounted onto slides, stained with thionin, and examined by microscopy (×40). Data from rats with the correct placement of the microdialysis probe (Fig. 1A) were used for statistical analyses ($n = 12$ for control group; $n = 12$ for clonidine-perfused group).

2.3. Microinjection

Each rat was bilaterally implanted with 25-gauge stainless steel guide cannulae 1.5 mm above the vBNST (−0.3 mm rostral, 1.6 mm lateral, 6.0 mm ventral to the bregma) (Paxinos and Watoson, 1998) under sodium pentobarbital anesthesia (50 mg/kg, i.p.). The rats were allowed to recover for at least 7 days and were handled 1–2 min each day for 3 consecutive days before the behavioral experiments. Microinjection was performed using 33-gauge stainless steel injection cannulae inserted bilaterally into the guide cannulae. The injection cannulae protruded 1.5 mm from the tip of guide cannulae to reach the vBNST. Injection cannulae were attached to a microinjection pump (CMA, Stockholm, Sweden) via PE 8 tubing. Clonidine or vehicle (phosphate-buffered saline) was bilaterally administered into the vBNST in a volume of 0.5 μl/side at a rate of 0.5 μl/min, and the injection cannulae were left in place for 1 min after the microinjection to prevent backflow. Schweimer et al. (2005) showed that intra-BNST injections of clonidine at a dose of 10 nmol/side suppressed the potentiation of startle responses by conditioned fear and light. Furthermore, Fendt et al. (2005) reported that intra-vBNST injections of clonidine at a dose of 5 nmol/side suppressed the TMT-induced freezing behavior in rats. Thus, in this study, doses of clonidine at 1 and 10 nmol/side were selected to examine its effect on pain-induced aversion.

2.4. CPA test

For the CPA test, we used a shuttle box composed of two equal-sized compartments (30 cm × 30 cm × 30 cm) with distinct visual and tactile cues (one compartment was black with a smooth floor and the other was white with
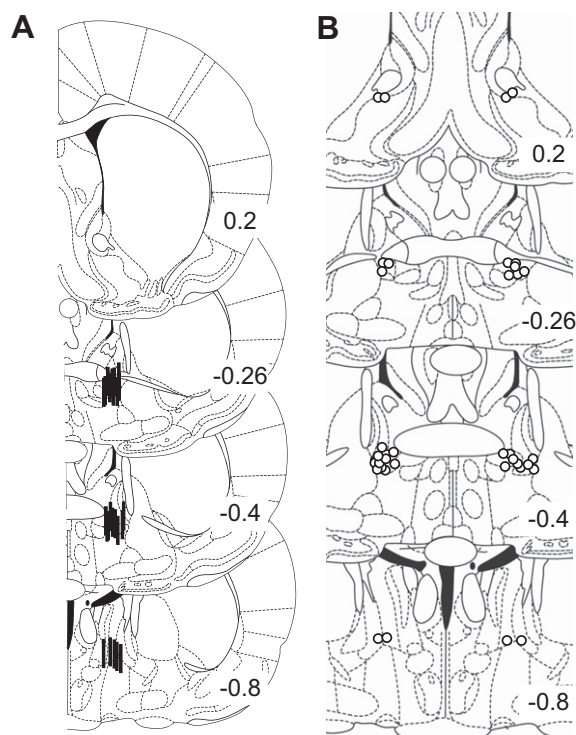

Fig. 1. Placement of the tips of the microdialysis probes and microinjection cannulae. (A) Black bars show the positions of the microdialysis probes. (B) Open circles show the positions of the tips of cannulae for microinjection of 10 nmol/side of clonidine in the CPA and formalin tests. The illustrations of coronal brain sections were taken from the atlas of Paxinos and Watoson (1998); 0.2, −0.26, −0.4, and −0.8 indicate distances (mm) from the bregma.

a textured floor) under dim illumination (25 ± 5 lux at the center of the box) (KN-80; Natsume Seisakusho, Tokyo, Japan). On day 1 (habituation session) and day 2 (preconditioning session), the rats freely explored the two compartments for 900 s, and the time spent in each compartment during the exploration period was measured automatically. Rats that spent more than 80% (>720 s) of the total time on one side on day 2, or that showed a difference of more than 200 s in the time spent on one side between days 1 and 2, were eliminated from the subsequent procedures. Additionally, following behavioral tests, histological analyses were performed (Fig. 1B), and data from the rats with misplacements of the bilateral microinjection cannulae were eliminated from the statistical analyses. Both before and after such eliminations, no significant difference ($P > 0.05$ ($n = 43$) and $P > 0.05$ ($n = 28$), respectively) was observed between the time spent in the black (454.1 ± 16.2 and 445.6 ± 16.1 s, respectively) and white (444.8 ± 16.3 and 453.6 ± 16.2 s, respectively) compartments, indicating no significant bias in compartment preference prior to conditioning. We designated the compartment in which each rat spent the majority of its time (>450 s) on day 2 (preconditioning session) as each animal's pain-paired compartment. Finally, in the CPA test, statistical analyses were performed with data from 28 rats. Black and white compartments were designated as pain-paired one for 13 and 15 rats, respectively. On day 3, conditioning was performed as follows: in the vehicle control session (conducted between 09:00 and 12:00), rats were given an intraplantar injection of saline (100 μl) into the left hind paw and immediately confined to the non-pain-paired compartment for 1 h. After at least 4 h, rats were injected with clonidine (1 or 10 nmol/side) or vehicle into the bilateral vBNST. Ten minutes after the intra-vBNST injection, in the pain-conditioning session (conducted between 14:00 and 18:00), rats were given an intraplantar injection of 2% formalin (100 μl) into the right hind paw and then confined to the pain-paired compartment for 1 h. On day 4, in the test session, rats were allowed to explore the two compartments freely, and the time spent in each compartment during the exploring period (900 s) was recorded. The CPA scores were calculated by subtracting the time spent in the pain-paired compartment during the test session from the time spent in this compartment during the preconditioning session.

2.5. Formalin test

Intraplantar formalin-induced nociceptive behaviors were measured in a blind manner, as described previously (Deyama et al., 2007a, 2008; Tanimoto et al., 2003). Briefly, each rat was placed in a Plexiglas cylinder (30 cm diameter; 30 cm high) for