NOCISTATIN EXCITES ROSTRAL AGRANULAR INSULAR CORTEX-PERIAQUEDUCTAL GRAY PROJECTION NEURONS BY ENHANCING TRANSIENT RECEPTOR POTENTIAL CATION CONDUCTANCE VIA $G_{\alpha q/11}$-PLC-PROTEIN KINASE C PATHWAY

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Abstract—Rostral agranular insular cortex (RAIC) projects to periaqueductal gray (PAG) and inhibits spinal nociceptive transmission by activating PAG-rostral ventromedial medulla (RVM) descending antinociceptive circuitry. Despite being generated from the same precursor prepronociceptin, nocistatin (NST) and nociceptin/orphanin FQ (N/OFQ) produce supraspinal analgesic and hyperalgesic effects, respectively. Prepronociceptin is highly expressed in the RAIC. In the present study, we hypothesized that NST and N/OFQ modulate spinal pain transmission by regulating the activity of RAIC neurons projecting to ventrolateral PAG (RAIC–PAG). This hypothesis was tested by investigating electrophysiological effects of N/OFQ and NST on RAIC–PAG projection neurons in brain slice. Retrogradely labeled RAIC–PAG projection neurons are layer V pyramidal cells and express mRNA of vesicular glutamate transporter subtype 1, a marker for glutamatergic neurons. N/OFQ hyperpolarized 25% of RAIC–PAG pyramidal neurons by enhancing inwardly rectifying potassium conductance via pertussis toxin-sensitive $G_{\alpha i/o}$. In contrast, NST depolarized 33% of RAIC–PAG glutamatergic neurons by causing the opening of canonical transient receptor potential (TRPC) cation channels through $G_{\alpha q/11}$-phospholipase C-protein kinase C pathway. There were two separate populations of RAIC–PAG pyramidal neurons, one responding to NST and the other one to N/OFQ. Our results suggest that $G_{\alpha q/11}$-coupled NST receptor mediates NST excitation of RAIC–PAG glutamatergic neurons, which is expected to cause the supraspinal analgesia by enhancing the activity of RAIC–PAG–RVM antinociceptive pathway. Opposite effects of NST and N/OFQ on supraspinal pain regulation are likely to result from their opposing effects on RAIC–PAG pyramidal neurons. © 2010 IBRO. Published by Elsevier Ltd. All rights reserved.

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Abbreviations: CeA, central amygdala nucleus; N/OFQ, nociceptin/orphanin FQ; NST, nocistatin; PAG, periaqueductal gray; PKC, protein kinase C; PLC, phospholipase C; PTX, pertussis toxin; RAIC, rostral agranular insular cortex; RVM, rostral ventromedial medulla; TRP, transient receptor potential; VGLUT1, vesicular glutamate transporter subtype 1.

Key words: nocistatin, nociceptin/orphanin FQ, rostral agranular insular cortex, periaqueductal gray, pyramidal neurons, TRPC channel.

Insular cortex receives nociceptive inputs and is associated with the affective aspects of pain (Treede et al., 2000; Ohara et al., 2005). Within the insular cortex, rostral agranular insular cortex (RAIC) is also involved in modulating spinal pain transmission and functions as the central action site of antinociceptive effect produced by opioid peptide, dopamine, or GABA (Ohara et al., 2005). Microinjection of μ-opioid receptor agonist morphine into RAIC or dopamine activation of D1 receptor within RAIC produced an analgesic effect by decreasing noxious stimulus-induced c-fos expression in spinal dorsal horn neurons and firing of spinal nociceptive projection neurons (Burkey et al., 1996, 1999). Augmented GABAergic transmission and resulting activation of $GABA_A$ receptors within RAIC has also been shown to cause antinociceptive effect by enhancing the descending inhibition of spinal nociceptive neurons (Jasmin et al., 2003). These findings suggest that RAIC contributes to μ-opioid receptor-, D1 receptor- or $GABA_A$ receptor-mediated supraspinal analgesia by activating a descending antinociceptive pathway that inhibits pain transmission in the spinal dorsal horn. In accordance with this hypothesis, anterograde and retrograde tracing studies showed that RAIC pyramidal neurons send a dense excitatory projection to ventrolateral periaqueductal gray (PAG) (Floyd et al., 2000; Jasmin et al., 2004), which is an essential component of brainstem descending antinociceptive circuitry (Fields, 2000). Analgesic effect produced by ventrolateral PAG excitation is mediated by an excitatory innervation from PAG to rostral ventromedial medulla (RVM), which projects to spinal dorsal horn and inhibits nociceptive transmission (Fields, 2000). Injection of naloxone into RAIC reversed antinociceptive effect induced by the systemic application of morphine (Burkey et al., 1996). Administration of D1 receptor antagonist SCH23390 into RAIC also caused a hyperalgesic effect, suggesting that dopamine acts tonically in the RAIC to inhibit spinal pain transmission (Burkey et al., 1999). Therefore, numerous analgesic drugs or neurotransmitters are likely to modulate spinal nociceptive transmission by affecting the activity of descending RAIC–PAG–RVM antinociceptive pathway that suppresses the activity of spinal nociceptive neurons.

Both nocistatin (NST) and nociceptin/orphanin FQ (N/OFQ) are derived from the precursor prepronociceptin/orphanin FQ (ppN/OFQ) (Mollereau et al., 1996; Nothacker et al., 1996; Okuda-Ashitaka et al., 1998; Okuda-Ashitaka and Ito, 2000). Despite being generated from the same precursor, NST fails to bind to N/OFQ receptor (NOP receptor), and specific binding sites of NST are expressed in the spinal cord and brain (Okuda-Ashitaka et al., 1998). Furthermore, NST and N/OFQ produce opposite biological effects in the CNS, and NST blocks several N/OFQ-mediated effects in the brain (Nicol et al., 1998; Hiramatsu and Inoue, 1999; Okuda-Ashitaka and Ito, 2000; Gavioli et al., 2002). In contrast with N/OFQ-induced supraspinal hyperalgesia (Meunier et al., 1995; Reinscheid et al., 1995; Mogil and Pasternak, 2001), i.c.v. application of NST produces an analgesic effect (Okuda-Ashitaka et al., 1998; Nakagawa et al., 1999). NST also antagonizes N/OFQ-mediated supraspinal pronociceptive effect (Okuda-Ashitaka et al., 1998; Okuda-Ashitaka and Ito, 2000; Liu et al., 2006). Therefore, NST acts as a functional antagonist of N/OFQ in modulating nociceptive transmission at the supraspinal level.

Prepronociceptin mRNA and NOP receptor mRNA are highly expressed in the RAIC (Anton et al., 1996; Boom et al., 1999; Witta et al., 2004). RAIC inhibits spinal nociceptive transmission by sending an excitatory projection to ventrolateral PAG and activating PAG–RVM descending antinociceptive pathway (Jasmin et al., 2004; Ohara et al., 2005). In the present study, we hypothesized that NST and N/OFQ modulate spinal pain transmission by regulating the activity of RAIC neurons projecting to ventrolateral PAG (RAIC–PAG) and that opposing effects of NST and N/OFQ on supraspinal pain modulation result from their opposite effects on the excitability of RAIC–PAG projection neurons. To test this hypothesis, we investigated electrophysiological effects of NST and N/OFQ on retrogradely labeled RAIC–PAG projection neurons in brain slice. Our results indicate that NST excites RAIC–PAG pyramidal neurons by causing the opening of canonical transient receptor potential (TRPC) cation channels via $G_{\alpha q/11}$-phospholipase C-protein kinase C pathway, which could lead to the activation of RAIC–PAG–RVM descending antinociceptive pathway. In contrast, N/OFQ hyperpolarizes RAIC–PAG pyramidal neurons by enhancing inwardly rectifying potassium conductance through pertussis toxin-sensitive $G_{\alpha i/o}$, which could inhibit the activity of RAIC–PAG–RVM pain-modulating circuitry.

EXPERIMENTAL PROCEDURES

Retrograde labeling of rat RAIC–PAG projection neurons

Animals were handled according to protocols approved by Animal Care and Use Committee of Chang Gung University. According to our previous study (Li et al., 2001a), RAIC neurons projecting to ventrolateral PAG were retrogradely labeled by carboxylate-modified yellow-green fluorescent microspheres (FluoSpheres, 0.04 μm; Molecular Probes, Eugene, OR, USA). Briefly, 3–4 weeks-old Sprague–Dawley rats were anesthetized with chloral hydrate and placed in a stereotaxic apparatus (David-Kopf). After drilling a hole at the coordinates for PAG, FluoSpheres suspen-

sion was stereotaxically injected into ventrolateral PAG (coordinates from lambda: anteroposterior, +0.6 mm; mediolateral, ±0.3 mm; and dorsoventral, −5.3 mm; injection volume=~40 nl). Three to four days after the injection, retrogradely labeled RAIC–PAG projection neurons in brain slice were identified and selected for whole-cell patch-clamp recordings as described below. To confirm the injection site in the PAG and visualize the distribution of FluoSpheres-labeled neurons in the RAIC, brain was dissected and fixed in the phosphate-buffered saline containing 4% paraformaldehyde. Subsequently, 100 μm vibratome sections of the PAG or RAIC were prepared and observed by the fluorescence microscopy.

Whole-cell patch-clamp recordings of RAIC–PAG projection neurons in brain slice

Whole-cell patch-clamp recordings of RAIC neurons in brain slice were performed according to our previous study (Yeh et al., 2005). Briefly, rats were terminally anesthetized with sodium pentobarbital and decapitated. The whole brain was quickly removed, and 250 μm-thick coronal brain slices were prepared by using a vibratome slicer (VT 1000S, Leica) in the ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 120, KCl 2.4, $MgSO_4$ 2, $NaHCO_3$ 26, $CaCl_2$ 2, NaH_2PO_4 1.25, and glucose 15. Before being used for patch-clamp recordings, brain slices were transferred to a holding chamber where they were submerged in the ACSF gassed with 95% O_2/5% CO_2 for 1 h.

Following the recovery period, a coronal brain slice containing RAIC was transferred to a recording chamber mounted on the stage of an upright microscope (Axioskop, Zeiss) and superfused continuously with ACSF pregassed at room temperature with 95% O_2/5% CO_2. RAIC neuron was visualized using a 40× water immersion objective (Zeiss) with the aid of differential interference contrast (DIC) optics and infrared light-sensitive camera (Newvicon C2400-07C, Hamamatsu). FluoSpheres-retrogradely labeled RAIC–PAG projection neurons were identified under the epifluorescence illumination (FITC filter) and selected for whole-cell patch-clamp recordings. Patch pipettes with a resistance of 3–4 MΩ were fabricated from hard borosilicate glasses using a pipet puller (P-87, Sutter). Patch electrodes were filled with the solution containing (in mM): KCl 70, KF 70, $MgCl_2$ 1, $CaCl_2$ 0.1, HEPES 10, EGTA 1.2, ATP 2 and GTP 0.3, pH=7.3. Nociceptin/orphanin FQ purchased from Tocris Bioscience (Bristol, UK) or rat nocistatin obtained from Phoenix Pharmaceuticals (Burlingame, CA, USA) was dissolved in the external solution and perfused to RAIC–PAG projection neurons in brain slice. Membrane currents or potentials recorded by the patch-clamp amplifier (Axopatch-200B, Axon Instruments) were filtered, digitized (Digidata, 1200, Axon Instruments) and stored for a later analysis. Series resistance was usually<12 MΩ, and the compensation circuitry of the amplifier was used to minimize the series resistance error. Liquid junction potentials were corrected as described previously (Li et al., 2001a). Holding potentials, data acquisition and analysis were controlled by software pCLAMP 7.0 (Axon Instruments). Prism program (GraphPad Software) was used to analyze the dose-response curve. Whole-cell patch-clamp recordings were performed at room temperature (25~26 °C).

Intracellular dialysis of anti-$G_{\alpha q/11}$ antibody or anti-TRPC antiserums

Rabbit polyclonal antiserum (QL) raised against the common C-terminal decapeptide of $G_{\alpha q}$ and $G_{\alpha 11}$ was obtained from Calbiochem (San Diego, CA, USA). This antibody has been used to block various $G_{\alpha q/11}$-mediated effects including phospholipase C activation, inositol (1,4,5)-trisphosphate (IP_3)-evoked calcium release and opening of non-selective cation channels (Wu and Wang, 1996; Li et al., 2001a,b). Polyclonal rabbit antiserums to TRPC1 and TRPC5 were obtained from Alomone laboratories