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Research Report

Local effects of octreotide on glutamate-evoked activation of Aδ and C afferent fibers in rat hairy skin

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ABSTRACT

The aim of the present study was to investigate whether local application of octreotide, an analogue of somatostatin, suppresses the glutamate-evoked activities of Aδ and C primary afferent fibers innervating dorsal hairy skin of the rat in vivo. The single unit activity of Aδ and C afferent fibers was recorded in isolated filaments from the dorsal cutaneous branches of the T9–T12 spinal nerves. Changes in discharge relative to baseline during injection of glutamate (0.3 mM, 10 μL) into the receptive field with pretreatment by octreotide (20 μM, 10 μL) were compared with injection after pretreatment with normal saline. Most of Aδ fibers (21/27, 78%) and C fibers (21/26, 81%) in the dorsal cutaneous branches were significantly activated by local injection of glutamate following saline injection. The mean discharge rates increased from 2.39±0.30 and 2.42±0.37 impulses/min to 12.79±2.04 and 13.56±2.56 impulses/min during injection of glutamate for Aδ and C fibers, respectively. After octreotide pretreatment group, glutamate increased the mean discharge rates from 1.93±0.38 and 2.25±0.29 impulses/min to 6.11±0.9 and 6.31±1.18 impulses/min in Aδ fibers and C fibers, respectively. The discharge rates during injection of glutamate after octreotide pretreatment were significantly lower than after normal saline pretreatment. The suppressive effect of octreotide was reversed by the somatostatin receptor antagonist cyclo-somatostatin. These results suggest that interactions between excitatory amino acid and inhibitory neuropeptides may contribute to sensory signaling in the peripheral nervous system.

1. Introduction

The neuropeptide somatostatin (SST) is a regulatory peptide widely distributed in the central and peripheral nervous systems. Its receptors are expressed in both neuronal and accessory cells, such as neuroendocrine and immune cells (Carlton et al., 2001a; Ferone et al., 2006). Behavioral studies in the rat indicate that local application of SST or its agonist octreotide (OCT) reduces nociception induced by formalin, capsaicin and carrageenan (Carlton et al., 2001a, 2004; Corsi et al., 1997). Electrophysiological studies also show that close arterial injection of SST inhibits mechanoreceptive primary afferent activity in response to noxious joint rotation in the normal and inflamed knee joint (Heppelmann and Pawlak,

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Abbreviations: c-SOM, Cyclo-somatostatin; GLU, Glutamate; NS, Normal saline; OCT, Octreotide; SST, Somatostatin; SSTR, Somatostatin receptor

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1997). In addition, local delivery of OCT in vitro inhibits the excitation of C-mechanoheat-sensitive fibers induced by bradykinin and capsaicin in glabrous skin (Carlton et al., 2001a, 2004). Our previous study show that OCT decreases the discharge rate of C fibers in the tibial nerve when evoked by non-noxious as well as noxious toe joint movements in the arthritic rat (Yao et al., 2008). These findings suggest that peripheral SST exerts an inhibitory effect on the activity of unmyelinated primary afferent neurons.

Glutamate is considered an important excitatory amino acid neurotransmitter in primary afferent neurons and can be released in the periphery during tissue injury or inflammatory conditions (Carlton, 2001; deGroot et al., 2000; Lawand et al., 2000). Several lines of evidence indicate that glutamate receptors are localized in the axonal membrane of peripheral nerves in the rat and human (Carlton et al., 1995; Coggeshall and Carlton, 1998; Du et al., 2003, 2006; Kinkelin et al., 2000). In the inflamed state, the number of glutamate receptors in axons of peripheral, unmyelinated afferents increases (Carlton and Coggeshall, 1999). In addition, local injection of glutamate receptor antagonists attenuates nociceptive behavior and the hyperalgesia that accompany inflammation or nerve injury (Ahn et al., 2004; Bursztajn et al., 2004; Davidson et al., 1997). Electrophysiological studies demonstrate that glutamate applied to the cutaneous receptive field of rat glabrous skin in vitro increases the activities of Aδ and C afferent fibers (Du et al., 2001). Our previous studies show that local injection of glutamate into cutaneous receptive field of sensory nerves also induces excitatory responses in Aδ and C fibers innervating the rat hairy skin in vivo (Cao et al., 2005; Tian et al., 2005). In addition, local treatment of the receptive field with ionotropic glutamate receptor antagonists blocks the enhanced spontaneous discharge induced by antidromic stimulation of adjacent afferents (Cao et al., 2007). Combined, these studies indicate that both endogenous and exogenous glutamate can activate primary afferent fibers.

Interactions between neuropeptides and amino acids have turned out to be a major regulatory mechanism underlying control of neuronal excitability and neuronal network activity in the central nervous system (Peineau et al., 2003). The aim of the present study was to determine whether, in the peripheral nervous system, interactions between inhibitory neuropeptides and excitatory amino acids also occur. We hypothesized that local application the neuropeptide SST would suppress the glutamate-evoked activity of fine sensory nerve fibers innervating the rat hairy skin.

2. Results

2.1. *Receptive properties of Aδ and C afferent fibers*

A total of 151 primary afferent units were obtained in this experiment, of which 75 were identified as Aδ fibers with a mean conduction velocity of 7.24±0.16 m/s and 76 as C fibers with a mean conduction velocity of 1.35±0.05 m/s. Both Aδ and C fibers exhibited very low background activity (2.28±0.21 impulses/min and 2.22±0.20 impulses/min, respectively). The receptive fields of these units were located in an area 0.5 to 2.5 cm lateral to the dorsal midline. Mean mechanical threshold was 0.49±0.01 mN for Aδ fibers and 0.68±0.01 mN for C fibers. Among the Aδ fibers tested 36% (27/75) were classified as mechanoheat-sensitive (AMH) units and the remaining 48 were classified as mechano-sensitive (AM) units. For C fibers 42% (32/76) were classified as mechanoheat-sensitive (CMH) units and the remaining 44 were classified as mechano-sensitive (CM) units. There was no significant difference in mechanical threshold between the AMH and AM units nor between the CMH and CM units ($P>0.05$).

2.2. *Group 1 and Group 2: Effects of glutamate (GLU) on activities of Aδ and C afferent fibers*

During and after either the first or second injection of normal saline (NS) into the receptive field of the control group (Group 1), no excitation of Aδ or C afferent fibers was observed ($P>0.05$, Fig. 1). In the NS+GLU group (Group 2), NS also had no excitatory effect on Aδ or C afferent fibers ($P>0.05$). However,

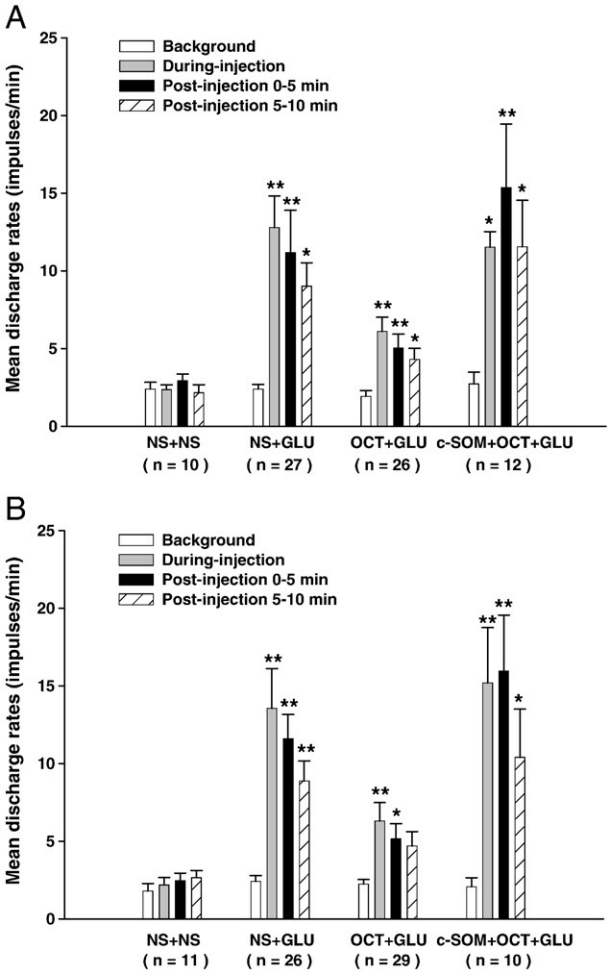

Fig. 1 – Mean discharge rates of Aδ (A) and C fibers (B) during and after glutamate injection into the receptive field compared with background activity in the different experimental groups. One way ANOVA test followed by Fisher LSD method was used. *P<0.05; **P<0.01, compared with the background activity. NS, normal saline; GLU, glutamate; c-SOM, cyclo-somatostatin; OCT, octreotide.

the discharge rates of both Aδ and C afferent fibers increased significantly during and after injection of glutamate (0.3 mM, 10 μL) compared with background discharge prior to any injection ($P<0.05$, Fig. 1). The response of a CMH unit to glutamate is shown in Fig. 2A.

Of the 27 Aδ fibers in the NS+GLU group 78% (21/27) were excited by glutamate including 8 AMH units and 13 AM units (Table 1). Data from the AMH and AM units were pooled because there was no significant difference in the proportion of these afferents which responded to glutamate injection. In addition, there was no significant difference in mechanical threshold between GLU-sensitive and non-sensitive fibers ($P>0.05$). Mean mechanical thresholds of GLU-sensitive Aδ fibers ($n=21$) and non-sensitive Aδ fibers ($n=6$) were 0.48±0.01 and 0.51±0.03 mN, respectively. The mean discharge rate of Aδ fibers in the NS+GLU group ($n=27$) increased from 2.39±0.30 impulses/min in background activity to 12.79±2.04 impulses/min during glutamate injection, was maintained at 11.16±2.74 impulses/min during the following 5 min, and dropped to 9.02±1.51 impulses/min during the second 5 min ($P<0.05$, Fig. 1A).

Among 26 C fibers in the NS+GLU group 81% (21/26) were excited by injection of glutamate including 11 CMH and 10 CM units (Table 1). Similar to Aδ fibers, the data of CMH and CM units were pooled. The mechanical thresholds of the GLU-sensitive ($n=21$) and non-sensitive ($n=5$) C fibers were not different (0.67±0.02 and 0.66±0.06 mN, respectively, $P>0.05$). Mean discharge rate of C fibers in the NS+GLU group ($n=26$) increased from 2.42±0.37 impulses/min in background activity to 13.56±2.56 impulses/min during injection of glutamate, was maintained at 11.61±1.55 impulses/min during the following 5 min, and dropped to 8.88±1.29 impulses/min during the second 5 min ($P<0.05$, Fig. 1B).

Table 1 – Proportion of Aδ and C primary afferent fibers responsive to glutamate in the 3 experimental groups.

Groups	AMH	AM	CMH	CM
NS+GLU	80% (8/10)	76% (13/17)	85% (11/13)	77% (10/13)
OCT+GLU	63% (5/8)	67% (12/18)	67% (8/12)	71% (12/17)
c-SOM+OCT+GLU	100% (4/4)	75% (6/8)	100% (4/4)	83% (5/6)

Fig. 3 (filled circles) shows the time course of discharge rate (placed in 1 min bins) induced by glutamate for the Aδ (Fig. 3A) and C fibers (Fig. 3B) compared with mean background activity. For both fiber types, discharge rates significantly

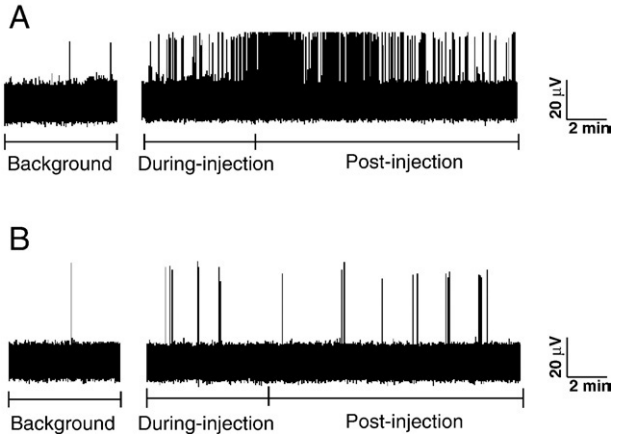

Fig. 2 – Original recording showing the discharge of two C fibers in the dorsal cutaneous branch of a spinal nerve in response to injection of normal saline plus glutamate (A) and octreotide plus glutamate (B) into the receptive field of each fiber. Glutamate increased the discharge when injected into the receptive field of a CMH unit (A) and octreotide partly prevented the glutamate-induced excitatory responses in another CMH unit (B). The conduction velocities of these two units were 1.37 and 0.83 m/s, respectively.

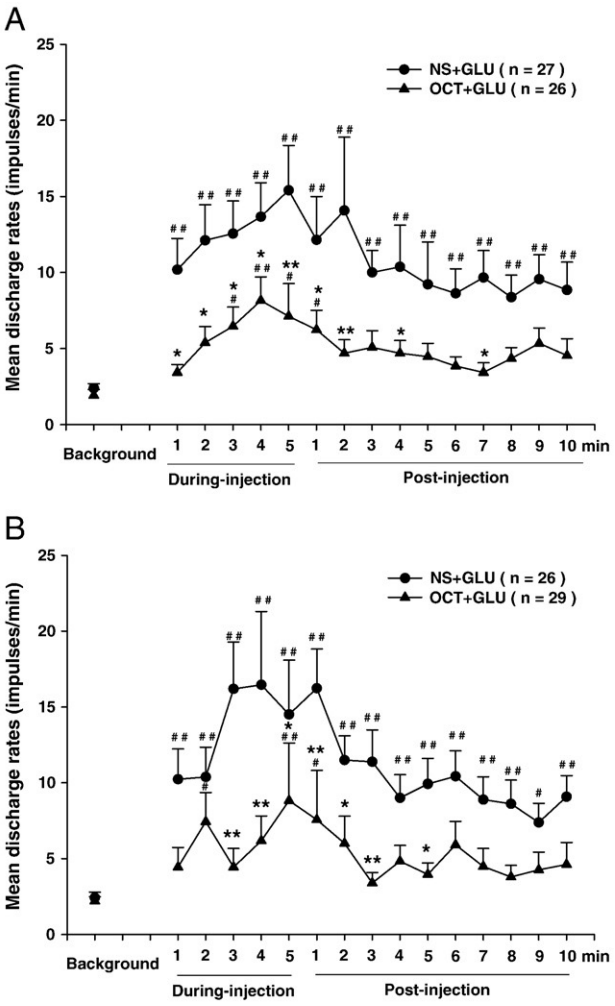

Fig. 3 – The time course of mean discharge rates of Aδ (A) and C fibers (B) in the dorsal cutaneous branches of spinal nerve during the periods of injection and post-injection of glutamate into the receptive fields. $^{\#}P<0.05$; $^{\#\#}P<0.01$, compared with the background activity in the same treatment group. $^{*}P<0.05$; $^{**}P<0.01$, compared with the same time interval in the NS+GLU group. Two Way RM ANOVA followed by Fisher LSD method was used. NS, normal saline; GLU, glutamate; OCT, octreotide.

increased over the 5 min during injection and over the 10 min following injection ($P<0.05$). Peak responses occurred at the fifth minute for Aδ fibers and the fourth minute for C fibers during injection (15.41±2.94 and 16.46±4.83 impulses/min, respectively, Fig. 3). These results are similar to previous reports (Cao et al., 2005; Tian et al., 2005).

2.3. *Group 3: Effects of OCT on glutamate-induced response*

OCT injection alone had no effect on the background activity of Aδ or C fibers in the OCT+GLU group (Group 3). However, even in the presence of OCT, glutamate increased the discharge rates of Aδ and C fibers both during and after its injection. Mean discharge rate of Aδ fibers increased from 1.93±0.38 impulses/min in background activity to 6.11±0.90 impulses/min during injection of glutamate, dropping to 5.03±0.89 impulses/min during the following 5 min, and dropping further to 4.30±0.71 impulses/min during the second 5 min ($n=26$, $P<0.05$, Fig. 1A). In the presence of OCT, discharge rate of Aδ fibers increased at the third, fourth and fifth minute during glutamate injection and at the first minute after injection compared with background activity ($P<0.05$, Fig. 3A). For C fibers in the OCT+GLU group ($n=29$), mean discharge rate significantly increased to 6.31±1.18 impulses/min during glutamate injection and to 5.15±0.97 impulses/min during the following 5 min compared with background activity (2.25±0.29 impulses/min, $P<0.05$), but the 4.70±0.92 impulses/min increase during the second 5 min interval was not significant ($P>0.05$, Fig. 1B). On the basis of 1 min bins, the discharge rate of C fibers was significantly increased only at the first and fifth minute during injection and the first minute after injection compared with the background activity ($P<0.05$, Fig. 3B). An example of CMH unit's activity in response to OCT+GLU is shown in Fig. 2B.

Aδ and C fibers had similar background discharge rates in both the OCT+GLU group and the NS+GLU group ($P>0.05$). In the OCT+GLU group, 65% of Aδ fibers (17/26, including 5 AMH, 12 AM units) and 69% of C fibers (20/29, including 8 CMH, 12 CM units) were excited by glutamate (Table 1). The activated proportions were lower than in the NS+GLU group (78% and 81% for Aδ and C fibers, respectively), but the differences were not statistically significant between the two groups ($P>0.05$). However, the magnitude of the increase in mean discharge rate for Aδ and C fibers in the OCT+GLU group during and after glutamate injection was significantly lower than that in the NS+GLU group ($P<0.05$, Fig. 4). On the basis of 1 min interval comparisons between the OCT+GLU and NS+GLU groups, there was no difference in the background activity for Aδ and C fibers between the two groups ($P>0.05$, Fig. 3). But the mean discharge rate of Aδ fibers in the OCT+GLU group was lower at each 1 min interval during the 5 min glutamate injection and the first, second, fourth, and seventh minute after injection of glutamate than the NS+GLU group ($P<0.05$, Fig. 3A). For the C fibers, the mean discharge rate in the OCT+GLU group was lower at the third to fifth minute during injection of glutamate, the first to third minute and the fifth minute after injection of glutamate ($P<0.05$, Fig. 3B). Taken together, these data indicated that OCT pretreatment reduced the excitation caused by glutamate in both Aδ and C afferent fibers.

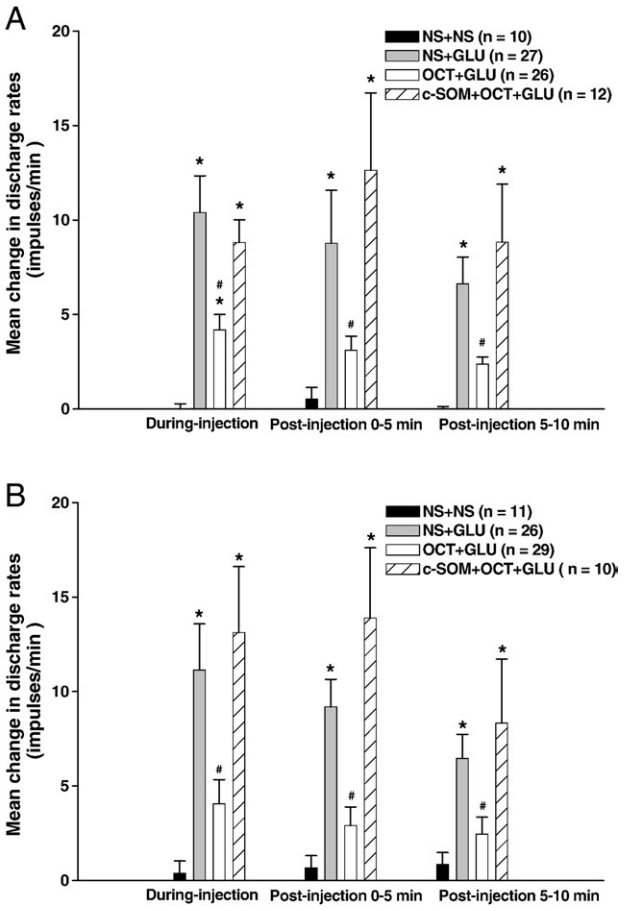

Fig. 4 – Comparison of changes (relative to their baseline) in mean discharge rate of Aδ (A) and C fibers (B) during injection and post-injection of glutamate into the receptive fields in the different experimental groups. *$P<0.05$, compared with the NS+NS group; #$P<0.05$, compared with the NS+GLU group. One Way ANOVA followed by Dunnett method was used to compare the difference in diverse experimental groups. NS, normal saline; GLU, glutamate; c-SOM, cyclo-somatostatin; OCT, octreotide.

2.4. *Group 4: Reverse effects of cyclo-somatostatin (c-SOM) on OCT-induced suppression*

Compared with background, the discharge rate of Aδ and C fibers dramatically increased during and after injection of OCT+c-SOM (a somatostatin receptor antagonist), and then gradually recovered to background by 10 min following injection. During and following the subsequent glutamate injection, the discharge rate of both Aδ and C afferent fibers significantly increased over background activity ($P<0.05$, Fig. 1). With c-SOM+OCT pretreatment, glutamate excited 83% (10/12) of Aδ fibers (including 4 AMH and 6 AM units) and 90% (9/10) of C fibers (including 4 CMH and 5 CM units) (Table 1). The activated proportions were higher compared with the NS+GLU group (78% and 81% for Aδ and C fibers, respectively), but not significantly ($P>0.05$). There was no significant difference in the increased discharge of either Aδ or C fibers during or after glutamate injection between the

NS+GLU and the (c-SOM+OCT)+GLU groups (Fig. 4). The results from Group 4 indicate that c-SOM antagonized OCT's suppressive effect on GLU's excitatory effect in both Aδ and C fibers.

3. Discussion

The most important findings from the present study are that local application of the SST neuropeptide analogue OCT suppresses the glutamate-induced excitatory responses of Aδ and C fibers in the rat hairy skin and that this effect is reversed by pretreatment with c-SOM, an SSTR antagonist. These findings indicate that sensory signaling by primary afferent neurons can be affected by interactions occurring between inhibitory neuropeptides and excitatory amino acids in the periphery through somatostatin and glutamate receptors. The presence of glutamate and somatostatin receptors in the periphery is supported by anatomical data showing that glutamate receptors (Bhave et al., 2001; Carlton et al., 1995, 2001c; Coggeshall and Carlton, 1998; Du et al., 2003, 2006; Walker et al., 2001) and the SSTR2a subtype (Carlton et al., 2001a) are expressed on afferent axons and in their peripheral terminals.

The primary afferent discharge of unitary Aδ and C fibers was increased by injection of glutamate into the cutaneous receptive field of sensory nerves in the present study. The mechanical act of injecting was not responsible for the response because no excitatory effect occurred when glutamate was replaced with normal saline. This result is similar to previous studies showing that Aδ and C afferent fibers are significantly activated by glutamate in vivo and in vitro (Cao et al., 2005; Du et al., 2001; Tian et al., 2005). In the present study, the glutamate-induced responses for some Aδ and C fibers may have been able to reach the painful levels. Both Aδ and C afferent fibers are responsible for nociceptive transmission. Microneurographic studies in humans demonstrate that the discharge rate of nociceptors needs to exceed a certain level for pain to be experienced (Koltzenburg, 1995; Torebjörk et al., 1984).

It has been shown that activation of peripheral ionotropic and/or metabotropic glutamate receptors with glutamate or its analogues results in the nociceptive behaviors (Carlton et al., 1995, 1998; Walker et al., 2001; Zhou et al., 1996). The concentration of peripheral glutamate increases following electrical stimulation of peripheral nerves and after subcutaneous injection of formalin into the rat hind paw (deGroot et al., 2000; Omote et al., 1998). Following complete Freund's adjuvant-induced inflammation, the proportions of ionotropic glutamate receptors expressed on both unmyelinated and myelinated nerves increase significantly (Carlton and Coggeshall, 1999). Our previous study has shown that pre-treating the receptive field with an ionotropic glutamate receptor antagonist blocks the enhanced spontaneous discharge evoked by antidromic stimulation of adjacent afferents (Cao et al., 2007), suggesting that endogenous glutamate released from neighboring peripheral nerves can activate primary afferent fibers. These findings indicate that the activation of peripheral glutamate receptors on the sensory nerve terminals contributes to the peripheral sensitization of pain, especially in the event of inflammation and neuropathic pain (Carlton, 2001).

In the present study pretreatment with c-SOM, an antagonist of the SSTR, reversed the OCT-induced suppression of glutamate's excitatory effect in Aδ and C fibers innervating the rat hairy skin. This indicated that OCT's local suppressive effect occurred through the activation of peripheral SSTRs. It was also reported that c-SOM may active both Aδ and C fibers by removing tonic inhibition of SSTRs in the rat hairy skin (Guo et al., 2008; Wang et al., 2009). It has been shown using immunohistochemical, PCR and *in situ* hybridization techniques that $SSTR_{1–4}$ are expressed in DRG neurons (Bär et al., 2004; Señaris et al., 1995). It is unknown whether all these SSTRs are transported to the peripheral sensory terminals, but $SSTR_{2a}$ subtype is localized on peripheral unmyelinated sensory axons (Carlton et al., 2001a). From the present study we cannot confirm which specific subtype of SSTRs contributed to the inhibition of the glutamate-induced responses because OCT can activate the $SSTR_2$, $SSTR_3$ and $SSTR_5$ subtypes and all SSTR subtypes are antagonized by c-SOM (Patel et al., 1995; Pintér et al., 2006). Specific agonists and antagonists for each SSTR subtype may be used in the future studies to identify the receptor mechanism involved in the neuropeptide-induced inhibition of glutamate-induced excitatory responses. To our knowledge, the interaction between SST and glutamate in the periphery has not been previously investigated. To some degree, results from the present study may explain the findings from previous studies demonstrating that SST has analgesic effects in rodents and humans by acting on peripheral SSTRs (Corsi et al., 1997; Helyes et al., 2004; 2000; Ji et al., 2006; Karalis et al., 1994; Olias et al., 2004; Paran et al., 2005; Silveri et al., 1994; Szolcsányi et al., 2004).

In the periphery, SST is present in a subpopulation of small diameter primary afferent neurons and in approximately 1/3 of fine cutaneous sensory axons (Carlton et al., 2003). Several lines of evidence show that peripheral local application of SST or SST analogues produces analgesic effects. Intraplantar injection of the SST analogue OCT reduces formalin-induced nociceptive behaviors and the responses of C fibers to noxious stimulation (Carlton et al., 2001a, 2003). Intraplantar injection of SCR007, a selective nonpeptide $SSTR_2$ agonist, significantly increases the nociceptive threshold (Ji et al., 2006). Inhibition of nociceptive transmission is an important mechanism underlying the analgesic effect of SSTR agonists (Pan et al., 2008). At the cellular level, activation of the SST receptor opens various K^+ channels and inhibits voltage-gated Ca^{2+} channels (Koch et al., 1988) leading to inhibition of both spike generation and neurotransmitter release (Weckbecker et al., 2003). SST may inhibit the release of pro-inflammatory neuropeptides from sensory terminals and thereby exert antinociceptive effects under inflammatory conditions through these mechanisms (Carlton et al., 2001a,b; Green et al., 1992; Grilli et al., 2004; Hathway et al., 2001; Pintér et al., 2006; Sawynok, 2003; Weckbecker et al., 2003). In addition, peripheral SSTRs are known to modulate nociceptors through phasic and tonic regulation of the peripheral TRPV1 receptor (Carlton et al., 2004). The present study may reveal another mechanism for SST's analgesic effect through suppression of peripheral glutamate receptors.

In summary, this study provides electrophysiological evidence that peripheral SSTRs may inhibit nociceptors through suppressing the glutamate-evoked excitatory responses in peripheral Aδ and C fibers. The results suggest that interactions between excitatory amino acid and inhibitory neuropeptides may play an important role in the peripheral nervous system. These findings provide a basis for the use of octreotide in the pharmacotherapy for acute and chronic pain of peripheral origin.

4. Experimental procedures

4.1. *Animals*

Thirty seven Sprague–Dawley rats weighing 210–280 g were employed in the present experiments. Animals were housed under a 12 h light–dark cycle, with food and water available ad libitum. The experiments were approved by the Institutional Animal Ethics Committee of Xi'an Jiaotong University and performed in accordance with the ethical guidelines of the International Association for the Study of Pain. Efforts were made to minimize the number of animals used and their suffering.

4.2. *Experimental preparation*

Animals were anesthetized initially with urethane (1.2 g/kg i.p.), then supplemental doses of urethane were given as needed in order to maintain areflexia as judged by the absence of voluntary movement or a paw withdrawal reflex. Hair was removed from the dorsal skin. A 10 mm left paramedian incision was made longitudinally from the T7 to L2 vertebra levels. This exposed the right dorsal cutaneous branches of T9–T12 spinal nerves without damaging their receptive fields. Under a dissecting microscope, each nerve was freed from the adjacent tissues for 2.5–3.5 cm and cut proximally at its junction with the spinal nerve. A pool was formed using the skin flaps and filled with warm mineral oil maintained at 37 °C over the exposed tissue. The nerves were desheathed and teased apart on a platform under a dissecting microscope. Small filaments from the dorsal cutaneous nerve branches were repeatedly split with sharpened watchmaker forceps until single unit activity was obtained.

4.3. *Recording and stimulation*

Single unit activity was recorded using platinum bipolar electrodes. Once an isolated unit was obtained, the unit's activity was amplified, filtered (30–3000 Hz) and displayed on an oscilloscope (VC-11, Nihon Kohden, Japan) for monitoring the shape and amplitude of the action potential's waveform. The signals were also fed into a computer system (micro 1401 Biological Signal Collecting and Analyzing System with Spike 2 software, Cambridge Electronic Design Limited, Cambridge, UK) that allowed the discharge to be monitored on-line and data to be analyzed off-line. To ensure that responses were from a single unit over the course of an experiment, the unit was discarded if another unit began to discharge with a waveform similar in amplitude to the original one. Following each experiment, a waveform analysis was performed based on the waveform's amplitude and shape to ensure that activity from the same unit had been recorded during all protocols.

Units were first identified by manual probing of the hairy skin with a blunt glass rod. Only units responding to mechanical probing with a clearly defined receptive field were studied in detail. The mechanical threshold of a unit was measured by applying von Frey monofilaments (Stoelting Company, Wood Dale, IL, USA) with increasing bending forces to the most sensitive area of the receptive field. The threshold force was defined as the minimum force (mN) that evoked a response in ≥50% of the trials for at least 6 trails (Cain et al., 2001). The range of von Frey filaments used in this study exerted bending forces from 0.1 to 42.3 mN. The most sensitive location of each unit's receptive field was marked with a felt tip pen and constituted the target for all drug injections.

Radiant heat was applied to the marked target using a custom-made lamp. Skin temperature was measured with a surface thermode and raised steadily from 32 °C to 53 °C over 20 s (Tian et al., 2005; Zhang et al., 2006a,b). A heat sensitive unit was defined as a unit that responded to the heat stimulation with an increase of at least two impulses during the 20 s compared with a similar time interval prior to heating (Leem et al., 1993).

To measure conduction velocity, electrical stimulation (0.5 ms, 0.1–1 mA) was applied to the unit's receptive field using silver bipolar electrodes. Conduction velocity was determined by dividing the latency of the action potential triggered on the oscilloscope by the distance between the stimulating and recording electrodes. Fibers conducting at a rate less than 2.0 m/s were classified as C fibers, those conducting between 2.0 and 30.0 m/s were classified as Aδ, and those with conduction velocities greater than 30.0 m/s were considered Aβ fibers (Horch et al., 1977). Aβ fibers were not tested in the present study. Units responding to both heat and mechanical stimuli were classified as mechanoheat-sensitive fibers (AMH for Aδ fibers and CMH for C fibers). Units responding only to the mechanical stimulus were classified as mechano-sensitive fibers (AM for Aδ fibers and CM for C fibers).

4.4. *Pharmacological intervention*

All drugs in the experiment were dissolved or diluted in NS. Glutamate, c-SOM (both purchased from Sigma Co., St. Louis, MO, USA) and OCT (Novartis Pharma Stein AG, Switzerland) were diluted to 0.3 mM, 128 μM and 20 μM, respectively. The dosage of glutamate, OCT and c-SOM was based on previous reports (Cao et al., 2005; Carlton et al., 2001a, 2004; Coggeshall et al., 1997; Du et al., 2001; Guo et al., 2008; Tian et al., 2005; Wang et al., 2009) and our preliminary data. A 28-gauge needle attached to a 50 μL Hamilton syringe via PE10 tubing was inserted subcutaneously at a target into the center of receptive field. Background spontaneous activity was recorded for 5 min and included in the analysis. Injections were controlled using a syringe pump (WZ-50, Medical Research Apparatus, Zhejiang University, Hangzhou, China). In order to minimize the stimulation of fluid pressure within the receptive field, the injection rate of all drugs was maintained at 2 μL/min.

To determine whether OCT has a receptor-specific inhibitory effect on glutamate-induced activity, the units were

randomly divided into four groups. (1) The control group (NS+NS group, 10 μL per injection): following a 5 min recording of background activity, NS (pH 7.4) was injected slowly over 5 min into the unit's receptive field. A second NS injection was made 10 min later. This interval helped ensure that discharge had returned to background levels. (2) NS+GLU group: the procedure was similar to the control group, but the second injection was 0.3 mM glutamate (10 μL) instead of NS. (3) OCT+GLU group: the difference between this group and the NS+GLU group was OCT, an SST analogue, which was injected instead of NS. (4) (c-SOM+OCT)+GLU group: this group determined whether the effect of OCT in the OCT+GLU group was SSTR specific. The first injection consisted of a cocktail containing 5 μL of 128 μM c-SOM (an SSTR antagonist) and 10 μL of 20 μM OCT. They were injected slowly over 7.5 min into the unit's receptive field. Following a 10 min interval, the second injection consisted of 10 μL of 0.3 mM glutamate. To avoid false negative reactions caused by tachyphylaxis, each unit was assigned to only one group. The responses of a unit to glutamate were recorded during the 5 min of injection of glutamate and 10 min following glutamate injection. If the unit began to discharge following administration of glutamate and its discharge rate increased to a value greater than or equal to the mean background discharge plus two times standard deviation of the whole sample (≥~6 impulses/min), the unit was considered as a glutamate-sensitive unit in the groups that included glutamate injection (Du et al., 2001; Tian et al., 2005).

4.5. *Statistical analysis*

All data were presented as mean±SEM. For comparison of the proportion of units activated by glutamate among the different treatments, chi-square test (χ^2 test) was used. A *t*-test was used to compare mechanical threshold between the different groups. One way ANOVA test followed by Dunnett method was used for statistical analysis to compare the differences in the increased discharge rates among the treatment groups. Two Way RM ANOVA followed by Fisher LSD method was used to compare the discharge during and after injection of drugs with the background activity in the same treatment group, and the difference in discharge rates between groups during the same time interval of the time course recording. $P<0.05$ level was considered to be statistically significant.

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REFERENCES

Ahn, D.K., Jung, C.Y., Lee, H.J., Choi, H.S., Ju, J.S., Bae, Y.C., 2004. Peripheral glutamate receptors participate in interleukin-1beta-induced mechanical allodynia in the orofacial area of rats. Neurosci. Lett. 357, 203–206.

Bär, K.J., Schurigt, U., Scholze, A., Segond Von Banchet, G., Stopfel, N., Bräuer, R., Halbhuber, K.J., Schaible, H.G., 2004. The expression and localization of somatostatin receptors in dorsal root ganglion neurons of normal and monoarthritic rats. Neuroscience 127, 197–206.

Bhave, G., Karim, F., Carlton, S.M., Gereau, R.W., 2001. Peripheral group I metabotropic glutamate receptors modulate nociception in mice. Nat. Neurosci. 4, 417–423.

Bursztajn, S., Rutkowski, M.D., Deleo, J.A., 2004. The role of the N-methyl-D-aspartate receptor NR1 subunit in peripheral nerve injury-induced mechanical allodynia, glial activation and chemokine expression in the mouse. Neuroscience 125, 269–275.

Cain, D.M., Khasabov, S.G., Simone, D.A., 2001. Response properties of mechanoreceptors and nociceptors in mouse glabrous skin: an in vivo study. J. Neurophysiol. 85, 1561–1574.

Cao, D.Y., Guo, Y., Zhang, Q., Tian, Y.L., Wang, H.S., Zhao, Y., 2005. Effects of glutamate on the afferent discharges of dorsal cutaneous sensory nerves in rats. Neurosci. Bull. 21, 111–116.

Cao, D.Y., You, H.J., Zhao, Y., Guo, Y., Wang, H.S., Arendt-Nielsen, L., Wang, H.L., Zhang, Q., 2007. Involvement of peripheral ionotropic glutamate receptors in activation of cutaneous branches of spinal dorsal rami following antidromic electrical stimulation of adjacent afferent nerves in rats. Brain Res. Bull. 72, 10–17.

Carlton, S.M., 2001. Peripheral excitatory amino acids. Curr. Opin. Pharmacol. 1, 52–56.

Carlton, S.M., Coggeshall, R.E., 1999. Inflammation-induced changes in peripheral glutamate receptor populations. Brain Res. 820, 63–70.

Carlton, S.M., Hargett, G.L., Coggeshall, R.E., 1995. Localization and activation of glutamate receptors in unmyelinated axons of rat glabrous skin. Neurosci. Lett. 197, 25–28.

Carlton, S.M., Zhou, S., Coggeshall, R.E., 1998. Evidence for the interaction of glutamate and NK1 receptors in the periphery. Brain Res. 790, 160–169.

Carlton, S.M., Du, J., Davidson, E., Zhou, S., Coggeshall, R.E., 2001a. Somatostatin receptors on peripheral primary afferent terminals: inhibition of sensitized nociceptors. Pain 90, 233–244.

Carlton, S.M., Du, J., Zhou, S., Coggeshall, R.E., 2001b. Tonic control of peripheral cutaneous nociceptors by somatostatin receptors. J. Neurosci. 21, 4042–4049.

Carlton, S.M., Hargett, G.L., Coggeshall, R.E., 2001c. Localization of metabotropic glutamate receptors 2/3 on primary afferent axons in the rat. Neuroscience 105, 957–969.

Carlton, S.M., Zhou, S., Kraemer, B., Coggeshall, R.E., 2003. A role for peripheral somatostatin receptors in counter-irritation-induced analgesia. Neuroscience 120, 499–508.

Carlton, S.M., Zhou, S., Du, J., Hargett, G.L., Ji, G., Coggeshall, R.E., 2004. Somatostatin modulates the transient receptor potential vanilloid 1 (TRPV1) ion channel. Pain 110, 616–627.

Coggeshall, R.E., Carlton, S.M., 1998. Ultrastructural analysis of NMDA, AMPA, and kainate receptors on unmyelinated and myelinated axons in the periphery. J. Comp. Neurol. 391, 78–86.

Coggeshall, R.E., Zhou, S., Carlton, S.M., 1997. Opioid receptors on peripheral sensory axons. Brain Res. 764, 126–132.

Corsi, M.M., Ticozzi, C., Netti, C., Fulgenzi, A., Tiengo, M., Gaja, G., Guidobono, F., Ferrero, M.E., 1997. The effect of somatostatin on experimental inflammation in rats. Anesth. Analg. 85, 1112–1115.

Davidson, E.M., Coggeshall, R.E., Carlton, S.M., 1997. Peripheral NMDA and non-NMDA glutamate receptors contribute to nociceptive behaviors in the rat formalin test. Neuroreport 8, 941–946.

deGroot, J., Zhou, S., Carlton, S.M., 2000. Peripheral glutamate release in the hindpaw following low and high intensity sciatic stimulation. Neuroreport 11, 497–502.

Du, J., Koltzenburg, M., Carlton, S.M., 2001. Glutamate-induced excitation and sensitization of nociceptors in rat glabrous skin. Pain 89, 187–198.

Du, J., Zhou, S., Coggeshall, R.E., Carlton, S.M., 2003. N-methyl-D-aspartate-induced excitation and sensitization of normal and inflamed nociceptors. Neuroscience 118, 547–562.

Du, J., Zhou, S., Carlton, S.M., 2006. Kainate-induced excitation and sensitization of nociceptors in normal and inflamed rat glabrous skin. Neuroscience 137, 999–1013.

Ferone, D., Boschetti, M., Resmini, E., Giusti, M., Albanese, V., Goglia, U., Albertelli, M., Vera, L., Bianchi, F., Minuto, F., 2006. Neuroendocrine-immune interactions: the role of cortistatin/somatostatin system. Ann. N. Y. Acad. Sci. 1069, 129–144.

Green, P.G., Basbaum, A.I., Levine, J.D., 1992. Sensory neuropeptide interactions in the production of plasma extravasation in the rat. Neuroscience 50, 745–749.

Grilli, M., Raiteri, L., Pittaluga, A., 2004. Somatostatin inhibits glutamate release from mouse cerebrocortical nerve endings through presynaptic sst2 receptors linked to the adenylyl cyclase–protein kinase A pathway. Neuropharmacology 46, 388–396.

Guo, Y., Yao, F.R., Cao, D.Y., Pickar, J.G., Zhang, Q., Wang, H.S., Zhao, Y., 2008. Somatostatin inhibits activation of dorsal cutaneous primary afferents induced by antidromic stimulation of primary afferents from an adjacent thoracic segment in the rat. Brain Res. 1229, 61–71.

Hathway, G.J., Humphrey, P.P., Kendrick, K.M., 2001. Somatostatin release by glutamate in vivo is primarily regulated by AMPA receptors. Br. J. Pharmacol. 134, 1155–1158.

Helyes, Z., Thán, M., Oroszi, G., Pintér, E., Németh, J., Kéri, G., Szolcsányi, J., 2000. Anti-nociceptive effect induced by somatostatin released from sensory nerve terminals and by synthetic somatostatin analogues in the rat. Neurosci. Lett. 278, 185–188.

Helyes, Z., Szabó, A., Németh, J., Jakab, B., Pintér, E., Bánvölgyi, A., Kereskai, L., Kéri, G., Szolcsányi, J., 2004. Antiinflammatory and analgesic effects of somatostatin released from capsaicin-sensitive sensory nerve terminals in a Freund's adjuvant-induced chronic arthritis model in the rat. Arthritis. Rheum. 50, 1677–1685.

Heppelmann, B., Pawlak, M., 1997. Inhibitory effect of somatostatin on the mechanosensitivity of articular afferents in normal and inflamed knee joints of the rat. Pain 73, 377–382.

Horch, K.W., Tuckett, R.P., Burgess, P.R., 1977. A key to the classification of cutaneous mechanoreceptors J. Invest. Dermatol. 69, 75–82.

Ji, G.C., Zhou, S.T., Shapiro, G., Reubi, J.C., Jurczyk, S., Carlton, S.M., 2006. Analgesic activity of a non-peptide imidazolidinedione somatostatin agonist: in vitro and in vivo studies in rat. Pain 124, 34–49.

Karalis, K., Mastorakos, G., Chrousos, G.P., Tolis, G., 1994. Somatostatin analogues suppress the inflammatory reaction in vivo. J. Clin. Invest. 93, 2000–2006.

Kinkelin, I., Bröcker, E.B., Koltzenburg, M., Carlton, S.M., 2000. Localization of ionotropic glutamate receptors in peripheral axons of human skin. Neurosci. Lett. 283, 149–152.

Koch, B.D., Blalock, J.B., Schonbrunn, A., 1988. Characterization of the cyclic AMP-independent actions of somatostatin in GH cells. I. An increase in potassium conductance is responsible for both the hyperpolarization and the decrease in intracellular free calcium produced by somatostatin. J. Biol. Chem. 263, 216–225.

Koltzenburg, M., 1995. The stability and plasticity of the encoding properties of peripheral nerve fibers and their relationship to provoked and ongoing pain. Semin. Neurosci. 7, 199–210.

Lawand, N.B., McNearney, T., Westlund, K.N., 2000. Amino acid release into the knee joint: key role in nociception and inflammation. Pain 86, 69–74.

Leem, J.W., Willis, W.D., Chung, J.M., 1993. Cutaneous sensory receptors in the rat foot. J. Neurophysiol. 69, 1684–1699.

Olias, G., Viollet, C., Kusserow, H., Epelbaum, J., Meyerhof, W., 2004. Regulation and function of somatostatin receptors. J. Neurochem. 89, 1057–1091.

Omote, K., Kawamata, T., Kawamata, M., Namiki, A., 1998. Formalin-induced release of excitatory amino acids in the skin of the rat hindpaw. Brain Res. 787, 161–164.

Pan, H.L., Wu, Z.Z., Zhou, H.Y., Chen, S.R., Zhang, H.M., Li, D.P., 2008. Modulation of pain transmission by G-protein-coupled receptors. Pharmacol. Ther. 117, 141–161.

Paran, D., Kidron, D., Mayo, A., Ziv, O., Chowers, Y., Caspi, D., Yaron, M., Paran, H., 2005. Somatostatin analogue treatment attenuates histological findings of inflammation and increases mRNA expression of interleukin-1 beta in the articular tissues of rats with ongoing adjuvant-induced arthritis. Rheumatol. Int. 25, 350–356.

Patel, Y.C., Greenwood, M.T., Panetta, R., Demchyshyn, L., Niznik, H., Srikant, C.B., 1995. The somatostatin receptor family. Life Sci. 57, 1249–1265.

Peineau, S., Potier, B., Petit, F., Dournaud, P., Epelbaum, J., Gardette, R., 2003. AMPA-sst2 somatostatin receptor interaction in rat hypothalamus requires activation of NMDA and/or metabotropic glutamate receptors and depends on intracellular calcium. J. Physiol. 546, 101–117.

Pintér, E., Helyes, Z., Szolcsányi, J., 2006. Inhibitory effect of somatostatin on inflammation and nociception. Pharmacol. Ther. 112, 440–456.

Sawynok, J., 2003. Topical and peripherally acting analgesics. Pharmacol. Rev. 55, 1–20.

Señaris, R.M., Schindler, M., Humphrey, P.P., Emson, P.C., 1995. Expression of somatostatin receptor 3 mRNA in the motorneurones of the rat spinal cord, and the sensory neurones of the spinal ganglia. Brain Res. Mol. Brain Res. 29, 185–190.

Silveri, F., Morosini, P., Brecciaroli, D., Cervini, C., 1994. Intra-articular injection of somatostatin in knee osteoarthritis: clinical results and IGF-1 serum levels. Int. J. Clin. Pharmacol. Res. 14, 79–85.

Szolcsányi, J., Bölcskei, K., Szabó, A., Pintér, E., Petho, G., Elekes, K., Börzsei, R., Almási, R., Szuts, T., Kéri, G., Helyes, Z., 2004. Analgesic effect of TT-232, a heptapeptide somatostatin analogue, in acute pain models of the rat and the mouse and in streptozotocin-induced diabetic mechanical allodynia. Eur. J. Pharmacol. 498, 103–109.

Tian, Y.L., Guo, Y., Cao, D.Y., Zhang, Q., Wang, H.S., Zhao, Y., 2005. Local application of morphine suppresses glutamate-evoked activities of C and Adelta afferent fibers in rat hairy skin. Brain Res. 1059, 28–34.

Torebjörk, H.E., LaMotte, R.H., Robinson, C.J., 1984. Peripheral neural correlates of magnitude of cutaneous pain and hyperalgesia: simultaneous recordings in humans of sensory judgments of pain and evoked responses in nociceptors with C-fibers. J. Neurophysiol. 51, 325–339.

Walker, K., Reeve, A., Bowes, M., Winter, J., Wotherspoon, G., Davis, A., Schmid, P., Gasparini, F., Kuhn, R., Urban, L., 2001. mGlu5 receptors and nociceptive function II. mGlu5 receptors functionally expressed on peripheral sensory neurones mediate inflammatory hyperalgesia. Neuropharmacology 40, 10–19.

Wang, J., Guo, Y., Cao, D.Y., Luo, R., Ma, S.J., Wang, H.S., Pickar, J.G., Zhao, Y., 2009. Tonic inhibition of somatostatin on C and Aδ afferent fibers in rat dorsal skin in vivo. Brain Res. 1288, 50–59.

Weckbecker, G., Lewis, I., Albert, R., Schmid, H.A., Hoyer, D., Bruns, C., 2003. Opportunities in somatostatin research: biological, chemical and therapeutic aspects. Nat. Rev. Drug Discov. 2, 999–1017.

Yao, F., Guo, Y., Lu, S., Sun, C., Zhang, Q., Wang, H., Zhao, Y., 2008. Mechanical hyperalgesia is attenuated by local administration of octreotide in pristane-induced arthritis in Dark-Agouti rats. Life Sci. 83, 732–738.

Zhang, Q., Zhao, Y., Guo, Y., Cao, D.Y., Tang, X.D., Tian, Y.L., Yao, F. R., Wang, H.S., 2006a. Activation and sensitization of C and Adelta afferent fibers mediated by P2X receptors in rat dorsal skin. Brain Res. 1102, 78–85.

Zhang, Q., Zhao, Y., Guo, Y., Cao, D.Y., Tian, Y.L., Yao, F.R., Wang, H.S., 2006b. Electrophysiological evidence for the interaction of substance P and glutamate on Adelta and C afferent fibre activity in rat hairy skin. Clin. Exp. Pharmacol. Physiol. 33, 1128–1133.

Zhou, S., Bonasera, L., Carlton, S.M., 1996. Peripheral administration of NMDA, AMPA or KA results in pain behaviors in rats. Neuroreport 7, 895–900.