Effects of Somatostatin on the Responses of Rostrally Projecting Spinal Dorsal Horn Neurons to Noxious Stimuli in Cats

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Somatostatin (SOM) is a widely distributed peptide in the central nervous system and exerts a variety of hormonal and neuronal actions. Although SOM is assumed to play an important role in spinal nociceptive processing, its exact function remains unclear. In fact, earlier pharmacological studies have provided results that support either a facilitatory or inhibitory role for SOM in nociception. In the current study, the effects of SOM were investigated using anesthetized cats. Specifically, the responses of rostrally projecting spinal dorsal horn neurons (RPSDH neurons) to different kinds of noxious stimuli (i.e., heat, mechanical, and cold stimuli) and to the Aδ- and C-fiber activation of the sciatic nerve were studied. Iontophoretically applied SOM suppressed the responses of RPSDH neurons to noxious heat and mechanical stimuli as well as to C-fiber activation. Conversely, it enhanced these responses to noxious cold stimulus and Aδ-fiber activation. In addition, SOM suppressed glutamate-evoked activities of RPSDH neurons. The effects of SOM were blocked by the SOM receptor antagonist cyclo-SOM. These findings suggest that SOM has a dual effect on the activities of RPSDH neurons; that is, facilitation and inhibition, depending on the modality of pain signaled through them and its action site.

Key Words: Somatostatin, Spinal cord, Dorsal horn, Nociception, Aδ-fiber, C-fiber, Pain

INTRODUCTION

Somatostatin (SOM), a cyclic tetradecapeptide, is widely distributed in the central nervous system and periphery. It acts as a neuromodulator that inhibits neuronal activity or modulates neurotransmitter release (Beitz et al, 1983; Patel, 1999). This peptide exists in organs processing nociceptive information, such as small dorsal root ganglion (DRG) cells (Rang et al, 1994; Helyes et al, 2000; Carlton et al, 2001), substantia gelatinosa neurons of the spinal dorsal horn (Hunt et al, 1981), intrinsic interneurons or relay neurons of the superficial dorsal horn (Lu and Ho, 1992; Mather and Ho, 1992), and midbrain periaqueductal gray that projects to the medullary nucleus raphe magnus (Beitz et al, 1985; Milhorn et al, 1987). However, the physiological function of SOM in the spinal nociceptive processing has not yet been fully elucidated. Pharmacological data in nociception are contradictory. When interpreted, SOM's modulatory role has been viewed as either facilitatory (pro-nociceptive) or inhibitory (anti-nociceptive) (Traub and Brozowski, 1996; Chapman and Dickenson, 1992; Song et al, 2002). As established in previous studies, two hypotheses have been proposed: 1) SOM might be involved in the transmission of nociceptive information, because of the following: First, SOM is released from small DRG neurons by various thermal stimulation (Kuraishi et al, 1985; Morton et al, 1989). Second, SOM is distributed in small primary afferent nerve fibers (Rang et al, 1984) and colocalized with substance P and CGRP (Garry et al, 1988; Hanesch et al, 1995). Finally, the spinal application of SOM enhanced pain behavior, such as facilitating the response to noxious thermal stimulation (Seybold et al, 1981; Wiesenfeld-Hallin, 1985, 1986; Morton et al, 1989; Kamei et al, 1993). 2) In contrast with pro-nociceptive action, SOM may also have anti-nociceptive effects. First, SOM exhibits depressant actions on the excitability of neurons, particularly in the spinal cord (Murase et al, 1982; Taddese et al, 1995; Kim et al, 2002; Jiang et al, 2003). Second, SOM superfusion or systemic SOM in vivo inhibits the thermal response of nociceptive spinal neurons (Sandkühler et al, 1990; Helmcen et al, 1995). Third, intrathecally applied SOM inhibits motor reflexes in response to noxious stimuli and reduces c-Fos expression and mechanical hyperalgesia in neuropathic pain model (Mollenholt et al, 1988; Tsui et al, 2002). Finally, SOM has been shown to be analgesic when given systemically to patients with cluster headache, or when given intrathecally to patients with cancer pain or postoperative pain (Sicuteri et al, 1984; Chrubasik et al, 1985; Meynadier et al, 1985; Penn et al, 1992; Paice et al, 1996). Collectively, these findings suggest that SOM plays a significant role

ABBREVIATIONS: SOM, somatostatin; DRG, dorsal root ganglion; RPSDH, rostrally projecting spinal dorsal horn; GLU, glutamate; cyclo-SOM, cyclo-(7-aminobutyryl-Phe-D-Trp-Lys-Thr(Bzl)); RF, receptive field.
in the transmission of nociceptive information. The inconsistency of the above mentioned experimental reports on the action of SOM in nociceptive processing and analgesic mechanisms might be due to diverse nociceptive processes and pain modality. Hence, the present study was taken to clarify this issue by examining the effects of SOM on the responses of RPSDH neurons to various noxious stimuli in cat.

METHODS

Animal preparation

The experiments were performed on 31 cats of either gender weighing 2.5 to 3.0 kg. After treatment with atropine sulfate (0.2 mg/kg, s.c.) and ketamine hydrochloride (30 mg/kg, i.m.), cats were anesthetized with α-chloralose (60 mg/kg, i.v.). Moreover, it was ventilated artificially with the end-tidal CO₂ level which was maintained between 3.5 and 4.5%. Rectal temperature was maintained at 37°C throughout the experiment, and arterial blood pressure was continuously monitored. A laminectomy on the spinal cord levels L2–S3 exposed the lumbosacral enlargement. Around the exposed spinal cord, a mineral oil pool was made. Afterwards, the pool temperature was maintained near body temperature. The left sciatic nerve was dissected free from the surrounding connective tissue and placed on the platinum tripolar stimulating electrodes.

Electrophysiology

Seven-barreled glass micropipettes assemblies were used for simultaneous recording from RPSDH neurons and microiontophoretic application of drugs. The low impedance (< 2 MΩ measured at 1 kHz) carbon fiber in the center barrel of the array served as an electrode for recording extracellular single-unit activities. The signals amplified through an AC amplifier (DAM 80, WPD) were fed into a window discriminator which was connected to a laboratory interface (CED 1401) and a personal computer to provide basis for sampling and analysis of the spontaneous and evoked neuronal activity.

Noxious stimulation and electrical stimulation

Mechanical stimuli were generated by manually squeezing the receptive field (RF) for 10 s using serrated forceps. The heat stimuli (60°C for 20 s, given at intervals of >15 min) were applied to the glabrous foot pad by a radiant heat source. The cold stimuli of -15°C, 10 s in duration, were delivered to the RF through contact of a piece of dry ice with the RF. The sciatic nerve was electrically stimulated to activate Aδ-fibers (single square wave pulses of 1 mA of 0.1 ms width) and C-fibers (a single pulse or a train of three square wave pulses of 10 mA of 0.5 ms width, 50 Hz). The intensity to activate C-fibers was determined to be a couple of hundred times greater than that of the Aα-β-fiber threshold strength. A δ- and C-fiber responses were identified through the latency of the responses. Since the length from the stimulating to the recording site was 15–20 cm, cellular activities appearing in less than 50 ms (conduction velocity, 3–30 m/s) were considered as Aδ responses, while those after 150 ms (0.3–1.3 m/s) were viewed as C-responses. Evoked responses were expressed as total number of impulses. Also, twenty sweeps were compiled as a peristimulus time histogram (bin width; 2 ms).

Identification of RPSDH neurons

The antidromic stimulation technique was employed to confirm the RPSDH neurons. After a laminectomy at cervical vertebrae, a bipolar electrode was placed on the contralateral ventrolateral funiculus at the C2 spinal cord segment for antidromic activation. The criteria for an antidromic activation were: 1) constant latency of the evoked response (100 μA and 1 Hz); 2) neuron’s ability to follow high-frequency stimulus trains (333 Hz, 3 pulses) with spikes; and 3) collision between the antidromic spike and the orthodromic action potentials evoked by natural stimulation to the RF.

Drugs and solutions

In this study, the drug concentration and pH were as follows: L-monoamine-glutamate (GLU, Sigma), 0.2 M, pH 8.5; somatostatin (SOM, Sigma), 0.1 M, pH 7.0; somatostatin receptor antagonist cyclo (7-aminohexanoyl-Phe-D-Trp-Lys-Thr(Bzl)) (cyclo-SOM; Sigma), 0.1 M, pH 7.0; and sodium chloride (NaCl, Sigma), 150 mM, pH 7.0. All these drugs were applied iontophoretically with cathodic currents, except GLU which was expelled through an anionic current. The retaining currents were kept at 8 nA, while the current neutralization via 150 mM NaCl-filled balancing barrel was used during all drug applications.

Statistical analysis

The data were expressed as means ± standard error. Differences in the data were evaluated by means of Student’s t-test. A p-value < 0.05 was taken as a statistically significant difference.

RESULTS

Identification of RPSDH neurons

The spinal dorsal horn neurons, located in the laminae II-VI of Rexed (200–3,000 μm), served as the basis for recording. These neurons had excitatory RFs at one or more toes of the ipsilateral hindpaw or footpad. The RPSDH neurons were identified by applying the antidromic stimulation to the cervical dorsal column (Fig. 1A).

Effects of SOM on the responses of RPSDH neurons to noxious heat, mechanical, and cold stimuli

Iontophoretically applied SOM (100 nA) was revealed to have no significant effects on the basal and innocuous touch-induced activities (Fig. 1B and 1C). Nevertheless, it altered the responses of RPSDH neurons to noxious stimuli: it decreased the number of action potentials induced by noxious heat and mechanical stimuli (Fig. 1B) and enhanced the neuronal firing elicited by cold stimulation (Fig. 1C). On the average, SOM suppressed the heat-evoked response to 47.7 ± 5.0% of the control (n=27, p < 0.05) and the noxious mechanical stimulus-evoked response to 65.5 ± 3.1% (n=33, p < 0.05). In contrast, the cold-evoked response was increased to 146.8 ± 15.9% of the control (n=8, p < 0.05).
Effects of SOM on the responses of RPSDH neurons to the activation of Aδ- and C-fibers in the sciatic nerve

These SOM effects could be associated with nerve fiber types which transmit nociceptive information or their functional properties. Taking this into account, the effects of SOM on the Aδ- and C-fiber-elicited responses of RPSDH neurons were investigated. Fig. 2 shows that SOM increased the Aδ-fiber-elicited response to 128.4±3.4% of the control in 8 of 13 cells tested (p<0.05). However, it did not exhibit any effect on the remaining five cells. On the other hand, SOM reduced the C-fiber-elicited response in all cells (n=9), tested to 57.7±8.3% (p<0.05) of the control. It was noted that SOM facilitated the responses of RPSDH neurons to cold stimuli in all of the 4 neurons, enhancing the Aδ-fiber-elicited responses.
Fig. 3. Blockade of SOM effect by SOM receptor antagonist, cyclo-SOM. The cyclo-SOM (100 nA) blocked the inhibitory effects of SOM (100 nA) on heat (59.2±9.8→80.8±11.5%, n=5) and squeeze (74.6±4.6→90.3±6.9%, n=6) as well as the facilitatory effect on cold stimulation (151.4±11.5→110.8±6.6%, n=4). In the case of activities by electrical stimuli such as Aδ- and C-response, SOM effect was also inhibited by cyclo-SOM (Aδ-response, 135.4±6.2→105.4±9.2%, n=5; C-response, 64.6±7.7→53.8±5.2%, n=5). Each bar graph represents mean value±standard error for SOM and cyclo-SOM effect on noxious stimuli. The asterisk shows significant difference in SOM effect (paired t-test, p<0.05).

A  

Glutamate  

SOM1  

SOM2  

B  

SOM  

Cyclo-SOM  

Fig. 4. Effects of SOM on glutamate-evoked activity of RPSDH neuron. Iontophoretical application of GLU (100 nA every 5 seconds) induced the activity of RPSDH neuron. SOM has inhibitory action on the GLU-evoked activity of RPSDH neuron in a dose dependent manner (SOM1, 100 nA; SOM2, 200 nA). This inhibitory effect of SOM was blocked by cyclo-SOM (100 nA). Each bar graph represents mean value±standard error for SOM and cyclo-SOM effect on glutamate-evoked activity of RPSDH neuron. Similarly, the asterisk shows significant difference in SOM effect (paired t-test, p<0.05).

DISCUSSION

SOM is present in small to medium size cells in the dorsal root ganglion and is colocalized with other neuropeptides, such as substance P (Tachtscher and Seybold, 1985). In addition, the SOM-immunoreactive cell bodies and neurites are found in the lamina II of the spinal dorsal horn (Stine et al., 1982). Therefore, it is generally thought that SOM may participate in pain signal processing. However, there is contradictory evidence with regards to the role of SOM in modulating the nociceptive transmission in the spinal cord. SOM elicits antinociceptive effects against acute noxious thermal and mechanical stimuli (Sandkühler et al., 1990) and attenuates the hyperalgesia in the formalin and carrageenan-induced inflammation model (Chapman and Dickens, 1992; Pinter et al., 2002). On the other hand, the intrathecal administration of SOM facilitates the flexion reflex to C-fiber input and noxious
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thermal stimulation (Seybold et al., 1981; Wisenfield-Hallin, 1985, 1986; Morton et al., 1988; Kamei et al., 1993ab). Also, the intrathecal administration of anti-SOM antisera attenuates hyperalgesia in experiments involving rats. Another effect is that it inhibits the response to thermal stimuli in normal and adjuvant inflamed rats (Ohno et al., 1988; Traub and Brozoski, 1996).

The present results demonstrate that iontophoretic application of SOM selectively suppressed the responses of RPSDH neurons to the noxious heat and mechanical stimuli and to C-fiber stimulation. The result in part contradicts with other reports that SOM is involved in mechanical or heat nociception (Kuraishi et al., 1985; Morton et al., 1989; Song et al., 2002). Nevertheless, it is in concordance with the previous reports that SOM has an inhibitory effect on dorsal horn neurons (Sandkühler et al., 1990; Helmcchen et al., 1995; Taddese et al., 1995).

An interesting feature of the present results is the facilitation of cold-evoked and Aδ-fiber-elicted responses by SOM. Sandkühler et al. reported that the responses to electrical stimulation of primary afferent Aβ- and Aδ-fibers, were at least not decreased by SOM superfusion, although C-fiber-elicited responses were blocked (Sandkühler et al., 1990). This discrepancy between Sandkühler's and our present results might be due to the difference in drug administration route (iontophoretic application in vivo vs. bath-application in vitro) or the difference of pain modality such as cold- and Aδ-response. Assuming that the cutaneous Aδ-nociceptors contribute to the sensation of cold pain (Fruhstorfer et al., 1974; Willis WD, Coggeshall, 1991; Simone and Kajander, 1997), the results imply that the response to the cold stimuli is associated with the increment of Aδ-fiber response by SOM.

The exact mechanism underlying the dual effect of SOM-inhibitory and excitatory-on the nociceptive transmission remains unknown. However, two possibilities can be suggested. The analgesic effect of SOM may be explained as follows. At the cellular level, it has been reported that an iontophoretic and bath application of SOM result in hyperpolarization and in reduction of the spontaneous firing of dorsal horn neurons in both neonatal (Murase et al., 1982) and adult rats (Yajiri et al., 1997). Also, SOM-14 could hyperpolarize cortical neurons of the CNS. This is made possible by increasing K+ current (Wang et al., 1989) and inhibiting a voltage-dependent Ca2+ current via a GTP-binding protein (Dichter et al., 1990; Kleuss et al., 1991). It was found that SOM inhibited the GLU-evoked response, indicating its inhibitory action on the excitability of postsynaptic neuron. Thus, SOM may directly act on the postsynaptic membrane and suppress the excitability of RPSDH neuron. This hypothesis is consistent with two recent studies (Kim et al., 2002; Jiang et al., 2003). They reported that SOM induced postsynaptic hyperpolarization via the activation of outward K+ current in superficial dorsal horn neurons.

However, the facilitating effect of SOM on nociception cannot be explained by the hyperpolarizing action at postsynaptic sites. SOM, which inhibits voltage-dependent K+ currents in neurons in CNS (Dichter et al., 1989) and colonic crypts (Sandile et al., 1999), may directly excite presynaptic neuron, resulting in the enhancement of glutamate release from primary afferent. It is also possible that SOM inhibits GABAergic interneurons involved in the descending inhibition of spinal nociceptive transmission, because GABA and SOM have been shown to be co-localized in numerous neurons throughout the CNS, and many of the SOM positive neurons in the superficial dorsal horn are likely to represent inhibitory interneurons. In turn, they inhibit the release of GABA and the interaction with the GABAA receptor to modulate responses to this inhibitory transmitter (Robbins, 1985; Dichter et al., 1990).

The present results suggest that SOM may suppress the responses of dorsal horn neurons to noxious heat and mechanical stimuli by blocking the C-fiber input via postsynaptic inhibition. On the other hand, it may facilitate the responses of dorsal horn neurons to noxious cold stimuli by enhancing the Aδ-fiber input via presynaptic excitation. Thus, SOM has a dual effect on the activities of RPSDH neurons; facilitation and inhibition. Such an effect is dependent on the modality of the pain signals transmitted. Furthermore, this dual effect of SOM might also be determined by the action site of SOM-pre- or postsynaptic SOM receptors.

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