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Research Report

Tonic inhibition of somatostatin on C and Aδ afferent fibers in rat dorsal skin in vivo

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ABSTRACT

The purpose of this study was to investigate the effect of somatostatin (SST) in peripheral nerve terminals using local application of the SST receptor (SSTR) antagonist cyclo-somatostatin (c-SOM) injected into the receptive fields of cutaneous afferent fibers innervating the dorsal hairy skin in anesthetized rats. Single unit activity was recorded in teased filaments from the dorsal cutaneous nerve branch. Recordings were obtained from 206 primary afferent fibers. They were classified as C ($n=70$), Aδ ($n=84$) or Aβ ($n=52$) fibers based upon their conduction velocity. For C and Aδ fibers, mean discharge rate increased and mechanical threshold decreased significantly to 10 μL of 12.8 and 128 μM injected subcutaneously, but not to 0.128 and 1.28 μM c-SOM injection. For Aβ fibers, no dose of c-SOM changed their discharge rate or their mechanical sensitivity. In control experiments, injection of normal saline (NS) had no effect on any of the units tested. Octreotide (20 μM, 10 μL), an SSTR agonist, successfully reversed the increase in discharge rates and the decrease in mechanical thresholds in C and Aδ fibers when it was pre-administrated into the receptive field before c-SOM injection. These results provide evidence that somatostatin tonically inhibits the peripheral nerve terminals of small-diameter cutaneous afferent fibers.

1. Introduction

Cell bodies of primary afferent neurons synthesize a variety of neuropeptides which are transported into their peripheral processes (Brimijoin et al., 1980; Gamse et al., 1982). Pro-inflammatory neuropeptides such as substance P sensitize the receptive endings of small-diameter afferents (Heppelmann and Pawlak, 1997a; Zhang et al., 2008) whereas anti-inflammatory neuropeptides such as somatostatin (SST) may regulate the sensitivity of these thin afferent fibers (Carlton et al., 2001a; Corsi et al., 1997; Heppelmann and Pawlak, 1997b). The actions of pro-inflammatory and anti-inflammatory peptides might neutralize each other in normal tissue.

Substantial evidence suggests that application of an anti-inflammatory peptide such as the SST receptor (SSTR) agonist octreotide (OCT) to inflamed tissue can inhibit nociceptive processing and result in pain relief (Karalis et al., 1994; Matucci-Cerinic et al., 1995; Szolcsányi et al., 1998a,b). Anatomical studies demonstrate that SST is localized in a subset of small-diameter dorsal root ganglion (DRG) cells (Hanesch et al., 1995; Hökfelt et al., 1976; McNeill et al., 1989; O'Brien et al., 1989; Price, 1985; Schulz et al., 1998), and that

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SSTRs are also present in DRG cells (Bär et al., 2004; Carlton et al., 2004; Schulz et al., 1998). Furthermore, the SST_{2a} receptor, a subtype of SSTR, has been found on unmyelinated sensory axons at the dermal-epidermal junction in rat glabrous skin (Carlton et al., 2001a). These anatomical findings provide us with a target to test the hypothesis that SSTRs maintain tonic inhibitory control over peripheral nociceptors via tonic release of endogenous SST from peripheral sensory terminals.

Recently, it has been shown that intraplantar injection of cyclo-somatostatin (c-SOM), an SSTR antagonist, results in nociceptive behaviors in vivo as well as increases activity of C mechanoheat-sensitive (CMH) units from the glabrous skin in an in vitro rat hindpaw preparation (Carlton et al., 2001b, 2004). A previous study from our lab indicates that SST also produces a receptor-mediated tonic inhibitory effect on the cross-excitation between dorsal cutaneous nerve branches in an in vivo rat model (Guo et al., 2008). The 10 times lower concentration of c-SOM compared with that of Carlton et al. (2001b) induced both an increase in discharge rate and a decrease in mechanical threshold of these cutaneous C fibers innervating the thoracic region of the rat. These findings intrigued us to further investigate the tonic inhibitory effect of SST on different types of peripheral afferent fibers in response to local injection of varying c-SOM concentrations in vivo.

2. Results

2.1. Characteristics of afferent fibers tested

Recordings were obtained from 206 primary afferent units including 70 C fibers, 84 Aδ fibers and 52 Aβ fibers. Mean conduction velocities of C, Aδ and Aβ fibers were 1.56±0.04 m/s (range 0.83–1.91 m/s), 10.74±0.56 m/s (range 2.26–26.40 m/s) and 39.51±0.75 m/s (range 30.00–58.72 m/s), respectively. The 3 types of cutaneous afferent fibers had very low background activity with mean spontaneous discharge rates of 1.76±0.09, 1.63±0.08, and 1.90±0.16 impulses/min (imp/min) for C, Aδ and Aβ fibers, respectively. Spontaneous discharge rates were not significantly different between the 3 fiber types ($P>0.05$, one way ANOVA). Table 1 summarizes the fiber types and their general properties.

2.2. c-SOM dose–response relationship for C and Aδ fibers

To determine the dose–response relationship of c-SOM-induced effects in afferent fibers, we injected c-SOM into each afferent's receptive field. The mean discharge rates evoked by 0.128, 1.28, 12.8 and 128 μM c-SOM were 1.75±0.13 ($n=8$), 3.19±0.76 ($n=10$), 7.63±1.54 ($n=16$), 15.63±3.79 ($n=10$) imp/min for C fibers, and 1.50±0.19 ($n=7$), 2.49±0.67 ($n=11$), 5.35±1.04 ($n=24$), 12.45±2.83 ($n=10$) imp/min for Aδ fibers as shown in Fig. 1. Increasing the dose of c-SOM increased the discharge rates of C and Aδ fibers monotonically but not the rates of Aβ fibers (Figs. 1A–C). Mean discharge rates of C fibers and Aδ fibers during injection were significantly higher than the pre-injection rates in response to 12.8 μM and 128 μM c-SOM but not 0.128 μM nor 1.28 μM c-SOM ($P<0.05$, paired t-test or Wilcoxon Signed Rank Test, Figs. 1A, B). Injection of normal saline (NS) into the receptive field had no effect on the discharge rate in any of the fiber types. Changes in C and Aδ fiber discharge rates caused by c-SOM for the 12.8 μM and 128 μM but not the 0.128 μM nor 1.28 μM c-SOM concentrations were higher than that caused by saline injection ($P<0.05$, one way ANOVA followed by Dunnett's post hoc test, Fig. 1D).

Fig. 2 shows original recordings from each of the 3 types of afferent fibers following injection of 12.8 μM c-SOM into their receptive fields. The increase in C and Aδ fiber discharge rate to the higher c-SOM concentrations (12.8 μM and 128 μM) usually peaked at the third minute during the injection and then gradually decreased over the following 2 min (see Fig. 3). Following injection of 0.128, 1.28, 12.8 and 128 μM c-SOM, the proportion of units considered responsive [discharge increased by at least 2 standard deviations from the mean background discharge, (see Experimental procedures)] were 0/8(0%), 3/10(30%), 9/16 (56%), 6/10(60%) for C fibers, and 0/7(0%), 2/11 (18%), 11/24(46%), 6/10(60%) for Aδ fibers, respectively.

In addition to the c-SOM-induced increase in discharge rate, changes in mechanical threshold were also observed for C and Aδ fibers following c-SOM injection into the receptive field but not for Aβ fibers (Figs. 4A–C). Mechanical thresholds of C and Aδ fibers were significantly lower than their pre-injection level following injection of 12.8 μM and 128 μM c-SOM ($P<0.05$, paired t-test) but not 0.128 μM nor 1.28 μM c-SOM. Injection of NS (10 μL) did not change the mechanical thresholds of C, Aδ or Aβ fibers. Based upon the dose–response relationship, 12.8 μM c-SOM was chosen for all further testing.

Table 1 – Receptive properties of primary afferent fibers.

	C fibers	Aδ fibers	Aβ fibers
Number of fibers	70	84	52
Conduction velocity (m/s)	1.56±0.04	10.74±0.56	39.51±0.75
Background activity (imp/min)	1.76±0.09	1.63±0.08	1.90±0.16
Mechanical threshold (mN)	0.73±0.03	0.49±0.02	0.29±0.01

Data are present as mean±SEM.

2.3. Local versus systemic effects

A control group was designated as rats receiving an injection of c-SOM outside of the recorded fiber's receptive field whereby c-SOM could be taken up by the circulation to act systemically. In this group, no significant differences were found in the discharge rates of C fibers ($n=6$) and Aδ fibers ($n=8$) during 5 min of injection (1.67±0.23 imp/min for C fibers; 1.75±0.21 imp/min for Aδ fibers) or 5 min after injection (1.78±0.18 imp/min for C fibers; 1.83±0.13 imp/min for Aδ fibers) compared with the pre-injection level ($P>0.05$, paired t-test or Wilcoxon Signed Rank Test). In addition, injection of c-SOM outside of the receptive field did not produce significant changes in the mechanical thresholds of these fibers ($P>0.05$, paired t-test or Wilcoxon Signed Rank Test). These data indicated that the effects of c-SOM application were mediated by a local mechanism.

2.4. OCT reversed the effects of c-SOM in C and Aδ fibers

To determine the effects of SSTRs on afferent fibers, OCT (20 μM, 10 μL) was pre-administrated into the receptive field