Neuropeptides 49 (2015) 47–54

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep

# Role of Somatostatin and Somatostatin Receptor type 2 in Postincisional Nociception in Rats

Pranav Prasoon [a], Rahul Kumar [a], Mayank Gautam [a], Ebin K. Sebastian [a], K.H. Reeta [b], Subrata Basu Ray [a,*]

[a] Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India
[b] Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, India

ARTICLE INFO

*Article history:*
Received 13 September 2014
Accepted 23 December 2014
Available online 30 December 2014

*Keywords:*
Analgesia
Expression
Neuropeptide
Pain
Rodent
Spinal cord

ABSTRACT

Somatostatin (SST) and the somatostatin receptor type 2 (sstr2) are expressed in the superficial part (Laminae I–III) of the dorsal horn of the spinal cord. Since the neurons in these laminae also receive nociceptive sensation from the periphery, it was hypothesized that both SST and sstr2 could be involved in the modulation of nociceptive transmission. To the best of knowledge, there are no studies on the involvement of SST and sstr2 in hind paw incision model in rats, which mimics postoperative pain in humans. Sprague-Dawley rats were subjected to hind paw incision under isoflurane anaesthesia and the resulting mechanical allodynia and thermal hyperalgesia were evaluated for 5 days. In another set of animals, the spinal cord was isolated at specified time intervals after incision and examined for SST and sstr2 expression using immunohistochemistry and immunoblotting procedures. Finally, nociceptive parameters were again evaluated in incised rats, which had received SST (400 μg/kg i.p. three times per day). Blood glucose level and locomotor activity were determined after SST treatment. Both allodynia and hyperalgesia were highest immediately after incision. Spinal SST expression increased at 2 h. A further increase was noted on day 3. Expression of sstr2 increased initially but decreased at day 1. These changes could be due to exocytosis of SST and internalization of the ligand–receptor complex. SST injection significantly attenuated mechanical allodynia but not thermal hyperalgesia. Significant change in blood glucose level or locomotor activity was absent. SST appears to contribute to postincisional pain. This finding could be of clinical relevance.



## 1. Introduction

The majority of the synapses in the central nervous system contain peptides, which regulate important physiological processes (Malavolta and Cabral, 2011). Somatostatin (SST) is a tetradecapeptide, which is widely expressed in the mammalian central and peripheral nervous systems (Carlton et al., 2004; Schindler et al., 1996; Schulz et al., 1998a; Shi et al., 2014). The corresponding high affinity somatostatin receptors are G protein-coupled receptors, which are divided into 5 subtypes (sstr1–5) (Lahlou et al., 2004). Activation of the receptors produces effects like inhibition of voltage-gated calcium channels and activation of inward rectifying potassium channels (Lahlou et al., 2004; Meriney et al., 1994; Murase et al., 1982). These result in neuronal hyperpolarization and decreased neurotransmitter release.

The sstr2 undergoes alternative splicing yielding two isoforms sstr2A (unspliced) and sstr2B (spliced) (Vanetti et al., 1993). The former is selectively expressed in the superficial layers of the dorsal horn in contrast to the latter, which is found diffusely in the gray matter (Schulz et al., 1998a). STT is mainly expressed in laminae II–III of the lumbar region of the spinal cord (Seybold and Elde, 1980). A different study has shown that SST is densely expressed within nerve fibres in the superficial layers of the dorsal horn including lamina I (Schulz et al., 1998b). The authors also observed that sstr2A expression is present in small round neuronal cell bodies and dendrites in close apposition to SST in the superficial laminae. Up-regulation of sstr2A occurs in neurons of lamina II following peripheral inflammation (Zhao et al., 2008). However, its expression during postincisional pain remains largely unknown.

The treatment of postoperative pain continues to be suboptimal (Wu and Raja, 2011). In a recent study based upon 121 patients in a Danish tertiary university hospital, almost 75% of the patients were treated with an opioid drug (commonly oxycodone or morphine) resulting in side effects (Mathiesen et al., 2012). Also, non-opioid analgesics like paracetamol and Ibuprofen were given insufficiently. The Anesthesia Patient Safety Foundation (APSF) recommends less use of opioids and more of non-opioidergic drugs in the treatment of postoperative pain. In this regard, SST or its long acting analogue octreotide could be a candidate drug, suitable for further investigations. It does not cross the blood–brain barrier and

No conflicts of interest exist in any form with the manuscript.

* Corresponding author. Department of Anatomy, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India.
*E-mail address:* raysb48@gmail.com (S.B. Ray).

http://dx.doi.org/10.1016/j.npep.2014.12.002
0143-4179/

so side effects related to the central nervous system can be avoided (Carlton et al., 2001a; Fricker et al., 2002). To the best of knowledge, there are no studies on the involvement of SST during postincisional pain in rats, which is a preclinical model of postoperative pain (Brennan et al., 1996).

In the present work, postincisional pain was determined using von Frey filaments (mechanical allodynia) and thermal escape behaviour (thermal hyperalgesia) over 5 days. Corresponding changes in the expression of SST and sstr2 in the spinal cord were observed. Antinociceptive effect of exogenously administered SST on pain behaviour was also noted. Finally, possible side effects of SST injection involving changes in blood glucose level or motor activity were evaluated.

## 2. Materials and methods

### 2.1. Animals

Approval for conducting the experiments was taken from the Institutional Animal Ethics Committee of the All India Institute of Medical Sciences, New Delhi. Experiments were performed on male Sprague-Dawley rats weighing about 250–270 g. Initially, 3–4 rats were kept in a cage under 12:12 h light and dark cycle with free access to food and water. The temperature was maintained between 22–25 °C. Initially, rats were kept in the laboratory for 3 days for acclimatization to the surroundings and gently handled to reduce stress. Baseline values of mechanical allodynia and thermal hyperalgesia were determined one day before plantar incision. Following incision, rats were kept singly in cages containing clean soft bedding made of cellulose (Alpha-dri, Shephard Speciality Papers, USA).

### 2.2. Procedure of hind paw incision

The rat model of hind paw incision was standardized by Brennan and his colleagues (Brennan, 2011; Brennan et al., 1996). Rats (n = 6) were anaesthetized with 4% isoflurane inhalation delivered through a nose cone. The plantar surface of the right hind paw was disinfected with 10% Povidone iodine solution followed by 70% isopropyl alcohol. A 1 cm long midline incision was made through the skin and later through the fascia on the plantar surface in a longitudinal manner, starting at 0.5 cm from the heel. The underlying flexor digitorum brevis muscle was elevated with curved forceps and incised longitudinally with the tip of a scalpel blade, without disturbing the origin and insertion. The limbs of forceps were introduced through the cut and gently separated. Finally, the skin was opposed by two mattress sutures using 4-0 polyamide (Ethicon®) with the knots placed on the lateral side. Antibiotic ointment was applied on the incision site after surgery. Rats were then transferred to the recovery chamber before being returned to the cages. A separate group of rats (n = 6) was treated with somatostatin injection, described later (subsection 2.6).

### 2.3. Behavioural testing for postincisional pain

Mechanical allodynia was determined by the "Up-down method" as described previously and represented as the 50% withdrawal threshold (g) (Chaplan et al., 1994). Animals were kept under transparent plastic enclosures (16 × 16 × 16 cm) on a floor made of wire mesh, which allowed access to the plantar surface of the foot from below. The rats were acclimatized for 15 minutes before testing. Paw withdrawal thresholds were measured using calibrated von Frey nylon filaments of specific sizes 3.61, 3.84, 4.08, 4.31, 4.56, 4.74, 4.93 and 5.18 (North Coast Medical Inc., San Jose, USA) before (basal) and after incision (2 h, 8 h and days 1, 3 and 5). The filaments were pressed on the medial side of the incision, close to the proximal end of the heel till it buckled. The pressure was maintained for 7–8 s. A positive response was defined as an abrupt withdrawal of the paw. In absence of a positive response to a particular filament, the next filament of higher size was used; in case of a response, the next lower filament was used. This was continued four times after the first filament that showed a withdrawal. No response to successive applications up to the heaviest filament (size 5.18) was taken as 15 g. Responses to successive applications till the lightest filament was recorded as 0.4 g. An interval of 2 min was maintained between successive applications. 50% withdrawal threshold was calculated using a specific algorithm (Chaplan et al., 1994).

Thermal hyperalgesia was determined by paw withdrawal test to radiant heat (Hargreaves et al., 1988) (Plantar Test apparatus, Ugo Basile, Italy). Rats were allowed to acclimatize in clear plastic enclosures (20 × 20 × 14 cm) placed on a special glass platform. The thermal source was positioned directly beneath the middle of the incision. Activation of the heat source triggered a timer that stopped when withdrawal of the paw was sensed by the motion detector. The length of time between the onset of the light beam and the foot-lift was defined as paw withdrawal latency. This was evaluated before and after incision (2 h, 8 h and days 1, 3 and 5). Baseline values were between 10–12 s. The cut-off value was 20 s. Each rat was tested 3 times at intervals of 2 min. The average of three trials was considered as the final value.

### 2.4. Immunohistochemistry for somatostatin (SST) and somatostatin receptor (sst2)

Rats (n = 36; 6 animals/group) at each time point (Control, 2 h, 8 h, days 1, 3 and 5) were deeply anaesthetized by pentobarbital injection (100 mg/kg i.p.). They were perfused transcardially with cold 0.1 M phosphate buffered saline (PBS) followed by cold 4% paraformaldehyde (PFA) in PBS. Lumbar spinal cords containing L4 and L5 segments were dissected out and the contralateral side demarcated by a groove using a very fine glass-capillary tube. Then, it was post-fixed in same paraformaldehyde solution for 2 days. The tissue was transferred firstly into 15% sucrose solution overnight followed by 30% sucrose solution for 24 hours at 4 °C for cryopreservation. Transverse sections (20 µm thick) of the spinal cord were then cut in a cryostat and floated in multi-vial trays containing

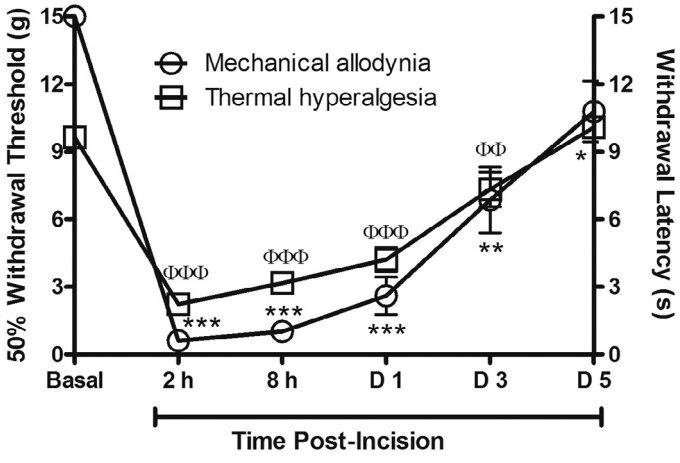


**Fig. 1.** Post-incisional nociception was determined before incision and at different time intervals after incision. Mechanical allodynia is represented as 50% withdrawal threshold (g) in the left Y-axis. Thermal hyperalgesia is represented on the right Y-axis as withdrawal latency (s). Basal values represent those before incision. Both mechanical allodynia and thermal hyperalgesia were maximum immediately after surgery (2 h). Later on, these decreased progressively so that thermal hyperalgesia had disappeared by day 5 although allodynia was still evident at this time interval. Each group has 6 animals. Values represented as mean ± sem. ***/ФФФ $p < 0.001$; **/ФΦ $p < 0.01$, * $p < 0.05$.