Inhibition of spinal nociceptive neurons by microinjections of somatostatin into the nucleus raphe magnus and the midbrain periaqueductal gray of the anesthetized cat

C. Helmchen, Q.-G. Fu, J. Sandkühler

Abstract

The effects of somatostatin (SOM) after intravenous application and intracerebral microinjection into the medullary nucleus raphe magnus (NRM) or into the periaqueductal gray (PAG) on the spinal nociceptive transmission was quantitatively studied in the anesthetized cat. Noxious heat-evoked responses of multireceptive lumbar spinal dorsal horn neurons were reversibly depressed to 56.6 ± 9.7% of the control after systemically applied SOM (7 μg/kg i.v.; 7 μg/kg per h infusion rate). At 11 of 14 brainstem microinjection sites in the NRM and PAG, SOM (2.5 μg/μl) attenuated the heat-evoked responses to 58.9 ± 6.2% (n = 5) (NRM) and 64.4 ± 6.3% (n = 6) (PAG) of the control. After microinjection, maximal inhibition was reached within 8–14 min (NRM) or 23–29 min (PAG), respectively. Inhibition was reversible within 60 min after the injection. Thus, SOM has an antinociceptive potency by activating descending inhibition of nociceptive dorsal horn neurons from the NRM and PAG.

Keywords: Somatostatin; Antinociception; Descending inhibition; Nucleus raphe magnus; Periaqueductal gray

The peptide, somatostatin (SOM), is thought to be a neuromodulator which affects spinal nociception [22]. SOM-containing neurons and binding sites have been found immunohistochemically in various brainstem structures including the nucleus raphe magnus (NRM) [5] and the midbrain periaqueductal gray (PAG) [3]. It is generally accepted that the NRM and PAG play a major role in the descending inhibition of spinal nociceptive dorsal horn neurons. Descending inhibition can be produced by focal electrical stimulation (reviewed in Ref. [12]) or by microinjections of morphine [13,14], bicuculline [23] or glutamate [21,23] into the NRM or PAG, respectively.

There is evidence that systemically, intrathecally or intraventricularly applied SOM can lead to a powerful antinociception in rats [16], mice [4,9] or cats [11] and analgesia in patients [6,18,24]. However, nothing is known about brainstem sites involved in antinociception by SOM (see review in Ref. [10]). Here we show that intravenous SOM (7–30 μg/kg) and SOM microinjected into the NRM and PAG attenuates the spinal neuronal response to noxious stimuli.

Experiments were performed on 19 female cats (2.4–3.6 kg). Surgical level of anesthesia was initiated with intraperitoneal sodium pentobarbital (40 mg/kg) and maintained by artificial ventilation with a gaseous mixture of 70% N₂O, 30% O₂ and halothane (0.2–0.7 vol.%). Blood pressure, central venous pressure, urinary output and rectal temperature were continuously monitored and kept within physiological limits as described previously [22]. The superficial peroneal and posterior tibial nerves were dissected free for bipolar electrical stimulation. The lumbar spinal cord was exposed by laminectomy from L₁ to S₁ and was covered by warm paraffin oil. Pial vessels on the surface of the spinal cord were monitored for...
changes in vascular tone by microscope. Extracellular recordings from dorsal horn neurons were performed using glass micropipettes filled with 3 M NaCl (5–15 MΩ). Neurons responded to hind limb nerve stimulation (square wave pulses of 2.0 V of 0.1 ms duration) being sufficient to activate Aβ- and Aδ-fibers and showed a late (>100 ms) discharge to electrical stimulation (square wave pulses of 25 V of 0.1 ms duration) sufficient to recruit C-fibers. All neurons were multireceptive in that they also responded to innocuous mechanical skin stimuli (brushing, tapping on skin). Noxious stimuli (radiant heat, 50°C or 52°C for 10 s, given at intervals of at least 3 min) were applied to the glabrous foot pad.

The head was fixed in a stereotaxic frame and craniotomies were performed to allow insertion of the guide cannulas (0.9 mm outer diameter) with their tips aiming 2 mm dorsal to the microinjection or electrical stimulation site. The stereotaxic atlas of Snider and Niemer was used [25]. The guide cannulas were placed vertically into the PAG and at an angle of 45° to the horizontal plane into the NRM ipsilateral to the recording site in the spinal cord. Electrical stimulation was applied by bipolar concentric electrodes (100 Hz of 1 ms monophasic pulses, 100 ms train duration, 3 times/s, 180–540 μA). SOM (2.5 μg dissolved in 1.0 μl physiological saline equivalent to 1.53 mM) was microinjected through cannulas (outer diameter 0.4 mm, extending the guide cannula by 2 mm) by pressure. Intravenous SOM was applied as a bolus injection (7, 15, 30, 50 μg/kg, dissolved in 1 ml of physiological saline) with subsequent continuous infusion at a rate of 7–50 μg/kg per h. In some experiments multibarrel glass micropipettes were used for microinjection and electrical stimulation at identical brainstem sites (tip diameter <40 μm). Efficacy of microinjections was analysed as change of spinal neuronal responses to noxious heat stimuli in percent of control; reduction to 75% or less of the control was considered to be effective. Three subsequent values, each taken after 3-min intervals, with the largest deviation from the control were averaged.

Animals were killed with pentobarbital. Stimulation and microinjection sites were electrolytically marked and histologically identified. Statistical comparisons were made using Student’s t-test for grouped or paired data, P < 0.05 was considered significant (two-tailed). The Pearson product-moment formula was used for correlation analysis. Mean values are given with standard errors.

Results were obtained from 33 neurons, 30 of which (91%) were located in dorsal horn laminae IV–VI of Rexed. All neurons were typical multireceptive or wide dynamic range units and had excitatory receptive fields at one or more toes of the ipsilateral hindpaw.

The effect of a single intravenous SOM injection (7, 15, 30, 50 μg/kg) was studied in 19 dorsal horn neurons in 19 cats (Table 1). During the time interval from 12 to 23 min after the injection of SOM (7 μg/kg), mean noxious heat-evoked responses of five dorsal horn neurons were depressed to 56.6 ± 9.7% of the control (Fig. 1A,B; P < 0.05). The inhibition is dose-dependent. Seven doses of 30 μg/kg SOM significantly attenuated heat-evoked responses (Fig. 1C) while 50 μg/kg SOM were ineffective, producing a U-shaped dose response function. Latency to inhibition to 75% of the control did not significantly differ between the effective doses (7, 15, 30 μg/kg; 7.4 ± 2.7, 18.2 ± 5.8, 7.5 ± 1.9 min). Duration of the inhibition was on average 21.4 ± 5.1 min (n = 16). The effect was reversible within 40 min.

Results for microinjections of SOM were obtained from 23 multireceptive spinal neurons recorded in 11 cats. Microinjections of vehicle into the NRM (n = 5) or PAG (n = 3) were ineffective. Electrical stimulation (300–550 μA) in the NRM attenuated the spinal neuronal responses to 32.5 ± 10.9% of the control, in the PAG to 44.9 ± 5.4%, respectively.

The effect of SOM microinjected at six different NRM sites was assessed in seven neurons (Table 1). SOM (2.5 μg) microinjections at different NRM sites attenuated discharges of five of the seven dorsal horn neurons investigated 8–14 min after the microinjection from 782 ± 72 imp/25 s to 461 ± 81 imp/25 s (i.e. 58.9 ± 6.2% of control, P < 0.02). In one case, two subsequent microinjections were performed with a time interval of at least 90 min in between to investigate the effect of SOM (2.5 μg) on two different nociceptive spinal neurons. Heat-evoked responses of both neurons were inhibited to

Table 1

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<th>Microinjections of somatostatin at different brainstem sites inhibits nociceptive spinal dorsal horn neuronal activity</th>
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Fig. 1. Systemically administered somatostatin depresses spinal nociception. (A) Mean neuronal responses of five dorsal horn neurons to noxious radiant skin heating evoked in 3-min intervals are shown as percent of control values (ordinate) before and after i.v. injection of SOM (17 µg/kg; 7 µg/kg/h infusion rate) for the time interval of 60 min after the injection (abscissa). Dotted line indicates time of i.v. injection. Vertical lines indicate SEM. (B) Effect of SOM (7 µg/kg i.v.) on noxious heat-evoked spinal responses of one dorsal horn neuron displayed as peristimulus time histograms (band width 1000 ms) before, 21 min after and 37 min after the injection of SOM. Intervals of skin heating are shown as horizontal bars. (C) Mean heat-evoked responses of dorsal horn neurons are expressed as total number of impulses and plotted on the ordinate in percent of control versus the dose of intravenous somatostatin (abscissa). Numbers in parentheses indicate the number of neurons investigated. Vertical bars represent SEM. *P < 0.05.

60.2% or 63.4%, respectively. The injection sites and the time course of the effect on spinal nociception of all five effective SOM microinjections (2.5 µg) are shown in Fig. 2. Latency to maximal inhibition was 8.8 ± 2.8 min. The inhibition was reversible within 30 min. We did not notice any change in heart rate or respiration. Electrical stimulation (400 ± 100 µA) at the SOM-sensitive NRM sites attenuated heat-evoked spinal neuronal discharges to 31.3 ± 16% of the control but this inhibition was not significantly correlated to the efficacy of the microinjection ($r_s = 0.662, P > 0.05$).

The ability of microinjected SOM (2.5 µg) given at eight different midbrain PAG sites to inhibit the heat-evoked discharges of eight spinal neurons was examined in eight cats. At different PAG sites (Fig. 3A) SOM attenuated spinal heat-evoked discharges of six neurons 23–29 min after the microinjection from 975 ± 106 imp/25 s to 628 ± 98 imp/25 s (64.4 ± 6.3% of control, $P < 0.001$, Fig. 3B). Latency of the time to maximal neuronal inhibition of dorsal horn neurons produced by SOM was 25.1 ± 7.2 min. Inhibition lasted on average 15 min and it was reversible in all cases within 60 min after the application. SOM administered in two sites lateral to the PAG failed to attenuate heat-evoked spinal neuronal responses (Fig. 3A). Within the PAG, latency did not correlate to the radial distance from the aqueduct ($r_s = 0.366, P > 0.05$). Moreover, there was also no significant correlation between the maximal inhibition of spinal neuronal heat-evoked responses and the radial distance from the cerebral aqueduct as can be appreciated in the scatter diagram in Fig. 3B ($r_s = 0.428, P > 0.05$). Electrical stimulation (514 ± 30 µA) was performed at four of the effective injection sites and attenuated spinal neuronal responses to 55.7 ± 11.7% of the control.

There was no effect on arterial blood pressure, neither after i.v. nor intracranial (i.c.) application of SOM. We did not notice any vasoconstriction microscopically after i.v. SOM. Postmortem histological examination of our brainstem slices after up to 24 h of survival did not reveal pathological features, in particular no signs of edema or ischemia.
jected at defined brainstem sites (NRM, PAG) can inhibit

Whereas 50 μg i.t. SOM is neurotoxic in mice, 8000 pg
toxic effect in rats were tolerated in mice and guinea pigs.

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concentration of 200-610 μM) causing vasoconstriction
of the capillaries and penetrate into the central nervous sys-
tem.

the effect in the PAG cannot probably be explained by
diffusion to the aqueduct since there was no correlation
between the latency and the radial distance from the
aquaduct.

SOM described before [7]. When applied by controlled
superfusion of the spinal cord at the recording site of no-
iceptive neurons in vivo, SOM at 1.53 mM, i.e. at the
same concentration used here, caused a selective depression
of nociceptive responses to noxious radiant heat but not
to innocuous mechanical stimuli [22].

Systemically given in clinical trials, SOM has been
shown to be analgesic in patients [18,24]. The site of ac-
tion remains unknown. Recently, we provided evidence
for a spinal site of action of SOM [22]. SOM-containing
neurons, SOM-binding sites and somatostatinergic affer-
ent and efferent neurons have been found in brainstem
areas known to activate descending inhibition by electro-
cal stimulation [3,5]. However, the poor blood–brain bar-
rier penetration and the very short half-life time in blood
(<2 min) raises questions about the pharmacological
mechanisms. Recently, systemic (s.c.) injections of vapre-
otide, a SOM-analog capable of penetrating the blood–
brain barrier, revealed a naloxone-reversible antinocicep-
tive effect at doses of 8–64 μg/kg, suggesting a central
opioidergic mechanism [4]. Direct evidence for a local
site of antinociceptive action of intracerebral SOM is pro-
vided by our data. There was a difference in latency be-
tween the antinociceptive effect of SOM when microin-
jected into the PAG and the NRM. The longer latency of
the effect in the PAG cannot probably be explained by
diffusion to the aqueduct since there was no correlation
between the latency and the radial distance from the
aquaduct.

Substances with excitatory effects are known to acti-
vate descending inhibition in the PAG and NRM, e.g.
glutamate [21,23], the peptide neurotensin [2] or norepi-
nephrine [10]. It is of particular interest that inhibitory
substances like SOM and morphine [13,14,19] can acti-
vate descending inhibition. PAG afferent neurons in-
volved in descending inhibition are thought to be subject
to a strong tonic GABAergic inhibition [23]. Morphine is
thought to produce descending inhibition by disinhibiting
rashespinal neurons underlying a tonic GABAergic con-
trol [8]. Bicuculline, a GABA antagonist, microinjected
into the PAG can be effective at PAG sites at which glu-
tamate, which indiscriminately excites all cell bodies, was
ineffective suggesting that the efficacy of the efferent
descending inhibition is determined by the net recruit-
ment of inhibitory and excitatory interneurons and the
converging influence on neurons that are selectively ac-
tive and inactive during descending inhibition (‘on-cells

The results show that intravenous SOM depresses re-
sponses of multireceptive spinal neurons to noxious skin
heating. This is the first study to show that SOM microin-
jected at defined brainstem sites (NRM, PAG) can inhibit
spinal dorsal horn neurons.

Due to the size (mol. wt. 1638) and its hydrophilic
properties, the peptide SOM is thought not to pass the
blood–brain barrier readily. Consequently, systemic ef-
facts of SOM have been attributed to metabolites with
a better capability of penetrating the blood–brain barrier,
as has been shown for vapreotide, an analog of SOM
[1]. Alternatively, SOM may enhance the permeability of
the capillaries and penetrate into the central nervous sys-
tem.

SOM has been shown to exhibit neurotoxic effects in
rats when applied intrathecally to the spinal cord (SOM
concentration of 200–610 μM) causing vasoconstriction
and severe neurological deficits and histological damage
with inflammatory cell reactions and necrosis [15,16].
However, considerable species specific toxic effects have
been found, i.e. Mollenholt et al. [16] showed that SOM
at doses more than 10 times higher than those producing
toxic effect in rats were tolerated in mice and guinea pigs.
Whereas 50 μg i.t. SOM is neurotoxic in mice, 8000 μg
cause neurotoxicity in cats [11]. Intrathecal doses below
15 μg reliably prolonged tail-flick latencies without evi-
dence of neurotoxicity [16]. In humans, intrathecal SOM
infusions of 50 μg/h (30.5 nmol/h) [6] and even 120 μg/h
[18] have been used without neurological deficits. We
used 1.53 nmol (2.5 μg), which is lower than any toxic
dose used in rats, mice or humans. The U-shaped dose
response function with the ineffective highest dose tested
confirms the unusual dose response characteristics of
SOM described before [7]. When applied by controlled
superfusion of the spinal cord at the recording site of no-
iceptive neurons in vivo, SOM at 1.53 mM, i.e. at the
same concentration used here, caused a selective depression
of nociceptive responses to noxious radiant heat but not
to innocuous mechanical stimuli [22].

Fig. 3. (A) Summary of all sites in the midbrain PAG at which SOM
(2.5 μg) was microinjected. Injection sites are superimposed on repre-
sentative coronal sections. Filled circles indicate effective sites, open
circles ineffective sites. (B) Inhibition of spinal neuronal responses of
six dorsal horn neurons to noxious skin heating does not depend on the
radial distance from the aqueduct. Mean responses are plotted versus
the radial distance from the aqueduct in mm (abscissa) after the micro-
injection of SOM. Each symbol represents the mean neuronal response
in the time interval of maximal depression (23–29 min after the micro-
injection into the PAG).

A 0.0-1.5
A 2.0-4.5

B

% of control

Response to skin heating

Radial distance from
aquaduct (mm)

2.0-2.5

0 0.4 0.8 1.2 1.6 2.0 2.5

and off-cells’ [19]). Since GABA and SOM have been shown to be co-localized in numerous neurons throughout the central nervous system, including the PAG, and since SOM is known to affect the release of GABA [20], we hypothesize that SOM inhibits GABAergic interneurons leading to descending inhibition of spinal nociceptive transmission.

In conclusion, several lines of evidence show that SOM, like morphine, has a spinal and supraspinal site of antinociceptive action and is also effective when administered systemically.

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