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Functional role of exogenous administration of substance P in chronic constriction injury model of neuropathic pain in gerbils

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Abstract

Substance P (SP) acts as a transmitter of nociception in both the peripheral and the central nervous system. Because the NK-1 receptors in gerbils are comparable to those in humans, gerbil models could be used to study the role of SP in neuropathic pain. A modification of the rat chronic constriction injury (CCI) model of neuropathic pain was produced in male gerbils by placing four loose chromic catgut ligatures around the sciatic nerve. This procedure clearly resulted in mechanical hypersensitivity. Intraplantar injections of SP and the selective NK-1 receptor agonist, [Sar^9-$Met(O_2)^{11}$]-substance P (Sar-SP), to the paw ipsilateral to the nerve injury and intrathecal administration of these peptides produced paw-lifting behavior in the CCI gerbils in thermoneutral conditions. In sham-operated and nonoperated controls, no such effects were observed. Systemic administration of the NK-1 antagonist R116301 attenuated the SP and the Sar-SP-induced paw-lifting behavior in the CCI gerbils indicating the role of NK-1 receptors in these effects. Intraplantar injection of the highest dose of SP (200 ng) to the paw contralateral to the CCI produced lifting of the paw ipsilateral to the injury, indicative for spinal mechanisms especially since administration of SP to the ipsilateral front paw or even intracardially did not have any effect at all. The SP-induced responses were not antagonized by the NMDA antagonist MK801. These results indicate that the peripheral and spinal SP reveal an increased reactivity in a neuropathic pain model. This increased pain sensitivity seems to involve spinal NK-1 mechanisms.

Keywords: Neurokinins; NK-1 receptors; Allodynia; Neuropathic pain; Chronic constriction injury

1. Introduction

Substance P (SP) is a well-established transmitter of nociception in both the peripheral and the central nervous system and its role in nociceptive and inflammatory pain has been studied extensively (Quartara and Maggi, 1998; Snijdelaar et al., 2000). In primary afferent nerves, SP is found primarily in the small, unmyelinated neurons. In the chronic constriction injury (CCI) model of neuropathic pain (Bennett and Xie, 1988), four loose chromic catgut ligatures are placed around the rat sciatic nerve. Constriction and perineural inflammation lead to behavioral symptoms of neuropathic pain. In rats, the CCI model has been described to lead to a loss of preferential large myelinated and unmyelinated fibers. (Carlton et al., 1991; Coggeshall et al., 1993; Bai et al., 1999). It could be expected that the role of SP would become more prominent in neuropathic pain states where myelinated fibers are primarily affected, resulting in an increase in the relative proportion of the remaining small and unmyelinated fibers. However, the course of the hyperalgesia in the rat CCI model is not clearly related to the proportion of large myelinated fibers in the affected nerve (Coggeshall et al., 1993). SP-like immunoreactivity and expression of preprotachykinin (PPT) mRNA in the dorsal horn of the spinal cord decrease after nerve injury (Garrison et al., 1993; Ghoul et al., 1993; Nahin et al., 1994; Xu et al., 1996; Malmberg and Basbaum, 1998). This can be explained at least in part by the nerve injury-induced loss of small fibers containing SP and does not necessarily reflect the situation in the remaining functional neurons. Complex changes in PPT mRNA and SP levels in dorsal root ganglion (DRG) neurons have been described after nerve injury (Herzberg et al., 1994; Marchand et al., 1994; Noguchi et al., 1994; Herzberg et al., 1996; Ma and Bisby,

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1998; McLachlan and Hu, 1998). Furthermore, changes in the effect of SP on the excitability of DRG neurons after axotomy have also been described. Indeed, changes in the SP levels in neuropathic conditions may be associated with or even initiated by alterations in the release of the peptide, in the NK-1 receptor expression (Malmberg and Basbaum, 1998), and in the functional coupling of the NK-receptors. Furthermore, recently, the NK-1 antagonist L-732 138 was demonstrated to overcome the CCI-induced tactile and cold nociceptive changes in rat (Cahill and Coderre, 2002).

These data demonstrate that SP is involved both in the induction and the maintenance of neuropathic pain and that specifically NK-1 antagonists seem to play some role in neuropathic pain. However, more data are needed to fully understand these mechanisms.

Marked species differences have been described in the pharmacology of the NK-1 receptors, necessitating the development of pain models in species other than rats and mice (Maggi, 1995) to fully evaluate the role of tachykinins in neuropathic pain. Based on binding studies and pharmacological experiments, the gerbil NK-1 receptor is similar to the human form (Beresford et al., 1991). Thus, gerbil models could be used to study the effects of SP and neurokinin receptor antagonists in neuropathic pain. In the present study, the CCI model of neuropathic pain (Bennett and Xie, 1988) has been adapted to gerbils. The aim of the study was to validate the CCI model in gerbils and to assess the effects of peripheral administration of SP in this model. The effects of spinal administration of SP were studied to find the mechanism of the allodynia-like behavior evoked by peripherally administered SP. Furthermore, the role of NK-1 receptors was evaluated testing the NK-1 agonist, [Sar^9-$Met(O_2)^{11}$]-substance P (Sar-SP), and the antagonist, R116301 (Megens et al., 2002).

2. Materials and methods

2.1. Animals

The modification of the CCI model of neuropathic pain (Bennett and Xie, 1988), described previously in rats, was produced in adult male gerbils [*Meriones unguiculatus*, Crl(MON)BR, Charles River Deutschland, Sulzfeld, Germany] weighing 60–75 g at the beginning of the experiment. The gerbils were housed individually in plastic cages under artificial lighting with a fixed 12:12-h light–dark cycle. Food and water were available ad libitum. Guidelines for animal research by the International Association for the Study of Pain (Zimmermann, 1983) were adhered to, and the study was accepted by the Institutional Ethical Committee.

2.2. The nerve injury model

The animals were anaesthetized with pentobarbital (60 mg/kg body weight), the sciatic nerve was exposed, and four loose chromic catgut ligatures (6/0 Chromic catgut, Ethicon, Somerville, NJ, USA) placed around the sciatic nerve. A sham operation was performed by exposing, but not ligating, the sciatic nerve. After checking for hemostasis, the muscle, the adjacent fascia, and the skin were closed with sutures.

2.3. Intrathecal catheterization

In a group of gerbils, after the CCI ligation, a PE-10 catheter was placed into the intrathecal space using the lumbar approach, as described earlier in rats (Boersma et al., 1992). To reduce the diameter of the catheter, 1 cm of the tip was stretched to double the length using hot water.

The dorsal aspect of L3-L6 vertebrae were exposed by skin incision and a small cut through the paravertebral muscles. The spinal process of the L5 vertebra was carefully removed, and the ligamentum flavum and the dura mater were penetrated at the base of L4 vertebra with a metal probe. The catheter was inserted 5 mm to the subarachnoideal space, parallel to the cord, and fixed with a drop of Histoacryl (B. Braun Surgical, Melsungen, Germany) glue and a suture. The wound was closed in layers. The extra epidural part of the catheter was tunneled subcutaneous to the neck of the animals. Approximately 3 cm of the catheter was left externally and the tip of the catheter was closed by melting.

2.4. The behavioral symptoms of neuropathic pain

Mechanical allodynia was assessed by placing the animals in cages with a metal mesh platform and touching the plantar surface of the paw with a metal probe (tip diameter 1 mm) connected to a pressure transducer (Somedic Sales, Hörby, Sweden) and increasing the force applied to the paw until the animal withdrew the paw (Moller et al., 1998). The animals were allowed to habituate to the testing chambers for 30 min before the first measurement. The mean of three consecutive readings on both hind limbs in each animal was used for analysis.

For testing cold and heat allodynia, animals were placed on metal plates in a transparent, circular Plexiglas cage with diameter of 190 mm. The temperature of the cold (-4 °C) and the warm ($+40$ °C) plate was selected on the basis of a preliminary trial comparing normal animals with CCI-ligated animals. For assessment of the spontaneous paw-lifting behavior, a thermoneutral ($+32$ °C) (Klir et al., 1990) metal plate covered with a soft paper tissue sheet was used.

The number of times that the animal lifted the left or the right hind limb off the platform and the duration of the lifts were separately recorded during a 5-min observation period. Limb movements that were considered a part of the animals' normal movement (walking) were not included in the assessment.

Experiments in normal animals indicated that gerbils have a lower threshold on cold stimulation as compared to other rodents. The behavioral assessments were performed 1

day before the CCI operation and 1, 3, 6, 8, 10, 13, 15, 17, 20, 30, and 35 days after the operation. Mechanical allodynia was extended up to 70 days after the operation.

2.5. Behavior produced by administration of SP

In a separate series of experiments, the effect of intraplantar administration of SP, vehicle (PBS), and of the selective NK-1 receptor agonist, Sar-SP, on paw-lifting behavior on a neutral plate were assessed in different groups of animals 7–11 days after the CCI operation. Dose response curves for peptides were constructed after intraplantar injections. These injections were all into the ipsilateral-operated hind paw using a volume of 50 μl and a 25-Gauge needle. Immediately after the injection, the animals were placed in an observation cage and the number and duration of paw lifts were recorded as described above.

The effect of intrathecal administration of different doses of SP was studied in an additional experiment using the same experimental conditions. The intrathecal injection volume was 10 μl. Observers followed a standard procedure for recording side effects and complications.

In order to evaluate the site of action of systemic SP, different groups of animals were studied after injection of 200 ng in the forepaw, the contralateral hind paw, and intracardially. For all these experiments, gerbils were only used once in a period of 7–14 days after surgery.

Additionally, to study the duration of the paw-lifting behavior after repeated administration of SP during 5–20 days of interval after the nerve injury, groups of gerbils were consecutively challenged on different days with an intraplantar injection of 200 ng of SP into the ipsilateral paw. Each animal was used between three and five times, with a washout of at least 3 days between subsequent testing. The experiments were performed as described above.

In separate experiments, the NK-1 antagonist R116301 in different doses of 0.16–10.0 mg/kg, and the NMDA antagonist MK-801 in doses of 0.01–0.63 mg/kg, were administered intraperitoneally 60 min before the SP challenge in order to study the effect of blocking these receptors on the SP-induced paw-lifting behavior. SP was then administered intraplantarly (200 ng) in the ipsilateral-operated paw or intrathecally (40 ng), and the paw-lifting behavior on the thermoneutral plate was assessed as in the previous experiments.

2.6. Drugs

SP, Sar-SP, and MK-801 were obtained from Tocris Cookson (Bristol, UK). R116301 was produced at PRD J&J, Beerse (Challet et al., 2001; Megens et al., 2002).

2.7. Blinding

All the experiments are blinded according to our standard procedures. Animals are recognized by a subcutaneous implanted system, which is blinded for the technician performing the behavioral testing. Different drugs and dosages are administered in a randomized way, blinded for the observer. The blinding code is only broken when all data are collected.

2.8. Statistical analysis

Results are presented as mean ± S.E.M. The Mann–Whitney Test, with Bonferroni correction for multiple comparisons where appropriate, was used for statistical analysis. Two-tailed significance level was set at $P < .05$.

3. Results

The CCI model in gerbils produced mechanical allodynia lasting over 55 days after the injury, as indicated by a reduction in paw withdrawal threshold to mechanical stimulation in the paw ipsilateral to the injury, but not in the paw contralateral to the injury or in either one of the paws of the sham-operated control animals (Fig. 1).

The CCI gerbils did not show specific signs of cold or heat allodynia. Similar paw-lifting behavior was observed on a cold (−4 °C), a warm (+40 °C), or a thermoneutral (+32 °C) plate (Fig. 2). On all three plates, the animals lifted selectively the paw ipsilateral to the nerve injury. As such, no distinction with regard to temperature could be made, and therefore the paw-lifting behavior probably indicates mechanical allodynia or spontaneous pain that is not related to the temperature of the platform per se. The sham-operated control animals did not show any of these behaviors.

Intraplantar injection of SP in the paw ipsilateral to the nerve injury caused a dose-dependent increase in the paw-lifting time in the CCI gerbils but not in sham-operated or nonoperated controls (Fig. 3).

In a separate experiment, the effect of the site of the SP administration was studied; the mean ± S.E.M. lifting time after injection of 200 ng of SP to the paw ipsilateral to the injury was 87.7 ± 11.6 s, whereas for a vehicle injection it was 20.7 ± 3.4 s ($n = 15$, $P = .002$). Injection of the same dose of 200 ng of SP to the paw contralateral to the CCI also produced a statistically significant increased lifting of the injured limb (92.7 ± 23 s for SP vs. 29.6 ± 7.1 s for vehicle, $n = 15$, $P = .04$). Administration of SP in the front paw ipsilateral to the nerve injury (12.3 ± 3.4 s for SP vs. 9.8 ± 2.4 s for vehicle, $n = 10$, $P = .7$) or intracardially (19.9 ± 7.2 s for SP vs. 9.8 ± 2.4 s for vehicle, $n = 10$, $P = .2$) did not reveal an increased reactivity.

The SP injection-induced paw-lifting behavior on the neutral plate was significantly stronger than the spontaneous paw-lifting behavior. The sham-operated and nonoperated controls did not show any paw-lifting behavior after intraplantar SP (50–200 ng) challenge over time.

In order to assess the SP effect over time, animals were repeatedly challenged with 200 ng SP at 5–20 days after

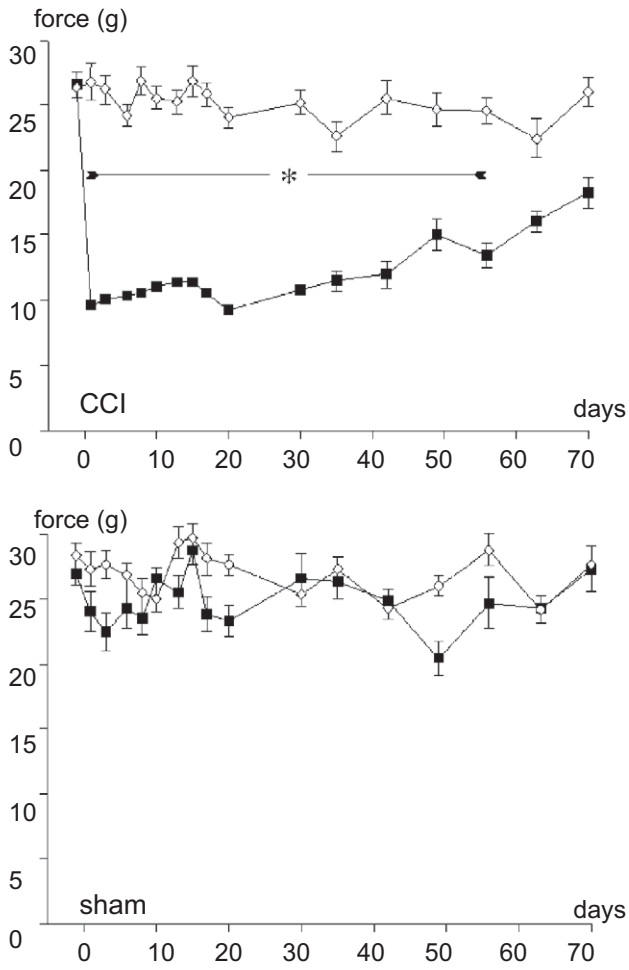

Fig. 1. Time curve of mechanical allodynia after CCI in gerbils. Mean ± S.E.M. force required to induce paw withdrawal response in CCI model of neuropathic pain (top panel, $n = 20$) and in sham-operated control animals (lower panel, $n = 15$) in the paw ipsilateral to the injury (filled squares) and in the contralateral paw (open diamonds) is plotted against time after the nerve injury. Asterisk (*) indicates statistically significant differences.

surgery. In all cases, a clear SP-induced lifting behavior of the injected paw was observed over the whole measured period. The amount of paw lifting was always larger than these of the non-CCI controls (Fig. 4).

Also, with Sar-SP, a dose-dependent increase in paw lifting of the operated paw was observed after the injections into the ipsilateral paw (Fig. 3).

Systemic administration of the NK-1 antagonist R116301 dose-dependently antagonized the effect of an intraplantar injection of SP (200 ng) (Fig. 5). Also, the paw-lifting behavior induced by intraplantar injection of Sar-SP (400 ng) was significantly attenuated by pretreatment with a dose of 10 mg/kg (Fig. 3). No side effects were reported.

Intrathecal administration of SP (2.5–40 ng, Fig. 6, top panel) produced a dose-dependent increase in lifting of the paw ipsilateral to the peripheral nerve injury but did not produce any pain-related behavior in sham-operated controls (data not shown). The paw-lifting behavior

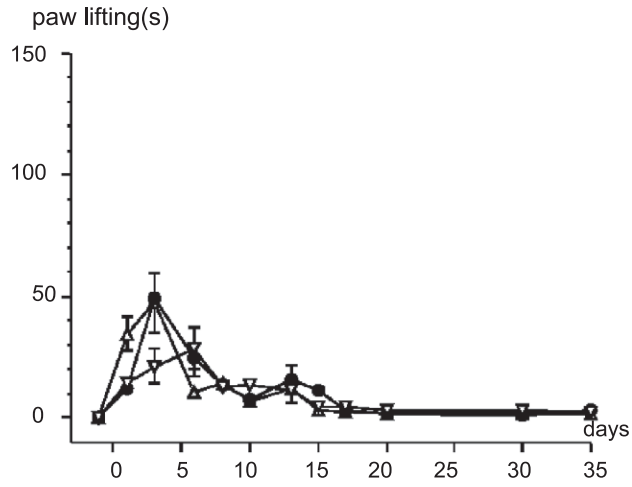

Fig. 2. Paw-lifting behavior after CCI in gerbils. Mean ± S.E.M., $n = 20$, duration of spontaneous paw lifting over a 5-min observation period on the cold (open triangles pointing down), neutral (filled circles), and warm (open triangles pointing up) plates are presented over time after the nerve injury.

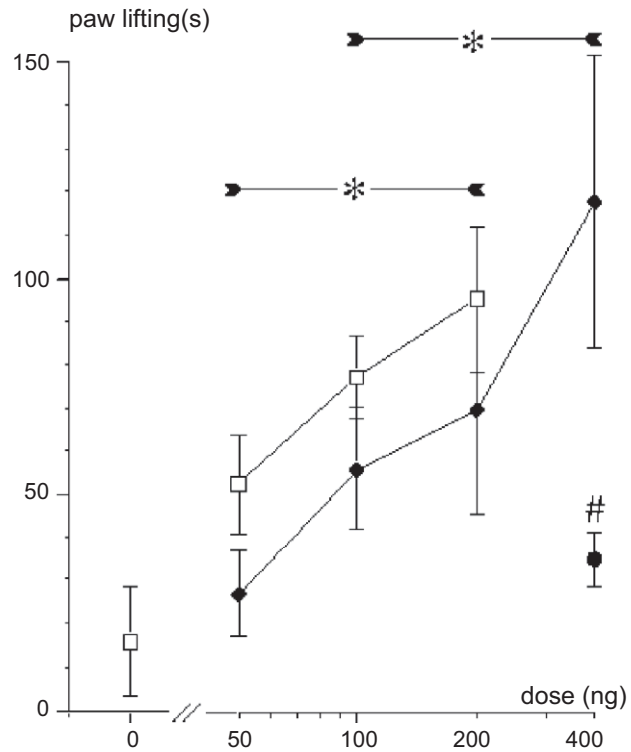

Fig. 3. Paw-lifting behavior induced by intraplantar injection of SP (open squares) and Sar-SP (filled diamonds) in CCI gerbils. Mean ± S.E.M., $n = 8–10$, duration of paw lifting during a 5-min observation period on a neutral plate immediately after the injection is presented against the dose. Asterisk (*) indicates statistically significant difference from the effect of vehicle. Additionally, the mean paw-lifting time resulting from intraplantar injections of Sar-SP (400 ng) after pretreatment with NK-1 antagonist R116301 (10 mg kg^{-1} ip, administered 60 min before the intraplantar injection) is shown (filled circles). Hash (#) indicates statistically significant difference to the effect of Sar-SP alone.

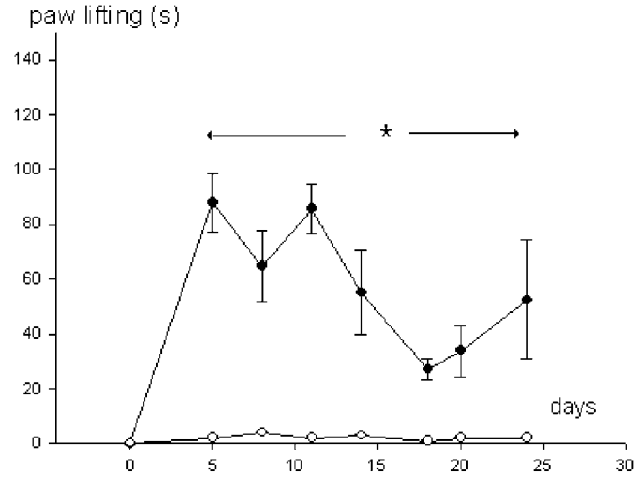

Fig. 4. The mean duration of paw lifting (± S.E.M., $n=8$) induced by intraplantar injection of SP (200 ng) (filled circles ●) and vehicle (open circles ○) during a 5-min observation period on neutral plate is plotted against time after the CCI nerve injury. In this experiment, the animals were used repeatedly for 3–5 times with a washout of at least 3 days between the injections. Asterisk (*) indicates statistically significant difference from the control.

induced intrathecally by 40 ng SP was antagonized by intraperitoneal administration of the NK-1 selective antagonist R116301 (Fig. 6, bottom panel).

In order to further evaluate the mechanism of the selectivity of the SP-induced paw lifting in gerbils, an antagonism study of the ipsilateral CCI paw was studied using an intraperitoneal treatment with the NMDA antagonist, MK801, at different doses, ranging from 0.01 to 0.63 m/kg. No antagonism of the SP-induced paw lifting was observed (data not shown). Higher doses of MK801 (up to 10 mg/kg) have intrinsic motor effects and do not allow further behavioral studies.

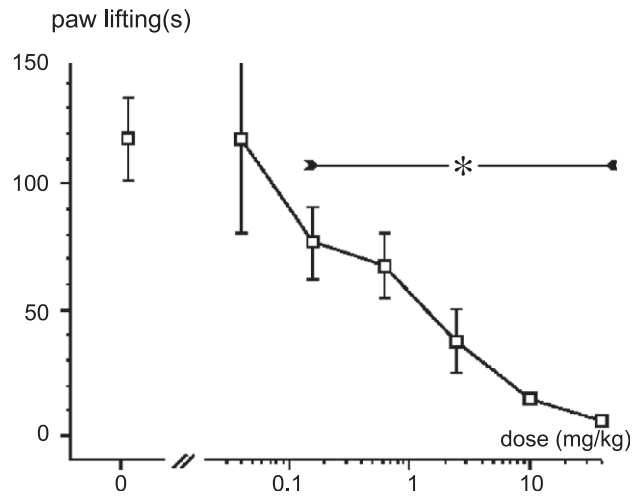

Fig. 5. Attenuation of intraplantar SP-induced paw-lifting behavior by the NK-1 antagonist R116301 (0.04–40 mg/kg ip, administered 60 min before the intraplantar injection). Mean ± S.E.M., $n=6$, duration of paw lifting during a 5-min observation period on a neutral plate is plotted against the antagonist dose. Asterisk (*) indicates statistically significant difference from the effect of vehicle.

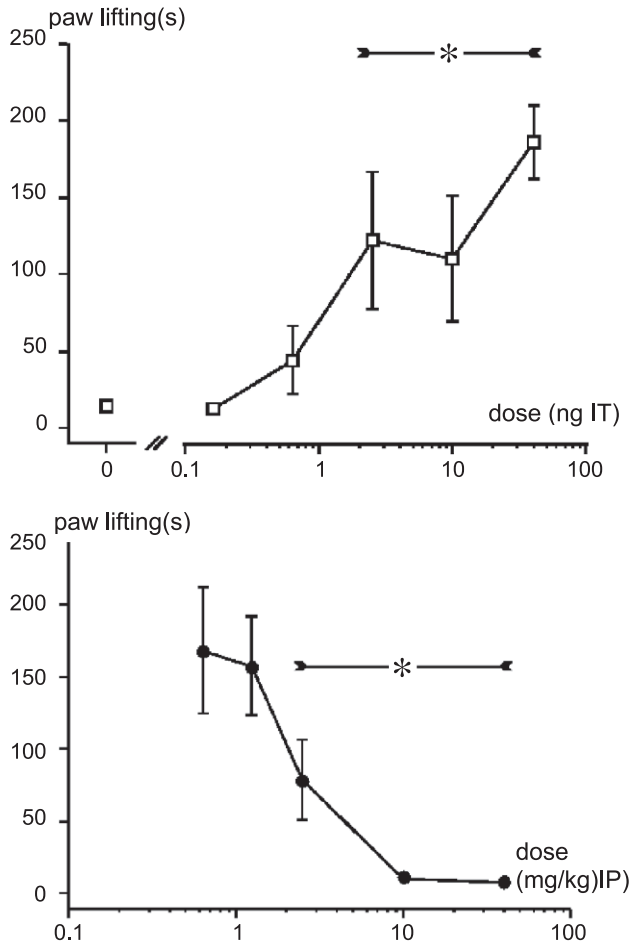

Fig. 6. The effect of intrathecal administration of SP on paw-lifting behavior on a neutral plate in CCI gerbils (top panel) and reversal of the effect of SP by NK-1 antagonist R116301 (bottom panel). Mean ± S.E.M., $n=7$ (open squares), duration of paw lifting during a 5-min observation period on a neutral plate immediately after the intrathecal injection of SP, and mean ± S.E.M., $n=7$ (filled circles), duration of paw lifting immediately after the intrathecal injection of SP (40 ng it) following pretreatment with different doses of R116301 administered intraperitoneally 60 min before the intrathecal injection is plotted against the dose. Asterisk (*) indicates statistically significant difference from control.

4. Discussion

The chronic constriction of the sciatic nerve in gerbils demonstrates clear signs of neuropathic pain behavior similar as seen in rats and described by Bennett and Xie (1988). A clear mechanical allodynia was present (Fig. 1). Whereas in the CCI model in rats, both cold and heat allodynia have been described (Bennett and Xie, 1988; Attal et al., 1990); no clear differentiations between temperature-induced allodynic responses were observed in gerbils. The initial increased paw lifting on the cold and warm plates disappeared over time and no differences among the three

temperature conditions were seen in our animals. The lack of thermal allodynia in gerbils could be related to the adaptations in thermoregulation that enable survival of this species in the extreme temperatures of a desert (Oufara et al., 1987; Klir et al., 1990). In order to evaluate the role of SP in the nociceptive changes following CCI, different challenge tests were performed.

Local administration of SP intraplantar into the hind paw of a CCI-affected hind limb induced an increased spontaneous paw lifting. This effect was dose dependent and exceeded the effects seen with vehicle as well as the effects seen after testing on cold or warm plates. Furthermore, the SP-induced hyperreactivity remained present for more than 20 days after surgery, a period in which intrinsic paw lifting due to CCI surgery, as well as paw lifting on the cold and warm plates, disappeared (see Figs. 2–4).

In order to further evaluate the functional site of action of this SP effect, different other SP challenge tests were performed. Injections of SP in the hind paw contralateral to the CCI resulted in a similar degree of spontaneous paw lifting as compared to the ipsilateral challenge. Because SP challenges in the ipsilateral front paw and intracardially did not result in an increased paw lifting, the SP-induced reactivity after subplantar ipsi- and contralateral paw injections is most likely spinal cord mediated. In order to further evaluate this, SP was injected spinally. Intrathecal injections of SP also produced a dose-dependent increase in the paw lifting of the CCI hind paw. The activity was comparable to the subplantar injections but occurred at lower concentrations of SP. As a result, it can be concluded that spinal mechanisms play a role in the SP-induced increased reactivity in CCI gerbils. Comparable data are described in rats (Aanonsen et al., 1992).

In previous experiments in nonneuropathic rats, injections of SP (10 ng–10 μg) into the plantar skin of the hind paws have been shown to produce mechanical hyperalgesia (Nakamura-Craig and Smith, 1989). After repeated administration of SP, the hyperalgesia was provoked with significantly lower SP doses (0.5–10 ng), suggesting a facilitation of the response (Nakamura-Craig and Smith, 1989). Injection of SP (0.2–20 μg) in the rat hind paw has also been reported to produce paw favoring, a response where the animal is "resting the paw lightly on the floor while sitting in rest position" 1–8 min after the injection (Hong and Abbott, 1994). Peripheral administration of 0.1 μg of SP has been reported to produce mechanical allodynia and hyperalgesia in rats, attenuated by local pretreatment with the NK-1 antagonist CP99,994-1 (Carlton et al., 1996). However, injection of SP to the receptive field of unmyelinated afferents produces only a weak short-lasting response in half of the neurons (Fitzgerald and Lynn, 1979) and a weak excitatory effect on some canine testicular polymodal receptors in an in vitro preparation (Mizumura et al., 1987), and SP has failed to activate or sensitize C-fibers significantly in other previous experiments (Lembeck and Gamse, 1977; Cohen and Perl, 1990). SP seems to have a role in the sensitization of CCI animals.

In order to further evaluate which tachykinin receptor might be involved in the SP-induced increased intrinsic nociceptive behavior in CCI gerbils, some additional pharmacological tests were performed.

First, it was demonstrated that Sar-SP produced comparable effects as SP in CCI gerbils when administered later in the ligated hind paw and intrathecally. Sar-SP is an NK-1 selective agonist that in most assays is approximately equipotent with SP and essentially inactive at both NK-2 and NK-3 receptors (Drapeau et al., 1987). The slightly lower potency of Sar-SP as compared to SP in the present study could indicate that a minor component of the paw-lifting behavior is meditated via receptors other than NK-1, or more likely, that components such as physicochemical properties of the solution or distribution of the peptide in the paw after the injection are different from that of SP.

Secondly, systemic administration of the centrally acting NK-1 selective antagonist R116301 (Romerio et al., 1999; Megens et al., 2002) dose-dependently attenuated the SP-induced paw lifting after intraplantar and intrathecal administration. This indicates that the SP-induced paw-lifting behavior was triggered via NK-1 receptors. R116301 also antagonized the Sar-SP-induced paw lifting in the CCI animals, again stressing the role of NK-1 tachykinin receptors in this phenomenon.

In general, there are conflicting data with regard to NK1 receptors in neuropathic pain. Mechanical allodynia after spinal nerve ligation injury is reduced in NK-1 receptor knockout mice (Mansikka et al., 2000), indicating that NK-1 receptors have a role in its development or maintenance. However, in another study, there were no differences between the NK-1 receptor knockout and the wild-type control animals in mechanical or cold allodynia after partial sciatic nerve ligation, suggesting that NK-1 receptors are not essential for mechanical allodynia resulting from this type of peripheral nerve injury in mice (Martinez-Caro and Laird, 2000). Several selective NK-1 receptor antagonists (Campbell et al., 1998; Coudore-Civiale et al., 1998; Gonzalez et al., 2000) have been reported to attenuate mechanical and thermal allodynia and hyperalgesia in rat and guinea pig models of neuropathic pain. NK-1 receptors seem to be involved in behavioral responses to high-intensity heat stimuli (Mansikka et al., 1999), but thermal hyperalgesia is not attenuated in NK-1 receptor knockout mice (Mansikka et al., 2000). Also in rats, a recent study using the NK-1 antagonist L-732,138 confirmed the role of NK-1 antagonists in neuropathic pain (Cahill and Coderre, 2002).

In clinical studies, failures have been reported with different NK-1 antagonists in neuropathic pain (Boyce and Hill, 2000).

Alternatively, to direct NK-1 receptor activation on the primary afferents, release of nociceptive transmitters (e.g., bradykinin, prostaglandin, and histamine) from nonneuronal structures in the skin (e.g., blood vessels, mast cells)

activated by the SP injection via NK-1 receptors on these structures could initiate the activation of the primary afferents in this model. This mechanism does not require the presence of NK-1 receptors in the peripheral terminals of the primary afferents. However, mast cell degranulation by SP appears to be a non-receptor-dependent response (Maggi, 1997) and would not be likely to be attenuated by NK-1 antagonists. Thus, the effect of peripherally administered SP in the present study could be explained either by direct activation of NK-1 receptors on the primary afferent neurons or by indirect activation of the primary afferent neurons via NK-1 receptor-mediated release of neurotransmitters from nonneuronal cells in the skin.

However, changes in the expression of the endogenous peptides do not seem to be sufficient to explain the increased effect of exogenous SP. In experiments where an immune challenge with keyhole limpet hemocyanin was used to produce an increase in the concentration of SP dialysate samples collected from the paw, the SP secretion response was increased and prolonged in neuropathic animals and the effect was reversed by the NK-1 receptor antagonist L-703,606. This could indicate that the neuropathic animals exhibited increased delayed-type hypersensitivity responses mediated by NK-1 receptors (Herzberg et al., 1994, 1996). Increased expression of NK-1 receptors on the primary afferent after nerve injury could lead to increased afferent barrage after the SP injection. This could be sufficient to initiate the paw-lifting behavior in the animals with nerve injury but not in normal control animals. Thus, increased response to the peripheral administration of SP at the level of the primary afferent neurons could explain the increased response to SP in CCI gerbils. Alternatively, nerve injury-induced central sensitization could lead to a comparable situation without any changes in the periphery.

In CCI gerbils, injection of SP to both the hind paw ipsi- and contralateral to the injury triggered lifting of the paw ipsilateral to the injury. SP injections to the forepaw ipsilateral to the nerve injury or intracardial administration of SP did not produce paw-lifting behavior. Hence, the paw-lifting behavior seems to be mediated via a segmental spinal mechanism triggered by the primary afferent activation. There is well-documented evidence for a variety of changes in the contralateral nonlesioned side following peripheral nerve injuries (Koltzenburg et al., 1999). In the rat CCI model, behavioral symptoms of neuropathic pain (Attal et al., 1994; Carlton et al., 1994) and neurochemical changes in the spinal cord (Wagner et al., 1993; Behbehani and Dollberg-Stolik, 1994; Hama et al., 1994) are frequently observed on the side contralateral to the injury, and peripheral vascular reactivity to SP has been reported to decrease bilaterally in CCI rats 2–5 weeks after the injury (Basile et al., 1993). Based on the findings of the present study, it is not possible to define the neurochemical mechanism of the paw-lifting behavior triggered by SP injection to the contralateral paw in the CCI gerbils. However, this can be interpreted as an indication that central, probably spinal plastic, changes underlie this response rather than increased excitability of the primary afferent neurons.

Intrathecal administration of SP (2.5–40 ng) produced paw lifting in CCI gerbils in doses, which were lower than those that triggered this behavior after intraplantar injections (50–200 ng). This effect was antagonized by R116301, indicating an NK-1 receptor-mediated mechanism. In previous behavioral studies in normal rats, higher concentrations of SP (100 ng–20 μg it) have been shown to produce short-lasting mechanical (Matsumura et al., 1985) and heat (Yasphal et al., 1982; Coderre and Melzack, 1991) hyperalgesia. In rat microdialysis experiments, intrathecal administration of SP leads to an increase in the spinal release of the excitatory amino acids aspartate and glutamate into the cerebrospinal fluid, and experimental peripheral neuropathy decreased the dose of SP required to trigger the excitatory amino acid release (Skilling et al., 1992). Neonatal capsaicin pretreatment blocks this SP-induced excitatory amino acid release in both neuropathic and control animals (Skilling et al., 1992), indicating a C-fiber-mediated mechanism probably relevant to pain processing. However, in the present study, pretreatment with NMDA-receptor antagonist MK-801 did not prevent the SP-induced paw-lifting behavior, indicating that excitatory amino acid activity mediated via NMDA receptors is not mainly involved.

Other possible spinal mechanisms include increased excitatory amino acid release mediated via postsynaptic AMPA receptors, increased release of peptide transmitters, including SP, the other neurokinins, and CGRP, and presynaptic modulations. An increase of NK-1 receptor-like immunoreactivity in dorsal horn of the spinal cord has been described in mice after partial ligation of the sciatic nerve (Malmberg and Basbaum, 1998). Since spinally administered SP triggered the paw-lifting behavior in neuropathic gerbils, it might also act as an excitatory transmitter at the spinal level mediating the paw-lifting response induced by peripheral SP injections. Under normal conditions, spinally administered SP selectively enhances C-fiber-evoked activity without affecting A-fiber-mediated activity (Kellstein et al., 1990). However, nerve injuries have been demonstrated to alter the expression pattern of SP in the DRG, and therefore this does not necessarily indicate a C-fiber-mediated mechanism in CCI gerbils.

In conclusion, CCI of the sciatic nerve produces long-term mechanical allodynia in gerbils. Peripheral administration of SP in the hind paws of the CCI animals bilaterally triggers paw-lifting behavior mediated via NK-1 receptors. Intrathecal administration of SP in CCI gerbils also brings about the same paw-lifting behavior. This behavior could be due to increased release of excitatory transmitters, including SP, in the spinal cord as a response to activation of the primary afferents in the injured nerve.

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