Inhibitory effects of tachykinin receptor antagonists on thermally induced inflammatory reactions in a rat model

O. Löfgren a, b, *, Y. Qi a, T. Lundeberg a, b

Abstract

Recent studies have proposed that activation of the sensory nervous system after thermal injury induces the release of vasoactive neuropeptides, including tachykinins which contribute to the local inflammatory reaction as well as to the nociceptive transmission at the spinal cord level. Effects of the tachykinins substance P and neurokinin A are mediated by the neurokinin 1 and 2 (NK1, NK2) receptors. The aim of the present study was to investigate the modulatory role of NK1 and NK2 antagonists on edema formation, and on hindpaw withdrawal latency to experimentally assess nociception. Thermal injury was inflicted on the anaesthetized rat by dipping the right hindpaw into hot water at 60°C for 20 s. The amount of edema formation was calculated by measuring the hindpaw volume with a plethysmograph before and during 420 min after scalding. In other studies scalding was inflicted under brief anesthesia, and hindpaw withdrawal latencies (HWL) to mechanical stimulation were recorded before injury and at 180 min after. The effect on edemic reactions of rats treated locally with NK1 and NK2 receptor antagonist were studied, as well as the effect of the same compounds on HWL after intrathecal injection. Scalding induced a progressive edema formation which was reduced significantly in rats treated with local injection of 100 nmol of NK1 and NK2 antagonists 45 min after the injury. The thermally induced inflammation was followed by significant decrease of the latency of hindpaw withdrawal response to mechanical stimulation. Intrathecal injection of 30 nmol of the same drugs 180 min after scalding was followed by significant increase in HWL. The results indicate that SP and NKA contribute to the inflammatory reactions after thermal injury and that the tachykinin receptor antagonists possess the ability to reduce both the local edemic reaction as well as the nociceptive transmission at the spinal cord level.

Keywords: Edema; Nociception; Sensory peptide antagonists

1. Introduction

Thermal injury such as scalding commonly results in the activation of nociceptive afferents. Following their activation, a release of neuropeptides from the peripheral nerve endings occurs, contributing to the inflammatory reactions. Among the neuropeptides released are the tachykinins including substance P (SP) and neurokinin A (NKA) [1, 2]. The release of SP and NKA induces microvascular reactions such as vasodilation and plasma extravasation which contribute to the edema formation [3, 4]. Furthermore, both neuropeptides are suggested to play a role in the transmission of nociceptive input at the spinal cord level [5]. The effects of SP and NKA are mediated by the neurokinin 1 and 2 (NK1, NK2) receptors [6]. Both receptors are widely distributed in the spinal cord as well as in peripheral tissue. The NK1 receptor is activated mainly by SP and the NK2 receptor by NKA. The expression of NK receptors has been shown to increase during an inflammatory process [7]. The role of tachykinins may be investigated through the use of selective peptide antagonists [8]. Recently, tachykinin receptor antagonists of nonpeptide origin have been synthesized and pharmacological potency is indicated [6].

The aim of the present study was to examine the contribution of tachykinins to thermally induced edema formation and the transmission of presumed painful sensation at the spinal cord level by using NK1 and NK2 antagonists.

* Corresponding author. Fax: +46-8-517-72505; e-mail: uke@kir.ks.se

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2. Material and methods

All experiments were performed on freely moving male Sprague–Dawley rats (300–350 g; ALAB, Stockholm). The rats were housed in cages with free access to food and water, and maintained in a room temperature of 24 ± 1°C with a 12 h light/dark cycle. On the day of experimentation the rats were anaesthetized (see below) and after completion of testing immediately killed by an overdose. All experiments were approved by the local ethical committee at Karolinska Institute.

2.1. Model of thermally induced inflammation and edema measurement

Eighteen rats were divided into three groups, according to drug treatment and a control group of 7 was given saline. The rats were first anesthetized with chloralhydrate (0.4 g/kg) i.p. The thermal injury was achieved by dipping one hindpaw into water at 60°C for 20 s while the other paw served as control.

Hindpaw volumes were measured with plethysmometer (Ugo Basil 7150) before scalding and then continuously at intervals for 7 h afterwards. Test substances were administered by subplantar injection 45 min after thermal injury.

2.2. Test of the latency to withdrawal response

Thermal injury was inflicted under brief ether anaesthesia. Forty rats were divided in four groups, according to treatment with different NK1 and NK2 antagonists and another 40 treated with saline also divided: i.e. for each group of rats treated with an active compound there was one of similar size given saline.

The Randall Selitto test (Ugo Basil 7200) was used to assess withdrawal latency to mechanical stimulation in awake animals. A wedge-shaped pusher with a loading rate of 48 g/s was applied to the dorsal surface of the manually handled hindpaw and the pressure required to initiate the struggle response was assessed. The hindpaw withdrawal latency (HWL) is expressed in seconds and paired t-test was used to determine differences in HWL after thermal injury was measured in seconds and paired t-test was used to determine differences in HWL after thermal injury. A two factor ANOVA was used to estimate the change of HWL according to drug treatment and thermal exposure conditions. A p value below the α level of 0.05 is defined as statistical significance.

3. Statistical analysis

The paw volume measured in milliliters is analyzed by ANOVA with repeated measures design. The analysis is carried out for the four independent groups according to drug treatment. Time and thermal exposure conditions are dependent factors and tests for interactional effects were done to find out if the changes over time were equal for all four groups and for scalded conditions versus control. Hindpaw withdrawal latency (HWL) after thermal injury was measured in seconds and paired t-test was used to determine differences in HWL between scalded/control. A two factor ANOVA was used to estimate the change of HWL according to drug treatment and thermal exposure conditions. A p value below the α level of 0.05 is defined as statistical significance.

4. Results

4.1. Effects of local injection of NK1 and NK2 antagonists on thermally induced edema formation

In all groups the preburn volume was the same and there was no difference between right and left hindpaws (Fig. 1). No edema formation was seen in the contralateral nonscaled paw during the time of recording. After scalding significant ipsilateral edema formation was noted in all groups during the observation period (p < 0.001). However, the plethysmographic volume measurements showed varying progressions of edema according to the treatment. The most prominent edema formation was observed in the group of rats which received saline. The mean increase of paw volume during 0–420 min was 1.9 ± 0.13 ml. Compared to the saline injected group, treatment with...
the NK1 antagonist resulted in significant reduction of edema formation over time ($p = 0.001$). Injection of the NK2 antagonist also produced a reduction of edemomic formation, but less pronounced ($p = 0.20$). However, administration of the nonpeptidergic NK1 antagonist had no effect on the edema formation ($p = 0.51$). The edema reaction was unrelated to initial paw volume. Such an effect has been allowed for by analyzing the correlation between initial value and change over time.

4.2. Effects of scalding on hindpaw withdrawal latency before and after intrathecal administration of NK1 and NK2 antagonists

180 min after scalding there was a significant decrease in HWLs of scalded paws ($p = 0.004$). However, the HWLs were significantly decreased bilaterally (Fig. 2). Then 60 min after intrathecal injections HWLs increased among rats which received the NK receptor antagonists (Fig. 3). Compared to the saline

![Fig. 1. Paw volume (ml) change over time, 0–420 min after scalding in four different groups treated with local injection of NK antagonists and saline. No. 25.](image1)

![Fig. 2. Changes in hindpaw withdrawal latency-HWL (s) to pressure after scalding of the right paw, 0–180 min. Figure illustrates four different groups before intrathecal injection with NK antagonists or saline. No. 80.](image2)
treated control group, the increase was significant in the group of rats treated with the NK1 antagonist \((p = 0.039)\). Treatment with the NK2 antagonist was followed by a less pronounced increase of HWL \((p = 0.58)\). But after the injection of NK1 nonpeptidergic antagonist the increase of HWL was significant \((p = 0.007)\). The effect of NK1 peptidergic and nonpeptidergic antagonist on HWLs was similar \((p = 0.97)\). In all groups treated with tachykinin antagonists, there was a tendency towards increases in HWLs on the contralateral side. (Fig. 3).

5. Discussion

The results of the present studies show that the administration of antagonists directed towards receptors of the sensory neuropeptides SP (NK1 receptor antagonist) and NKA (NK2 receptor antagonist) influence thermally induced inflammation. Unilateral scalding of the hindpaw was followed by a progressive ipsilateral edema formation which was significantly reduced by local treatment with the NK1 antagonist. Administration of the NK2 antagonist also reduced the edema formation, although to a lesser extent. After scalding there was a bilateral decrease in hindpaw withdrawal latency to mechanical stimulation with the decrease more pronounced on the thermally injured paw. Intrathecal administration of the NK1 antagonist as well as the nonpeptidergic NK1 antagonist significantly increased the withdrawal responses.

The localized edema reaction after thermal injury is initiated by an early fall in interstitial hydrostatic pressure followed by transcapillary fluid shift [9] and the release of different inflammatory mediators [10]. In previous studies it has been reported that the sensory nervous system contributes to inflammatory processes, and collaborators have reported that SP is released locally after scalding [11]. We have recently reported that another tachykinin, NKA, is released into the subcutaneous space of the paw after scalding [2]. Pretreatment with the neurotoxin capsaicin or with a substance P antagonist decreases plasma extravasation after scalding [12, 13]. In other studies, using an online laser Doppler technique, we reported a biphasic perfusion increase during the initial phase of the edema formation [14] which can be significantly reduced by pretreatment with NK1 and NK2 antagonists [15]. This is in accordance with the present finding where the NK1 antagonist significantly reduced the edema formation after thermal injury and the NK2 antagonist had a similar but less pronounced effect.

Thermal injury results in the activation of primary nociceptive afferents followed by the release of SP and NKA at the dorsal horn of the spinal cord [4]. Duggan et al. suggested that NKA is especially involved in the transmission of noxious thermal stimuli [16]. In present studies intrathecally administered NK1 or NK2 peptidergic receptor antagonists resulted in an increase of hindpaw withdrawal responses, an effect most apparent after treatment with NK1 antagonists. Since there are drawbacks in the use of receptor antagonists of peptidergic origin, different nonpeptidergic antagonists have been developed during the last decade [6]. Therefore it was decided also to investigate the effect of a nonpeptidergic NK1 receptor antagonist in the present study. The result was a significant increase in hindpaw withdrawal latency, equal in effect to the peptidergic one.

As described above, unilateral scalding to the hindpaw resulted in an ipsilateral edema formation and a bilateral decrease in hindpaw withdrawal latencies to mechanical pressure. This is in line with other results, indicating a communication of sensory activity from one side to the other [17, 18]. As earlier mentioned it has been proposed that primary sensory neurons...
contributes both to the local inflammatory reaction and the transmission of nociceptive information centrally [19]. The neurogenic contribution to the peripheral inflammatory reaction has been attributed to local axon reflexes and dorsal root reflexes, resulting in the release of sensory neuropeptides [20]. Recently it has also been shown that excessive nociceptive activation at the central terminals of primary afferents results in an interneuronal circuitry which may be followed by increased activity in contralateral nociceptive terminals [20]. Taken together, the results show that unilateral thermal injury causes an unilateral edema formation which is a part of a local defense reaction, whereas the bilateral increase of withdrawal latency is a sign of a general hyperalgesic response.

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