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TRPV1 desensitisation and endogenous vanilloid involvement in the enhanced analgesia induced by capsaicin in inflamed tissues

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Abstract

The intraplantar acute administration of 10 μg of capsaicin to mice which had received complete Freund's adjuvant (CFA) 1 week before inhibits the thermal inflammatory hyperalgesia it induces and even produces a long-lasting analgesia for at least 2 weeks. In this study, we show that the administration of capsaicin (10 μg) also reduces the immediate licking behavior evoked by the intraplantar administration of a lower dose of capsaicin (0.1 μg), the duration of this inhibitory effect being greater in CFA-inflamed mice (at least 2 weeks) than in non-inflamed animals (less than 4 days). Since this reduction of capsaicin-induced licking behavior may be interpreted as a consequence of the transient receptor potential vanilloid 1 receptor (TRPV1) unresponsiveness, we conclude that the administration of 10 μg of capsaicin into inflamed tissues can render the TRPV1 desensitised. We next explored whether endogenous vanilloids released during inflammation contribute to maintain the analgesia triggered by exogenous capsaicin. The acute administration of capsazepine (10 μg; intraplantarly (i.pl.)) abolished the analgesic effect induced by the injection of capsaicin 1 week before in inflamed mice. From these results, it may be proposed that the maintenance by endovanilloids of the TRPV1 desensitisation induced by capsaicin could contribute to prolonging the analgesic effect induced by this agonist in inflamed tissues.

Keywords: TRPV1 (VR1); Inflammation; Hyperalgesia; Pain; Capsazepine; Mouse

1. Introduction

The transient receptor potential vanilloid 1 receptor (TRPV1), formerly called vanilloid receptor type 1 (VR1), is a non-selective cation channel with high Ca^{2+} permeability, activated by noxious heat and sensitised by acid [5], whose activation produces immediate nociceptor excitation [3]. It is also well established that the tonic stimulation of TRPV1 can lead to analgesic effects through receptor desensitisation [14] or even the degeneration of nociceptive fibres [10,21]. Both mechanisms may help to explain the use of the TRPV1 agonist capsaicin in the management of certain painful pathologies, such as neuropathies [24].

Moreover, the analgesic properties of TRPV1 agonists have been demonstrated experimentally in inflammatory processes. Thus, the systemic administration of resiniferatoxin prevents either complete Freund's adjuvant (CFA)- [28] or carrageenan-induced [20] thermal hyperalgesia in rats and the local injection of capsaicin abolishes thermal hyperalgesic responses induced by both agents in mice [17]. Apart from exhibiting a high efficacy in the relief of inflammatory hyperalgesia, it might be remarked that the analgesic effect induced by TRPV1 agonists in inflamed animals is rather long lasting [17,28], being considerably longer than that observed in non-inflamed animals [17]. In fact, a detailed analysis of the above mentioned data reveals that the analgesic effect induced by a single dose of a TRPV1 agonist lasts approximately the same time as the hyperalgesia observed in solvent-treated inflamed animals [17,28], in such a way that the persistence of the analgesic effect of the TRPV1 agonist seems to be adjusted to the underlying pathology.

On the other hand, the endogenous vanilloid system is clearly involved in the development and maintenance

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of inflammatory hyperalgesia. In fact, the release during inflammation of protons or several molecules – globally called endovanilloids – able to stimulate TRPV1 [12,15,26] or contribute, such as bradykinin, neural growth factor or ATP, to receptor sensitisation [19,22] has been repeatedly reported. Furthermore, the up-regulation of TRPV1 in inflamed tissues has been also proven [1,4,25] and a correlation between TRPV1 expression and thermal inflammatory hyperalgesia has been established in rats [13]. These data make it understandable that either the blockade of TRPV1 with antagonists [9,27] or the deletion of its expression in knockout mice [5,8] induces a considerable reduction of inflammatory hyperalgesia.

We explore here whether the long-term analgesia induced by capsaicin in inflamed mice is accompanied by the TRPV1 unresponsiveness and, if so, whether the endovanilloids released during inflammation could be involved in this process. With this aim in mind, we initially tested if the acute nociceptive licking behavior induced by a low dose of capsaicin is also decreased after the previous administration of an analgesic dose of capsaicin. We have therefore studied whether the administration of the TRPV1 antagonist capsazepine could block the prolongation of capsaicin analgesia in inflamed tissues once such is established.

2. Material and methods

2.1. Animals

Forty to fifty days old Swiss male mice, from the Animalario of the Universidad de Oviedo (Reg. 33044 13A) exposed to a 12-h light/12-h dark cycle and with free access to water and food, were used. Experiments were conducted according to ethical guidelines [29] and approved by the Comité Ético de Experimentación Animal de la Universidad de Oviedo (Spain).

2.2. Drugs

Capsaicin (Tocris), capsazepine (Tocris) and complete Freund's adjuvant (CFA; Sigma; 30 μl) were intraplantarly (i.pl.) administered into the right hind paw of mice. Capsaicin was dissolved in 25 μl of 10% DMSO at the highest dose used and capsazepine in the same volume of 15% DMSO. In all cases, control groups received an injection of the corresponding solvent.

2.3. Nociceptive measures

2.3.1. Measure of the thermal sensitivity in the unilateral hot plate (UHP) test

As previously described [16], mice were gently restrained with the plantar side of one hind paw placed on the hot plate surface ($53 \pm 1\,^{\circ}C$) and the latency for paw withdrawal was manually recorded with a chronometer. Only the clear unilateral withdrawal of the paw was taken into account, discarding the unspecific generalised struggle observed in some cases. Two measures at 3-min intervals were taken in each trial and means were considered. A cut-off of 25 s was established in order to prevent tissue damage.

2.3.2. Measure of capsaicin-induced licking behavior

Mice were placed into transparent plastic cages, and after a 10 min habituation period they were i.pl. injected with 0.1 μg of capsaicin solved in 25 μl of saline. Immediately after the injection of capsaicin, the animals were returned to the observation cages and the time spent in licking the injected paw during a 10 min period was manually recorded with a chronometer.

2.3.3. Measure of the mechanical sensitivity by the von Frey test

Mechanical allodynia was assessed by applying the von Frey filaments (Stoelting) to the plantar side of the paws. Mice were placed on a wire mesh platform, covered with transparent plastic containers and a 15-min period was allowed for habituation. Based on a previously described method [7], the testing procedure randomly started by the inflamed or non-inflamed paw, and following the up and down method an increased or decreased force was applied when a negative or positive response (paw lifting, "shaking" or licking) was obtained. The following von Frey filaments were selected to be tested (1.65, 2.83, 3.22, 3.61, 4.08, 4.56, 4.93, 5.88), and starting with the 3.61 filament, six measures were taken in each animal.

2.4. Statistical analysis

The mean values (and their corresponding S.E.) of the withdrawal latencies obtained by the UHP test, the time spent in licking behavior and the mechanical threshold values obtained by the von Frey test were calculated for each group. When thermal withdrawal latencies of more than two groups were compared, an initial one-way analysis of variance (ANOVA) followed by the Newman–Keuls test was performed. In this case, the theoretical and the obtained *F*-values are shown as follows: [*F*(degrees of freedom, theoretical value) = obtained value, level of significance]. When comparisons were made of the withdrawal latency values or of the licking time values between two groups, the Student's *t*-test for unpaired data was applied. In this case, the values are shown as follows: [*t*(d.f., theoretical value) = obtained value; level of significance].

In order to compare the mechanical threshold values obtained by the von Frey test, an initial Kruskal–Wallis test followed by the Mann–Whitney's *U*-test was performed. For the Kruskal–Wallis test, the theoretical value corresponding to the degrees of freedom is shown together with the obtained value and the level of significance: [*H*(d.f., theoretical value) = obtained value, level of significance]. In the case of the Mann–Whitney's *U*-value obtained, the number