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Antinociceptive activity of ricinoleic acid, a capsaicin-like compound devoid of pungent properties

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Abstract

The antinociceptive effect of ricinoleic acid ([*R*-(*Z*)]-12-hydroxy-9-octadecenoic acid) in comparison with capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide) has been investigated in several "in vivo" tests. Acute topical application of capsaicin, but not ricinoleic acid, produced by itself an hyperalgesic effect detected as a decrease in paw withdrawal latency in response to a painful (heat) stimulus in mice. Capsaicin, but not ricinoleic acid at any dose tested, showed an irritant effect in the wiping test in guinea pig conjunctiva after local application and in the paw licking test in mice after intradermal injection. Whereas acute application of ricinoleic acid or capsaicin decreased paw withdrawal latency to heat in the presence of a pre-existing inflammation (injection of carrageenan in the mouse paw), the repeated local treatment for 8 days with either compounds markedly increased paw withdrawal latency. In a chronic model of inflammation (complete Freund's adjuvant arthritis in mice), the repeated topical and intradermal treatments with both ricinoleic acid and capsaicin increased paw withdrawal latency to heat, the antinociceptive effect of ricinoleic acid being more persistent than that of capsaicin. Antinociceptive effect of 8 days of treatment with ricinoleic acid and capsaicin was observed in acetic acid-induced writhing in mice, capsaicin-induced foot licking in mice and capsaicin-induced wiping movements in guinea pig conjunctiva. A decrease of substance P tissue levels in the mouse paw was found after repeated treatment with ricinoleic acid. In conclusion, ricinoleic acid seems to be a new antinociceptive agent lacking the pungent and acute hyperalgesic properties of capsaicin.

Keywords: Capsaicin; Nociception; Pain; Ricinoleic acid; Substance P; Tachykinin; (Guinea pig); (Mouse)

1. Introduction

It was Jancsó (1960) who first showed that capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide), the pungent principle present in a wide variety of red peppers of the genus *Capsicum*, is able to stimulate sensory nerve fibers and to produce a subsequent long-lasting antinociceptive effect (desensitization). The latter is the most remarkable pharmacological property of capsaicin; in fact, many chemical substances including several mediators of inflammation are capable of stimulating afferent nerves (see Maggi, 1991a; Szolcsányi, 1993 for review) and possibly to induce desensitization (or tachyphylaxis) upon repeated application. On the other hand, the repeated application of maximally effective concentrations of capsaicin (or its analogues) to the peripheral endings of neuropeptide-containing primary afferent neurons induces a desensitizing effect: in this condition, the sensory nerves become unresponsive not only to subsequent applications of capsaicin itself but also to a variety of other chemical or thermal stimuli (Gamse, 1982). Over the last two decades, capsaicin has been utilized to study neurogenic inflammation and the neurophysiology of pain. Capsaicin binds to a "vanilloid" receptor (VR1, so called because of the vanillyl moiety present in many capsaicin analogues; see Szallasi, 1994 for review) expressed on certain sensory neurons (Caterina et al., 1997). The binding of capsaicin or its analogues to the receptor induces the influx of cations and the concomitant activation of pain or reflexes (sensory

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function) along with the release of neuropeptides in the sensory nerve endings (efferent function or neurogenic inflammation). The desensitization caused by the application of high doses of capsaicin is usually accompanied by a depletion of neuropeptides in the sensory nerves (Holzer, 1991). Clinically, the overall effect produced by the application of capsaicin is pain relief. Proof of clinical efficacy of topical capsaicin has been obtained in painful diabetic neuropathy (The Capsaicin Study Group, 1991), neuropathic pain in cancer patients (Ellison et al., 1997), postherpetic neuralgia, post-mastectomy pain syndrome and arthritis (see Maggi, 1991b; Watson, 1994 for review). Although capsaicin is certainly useful in different forms of neuropathic pain and other disorders, its utility has been limited by a troublesome adverse reaction which almost invariably accompanies its use. This reaction is a localized stinging and burning sensation, which can be quite severe, and is acutely produced by the topical application of capsaicin to skin or mucous membranes or on injection into tissues such as dermis, the cerebrospinal canal or into blood vessels.

Ricinoleic acid ([*R*-(*Z*)]-12-hydroxy-9-octadecenoic acid) is the main active component of castor oil and it is well known for its laxative properties (Beubler and Juan, 1979). The most studied pharmacological property of ricinoleic acid is its secretory action that occurs in the small intestine and colon of many mammals, including man (Ammon et al., 1974; Cline et al., 1976).

Recently, we have gathered evidence that chronic local treatment with ricinoleic acid antagonizes neurogenic inflammation and that ricinoleic acid exhibits pharmacodynamic properties resembling that of capsaicin (Vieira et al., submitted). The aim of this paper has been to further characterize ricinoleic acid pharmacology by comparing its nociception-affecting activities with those of capsaicin. Interestingly enough, ricinoleic acid has been proven to produce relevant antinociceptive activities probably through the desensitisation of capsaicin-sensitive nerves, but, at variance with capsaicin, it is devoid of acute irritant and pungent effects.

2. Materials and methods

2.1. Experimental animals

Male albino Dunkin–Hartley guinea pigs (250–350 g) from Charles River (Calco, Italy) and male Swiss mice (20–25 g) from Harlan Nossan (Correzzana, Milan, Italy) were used. The animals were fed with a standard diet and water ad libitum. The experiments have been performed in concordance with the "Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985) and in accordance to the Italian Health Ministry guidelines for the care and use of experimental animals.

2.2. Paw withdrawal latency test in mice

Maximal applicable doses of ricinoleic acid (10 mg/mouse; 99% pure from Sigma (St. Louis, MO, USA) diluted with peanut oil also from Sigma) or capsaicin (0.1–1 mg/mouse; from Serva (Germany), dissolved in a solution with 10% ethanol, 10% Tween 80 and 80% saline) were topically applied on ventral surface of right paw. Paw withdrawal latency was measured 3 h after the topical application in response to a painful stimulus (radiant heat) according to Hargreaves et al. (1988) who used a plantar test apparatus (Basile, Varese, Italy). Animals were placed in plastic cages ($22 \times 17 \times 14$ cm; length $\times$ width $\times$ height) with a plexiglass floor. After the 10-min habituation period, the plantar surface of the hind paw was exposed to a beam of radiant heat through the plexiglass floor. The radiant heat source consisted of an infrared bulb (Osram halogen-bellaphot bulb 8 V, 50 W). A photoelectric cell detected light reflected from the paw and turned off the lamp when paw movement interrupted the reflected light. The paw withdrawal latency was automatically displayed to the nearest 0.1 s; cut-off time was 23 s in order to prevent tissue damage.

In other experiments, single or repeated (8 days) topical applications of ricinoleic acid (900 μg/mouse) or capsaicin (90 μg/mouse), doses chosen in preliminary experiments, were applied by rubbing on the ventral surface of the right paw and, 30 min later, hindpaw oedema was induced by the intraplantar injection of carrageenan (type II, Sigma; 300 μg in 0.1 ml of sterile phosphate buffer solution without sodium bicarbonate). The extent of oedema (mm) was measured in the following 6 h by a microcalyper whereas 3 h after carrageenan injection, the paw withdrawal latency to a painful stimulus (heat) was measured as described above.

In Freund's adjuvant experiments, the repeated local application (8 days) of ricinoleic acid (900 μg/mouse) or capsaicin (90 μg/mouse) or the repeated (4 days) intradermal administration of the water-soluble potassium salt of ricinoleic acid (potassium [*R*-(*Z*)]-12-hyroxy-9-octadecenoate or MEN 11938 from Menarini Research (Pomezia, Italy); 30 μg/mouse) or capsaicin (3 μg/mouse) on the ventral surface of the right paw was tested in the presence of complete Freund's adjuvant (30 μl; Sigma), injected in the hindpaw at the 1st day. The withdrawal latency to a painful stimulus (heat) was monitored once a week for 3 weeks as described above.

2.3. Wiping test in guinea pigs

The irritant effect (induction of wiping movements) of ricinoleic acid or capsaicin was assessed by applying ricinoleic acid (1, 10 and 100 μg/guinea pig) or capsaicin (0.1, 1 and 10 μg/guinea pig) in a volume of 100 μl on the guinea pig conjunctiva and the number of wiping

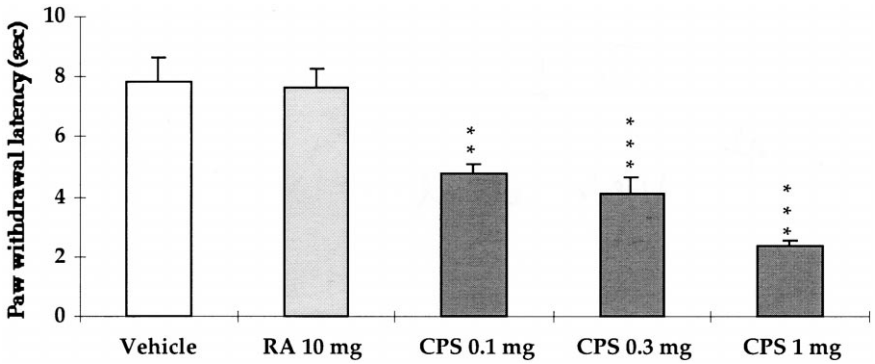

Fig. 1. Effect of topical application of ricinoleic acid (RA, 10 mg/mouse) or capsaicin (CPS, 0.1–1 mg/mouse) on paw withdrawal latency to a painful stimulus (radiant heat) in non-inflamed paws. Paw withdrawal latency was measured 3 h after the topical application of both compounds. Data are mean ± S.E.M. $N = 8–10$ for each group. $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. vehicle.

movements was recorded during the 30-s period that followed the application.

In other experiments, guinea pigs were treated topically, on the conjunctiva of the right eye, twice a day for 8 consecutive days with different doses of ricinoleic acid (1, 10 and 100 μg/guinea pig). On the 9th day, guinea pigs were exposed to a fully pain-inducing dose of capsaicin (10 μg/guinea pig) and then the number of wiping movements was recorded during the 30-s period that followed the application.

2.4. Hindpaw licking test in mice

The animals were placed individually for adaptation, 20 min before testing, in transparent glass boxes (20 cm diameter) which thereafter served as observation chamber. To assess whether the peripheral application of MEN 11938 or capsaicin induces stimulation of nociceptive neurons sufficient to produce overt behavioral pain, MEN 11938 (10, 30 and 100 μg/mouse) or capsaicin (1, 3 and 10 μg/mouse) was intradermally injected in a volume of 20 μl in the plantar surface of the right hindpaw. Duration of licking behavior was measured during the first 5 min following the administration of both compounds (Santos and Callixto, 1997). In other experiments vehicle, MEN 11938 (10, 30 and 100 mg/kg) or capsaicin (1, 3 and 10 mg/kg) was injected intradermally once daily for 4 consecutive days in the mice hindpaw. On the 5th day, capsaicin (1.6 μg/mouse) was intradermally injected to

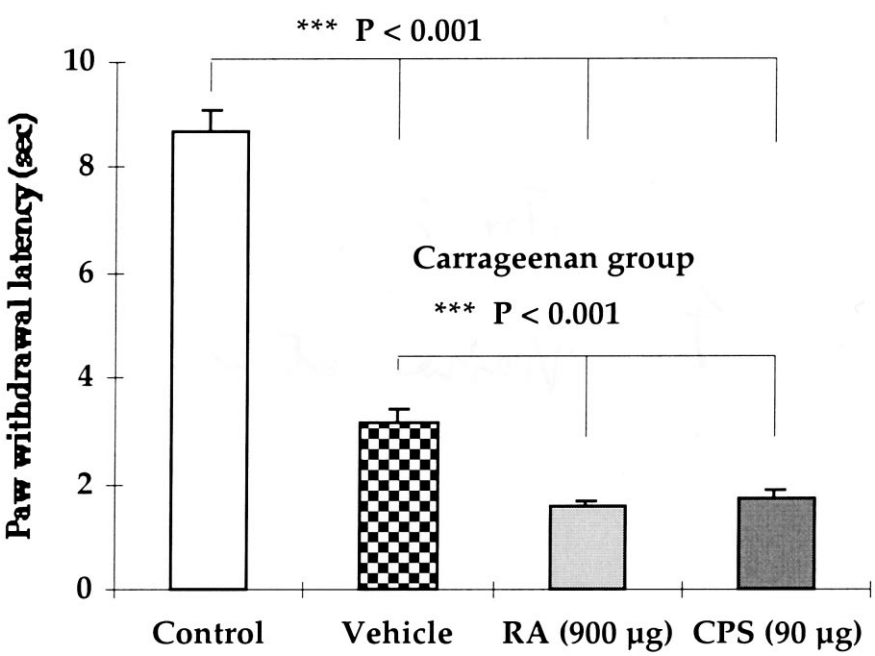

Fig. 2. Effect of acute topical application of ricinoleic acid (RA, 900 μg/mouse) or capsaicin (CPS, 90 μg/mouse) on paw withdrawal latency in carrageenan-induced hindpaw oedema in mice. Paw withdrawal latency was measured 3 h after carrageenan injection. Data are mean ± S.E.M. $N = 8–10$ for each group. $^{***}P < 0.001$ vs. vehicle or untreated (control) group.

all the groups and duration of licking behavior was measured as described above.

2.5. Acetic acid-induced writhing test in mice

MEN 11938 (30–100 mg/kg, i.p.) and capsaicin (1–5 mg/kg, i.p.) were administered once daily for 8 days and then, on the 9th day, abdominal writhes were induced administering i.p. 0.6% acetic acid in a volume of 10 ml/kg. The number of writhes was monitored during the first 10 min following acetic acid injection. Control group received the vehicle alone.

2.6. Tissue substance P measurement

Ricinoleic acid (900 μg/mouse) or capsaicin (90 μg/mouse) were applied topically on the ventral surface of the right paw for 8 days. On day 9, oedema was induced by carrageenan, and the animals were then sacrificed. For tissue peptide measurements, the paws were rapidly removed and weighed. The samples were extracted with 3 ml of 2 N acetic acid at 95°C for 15 min. They were then homogenized and centrifuged at $10{,}000 \times g$ for 30 min at 4° C, the supernatants were freeze-dried and stored at −20°C until their content of substance P-like immunoreactivity was determined by radioimmuno assay. Lyophilized samples were reconstituted in 10 ml of 50 mM phosphate buffer (pH 7.4), containing 0.3% bovine serum albumin and 10 mM EDTA. The radioimmuno assay for substance P-like immunoreactivity was based on scintillation proximity assay technology, as previously described (Lippi et al., 1998). The incubation mixture was composed of 100 μl of the reconstituted sample (diluted 1:30), 100 μl of ^{125}I-labeled tracer (about 6,000 cpm), 100 μl of diluted antiserum (1:100,000 for substance P) and 50 μl of scintillation proximity assay protein A reagent. After an overnight incubation at room temperature under gentle agitation, the samples were counted in a β-scintillation counter (2200CA, Canberra-Packard). [^{125}I]-Bolton Hunter substance P and scintillation proximity assay reagents were from Amersham (UK). The rabbit anti-substance P serum (RAS 7451, cross-reactivity < 5% with substance P 7–11, < 0.01% with neurokinin A) was from Peninsula Laboratories (St. Helens, UK).

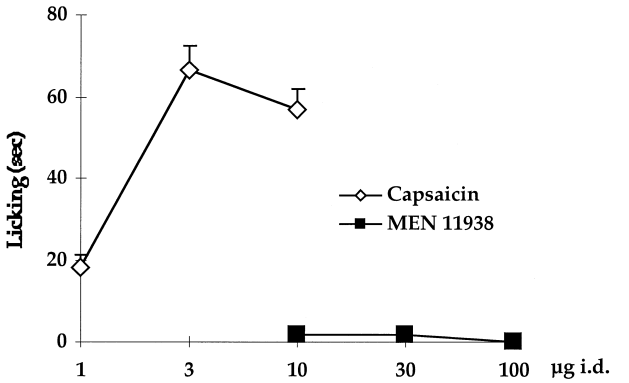

Fig. 4. Pain induction by potassium salt of ricinoleic acid, MEN 11938 (10–100 μg/mouse), and capsaicin (1–10 μg/mouse) injected intradermally in the hindpaw. Duration of licking behavior was measured during the first 5 min that followed the administration. Data are mean ± S.E.M. $N = 6–9$ for each group.

2.7. Statistical analysis

Data in the text, figures and tables are means + S.E.M. Statistical significance between groups was assessed using

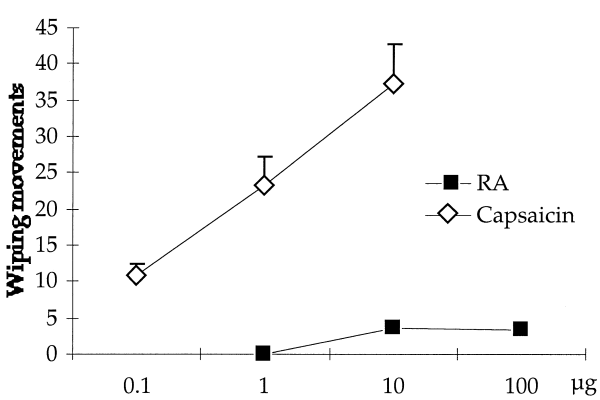

Fig. 3. Acute irritant effects of ricinoleic acid (RA, 1–100 μg/guinea pig) and capsaicin (0.1–10 μg/guinea pig) applied topically on the conjunctiva. The number of wiping movements was detected in the 30-min period that followed the application. Data are mean ± S.E.M. $N = 5–8$ for each group.

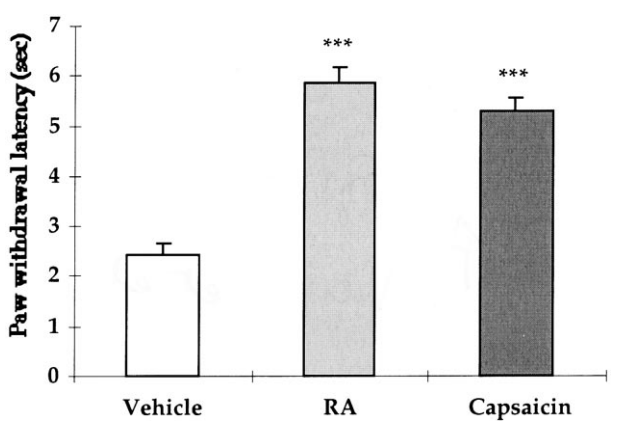

Fig. 5. Effect of repeated (8 days) topical application of ricinoleic acid (RA, 900 μg/mouse) or capsaicin (90 μg/mouse) on paw withdrawal latency in carrageenan-induced hindpaw oedema in mice. Carrageenan was injected at the 1st and at the 9th days. Paw withdrawal latency was measured at 3 h after the second injection of carrageenan. Data are mean ± S.E.M. $N = 8–10$ for each group. $^{***}P < 0.001$ vs. vehicle group.

Table 1
Paw withdrawal latency (s) to a painful stimulus (heat) in Freund's adjuvant-treated mice

	1st week	2nd week	3rd week
Local application for 8 days			
Vehicle	n.t.	2.63 ± 0.27	4.24 ± 0.49
Ricinoleic acid (900 μg/mouse)	n.t.	6.48 ± 0.54[a]	7.54 ± 0.94[b]
Capsaicin (90 μg/mouse)	n.t.	5.95 ± 0.51[a]	6.14 ± 0.68
Intradermal treatment for 4 days			
Vehicle	3.04 ± 0.15	2.83 ± 0.3	4.39 ± 0.44
MEN 11938 (30 μg/mouse)	5.86 ± 0.62[a]	6.83 ± 0.41[a]	8.23 ± 0.79[a]
Capsaicin (3 μg/mouse)	5.57 ± 0.49[a]	6.13 ± 0.71[a]	6.89 ± 0.54[b]

Animals were treated by local application of ricinoleic acid (900 μg/mouse) or capsaicin (90 μg/mouse) or intradermally for 4 days with the potassium salt of ricinoleic acid MEN 11938 (30 μg/mouse) or capsaicin (3 μg/mouse). Paw withdrawal latency was monitored once a week, 7, 14 or 21 days after the injection of Freund's adjuvant.
Data are mean ± S.E.M. $N = 8–10$ for each group.
n.t. = not tested.
[a] $P < 0.01$ vs. respective vehicle group.
[b] $P < 0.05$ vs. respective vehicle group.

one-way analysis of variance (ANOVA) followed by Bonferroni's test.

3. Results

3.1. Acute hyperalgesic effects of capsaicin and ricinoleic acid

3.1.1. Hyperalgesic effect of a topical treatment with capsaicin and ricinoleic acid in the mouse paw

A single topical application of maximal administrable doses of ricinoleic acid (10 mg/mouse) or capsaicin (0.1–1 mg/mouse) on the ventral surface of non-inflamed mice paw did not produce any skin reddening or paw oedema. When exposed to a painful stimulus (radiant heat), only the capsaicin-treated group showed a significant dose-related hyperalgesic effect (Fig. 1).

3.1.2. Hyperalgesic effect of a topical treatment with capsaicin and ricinoleic acid in the mouse paw after oedema induction by carrageenan

The oedema thickness reached the maximum value at the 3rd hour after the carrageenan injection (0.5 + 0.09 mm). At this time, the paw withdrawal latency to heat was markedly reduced by carrageenan as compared to control group (Fig. 2). The topical treatment with ricinoleic acid (900 μg/mouse) and capsaicin (90 μg/mouse) significantly reduced the reaction time to heat as compared to that observed in the group treated with the vehicle (Fig. 2).

3.1.3. Irritant (pain-inducing) effect of capsaicin and ricinoleic acid on guinea pig conjunctiva

Topical application of capsaicin in the conjunctiva induced a potent and dose-dependent (0.1, 1 and 10 μg/guinea pig) pungent effect seen as induction of wiping movements, whereas ricinoleic acid, up to 100 μg/guinea pig, did not induce effect (Fig. 3).

3.1.4. Irritant (pain-inducing) effect of capsaicin and ricinoleic acid after intradermal injection in mice

The intradermal administration of capsaicin (1–10 μg/mouse) induced pain, as seen by licking behavior directed to the injected hindpaw, whereas intradermal administration of the water-soluble ricinoleic acid potassium salt, MEN 11938 (up to 100 μg/mouse) showed no effect (Fig. 4).

3.2. Antinociceptive effect of ricinoleic acid and capsaicin after repeated treatments

3.2.1. Antinociceptive effect of repeated topical treatments with ricinoleic acid and capsaicin in carrageenan-induced paw oedema in mice

Repeated (8 days) topical treatment with ricinoleic acid (900 μg/mouse) or capsaicin (90 μg/mouse) resulted in

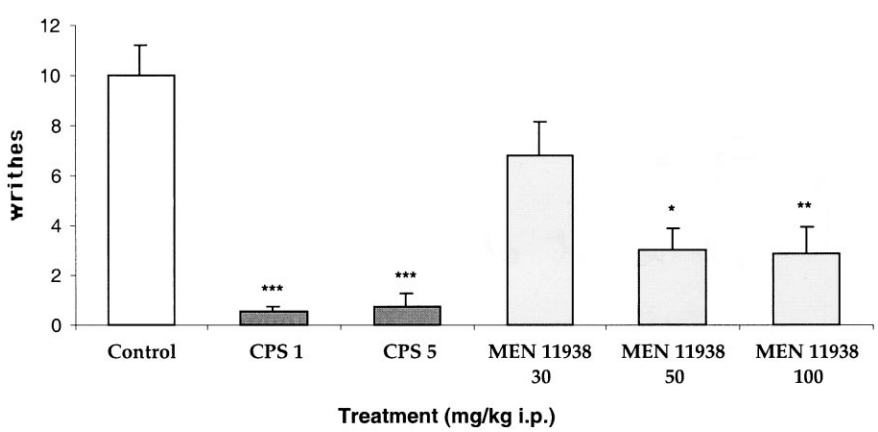

Fig. 6. Effect of repeated (8 days) i.p. treatment with the potassium salt of ricinoleic acid, MEN 11938 (30–100 mg/kg i.p.) and capsaicin (CPS, 1 and 5 mg/kg i.p.) on 0.6% acetic acid-writhing test in mice. Control group received the vehicle alone. Data are mean ± S.E.M. $N = 8–10$ for each group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. control group.

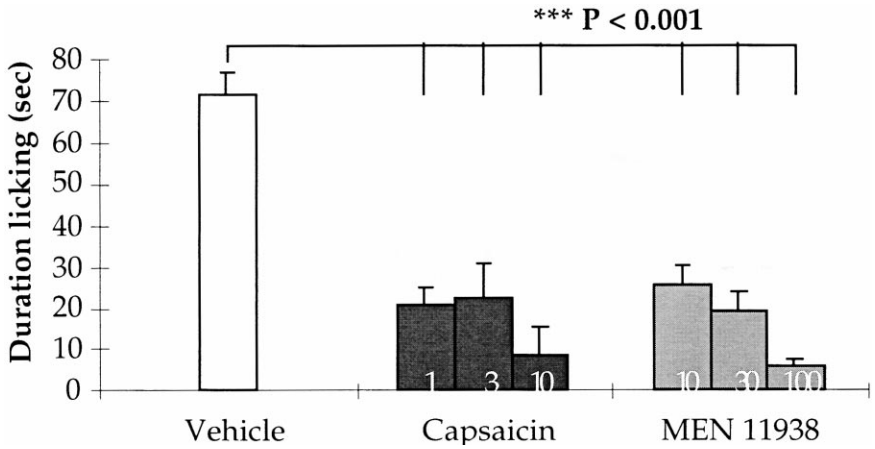

Fig. 7. Desensitizing effects of the potassium salt of ricinoleic acid, MEN 11938, and capsaicin. MEN 11938 (10, 30 and 100 mg/kg) and capsaicin (1, 3 and 10 mg/kg) were injected intradermally in hindpaw mouse for 4 consecutive days. At the 5th day, capsaicin (1.6 μg/mouse) was intradermally injected to all the animals and duration of licking was measured during the first 5 min following the administration. Data are mean ± S.E.M. $N = 6–9$ for each group. $^{***}P < 0.001$ vs. vehicle group.

a marked prolongation of the paw withdrawal latency to heat as compared to that observed in the group treated with the vehicle (Fig. 5).

3.2.2. Antinociceptive effect of repeated topical or intradermal treatments with ricinoleic acid and capsaicin in Freund's adjuvant-induced oedema in mice

As shown in Table 1, the paw withdrawal latency to a painful stimulus in Freund's adjuvant treated mice treated with both topical ricinoleic acid (900 μg/mouse) and capsaicin (90 μg/mouse) for 8 days was increased over at least 2 weeks.

The intradermal administration of MEN 11938 or capsaicin exerted a significant antinociceptive effect that lasted for at least 3 weeks (Table 1). With either route of administration, the antinociceptive effect of ricinoleic acid was greater than that of capsaicin at the 3rd week (Table 1).

3.2.3. Antinociceptive effect of repeated treatments with ricinoleic acid and capsaicin in acetic acid-induced writhing test in mice

Either MEN 11938 (30–100 mg/kg, i.p.) or capsaicin (1–5 mg/kg, i.p.) administered for 8 consecutive days induced a significant inhibition of the acetic acid-induced writhing responses (Fig. 6). The antinociceptive effect of MEN 11938 was lesser than that of capsaicin.

3.2.4. Desensitizing effect of ricinoleic acid and capsaicin after repeated intradermal injections. Inhibition of capsaicin-induced foot licking in mice

Repeated intradermal treatment with both MEN 11938 (10–100 mg/kg) or capsaicin (1–10 mg/kg) given for 4 days produced a dose-dependent inhibition of capsaicin-induced foot licking (Fig. 7). These findings showed a cross-desensitization between capsaicin and ricinoleic acid, being ricinoleic acid 10 times less potent on a milligram basis as compared to capsaicin.

3.2.5. Antinociceptive effect of repeated topical applications with ricinoleic acid in the guinea pig conjunctiva. Inhibition of capsaicin-induced wiping.

As shown in Fig. 8, the 8-day twice daily treatment with different doses of ricinoleic acid (1, 10 and 100 μg/guinea pig) resulted in a marked dose-dependent inhibition of capsaicin-induced wiping.

3.3. Effect of repeated topical applications with ricinoleic acid and capsaicin on the paw levels of substance P in carrageenan-induced oedema in mice

The levels of substance P were not different in carrageenan-inflamed as compared to non-inflamed paws (Table 2). The repeated (8 days) local application of ricinoleic

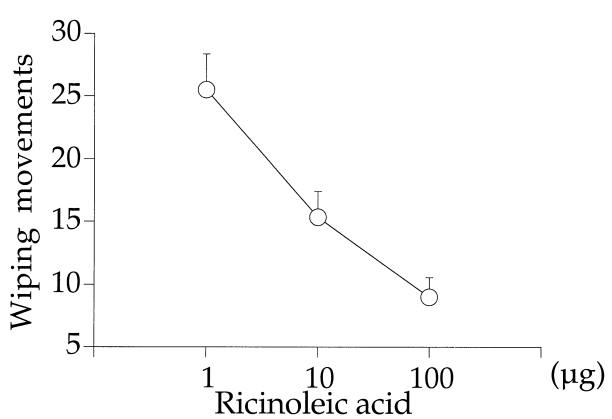

Fig. 8. Antinociceptive effect of repeated topical application of ricinoleic acid (1–100 μg/guinea pig) on wiping induced by 10 μg/guinea pig of capsaicin in the conjunctiva. Ricinoleic acid was applied twice a day for 8 days and, at the 9th day, the animals were exposed to capsaicin. Data are mean ± S.E.M. $N = 5$ for each group.

Table 2
Effect of ricinoleic acid (900 μg/mouse) or capsaicin (90 μg/mouse) applied topically on the ventral surface of the right paw for 8 days on substance P-like immunoreactivity (SP-LI) levels in mice paw

Groups	SP-LI (pmol/g tissue)
Non-inflamed paw	1.09 ± 0.34
Carrageenan + vehicle	1.30 ± 0.28
Carrageenan + ricinoleic acid	0.54 ± 0.12[a]
Carrageenan + capsaicin	0.73 ± 0.14

At day 9, oedema was induced by carrageenan.
Data are mean ± S.E.M. $N = 8–10$ for each group.
[a] $P < 0.05$ vs. carrageenan + veihicle group.

acid, at doses able to produce marked antinociceptive effects (see above), reduced by 58% substance P-like immunoreactivity in the inflamed paw of mice (Table 2). Repeated topical capsaicin administration did not significantly reduce substance P paw levels although a trend of reduction was found (Table 2).

4. Discussion

The main result of this study is the demonstration that unlike capsaicin, the local application of ricinoleic acid is devoid of irritant (pain-inducing) or nociceptive effects in guinea pig conjunctiva or mouse hindpaw whereas the repeated administration of ricinoleic acid exerts a potent and long-lasting antinociceptive effect in several tests. The antinociceptive effect of ricinoleic acid was 10 times less potent on milligram basis as compared to that of capsaicin.

Part of the effects of ricinoleic acid is similar to those of capsaicin, a pungent component of the fruits of the *Capsicum* plants that produces marked alterations in the function of unmyelinated sensory afferents C-polymodal nociceptors (Maggi and Meli, 1988). Capsaicin is known to induce neurogenic inflammation (e.g., vasodilation and protein extravasation) due to an acute release of bioactive peptides, such as substance P, neurokinin A and calcitonin gene-related peptide from sensory nerve terminals (Holzer, 1991).

On acute application, ricinoleic acid seems to determine a mild degree of stimulation of sensory nerves. It is very interesting to note that, at any dose tested acutely, ricinoleic acid was devoid of pungent properties characteristic of capsaicin and it did not exert any hyperalgesic effect toward heat or chemical painful stimuli. In patch clamp experiments (Vieira et al., submitted), contrary to capsaicin, ricinoleic acid did not induce any change in excitatory inward current in isolated dorsal root ganglia expressing preferentially VR1. A similar effect was recently reported for scutigeral, a triprenyl phenol of fungal origin belonging to a new class of vanilloids (Szallasi et al., 1999). Capsaicin and heat in the noxious range activates VR1 (Caterina et al., 1997) although it is not clear if directly or through other thermally sensitive molecules. In particular in the skin, more than in other tissues, temperature and low pH seem to play an important role in the activation of VR1 related to pain induction (Del Bianco et al., 1996). In our experimental conditions, ricinoleic acid seems unable to affect by itself VR1 but to reduce the excitatory effect produced by capsaicin. In fact, ricinoleic acid reduced by 25% the release of calcitonin gene-related peptide induced by capsaicin from rat skin flaps (Reeh et al., unpublished observations).

In repeated treatment studies, ricinoleic acid induces a condition of desensitization of capsaicin sensitive nociceptive neurons following its application to the peripheral receptive field of the afferent neurons. It was able to reduce the paw licking and the conjunctiva wiping induced by capsaicin whose capability to cause nociception is mediated by a selective site of action as shown in mice lacking the capsaicin receptor (Caterina et al., 2000). This sustained antinociceptive effect, which anticipates, by analogy with capsaicin itself, a potential antinociceptive effect on neuropathic pain in humans, is not accompanied by the marked pain-inducing effect, which is typical of the initial stage of capsaicin action as discussed above.

Experimental evidence suggests that substance P can be released either from peripheral nerve terminals of primary afferent fibers to produce inflammation or from the central terminals of these fibers to modulate nociceptive transmission at the level of the spinal cord horn (Otsuka and Yanagisawa, 1987). It has been shown that an increased release of substance P from dorsal horn of the spinal cord followed heat (Duggan et al., 1988) and chemical (Donnerer et al., 1992) stimulation, and that the expression of the tachykinin NK_1 receptor was significantly increased in inflamed hindpaws (McCarson, 1999). On the other hand, the tachykinin NK_1 receptor antagonists CP-96,345 (Birch et al., 1992; Sakurada et al., 1993; Traub, 1996) and CP 122,721 (our unpublished observation) produced antinociceptive and anti-oedema effects and in mice lacking preprotachykinin A, the gene encoding substance P and neurokinin A, the behavioral response to mild painful stimuli was intact whereas they had reduced the response to moderate to intense pain (Cao et al., 1998).

The observation that ricinoleic acid, at least in part, was able to determine a peripheral depletion of substance P in inflamed paws seems to indicate that the reduction of substance P in the periphery by topical application is not related to its effect on “important pain neurotransmitter” in the spinal cord. In particular, the reaction time is increased by ricinoleic acid in model of chronic inflammation such as that induced by complete Freund’s adjuvant. In this latter experimental setting, it has been shown that VR1 is a critical mediator of the thermal nociception (Caterina et al., 2000). Chemical (capsaicin- and acetic acid-induced) and thermal pains were reduced by ricinoleic acid at extent similar to capsaicin.

Although the knowledge of the nociceptor site of action of ricinoleic acid is not known so far, we anticipate that

ricinoleic acid will represent a useful alternative to capsaicin in those human diseases in which capsaicin has been shown to be effective in the treatment of neuropathic pain.

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