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An examination of neurogenic mechanisms involved in mustard oil-induced inflammation in the mouse

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Abstract

The mechanisms by which topical mustard oil causes vasodilatation in the mouse were investigated using the tachykinin NK_1 receptor antagonist SR140333 and the calcitonin gene-related peptide (CGRP) antagonist BIBN4096BS, alongside αCGRP or NK_1 receptor knockout mice. Blood flow was assessed by laser Doppler flowmetry and plasma extravasation by ^{125}I-albumin accumulation. Mustard oil produced significant plasma extravasation and vasodilatation in wild type mice, although the plasma extravasation was less than that seen with capsaicin whilst the vasodilatation was greater. The plasma extravasation was abolished in tachykinin NK_1 knockout mice, whilst the vasodilatation was enhanced. BIBN4096BS was unable to inhibit the vasodilatation in wild type mice but abolished it in the NK_1 knockout mice. In αCGRP knockout mice, mustard oil also caused plasma extravasation and vasodilatation, which were both inhibited by treatment with SR140333. These data suggest that both a tachykinin NK_1 receptor agonist and a CGRP agonist are active as vasodilators, producing redundancy, requiring blockade of both mediators to prevent vasodilatation.
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1. Introduction

Neurogenic inflammation occurs when stimulated sensory neurons release proinflammatory neuropeptides leading to localized vasodilatation and plasma extravasation (Lembeck and Holzer, 1979; Lundberg et al., 1985; Brain et al., 1985). Studies, primarily carried out in the rat, indicate that these vascular changes are caused by the neuropeptides substance P and calcitonin gene-related peptide (CGRP). Substance P increases microvascular permeability, leading to oedema formation (Lembeck and Holzer, 1979; Lembeck et al., 1992), whilst CGRP dilates local arterioles (Brain et al., 1985; Gamse and Saria, 1987; Escott and Brain, 1993). However, our studies in the mouse suggest that, in this species, the actions of substance P and CGRP are not so clearly separated. Although substance P remains the sole mediator of plasma extravasation, both CGRP and substance P contribute to the control of neurogenic vasodilatation (Grant et al., 2002).

Capsaicin, a chemical activator of sensory neurons, is commonly used in the study of neurogenic inflammation. Mustard oil (allyl isothiocyanate) is also believed to stimulate sensory neurons to produce an inflammatory response (Inoue et al., 1997; Banvolgyi et al., 2004). Capsaicin has, however, been studied in much greater depth, and these studies have been aided by the identification and cloning of a receptor for capsaicin, transient receptor potential vanilloid 1 (TRPV1; Caterina et al., 1997). In contrast, the cellular targets and mechanisms of action of mustard oil are still not fully elucidated, although a

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recent report indicates that ankyrin transmembrane protein 1 (ANKTM1), a member of the transient receptor potential family of ion channels, may be activated by mustard oil (Jordt et al., 2004). Topical application of mustard oil in experimental animals caused inflammation, comprising both vasodilatation and oedema formation. This inflammatory response is considered to occur as a result of the release of mediators from capsaicin-sensitive sensory neurons (Jancso et al., 1967, 1977). Depletion of sensory neuropeptides by pretreatment with capsaicin causes a decrease in the response to mustard oil in the mouse ear (Inoue et al., 1997), rat bladder (Patacchini et al., 1990) and rat skin (Holzer and Jocic, 1994). These data support the hypothesis that mustard oil produces its effects through activation of capsaicin-sensitive primary afferent fibres and consequent neuropeptide release.

Although there is good evidence that both mustard oil and capsaicin exert their inflammatory effects via activation of nociceptive fibres, not all their actions are identical. The inflammatory response produced by capsaicin shows a prolonged desensitization to further inflammatory stimuli after the initial inflammation. However, repeated application of mustard oil to the mouse ear does not lead to a desensitization of the plasma extravasation response (Inoue et al., 1997; Banvolgyi et al., 2004). The interaction of mustard oil with the sensory fibres also seems to occur through a different mechanism to capsaicin. Capsaicin causes neuronal depolarization by activation of TRPV1 receptors (Caterina et al., 1997), which can be blocked by the dye ruthenium red, preventing fibre depolarization. In contrast, the effects of mustard oil were unaffected by ruthenium red in the mouse ear (Inoue et al., 1997). The absence of desensitization and inability of ruthenium red to fully inhibit the mustard oil response suggest that it does not activate sensory fibres via TRPV1 receptors, so the identification of ANKTM1 as a molecular target for mustard oil (Jordt et al., 2004) is timely.

The inflammatory response produced by mustard oil also differs from that to capsaicin. Tachykinin NK_1 receptor antagonists inhibit the plasma extravasation produced by mustard oil in rat skin (Lembeck et al., 1992) and in the mouse ear (Inoue et al., 1997; Banvolgyi et al., 2004), implicating substance P as the mediator of plasma extravasation. The oedema produced by capsaicin in the mouse ear is inhibited by the histamine H_1 receptor antagonist chlorpheniramine or the 5-HT_2 receptor antagonist LY 53857 (Inoue et al., 1995), implicating release of mast cell amines in the plasma extravasation, in addition to the vascular effects of substance P. In contrast to capsaicin, mustard oil-induced plasma extravasation is not inhibited by chlorpheniramine or LY 53857 in the mouse ear (Inoue et al., 1997), suggesting that mast cell activation is not involved in this response. Few studies have examined the mechanisms by which mustard oil induces neurogenic vasodilatation. It is inhibited by the nitric oxide synthase inhibitor NG-nitro-L-arginine methyl ester (L-NAME) in the rat paw (Lippe et al., 1993; Holzer and Jocic, 1994), although L-NAME did not affect the hyperaemia produced by injection of CGRP or electrical stimulation of the saphenous nerve (Holzer and Jocic, 1994). The precise mechanisms by which mustard oil stimulates vasodilatation remain unclear. Further complexity in the inflammatory activity of mustard oil has recently been revealed by Banvolgyi et al. (2004), who demonstrate that prolonged treatment of mouse ear skin with mustard oil produces an oedema that is insensitive to capsaicin desensitization or tachykinin NK_1 receptor antagonist treatment, implicating a nonneuronal mechanism in addition to the release of neuropeptides from sensory neurons. Overall, it is apparent that although the inflammatory response produced by both capsaicin and mustard oil is primarily neurogenic in nature, there are also key differences in both the mechanisms of sensory fibre activation and the inflammatory mediators involved.

Mustard oil was used in this study as an alternative to capsaicin in an attempt to further define the neurogenic inflammatory response in the mouse. Although differences between these two mediators have been identified, as described above, the majority of their inflammatory activity is due to activation of sensory nerves leading to a release of inflammatory neuropeptides, probably substance P and CGRP. The mechanisms of neurogenic vasodilatation in the mouse remain poorly defined, although evidence from our laboratory suggests that both CGRP and substance P contribute (Grant et al., 2002). This study was based around the use of antagonists to both the tachykinin NK_1 and CGRP receptor, along with mice in which the genes for αCGRP or the NK_1 receptor were deleted, to provide a greater understanding of the relative contributions of substance P and CGRP to the vasodilatation produced by topical mustard oil application.

2. Methods

The experiments were carried out under the Animals (Scientific Procedures) Act, 1986, UK and adhere to the EU guidelines for the use of experimental animals. They were approved by the Guy's Campus Ethical Committee, KCL. After completion of experiments, the animals were killed by cervical dislocation. C57BL/6 mice were obtained from Charles River, UK. Wild type and tachykinin NK_1 receptor knockout Sv129+C57BL/6 (Bozic et al., 1996) mice were a gift from Dr. N. Gerard, Perlmutter Laboratory, Children's Hospital, Boston, MA, then bred in house. CGRP knockout C57BL/6 mice (Salmon et al., 1999) were also used. Mice of both sexes (20–30 g) were used in this study. All were maintained on normal diet, with free access to food and water, in a climatically controlled environment. Both strains displayed normal growth and behavioral characteristics. Transgenic mice were sex-and age-matched to within 14 days. Appropriate

anaesthesia was induced using urethane (2.5 mg/g; i.p.) and maintained throughout all procedures.

2.1. Measurement of plasma extravasation in the ear

Plasma extravasation was measured by the extravascular accumulation of [^{125}I]-labelled bovine serum albumin (45 kBq) injected intravenously into the tail vein and flushed through with saline, as previously described (Cao et al., 1999). After 5 min, capsaicin (10 μl of 10 mg/ml in ethanol), mustard oil (10 μl of 1% v/v in paraffin) or vehicle control was applied topically to each side of the ears. Plasma extravasation was allowed for up to 1 h, during which time blood flow measurements were made, if required. A blood sample (0.3–0.7 ml) was then taken by cardiac puncture, and the animal was killed by cervical dislocation. The blood samples were centrifuged at 6000 ×*g* for 4 min, after which plasma was taken for measurement of plasma radioactivity in a gamma counter (1260 Multigamma II, EG and G Wallac, UK). The ears were removed and weighed, and their radioactivity was measured. Plasma extravasation in the ears was expressed as microliter of plasma accumulated per gram of tissue.

2.2. Assessment of cutaneous blood flow in the ear

A laser Doppler probe (P1 probe, Moor Instruments, UK) was placed on each ear, and blood flow was recorded for a 5 min period to ensure stability. Recordings were made via a two-channel laser Doppler flowmeter (Moor Instruments) connected to a MacLab (ADInstruments, UK). Mustard oil (10 μl of 1% v/v solution in paraffin) was applied externally to both surfaces of one ear (i.e., 20 μl per ear in total) and paraffin (vehicle control) to both surfaces of the contralateral ear. Blood flow was then assessed for a period of 1 h, as previously described (Grant et al., 2002). Blood flow data were recorded in arbitrary flux units, which were proportional to the blood flow through the ears. They are expressed as the area under the recorded flux vs. time trace for the entire recording period (mm^2). In some cases, the mean flux values themselves were plotted against time to give a representation of the time course of the vasodilatation response.

2.3. Effect of test compounds on plasma extravasation and vasodilatation in the ear

Antagonists were injected intravenously, along with [^{125}I]-bovine serum albumin solution (0.1 ml total volume i.v. into tail vein), 5 min prior to the start of the measurement period. Plasma extravasation and blood flow measurements were then carried out as previously described. The tachykinin NK_1 receptor antagonist SR140333 was used at a dose of 480 nmol/kg (Cao et al., 2000). The nonpeptide CGRP receptor antagonist BIBN4096BS was administered at doses of 0.3 (350 nmol/kg) or 3 mg/kg (3.5 μmol/kg), as although maximal inhibition was found at 0.03 mg/kg in the marmoset, BIBN4096BS is up to 200-fold less potent at nonprimate receptors (Doods et al., 2000).

2.4. Materials

The following drugs were used in this study: [^{125}I]-bovine serum albumin was purchased from ICN, U.K. Capsaicin, bovine serum albumin and urethane were obtained from Sigma. Capsaicin was dissolved in ethanol and urethane in saline. Mustard oil (allyl isothiocyanate) was from Fluka Chemika, Switzerland. SR140333 ((S)1-(2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl)-4-phenyl-1-azoniabicyclo[2.2.2]octane chloride) was a gift from Dr X. Emonds-Alt, Sanofi, Toulouse, France. SR140333 was dissolved in 50 μl of ethanol and then diluted to the final volume with saline. BIBN4096BS (1-Piperidinecarboxamide, *N*-[2-[[5-amino-1-[[4-(4-pyridinyl)-1-piperazinyl]carbonyl]pentyl]amino]-1-[(3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2H)-quinazolinyl)-, [R-(R*,S*)] was a gift from Dr M. Schindler, Boehringer-Ingelheim, Germany. It was dissolved in a minimum volume of HCl (1 N), made up to the required final volume with saline and then titrated with NaOH (1 M) to return the pH to neutral.

2.5. Statistical analysis

Blood flow was assessed as the mean area under the recorded flux vs. time curve for the 60-min recording period (mm^2). Plasma extravasation was expressed as microliter of fluid per gram of tissue. Statistical analyses were carried out by analysis of variance (ANOVA) followed by Dunnett's test for the plasma extravasation time course data. Comparisons between treated and untreated mice were carried out by unpaired *t* tests, and by paired *t* tests when comparing treated and control ears on one animal. Results were all expressed as mean±S.E.M.

3. Results

Initially, the magnitude and time course of the plasma extravasation induced by topical mustard oil and capsaicin were compared. Topical application of mustard oil (20 μl of 1% solution) to the ears of wild type mice produced significant plasma extravasation, although it was reduced (approximately 66% less at the 60-min time point) compared to that to capsaicin (Fig. 1). In addition to the difference in the magnitude of the neurogenic response to mustard oil, compared to capsaicin, a difference in the time course was also revealed. The plasma extravasation in response to capsaicin increased throughout the 60-min measurement period. In contrast, the plasma extravasation stimulated by mustard oil reached its maximum value within 10 min and

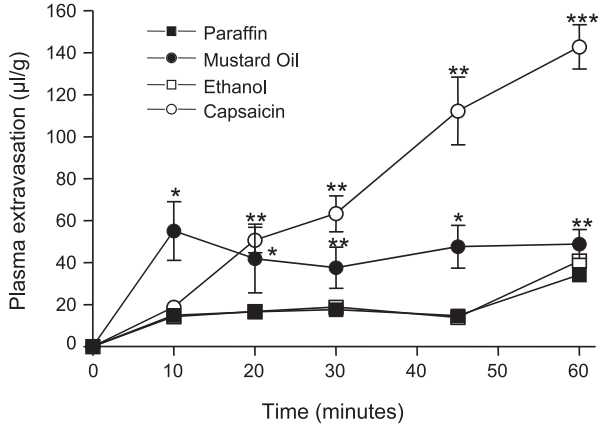

Fig. 1. The time course of the plasma extravasation response to topical mustard oil (1%), capsaicin (10 mg/ml) and their respective controls measured in the ears of Sv129+C57BL/6 wild type mice. Results are expressed as microliter of fluid accumulated per gram of tissue, mean ±S.E.M., n=5–9. *P<0.05, **P<0.01, ***P<0.001 compared to ethanol/paraffin-treated ears.

then remained constant for the remainder of the experiment. Expression of cutaneous blood flow as mean Doppler flux (Fig. 2) shows that the increase in blood flow caused by mustard oil also occurs more rapidly than that to capsaicin and is of a greater magnitude. The vasodilatation to mustard oil reaches a maximum value within 5–10 min, whereas the vasodilatation to capsaicin is only maximal after 15 min.

The effects of mustard oil (1%) on plasma extravasation and blood flow in both tachykinin NK_1 receptor wild type and knockout mice over 60 min are summarised in Figs. 2 and 3. Mustard oil did not induce plasma extravasation in the ears of tachykinin NK_1 receptor knockout mice (Fig. 3A). Concurrent measurement of the increased blood flow produced by mustard oil showed that a significant increase was produced in both wild type and knockout mice

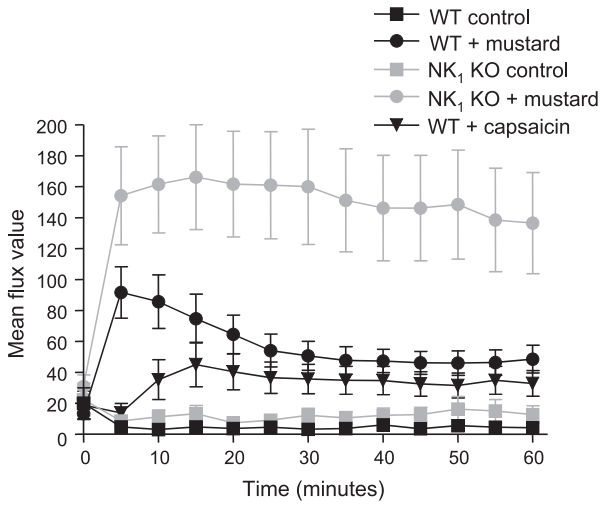

Fig. 2. The effect of topical mustard oil (1% solution) on blood flow changes in the ears of Sv129+C57BL/6 wild type and tachykinin NK_1 receptor knockout mice, measured over a 60-min period. Data from wild type mice treated with capsaicin are shown as a comparison. Results are expressed as measured flux value, mean±S.E.M., n=8.

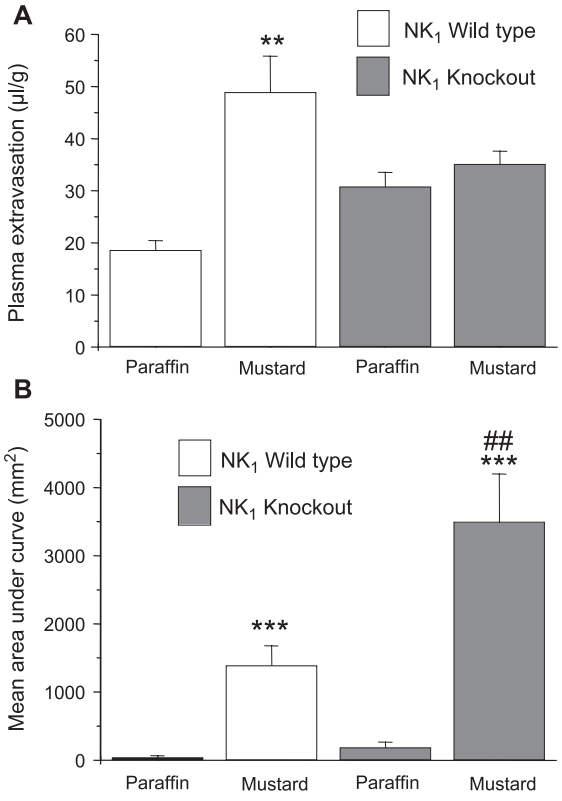

Fig. 3. Comparison of effects of topical mustard oil (1%) or paraffin (vehicle control) on (A) plasma extravasation and (B) blood flow in the ears of wild type and tachykinin NK_1 receptor knockout Sv129+C57BL/6 mice. Results are expressed as (A) microliter of fluid accumulated per gram of ear tissue and (B) area under the flux curve, mean±S.E.M., n8, **P<0.01, ***P<0.001 compared to paraffin-treated control. ##=P<0.01 compared to mustard oil-treated wild type.

(Fig. 3B). The increase in blood flow was significantly greater in tachykinin NK_1 receptor knockout mice than in their wild type counterparts.

Pretreatment of wild type mice with the CGRP antagonist BIBN4096BS (0.3 or 3 mg/kg) had no effect on the vasodilatation induced by mustard oil (Fig. 4A). However, in tachykinin NK_1 receptor knockout mice, BIBN4096BS (0.3 mg/kg) substantially inhibited the response produced by the same dose of mustard oil (Fig. 4B). A small but significant residual vasodilatation to mustard oil remained in the presence of BIBN4096BS (0.3 mg/kg) but was abolished by a dose of 3 mg/kg.

The response to mustard oil of mice in which the gene for αCGRP was disrupted was also studied to test whether release of CGRP is essential for neurogenic vasodilatation in the mouse. The tachykinin system in these mice remains intact, and so, as expected, mustard oil (1%) produced significant plasma extravasation 60 min after application (Fig. 5A). This plasma extravasation was not reduced compared to the response seen in wild type C57BL/6 mice. A significant vasodilatation to mustard oil was also seen in these mice (Fig. 5B), raising the possibility that substance P is mediating neurogenic vasodilatation. The mice were pretreated with the tachykinin NK_1 antagonist SR140333

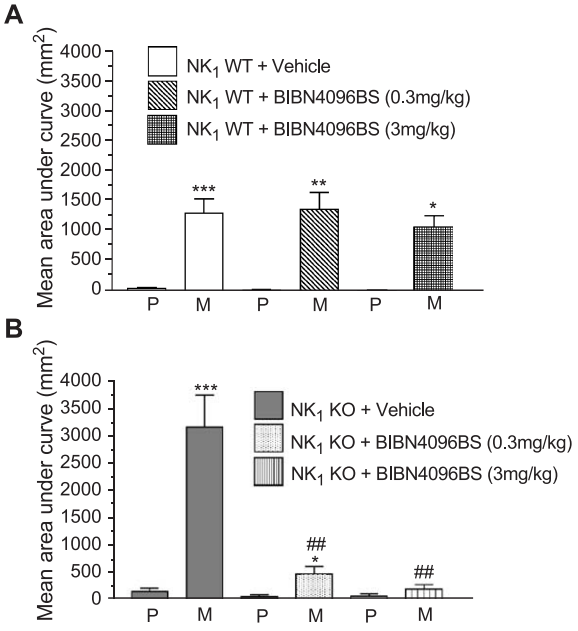

Fig. 4. Comparison of the effects of mustard oil (M; 1%) or paraffin (P) in the presence or absence of BIBN4096BS (0.3 or 3 mg/kg) on blood flow in the ears of (A) wild type and (B) tachykinin NK_1 receptor knockout Sv129+C57BL/6 mice. Results are expressed as area under the flux curve, mean±S.E.M., n=3–9. *P<0.05, ***P<0.001 compared to paraffin-treated controls. ##=P<0.01 compared to vehicle-treated control.

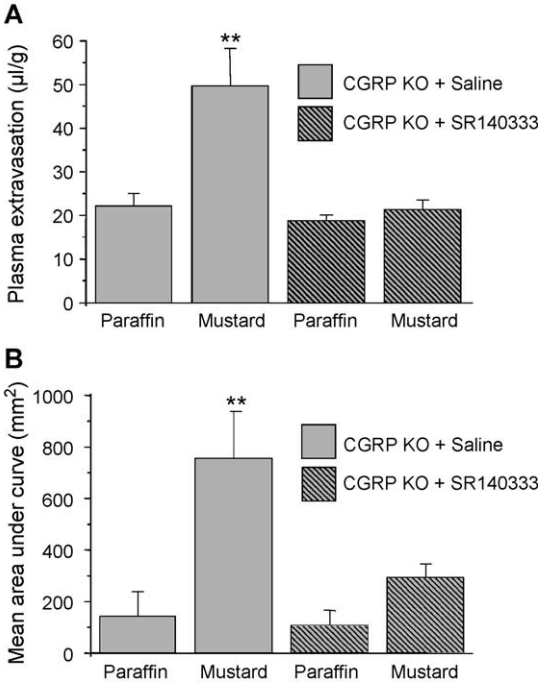

Fig. 5. Comparison of effects of topical mustard oil (1%) on (A) plasma extravasation and (B) blood flow in the ears of αCGRP knockout C57BL/6 mice in the presence of SR140333 or saline (vehicle control). SR140333 was administered at a dose of 480 nmol/kg. Results are expressed as (A) microliter of fluid accumulated per gram of ear tissue and (B) area under the flux curve, mean±S.E.M., n=4–8. **P<0.01 compared to paraffin-treated ear.

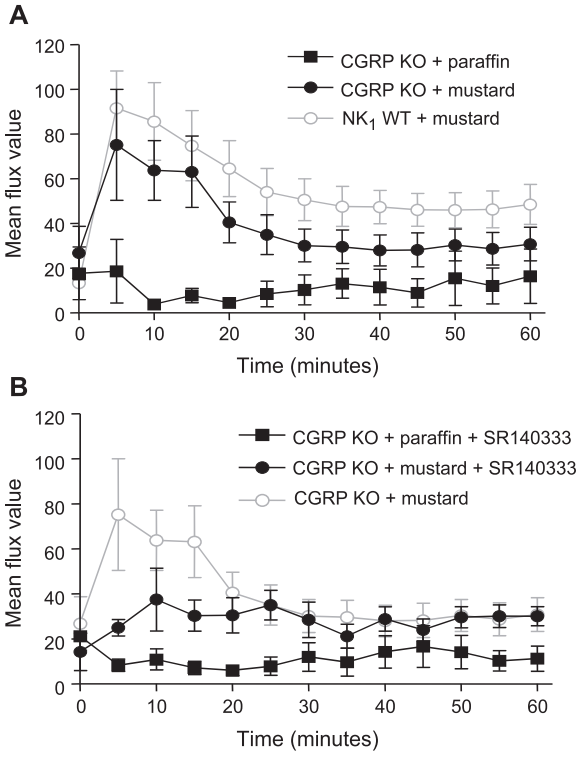

Fig. 6. Effect of topical mustard oil (1% solution) on blood flow to the ears of αCGRP knockout mice in (A) the absence or (B) presence of SR140333 (480 nmol/kg), measured over a 60-min period. Data for mustard oil-treated tachykinin NK_1 receptor wild type mice are shown as a comparison. Results are expressed as measured flux value, mean±S.E.M., n=4–8.

(480 nmol/kg) before application of mustard oil, to block any effect of a tachykinin NK_1 receptor agonist. As expected, this abolished the plasma extravasation (Fig. 5A). In addition, rather than potentiating the neurogenic vasodilatation, as is seen in tachykinin NK_1 receptor knockout mice (Fig. 3), it produced an inhibition of the response, almost totally blocking it (Fig. 5B). This suggests that the neurogenic vasodilatation in these mice is produced by release of substance P or the related tachykinin neurokinin A from the sensory neurons.

The similarity in shape between the changes in measured flux produced by mustard oil in Sv129+C57BL/6 wild type mice and those in αCGRP knockout mice are apparent from a graph of mean flux value against time (Fig. 6A). A peak response is reached within the first 5 min, and then the increased blood flow declines until around 30–40 min when it stabilizes for the remainder of the measurement period. In contrast, after treatment with SR140333 (Fig. 6B), there is no initial peak in the blood flow. It remains relatively constant at a level not much greater than that seen in the control ear for the whole period of study.

4. Discussion

The mustard oil-induced inflammatory response is generally believed to occur as a result of activation of

sensory nerves and the release of inflammatory neuropeptides (Jancso et al., 1967, 1977; Inoue et al., 1997). The experiments in this study were performed to determine whether the inflammatory response to mustard oil is mechanistically similar to that produced by capsaicin, a well-characterised stimulant of the neurogenic inflammatory response, in the mouse ear. The mediator of mustard oil-induced vasodilatation was a particular focus, as mustard oil has been primarily considered in terms of its ability to stimulate plasma extravasation (Lembeck et al., 1992; Inoue et al., 1997), and mustard oil-induced vasodilatation in the mouse has not previously been studied. A potential regulatory role for substance P in mustard oil-induced vasodilatation, as is seen with capsaicin-induced neurogenic vasodilatation (Grant et al., 2002), was also investigated.

The inflammatory response induced by mustard oil differs from that to capsaicin in both its time course and magnitude. Both the plasma extravasation (Fig. 1) and the increase in blood flow (Fig. 2) occur more rapidly after mustard oil application than after capsaicin. However, the magnitude of the plasma extravasation produced over 60 min by mustard oil is less than that to capsaicin (Fig. 1), whilst the magnitude of the increase in blood flow over the same period is much greater (Fig. 3B). Capsaicin and mustard oil are both thought to produce their inflammatory effects via activation of sensory neurons, leading to the release of the vasoactive peptides substance P and CGRP (Lembeck and Holzer, 1979; Lundberg et al., 1985; Brain et al., 1985), as responses to both are ablated following sensory denervation (Jancso et al., 1977; Inoue et al., 1997). It is thus unlikely that differences in the cellular targets or identities of released peptides contribute to the differences in the inflammatory response. It has been suggested that mustard oil can produce nonneurogenic effects (Banvolgyi et al., 2004), although they occur over a time scale of several hours after treatment and so are not relevant to the models used in this study. Thus, the differences in capsaicin and mustard oil responses are most likely to be due to differences in the time course and mechanism by which these molecules interact with the sensory fibres.

The lesser response to mustard oil in these mice may imply that mustard oil is less potent than capsaicin as a stimulator of plasma extravasation. However, plasma extravasation to capsaicin seems to be an “all-or-nothing” response (Grant et al., 2002), whereas plasma extravasation to mustard oil, assessed by Evans blue accumulation, shows a high degree of dose-dependence (Inoue et al., 1997), so a greater concentration of mustard oil may have evoked a greater response. The reason that capsaicin-induced plasma extravasation increases throughout the 60 min whereas mustard oil-induced plasma extravasation reaches a maximum within 10 min is also unclear. Confirmation of the importance of substance P in the plasma extravasation produced by both mustard oil (Fig. 3A) and capsaicin (Grant et al., 2002) is given by the absence of any increase in plasma extravasation in tachykinin NK_1 receptor knockout mice. The fact that both compounds stimulate sensory neurons to release a tachykinin NK_1 receptor agonist indicates that the differences in time course of the plasma extravasation must be due to different temporal patterns of activation of sensory neurons or release of the agonist. The lack of desensitisation of the oedema response to mustard oil (Inoue et al., 1997; Banvolgyi et al., 2004) suggests that it is independent of TRPV1 receptor activation. Instead, a receptor with different kinetic properties to TRPV1, possibly ANKTM1 (Jordt et al., 2004), may mediate the actions of mustard oil. Alternatively, the faster onset of mustard oil induced inflammation may indicate that it penetrates the skin and reaches the nociceptive fibres faster than capsaicin, or a combination of these mechanisms may apply.

The neurogenic inflammation induced by mustard oil is similar to capsaicin (Grant et al., 2002) in that, in tachykinin NK_1 receptor knockout mice, plasma extravasation is entirely absent, whilst vasodilatation greatly increases compared to wild type mice (Fig. 3). This supports our hypothesis that the tachykinin receptor plays a direct role in the control of neurogenic vasodilatation (Grant et al., 2002). Treatment with the CGRP antagonist BIBN4096BS at a dose up to 3 mg/kg was unable to inhibit the increase in blood flow produced by mustard oil in wild type mice (Fig. 4A), suggesting that, as with capsaicin (Grant et al., 2002), the primary neurogenic vasodilator in the wild type mice is not CGRP. By comparison, in the absence of tachykinin NK_1 receptors in tachykinin NK_1 receptor knockout mice, BIBN4096BS (0.3 mg/kg) was able to significantly inhibit the mustard oil-induced increase in blood flow (Fig. 4B). A small but significant vasodilatation remained, but this was abolished by treatment with BIBN4096BS (3 mg/kg; Fig. 4B). This reflects the situation seen with capsaicin, where the neurogenic vasodilatation was only susceptible to inhibition by a CGRP antagonist in tachykinin NK_1 receptor knockout mice or wild type mice treated with a tachykinin receptor antagonist (Grant et al., 2002). Conversely, a tachykinin NK_1 receptor antagonist was only able to inhibit vasodilatation once the CGRP component had been removed (Fig. 5).

The identity of the mediator responsible for the increase in blood flow in the wild type mice and the nature of any interaction between mediators still remain unclear. The simplest explanation is that both a tachykinin NK_1 agonist and a CGRP agonist are active as vasodilators, producing redundancy, such that there is no functional alteration in the response if only one mediator pathway is blocked. The graph of flux vs. time for the mice treated with SR140333 (Fig. 6B) lacks the initial peak seen in mice in the absence of antagonist, suggesting that this peak is due to vasodilatation caused by tachykinin NK_1 receptor activation. Abolition of the increase in blood flow suggests that the mustard oil-induced increase in blood flow in the CGRP

knockout mice, and by extension in wild type mice, where the time course and magnitude of the response is similar, is primarily dependent on activation of tachykinin NK_1 receptors by substance P or neurokinin A. However, this does not explain the increase in vasodilatation to mustard oil observed in tachykinin NK_1 receptor knockout mice. The increased blood flow after removal of the tachykinin NK_1 receptor may be produced by a greater release of CGRP under these circumstances.

Overall, these data show many similarities in the inflammatory responses produced by topical application of capsaicin or mustard oil. Both produce a combination of plasma extravasation and an increased blood flow. The plasma extravasation in response to both stimuli is dependent on the activation of tachykinin NK_1 receptors. In wild type mice, the presence of functional tachykinin NK_1 receptors seems to inhibit the release of CGRP, leading to vasodilatation primarily mediated by a tachykinin NK_1 agonist. This may be related to our finding that tachykinin NK_1 receptors on the peripheral terminals of sensory neurons modulate the release of substance P in the mouse (Lever et al., 2003). Activation of these neuronal tachykinin NK_1 receptors may inhibit release of CGRP, either from the same neurons as the tachykinin NK_1 receptor agonist or from a distinct subset of CGRPergic neurons. When tachykinin NK_1 receptors are blocked or deleted, there is an increase in the vasodilatation, which is sensitive to CGRP antagonists, suggesting that CGRP replaces the tachykinin NK_1 agonist as the primary mediator of neurogenic vasodilatation. This could explain the greater size of the blood flow responses in these animals, as CGRP is a more potent dilator than substance P (Brain et al., 1985).

In conclusion, the neurogenic inflammatory response to mustard oil is produced by release of the same mediators as capsaicin. Substance P is responsible for both the vasodilatation and plasma extravasation in wild type mice, whilst CGRP replaces substance P as the primary vasodilator when tachykinin NK_1 receptors are deleted or blocked pharmacologically. The differences between activation of acute neurogenic inflammation by either mustard oil or capsaicin described here are probably due to a different rate or mechanism of activation of sensory neurons, leading to a different pattern of neuropeptide release. The nature of the mechanism of action of mustard oil remains to be determined, although the recently described effects of mustard oil on the ANKTM1 receptor (Jordt et al., 2004) suggest that this may be a plausible candidate for a molecular target for mustard oil on sensory neurons.

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