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Mustard oil excites but does not inhibit nociceptive dorsal horn neurones in the rat: a presumed effect on A-delta fibres

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1 Mustard oil was applied topically in concentrations of 10–20% to the excitatory and inhibitory nociceptive receptive fields in glabrous and hairy skin of the anaesthetized rat while recording the activity of nociceptive dorsal horn neurones. The noxious stimulus was radiant heat which is known to activate C-fibres in glabrous and hairy skin.

2 Mustard oil had little effect when applied to glabrous skin and this was attributed to poor penetration of the skin.

3 Mustard oil excited cells in the dorsal horn which were excited by noxious heat in the receptive field on hairy skin.

4 Mustard oil excited cells in the dorsal horn which were inhibited by noxious heat in the receptive field on hairy skin.

5 Inhibitory effects of mustard oil were never seen, even when applied to receptive fields in which noxious heating caused inhibition.

6 The excitatory effects of mustard oil on cells inhibited by noxious heating of the skin are attributed to the reported activation of A-delta fibres which probably masked any C-fibre activation.

Introduction

The chemical irritants capsaicin (8-methyl-N-vanillyl-6-noneamide) and mustard oil (3-isothiocyanato-prop-1-ene) produce a burning sensation when applied to the skin. Capsaicin causes a degeneration of C-fibres when given systemically to neonates (Jancso *et al.*, 1977; 1980). It only affects polymodal nociceptive C-fibres when it is given over a long period (Lynn & Pini, 1984) but when applied directly to the saphenous nerve, it also acutely blocks nervous conduction in C-fibres and in larger diameter fibres, although the effect on the latter is transient (Baranowski *et al.*, 1986). The C-fibres are initially excited, before they are desensitized (Kennins, 1982). There is less evidence available concerning the action of mustard oil. However, in a small sample of single fibre recordings topical application activated only C-fibres (Woolf & Wall, 1986).

We have recently shown (Pini & Ryall, 1986; Ryall & Pini, 1986) that noxious radiant heating of the hind limb of the rat and cat not only excites but also inhibits via a γ-aminobutyric acid (GABA)ergic mechanism the firing rate of convergent dorsal horn neurones in the spinal cord. Unlike the excitation, the inhibition is unaffected by the systemic administration of opiates (Harris & Ryall, 1986; 1988).

Since the primary afferent fibres involved in both the excitatory and inhibitory responses to noxious heat are believed to be C-fibres, both types of response should be reduced by capsaicin. In a preliminary study with topical application of capsaicin to glabrous or hairy skin or by direct application to the sciatic nerve (unpublished data), we were unable to show an unequivocal change in either the excitatory or inhibitory responses to noxious heating. This failure was attributed, in part, to poor penetration of the agent to the receptors, coupled with difficulties in recording from a single neurone over several hours and the fact that no recovery from the drug action could be expected.

The alternative approach, described in this paper, was to determine whether the topical application of mustard oil to the cutaneous receptive field could evoke both the inhibition and the excitation of the dorsal horn neurones.

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Some of the data were presented to a meeting of the British Pharmacological Society (Harris & Ryall, 1987).

Methods

Experiments were performed on seventeen male Wistar rates (Bantin & Kingman, Hull) anaesthetized with thiobutabarbitone (Inactin, Byk; 100–118 mg kg^{-1} i.p.) and supplemented with intravenous administration as required. Rectal temperature was monitored and maintained at 37 ± 1°C with a thermostatically controlled heating pad. The spinal cord was exposed by a laminectomy over the lumbar vertebrae (L1–L3) and the cord was transected in the lower thoracic region. The dura mater was carefully opened and the exposed cord was covered in a pool of paraffin oil.

Recordings were made from nociceptive dorsal horn neurones in laminae III to V with glass microelectrodes filled with 4 M NaCl. The resistances were within the range of 2–5 MΩ. Extracellular recordings of action potentials were monitored on an oscilloscope. They were discriminated from the background activity and counted by a ratemeter which gave a continuous record of firing frequency on a chart recorder.

Cells were characterized according to their responses to noxious and non-noxious stimuli from ipsilateral receptive fields on the glabrous and hairy skin on the hind limb. Both excitatory and inhibitory receptive fields have been demonstrated on the same neurone (Pini & Ryall, 1986 and unpublished data). The nociceptive receptive fields were therefore carefully mapped on the skin in order to apply mustard oil to specific parts of the field.

Noxious stimuli consisted of radiant heat pulses of 15 to 30 s in duration from a 100 W projector lamp, focused by a parabolic reflector. The pulses were applied to the ipsilateral plantar surface of the foot or the hairy skin of the inner aspect of the thigh, at temperatures between 45–52°C which were under feedback control from a thermistor placed in the centre of the heated area, as previously described (Clark & Ryall, 1983; Harris & Ryall, 1987).

Mustard oil (BDH) was diluted to 10% and 20% with paraffin mineral oil and painted on to a small area of the ipsilateral receptive field with a fine brush: this restricted the spread of the oil to an area which was smaller than the receptive field of the neurone under study.

Results

Mustard oil was applied topically to the receptive fields of nociceptive dorsal horn neurones in an attempt to correlate the responses to mustard oil with excitatory or inhibitory responses obtained with noxious radiant heating of different areas of the skin.

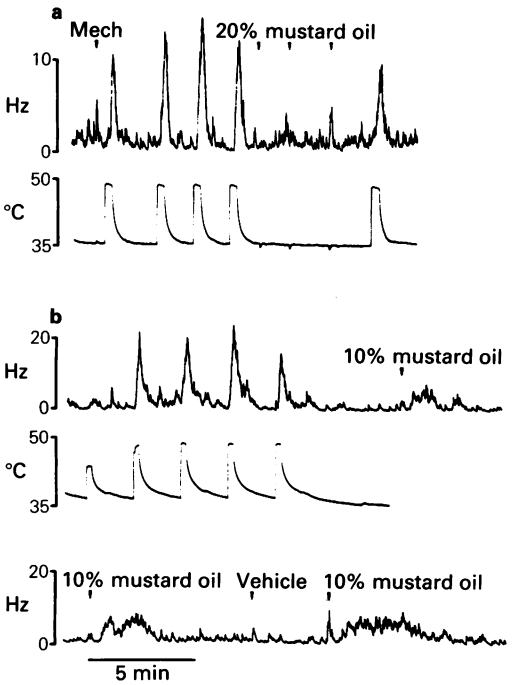

Figure 1 Effects of topical applications of mustard oil to two dorsal horn neurones with excitatory receptive fields on the glabrous skin of the foot (a) and the hairy skin of the thigh (b). The frequency of firing is shown in Hz. Skin temperature at the heated area is plotted in °C in (a) and when the skin was heated in (b). The response to light touch is shown in (a) (Mech). On glabrous skin, the slight increase in firing after the application of 20% mustard oil is due to mechanical stimulation during the application. On hairy skin (b), 10% mustard oil produced a reproducible delayed activation of the cell which lasted for about 3 min. The threshold for excitation by radiant heat was about 45°C, which is just above the noxious threshold. This was typical of the cells examined. The effect of vehicle alone is shown in (b) (Vehicle).

Mustard oil at 10 and 20% dilutions, applied to the receptive fields on the glabrous skin, had minimal effects on the firing rates of two cells activated or on six cells inhibited by noxious radiant heat (Figures 1a and 2a).

The lack of effect of the mustard oil on the glabrous skin could be attributed to a failure of mustard oil to penetrate the skin adequately. Subsequently, we performed similar experiments on the thinner, hairy skin of the thigh. Noxious radiant heating of

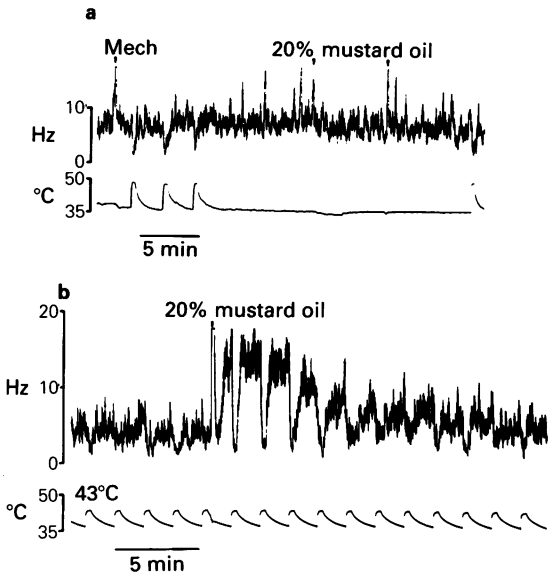

Figure 2 Effect of topical applications of mustard oil to two dorsal horn neurones with inhibitory receptive fields on the glabrous skin of the foot (a) and the hairy skin of the thigh (b). The frequency of firing is shown in Hz. Skin temperature at the heated area is plotted in °C. There was no effect when the oil was applied to glabrous skin (a). On hairy skin (b), the application of mustard oil increased the firing rate to about 15 Hz which slowly recovered over a period of 20–30 min. During the whole of this period the inhibitory responses to noxious heating of the skin were more prominent than before. In (b) the temperature was set to be just threshold for the inhibitory response during skin heating.

the hairy skin produced either excitatory or inhibitory responses on dorsal horn neurones. The areas producing inhibition usually extended over a large part of the skin of the thigh and it was only rarely possible to demonstrate excitation from a surrounding area, despite thorough searching with the stimulus. Administration of the vehicle produced only a slight, transient activation of cells (Figure 1b) as a result of the activation of mechanoreceptors as the liquid was applied to the skin.

The effects of mustard oil at 10 and 20% applied to the receptive fields in hairy skin of cells recorded in the dorsal horn are summarized in Table 1 and illustrated in Figures 1b and 2b.

On four cells excited by noxious heat, a 10% mustard oil solution applied to the excitatory receptive field on hairy skin increased the firing rate of three of them and had no effect on the other. Figure 1b shows such an excitation by 10% mustard oil. There was a delay of 30–60 s before any effect of the irritant oil was seen and the effects lasted for 4–5 min.

A stronger solution (20%) of mustard oil on the excitatory field excited all three of the cells on which it was tested. The responses to the 20% solution had a faster onset of action than the 10% solution and lasted for a longer period of time, between 10–20 min.

On four dorsal horn neurones that were inhibited by noxious heating of the hairy skin, 10% mustard oil, applied only to the inhibitory receptive fields, excited one of these cells but had no effect on the other three.

The application of 20% mustard oil produced more convincing effects and repeatedly increased the firing rates of five of the six cells which were inhibited by noxious heating (Figure 2b). In Figure 2b the inhibitory responses to noxious heat were visible before the application of mustard oil, but were far more evident when superimposed on the excitation produced by the topical application of the oil to the cutaneous inhibitory receptive field. Most importantly, on no occasion was a decrease in the firing rate seen following the topical application of mustard oil, even when recordings were continued for up to two hours after the application of mustard oil.

It is evident that there was no correlation between the ability of mustard oil to excite nociceptive neurones and the manner in which they responded to noxious heating of the skin and that the oil never caused inhibition.

Table 1 Effect of mustard oil on dorsal horn cells

	Concentration of mustard oil						
	10%	*20%*	*10%*	*20%*	*10%*	*20%*	
	Effect of mustard oil						
Effect of heat on firing rate	Excitation		No effect		Inhibition		
	Number of cells						*Total*
Excitation	3	3	1	0	0	0	7
Inhibition	1	5	3	1	0	0	10
Totals	4	8	4	1	0	0	17

The oil was applied to the receptive fields on hairy skin.

Discussion

Unlike capsaicin, which failed to show any convincing effects when applied to hairy or glabrous skin or directly to the sciatic nerve in our preliminary study (unpublished data), mustard oil produced repeatable and convincing effects, particularly on hairy skin: the effects were less pronounced on glabrous skin. However, the only effects observed were excitatory in nature and were relatively short lasting and rapid in onset. In particular, mustard oil never caused inhibition, even when the agent was administered to parts of the cutaneous receptive field which caused only inhibition when activated by noxious radiant heat. It is unlikely that the oil spread to adjacent excitatory parts of the receptive field because it was applied to a small region with a fine brush, rather than being applied in a droplet, and in many instances no excitatory adjacent field could be demonstrated, despite a thorough search.

It was therefore rather disappointing that mustard oil did not behave as we expected of an agent which should selectively excite cutaneous C-fibres, with minimal or no excitation of A-fibres (Woolf & Wall, 1986) and so cause excitation of nociceptive dorsal horn neurones when applied to an excitatory region of the receptive field, but inhibition when applied to an inhibitory part of the field.

Woolf & Wall examined the effect of mustard oil on eight A fibres. During the course of our study Heapy *et al.* (1987) found that the topical application of 5% mustard oil in paraffin, a concentration even less than that used in our experiments or in those of Woolf & Wall, caused an increase in activity in both A-delta and in C-fibres in multi-unit recordings. The increase in A-delta activity was fairly transient but the increase in C-fibre activity was prolonged. Thus the selectivity of mustard oil for C-fibres is not as good as we had anticipated when we began our experiments.

The time course of the excitation of dorsal horn neurones by mustard oil in our experiments is more consistent with the demonstrated (Heapy *et al.*, 1987) activation of A-delta fibres than with excitation of C-fibre afferents. It is therefore probable that the excitatory effect which we observed was due predominantly to the activation of the myelinated afferent fibres by mustard oil. This may have masked any excitation of slower onset due to activation of the C-fibres.

The situation with the expected inhibitory action of mustard oil is more complex and the effect was opposite in direction to that which we had anticipated. We believe (Pini & Ryall, 1986; Ryall & Pini, 1986 and unpublished) that the inhibitory effect of noxious radiant heat on the dorsal horn neurones is due mainly to activation of C-fibres which relay with GABAergic interneurones, which in turn synapse on the deeper dorsal horn cells. Activation by mustard oil of A-delta fibres, with excitatory pathways to the dorsal horn neurones, may have masked any inhibition due to activation of C-fibres with inhibitory connections to the cells. Alternatively, it is possible that different populations of C-fibres are activated by heat stimuli and by mustard oil. However, this would necessitate the postulate, which is in our view improbable, that those fibres excited by the chemical irritant are always excitatory to the dorsal horn neurones whereas those excited by noxious heat may cause either excitation or inhibition.

An interesting conclusion from these experiments, taken in conjunction with the effect of mustard oil on A-delta fibres (Heapy *et al.*, 1987), is that pain caused by the activation of A-delta afferents may be attenuated under some circumstances by coincident activation of C-fibre nociceptors at the same location in the receptive field.

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