fore treatment. The hypoalgesic method was administered just after the baseline VAS rating of the Von Frey filament had been performed.

After being seated, video or N₂O was administered (except in the control situation) and after a few minutes of adaptation the scaling treatment began. Halfway through and immediately after scaling was completed in one quadrant, the patients scored their perceived pain and unpleasantness from the scaling on 100-mm VAS. The VAS were all labelled with the statements “not at all painful” and “extremely painful” and, “not at all unpleasant” and “extremely unpleasant” in either end. In addition, the patient was asked the question “Did the nitrous oxide/video glasses have any effect on your overall experience of the dental treatment situation.” The patient could choose between the pre-stated answers: “positive effect,” “no effect,” or “negative effect.” The same dental hygienist performed the treatment in all patients and both sessions. This dental hygienist was not informed about the nature of the experiment or otherwise involved in this. The data were analysed using a mixed model with random differences between persons and between days within a person, and systematic differences between the treatment sequences, the four quadrants, and the first and second sessions, as well as the fact that each subject has two video observations. The effect of the method was defined as the difference in VAS score of the pain intensity during and before treatment (before–during). The level of significance was \( p < 0.05 \).

3. Results

There was no difference in the scores on the Corah dental anxiety scales between the two sessions (first session: mean 7.4, SD 2.4 and second session: mean 7.2, SD 2.4). There were similarly no significant differences between sessions, neither for the baseline VAS scores of pain intensity (\( p = 0.23 \)) nor the VAS scores during treatment with video glasses (\( p = 0.39 \)) using the Von Frey filament as the painful stimulus.

Table 1 shows the VAS scores for pain intensity and unpleasantness induced by the dental scaling procedure. No significant hypoalgesic effect was found of video glasses on the perceived pain (\( p = 0.85 \)) or unpleasantness (\( p = 0.73 \)) nor of N₂O (\( p = 0.69 \) and \( p = 0.51 \), respectively) compared with the control situation. There was similarly no significant difference between VAS scores in the video glasses and N₂O situation (\( p = 0.48 \) for pain, \( p = 0.58 \) for unpleasantness). Thus, the null hypothesis could not be rejected.

The perceived pain intensity of the Von Frey filament stimulus found during the administration of both video glasses and N₂O was significantly lower compared with the control situation (\( p = 0.008 \) and \( p = 0.001 \), respectively) (Table 2). No statistically significant difference in perceived pain intensity was found between the N₂O and the video situation for the Von Frey stimulus (\( p = 0.07 \)). Additionally, no gender difference was found in treatment effects, but males generally rated the perceived pain intensity of the baseline Von Frey filament stimulus significantly higher than females (\( p = 0.003 \)). The difference between the baseline pain rating of the Von Frey filament and the rating during the three treatment situations (including the control) was greater with increasing age, i.e., with a non-dental pain stimulus the treatment effect in all three groups (video glasses, nitrous oxide, and control) were significantly correlated to the age of the subjects (Spearman’s \( r = 0.47 \); \( p = 0.016 \). No other age-related effects were detected.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The VAS scores for pain intensity and unpleasantness induced by dental scaling rated immediately after the treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dental pain ( N = 26 )</td>
<td>Video</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>VAS pain</td>
<td>16.9</td>
</tr>
<tr>
<td>VAS unpleasantness</td>
<td>13.2</td>
</tr>
</tbody>
</table>

There were no significant differences between the treatments (\( p > 0.05 \)).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Baseline skin stimulation with Von Frey filaments to the upper lip before treatment and the same stimulus during the treatment sequences (video, control, and nitrous oxide)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Von Frey filament ( N = 26 )</td>
<td>Video</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>VAS baseline</td>
<td>38.1</td>
</tr>
<tr>
<td>VAS during treatment</td>
<td>25.2</td>
</tr>
<tr>
<td>VAS difference (effect)</td>
<td>12.9*</td>
</tr>
</tbody>
</table>

* \( p = 0.008 \).  
\( p = 0.001 \) (compared to control).
To the question if the N₂O and video distraction had any effect on their overall experience of the dental treatment situation, 21 of the 26 patients stated a positive effect in the video situation, 1 stated no effect, and 4 stated a negative effect (confidence interval 6–20 for \( p = 0.01 \) when \( N = 26 \)). In the N₂O situation, 17 stated a positive effect, 2 no effect, and 7 a negative effect of N₂O.

4. Discussion

In the present study, the treatment was provided in two clinical sessions one week apart. This might introduce a source of error if there were differences in the patient’s level of anxiety between the two sessions. The Corah anxiety scale was therefore used to determine any such differences in levels of dental anxiety between the two sessions. The patients’ dental anxiety level observed in the present study was in line with the mean anxiety score of 7.2 on the Corah dental anxiety scale recorded for 750 patients from dental school clinics, and far below the mean of 17, which has been reported for a group of dental phobics (Corah et al., 1978). The influence of dental anxiety/phobia is therefore thought to be very low in this study.

The Von Frey filaments were included in the study to obtain a standardised, non-dental pain stimulus to the skin. At baseline, an individual patient score around 50 on the 100-mmVAS for perceived pain was desired. The finding of a hypoalgesic effect of the video glasses and of N₂O to the painful skin stimuli evoked by the Von Frey filament is comparable to previous findings of an effect of video glasses on skin pain in cold pressor tests and burn wound care (Bentsen et al., 1999, 2000; Hoffman et al., 2000a,b). These findings suggest that patients’ sensitivity to cutaneous stimuli may be influenced by distraction methods.

In a recent study on anxiety and behaviour during dental treatment in children (Sullivan et al., 2000), no effect of glasses imparting virtual reality scenes was found. Others have been able to distract patients to some extent with video-comedy and -games in a dental filling situation (Seyrek et al., 1984). The lack of effect of video and N₂O on the perceived pain and unpleasantness during ultrasonic scaling in the present study is, on the other hand, consistent with the findings in a recent clinical study by the authors (Bentsen et al., 2001). In this study, the pain was induced by preparation for a dental filling. The pain induced by cavity preparation was of moderate intensity (mean VAS in the control situation = 35) compared with the low intensity of pain produced by dental ultrasonic scaling (mean VAS in the control situation = 15). In experimental cold pressor pain, the pain perceived by the volunteers was considerably stronger (mean VAS in the control situation = 52). Thus, in both clinical treatment situations, there was no effect on dental pain of the video glasses, but the intensity of pain was lower than in the experimental settings. Inhalation of N₂O in combination with oxygen has for a long time been known to provide pain relief and we had therefore expected a hypoalgesic effect of N₂O. The pain perception during N₂O administration was, however, not significantly different from that seen under the influence of video glasses. Nor was there any effect of N₂O administration or video glasses compared with the control situation. Previously, some studies have shown a significant effect of N₂O on pain tolerance and on pain threshold with electrical stimulation of the dental pulp (Dworkin et al., 1983) and on tactile stimulation with Von Frey filaments and pressure pain to the skin (Sibut et al., 1999) while others have failed to show a significant difference between compressed air and N₂O on labour pain (Carstontiu et al., 1994). As mentioned by Sprehn et al. (1994), there are only a limited number of controlled, clinical studies on the pain alleviating effect of N₂O, which is quite surprising in consideration of the widespread use of the method.

We can speculate only on the physiological mechanism that no effect of video glasses was found on pain when the painful stimulus was dental scaling whilst an effect could be demonstrated with painful skin stimuli. The general lack of a hypoalgesic effect of video glasses in dental treatment situations (Bentsen et al., 2001; Sullivan et al., 2000) together with a significant effect on cold pressor pain (Bentsen et al., 1999, 2000) and clinical skin pain (Hoffman et al., 2000a,b) could therefore suggest that dental pain is more difficult to manage than, e.g., skin pain. Both sensory-disterminative components of the pain experience in addition to affective-motivational aspects could underlie such differences. It is likely that scaling of the dental surface in the present study will have activated free nerve endings in several oral tissues including the tooth pulp, periodontal ligament, and gingiva. These free nerve endings could have nociceptive function and in particular be associated with Aδ-mechano-thermal and polymodal C-fibres (Matthews and Sessle, 2001; Sessle, 1987). The properties of some of these nociceptive fibres could be different from those in spinal nerves (Sessle, 1987). Furthermore, the tooth pulp is extremely richly innervated with free nerve endings and previous experiments have demonstrated a strong temporal and spatial summation of painful stimulation of the tooth pulp (Brown et al., 1985; Virtanen et al., 1987). Finally, there is a disproportionately large representation of the orofacial region in higher levels of the somatosensory system (Sessle, 1987). In addition to these suggestions, a number of differences in the affective-motivational responses between clinical dental pain and experimental pain (cold pressor pain in the arm and mechanical stimulation of the skin) may help explain the differential effect of the video glasses.
Suppression of motor evoked potentials in a hand muscle following prolonged painful stimulation

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Abstract

Earlier investigations have shown that stimulation of peripheral afferent nerves induces prolonged changes in the excitability of the human motor cortex. The present study compared the effect of experimental pain and non-painful conditioning stimulation on motor evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) in the relaxed first dorsal interosseous (FDI) and flexor carpi ulnaris (FCU) muscles. The MEPs were measured in 10 healthy subjects, and stimulus–response curves were generated before and after each of four stimulation paradigms conducted in random order on separate occasions: (a) control; (b) "dual stimulation" consisting of electrical stimulation of the FDI motor point paired with TMS; (c) painful infusion of hypertonic saline in the FDI muscle; and (d) pain combined with dual stimulation. There were no significant changes in FDI MEPs following the control paradigm, and dual stimulation induced an increase in the FDI MEPs only inconsistently. In contrast, the painful stimulation and the combined pain and dual stimulation paradigms were followed by significant suppression of the FDI MEPs at higher stimulus intensities. No changes were observed in the FCU MEPs following the four paradigms. In two additional subjects, the responses evoked in FDI by direct stimulation of the descending corticospinal tracts were significantly depressed following painful stimulation of the FDI, although the unar-evoked M-waves remained constant. It is concluded that muscle pain is followed by a period with profound depression of MEPs amplitudes in the resting muscle, but that these changes are at least in part due to a lasting depression of the excitability of the motoneurons in the spinal cord. Hence, painful stimulation differs from non-painful, repetitive stimulation, which facilitates the corticomotor pathway.

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Keywords: Cortical–spinal plasticity; Muscle pain; Human

1. Introduction

The organisation of the human motor cortex is known to change following various interventions that alter sensory inputs. Studies using transcranial magnetic stimulation (TMS) to monitor corticospinal excitability have shown that interventions such as amputation, immobilisation, and the learning of simple motor tasks can induce changes in both the excitability of the motor cortex and the topography of the corticospinal projection to individual muscles (Cohen et al., 1991; Liepert et al., 1995, 1999; Ridding and Rothwell, 1995; Zanette et al., 1997). Similar changes can also be induced by direct stimulation of peripheral afferent nerves with and without concurrent pairing with TMS (Hamdy et al., 1998; McKay et al., 2002; Ridding et al., 2000, 2001; Ridding and Taylor, 2001).

A number of studies have shown changes in the motor evoked potentials (MEPs) that are paired with brief, painful stimuli. It has been reported that painful stimulation of the ipsilateral finger profoundly inhibits MEP amplitude in the thenar eminence muscle but augments the response in the ipsilateral biceps brachii

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Brief, painful conditioning stimuli applied to the skin of one hand inhibit the MEPs evoked by TMS in both the contralateral and ipsilateral first dorsal intersosseus (FDI) muscles (Valeriani et al., 1999, 2001). Romaniello et al. (2000) observed no significant effect of experimental muscle pain on MEPs in the contracting masseter; however, maintaining a voluntary contraction at a target level will mask changes in corticobulbar excitability. Recently, two studies have indicated inhibitory effects of tonic muscle and skin pain on MEPs in small hand muscles (Farina et al., 2001; Le Pera et al., 2001), but the relation to TMS intensity, and the duration and intensity of the painful stimulation remain unclear. For example, the electrical stimulation that was used in the earlier studies to induce cortical reorganisation was intended to be non-painful and to activate large afferent (and efferent) fibres, although there was no effective control of the fibre types activated. The facilitation of MEPs is quite variable between subjects. It is possible that this variability is the result of lack of control of the peripheral afferent fibre groups that are activated by the stimuli, which are sometimes perceived to be uncomfortable.

The aim of the present study was to determine whether a prolonged, deep painful stimulus induces changes in the excitability of the cortico-motoneuronal projection that outlast the painful sensation, reflecting a more prolonged resetting of corticospinal excitability. Such mechanisms could underlie the inhibition of dynamic movements like mastication (Svensson et al., 1996), gait (Arendt-Nielsen et al., 1996; Graven-Nielsen et al., 1997), and shoulder movements (Madeleine et al., 1999) during experimental muscle pain, or in chronic pain syndromes (for a review see Svensson and Graven-Nielsen, 2001). The effect of painful stimulation was also compared with non-painful stimulation of peripheral afferent nerves with concurrent pairing with TMS.

2. Materials and methods

2.1. Subjects

Ten neurologically normal, right-handed subjects (two women, eight men; age range 19–54 years, mean 34.3 ± 4.0 years) volunteered for this study. Two women (ages 36 and 20 years) participated in a subsequent control experiment. The Adelaide University Committee on the Ethics of Human Experimentation approved the experimental protocols and all subjects gave their informed consent in accordance with the Helsinki declaration.

Subjects were seated comfortably with the right hand relaxed throughout the procedures. The surface electromyograms (EMG) of the FDI and flexor carpi ulnaris (FCU) muscles of the right hand were recorded. The signals were amplified in the bandwidth 20 Hz to 1 kHz, sampled at 5kHz and averaged off-line (Cambridge Electronic Design, UK).

TMS was delivered with a figure-8 coil (external wing diameter 9 cm) over the left hemisphere. The coil of the Magstim 200 magnetic stimulator (Magstim, Dyfed, UK) was oriented 45° oblique to the sagittal mid-line so that the induced current flowed in a plane perpendicular to the estimated alignment of the central sulcus. The scalp site at which the response was evoked in FDI at the lowest stimulus strength was determined, and the response threshold (RT) was defined as the stimulus intensity at which 5 out of 10 stimuli at that optimal site evoked a MEP of at least 50 μV amplitude in the relaxed FDI muscle. Stimulation of this scalp site also evoked MEPs in FCU. Stimulus–response curves were constructed in steps of 5% of maximum stimulator output from below RT to the intensity where MEP amplitude became maximal. Eight trials were averaged at each intensity, and the inter-stimulus interval was 10 s. Only trials in which the muscle was completely relaxed were included in the further analyses.

2.2. Stimulus paradigms

Four different stimulation paradigms were tested on each subject at intervals of at least one week in randomised order; each lasted for 30 min. These were: (a) Control: subjects were seated comfortably and allowed to rest but not to doze for the 30 min. (b) Dual stimulation: electrical stimulation of the motor point of FDI was paired with TMS over the contralateral FDI scalp site. Motor point stimulation consisted of trains of 10 Hz, 1 ms square waves for 500 ms, delivered at one train per 10 s. The stimulus intensity was set just below the threshold for pain. TMS (intensity 115% RT with figure-8 coil over the optimal scalp site for FDI) was given 25 ms after the onset of the train to coincide with the arrival of the afferent volley at the cortex (Stefan et al., 2000). The TMS-plus-motor point stimulation is referred to as “dual” stimulation (for more details see McKay et al., 2002; Ridding and Taylor, 2001). (c) Muscle pain: a sterile solution of hypertonic saline (5.8%) was infused continuously into FDI with a computer-controlled pump using the protocol described by Svensson et al. (1998). Subjects continuously rated their pain intensity on an electronic 0–10 cm visual analogue scale (VAS) ranging from “no pain” to “most pain imaginable”. (d) Muscle pain plus dual stimulation: the infusion of hypertonic saline into the FDI was combined with the dual stimulation paradigm.

Following each of these paradigms, the subject remained at rest for 10 min before stimulus–response curves were constructed; this delay was sufficient for the painful sensation to dissipate following the cessation of the saline infusion paradigms. Thus, the interval between the two stimulus–response curves was 40 min.
2.3. M-wave and descending tract stimulation

In two subjects, the location of the changes in excitability was investigated by measuring the response evoked in FDI by stimulation of the descending motor tracts as well as the M-wave evoked by supra-maximal ulnar stimulation at the wrist. These procedures test for changes in excitability of the motoneurone pool. The descending motor tracts were activated near the level of the foramen magnum by TMS delivered through a double-cone coil (Taylor et al., 2000; Ugawa et al., 1996). The coil junction was held over the inion so that coil current flowed in a downward direction at the junction, and the intensity was adjusted to evoke the largest possible response in FDI. Five responses were averaged prior to and 10 min after the cessation of the painful infusion of hypertonic saline into the FDI in these two subjects.

2.4. Analysis of MEPs

The peak-to-peak amplitude and onset latency of the MEPs in FDI and FCU were determined. The stimulus–response curves in the four conditions were analysed in a repeated measure ANOVA model with the factors stimulus intensity (8–10 levels) and time (two levels: before and after). The paradigms were directly compared in a two-way ANOVA model with condition as one factor (four levels) and differences between before and after values at each intensity as the other factor (8–10 levels). Post hoc comparisons were performed with Tukey honest significant difference tests with $P < 0.05$ accepted as significant. The peak pain (cm on the VAS) and area under the curve (cm s) were determined from the individual VAS recordings and compared between the two painful paradigms with a paired $t$ test.

3. Results

3.1. General features

The electrical stimulation of the FDI motor point was well tolerated by the subjects: in all cases, subjects affirmed that the stimulus was not painful. In contrast, the infusion of hypertonic saline into FDI evoked a fairly intense sensation of pain with a peak on the VAS of $5.4 \pm 0.5$ cm (means ±SEM) and an area under the curve of $7648 \pm 571$ cm s (Fig. 1). The intensity of sensation was not significantly different when the infusion was paired with motor point stimulation: the peak pain scores ($4.6 \pm 0.5$ cm; paired $t$ test: $P = 0.143$) and areas under curves ($6357 \pm 706$ cm s; paired $t$ test: $P = 0.106$). The subjects described the FDI pain using words such as “dull,” “aching,” and “tender” and indicated that it often spread to the dorsum and palm of the hand. In all subjects, the VAS scores indicated that the pain had disappeared within 10 min of cessation of the infusion, and this was confirmed by direct questioning.

The onset latencies of the MEPs (means ±SEM) were $23 \pm 1$ ms in FDI and $17 \pm 1$ ms in FCU. Latency was not influenced by the experimental paradigm (ANOVA: $F < 1.688$; $P > 0.193$) or time (ANOVA: $F < 1.345$; $P > 0.276$). The RT expressed in percentage of the maximum stimulator output ($35 \pm 2\%$) also did not change between the experimental paradigms (ANOVA: $F = 0.783$; $P = 0.514$).

The stimulus–response curves made before the intervention were reproducible across the four testing sessions, which indicates firstly that averaging eight trials gives a reproducible result, and secondly that there was no carry-over effect on excitability following any of the interventions.

3.2. Conditioning of MEPs

In the control paradigm, the statistical analysis of the amplitudes of the MEPs in FDI showed no significant effect of time (ANOVA: $F = 0.278$; $P = 0.620$), but a strong effect of stimulus intensity (ANOVA: $F = 18.977$; $P < 0.0001$) (Figs. 2 and 3). There was no significant interaction between time and stimulus intensity (ANOVA: $F = 0.563$; $P = 0.819$).

The grouped data for FDI for all 10 subjects in the four paradigms tested are shown in Fig. 2. The dual stimulation paradigm did not result in a significant main effect of time on the MEPs in FDI (ANOVA: $F = 1.135$; $P = 0.35$). Again there was a significant effect of stimulus intensity (ANOVA: $F = 21.309$; $P < 0.0001$) and no significant interaction between time and stimulus intensity (ANOVA: $F = 0.981$; $P = 0.469$).

The pain paradigm was associated with a significant effect of time on the MEPs in the FDI muscle (ANOVA: $F = 20.531$; $P = 0.020$), a significant effect of stimulus
Fig. 2. Stimulus–response curves for FDI in the four conditioning paradigms. Grouped data showing the mean (and SEM) peak-to-peak amplitudes of MEPs in FDI in 10 subjects. TMS intensity is expressed as percentage of the response threshold (% RT). The filled circles (*) show MEP amplitudes before the intervention and the open circles (o) 10 min after the pain induced by the infusion had abated. There was a significant main effect of stimulus intensity in all paradigms whereas only the pain and the combined pain-plus-paired stimulation paradigms induced significant changes in MEP amplitudes. The TMS intensities at which significant changes (Tukey: $P < 0.05$) were seen after the intervention are shown by asterisks (*).

Fig. 3. Stimulus–response curves for FCU in the four conditioning paradigms. Grouped data showing the mean (and SEM) peak-to-peak amplitudes of MEPs in FCU in 10 subjects. TMS intensity is expressed as percentage of the response threshold (% RT). The filled circles show MEP amplitudes before the intervention and the open circles after the intervention. A significant main effect of stimulus intensity and no effect of the intervention was seen in all paradigms.
intensity (ANOVA: \( F = 22.621; P < 0.0001 \)) and a significant interaction between time and stimulus intensity (ANOVA: \( F = 4.664; P = 0.0008 \)). Post hoc tests showed that the MEPs evoked by stimulus intensities of 125–140% RT were significantly smaller after the painful intervention (Tukey: \( P < 0.05 \)).

The paradigm which combined pain and dual stimulation was also associated with a significant main effect of time on the MEPs in the FDI muscle (ANOVA: \( F = 7.086; P = 0.037 \)), a significant effect of stimulus intensity (ANOVA: \( F = 36.327; P < 0.0001 \)) and a significant interaction between time and stimulus intensity (ANOVA: \( F = 6.796; P < 0.0001 \)). Post hoc tests demonstrated that the MEPs evoked by stimulus intensities of 120–140% RT were significantly smaller following this intervention (Tukey: \( P < 0.05 \)).

There was a significant interaction between time and stimulus intensity between the before and after measures of MEPs in the four different paradigms (ANOVA: \( F = 22.263; P = 0.002 \)). The two paradigms involving pain did not differ from each other, but differences in the amplitude of the MEPs were smaller (negative = suppression) at intensities above 125% RT compared to the dual stimulation and control paradigms.

Because previous studies have shown a significant increase in the amplitude of MEPs evoked at TMS intensities of 115% RT following similar dual stimulation paradigms, we tested for this effect. At this intensity, the amplitude of the MEPs in the FDI was greater in 8 of 10 subjects but this increase was not significant for the overall group.

The data that were recorded simultaneously in FCU are summarised in Fig. 3. The two-way analyses of the amplitudes of the FCU MEPs all showed significant effects of stimulus intensity (ANOVA: \( F > 11.654; P < 0.0001 \)) with no significant effects of time (ANOVA: \( F < 5.058; P > 0.087 \)) and no significant interactions (ANOVA: \( F < 0.469; P > 0.729 \)).

### 3.3. Descending tract stimulation

Unfortunately, magnetic stimulation of the descending tract does not evoke muscle responses in all subjects. We tested seven subjects with this method and found responses in only two of these. Hence, it was possible to test the excitability of the motoneuronal pool only in these two subjects with this method. The painful infusion of hypertonic saline into FDI was given as before. The peak VAS pain was 8.5 and 7.0 cm and the area under the pain curve 12102 and 9923 cm$^2$s, respectively.

Ten minutes after the painful infusion, the amplitudes of the responses evoked in FDI by both the TMS and

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**Fig. 4.** Responses evoked in FDI of two subjects by TMS and descending tract stimulation, and the M-waves before and after a prolonged painful infusion into the muscle. The fine lines show the before-pain responses and the thicker lines the responses 10 min after a 30-min painful infusion. Upper panels, MEPs evoked by TMS; middle panels, responses evoked by descending tract stimulation; lower panels, M-waves evoked by supramaximal ulnar stimulation at the wrist.
the descending tract stimulation were significantly suppressed (Fig. 4). The MEPs were reduced to 20% and 70% of their pre-pain amplitudes, and the responses evoked by descending tract stimulation to 30% and 70% of their pre-pain amplitudes, respectively. No change in M-waves was observed.

4. Discussion

This study arose from earlier observations that long periods of electrical stimulation of peripheral nerves can induce prolonged increases in the excitability of the motor cortex (McKay et al., 2002; Ridding et al., 2001). This facilitation of MEPs is quite variable between subjects, however, and indeed the MEPs were depressed following stimulation in a minority of subjects in an earlier study (C.S. Charlton, M.C. Ridding, P.D. Thompson, and T.S. Miles, unpublished observations, 2002). This raised the possibility that this depression could be the result of stimulus intensities that were high enough to activate nociceptive pathways, albeit unintentionally.

This hypothesis was confirmed in the present study by the observation that, after the pain induced by a period of painful stimulation of FDI had subsided, the amplitude of the MEPs evoked in FDI by TMS remained suppressed for more than 10 min. The responses of FCU, which was not subject to painful stimulation, were unaffected, indicating a topographical specificity in the response.

The effect of painful stimuli on MEPs has been the subject of a number of investigations. In those earlier studies, brief or tonic painful stimuli were used to condition the MEP in various muscles including FDI (e.g., Farina et al., 2001; Koller et al., 1998, 2001; Le Pera et al., 2001; Valeriani et al., 1999). Most of these studies report that painful stimuli suppress MEPs in the hand muscles for periods of up to 200 ms or several minutes. The duration of excitability changes as well as the level of the suppression (spinal or supraspinal mechanisms) has been extensively discussed. Furthermore, the function of the muscle and the site of the painful conditioning stimulus may determine whether excitatory or inhibitory responses are produced (Koller et al., 2001; Valeriani et al., 2001). However, the present investigation clearly demonstrates that the suppression of FDI MEPs continued for at least 10 min after the painful stimulation had stopped. This indicates that the mechanism underlying the present observations has a time course of many minutes, which is much longer than that of the synaptic interactions that account for the suppression seen in the conditioning-testing experiments (e.g., Valeriani et al., 1999, 2001). That is, prolonged painful stimulation induces plastic changes in the excitability of neural circuits which outlast the condition-

ing stimulus, in a manner that appeared initially to be analogous to the prolonged facilitation that is induced by non-painful stimulation.

The prolonged facilitation that is induced by non-painful stimulation originates in supraspinal, presumably cortical areas (Hamdy et al., 1998; McKay et al., 2002; Ridding et al., 2000, 2001; Ridding and Taylor, 2001; Stefan et al., 2000). In the present experiments, the responses evoked in FDI by descending tract stimulation by both the TMS and stimulation of the descending tracts were significantly suppressed in the two subjects tested with this method. These observations suggest that the prolonged depression of the MEPs induced by the 30-min painful stimulus was due at least in part to changes in spinal motoneuronal excitability. This preliminary finding is in agreement with observations of decreases in H-reflexes following experimental muscle pain (Le Pera et al., 2001), but not with the lack of changes in F-waves and H-reflexes seen following capsaicin-induced pain (Farina et al., 2001). It has been suggested that painful stimuli inhibit cortical excitability in an early phase and that the spinal motoneuronal excitability is depressed in a later phase (Le Pera et al., 2001). The present result extends the findings from Le Pera et al. (2001) by the demonstration of a differential effect of the deep painful stimulus on the MEPs evoked by different intensities of the TMS. Thus, the largest inhibitory effects were observed at higher TMS intensities (>120% RT). Larger motoneurons are recruited into the MEP by higher intensity TMS; hence, it is likely that the inhibition induced by the prolonged, deep noxious stimulus induces prolonged inhibition preferentially in larger motoneurons.

It has been shown in numerous animal models that nociceptive inputs can induce prolonged changes in the excitability of spinal cord circuits through long-term synaptic potentiation (LTP); this mechanism is believed to underlie a number of pain-related phenomena such as central hyperexcitability (see Sandküler, 2000 for review). It is therefore possible that the depression of MEPs induced by the 30-min painful infusion is an indirect result of the operation of pain-induced LTP. The induction of spinal LTP depends on the type, intensity, and duration of the nociceptive input (Liu and Sandküler, 1997, 1995). This could account for the variability of the effect of experimental pain on various reflexes and responses (e.g., Farina et al., 2001; Le Pera et al., 2001; Matre et al., 1998; Rossi et al., 1999a, b; Rossi and Decchi, 1997). That is, the effect of pain may depend inter alia on the timing of testing of the response in relation to the onset of the pain, and on the nature and intensity of the pain.

The depression of the MEPs evoked by TMS in the present study reflects depression of motoneuronal excitability; however, it was not possible to determine whether the nociceptive input also induced a concurrent
depression or facilitation of the motor cortex. There was no significant difference between the two pain paradigms, although the grouped data in Fig. 2 suggest that the MEPs tended to be less depressed when the motor point stimulation was given concurrently with the pain.

In earlier studies, prolonged facilitation of MEPs in the target muscle was induced by peripheral nerve stimulation both with and without concurrent TMS (Hamdy et al., 1998; Ridding et al., 2000, 2001; Ridding and Taylor, 2001; Stefan et al., 2000). This was not observed for the pooled data in the present study. However, the FDI MEPs were facilitated at the TMS intensity used to test corticospinal excitability in the earlier studies (115% RT) in 8 of the 10 subjects. This difference may reflect the weaker stimulus intensity used in the present study. In our earlier studies, the intensity was always set to evoke a visible twitch in the target muscle(s): this stimulus was, on occasion, mildly painful. In the present study, care was taken to keep the stimulus intensity below that which was perceived to be painful, and it did not elicit a visible contraction of the FDI in all subjects.

It is concluded that prolonged, deep painful inputs induce prolonged depression of the MEPs evoked by TMS in the target muscle but not nearby muscles, and that this effect is mediated at least in part at the spinal level.

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References


The significance of A-δ and C fibres for the perception of synthetic heat

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Abstract

Synthetic heat is a perception of strong, but not painful, heat arising when skin is stimulated by an alternating pattern of adjacent cold and warmth. This study examines the contribution of different classes of nerve fibres to this perception. In 40 subjects changes in synthetic heat and thermal perceptions were studied during a 30-min ischaemic nerve block in one reaction time, and one threshold determination task. Synthetic heat stimuli were described as hot or warm, but not as painful, and were preceded by a transient cold. Reaction times for synthetic heat stimuli did not differ from those for cold stimuli. Thresholds for synthetic heat and thermal stimuli were similar. During A fibre nerve block the perception of synthetic heat lost the cold component whereas the frequency of hot and warm descriptors did not change. The perception of cold stimuli changed, such that pure cold was replaced by dysesthetic descriptors. Reaction times and thresholds for thermal and synthetic heat stimuli increased equally during the nerve block. It is concluded that the perception of synthetic heat most likely arises from the fusion of signals dependent on unmyelinated low threshold cold and warm receptors. It is not dependent on A-δ cold fibres, and a contribution of nociceptors is quite unlikely. The possibility of a psychological contribution at the perceptual level is discussed.

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Keywords: Synthetic heat perception; Perception threshold; Reaction time; Ischaemic block; Low threshold thermoreceptors

1. Introduction

Interaction of conflicting stimuli in a sensory channel may lead to peculiar perceptions. A long known example of such a perception is the Thunberg’s phenomenon (Thunberg, 1896). Stimulation of the skin by an alternating pattern of cold and warmth elicits—after a transient cold—a perception of strong, but not painful, heat (cf. Alrutz, 1898; Burnett and Dallenbach, 1927; Cutolo, 1918; Ferrall and Dallenbach, 1930; Gritman and Dallenbach, 1929; Green, 1977; Jenkins, 1938; Sullivan and Verda, 1936; Thunberg, 1896). Such perceptions of heat evoked by opposing thermal stimuli presented to adjacent skin areas were later called synthetic heat (Ferrall and Dallenbach, 1930; see also Green, 1977). Despite numerous studies on this phenomenon a satisfactory explanation of synthetic heat has not been established during the 100 years since it was discovered by Thunberg. A few theories have been proposed that would account for synthetic heat. Alrutz (1898) tried to explain the perception as being caused by the activation of cold receptors at high temperatures (paradoxical cold) together with the warm receptors. Craig and Bushnell (1994) for the first time reported synthetic heat to be described as painful, and proposed the perception to be the result of an interaction of cold sensitive polymodal nociceptors and A-δ cold receptors.

As in man unmyelinated cold-sensitive polymodal nociceptors respond only to temperatures below 20°C (Campero et al., 1996) whereas Thunberg in his original
study used cold of 24°C, it is unlikely that nociceptors are essential for the generation of synthetic heat. Knowledge of cutaneous thermoreception is still incomplete, but there is some evidence that low threshold cold receptors have myelinated as well as unmyelinated axons. Unmyelinated low threshold cold receptors have been detected in several species (see Hensel et al., 1960; Hensel, 1966, 1981), and recently they were also discovered in man (Campero et al., 2001). Thermal reaction time studies have indicated that in humans cold sensation is mediated by myelinated and warm sensation by unmyelinated afferent fibres (Fruhstorfer, 1976; Fowler et al., 1988; Susser et al., 1999; Yarnitsky and Ochoa, 1991b). Additionally, selective nerve blocks have demonstrated that with innocuous cold stimulation myelinated axons alone mediate pure cold sensations (Fruhstorfer et al., 1974) whereas unmyelinated axons alone elicit dysesthetic sensations (Fruhstorfer, 1984; Wahren et al., 1989; Yarnitsky and Ochoa, 1990). As a consequence, signals mediated by unmyelinated low threshold cold receptors (Campero et al., 2001) and warm receptors together could synthesize the heat perception. Thunberg was not convinced that the 'cold nerves' contributed to the synthetic heat. Therefore he suggested that '...an investigation on a part of the skin affected by cold anaesthesia would settle the matter; if the perception of 'hot' in such an experiment did not lose any of its usual characteristics, the latent perception of cold would be proven unimportant' (Thunberg, 1896, p. 494, translated from Swedish).

This study tests Thunberg's hypothesis and examines possible changes in synthetic heat and thermal perception along a selective block of myelinated nerve fibres. If unmyelinated cold receptors were essential for the generation of synthetic heat whereas myelinated cold receptors (A fibres, the 'cold nerves') were not, then synthetic heat perception should not be affected by the nerve block. This hypothesis was tested in one reaction time task and in one threshold determination task. Ischaemic nerve block was used because it can easily be applied and produces anaesthesia of the whole hand thus allowing thermal stimulation of a sufficiently large area of the thenar eminence. Perceived quality of synthetic heat was carefully recorded from all participants during both experimental tasks.

2. Methods

2.1. Subjects

40 healthy women and men (15 female, 25 male; mean age 23.7 years, range 18–32 years; mostly medical students) took part in this study which was carried out at the Institute of Physiology, Marburg, and which had been approved by the local ethical committee. Subjects had no previous experience of synthetic heat stimuli.

They were paid for their participation and gave their informed consent. Within a period of two weeks they underwent two different tests, for which they received verbal and written instructions. During a test the subject was comfortably seated with his hand on the stimulator.

2.2. Reaction time test

2.2.1. Procedure

Before and during an ischaemic nerve block, cold and synthetic heat pulses were delivered to the left thenar eminence and reaction times were recorded together with the quality of the elicited sensations. Cold and synthetic heat stimuli were alternately presented at a rate of 1/min. The subject had to press a button as soon as he or she perceived the stimulus and had to state the quality of the sensation by selecting verbal descriptors from a list (cold, icy, stinging, burning, hot, warm, pain, other, and neutral). After 6 min baseline recording the test was continued for 30 min under ischaemic nerve block. The block was initiated by draining the arm from blood (lifting, elastic bandage) and rapidly inflating a blood pressure cuff around the upper arm to 50 mm Hg above systolic pressure. Subjects were instructed not to move any muscles in the blocked arm. In the reaction test, loss of touch sensibility, motor paralysis, and the intensity of cuff pain were additionally monitored.

2.2.2. Stimulator and stimulus characteristics

Water from three thermostats set to 15, 30, and 45°C continuously circulated with a high flow through an epoxy block which had six bronze plates of 35 × 8 × 0.8 mm arranged in parallel on its surface (see Fig. 1A; principle of function according to Fruhstorfer and Detering,

Fig. 1. Outline of stimulators used for the reaction task (A) and the threshold task (B) together with schematic drawings of pure (a) and mixed (b) temperature stimuli (calibration is for both stimulators; temperature was measured at the skin-thermode interface). In the reaction task, maximum temperature of the stimulator was 38.5°C for the longest stimuli (5 s) and correspondingly smaller for shorter ones. In the threshold task, possible peak temperature was limited to 53.5°C.
1974). Between stimuli, 30 °C water was directed to all six plates whilst 15 and 45 °C water just passed the thermode. For a cold pulse 15 °C water was directed to all six plates causing a rapid fall of their temperature. For a synthetic heat pulse water of 15 and 45 °C was directed to every other plate resulting in a rapid warm/cold stimulus (synthetic heat). Within the thermode water flow was diverted by back pressure valves and the three circulations always returned to their correct thermostats. The temperature change occurred without noise or vibrations of the thermode. Each stimulus was preceded by a warning beep at an interval of 1 s. The stimulus was turned off by the subject's reaction or after 5 s, and reaction time was recorded (time between onset of temperature change and push of button; resolution 1 ms). With the load of the hand the temperature at the skin-thermode interface reached an amplitude of ±7.5 °C after 1 s, and a maximum of ±8.5 °C after 5 s. As the duration of a stimulus depended on reaction time, its maximum temperature varied accordingly. Stimulation and data recording were computer controlled.

2.3. Threshold determination test

2.3.1. Procedure

Before and during an ischaemic nerve block thresholds for cold, warmth, and synthetic heat stimuli were determined by the Marstock method (Frühstorfer et al., 1976). Temperature ramps were delivered to the right thenar eminence by a Peltier thermode and thresholds were recorded together with the quality of the elicited sensations. Groups of either two pairs of cold and warmth or of four synthetic heat stimuli were alternately presented by the operator at a rate of 1/min (Fig. 1B). The subject responded to the perception of a thermal sensation by pressing a button that reversed the temperature change. After each stimulation the subject had to state perception quality by selecting a verbal descriptor from a list (see above). After recording baseline values for cold, warmth, synthetic heat and additionally for heat pain, the test was continued for 30 min under ischaemic nerve block.

2.3.2. Stimulator and stimulus characteristics

Ten ceramic Peltier modules (8.5 × 13.0 mm) were arranged to form a rectangular surface of 47 × 26 mm (Fig. 1B). The individual modules could change temperature either uniformly in the same direction (cold or warm) or alternately in the opposite direction to give a chequered warm/cold stimulus (synthetic heat). Temperature was measured by thermocouples on the surface of two opposite modules and continuously recorded on paper. Between the stimuli the adapting temperature was 27 °C; during a stimulus temperature changed at a rate of 3.5 °C. This high rate had to be used in order to achieve an approximately linear gradient over the wide temperature range to be studied. As a consequence, stimuli were rather short and the measured thresholds were unusually high. For each stimulus, means were calculated from the recorded cold and warmth values.

2.4. Statistics

The statistics of this study are mainly descriptive. To estimate the differences expected in the frequency of the verbal descriptors cold and hot between the pure cold and synthetic heat stimuli in both test situations, the Wilcoxon Signed Rank Test was calculated. The overall threshold for acceptance was set to \( p \leq 0.01 \) correcting the levels of the individual comparisons. As the descriptors pain, other and neutral were rarely used they were omitted from further analysis.

3. Results

3.1. General findings

All subjects completed both tests and except for two cases they could feel even the last stimuli. In the reaction task, slight paraesthesias appeared during the first 2–4 min. In 24 subjects paraesthesias were followed by cuff pain, which was only slight in 9 subjects, but became severe in 15 others. Touch was lost in the fingers on average after 20 min and at the wrist 3 min later. At the end of the session, hand and fingers were paralyzed. Reperfusion of the arm elicited paraesthesias of extreme intensity and often the feeling of painful muscle cramps.

3.2. Reaction task

Before the block, reaction times to cold and to synthetic heat did not differ and they continued to be the same during the block when both became gradually longer (Fig. 2). Temporal scatter of reaction times increased significantly with the loss of pure cold sensation.
Before the block the cold stimulus was described as cold or icy except for three individuals, who perceived it as hot or burning. The synthetic heat stimulus was described as hot or warm, and in 17 subjects it was preceded by a transient cold sensation. Slight burning afferent sensations often followed the synthetic heat stimuli. During the block, cold sensations to both stimuli became less frequent, and on average after 13 min, cold was not perceived any longer. Instead, the cold stimulus was described as icy, stinging, burning, or hot. When cold sensation was lost, perception of synthetic heat was still unchanged; only during the last 10 min of an experiment hot was less often perceived and burning and stinging sensations appeared (Fig. 3).

3.3. Threshold task

In the beginning of nerve block, thresholds for cold and warmth did not differ from those for synthetic heat. During the block, all thresholds increased steadily and continued to be similar until the end of the block (Fig. 4). During the last third of the experiment warm threshold and the warm side of synthetic heat threshold of 22 subjects reached the pre-block heat pain threshold (but see Fig. 5 for quality of perception).

In the beginning of the block, nearly half of the subjects described some of the warmth stimuli at threshold as hot. During nerve block the frequency of warm perceptions decreased whereas the frequency of hot remained the same; dysesthetic sensations like burning and stinging became more frequent. Cold stimuli at threshold were perceived as cold by all subjects. During nerve blocks the frequency of cold perceptions was drastically reduced.
and they had disappeared or become dysaesthetic after about 12 min (Fig. 5). Synthetic heat stimuli at threshold (baseline recordings) were generally described as hot or warm, and often were preceded by a transient cold. During nerve block the frequency of hot and warm descriptors of synthetic heat did not change whereas after 8 min the preceding cold had disappeared. During the second half of an experimental session dysaesthetic sensations appeared (Fig. 5).

3.4. Descriptor profiles

In both tasks, the frequency of the descriptors cold and hot differed significantly between pure cold and synthetic heat stimuli (Wilcoxon Signed Rank Test). In the threshold task, the use of cold was more frequent for cold than for synthetic heat stimulations \( (p = 0.004) \), and the use of the word hot was more frequent for synthetic heat than for cold stimulations \( (p < 0.001) \). In the reaction time task, the use of cold was more frequent for cold than for synthetic heat stimulations \( (p = 0.001) \), and the use of the word hot was more frequent for synthetic heat than for cold stimulations \( (p < 0.001) \). If all descriptors for cold and synthetic heat stimuli were assembled at the moment when pure cold was lost, the descriptor profiles for synthetic heat stimuli differed from those for cold stimuli by a higher frequency of hot and warm (by a factor of 1.8 and 2.2, respectively) and a lower frequency of cold, icy, and stinging (by a factor of 0.3, 0.1, and 0.4, respectively). The use of the descriptor for pain was negligible (see Figs. 3 and 5).

3.5. Further observations

In the reaction task, data from seven subjects with intense cuff pain were compared to the data of seven subjects without cuff pain. Neither reaction times throughout the experiment nor descriptors at min 19/20 differed between these groups.

4. Discussion

4.1. The perceptual quality of synthetic heat

This study shows that during selective ischaemic block of myelinated nerve fibres the perception of pure cold to innocuous cold stimuli is lost whereas the hot perception to chequered warm/cold stimuli (i.e., synthetic heat) remains, occasionally accompanied by the descriptors 'burning' and 'stinging'. The analysis of the verbal descriptors chosen to describe the quality of the perception of synthetic heat shows that the use of the descriptor 'hot' dominates, whereas the use of the descriptor 'pain' is negligible (Figs. 3 and 5). Thus, the results of this study support Thunberg's assumption that 'cold nerves'
(i.e., axons mediating pure cold sensations) are not essential for the generation of synthetic heat. This study further shows that synthetic heat sensation can be elicited in an innocuous temperature range (21–39 °C in the reaction time test), and that the perception is described merely as 'hot', and not as 'pain' (Figs. 3 and 5). In this restricted temperature range an activation of nociceptors is quite unlikely (Campero et al., 1996; Tillman et al., 1995; Torebjörk et al., 1984). Consequently, this study does not support the assertion by Craig and Bushnell (1994) that synthetic heat reflects a central unmasking of a cold-activated nociceptive channel.

When pure cold perception is lost but warmth is still perceived, innocuous cold stimuli are described as icy, stinging, burning, or hot. Comparable dysaesthetic changes of cold perception have been observed during prolonged nerve block (>30 min) by ischaemia (Yarnitsky and Ochoa, 1990) or compression (Wahren et al., 1989). In both studies a separate cold pain threshold was recorded at temperatures that always were lower than the cold threshold. Therefore the assumption seems to be justified that there are two classes of unmyelinated cold sensitive receptors, one of which is specific and has a low threshold whereas the other is a cold sensitive nociceptor activated at temperatures below 20 °C. Low threshold cold receptors with unmyelinated axons supplying the hairy skin of man have recently been detected by Campero et al. (2001). It is conceivable that the signals from these receptors are normally prevented to enter awareness by inhibition generated by myelinated cold receptors and that their signals might be used for other tasks, for example, thermoregulation.

Synthetic heat clearly differs from dysaesthetic cold in that it can be elicited when myelinated fibres are conducting. In both tasks its main descriptors were 'hot' and 'warm' whereas the descriptor 'icy' is missing. Before the nerve block, synthetic heat was often preceded by a transient cold perception that disappeared when pure cold perception to cooling was lost during the nerve block. Throughout the nerve block, thresholds for synthetic heat stimuli closely followed those for warm and cold stimuli, which remained within an innocuous range during the first 20 min. Also reaction time to synthetic heat stimuli closely paralleled reaction times to cold throughout the nerve block. The finding that reaction times agree already in the unblocked state can be explained by the subjects responding to the signal mediated by the fast cold afferents. The rise in thresholds and increase in reaction times observed during the nerve block can be attributed to the progressive loss of conducting axons, which is compensated for by temporal summation (Yarnitsky and Ochoa, 1990). Thus, it can be concluded that synthetic heat can be elicited in an innocuous temperature range and does not depend on the activation of cold receptors with myelinated axons; however, it apparently depends on the activation of unmyelinated low threshold cold and warm receptors.

4.2. The neural correlates of cold, warmth, and heat perception

It is accepted that cold and warmth sensations are generated by the activation of distinct sensory channels. It is tempting to assume that a further channel mediates the sensation of hot. Already 100 years ago, Alrutz (1898) argued that ‘...hot is a simple sensation, which generally cannot be cut up or analyzed in component parts...’ and he believed that it originates from the fusion of warmth and paradoxical cold. According to our present knowledge, signals from low threshold warm receptors and heat sensitive polymodal nociceptors (Torebjörk et al., 1984) might have access to this assumed sensory channel and together they may elicit the sensation of hot in the restricted temperature range where both receptor types are active; above this range burning and stinging pain will predominate. If signals from unmyelinated low threshold cold receptors could enter this channel, this would explain the sensation of synthetic heat. There is indirect evidence that signals from widely separated thermoreceptors (>10 cm) may converge in this channel (Alston, 1920; Green, 1977). The dysaesthetic sensations elicited by innocuous cold stimuli in patients with a loss of myelinated low threshold cold receptors occurring in neuropathies of various origins (e.g., Hansson et al., 1991; Yosipovitch et al., 1995) would consequently correspond to the dysaesthetic cold observed during selective block of myelinated axons. Whilst the phenomenon of dysaesthetic cold seems to be caused by a lack of inhibition normally exerted by myelinated cold receptors, the present data rather support the assumption that the phenomenon of synthetic heat is the result of a synthesis of signals from unmyelinated low threshold cold and warm receptors. The paradoxical heat sensation which is elicited in preheated skin areas by cooling and which is mediated by unmyelinated fibres might be a related phenomenon (Susser et al., 1999).

4.3. Important methodological issues in the present and in previous experiments on the perception of synthetic heat

4.5.1. The nerve block

Certain methodological concessions had to be made in this experiment, that might have influenced the results. Ischaemic nerve block was used although it had been objected that this method besides blocking nerve fibres additionally affects excitability of peripheral receptors (Wahren et al., 1989). Changes in thermal sensitivity, however, are principally the same during nerve blocks caused by compression or ischaemia (Fruhstorfer, 1984; Wahren et al., 1989; Yarnitsky and Ochoa, 1990, 1991a,b). Due to the fast temperature change in the threshold task, thresholds for warmth and synthetic heat were high from the beginning. During the block
they tended to rise, and in half of the subjects the pre-
block heat pain threshold was reached. As the heat pain
threshold has been found to be rather stable during is-
chaemic nerve block (Yarnitsky and Ochoa, 1991a), it
could be suspected that the appearance of burning and
stinging perceptions during the last third of the thresh-
old experiment was caused by nociceptor activation.
However, in the reaction task burning and stinging de-
scriptors became even more frequent though the stim-
ulus never exceeded the range 21–39 °C. Receptors
responding to cooling in an innocuous temperature
range above 20 °C can hardly be called nociceptive,
nevertheless their activation is essential for synthetic
heat. Furthermore, during conduction block of myel-
nated cold receptor axons, a cold stimulus does not
produce synthetic heat but a distinct dysaesthetic cold
perception clearly differing in its descriptor profile.
Therefore synthetic heat should be generated by the
coactivation of unmyelinated low threshold warm and
cold receptors, and its characteristic descriptor is hot.

4.3.2. The apparatus

A comment on the stimulation methods used in the
present, as well as in the previous experiments on syn-
thetic heat is necessary. In the present experiments, syn-
thetic heat was produced by simultaneous warm and cold
stimulation by (a) water circulating with a high flow
through an epoxy block with six bronze plates of 2.8 cm²
(reaction time test; see Fig. 1), and (b) 10 ceramic Peltier
modules (8.5 × 13.0 mm), arranged to form a rectangular
surface of 122 cm² (threshold test; see Fig. 1). In the his-
tory of research on the synthetic heat phenomenon, var-
ious apparatus have been used to produce simultaneous
warm and cold stimulation (cf. Thunberg, 1896; 5.5 cm
diameter double brass spirals; Cutolo, 1918: 53.2 cm²
glass capillary tubes; Burnett and Dallenbach, 1927;
64 cm² copper tubing grill; Gritman, 1929: 64 cm² cot-
er tubing grill; Ferrall and Dallenbach, 1930: 64 cm²
copper tubing grill; Sullivan and Verda, 1930; cold and warm currents of air flowing through copper and pyrex tubes, respectively; Jenkins, 1938; four
rectangular grills of 7.5, 8.25, 19.74, and 47.5 cm²; Green,
1977: 2.15 cm² Peltier thermode), however, all methods
succeeded to evoke the same heat perception when ap-
plying warm and cold temperatures of approximately 40
and 20 °C, respectively. This replicability over different
laboratories, using more or less sophisticated apparatus
over the years, shows that the perception of synthetic heat
is a very robust phenomenon. Only Craig and co-workers
(Craig and Bushnell, 1994; Craig et al., 1996), using a
280 cm² thermode of parallel silver bars have reported
that synthetic heat is described as painful.

4.3.3. Instructions

A crucial methodological difference between the
present (and previous) experiments, and the study by
Craig and Bushnell (1994), lies in the instructions
to participants. In the present experiment on synthetic heat
the participants were instructed to describe the evoked
perception for warm, cold, and simultaneous warm and
cold stimulation by choosing descriptors from a list
(cold, icy, stinging, burning, hot, warm, pain, other,
neutral), or spontaneously describe the perception (re-
response alternative: other). The perception of simulta-
nous warm and cold stimulation (synthetic heat) was
consistently described as ‘hot’ (Figs. 3 and 5). The same
approach of assessing perceptual quality (by similar list
of descriptors, always including the category ‘other’) has
recently been shown to yield very useful qualitative in-
formation on tactile, cold, and warmth perception in
patients with neuropathic pain (Berglund et al., 1997)
and fibromyalgia (Berglund et al., 1999) and in healthy
subjects (Harju, 2000).

In all previous experiments on synthetic heat, the
participants were also asked to spontaneously describe
the perception evoked by simultaneous warm and cold
stimulation. The perception was consistently described
as ‘warm,’ ‘hot,’ or ‘heat’. Green (1977), for example, in
his experiment on synthetic heat was especially aware of
the biasing effect of instructions, and included no men-
tion of sensations of heat in his instructions to partici-
pants, because it might have biased their judgements.
However, the participants still volunteered comments
indicative of the perception of ‘heat’, or described the
sensation as ‘first feeling cold’, then turning ‘very warm’
or ‘hot’, which is characteristic of synthetic heat (e.g.,
Green, 1977, p. 336). Importantly, in the study by Jen-
kins (1938), the perception was described merely as
‘warm’ by all 126 (untrained) participants. In contrast,
in the experiment by Craig and Bushnell (1994) partic-
ipants were given a list of 15 words from the McGill
Pain Questionnaire (Melzack, 1975), and a common
definition of pain as ‘any uncomfortable sensation, such
as pricking, stinging, or burning, even if the stimulus is
tolerable’ (p. 254, footnote No. 8). These instructions
resulted in more pain-related descriptors to simultaneous
warm and cold stimulation than to warm and cold in
isolation, and sensations of painful heat (p. 253).

4.4. Synthetic heat as a model for pain, and its relation
to cortical activation

It has to be pointed out that the aim of the early ex-
periments on synthetic heat was in fact to establish
whether the perception of ‘heat’ belongs to a separate
sensory modality, or is a fusion of warmth and cold
(cf., Alruiz, 1898; Burnett and Dallenbach, 1927;
Cutolo, 1918; Ferrall and Dallenbach, 1930; Green,
1977; Gritman, 1929; Jenkins, 1938; Sullivan and Verda,
1930; Thunberg, 1896). In none of these studies was the perception of synthetic heat ever
discussed in terms of a painful perception.
The results from the perceptual analyses of the present, as well as the large number of previous studies, show that synthetic heat is not perceived as painful, which has to be acknowledged. However, Craig et al. (1996) presented further evidence from a PET study in favor of their neurophysiological/neurobiological interpretation of the ‘thermal grill illusion’. They demonstrated that the thermal grill illusion produces activation in the anterior cingulate cortex (ACC), whereas its component warm and cool stimuli do not (p. 258), and that ACC is also activated by noxious heat or cold. They further concluded that ACC appears to be selectively associated with the perception of pain (p. 258), that their results confirm a special role of the ACC in pain, and that the cortical locus of unmasked activity is in the ACC (p. 260).

Absolutely crucial to the hypothesis that synthetic heat is related to pain because it activates the ACC, is the fact that the ACC has recently been shown to be involved also during conditions that elicit response competition (Carter et al., 1998), in the response to incongruent (confusing) stimuli, which is consistent with a role in performance monitoring, such as in the occurrence of errors, or in the presence of a response conflict (McDonald et al., 2000), as well as during performance of tasks requiring effortful thought (Davis et al., 2000). All of these cognitive operations are most likely to occur while processing such surprising stimulation as simultaneous warm and cold temperatures. Furthermore, a recent PET study on chronic pain by Kupers et al. (2000) shows no increase in ACC activation, supporting the notion that there are important differences in the cerebral processing of acute and chronic pain.

The entire cingulate cortex has also been proposed as one of the brain areas essential for extended (i.e., the uniquely human function of) consciousness (e.g., Damasio, 1999, p. 181), and because of the massiveness of its somatosensory inputs, it was described by Damasio (1999, pp. 260–264) as an area giving rise to the most integrated view of the entire body state of an organism at any given time. In the light of the results from the present experiment, of the results from the previous studies on synthetic heat, and of the results from the studies on ACC activity in various cognitive tasks, it is not appropriate to refer to synthetic heat as a model for pain.

4.4.1. Potential psychological contribution to the perception of synthetic heat

The possibility of a psychological contribution to the perception of synthetic heat will shortly be discussed here although the present experiment does not allow any conclusions on this issue. Perceptual paradoxes can be produced by unphysiological, previously unexperienced, or conflicting, signals arriving from different sensory channels, and by the acceptance of false assumptions (e.g., Gregory, 1993; Heller and Schiff, 1991; Katz, 1989; Weber, 1996). According to Gregory (1993, p. 55) all perceptions are ‘computed’ from probabilities of the presence of objects. Katz’s (1989) concept of a ‘temperature gestalt’ may also be interesting in relation to the perception of synthetic heat since it can be impressed on memory and later aid the recognition of objects. Furthermore, when the senses are confronted with a conflict we may rely on the sense that is most likely to provide good information, as stated by the concept of ‘intersensory dominance’ (Freidus, 1974; Welch and Warren, 1980; see also Heller and Schiff, 1991, p. 116). In summary any perception may depend on which sense appears as appropriate for perceiving an attribute.

5. Conclusions

The most probable physiological basis of synthetic heat is that it is mediated by unmyelinated low threshold cold and warm fibres. The phenomenon is not dependent on cold Δ fibres. A contribution of low threshold nociceptors is unlikely for several reasons. A partly different conceptual hypothesis with a psychological mechanism contributing to the perception of synthetic heat is discussed.

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References

Experimental muscle pain provokes long-lasting alterations of thermal sensitivity in the referred pain area

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Abstract

This study explores thermal sensitivity and thermal nociception for signs of central sensitization in the area of referred muscle pain. Two groups of 24 healthy subjects (ss) each, with mean ages of, respectively, 27 and 55 years, were first trained in quantitative sensory testing and pain rating. Then, in a second session, referred pain was evoked by injection of 6\% hypertonic saline into the infraspinatus muscle. Cold and warm thresholds, synthetic heat threshold (SHT – evoked by an alternating pattern of adjacent cold and warmth), and thermal pain thresholds were measured within the referred pain area at a rate of 1/20 min for 60–120 min. All ss of both groups experienced referred pain mostly in the upper arm and of medium intensity. Pain lasted for approximately 12 min with a shorter duration in the older group \((p < 0.02)\). The cold threshold increased significantly \((p < 0.001)\), and the warm threshold slightly, after the injection and remained high for the whole observation period (i.e. lower and higher temperatures were necessary to elicit cold and warmth, respectively). Threshold recovery was more delayed in the older age group. Of those 28 ss in whom cold pain threshold could be followed during the whole observation period, 18 ss showed an immediate threshold decrease of average 6 \({}^\circ\text{C}\) which outlasted the observation period. Four ss responded with a threshold increase. Heat pain thresholds were not affected in the referred pain area. Average synthetic heat threshold did not change; there were, however, distinct and lasting individual threshold shifts in either direction. Six with lowered cold pain thresholds or evident threshold shifts for synthetic heat had also higher pain ratings. The results demonstrate that experimental muscle pain can induce long-lasting changes in thermal sensitivity and nociception. The unexpected cold threshold increase may tentatively be explained as an expression of long-term depression. The decrease of cold pain threshold or SHT in subgroups of ss may indicate central sensitization. However, the observed changes in this experiment do not provide an unambiguous indicator for central sensitization which seems to be rather individual and might depend on pain intensity and proneness to express central mechanisms of sensitization. Therefore in clinical pain states the individual pattern of sensory abnormalities has to be analysed and interpreted in addition to the pain parameters to assess central involvement.

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Keywords: Muscle pain; Referred pain; Thermal pain; Thermosensitivity; Synthetic heat

1. Introduction

Experimental muscle pain with a diffuse localization and referral was initially described by Kellgren (1938). In his basic studies hypertonic saline was used to induce referred pain and through the years this has remained a reliable method (Arendt-Nielsen, 1997; Feinstein, 1954; Graven-Nielsen et al., 1997; Inman and Saunders, 1944; Leffler et al., 2000). The mechanisms underlying referred pain are still incompletely understood (Coderre et al., 1993; Wall, 1993) and several possible mechanisms have been proposed (MacKenzie, 1893; Mense, 1994; Ruch, 1949; Sinclair et al., 1948). The theories assume that dorsal horn neurones receive convergent input from various tissues, and higher centres misinterpret the actual source of input. The experience of referred pain is also modified at the thalamic level (Akparian et al.,...
Various and partly inconsistent somatosensory alterations in the referred pain area have been reported including hypoaesthesia (Feinstein et al., 1954) and hyperphenomena (Steinbrocker et al., 1953). The possibility to use quantitative sensory testing (QST) to document and analyse these sensory alterations could give more insight into their nature and the underlying mechanisms. An examination of thermal perception thresholds would offer the opportunity to study four sensory channels simultaneously (cold, warmth, cold pain, and heat pain). If additionally synthetic heat is included, one could also gain insight into central processing of information stemming from cold and warm channels. Synthetic heat is a peculiar hot perception evoked by adjacent simultaneous stimulation with innocuous cold and warmth as originally described by Thunberg (1896). A central modulation of either the warm or the cold sense, or of both, could be expected to produce changes in threshold or quality of synthetic heat as an indicator of central modulation.

The aim of the present study was to record in two age groups of healthy subjects (ss) thermal perception thresholds at the site of referred pain during and after experimental muscle pain to explore sensory alterations as potential indices of central sensitization. The recording was made in the area of referred pain rather than in the primary area to avoid possible 'contamination' of the results from peripheral sensitization. Clinically useful indicators of the complex mechanisms of central sensitization would be important for an improved treatment according to the principle of a mechanism-based therapy (Woolf et al., 1998) and for the possibility of a more specific selection of pharmacotherapy.

2. Materials and methods

2.1. Subjects (ss)

Two groups differing in age and each consisting of 24 healthy volunteers were drawn from students and hospital staff. The age of the younger group was 20–35 years (average age 26.6 years) and that of the older group 49–64 years (average age 55.2 years). There were 13 men and 11 women in the younger and 11 men and 13 women in the older group. The local ethical committee had approved the study, and informed consent was obtained from each participant. Before recruiting a subject, the shoulder muscles were examined by manual palpation to exclude ss with sore or tender muscles. Each subject attended two sessions: during the first he/she was trained in quantitative sensory testing (QST) and in pain intensity rating using pressure pain as a model, whereas the second was the actual experimental session.

2.2. Sensory testing

In the training session warm, cold, thermal pain, and synthetic heat thresholds were measured on hands and both upper arms with the Marstock method (Fruhstorfer et al., 1976) using a 25 × 50 mm thermode. This method is reaction-time-dependent (Yarnitsky and Oehlau, 1990), but enables recording of the perceptual quality of each evoked sensation due to the slightly suprathreshold stimulus (Lindblom, 1994). Initial temperature was set to 31°C, and during stimulation temperature could vary between 5 and 55°C. Thresholds were determined in the same order: cold (CT), warmth (WT), cold pain (CPT), and heat pain threshold (HPT). Rate of temperature change was 1°C/s for cold and warmth, 1.5°C/s for heat pain, and about 2°C/s for cold pain. For CPT and HPT the ss were carefully instructed to indicate the threshold and not the tolerance levels. Three measurements were performed for CT, WT, CPT, and HPT and the average of the last two recordings was calculated as the threshold value. For synthetic heat stimuli a similar thermode of identical size was used in which every other of the 36 elements changed temperature in the opposite direction to produce a checkerboard pattern of adjacent cold and warmth. Rate of the temperature changes in either direction was 2–2.5°C/s.

Synthetic heat threshold (SHT) was measured between the two opposite temperatures, and the average of the last two of three stimuli was taken. Pressure pain stimuli, used in the training session, were delivered by an algometer (Somedic Production, Sollentuna, Sweden) to the medial part of the thigh above the knee, which appeared to be palpatory more sensible. Pain thresholds were determined and the intensity of the stimuli was rated on a 0–10 numerical rating scale (NRS: 0 = no pain, 10 = worstimaginable pain). The values obtained were not further analysed. Skin temperature was measured by infrared thermometer Tempet® (Senselab, Stockholm, Sweden).

During the experimental session, skin temperature and QST were first assessed on both upper arms. Then experimental muscle pain was elicited and ss had to rate pain intensity every minute. Immediately following pain provocation skin temperature and QST measurements were repeated on the pain side. Thereafter, measurements were repeated on the pain side every 20 min. During this period SHT was measured alternately before or after the thermal thresholds. The younger group was followed up for 60–80 min and the older group for 60–120 min. Some single cases were followed up for 3–6 h and two cases had an additional control the next morning. In 18 older ss skin temperature and QST were also measured on the normal side 50 min after induction of muscle pain. The skin temperature in the pain area gradually fell during the experiment by 1.0 ± 1.0°C in the younger and 0.7 ± 1.2°C in the older group. No
statistical covariance existed between the increase of CT and the fall of skin temperature.

2.3. Provocation of experimental muscle pain

Muscle pain was induced by injection of 6% hypertonic saline into the infraspinatus muscle. A 27 G Spinoe® needle (Braun) was used for the injection. The injection side was randomly selected and activation of cutaneous nociceptors was minimized by skin anaesthesia with EMLA® (Astra, Södertälje, Sweden) one hour before injection. Despite EMLA® anaesthesia, 3 younger and 6 older ss felt slight momentary pain when the injection needle penetrated the skin. The injection of 1ml hypertonic saline was given 2-3 cm below the highest point of spina scapulae and the intramuscular depth of injection was 2-3 cm. During the injection, an ultrasound scanner (Aloka Echo Camera, Tokyo, Japan) was used to confirm and document the intramuscular localization (Fig. 1). If pain intensity stayed below 6 NRS units, an additional bolus injection of 0.5 ml was given. Time between these two injections usually was 1-2 min. All injections were done by one of us (B.T.).

2.4. Statistics

Statistics of this explorative study are mainly descriptive. One-way ANOVA with repeated measures was used for analysis of CT, WT, HPT, and SHT. CPT data were analysed by Friedman analysis of variance by ranks. Mann–Whitney U test was used for comparison of pain parameters between groups. Thermal thresholds were analysed using two-way repeated measures ANOVA with one within factor (time) and one between factor (group). As no relationship between thresholds and skin temperature on the pain side could be demonstrated no adjustment for the temperature was needed. In the results, means (± SD) or, if appropriate, medians (range) are given together with the p-values of conspicuous differences.

3. Results

3.1. Experimental pain

The injection of hypertonic saline provoked pain in all ss. Thirty three ss reported only referred pain whereas 5 ss also reported local pain at the injection site. 15 ss reported local muscle pain at the injection site from where it in 10 ss moved to the region to which it was later referred. The location of the referred pain was in most ss the proximal upper arm, and only in 4 ss the location was more cranially over the lateral portion of the trapezius muscle (Fig. 2).

In the younger group the referred pain appeared with a median latency of 30 s (1.5–120 s), whereas in the older group median latency was 40 s (10–300 s; p = 0.03). In both groups peak pain intensity varied from 0.5 NRS to 9 NRS units with similar mean intensities of 6.5 ± 1.7 and 6.0 ± 1.9 NRS units, respectively. Autonomic signs like pallor or sweating occasionally accompanied strong pain. Pain duration differed between the age groups (p < 0.02) with a median duration of 13 min (8–42 min) in the younger and of 11 min (1–27 min) in the older group (Fig. 3). There was no gender difference in pain intensity or duration. The most common pain descriptors were, with some overlap, pressure (32 ss), dull ache (25 ss), a feeling of a deep bursting (14 ss), and cramp (8 ss). Eleven ss described a radiation of the referred pain down the arm and 4 ss a feeling of heaviness. After the provoked pain had faded, all ss were without complain but some ss felt a slight heaviness of the arm or tenderness during arm movements.

Fig. 1. Ultrasound documentation of intramuscular hypertonic saline injection at the depth of slightly more than 2 cm from skin level (upper border of the picture). The scapula is seen near the bottom of the picture with the round shadow of the bolus above it.

Fig. 2. Referred pain was localized to the proximal upper arm for all but 4 ss, where the localization was the lateral portion of the trapezias muscle. The localization was the frontal side of the upper arm (14 ss), over deltoides muscle (20 ss), and the dorsal part (10 ss). X indicates the site of hypertonic saline injection.
3.2. Sensory thresholds

3.2.1. Cold perception threshold (CT)

Following the saline injection CT increased in both groups, on average by $2.4 \pm 2.8^\circ C (p < 0.001)$, and remained high during the whole observation period (Fig. 4). This unexpected decrease in sensitivity to cold implied that CT was found at a lower temperature than baseline. The general course of the CT increase was similar in both groups but in the older group the threshold deviation reached its maximum later and lasted longer. In 14 younger ss CT recovered or tended to recover during the observation period, whereas only 4 older ss showed threshold recovery during the observation period although this had been longer. In those 6 older ss who were followed up for a longer time the rise in CT was maintained for at least another 3–6 h. Two ss with such a lasting CT increase had, however, normal thresholds the next morning.

3.2.2. Warm perception threshold (WT)

Concomitantly with the CT increase WT described a minor increase of $0.8 \pm 2.7^\circ C$ (Fig. 4).

3.2.3. Cold pain threshold (CPT)

Out of the 48 ss CPT could only be assessed in 28 ss since it was mostly below or at the lower temperature limit of the apparatus ($5^\circ C$). A majority of 18 of these 28 ss CPT displayed an increased sensitivity with a threshold decrease with a maximal average deviation of $6.2 \pm 3.4^\circ C$ from the baseline threshold after 20 min. This increase in cold pain sensitivity lasted throughout the observation period (Fig. 5). As a group the 18 ss who displayed the decrease in CPT rated peak pain intensity somewhat higher than the group average but no other relations could be detected. Among the remaining 10 ss CPT either increased (4 ss) or did not change (6 ss).

3.2.4. Heat pain threshold (HPT)

There were no systematic or conspicuous changes in average HPT in any of the groups. Individual variations (increase 26 ss; decrease 22 ss) were mostly less than $2^\circ C$ and were often paralleled by similar changes in WT.

3.2.5. Synthetic heat threshold (SHT)

During the training procedure and measurements before the saline injection, SHT was recorded altogether 190 times with a mean value of $7.5 \pm 1.8^\circ C$. For the first stimulus of a series, ss often reported a short cold sensation preceding the heat sensation. Experimental pain did not change average SHT although individual changes in either direction were partly large: in 14 ss the threshold was lowered by at least $1^\circ C$ for more than 1 h whereas in 14 other ss the opposite happened (Fig. 6). No relations of SHT changes to any of the other threshold changes could be detected. Again, ss with
larger threshold deviations rated experimental pain higher. For the young group there was even some correlation between maximal pain intensity and decrease of SHT at injection ($p = 0.09$) which was significant at 20 min ($p < 0.04$) and 40 min ($p < 0.02$), notably in spite of the simultaneous increase in CT.

3.2.6. Thresholds on the contralateral side

In those 18 ss in whom QST was repeated on the contralateral side to the saline injection after 50 min, the skin temperature had fallen during the course of the experiment with $0.8 \pm 1.0^\circ$C which is a similar value as on the pain side. CT was increased by $0.7 \pm 0.8^\circ$C and there was a statistical correlation to the fall of skin temperature, $p < 0.04$, (cf Methods). All other sensory thresholds were identical with the basis values.

4. Discussion

The injection of 6% saline into the infraspinatus muscle provoked referred pain in all ss which was mostly of medium intensity and lasted about 12 min. According to the convergence projection theory referred pain should be segmentally distributed (Ruch, 1949), but experimental studies have also demonstrated a distal (Kellgren, 1938) or sometimes a rostral spread (Mense, 1994; Torebjörk et al., 1984). Since the infraspinatus muscle is innervated by the C4–C5 segments, all referrals in this study can be regarded as intrasegmental.

The main aim of the study was to explore perception threshold changes within the area of referred pain, which would indicate central sensitization. CPT was lowered in the majority of those ss in which it could be measured (increased sensitivity), while WT and especially CT were found to be increased (decreased sensitivity). HPT was not changed and SHT showed individual deviations in either direction. As ss with marked decrease of SHT or lowered CPT had on average more pain one might assume that experimental pain was not intense enough or did not last long enough to produce clearer signs of sensitization. Further, individual ss could differ in their proneness to develop central sensitization. This would correspond with the authors’ clinical observations that after nerve or tissue injury some but not all patients develop signs and symptoms of central sensitization. Although generally hyperalgesia is described as the typical finding of referred pain (Coderre et al., 1993; Vecchiet al., 1993; Wall, 1993), modality-specific differences do exist. Thus, pressure pain thresholds have indicated hyperalgesia (Graven-Nielsen et al., 1997) or no significant change (Leffler et al., 2000). Threshold changes in other modalities are inconsistent. For touch both hyperaesthesia (Steinbrocker et al., 1953) and hypoaesthesia (Feinstein et al., 1954) have been described. For the thermal senses a minor hypoaesthesia has been observed (Leffler et al., 2000). Finally, both negative and positive signs with paraesthesia may occur in clinical muscle pain conditions (Hansson and Lindblom, 1993). Thus, a single stereotyped pattern of threshold changes as a sign of central sensitization probably does not exist. More likely threshold changes vary with the character of the pain stimulus and the specific proneness of the individual.

A striking feature of all threshold changes observed in this study is their long duration, which considerably outlasted the experimental pain. Because of this long
duration it is unlikely that thermal sensitivity was merely masked by the experimental pain. Similarly, a uniform change in reaction time due to the pain cannot have caused thermal sensitivity changes in the opposite direction. Therefore these threshold changes can rather be regarded as an indicator of a long-term central modulation of information processing caused by the induced muscle pain. The long duration of the observed threshold alterations and particularly the CT increase suggests long-term potentiation of synaptic strength or long-term depression as the underlying cause (Sandkühler, 2000). The individual effectiveness of the endogenous antinoceptive system may have modulated the expression of this process generating long-term depression in the cold channel and potentiation in the cold pain channel.

All subjects in the present study experienced synthetic heat sensation as has been described in the literature e.g. by Green (1977). The present results are compatible with synthetic heat being a complex perception. The transient cold sensation often preceding the first stimulus indicates conduction in cold-sensitive myelinated Aδ nerve fibres (Iggo, 1969) which have higher conduction velocity than the warm-sensitive C fibres. Qualitatively different cold evoked perceptions have been demonstrated in studies with compression block (Fruhstorfer, 1984; Wahren et al., 1989; Yarnitsky and Ochoa, 1990). When the conduction of myelinated cold fibres is blocked, non-noxious cold and warm stimulation still evokes the dysesthetic synthetic heat sensation, apparently mediated by low threshold cold and warm fibres (Fruhstorfer, 1984; Fruhstorfer et al., in press). C fibres responding to innocuous cold have recently been discovered in man by Campero et al. (2001). These fibres are supposed to contribute little to the normal cold sensation but could be important for the sensation of synthetic heat.

A recent positron emission tomography study has demonstrated that the chequered WC stimulus produces activation in the anterior cingulate cortex, while its components of warm and cool in isolation do not (Craig et al., 1996). A study performed by functional magnetic resonance imaging has shown that the anterior cingulate cortex is found to be active in evaluative processes, when control needs to be more strongly engaged e.g. cognitive control of the perception of conflicting stimuli (MacDonald et al., 2000). SHT is apparently an unphysiological conflicting blend of stimuli, which might produce the peculiar 'unphysiological' perception of heat.

The changes of SHT were diverse. During the short pain period some ss with more intense pain, experienced a decrease of SHT. It is not excluded that SHT might prove to be a valuable tool in the assessment of central sensory processing.

The differences between the two age groups were only small; older ss experienced experimental pain of a shorter duration though with similar intensity, and thermal threshold changes in the referred pain area lasted longer. Central processing of pain does change with age as studies on temporal summation of pain have shown (Harrisk et al., 1996).

The results of this study demonstrate that short-lasting muscle pain may provoke dynamic changes of thermal thresholds and thermal pain thresholds. However, the recorded changes do not provide an unambiguous indicator for central sensitization. Additional assessment of suprathreshold phenomena like altered stimulus intensity/perception magnitude relation (Berglund et al., 2001) or abnormal temporal summation of pain (Eide, 2000) may provide further clues but not the definite answer to the complex phenomenon of central sensitization.

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Induction of non-painful and painful intestinal sensations by hypertonic saline: a new human experimental model

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Abstract

Background and aims. To develop a pain model for chemical stimulation of the human gut.

Methods. In a double-blind experimental study 10 subjects with a previously performed sigmiodostomy were randomised to receive injections with either isotonic or hypertonic saline in the colonic mucosa. In the hypertonic experimental arm, 0.1 ml of 0.9%, 2%, 4%, and 6% and 0.2 ml of 2% and 4% saline were given. In the placebo arm, six 0.9% saline injections of the same quantities were given. In a separate experiment 0.8 ml 4% saline was infused into the mucosa by a pump over a period of 2 min. The pain intensity was rated on a 0–10 visual analogue scale with 5 as the pain threshold.

Results. The hypertonic saline injections resulted in local as well as referred non-painful and painful sensations in 9 out of the 10 subjects. The evoked sensations were mostly described as a smarting sensation with an intensity of median 1 (range 0–5.6) for 0.1 ml 2% saline to median 2.9 (range 0–6.2) for 0.2 ml 4% saline. Seven subjects reported referred sensations to the abdominal skin. Continuous infusion of 4% saline resulted in a consistent sensory response in all subjects, with a median intensity of 4.1 (range 2.1–8.1). This sensory intensity was reproducible in 70% in a retest experiment after median 7 weeks. In the placebo arm a total of 70 isotonic saline injections only resulted in inconsistent, short-lasting non-painful sensations in three subjects.

Conclusion. The model represents a safe method for direct chemical activation of the sensory endings in the human gut. The model may be used for pharmacological screening of analgesics and for basic investigations in patients suffering from gastrointestinal diseases.

Keywords: Experimental; Pain; Sensation; Gut; Saline

1. Introduction

Since pain related to the gut probably is the most common symptom in gastroenterology, characterisation and knowledge of the mechanisms behind the sensory experience is of major importance in the diagnosis and assessment of organ dysfunction and management of pain. Studies focusing on the development and manifestation of pain are therefore highly needed. In the clinic, however, the study of pain is confounded with individual emotional, social, and cognitive factors. Experimental pain models are tools for characterisation and understanding of pain mechanisms with minor interference from individual reactions to pain. In such models the investigator is able to control the stimulus intensity, duration, and modality, and the output can be assessed quantitatively or qualitatively (Arendt-Nielsen, 1997). In comparison with the somatic system, experimental visceral models have been troublesome to develop in man, mainly due to the localisation of the organs. Most human models used short-lasting mechanical (Arendt-Nielsen, 1994; Whitehead and Delvaux, 1997) or electrical stimuli (Arendt-Nielsen, 1994;
Drewes et al., 1997), but these methods are not very natural, and they have methodological limitations. Chemical stimuli with direct effect on the afferent nerves may reflect the clinical situation with inflammation and trauma to the gut. In animal research stimulation with numerous substances (bradykinin, serotonin, lactic acidic, and basic solutions, etc.) has been applied in different visceral organs (Arendt-Nielsen, 1994; Ness and Gebhart, 1990). In man chemicals have been used successfully as experimental stimuli in the skin (Andersen et al., 1995; Arendt-Nielsen, 1994) and muscle (Babenko et al., 1999), but only few studies were performed in the visceral organs (Ness and Gebhart, 1990). Hypertonic saline has been used extensively for eliciting muscle pain in man. The induced pain, which is related to the concentration and volume of the saline, evokes a response comparable to clinical muscle pain with localised and referred pain phenomena (Arendt-Nielsen et al., 2000). Injections of hypertonic saline in the muscle give a direct excitation of the afferents, as the drastic elevation of the extracellular sodium concentration leads to sodium influx through the cell membrane and depolarisation of the nerve (Graven-Nielsen et al., 1997). Therefore, the model may be suitable for studying basic sensory mechanisms in the gut by direct activation of sensory nerves in the mucosa.

We hypothesised that hypertonic saline, injected into the gut mucosa in increasing concentrations and volumes, would evoke a consistent sensory response. Thus, the aims of the study were (1) to study the effect of bolus injections of saline in increasing concentrations (0.9%, 2%, 4%, and 6%) and volumes (0.1 and 0.2 ml) on the intensity and duration of the evoked sensory response, and (2) to test the effect of continuous infusions of 4% saline over a period of 2 min.

2. Materials and methods

2.1. Pilot investigations

In seven subjects with sigmoidostomies (see below for description of operative technique) different combinations of iso- and hypertonic (2–8%) saline were injected. The needle was inserted a few millimeters into the mucosa tangential to the surface of the stoma (Fig. 1), and after each injection the needle was withdrawn. A burning sensation or pain was reported after hypertonic saline injections, but repeated injections with isotonic saline also caused a moderate pain in three subjects. Consequently, we speculated that the repeated injections with removal of the needle might cause tissue trauma and bleeding followed by subsequent release of algogenic substances. Moreover, two subjects reported pain following an injection of isotonic saline shortly after previous painful injections of hypertonic saline. Therefore, the possibility of nociceptor activation by a local distension of an area of the gut wall could not be excluded. The protocol was therefore designed as follows: (1) To avoid tissue trauma, the needle was kept in situ during the injection series. (2) Different concentrations (0.9%, 2%, 4%, and 6%) and volumes (0.1 and 0.2 ml) of hypertonic saline were given in one experiment. (3) To investigate a “volume effect” repeated injections with isotonic saline were given in a separate experiment. (4) To investigate the effect of continuous infusion a separate experiment was performed, where hypertonic saline was given with an infusion pump. The infused volume and time was chosen according to four pilot experiments with different infusion rates using 0.4–1.2 ml of 4% saline. All experiments evoked a sensation above 3 on the VAS (see below), but there was no additional effect of the volumes above 0.8 ml. To avoid potential tissue damage we therefore decided to give 0.8 ml in the final protocol.

2.2. Subjects

Ten patients (eight males and two females) with a sigmoidostomy agreed to participate in the study. The sigmoidostomy was constructed in a standardised manner. Through a midline incision the sigmoid and descending colon were mobilised. The oral part of the rectum was divided, and after resection of the diseased sigmoid segment a colostomy of the proximal sigmoid colon was performed in the left abdominal quadrant. The median age of the patients was 66 years (range 43–74), and the time from the operation to the experiment.
was median 9.5 months (range 3–61). Seven patients were operated for cancer in the rectum, two for perforation of the sigmoid colon due to diverticulitis, and one for incontinence. The stoma was well functioning, and none of the patients had other gastrointestinal complaints. Two subjects had an intermittent intake of diuretics and hypnotics, but the remaining patients took no medication. One subject had intermittent low back pain, but no back pain was reported during the experiments. Otherwise none of the subjects had any complaints of pain and as they all had a normal function of the stoma they were considered clinically comparable.

For the experiment with continuous infusion of 4% saline (see below) the same 10 subjects were asked to participate. One patient did not enter the study, as he had intestinal continuity re-established before this part of the protocol was carried out, and two did not want to participate for personal reasons, leaving seven patients for the experiment.

The local Ethics Committee approved the protocol, which fulfilled the recommendations of the Helsinki II Declaration.

3. Experimental protocol

3.1. Experiment I (bolus injections of either isotonic or hypertonic saline)

The patients were admitted to the outpatient department at two occasions at least one week apart (median 14 days (range 7–72)). They were randomised to receive either hypertonic (2–6%) or isotonic (0.9%) saline in six consecutive injections at each occasion. The patients were informed that they would receive a series of either hypertonic or isotonic saline injections, but they were not aware of the volume or the concentrations. To ensure blinding the syringes with saline were prepared before the patient entered the laboratory, and neither the patient nor the investigator knew if the experiment was to be carried out in the hypertonic or the isotonic arm. Before the injections the stoma was cleaned with water and a swap with ethanol. The stoma projects several (typically 2–3) centimeters outside the abdominal wall and the injections were given in the gut mucosa in an area, where the stoma was visible, and outside the abdomen. Thus, we avoided the injections to get into contact with the somatic nerves.

3.1.1. 1A. Bolus injections with hypertonic saline—effect of concentration and volume

For the injections with 0.1 ml saline, a 26G needle was inserted into the left side of the stoma. The direction of the needle was tangential to the gut surface with the tip pointing to the left abdominal side—Fig 1. The subsequent injections contained 0.1 ml 0.9%, 2%, 4%, and 6% saline, followed by 0.2 ml 2% and 4% saline. The first injection (0.9% saline) was given approximately 12 mm into the gut. The needle was left in situ, but withdrawn during the three subsequent injections, which were given approximately 10 mm (2% saline), 8 mm (4% saline), and 6 mm (6% saline) into the tissue. For the experiment with 0.2 ml injections the needle was inserted at a new position in the bottom of the stoma. The tip was pointing tangential to the surface of the gut and towards the left inguinal region. In this experiment 0.2 ml of saline was injected approximately 12 mm (2% saline) and 8 mm (4% saline) into the tissue with the needle left in situ until the end of the experiment. A period of at least 5 min was interspersed between the injections.

3.1.2. 1B. Bolus injections with isotonic saline

Six injections with 0.9% saline were given, including the same volumes as those in the hypertonic experiment, and the same protocol was followed. The experiment was separated at least one week from the hypertonic injections, and it was performed either before or after, depending on the randomisation.

3.2. Experiment II: continuous infusion of 4% saline

After experiment I it was evident, that the bolus injections most frequently resulted in relative low-intensity sensations. To develop a model where a sensory response of higher intensity was more consistently evoked, we investigated the effect of continuous infusions with hypertonic saline. In this experiment 0.8 ml of 4% saline was infused over a period of 2 min with an electromechanical pump (type 111, Ole Dich Instrumentmakers ApS, Hvidovre, Denmark). The needle was inserted 10 mm into the left side of the stoma. As in experiment I the needle was tangential to the gut surface with the tip pointing to the left abdominal side. The experiment was repeated after median 7 weeks (range 2–8) to investigate the test–retest reproducibility. The subject was not aware of the saline concentration, but due to the relatively high volume infused (and theoretical risk of tissue damage) it was not possible to repeat the experiment with other concentrations. Placebo injections were not given, as experiment I included several series of isotonic injections without consistent sensory responses.

3.2.1. Assessments of local and referred sensations

The sensory intensity was assessed continuously during the experiments using an electronic visual analogue scale (VAS) (The Noxiest Institute A/S, Biomedical Engineering, Aalborg, Denmark). The scale provided the possibility of giving the following measurements: time from start of the injection to the first sensation, mean sensory intensity, maximal sensory intensity, duration of the sensation, and area under the
VAS curve as a function of time. Sensory assessment on a VAS can be difficult, especially in visceral pain, which is diffuse and difficult to characterise. Therefore the patients were trained in assessment of sensation of deep pressure at the muscles on the right forearm several times before the injection of the substance.

Although still debated, most sensory afferents in the gut are probably polymodal. Whether or not afferents specific for high- and low intensity stimuli exist, is also a matter of debate, and in contrast to afferents to the skin, most visceral afferents probably encode both non-painful and painful sensations (Mayer and Gebhart, 1994; Su and Gebhart, 1998). We therefore decided to use a continuous scale ranging from non-painful to painful sensations. This scale has proven to be robust in previous experiments with evoked visceral sensations in our laboratory (Drewes et al., 2002). The intensity of the non-painful sensations was scored from 1 to 5, where the following descriptions were added to facilitate the ratings: 1 = vague perception of mild sensation; 2 = definite perception of mild sensation; 3 = vague perception of moderate sensation; 4 = definite perception of moderate sensation, and 5 = discomfort and first sensation of pain (pain threshold). The descriptors were chosen according to earlier studies using balloon distension in the intestine (Serra et al., 1995). For the painful sensations the patients used the scale from 5 to 10, anchored at 5 = pain threshold to 10 = unbearable pain. This part of the scale was red to clearly separate the non-painful from the painful range of sensations. To facilitate the scoring, the following anchor words were used: 6 = slight pain, 7 = moderate pain, 8 = medium pain intensity, 9 = intense pain, and 10 = unbearable pain. These words were previously found to be equidistant from 1 to 5 on the pain scale (Drewes et al., 1993). Consequently, when the subject reported that the injections resulted in pain and/or severe discomfort (above 5 on the non-painful scale), they were asked to score the intensity from 5 to 10. The use of VAS had previously been demonstrated to be useful to assess painful visceral stimuli to electrical current and distension in the stomach and small and large intestine (Arendt-Nielsen et al., 1997a; Drewes et al., 1997, 1999a, b).

The sensory rating was performed continuously during the experiment. After the injections the subjects were allowed at least 3 min to rate the sensations. If any sensation was evoked, the VAS recording was continued, until all sensations had disappeared. After the experiment the sensations were described qualitatively. The subjects were asked to assign the non-painful feelings to one of the following six sensations: fullness, colicky, warmth, stinging, pressure, and others (Serra et al., 1995). For qualitative descriptions of the painful sensations the Danish version of the McGill Pain Questionnaire (Drewes et al., 1993) was used. After each injection the patients were asked about any referred sensation. The area was marked with a pen, transferred to a transparent paper, digitised (ACCEAD D900+ Digitizer, Taiwan), and the area calculated electronically (Sigma-Scan, Jandel Scientific, Canada).

4. Statistics

Data are presented as median and (range). Data were analysed using analysis of variance (ANOVA), and Spearman's test was used for correlations. SPSS v. 10.0 was used as software package. $P < 0.05$ was considered significant.

5. Results

All patients went through with the experiments. No autonomic side effects were observed after the injections. After finishing the pilot series, one subject had a small ulcer, probably due to bleeding after the repeated injections. Otherwise no adverse effects were detected, and no evidence of local skin inflammation was observed. No changes in the frequency or volume of the output from the stoma were seen.

During the pilot experiments bolus injections were given to seven subjects (and repeated again in two subjects). This resulted in a non-painful pressing or burning sensation in five experiments, and in moderate pain (VAS = 6 and 7) in four experiments. The infusion of 4% saline resulted in a consistent sensory response in all four subjects with VAS ratings from 3 to 7.

5.1. Experiment I

5.1.1. IA. Effect of concentration and volume of bolus injections with hypertonic saline

Most sensations were located within the non-painful range, and only three subjects reported painful sensations. The non-painful sensations were described as taut in five subjects, smart in four subjects, and warm in one. The dominant painful sensations were smart, pricking, and taut in the three subjects.

The median intensity of the different concentrations and volumes of saline is seen in Table 1. In general there was an increasing sensation intensity following the increasing concentrations and volumes, but due to dropout of the subjects with the highest pain intensity, the median intensity decreased for the last injection in each series. As a VAS of 1, corresponding to "a vague perception of mild sensation," is difficult to assess for the patients, the sensation onset, time to peak sensation, and duration of the sensory response was only reported for those with scores higher than 1 (Table 1). There was a high inter-individual variation, but typically the first sensation was reported after more than 20s with a total
Table 1
Characteristics of the sensory response to bolus injections of the different concentrations and volumes of hypertonic saline (experiment IA, n = 10), and continuous infusion of 0.8 ml 4% saline for two minutes (experiment II, n = 7)

<table>
<thead>
<tr>
<th>Experiment IA</th>
<th>Max VAS</th>
<th>Number having VAS&gt;1*</th>
<th>Sensation onset (s)**</th>
<th>Time (s) to peak sensation**</th>
<th>Duration (s) of sensation**</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 ml, 2%</td>
<td>1 (0-5.6), n = 10</td>
<td>5</td>
<td>39 (4-92)</td>
<td>209 (72-280)</td>
<td>263 (43-568)</td>
</tr>
<tr>
<td>0.1 ml, 4%</td>
<td>2.1 (0-7.0), n = 10</td>
<td>5</td>
<td>25 (10-146)</td>
<td>59 (14-246)</td>
<td>189 (83-378)</td>
</tr>
<tr>
<td>0.1 ml, 6%</td>
<td>1.0 (0-5.1), n = 9</td>
<td>4</td>
<td>53 (10-126)</td>
<td>63 (20-175)</td>
<td>372 (9-200)</td>
</tr>
<tr>
<td>0.2 ml, 2%</td>
<td>2.9 (0-62), n = 9</td>
<td>4</td>
<td>26.5 (7-80)</td>
<td>82.5 (57-108)</td>
<td>147.6 (23-280)</td>
</tr>
<tr>
<td>0.2 ml, 4%</td>
<td>1.4 (0-6), n = 7</td>
<td>4</td>
<td>45.5 (3-130)</td>
<td>102.5 (83-170)</td>
<td>134 (47-293)</td>
</tr>
<tr>
<td>Experiment II</td>
<td>0.8 ml, 4% retest</td>
<td>4.1 (2-8.1), n = 7</td>
<td>60 (30-301)</td>
<td>190 (58-320)</td>
<td>171 (40-341)</td>
</tr>
<tr>
<td>0.8 ml, 4% retest</td>
<td>4.1 (2-8), n = 7</td>
<td>7</td>
<td>84 (28-338)</td>
<td>154 (80-400)</td>
<td>271 (108-513)</td>
</tr>
</tbody>
</table>

The continuous infusion was repeated after median 7 weeks (last row-retest). VAS = visual analogue scale ratings with 5 as the pain threshold. Max VAS is the maximal sensory rating during the experiment. Sensation onset is the time from injection to any sensation was reported. Time to peak sensation is the time from the injection to the maximal VAS rating. Duration of sensation is the time from onset of sensation until any sensation has disappeared.

*In experiment IA one subject did not have injections with 0.1 ml 6% and 0.2 ml 2% saline, and three subjects were not willing to have injections with 0.2 ml 4% saline due unpleasantness associated with the previous injections.

**Data in experiment IA are only given for subjects reporting a sensation >1 on the VAS.

duration of more than 2 min. An example is shown in Fig. 2.

For the three hypertonic 0.1 ml injections there were no differences between sensory intensity ($F = 0.48; P = 0.62$), sensory onset ($F = 0.27; P = 0.77$), time to peak VAS ($F = 2.47; P = 0.12$), sensory duration ($F = 3.5; P = 0.06$), or area under the VAS curve ($F = 1.58; P = 0.23$). There was a significant correlation between the area under the VAS curve and the maximal sensation (Spearman's $\rho = 0.87, P < 0.001$).

Comparison between the 0.1 and 0.2 ml injections showed no differences with regard to maximal sensory intensity ($F = 0.63; P = 0.43$), sensory onset ($F = 0.062; P = 0.967$), time to peak sensation ($F = 2.1; P = 0.17$), sensory duration ($F = 2.5; P = 1.3$), or area under the VAS curve ($F = 0.09; P = 0.78$).

5.1.2. IB. Comparison between bolus injections of hypertonic and isotonic saline

The first isotonic injection in the hypertonic series evoked a non-painful sensation in one subject (maximal VAS rating = 3.6), whereas no sensation was reported in the remaining nine patients.

The six repeated isotonic injections did only evoke a sensory response in three subjects. The ratings varied between 1.9 and 7.4 on the VAS. The high sensory intensities were all reported by the same subject. For this person the sensation to hypertonic saline was consistently higher compared with the corresponding placebo injections. In general the sensory response to the isotonic injections was immediate and very short lasting (less than 10 s), and it was probably caused by a mechanical effect on the receptors.

Comparing the maximal sensory response in the hypertonic and isotonic series, the effect of hypertonic saline was significant ($F = 21.2; P < 0.001$). There was also a significant effect for the hypertonic saline injections compared to placebo on the effect on pain onset, duration, time to peak VAS, and area under the VAS curve (data not shown).

5.1.3. Referred area

Referred sensations to hypertonic saline injections were seen in seven subjects. The sensations were reported around the stoma with a distance from the centre of the stoma to the centre of the referred area of median 4 cm$^2$ (2.5-5.5). The size of the referred pain area was median 16 cm$^2$ (1.5-11.3). No correlation was found between the referred areas and sensory intensity (Spearman's $\rho = 0.21, P = 0.5$) or area under the VAS curve (Spearman's $\rho = 0.45, P = 0.14$).

Fig. 2. The typical sensory response (dotted line) to a bolus injection with 0.1 ml 0.4% saline into the gut mucosa in a patient with a sigmoidostomy. The second curve (normal line) shows the typical sensory response to a continuous infusion with 0.8 ml 4% saline for 2 min. VAS = visual analogue scale ratings with 5 as the pain threshold.
5.2. Experiment II

5.2.1. Continuous infusion of 4% saline for 2 min

The pump infusions resulted in a consistent sensory response in all subjects. Four of the injections resulted in a painful response. The data for the VAS ratings from the first and second injections are given in Table 1. Fig. 2 shows a typical time course of the sensory response in a patient. Five subjects reported referred sensations to the skin around the stoma, whereas the sensation was only felt inside the stoma in two patients. The area of the referred sensations was median 24 cm² (13–30.5). The maximal VAS ratings in the test–retest experiments are shown in Fig. 3. The median ratings were the same in both experiments, but since one subject reported an increase and another a similar decrease from the first to the second experiment, the correlation was not significant (Spearman’s \( r = 0.42, P = 0.35 \)). The time to the onset of the evoked sensations was reproducible from the first (60 s (30–301)) to the second experiment (84 s (28–338)) (Spearman’s \( r = 0.82, P = 0.023 \)), whereas no correlation was found between the duration of the evoked sensation in the two experiments (first experiment: 171 s (40–341); second experiment: 271 s (108–513); Spearman’s \( r = 0.31, P = 0.54 \)). In the five subjects who reported referred sensations, the retest experiment evoked the sensations in the same or a very adjacent area of the skin.

6. Discussion

A new experimental model for chemical gut pain in humans is presented. Injections of hypertonic saline in the colonic mucosa resulted in deep as well as referred non-painful and painful perceptions. The evoked perceptions were mostly described as a smarting sensation with referred spreading to the lower abdominal wall. No evidence of tissue damage or long-term effects on sensation or motility was found. Compared to isotonic saline, the hypertonic saline injections evoked a higher sensation intensity, duration, and area under the VAS curve. Finally, continuous infusion of hypertonic saline for 2 min resulted in a consistent sensory response in all subjects.

6.1. Comparison with previous models to evoke sensations in the gastrointestinal tract

Most visceral models to evoke non-painful and painful sensations are based on non-physiological stimuli such as distension with balloons (Drewes et al., 2001; van der Schaar et al., 1999; Whitehead and Delvaux, 1997) or electrical current (Arendt-Nielsen et al., 2000). Electrical current activatesafferent nerves non-specifically in different layers of the gut and may not be suitable for studying specific nociceptive pathways. Distension with a balloon is a relatively natural stimulus, as pain is probably released by distension of the gut (Ness and Gebhart, 1990) in many diseases. These models have, however, several limitations, since the balloons deform during the distension, the response is motility dependent, and measurement of pressure and volume is not very reliable as measures of the mechanical forces applied to the gut wall (Gregersen and Kasab, 1996). Some of these limitations may be overcome by newer methods such as impedance planimetry, where cross-sectional area, tension, strain, etc., can be measured (Drewes et al., 2001; Gregersen, 1998; Gregersen et al., 1999). The clinical relevance of a mechanical stimulus can, however, be questioned, as pain in most gastrointestinal diseases is not related to distension of the gut. Furthermore, in diseases with mechanical obstruction such as appendicitis, distension co-exists with inflammation, which is probably more important in the activation of nociceptors. Chemical stimuli of the gut are more natural and are believed to be close to the ideal experimental visceral pain stimulus. In contrast to electrical stimuli, chemical stimuli most likely activate C-fibres more frequently than myelinated fibres (Longhurst, 1995), and this may be an advantage in basic studies. In animals, several algogenic substances, such as acidic and basic solutions, bradykinin, etc., have been applied to exposed visceral surfaces or injected into the blood supply of various organs, but due to the potential harmful effects in man only a few studies exist.

Experimental visceral pain in humans by injection of bradykinin was investigated by Lim et al. (1967), who injected the substance into the peritoneal cavity. Bradykinin injections may, however, lead to reflex contractions of the organs, which can affect the assessment of pain (Bhoola et al., 1992). In a recent study (Louvel et al., 1996) pain was evoked in patients with irritable bowel syndrome (IBS) by injecting glycerol into the large intestine. Patients with IBS may, however, suffer from visceral hyperalgesia and central neuroplas-
tic changes (Rössel et al., 1999), and the interpretation with relation to basic pain mechanisms can be confounded. Correspondingly, injecting glycerol into the rectum in healthy controls did not evoke pain, although hypersensitivity to distension was induced (Bouin et al., 2001). In the oesophagus infusion with 0.1 N HCl has been used in clinical and experimental studies (Bernstein, 1958; Sarker et al., 2000) and has been shown to be of value when identifying subjects with pre-existing increased sensitivity (Fass et al., 1998). In normal subjects, however, pain or unpleasant sensations typically are not experienced following a standardised test (Bernstein, 1958; Hu et al., 2000). As the infusion affects a variable area of the mucosa, it is difficult to control, and the test itself does not fulfil the requirements of a well-defined experimental stimulus (Arendt-Nielsen, 1997). Other preliminary human studies applied algogenic substances to the mucosa in selected patients with pre-existing gastro- or colostomies, but the results were not consistent (Hardy et al., 1952; Ness and Gebhart, 1990; Wolf and Wolf, 1993). Our current attempt to search for other possibilities of chemical stimuli to evoke gut sensations therefore seems highly relevant. It should be stated, however, that the response to the saline injections might differ from the response to other stimuli. In future studies the psychophysical and neurophysiological responses to saline injections should therefore be compared with, e.g., electrical and mechanical stimuli.

6.2. Advantages of the current model

A reliable experimental pain stimulus must be standardised and controllable with regard to pain intensity and duration, and dose-dependent increases in sensory intensity should preferably be demonstrated. In the current study we injected hypertonic saline, since this stimulus has previously proved to fulfil these features when inducing experimental muscle pain (Arendt-Nielsen et al., 2000; Graven-Nielsen, 1997). As visceral pain may be a very unpleasant sensation with autonomic side effects, the model should be carefully tested with respect to the above parameters. Hypertonic saline has previously been shown to induce pain-like behaviour in animals, when injected into arteries supplying the external surface of visceral structures (Arendt-Nielsen et al., 1994), but until today observations in humans have not been reported. In the muscle this chemical pain model has several advantages compared to other experimental models, as the stimulus seems to give a consistent sensory response, with an intensity that correlates with the volume, infusion speed, and concentration of the saline (Arendt-Nielsen et al., 2000; Graven-Nielsen et al., 1997). Thus, the model has given important information about basic pain mechanisms in the muscles. In the current study the injections were given in the mucosa to mimic most inflammatory gut diseases, which start at the luminal site. The successive injections resulted in a sensory response in most subjects, although it was only painful in approximately 50%. The sparse innervation by sensory afferents in the gut may explain this, as the injections with 0.1-0.2 ml may only affect a few sensory afferents, and central summation of the input from several neurones probably is necessary to evoke pain. Moreover, the peripheral termination of sensory afferents in the gut is still debated, as mucosal afferents may only constitute a minor population of the sensory afferents in the colon. Thus, in the colon of the cat sensory afferents were shown to be predominant in the serosa (Jänig et al., 1993). On the other hand, most animal experiments have shown mucosal afferents present below the epithelium throughout the gastrointestinal tract, and mechanical as well as chemical stimuli, applied at the luminal site, were able to activate afferent fibres in the ventral root (Sengupta and Gebhart, 1994; Su and Gebhart, 1998). Accordingly, Lembo et al. (1994) was able to decrease the response to slow ramp distensions by applying lidocaine to the mucosa in human experiments. In the current study the sensory response was evoked, before any diffusion of the saline could have entered the serosa. We aimed at giving the injections into the mucosa, although it cannot be excluded, that a small amount of saline might have entered the submucosa or the circular muscle layer, either directly by the injection or by diffusion. The existence of sensory afferents in the mucosa was therefore clearly demonstrated, and the model might serve as a potent instrument in the study of the anatomy and physiology of the afferent innervation of the colon in man.

The sensation following successive injections was variable, but in general there was a correlation between the intensity and the duration of the response. The pump infusion evoked a sensory response in all patients. The onset time was reproducible, and the maximal pain intensity was the same in 5 out of the 7 subjects, showing the method to be valuable in experiments, where pain is modulated by, e.g., analgesics. No placebo infusions were given with the pump, since the pilot experiment showed no consistent effect of isotonic saline infusions, and the successive isotonic injections with a total of 0.8 ml in experiment I gave no consistent sensory responses.

The evoked sensation was clinically relevant with both local and referred sensations in 7 subjects. Referred pain is evoked in 20–85% after hypertonic saline injections in the muscle (Arendt-Nielsen et al., 2000), which is consistent with our results. Compared to other visceral models using distension (Munakata et al., 1997; Ness et al., 1990) and electrical stimuli (Arendt-Nielsen et al., 1997a; Drewes et al., 1999b) the referred areas were not very large, but they corresponded very well with the low stimulus intensity in the current study. After the sensation disappeared, no adverse effects re-
lated to sensation or motility were reported, and the model is therefore considered safe as an experimental stimulus.

6.3. Limitations of the model

The main limitation of the model is that the number of subsequent stimuli or the infused volume is limited due to the potential risk of tissue damage, when large volumes of hypertonic saline are injected. In the current experiment, however, 0.8 ml was given without observing side effects such as bleeding, ulcers, or motility disturbances. Furthermore, in pilot experiments we have given up to 1.6 ml 6% saline without side effects or visual evidence of tissue damage, and it is probably safe to give even larger volumes. Recently, animal studies showed that hypertonic saline in the current concentrations caused no toxicity to skeletal muscles (Svendsen et al., 2001). Correspondingly, several hundred intramuscular injections with hypertonic saline have been given in our laboratory without side-effects, except for a few cases of hematomas caused by the injection needle (Svensson, 2000), thus proving such injections to be safe.

Another limitation is that only half of the subjects reported pain, although unpleasantness was clearly demonstrated. This could be related to the low intensity of the stimulus. The data do not support the use of higher concentrations of saline. In the muscles infusion speed may interfere with the results (Graven-Nielsen et al., 1997), but our pilot experiments did not suggest, that this would increase the sensory intensity. Thus, future experiments should probably use higher volumes, preferably given with the infusion pump.

We were able to demonstrate an increased response to increasing concentrations and volumes in many patients, although the stimulus–response function was not as consistent as for studies of hypertonic saline injections in muscles (Graven-Nielsen et al., 1997). Due to the complex nervous innervation and corresponding diffuse sensation evoked by stimulation of the viscera, the same consistent stimulus–response functions, as seen in somatic tissue, can probably not be obtained in the gut mucosa. For other visceral stimuli such as distension and electrical stimuli, stimulus–response functions have been demonstrated. In distension models, however, the response may be related to motility, as, e.g., contractions against the balloon result in varying responses, which may not be reproducible, and different responses to continuous electrical stimuli have also been demonstrated, for example in the stomach (Drewes et al., 1999a).

In these experiments patients with a stoma were selected due to the easy access to the gut. Because this was the first experiment, several arrangements with regard to security were made. Consequently, the model gave us the possibility of inspecting the gut directly during and after the injections, and the patients could observe, whether or not harmful long-term effects occurred during the first days after the injections. The results in the current study cannot be transferred directly to subjects with a normal continuation of the gut, but it is assumed that the nervous innervation in the stoma is the same for the intact gut, and in future experiments the model should be refined and extended to non-operated subjects and patient groups. The same technique could be applied via endoscopy.

As the experiment was blinded, we believe that any psychological factors would have affected the response to iso- and hypertonic saline to the same degree. Only a few subjects had a consistent sensory response to the isotonic saline injections, and in that case it was very short lasting. There were no long-lasting responses following the isotonic injections. As the typical response to hypertonic saline injection was long lasting and seen after a latency of more than 25 s, the effect of psychological parameters could probably not have any major effect on the results.

Objective assessments with, e.g., neurophysiological or autonomic parameters were not made in the current study. Such recordings could give valuable information about different peripheral and central responses to noxious stimuli, but we believe that the nature (latency, intensity, etc.) of the stimuli remained to be determined before such a comprehensive set-up could be introduced in the experiment. The neurophysiological methods that have been used to assess visceral pain intensity in man are evoked brain potentials and the nociceptive reflex. The experiments are very difficult to perform and we did not want to confound the valuable psychophysical data we obtained from the individual subjects by other methods for pain assessment. In the current experiment, there was also a long latency and slow increase in sensory intensity to the saline injections. Hence, methods such as evoked brain potentials could not be used as they demand a well-defined, phase-locked stimulus to give reproducible results to a repeated series of stimuli. The nociceptive reflex can be modified by visceral stimuli. Bouhassira et al. (1998) showed that tonic distension of the stomach and rectum caused inhibition of the reflex, whereas phasic mechanical stimuli of the rectum resulted in more complex modulations. The reflex may probably be the best objective measurement to assess the effect of chemogenic visceral pain in human studies, but eliciting the reflex is also painful and we did not want to expose the subjects to this additional burden. Autonomic responses such as changes in heart rate, respiration, electromyographic activity, and skin resistance may also be used as indirect measures of visceral nerve activity. Animal experiments have shown that stimulation of visceral nerves results in autonomic reflexes via relays in prevertebral ganglia and impulse transmission at higher levels in the CNS (Jänig and
Häbler, 1995). More advanced methods such as spectral analysis of heart rate variability have been used to demonstrate increased sympathetic activity in patients with functional disorders of the gut (Karling et al., 1998; Tougas et al., 2001), but to our knowledge autonomic reactions have not been systematically used as a correlate of pain intensity related to the human gut. Autonomic responses may be important to give more basic information about the integrity of the pain system following experimental pain stimuli and could be included in future studies.

6.4. Physiological mechanisms

The mechanisms, by which hypertonic saline activates sensory afferents, are not known. In the muscles hypertonic saline injections excite non-nociceptive afferents together with myelinated and non-myelinated nociceptive nerve fibres (Mense, 1993). The non-sensory manifestations are, however, not demonstrated to a detectable degree, and in general it is assumed, that saline-excitation of other receptors than nociceptors does not have a major influence on the sensory manifestations (Graven-Nielsen, 1997; Svensson, 2000). Stimulation of axotomised afferent nerve fibres demonstrated an excitatory effect of hypertonic saline on a significant proportion of C-fibres in the muscles (Michaelis et al., 1997). Correspondingly, the dominant sensation following hypertonic saline injections in the muscle is a deep, diffuse pain after some latency, corresponding well with a major activation of C-fibres (Drewes et al., 1997; Graven-Nielsen, 1997; Svensson, 2000). The same kind of sensation was demonstrated in the current study, where no motility changes were observed. The rather long latency and words used to characterise the pain mimics to some degree also the “second” burning pain response seen to C-fibre activation of the skin. This contrasts the response to, e.g., electrical stimuli of patients with a stoma described as an immediate feeling of “shooting and pricking” pain (Arendt-Nielsen et al., 1997b). Thus, the hypertonic saline probably activates predominantly uncoupled C-fibres in the gut, responsible for mediating the sensation to the central nervous system. The physiological mechanisms are close to those seen, when natural stimuli such as inflammation and ischemia affect the gut from the luminal side. This is in contrast to the non-specific electrical stimuli and the mechanical stimuli, which mainly activate deeper receptors (Sengupta and Gebhart, 1994). Therefore, the model may be more suitable to mimic the clinical pain in diseases such as appendicitis, ulcerative colitis, etc.

In future experiments the model could be refined and extended to non-operated subjects and patient groups. The same technique could be applied via, e.g., endoscopy in areas of the gut such as the rectum and sigmoid colon. The response to a chemical stimulus in patients with visceral hyperalgiesia may be more pronounced compared to healthy controls and such studies could be of major basic interest. As the hypertonic saline stimuli are thought predominantly to affect C-fibres the response to, e.g., pharmacological treatment could be compared with other stimuli such as mechanical and electrical stimuli that affect both C and A fibre populations (Ness and Gebhart, 1990) and this may give more differentiated information about the visceral pain system.

7. Conclusion

In conclusion, the present experimental model may give the basis for studies of "true" visceral sensory afferents in man. The injections can be given in different layers of the gut, and therefore the model provides an opportunity to increase our knowledge on the basic anatomy and physiology of visceral nerve pathways. Clinically, inflamed viscera are the most common sources of abdominal pain in non-functional diseases, and chemical stimuli mimic gastrointestinal illness to a greater extent than the existing human models. This makes the experimental model of interest in future experiments with, e.g., pharmacological modulation of pain from the human gut.

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Experimental pain by ischaemic contractions compared with pain by intramuscular infusions of adenosine and hypertonic saline

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Abstract

Deep tissue pain can be related to reduced muscle blood flow, which comprises the metabolic demand under muscle work. The tissues and receptors involved in nociception after ischaemic muscle contractions are not known. The concentration of adenosine is increased after ischaemic contractions and might act as an algicic substance. In 15 subjects, adenosine, hypertonic saline (algesic), and isotonic mannitol (placebo) were infused into the tibialis anterior muscle and compared with the pain caused by ischaemic contractions. The muscle pain intensity (visual analogue scale; VAS), distribution, and quality were assessed. Pressure pain thresholds were recorded to assess the deep tissue sensitivity. Adenosine did not induce more pain than the placebo. The maximal VAS score after hypertonic saline and ischaemic contractions was higher compared with adenosine/placebo infusions. The duration and area of pain were significantly increased after hypertonic saline infusions compared with ischaemic contractions. Higher scores on the McGill pain questionnaire were given to the "stabbing", "burning", "heavy", and "exhausting" word categories after ischaemic contractions, and "cramping" was rated higher during hypertonic saline-induced muscle pain compared with ischaemic contractions. During hypertonic saline infusions, the pressure pain threshold was decreased compared with before and immediately after the pain had vanished. The present study shows that pharmacological levels of adenosine in skeletal muscle did not induce pain. Excitation of muscle nociceptors by hypertonic saline evoked hyperalgesia, larger areas of pain, and a different quality of pain compared with ischaemic contractions, suggesting that the pain after ischaemic contractions is mediated by other populations of nociceptors in muscle and/or other tissues than excited by hypertonic saline.

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Keywords: Experimental muscle pain; Referred pain; Pressure algometry; Ischaemic pain; Adenosine

1. Introduction

In various musculoskeletal pain conditions such as the compartment syndrome the main cause of the deep tissue pain is related to reduced blood flow which compromises the metabolic demand under muscle work (Matsen, 1979; Martens et al., 1984; Martens and Moeysornoos, 1990). In experimental settings, repeated contractions of limb muscles under ischaemia by application of a tourniquet produce a deep pain sensation with moderate to high pain intensity (Harpuder and Stein, 1943; Lewis, 1932; Mills et al., 1982; Vecchiet et al., 1987).

The involved mechanisms of deep pain after ischaemic contractions are not fully understood. Lewis (1932) suggested that the pain induced by ischaemic contractions might be due to a physical or chemical mechanism determined as "factor P". "Factor P" is formed under ischaemic contractions and remains unchanged or accumulates with each contraction under circulatory arrest.
(Lewis, 1932). Accumulation of various substances (i.e. potassium, adenosine, lactate) has been suggested to excite muscle nociceptors or sensitise nociceptors to respond to muscle contractions that are normally non-painful (Mense and Simons, 2001; Newham and Mills, 1999). The muscle sensitivity during pain evoked by ischaemic contractions has however not previously been assessed.

Progressively increased work rate escalates the concentration of skeletal muscle interstitial adenosine (Hellsten et al., 1998; Costa et al., 1999) and a further increase is found during exercise combined with ischaemia (Costa et al., 1999). In animals, adenosine excites joint nociceptors (Dowd et al., 1998), and therefore adenosine might be a candidate for exciting muscle nociceptors. Actually, adenosine antagonists delay the development of pain experienced after ischaemic contractions (Jonzon et al., 1989; Myers et al., 1997). Intramuscular injections of adenosine induce distal limb pain (Sylven et al., 1988). Also adenosine applied on human blister base preparation induces pain (Bleehen and Keele, 1977). Thus, it is likely that adenosine has a role in muscle pain and muscle hyperalgesia. If this is the case, a dose-response relation may be expected.

Intramuscular injections of algogenic substances such as capsaicin, bradykinin, serotonin, potassium chloride, levo-ascorbic acid, acid phosphate buffer, and hypertonic saline have been used to induce muscle pain and thereby excitation of muscle nociceptors; for reviews see Arendt-Nielsen (1997), Graven-Nielsen and Mense (2001), and Graven-Nielsen et al. (2001). In the present study, hypertonic saline-induced muscle pain was used as an active control.

The aim of the present study was to investigate if pain by ischaemic contractions can be explained by: (1) an algogenic effect of adenosine, (2) excitation of muscle nociceptors alone, and/or (3) sensitised muscle nociceptors. In a placebo-controlled manner, the pain intensity, pain quality, and sensitivity to pressure were compared during intramuscular (i.m.) infusion of adenosine or hypertonic saline, or after ischaemic exercise-induced pain.

2. Materials and methods

2.1. Subjects

Fifteen non-medicated subjects participated in this study (7 male, 8 female; mean age: 29 years; range 23–40 years). Palpation of the test sites was performed to exclude subjects with signs of local tenderness or sore deep tissues. The study was conducted in accordance with the Declaration of Helsinki, approved by the local Ethics Committee, and written informed consent was obtained from all participants prior to inclusion.

2.2. Protocol

The subject was positioned in a supine position in a bed. The subjects received five infusions (three doses of adenosine, hypertonic saline, and isotonic mannitol as placebo) and one trial with ischaemic-contraction induced pain. The experiment comprised three sessions separated by minimum one week. In each session, one intervention (infusions or ischaemic contractions) was performed in each tibialis anterior muscles (in total six interventions). All interventions were applied in a random sequence.

2.3. Ischaemic contraction pain

Ischaemic muscle pain was induced by application of a tourniquet (inflated to 300 mmHg) proximal to the knee. Before application of the tourniquet, the leg was raised to a vertical position for 2 min to drain the leg. A load of 3 kg was then strapped to the foot as distally as possible to resist dorsal flexion of the foot. This load is approximately 5–10% of the maximal contraction effort in dorsiflexion (Stoll et al., 2000). Subsequently, the subject completed 45 contractions (dorsiflexion) over 90 s (2 s contraction and 2 s rest). Five additional contractions were performed 3 min after the first bout of contractions. The tourniquet was kept inflated for 6 min.

2.4. Intramuscular infusions

Infusions of adenosine, isotonic mannitol (placebo), and hypertonic saline were accomplished with a computer-controlled syringe pump (IVAC, model 770) and a 10 ml plastic syringe. A tube (IVAC G3030, extension set with polyethylene inner line) was connected from the syringe to a disposable needle (27G, 20 mm). The infusion site was 15 cm distal to the apex patellae and the infusion depth was approximately 20 mm. Infusions of adenosine (0.75 mg/ml, 2.5 mg/ml and 5 mg/ml) in isotonic mannitol, isotonic mannitol (50 mg/ml) and hypertonic saline (58.5 mg/ml) were performed over 6 min with an infusion rate of 30 ml/h (i.e., 3 ml). The various adenosine and mannitol solutions were prepared from 5 mg/ml adenosine (Item Development AB, Stocksund, Sweden), 150 mg/ml mannitol (Fresenius Kabi, Uppsala, Sweden), and isotonic saline.

2.5. Assessment of muscle and referred pain

The pain intensity was continuously scored on a 10-cm electronic visual analogue scale (VAS) where 0 cm indicated “no pain” and 10 cm “most intense pain”. The pain intensity was sampled every 5 s by the computer. The area under the VAS-time curve (VAS area), the maximum pain intensity rating (VAS peak), time to VAS peak (from initialisation of recording), and the
onset (first time VAS > 0) and duration of pain were determined.

The subject drew the distribution of pain on an anatomical map. The circumference of the pain areas was scanned (Snap Scan 310, Agfa, Belgium), and the area calculated (SigmaScan Pro). If no pain was perceived, the pain area was included in the statistics as zero. Pain around the injection site was defined as local pain. Referred pain was defined as pain occurring outside and remote from the local pain area. Frequently, experimental muscle pain induced in the tibialis anterior muscle results in widespread pain including the typical area of referred pain; frontal aspect of the ankle (Graven-Nielsen et al., 1997b). In conditions with widespread pain from the local to the typical referred pain area, the division between local and referred pain cannot be established, and the entire pain area is, therefore, defined as local pain. The proportions of subjects, who indicate pain (1) around the tibialis anterior muscle, and (2) in the typical referred pain area, were determined. The borderline between the two regions was defined as a line connecting the lateral and medial malleolus (Fig. 3B).

The quality of pain was assessed by a Swedish version of the short form McGill pain questionnaire (MPQ) (Burckhardt and Bjell, 1994). This included 15 words, which were numerically rated with 0 (none), 1 (mild), 2 (moderate), or 3 (severe).

2.6. Assessment of sensitivity to pressure

Pain sensitivity to pressure was examined 18 cm from the apex patella. Pressure pain thresholds (PPTs) were determined with an electronic pressure algometer (Somedic AB, Farsta, Sweden) equipped with a 1 cm² probe. Force was applied with approximately 50 kPa/s in a simple, continuously ascending series. The subject was instructed to press a button at the moment that the pressure stimulation elicited "just noticeable" pain. The mean of three measurements with a 25 s interval defined the PPT. PPTs were recorded two times before, 2 min and 5 min after intervention start, immediately after deep tissue pain had vanished, and finally 10 min after the pain had vanished. During and post-intervention recordings were normalised to the mean of pre-recordings.

2.7. Statistical analysis

The non-parametric Friedman analysis of variance for repeated measures was used for the analysis of VAS, MPQ, and pain area parameters. When this test gave significant results, it was followed by the non-parametric Student–Newman–Keuls (SNK) test. For PPTs a two-way analysis of variance (ANOVA) for repeated measures with time (before, during, and after) and intervention (the different infusions and ischaemia) as factors was used. If significant, the parametric Student–Newman–Keuls (SNK) test was used for post-hoc test and to correct for multiple comparisons. Spearman's correlation coefficients were used to describe the correlation between parameters. The data were presented as means and standard error of the means (SE). The significance was accepted at $P < 0.05$.

3. Results

3.1. Pain intensity

The VAS–time profiles were significantly different among the six interventions (Fig. 1). Quantitative data from the VAS–time profiles showed that the VAS area was significantly larger, and the duration of pain significantly prolonged after hypertonic saline-induced muscle pain compared with all other interventions (Table 1; Friedman: $P < 0.011$; SNK: $P < 0.05$). After ischaemic contractions, the VAS area was significantly larger than adenosine and mannitol (placebo) infusions (SNK: $P < 0.05$). The VAS peak after hypertonic saline and ischaemic contractions was similar and both were significantly higher than for infusion of adenosine and mannitol (SNK: $P < 0.05$).

The relation between VAS peaks and VAS onset (Fig. 2A) illustrates that the adenosine infusions have a delayed VAS onset and a lower VAS peak compared with ischaemic contractions and hypertonic saline-induced pain. Between three and six subjects did not perceive pain after each of the three doses of adenosine. As the

![Fig. 1. The mean VAS profiles after ischaemic contractions and infusion of hypertonic saline, adenosine, and isotonic mannitol (placebo). The bars below the abscissa illustrate the time for infusion, inflation of tourniquet, and periods for ischaemic contractions.](image-url)
Table 1
Mean (± SE) VAS parameters after infusion of hypertonic saline, placebo (mannitol), adenosine, and after ischaemic contractions

<table>
<thead>
<tr>
<th></th>
<th>VAS area (cm²)</th>
<th>VAS peak (cm)</th>
<th>VAS onset (s)</th>
<th>VAS duration (s)</th>
<th>VAS peak time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline, 58.5 mg/ml</td>
<td>2867.3 ± 326.0a</td>
<td>7.2 ± 0.5a</td>
<td>19.6 ± 4.2</td>
<td>670.4 ± 52.2a</td>
<td>330.0 ± 29.1</td>
</tr>
<tr>
<td>Ischaemic contractions</td>
<td>1357.2 ± 197.0d</td>
<td>6.4 ± 0.3b</td>
<td>28.3 ± 6.7</td>
<td>474.3 ± 29.2</td>
<td>343.7 ± 15.5</td>
</tr>
<tr>
<td>Mannitol, 50 mg/ml</td>
<td>613.1 ± 168.2</td>
<td>1.9 ± 0.4</td>
<td>52.5 ± 12.0 (n = 12)</td>
<td>420.7 ± 72.8</td>
<td>235.4 ± 29.1 (n = 12)</td>
</tr>
<tr>
<td>Adenosine, 0.75 mg/ml</td>
<td>635.2 ± 236.9</td>
<td>2.3 ± 0.6</td>
<td>92.1 ± 18.0 (n = 12)</td>
<td>362.7 ± 96.7</td>
<td>247.9 ± 31.1 (n = 12)</td>
</tr>
<tr>
<td>Adenosine, 2.5 mg/ml</td>
<td>492.0 ± 197.9</td>
<td>1.4 ± 0.4</td>
<td>144.0 ± 55.2 (n = 10)</td>
<td>361.0 ± 123.4</td>
<td>363.0 ± 75.0 (n = 10)</td>
</tr>
<tr>
<td>Adenosine, 5 mg/ml</td>
<td>356.8 ± 95.2</td>
<td>1.2 ± 0.3</td>
<td>110.6 ± 29.5 (n = 9)</td>
<td>405.3 ± 110.8</td>
<td>507.2 ± 86.7 (n = 9)</td>
</tr>
<tr>
<td>P-value Friedman</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.051</td>
<td>0.011</td>
<td>NS</td>
</tr>
</tbody>
</table>

Significant difference compared with all interventions (a) or compared with adenosine and placebo infusions (b) is illustrated (SNK: P < 0.05). NS: not significant.

time for onset of pain is not defined in these subjects, a comparison of onset across all interventions is not reliable. However, all subjects experienced pain after at least one of the three adenosine infusions, and the minimum VAS onset from these recordings was extracted (85.3 ± 16.3 s). The minimum VAS onset after adenosine compared with hypertonic saline, ischaemic contractions, and mannitol showed that adenosine infusions had significantly longer VAS onset compared with the other interventions (Friedman: P < 0.001; SNK: P < 0.05). Moreover, hypertonic saline and ischaemic contractions had faster VAS onset than mannitol (SNK: P < 0.05). Together these findings indicate that pain induced by adenosine most likely is delayed compared with mannitol, hypertonic saline, and ischaemic contractions.

3.2. Pain distribution

In general, the pain distribution (Fig. 3A) was characterised by local muscle pain around the injection site and referred deep pain to the anterior part of the ankle and foot. The total area (local plus referred pain area) of pain was significantly increased for hypertonic salinel-induced pain compared with the pain after ischaemic contractions, and both conditions evoked total pain areas that were significantly larger than after adenosine and mannitol infusions (Table 2; Friedman: P < 0.001; SNK: P < 0.05). The same was found for the local pain area (Table 2). The total area of pain was significantly correlated to the VAS area and VAS peak (Fig. 2B, R > 0.83, P < 0.0001). Only few subjects met the criterion for referred pain (i.e. a distinct pain area not included in the local pain area; Table 2). Nonetheless, about 80% of the subjects experienced pain in the ankle and foot area after both hypertonic saline infusions and ischaemic contractions (Fig. 3B).

3.3. Pain quality

Ischaemic contractions produced significantly higher ratings of the "stabbing", "burning", "heavy", and "exhausting" word categories from the MFQ compared with all the intramuscular infusions (Fig. 4; Friedman: P < 0.005; SNK: P < 0.05). "Cramping" was rated significantly higher after the hypertonic saline infusion compared with all other interventions and significantly higher for ischaemic contractions compared with the adenosine and mannitol infusions (Friedman: P < 0.001; SNK: P < 0.05).
Fig. 3. (A) The individual pain drawings after ischaemic contractions and infusion of hypertonic saline, adenosine, and mannitol (placebo). Right leg drawings have been transposed to the left leg. (B) The proportions of subjects, who indicate pain around the tibialis anterior muscle (filled bars) and in the typical referred pain area (open bars), are shown. The insert illustrates the borderline between the two regions.

Table 2
Mean (±SE) area of local and referred pain after infusion of hypertonic saline, placebo (mannitol), adenosine, and after ischaemic contractions

<table>
<thead>
<tr>
<th></th>
<th>Local pain area (AU)</th>
<th>Referred pain area (AU)</th>
<th>Total pain area (AU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertonic saline, 58.5 mg/ml</td>
<td>912.1 ± 346.8 (13)</td>
<td>100.6 ± 46.9 (5)</td>
<td>1012.7 ± 316.6*</td>
</tr>
<tr>
<td>Ischaemic contractions</td>
<td>675.2 ± 171.0 (13)</td>
<td>39.9 ± 36.7 (3)</td>
<td>715.1 ± 108.1*</td>
</tr>
<tr>
<td>Mannitol, 50mg/ml</td>
<td>116.3 ± 38.1 (10)</td>
<td>82.1 ± 73.1 (2)</td>
<td>198.4 ± 73.7</td>
</tr>
<tr>
<td>Adenosine, 0.75mg/ml</td>
<td>154.9 ± 70.9 (11)</td>
<td>23.5 ± 16.6 (3)</td>
<td>178.5 ± 70.7</td>
</tr>
<tr>
<td>Adenosine, 2.5mg/ml</td>
<td>75.6 ± 28.5 (9)</td>
<td>26.0 ± 17.7 (3)</td>
<td>101.6 ± 35.3</td>
</tr>
<tr>
<td>Adenosine, 5mg/ml</td>
<td>54.7 ± 24.6 (5)</td>
<td>60.5 ± 32.0 (6)</td>
<td>115.2 ± 37.1</td>
</tr>
<tr>
<td>P-value Friedman</td>
<td>&lt; 0.001</td>
<td>NS</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Pain areas are given in arbitrary units (AU). The number of subjects developing either local or referred pain is indicated in parenthesis. Significant difference compared with all interventions is illustrated (*, SNK: P < 0.05).

NS: not significant.

3.4. Pressure algometry

During infusion of hypertonic saline the PPTs decreased significantly compared with all other interventions and compared with pre- and post-recordings (Fig. 5; ANOVA: df = 20; F = 4.10; P = 0.0001; SNK: P < 0.05). In this condition, the absolute decrease in PPT was significantly correlated to the muscle pain intensity (VAS area: R = 0.42; P = 0.025); i.e. subjects who scored high pain intensities also showed the most pronounced hyperalgesia to pressure. In contrast, the PPTs increased 2 min after the ischaemic contractions were initiated (SNK: P < 0.05) compared with the other interventions.

4. Discussion

The present study shows that i.m. infusion of adenosine in different doses does not induce more muscle pain than placebo. Excitation of muscle nociceptors by infusion of hypertonic saline induced more pain than after ischaemic contractions. The quality of pain induced by ischaemic contractions was characteristically different compared with muscle pain evoked by hypertonic saline. Together these findings indicate that the pain after ischaemic contractions is probably mediated by other populations of nociceptors in muscle and/or other tissues than excited by hypertonic saline.
4.1. Muscle pain by algesic substances

Intramuscular infusion of hypertonic saline produced moderate to high intensity of muscle pain frequently associated with referred pain as also reported before; for a review see Graven-Nielsen et al. (2001). The quality of hypertonic saline-induced muscle pain was mainly described as a cramping sensation that is different from cutaneous pain, which has a burning and sharp quality (Bonica, 1990).

Hypertonic saline produced hyperalgesia to pressure during the infusion. Immediately after the pain had vanished, no hyperalgesia could be detected. Previous findings on muscle sensitivity in hypertonic saline-induced muscle pain areas are not clear as hyperalgesia (Feinstein et al., 1954; Jensen and Norup, 1992; Svensson et al., 1995; Vecchiet et al., 1988), hypoalgesia (Arendt-Nielsen et al., 1997), or hyperalgesia after both hypertonic and isotonic saline (Graven-Nielsen et al., 1998a) have been detected. The muscle pain intensity might be of importance since a correlation between the degree of hyperalgesia and pain intensity was found in the present study. Animal studies have shown that peripheral sensitisation of muscle nociceptors by algesic substances is active for several minutes (Mense, 1993). The immediate reversibility of hyperalgesia when pain vanishes indicates that it is not a typical peripheral sensitisation (i.e. release of algesic substances) which is responsible for the hyperalgesia during hypertonic saline-induced muscle pain. The necessary ongoing pain for hyperalgesia to pressure might alternatively reflect a central mechanism. Assuming that hypertonic saline and...
pressure excite two different pools of muscle nociceptors (the pressure site and injection site are separated by 3 cm), spatial summation of nociceptive activity at a central level may be important. Previously, hyperalgesia was detected at the injection site but not 4 cm distal to the injection site during experimental muscle pain by 0.5 ml hypertonic saline (Graven-Nielsen et al., 1998b). The contrasting finding of hyperalgesia 3 cm distal to the injection site in the present study is probably explained by the larger infusion volume (3 ml) which might spread to the muscle tissue below the pressure assessment site and thereby sensitise the muscle nociceptors which also respond to pressure. Finally, in the present study two conditions with moderate to high pain intensity (hypertonic saline infusion and ischaemic contractions) produced opposite effects on pressure pain thresholds in contrast to what might be expected if ongoing pain is a distracting factor.

Intramuscular infusion of adenosine did not induce more pain than a similar volume of isotonic mannitol. Intradermal (Pappagallo et al., 1993), intradermal or intravenous (Crea et al., 1999; Lagerqvist et al., 1990, 1992; Sadigh-Lindell et al., 2001; Sylven et al., 1988, 1989) and blister application (Bleehen and Keele, 1977) of adenosine have been used to induce pain in humans. Nonetheless, adenosine does not evoke pain when injected in veins (Klement and Arndt, 1992). Three subtypes of adenosine receptor (A1, A2, and A3) at peripheral nerve terminals have been described (Sawynok, 1998). These subtypes have either nociceptive (A2, A3 via release of histamine and serotonin) or antinociceptive (A1) effects in rodents, but there are contrasts when comparing with human studies. The pain induced by intradermal or intravenous injections of adenosine is significantly reduced by bapineuline, a selective A1 receptor antagonist (Gasparo et al., 1995; Pappagallo et al., 1993); i.e. adenosine-induced pain is probably mediated by A1 receptors in humans. No significant pain was induced by intramuscular infusions of adenosine diluted in isotonic mannitol, compared with isotonic mannitol alone even though the doses were comparable to (or higher) what has previously been applied for intravenous, intraarterial, and intradermal administrations. Isotonic mannitol alone induced higher levels of pain than expected. On average, the VAS peak after infusion of mannitol (1.9 cm) seems higher than the pain induced by infusion of isotonic saline (VAS below 1 cm) as reported in previous studies (Babenko et al., 1999; Graven-Nielsen et al., 1998a,b; Svensson et al., 1998; Wang et al., 1999). The applied mannitol has the same osmolarity and pH levels as isotonic saline so the mechanism for excitation of muscle nociceptors by mannitol is not clear. Interestingly, those subjects developing pain during adenosine infusions showed significantly prolonged onset of the pain compared with infusion of mannitol. It is important to note that this delayed onset of pain is based on a pooled evaluation of all adenosine concentrations and could not be established within each concentration of adenosine. An immediate analgesic effect of adenosine might explain the prolonged time before onset of pain induced by mannitol. Hypothetically, there might be a competitive action between mannitol, which excites muscle nociceptors and an antinociceptive effect of adenosine. On the other hand, i.m. infusion of adenosine initiates the release of the algogenic substance bradykinin in animals (Boix and Knardahl, 2000). Intradermal injections of bradykinin have been found effective in exciting and sensitising nociceptors in animals (Mense, 1993). In humans, however, the potency of bradykinin alone is limited, but when injected together with serotonin, moderate levels of pain and hyperalgesia to pressure were found (Babenko et al., 1999).

4.2. Ischaemic contraction evoked pain

Previous studies have shown that the interstitial concentration of adenosine in resting muscle is in the range of 0.22–0.29 μmol/L with a progressive increase depending on the exercise level (Hellsten et al., 1998; Costa et al., 1999) and a fivefold increase (>1.5 μmol/L) during low-level ischaemic contractions (Costa et al., 1999). In the present study, the concentration of infused adenosine was in the range of 3–19 mmol/L assuring that the intramuscular concentration of adenosine is higher than what is obtained after ischaemic exercise. It is probably only a relatively small volume of muscle tissue in which the adenosine concentrations are increased and not directly comparable to an ischaemic muscle in which a large volume of muscle tissue has increased adenosine levels. Nonetheless, experimentally elevated adenosine levels did not induce muscle pain. This strongly suggests that increased muscle interstitial adenosine concentrations alone are not the main pain-producing factor in ischaemic contractions.

The pain quality after ischaemic contractions was described by “cramping” on the MPQ similar to hypertonic saline-induced muscle pain, but the words “stabbing”, “burning”, “heavy”, and “exhausting” were rated significantly higher than hypertonic saline-induced muscle pain. Especially, the “stabbing” and “burning” pain qualities indicate that ischaemic pain is not exclusively muscle pain since these words categories are frequently used for more superficial pain conditions (Graven-Nielsen et al., 1997a; Witting et al., 2000). Intravenous administrations (e.g. propofol) are known to induce pain with a burning quality (Eriksson et al., 1997) which suggests that the ischaemic pain might originate from the vascular system in addition to the pain mediated by muscle nociceptors. However, to qualify such a suggestion the algic substances must diffuse from the muscle to other tissues (e.g. skin or
vascular systems) as short time ischaemia without contractions do not induce pain. Alternatively, the ischaemic pain might be mediated by a subset of nociceptors that are not excited by i.m. hypertonic saline. Another possibility is that the increase in intramuscular temperature during exercise might excite heat sensitive muscle nociceptors. A recent study showed that 48°C isotonic saline injected intramuscularly induced muscle pain characterised by “thermal, hot”, and “dullness” words (Graven-Nielsen et al., 2002). The “thermal, hot” words were not selected after injection of hypertonic saline (Graven-Nielsen et al., 2002) similar to the present study. Exercise related increase in intramuscular temperature is typically in the range of 2–3°C with a long recovery period (Allsop et al., 1990), and combined with potential receptor sensitisation by substances released due to ischaemic contractions, this might excite the heat sensitive muscle nociceptors. In summary, it is likely that the subset of nociceptors excited by hypertonic saline is not exclusively excited by ischaemic contractions where other nociceptor populations (in muscle and/or other tissues) might be additionally excited. In line with this, only a fraction of muscle nociceptors responded to ischaemic contractions (Mense and Stahnke, 1983).

The models of pain by ischaemic contractions and infusion of hypertonic saline are characteristically different in the volume of stimulated tissue where spatial summation of neural activity might be important. Intraneural microstimulation of muscle nociceptive afferents causes muscle pain that is dependent on the number of stimulated afferents; i.e. spatial summation (Simone et al., 1994). Infusion of hypertonic saline is effective in a relatively small volume of the muscle (Graven-Nielsen et al., 1997c) in contrast to the ischaemic contractions that probably affect the whole muscle and eventually other tissues. Assuming that the pain induced by ischaemic contractions is mediated by muscle nociceptors and that these nociceptors are also excitable by hypertonic saline, then, by spatial summation, pain intensity should be higher after ischaemic contractions than after infusion of hypertonic saline. This is however in striking contrast to the present findings where hypertonic saline induced more pain than ischaemic contractions. One reasonable explanation may be that the nociceptors excited by ischaemic contractions respond with a lower firing frequency compared with the nociceptors excited by hypertonic saline. Finally, it should be noted that the intensity of pain by ischaemic contractions naturally depends on intensity and duration of the contractions.

Sensitisation of muscle nociceptors to respond to muscle contractions that are normally non-painful has been suggested as another mechanism involved in pain after ischaemic contractions (Mense and Simons, 2001; Newham and Mills, 1999). However, the pressure pain thresholds after ischaemic contractions were not decreased as expected in case of receptor sensitisation. In contrast, the first assessment immediately after the ischaemic contractions was performed showed increased pressure pain thresholds. Potentially, the tourniquet block may evoke hypoalgesia to pressure, but this has only been reliably detected after 15 min with ischaemia (Laursen et al., 1999) in contrast to the hypoalgesia detected after 2 min with ischaemia in the present study. Previously, hypoalgesia to pressure has been reported during and immediately after an isometric contraction (Kosek and Ekholm, 1995).

5. Conclusion

Intramuscular infusions of adenosine, far above in vivo concentrations during ischaemic contractions, did not produce more pain than control infusions indicating that adenosine is probably not involved in the pain evoked by ischaemic contractions. Thus, the substance mediating the pain (“factor P” according to Lewis (1932)) still needs to be determined. Hyperalgesia to pressure was not detected after ischaemic contractions suggesting that muscle nociceptors are not likely to be sensitised. In contrast, muscle pain evoked by hypertonic saline produced hyperalgesia to pressure. The degree of hyperalgesia was correlated to the pain intensity. Moreover, the typical quality of muscle pain alone was not comparable to the pain evoked by ischaemic contractions. Together these results indicate that the subset of nociceptors excited by hypertonic saline is not exclusively excited by ischaemic contractions where other nociceptor populations (in muscle and/or other tissues) might be additionally excited.

Acknowledgments

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