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RESEARCH ARTICLE

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Temporal summation of pain from skin, muscle and joint following nociceptive ultrasonic stimulation in humans

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Abstract This study investigated the phenomenon of temporal summation in response to repetitive focused ultrasound stimulation of skin, muscle and joint in human volunteers. Stimulation was carried out using a custom-designed, focused ultrasonic stimulator with a resonant frequency of 1.66 MHz. A series of stand-off attachments were used to ensure that the focal region of the ultrasound beam projected either cutaneously, within the distal interphalangeal joint of the index finger, or within the first dorsal interosseous muscle. Stimulation was carried out using single pulses and trains of five pulses of different pulse durations (25 ms, 50 ms, 75 ms, 100 ms), and using single pulses and trains of five pulses (50 ms duration) at different frequencies (0.5 Hz, 1 Hz, 2 Hz, 3 Hz, 4 Hz, 5 Hz). Tactile perception thresholds, pain thresholds and summation pain thresholds were recorded. Temporal summation of pain could be elicited by stimulation of both skin, joint and muscle, although the influence of temporal summation appeared to be more pronounced for muscle stimulation. Muscle stimulation also required greater ultrasound intensity compared with joint and skin stimulation. Temporal summation could not be elicited by tactile, low-intensity stimulation. Focused ultrasound is a potent, noninvasive technique with which to investigate temporal summation from somatic structures. A number of factors may account for the higher intensities required to elicit pain in muscle and the increased rate of temporal summation. It is clear, however, that if temporal summation is more pronounced in muscle than other tissues then this may be an important factor contributing to pain in musculoskeletal syndromes.

Keywords Muscle pain · Skin pain · Joint pain · Focused ultrasound · Temporal summation

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Introduction

Pain from deep somatic tissue is a major clinical problem and yet the basic mechanisms involved in muscle and joint pain are still not understood. Experimental pain studies with standardised induction and assessment of pain in healthy subjects can give new information on basic mechanisms involved in pain from deep structures (Arendt-Nielsen 1997; Graven-Nielsen et al. 2001). There are a limited number of models that can be used to study muscle pain in humans. Available methods include injection of hypertonic saline or endogenous algesiogenic substances and intramuscular electrical stimulation (Kellgren 1938; Jensen and Norup 1992; Zhang et al. 1993; Arendt-Nielsen et al. 1997; Graven-Nielsen et al. 1997; Rossi and Decchi 1997; Svensson et al. 1997; Babenko et al. 1999; Laursen et al. 1999; Stohler and Kowalski 1999; Witting et al. 2000). The disadvantage of the aforementioned methods is that they involve invasive procedures. An alternative method is stimulation with focused ultrasound, which has been used to induce joint and skin pain (Gavrilov et al. 1977; Tsirulnikov et al. 1986; Wright and Davies 1989; Wright et al. 1993).

The transducer for ultrasound stimulation is located externally but as a result of focusing the beam, the energy can be applied maximally to the deeper tissues, thereby selectively activating nociceptors in deep structures (for reviews, see Davies et al. 1996; Gavrilov et al. 1996). The distance from the ultrasound transducer to the focal region is accurately defined. By appropriate adjustment of this distance, it is possible to focus the main ultrasound energy in the target tissue and thereby use ul-

trasound to stimulate the skin, joints and other subcutaneous structures (Gavrilov 1984; Gavrilov et al. 1977; Tsirulnikov et al. 1986; Wright and Davies 1989; Wright et al. 1993). The diameter of the focal region varies between 6.4 and 1.1 mm for ultrasound transducers, with resonant frequencies between 0.48 and 2.67 MHz (Gavrilov et al. 1977). This suggests that for transducers with a resonant frequency in excess of 1.5 MHz the focal region will be relatively small and facilitate stimulation of specific target tissues. Gavrilov et al. (1977; Gavrilov 1984) report that, while ultrasound may elicit a variety of sensations when used to stimulate skin, stimulation of deeper structures predominantly elicits only a report of pain. As such, ultrasound constitutes a noninvasive means of producing a relatively pure pain sensation in deep tissue structures. However, whether it is possible to induce muscle pain using focused ultrasound stimulation has not been demonstrated explicitly.

The phenomenon that a single nociceptive stimulus by repetition causes exaggerated perceptions of human pain is called temporal summation, which is assumed to be related to the wind-up that can be measured in animal dorsal horn neurons (Arendt-Nielsen et al. 1994; Price et al. 1994; Ren 1994; Arendt-Nielsen and Petersen-Felix 1995). The relationship between temporal summation and wind-up is supported by the finding that both are inhibited by blocking the NMDA receptor (Dickenson and Sullivan 1987; Price et al. 1994; Arendt-Nielsen et al. 1995, 1996; Andersen et al. 1996). Temporal summation has been demonstrated for repetitive electrical and thermal cutaneous stimulation, saline infusion in muscles, electrical stimulation of muscles and electrical stimulation of visceral afferents (Price et al. 1994; Frøbert et al. 1995; Arendt-Nielsen et al. 1997; Graven-Nielsen et al. 1997; Svensson et al. 1997). To date, there has been no study evaluating the temporal summation phenomenon following stimulation of articular or muscle nociceptors by noninvasive techniques.

The aims of the present study were: (1) to assess focused ultrasound as a new noninvasive muscle pain stimulus, (2) to systematically evaluate the effect of stimulus duration on pain perception, and (3) to investigate the phenomenon of temporal summation to repetitive focused ultrasound stimulation of skin, joint and muscle.

Materials and methods

Subjects

The study included 15 healthy subjects (12 men, 3 women) with a mean age of 24 years 6 months (range 21 years 2 months–42 years 9 months). All subjects participated in two separate experiments at least 1 week apart. They were not experiencing any ongoing pain in the hand or arm at the time of the experiment, and subjects with any history of significant pain or surgeries affecting the upper limb were excluded from the study. The subject population did not include any authors of the study and subjects were not informed about the specific hypotheses being tested. Subjects gave their informed written consent prior to inclusion in the study, which had received approval from the local ethics committee and was performed in accordance with the Declaration of Helsinki.

Focused ultrasound stimulation

Ultrasonic stimuli were delivered via a computer-controlled ultrasonic stimulator. This consisted of three main elements: a pulse generator (Philips PM 5138; Germany) externally controlled by a computer, a radio-frequency power amplifier (A300; Electro Navigation Industries, USA) and a focused ultrasonic transducer (Queen's University, Northern Ireland) with a resonant frequency of 1.66 MHz. The function generator produced a 1.66-MHz sine-wave pulse with amplitude, duration and repetition frequency determined by the computer. The power amplifier provided 55-dB amplification of the output signal from the function generator and generated the drive signal that was applied to the ultrasound transducer. Ultrasonic stimuli were delivered as single pulses or trains of five pulses with different durations and interstimulus intervals. Adjusting the amplitude of the sine-wave pulse controlled the intensity of the ultrasonic stimulus. The stimulation intensity could be adjusted in increments of 0.01 arbitrary units (AU) up to a maximum of 1 AU.

The transducer consisted of a circular, concave piezoceramic disc with a diameter of 50 mm. A series of stand-off attachments were used in conjunction with the ultrasound transducer to control the depth to which the focal region of the ultrasound beam penetrated into the tissues tested. The stand-offs were filled with water at room temperature, to provide ultrasonic coupling between the transducer and the subjects' skin. The aperture of each of the stand-offs was the same (15 mm), ensuring that the water surface in contact with the skin was constant for each form of stimulation. By adjusting the stand-off attachments, the distance from the transducer to the focal region of the ultrasound beam was changed and it was possible to project the focal region to the optimal depth within the target tissue. For skin stimulation, the stand-off was adjusted to the upper limit of the focal region, whereas for joint stimulation and muscle stimulation the stand-offs were adjusted such that the focal region projected 5 mm and 7–8 mm below the skin, respectively. Because of the focused nature of the beam, this ensured that maximum ultrasonic intensity occurred within the deep tissues rather than the overlying skin.

Cutaneous stimuli were applied to the skin on the palmar surface of the distal end of the index finger. Articular stimuli were applied to the distal interphalangeal joint of the index finger with the beam being projected from the palmar surface of the finger. Muscular stimuli were delivered to the first dorsal interosseous muscle by projecting the ultrasound beam upwards from the palmar surface of the hand between the first and second metacarpal bones. The focal region of the beam was projected into the central portion of the muscle to avoid stimulating the adjacent metacarpal bones. The metacarpal bones were positioned to either side of the aperture to avoid direct stimulation by the ultrasound beam. At the cutaneous test site, it was possible to induce both tactile and pain sensations depending on the intensity of stimulation. At the other sites the predominant sensation reported was pain. The only extraneous sensation reported by some subjects was that stimulation of the first dorsal interosseous muscle occasionally produced a low-intensity warm sensation on the dorsal skin surface. Minor adjustments were made to finger position to ensure that subjects clearly felt pain in the target tissue and only that location. Subjects were encouraged to keep their hand relaxed and to maintain a constant position during stimulation.

Psychometric parameters

Tactile thresholds, pain thresholds for single stimuli and summation pain thresholds for trains of five stimuli were determined for all subjects. Tactile threshold was determined as the minimum intensity required to obtain a sensation of touch or very light pressure on the skin for both single pulses and pulse trains. Pain threshold was defined as the minimum intensity eliciting pain with a single stimulus pulse. Summation pain threshold was the minimum intensity required to elicit pain at the end of a series of five pulses (Arendt-Nielsen et al. 1994, 1997).

The subjects completed a Danish version of the McGill pain questionnaire (MPQ; Drewes et al. 1993) to provide a qualitative description of the pain sensations induced in each of the tissues. MPQ data were collated as category use profiles for each of the 20 subcategories (Melzack 1975; Parker et al. 1988). In addition, unpleasantness was scored on a visual analogue scale (VAS) anchored with the terms "not unpleasant at all" and "extremely unpleasant."

Protocols

Experiment 1 (effect of stimulus duration and target tissue)

The aim of this study was to evaluate the effect of stimulus duration on pain perception. Skin, muscle and joint were stimulated with focused ultrasound at four different pulse durations (25 ms, 50 ms, 75 ms, and 100 ms). Thresholds were determined for single stimuli and trains of five stimuli (2 Hz). For each subject the order of tissue stimulated and the order of pulse duration were randomised. Randomisation was accomplished by drawing lots and determining a testing sequence prior to the start of the experiment. In all cases single-pulse thresholds were determined before summation thresholds. When testing cutaneous sensitivity, determination of tactile thresholds was carried out before determination of pain thresholds. All measures were obtained in duplicate with an interval of approximately 2–3 min between test sequences. Following stimulation of each tissue, subjects were asked to complete the MPQ and unpleasantness VAS.

Experiment 2: temporal summation

The aim of this study was to evaluate temporal summation in each of the three tissues across a range of pulse-train frequencies (0.5 Hz, 1 Hz, 2 Hz, 3 Hz, 4 Hz, 5 Hz) at a constant pulse duration of 50 ms. Skin, joint and muscle were stimulated in a random sequence. Thresholds were determined for single stimuli and trains of five stimuli. The order of tissue stimulated and stimulation frequency were randomly varied. All measures were obtained in duplicate and the MPQ and unpleasantness VAS were administered following stimulation of each of the three tissues.

Data analysis

Mean thresholds were obtained from the duplicate measures. The data are presented as mean and standard error (SE). In experiment 1, thresholds were analysed using two 3-way ANOVAs with main effects of pulse duration (25 ms, 50 ms, 75 ms, 100 ms), pulse pattern (single pulse, train of five pulses) and tissue (skin, joint, muscle) or stimulus type (tactile, pain). In experiment 2, thresholds were analysed using two 2-way ANOVAs with main effects of pulse-train frequency (single, 0.5 Hz, 1 Hz, 2 Hz, 3 Hz, 4 Hz, 5 Hz) and tissue (skin, joint, muscle) or stimulus type (tactile, pain). VAS data were evaluated using a 1-way ANOVA and MPQ data were analysed using chi-square tests. The ANOVAs were followed by post hoc least square means (LSM) pairwise comparisons as required. Bonferroni-corrected *P*-values were used for all pairwise comparisons. Analyses were conducted using SAS version 8.1; a *P*-value of 0.05 was used for all tests.

Results

Effect of stimulus duration on pain thresholds

Pain thresholds to focused ultrasound in joint and skin tissue were successfully recorded in all subjects. For single stimulation of muscle tissue, maximum stimulus intensity did not elicit pain in 11, 8, 4 and 3 subjects for stimulus durations of 25 ms, 50 ms, 75 ms and 100 ms, respectively. For train stimulation of muscle tissue, only the duration at 25 ms did not induce pain at maximum stimulus intensity in seven subjects.

Significant reductions in pain threshold were apparent for single-pulse and pulse-train stimulation with increasing pulse duration, although an interaction was found between tissue type, pulse duration and single versus train mode (Fig. 1; ANOVA: $F_{6,204}$=2.85, P<0.011; LSM: P<0.005). In general a progressive decrease in thresholds was found with increased stimulus duration (LSM: P<0.008). For all tissues, pain thresholds for single pulses at all durations were significantly greater than pain thresholds for trains of five pulses (LSM: P<0.0001). Pain thresholds were significantly greater (LSM: P<0.0001) for muscle stimulation compared with both joint stimulation and skin stimulation for all pulse dura-

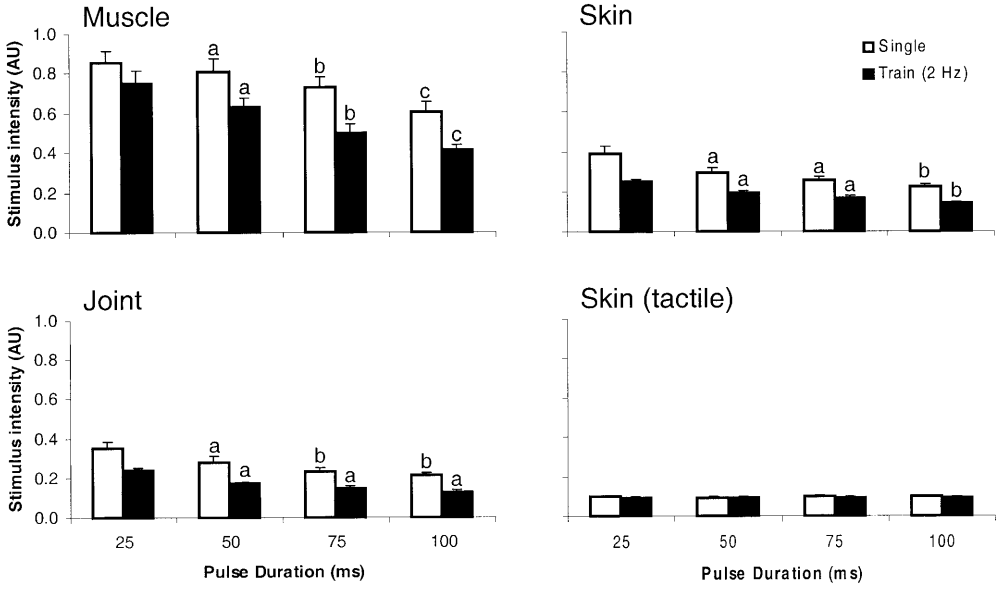

Fig. 1 Mean (± SE) pain and tactile thresholds for focused ultrasound stimulation with different pulse durations. Pain thresholds for single-pulse (*open bars*) and pulse-train (*solid bars*, 2 Hz) stimulation of skin, joint and muscle are shown. In addition thresholds for tactile perception from single-pulse (*open bars*) and pulse-train (*solid bars*, 2 Hz) stimulation of skin are shown. Significant differences within the same tissue and stimulus mode (single or train) compared with 25-ms duration (*a*), 25-ms and 50-ms durations (*b*) and 25-ms, 50-ms and 75-ms durations (*c*) are shown (post hoc least square means (LSM) pairwise comparison: P<0.002). (*AU* arbitrary units)

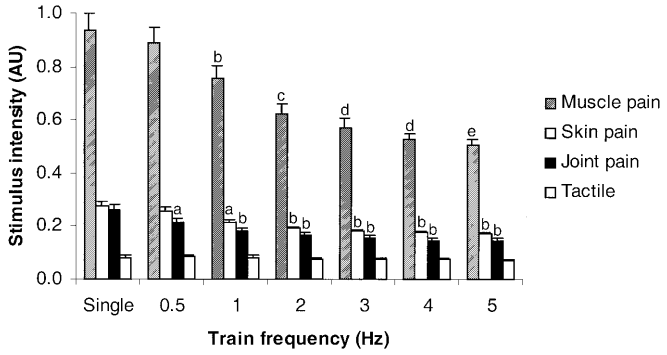

Fig. 2 Mean (± SE) pain and tactile thresholds for focused ultrasound stimulation as single-pulse or pulse-train stimulation at a range of different frequencies (0.5 Hz, 1 Hz, 2 Hz, 3 Hz, 4 Hz, 5 Hz). Pain thresholds from stimulation of skin (*open bars*), joint (*solid bars*) and muscle (*hatched bars*) are shown. Tactile perception thresholds are also shown (*grey bars*). Significant differences within the same tissue compared: with single stimulus (*a*), single stimulus and 0.5-Hz train (*b*); single stimulus, 0.5-Hz and 1-Hz trains (*c*); single stimulus, 0.5-Hz, 1-Hz and 2-Hz trains (*d*); and single stimulus, 0.5-Hz, 1-Hz, 2-Hz and 4-Hz trains (*e*) are shown (LSM: $P<0.005$)

tions, for both single pulses and pulse trains. There were no significant differences between thresholds for skin and joint stimulation at any pulse duration.

Temporal summation pain thresholds

Pain thresholds to single and pulse trains were detected in skin and joint tissue in all subjects. Maximum-intensity single-pulse stimulation and pulse-train stimulation at 0.5 Hz focused into muscle tissue did not induce pain in seven and six subjects, respectively.

Analysis of variance showed a significant interaction between tissue stimulated and pulse-train frequency (Fig. 2; ANOVA: $F_{12,150}=38.66$, $P<0.0001$; LSM: $P<0.005$). Overall there were significant reductions in pain thresholds for increased pulse-train frequencies (LSM: $P<0.002$). There were significant differences in pain thresholds (LSM: $P<0.0001$) between muscle and skin and between muscle and joint at all frequencies. There was no significant difference between pain thresholds for skin and joint at any of the frequencies tested.

For further analysis of temporal summation, the absolute difference in pain thresholds between tissues was eliminated by normalising to the pain thresholds obtained for pulse trains at 0.5 Hz (Fig. 3). For the subjects where it was not possible to determine the summation pain threshold, maximum intensity (1 AU) was used in the analysis, because this gives the most conservative estimation. A significant interaction between tissue and stimulus frequency (ANOVA: $F_{10,140}=4.21$, $P<0.0001$) was found, showing that temporal summation thresholds for 2 Hz or more in muscle were lower than those for skin and joint (LSM: $P<0.008$).

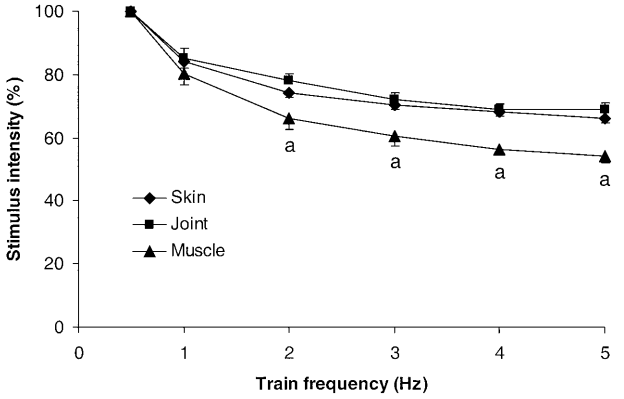

Fig. 3 Mean normalised pain thresholds (± SE) for focused ultrasound stimulation at a range of pulse-train frequencies (0.5 Hz, 1 Hz, 2 Hz, 3 Hz, 4 Hz, 5 Hz). Significant differences compared with skin and joint tissue are shown (*a*; LSM: $P<0.008$)

Tactile versus pain thresholds for skin stimulation

For cutaneous stimulation, ANOVA showed significant interactions between threshold type (tactile, pain) and pulse duration (Fig. 1; ANOVA: $F_{3,140}=52.4$, $P<0.0001$) or train frequency (Fig. 2; ANOVA: $F_{6,84}=48.3$, $P<0.0001$). These interactions showed, however, no significant effect of pulse duration or train frequency on tactile perception thresholds but reflected the findings on skin pain thresholds (see previous sections). Tactile perception thresholds and skin pain thresholds were significantly different (LSM: $P<0.008$) at all durations and frequencies tested.

Pain quality and unpleasantness

Category utilisation profiles for the MPQ (Fig. 4) showed some distinctions between the tissues stimulated in the sensory categories of the questionnaire. Significant differences between skin, muscle and joint were demonstrated for four categories. Muscle pain appeared to be different from joint and skin pain in the spatial ($\chi^2=8.96$; $P=0.011$), incisive pressure ($\chi^2=8.64$; $P=0.013$) and thermal, hot ($\chi^2=15.59$; $P=0.0004$) categories. Skin pain differed from joint and muscle pain in the dullness category ($\chi^2=13.48$: $P=0.001$).

VAS ratings of the unpleasantness of the induced pain were 2.92±0.29 cm, 3.49±0.38 cm and 2.81±0.29 cm for skin, joint and muscle tissue, respectively, and were not significantly different or dependent on stimulus duration or frequency.

Discussion

This study provides the first comparison between pain thresholds and summation pain thresholds for skin, muscle and joint tissues using the same noninvasive stimulus modality for each of the tissues. As a result, it provides some distinctions between tissues in terms of how pain

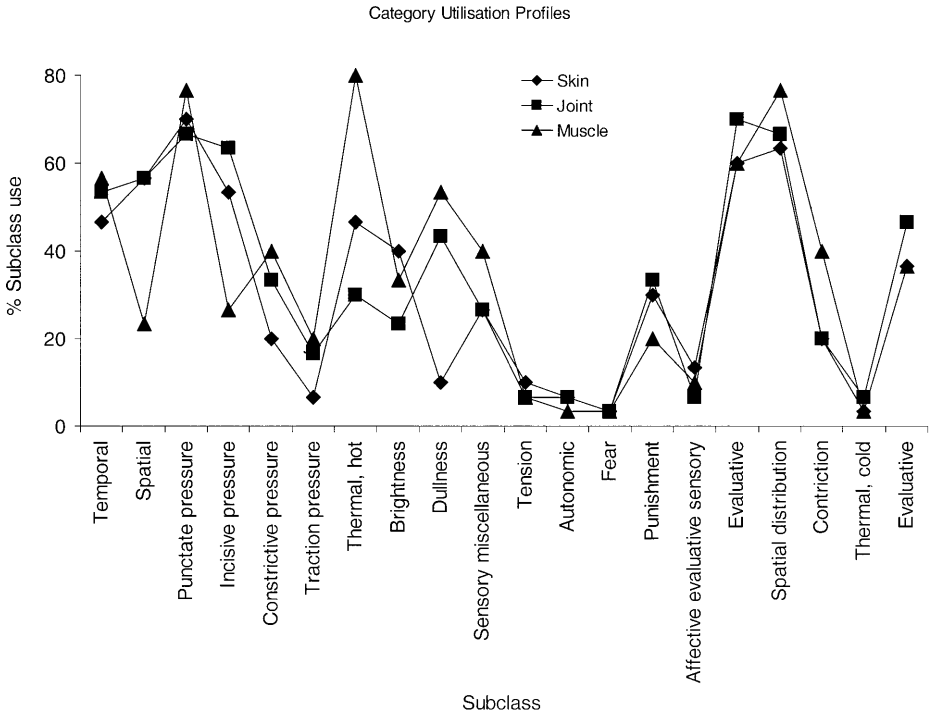

Fig. 4 Category use profiles for responses on the subclasses of the McGill pain questionnaire

perception is influenced by temporal summation. The study demonstrates differences in the ultrasound energy required to elicit pain in each of the tissues. In particular, higher intensities are required to elicit pain in muscle. A variety of reasons may account for this difference. The results also suggest that temporal summation is more potent for muscle pain than either skin or joint pain. This effect was apparent even when the data were normalised to the intensity required to elicit pain at 0.5 Hz. Focused ultrasound stimulation appears to be a potential method for further experimental pain studies.

Focused ultrasound stimulation

One of the advantages of ultrasound as an experimental pain stimulus is that the beam can be focused at different depths, resulting in high-intensity energy in deep tissues while having relatively low incident energy when penetrating the superficial tissue. This means that ultrasound can be used to target and induce pain in selected deep-tissue structures. Gavrilov and colleagues were amongst the first to study the parameters of stimulation for inducing pain in skin and subcutaneous tissues using focused ultrasound (Gavrilov et al. 1977; Gavrilov 1984; Tsirulnikov et al. 1986). They demonstrated that, while it was possible to produce a range of sensations including heat, cold, itch and tickle in skin and immediate subcutaneous tissue, in deeper tissues the predominant sensation encountered was pain. It was possible to induce pain in many different structures in the upper limb including the soft tissues of the hand and forearm, the bones of the fingers and the small joints of the hand (Gavrilov et al. 1977; Gavrilov 1984; Tsirulnikov et al. 1986). Pain was produced more readily by stimulation at lower ultrasound frequencies (e.g. 0.48 MHz) and with stimuli of longer duration (up to 100 ms; Gavrilov et al. 1977) similarly to the present findings, which show a negative correlation between pulse duration and pain threshold as also seen for other stimulation modalities.

It is not clear from previous studies (Gavrilov et al. 1977; Tsirulnikov et al. 1986) whether it is possible to use ultrasound to induce muscle pain. The present study shows clearly that it is possible to use focused ultrasound as a nociceptive stimulus for stimulating muscle tissue. However, it also shows that the intensity of ultrasound required to induce muscle pain, and the duration of stimulation required are much greater than for other tissues. It is acknowledged that increased ultrasound energy delivered to muscle tissue does not necessarily mean that increased energy is required to activate muscle nociceptors.

Several subjects noted a specific side-effect of the high-power levels used for muscle stimulation. Dissipation of the ultrasound energy within the tissues resulted in the perception of heat in the skin on the dorsal aspect of the hand (opposite to the side of the hand on which the stimulator was located). At no time was this sensation described as burning pain and for most individuals it was classified as pleasant warmth.

The mechanism behind excitation of nociceptors by focused ultrasound (1.66 MHz) is not clear. For stimulus durations shorter than 100 ms, it has been suggested that it is mainly due to mechanical factors, but that thermal factors cannot be neglected (Davies et al. 1996). It has been estimated that focused ultrasound stimulation (2.67 MHz, 100 ms duration) gives a similar amplitude of particle displacement and temperature elevation at

pain thresholds for skin and soft tissues, respectively (Davies et al. 1996). The velocity of ultrasound in skin and muscle tissue is comparable, but the attenuation of the ultrasound energy may be less in muscle than in skin (Goss et al. 1978). This might result in more ultrasound energy being transmitted through muscle without actually being absorbed by the tissue and may account for the phenomenon of ultrasound energy being absorbed and eliciting a warm sensation in the skin on the surface distant from the transducer. This effect did not occur with skin or joint stimulation, suggesting that more of the energy may be absorbed by those tissues. Assuming the same threshold for excitation of nociceptors in both muscle and skin, no difference in pain thresholds was anticipated in contrast to the actual findings. A potential explanation of the difference in pain thresholds among tissues might be a relatively higher density of nociceptors in joint and skin compared with muscle tissue. As an example the capsules of the joints have been reported to be more pain sensitive than muscle tissue (Inman and Saunders 1944), in line with the present findings. More detailed studies would be required to determine whether the differences reported between tissues reflect differences in the capacity of each of the tissues to absorb ultrasound energy or differences in the relative density of nociceptive endings.

The MPQ data for this study highlighted some distinctions between the sensory aspects of pain induced in each of the tissues. Muscle pain appeared to be different from joint and skin pain in the spatial, incisive pressure and thermal, hot categories of the MPQ. This may reflect the perception of muscle pain as a duller more diffuse pain. Selection of thermal/hot descriptors may reflect the extraneous sensation reported by some subjects, who reported a warm sensation specifically in the skin on the dorsal surface of the hand. All subjects could clearly differentiate this sensation from the pain that they were experiencing in the first dorsal interosseous muscle at the same time. It might be possible to avoid this thermal sensation by using a more tightly focused ultrasound beam. This, however, would necessitate using a higher-frequency, higher-intensity transducer. Skin pain appeared to be different from joint and muscle pain in the dullness category. This may reflect the perception of skin pain as a less diffuse sensation than pain from the deeper tissues. All of the tissues stimulated produced similar responses in the non-sensory categories of the MPQ. In common with previous studies (Crockett et al. 1977; Wright 1997), the experimental stimuli used in this study resulted in comparatively low utilisation of the affective categories of the MPQ. Further studies could compare the quality of pain induced by ultrasound stimulation with the quality of clinical pain in disease states affecting each of the tissues as a means of validating the experimental model. This has been done previously for joint pain, with results suggesting that ultrasound-induced joint pain is described using a similar array of MPQ descriptors to those selected to describe arthritic hand pain (Wright 1997).

Temporal summation

A distinction between temporal summation in muscle and skin was anticipated on the basis of previous research using repetitive electrical stimulation (Arendt-Nielsen et al. 1997). Also the summation of muscle pain caused by electrical stimuli was found to be more inhibited by i.v. remifentanil hydrochloride than similar cutaneous stimuli (Curatolo et al. 2000), indicating the difference in pain summation from the two tissues. It was anticipated, however, that stimulation of joint tissue would also exhibit marked temporal summation. Temporal summation may equate to the initial part of the wind-up phenomenon that has been demonstrated in spinal cord neurons by a number of researchers. Stimulation of Aδ- and C-fibre afferents at frequencies in excess of 0.5 Hz evokes slow excitatory postsynaptic potentials, with resultant cumulative depolarisation in dorsal horn neurons (Sivilotti et al. 1993). Repetitive stimulation at C-fibre strength leads to action potential wind-up in a subpopulation of dorsal horn neurons that exhibit rapidly progressive cumulative depolarisation (Sivilotti et al. 1993). Wall and Woolf (1984) have demonstrated that electrical stimulation of C-fibre afferents from muscle produce a more prolonged increase in the excitability of the flexion reflex than stimulation of cutaneous C-fibre afferents. In a subsequent study they have also demonstrated differences in the capacity of mustard oil to evoke prolonged facilitation of the flexion reflex depending on the tissue stimulated (Woolf and Wall 1986). In that study intra-articular stimulation produced the most sustained facilitation effect. It is apparent that stimulation of C-fibre afferents from deep tissues (muscle and joint) may have a more profound influence on spinal neurons than stimulation of cutaneous C fibres. The data obtained in our study, however, suggest to the contrary that the pattern of temporal summation in joints shows greater similarity to that in skin than the pattern in muscle tissue. The distinction between joint and muscle was maintained even when the data were normalised in order to account for the substantial differences in intensity required to elicit pain in each of the tissues. This distinction may relate to differences in compliance between the tissues and consequent differences in the relative absorption of ultrasound between muscle and joint (see previous section). It would be necessary to use a different experimental paradigm to capture differences in the duration of sensitisation between tissues.

In most cases the effects of temporal summation were apparent with stimulation using pulse trains at frequencies of 1 Hz and 2 Hz. Increasing stimulus frequency beyond 2 Hz did not result in a further reduction in pain threshold for skin and joint stimulation. Only muscle tissue showed significant changes in summation pain threshold when stimulus-train frequency was increased beyond 2 Hz. Stimulus parameters using a pulse duration of more than 25 ms are necessary to effectively stimulate muscle tissue. Pulse durations of 50 ms or more and a 2-Hz pulse train would appear to be appropriate for fur-

ther studies using this methodology. A stimulation frequency of 2 Hz has been used previously for electrical stimulation of muscle tissue (Arendt-Nielsen et al. 1997; Sörensen et al. 1998; Graven-Nielsen et al. 2000).

Even though it is not fully clarified whether the thermal modality is important in exciting nociceptors during focused ultrasound stimulation, an increased heat accumulation capacity for muscle tissue could explain the increased potency for temporal summation in muscle versus skin and joint tissue. Laser stimulation of in vivo muscle tissue produced a temperature increase from baseline to more than 25°C immediately post-stimulus, and after 150 ms the temperature was less than 15% of peak post-stimulus temperature (Brugmans et al. 1991). The temperature elevation in skin and soft tissues after short-duration focused ultrasound stimulation at pain threshold has been estimated to be less than 4°C (Davies et al. 1996), suggesting that the worst-case heat accumulation 150 ms after each focused ultrasound stimulus is less than 0.6°C. Thus, for train frequencies of less than 3 Hz, the difference in temporal summation from different tissues probably is related to a difference in the central integrative mechanisms and is most likely not due to heat accumulation. A minor heat accumulation in muscle tissue cannot be excluded for train frequencies at 3 Hz or higher. This may account for the progressive decrease in summation pain thresholds for train frequencies at 3 Hz or higher in muscle tissue, which is not found for joint and skin stimulation.

Tactile sensation

This study confirmed the findings of previous studies, indicating that it is possible to induce a tactile or touch sensation in the skin using focused ultrasound (Gavrilov et al. 1977; Gavrilov 1984). In this study tactile sensations were consistently elicited with low-intensity ultrasonic stimulation. Tactile stimulation did not appear to exhibit temporal summation for train stimulation, and the pattern of response for tactile stimulation was distinctly different from that for painful stimulation. Neither did the pulse duration make any difference to thresholds for tactile sensation. This contrasts with previous studies using electrical stimulation where temporal summation of nonpainful stimulation has been demonstrated across a similar frequency range (Arendt-Nielsen et al. 2000). One reason for this distinction may be that the stimuli used in this study were at perception threshold only, whereas the electrical stimuli were above perception threshold but did not exceed pain threshold.

Conclusion

Focused ultrasound can be used as a noninvasive method to induce pain in a variety of tissues, including muscles. The intensity required to induce pain in muscle is significantly greater than in other tissues, which could be due to a number of potential differences between the tissues. Studies specifically testing the capacity to activate nociceptors in skin, joint and muscle using ultrasound stimulation would be an important next step to determine the reasons for the differences in the capacity of ultrasound stimulation to elicit pain in different tissues. Temporal summation is a feature of ultrasound-induced pain in all tissues tested. The summation phenomenon does not appear to occur at tactile intensities. Temporal summation seems to be a more distinct phenomenon in muscle compared with both skin and joint. This may be due to a difference in the central integrative mechanisms. The methodology has potential for further research investigating muscle sensitivity in clinical disorders and investigating the effects of drugs that might act on the process of central summation in muscles.

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