ELSEVIER

Brain Research 853 (2000) 275–281

BRAIN RESEARCH

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Research report

Duration and distribution of experimental muscle hyperalgesia in humans following combined infusions of serotonin and bradykinin

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Accepted 26 October 1999

Abstract

The present study examined distribution and duration of muscle hyperalgesia to pressure stimuli after intramuscular bolus-infusions of serotonin (5-HT, 20 nmol) and bradykinin (BKN, 10 nmol) in 10 volunteers. Infusions were given into the tibialis anterior (TA) muscle over 20 s with an inter-infusions interval of 3 min. Infusions of isotonic saline (NaCl, 0.9%) were given as control. Pain intensity was continuously scored on a visual analogue scale (VAS), and subjects drew the distribution of the pain areas on an anatomical map. Pressure pain thresholds (PPTs) were assessed with an electronic algometer at the injection site (10 cm below the patella), 2, 5, and 10 cm distal from the injection site, and at the ankle. Control assessments of PPTs were done at the contralateral TA and ankle. Skin sensibility was assessed with a Von Frey hair at the same sites. All measurements were done before and 5, 20, 40, and 60 min after infusions. The VAS-peak after BKN was significantly higher ($P < 0.05$) compared with 5-HT and the second infusion of NaCl. The duration of the increase in VAS after 5-HT + BKN was significantly longer ($P < 0.05$) compared with the infusions of NaCl. The local pain area after infusion of BKN was significantly larger ($P < 0.05$) compared with 5-HT and control infusions. Cutaneous sensibility to tactile stimuli was not affected by any of the combinations. PPTs at the injection site and 2 cm (5, 20, and 40 min) were significantly decreased ($P < 0.05$) after 5-HT + BKN compared with baseline and isotonic saline. In addition, PPTs were significantly decreased ($P < 0.05$) after 5-HT + BKN at 5 cm (5 and 20 min) and 10 cm (5 min). Serotonin may enhance the effect of bradykinin in producing experimental muscle pain and muscle hyperalgesia to mechanical stimuli. The combination of serotonin and bradykinin can produce muscle hyperalgesia, lasted for up to 40 min and located within the muscle. No widespread hyperalgesia to the ankle and other leg (tested at 10 cm below the patella and ankle) was observed suggesting a predominant peripheral origin of the experimentally induced hyperalgesic stage.

Keywords: Serotonin; Bradykinin; Sensitization; Muscle hyperalgesia

1. Introduction

Muscle pain is perceived as an aching and cramping sensation that is difficult to localise, and which is often referred to other somatic structures [8,47,50]. The relationship between muscle pain duration and the spread of pain, hyperalgesia, and allodynia is unknown, but is of significant clinical interest. Delay in alleviation of muscle pain could result in spread of muscle pain, hyperalgesia, and allodynia to other parts of the body [7]. In many cases of muscle pain, particularly in chronic muscle pain and hyperalgesia, sensitization of dorsal horn neurones could play an important role [10]. Thus, the excitability of dorsal horn neurones can be changed for prolonged periods by both descending mechanisms and nociceptive input from the periphery [1,24,38,49]. An acute painful stimulus to a muscle is likely to increase the responses of dorsal horn neurones or even lead to the appearance of new or extension of existing receptive fields [26,27]. Longer-lasting nociceptive input from peripheral tissue has been suggested to lead to marked changes of the circuitry in the dorsal horn [37]. Changes in the peripheral nervous system following tissue injury result in increased nociceptor sensi-

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0006-8993/00/$ - see front matter
PII: S0006-8993(99)02270-2

tivity at the site of injury: peripheral sensitization [5,15,42]. The sensitized nociceptors exhibit spontaneous activity, lowered thresholds, and increased responsiveness to suprathreshold stimuli. The peripheral sensitization on an injured area is the basis of primary hyperalgesia [31]. Around the primary site of injury, an enlarged area of increased sensitivity to noxious and non-noxious stimuli soon develops: secondary hyperalgesia [23,36]. This phenomenon is caused by a state of increased excitability of dorsal horn neurones [9,33,46]. Manifestation of muscle hyperalgesia to distant areas might share some features with secondary hyperalgesia after cutaneous C-fibre stimulation [1,24]. Effective stimulants of free nerve endings in skeletal muscle are endogenous pain-producing substances such as bradykinin (BKN), serotonin (5-HT), and potassium ions [19,32]. Under pathological conditions (lowering of pH, ischaemia, blood clotting, and vascular damage), BKN is cleaved from plasma proteins, and 5-HT is released from platelets. Furthermore, many muscle nociceptors respond to both noxious local pressure and injections of BKN [39]. Prostaglandins and 5-HT have been shown to sensitize nociceptive muscle afferents to BKN [40]. This process of sensitization probably accompanies many types of tissue lesions and is the best-established peripheral mechanism explaining the clinical symptoms of tenderness and hyperalgesia [38]. In our previous studies [2,3], we have shown changes in muscle sensibility to mechanical stimuli after infusions of one substance (5-HT, BKN, SP) or combinations of 5-HT and BKN. It is likely that induced muscle hyperalgesia after combined infusions of 5-HT and BKN may be localized within the tested muscle. The aims of the present study were to assess the distribution and duration of experimentally induced muscle hyperalgesia by two subsequent intramuscular infusions of 5-HT and BKN that can help to understand the neurophysiological mechanisms behind this phenomenon.

2. Material and methods

2.1. Subjects

A total of 10 healthy men (mean age: 21.1 years, range: 20 to 24 years) without a history of musculoskeletal disorders or allergic disease participated. The study was single-blind and comprised two sessions. All subjects had previously participated in experimental muscle pain studies. Written informed consent was obtained from all participants before inclusion. The experiments were performed in accordance with the Declaration of Helsinki and approved by the local Ethics Committee.

2.2. Induction of muscle pain

Two subsequent bolus infusions (0.5 ml) of the sterile substance (Clinalfa, Switzerland), 5-HT (20 nmol, concentration 40 μM), and BKN (10 nmol, 20 μM) dissolved in isotonic saline were given intramuscularly. The substances were administered over 20 s with an interval of 3 min between infusions. Isotonic saline infusions were used as control on a separated session. Infusions were given into the anterior tibialis (TA) muscle on the right leg, 10 cm from the lower border of the patella (depth: 2 cm). Both of the infusions were given through the same needle, and thus the substances were delivered to the same spot of tissue. The two sessions (active and control) were separated by a minimum of 1 week.

2.3. Assessment of muscle pain

The substance-induced pain intensity was continuously scored by the subjects on a 10-cm electronic visual analogue scale (VAS) where 0 cm indicated ''no pain'' and 10 cm ''worst pain imaginable''. The VAS was recorded on a computer (1-s sampling interval) until the pain vanished. Extracted parameters from the VAS time curve were VAS-peak, onset, and offset. The quality of the pain was described with a Danish version of the McGill Pain Questionnaire (MPQ) [13]. The subjects drew the distribution of the pain areas on an anatomical map after the first and second infusion. The circumference was digitised (ACECAD D9000 + digitizer), and the area calculated (Sigma-Scan, Jandel Scientific). The local pain area was defined as pain located around the injection site and the referred pain area as pain outside the boundaries of the local pain area. If pain was not perceived, the pain areas were included in the statistics as zero.

2.4. Assessment of somatosensory sensibility

Pressure pain threshold (PPT) was determined with an electronic pressure algometer (Somedic, Sweden) mounted with a 1-cm^2 probe. The PPT was obtained as the mean of three trials with a minimum 30-s interval between each trial. The pressure intensity was increased (50 kPa/s) until the subject perceived a sensation of pressure pain and pressed a button. PPTs were determined at the injection site, 2, 5, 10 cm distal from the injection site, at the ankle, and at the contralateral leg and ankle as control. The determination of PPTs was done before infusions (pre-infusion) and 5, 20, 40, and 60 min after the second infusion. The PPTs after infusions were normalized (% change) to pre-infusion PPTs for statistical evaluation. The average level of reduction was calculated in order to get an estimate of the number of subjects reporting decreases in PPT at the different sites at the different time points. The cutaneous sensibility to a Von Frey hair (Stoelting, USA; corresponding to a bending force of 279.4 g) was assessed on a 10-cm sensory scale where 0 indicated ''no sensation'', 5 indicated ''pain threshold'', and 10 represented ''worst pain imaginable''. The sites for assessment were the same as for the PPT (at the infusion site, 2, 5, 10

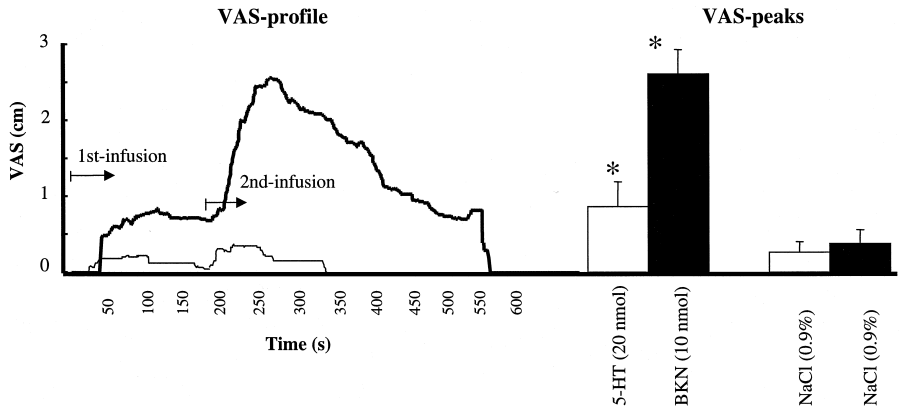

Fig. 1. The mean ($n = 10$) VAS-profile (10-cm scale) after infusions of (5-HT + BKN) (thick line) and (NaCl + NaCl) (thin line). The mean (+S.E., $n = 10$) VAS-peaks after the first (5-HT and NaCl: open bars) and second (BKN and NaCl: black bars) infusions for both of combinations. The VAS-peak was significantly higher (* SNK: $P < 0.05$) after BKN compared with 5-HT and the second NaCl-infusion. The VAS-peak after 5-HT was significantly higher (* SNK: $P < 0.05$) compared with the first infusion of NaCl.

cm from the injection site, at the ankle, and at the contralateral leg and ankle). Assessment of cutaneous sensibility was done before infusion (pre-infusion) and 5, 20, 40, and 60 min after the infusion.

2.5. Assessment of the biological activity of the substances

Intradermal injections (0.05 ml) with the same substances as given intramuscularly were tested on the volar side of the right forearm after the experiment to evaluate the biological activity (flare and wheal reactions) of the substances [28]. The wheal and flare areas were measured 10 min after injection and calculated (Sigma-Scan, Jandel Scientific).

2.6. Statistics

Analysis of variance (ANOVA) for repeated measures was used. The different factors were time (before and 5, 20, 40, and 60 min after infusion), combination (5-HT + BKN and NaCl + NaCl), substance (BKN and 5-HT), and infusion sequence (first infusion and second infusion). Post-hoc tests were performed with Student's–Newman–Keuls test to correct for multiple comparisons. A significance level of $P < 0.05$ was accepted. Data are presented by mean and standard error (S.E.).

3. Results

3.1. Substance-induced muscle pain

Generally, the isotonic saline infusions evoked minimal pain intensity (Fig. 1). After infusion of BKN in the combination (5-HT + BKN), the pain intensity (VAS-peak) was significantly higher (two-way ANOVA: $P < 0.0003$; SNK: $P < 0.05$) compared with the pain intensity after 5-HT and the second infusion of NaCl (2.6 ± 0.3 vs. 0.8 ± 0.3 cm and 0.3 ± 0.1 cm) (Fig. 1). The VAS-peak after infusion of 5-HT was significantly higher (SNK: $P < 0.05$) compared with the first infusion of NaCl (0.8 ± 0.3 vs. 0.2 ± 0.1 cm) (Fig. 1). The onset of pain after 5-HT (39.0 ± 7.8 s; $n = 8$) was not different from the first infusion of NaCl (24.5 ± 9.5 s; $n = 2$). The offset of the induced muscle pain was significantly longer (One-way ANOVA: $P < 0.0173$; SNK: $P < 0.05$) after BKN infusion (565.4 ± 61.1 s; $n = 10$) with a prior 5-HT treatment compared with the second infusion of NaCl (346.5 ± 118.4 s; $n = 2$). The words selected by a minimum of 30% of the subjects after the infusions were for 5-HT: ''tender'' (30%); for BKN: ''sharp'' (50%), ''pressing'' (30%), ''hot'' (30%), ''taut'' (40%), ''tight'' (40%); no words were selected after the infusions of NaCl.

3.2. Local and referred pain areas

The local pain areas were significantly larger (two-way ANOVA: $P < 0.0056$; SNK: $P < 0.05$) after infusions of BKN compared with 5-HT and the second infusion of NaCl (2.8 ± 0.7 vs. 0.4 ± 0.2 and 0.2 ± 0.1 arbitrary units on the anatomical map) (Fig. 2). Two out of 10 subjects reported referred pain to the frontal aspect of the ankle after the combination of (5-HT + BKN) (Fig. 2).

3.3. Somatosensory sensibility

The PPT was significantly decreased (three-way ANOVA: $P < 0.0001$; SNK: $P < 0.05$) at the injection site and 2 cm distal from the injection site (SNK: $P < 0.05$; 5, 20, and 40 min) after the combination (5-HT + BKN) compared with pre-infusion PPT and PPTs after NaCl-combination (Fig. 3). The PPTs were also significantly decreased at 5 cm (SNK: $P < 0.05$; 5 and 20 min) and 10 cm (SNK: $P < 0.05$; 5 min) (Fig. 3). All the subjects

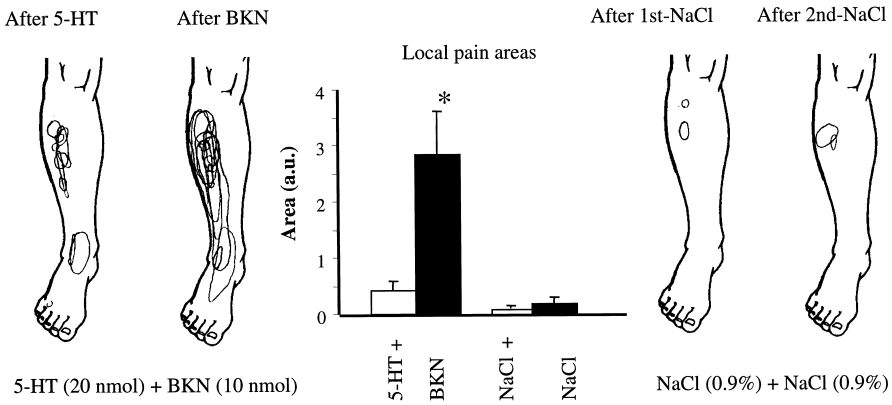

Fig. 2. The distribution of local and referred pain areas (drawn by the ten subjects) after the combinations (5-HT + BKN) and (NaCl + NaCl). The mean (+S.E., $n = 10$) local pain areas (in arbitrary units; calculated from the drawings) after the first (open bars) and second (black bars) infusions for the combinations (5-HT + BKN) and (NaCl + NaCl). The local pain areas after BKN were significantly larger (*SNK: $P < 0.05$) compared with the local pain areas after 5-HT and the second infusion of NaCl.

($n = 10$) reported a decreased PPT (a decrease of $\geq 12\%$ compared with baseline) at the injection site (5-min post). At 2 cm from the injection site, the decrease of PPT tended to be more pronounced and to last longer in 80% of the subjects (Figs. 3 and 4). The PPTs were not affected significantly at the ankle and at the contralateral leg (at 10 cm below the patella) and ankle (Fig. 3). The skin sensitivity was not affected by the different infusions.

3.4. Biological activity of the substances

All substances were found biologically active at the time of injection. The wheal area reaction (0.60 ± 0.05

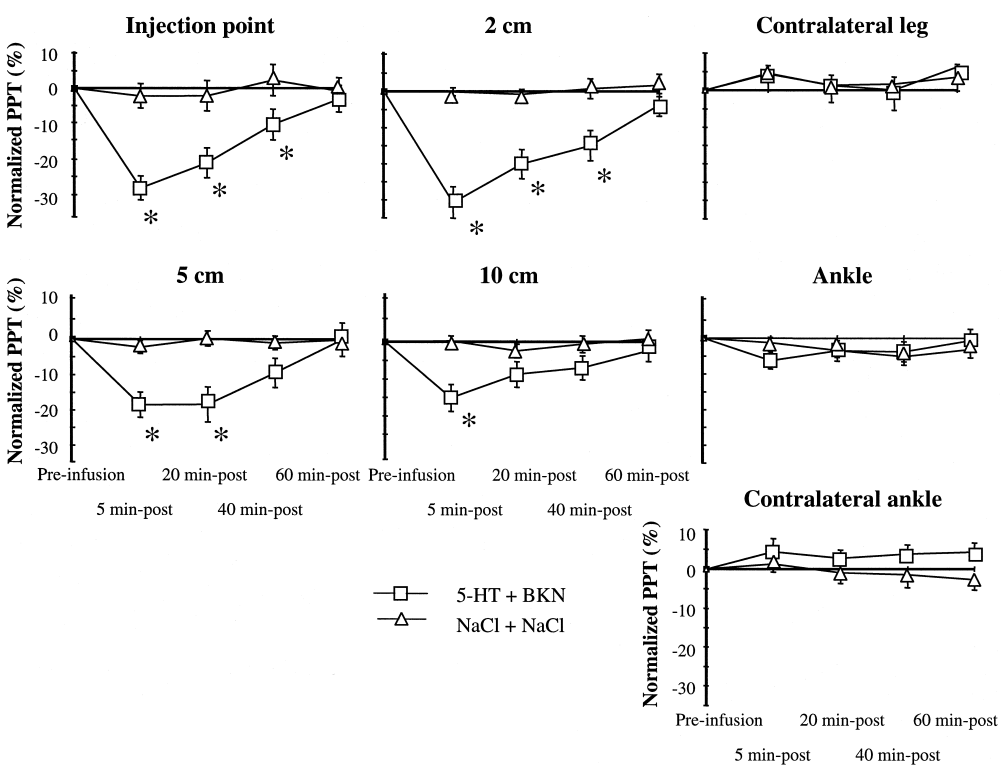

Fig. 3. Mean (±S.E., $n = 10$) PPTs normalised (%) to the pre-infusion PPTs at the injection site, 2, 5, and 10 cm distal from the injection site, at the ankle, contralateral leg (TA muscle; 10 cm below the patella), and contralateral ankle after combinations of isotonic saline (triangles) and (5-HT + BKN) (boxes). The PPT was significantly decreased after the combination (5-HT + BKN) at the injection site and 2 cm (*SNK: $P < 0.05$; 5, 20, 40 min) compared with the pre-infusion PPT and PPTs after (NaCl + NaCl). At 5 cm (5 min and 20 min) and 10 cm (5 min) from the injection site, the PPTs were also significantly decreased (*SNK: $P < 0.05$) after (5-HT + BKN).

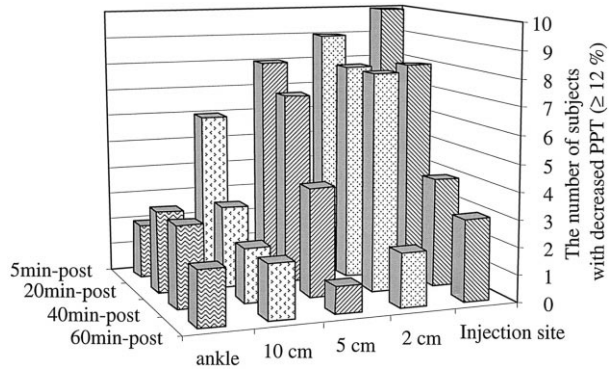

Fig. 4. The number of subjects ($n = 10$) reported a decreased PPT (a decrease of $\geq 12\%$ compared with baseline) measured at the injection site, 2, 5, and 10 cm distal from the injection point, and ankle over 60 min after the combination (5-HT + BKN).

cm^2) was evaluated after BKN (0.05 ml, 20 μM) and (0.23 ± 0.03 cm^2) after 5-HT (0.05 ml, 40 μM). Flare reaction (5.75 ± 0.81 cm^2) was observed only after intradermal injections of 5-HT (0.05 ml, 40 μM).

4. Discussion

The present study showed a local distribution of muscle hyperalgesia after the combination of 5-HT + BKN within the TA muscle with a duration of up to 40 min. This seemed to reflect a peripheral sensitization within the muscle.

4.1. Duration of the induced muscle hyperalgesia

In clinical practice, the hyperalgesic phenomenon is generally detected at the time of the painful event and long outlasts the spontaneous pain. A previous human experimental study [28] has shown muscle hyperalgesia after 5-HT + BKN combined infusions, but PPTs were measured only for 10 min. In the present study, muscle hyperalgesia was observed after a short-lasting noxious input induced by 5-HT + BKN and lasted up to 40 min. This finding is comparable to some aspects of the animal data [27], where the mechanical threshold in the muscle was decreased after the second chemical stimulation by BKN for more than 30 min. However, the induced activation of muscle nociceptors by the BKN injections usually lasts a few minutes [39]. In our previous human study [2], a single injection of BKN did not cause any manifestation of significant sensory changes in the TA muscle. It is not clear whether the sensitization outlasts the direct effect of inflammatory substances or whether there is a long-lasting change in the response characteristics after the stimulus. Most likely, the peripheral nociceptive stimuli and the stimulus duration are defined by interactions between endogenous substances with possible consequent cascades. BKN may induce secondary effects via the release of prostaglandins from sympathetic efferent fibres, cytokines from a variety of cells, histamine, and other inflammatory mediators from degranulated mast cells [12,35]. Furthermore, many peptides (substance P, tachykinins, calcitonin gene-related peptide, etc.) are contained within nociceptive afferents and are released into peripheral tissues when these fibers are activated [35,38]. There is also a dynamic chemical exchange between secretions produced by the afferents and the chemical substances circulating in the surrounding tissues. This may lead to neurogenic inflammation [6], changes in blood flow, and other effects during inflammation [4,34] depending on the peptide released. There may be a great potential for synergistic interaction and signal modulation between different substances and between neural (sensory and sympathetic nerves) and non-neural systems (immune cells) [11]. Finally, peripheral stimuli can initiate profound and prolonged changes in the excitability of central neurones with consequently produced sensory modifications like long-lasting and distributed hyperalgesia [52]. The triggered hyperalgesia by 5-HT and BKN is suggested to be the result of peripheral sensitization from muscle nociceptors.

4.2. Distribution of induced muscle hyperalgesia

Many studies have followed the evolution of pain after the injections of algogenic substances like hypertonic saline (NaCl 6%) into deep tissue [17,21,25,29,36,45,48]. Three sensory manifestations of muscle pain are generally reported: local pain, referred pain, and occasionally muscle hyperalgesia [7,50]. The question is still how can muscle hyperalgesia spread to distant areas and what allows the hyperalgesia to persist [47,50]. In the present study, the infusions of 5-HT and BKN induced pain in the TA muscle, and the distribution of pain was similar in the different subjects (see also Ref. [3]). The induced muscle pain was associated with development of muscle hyperalgesia mainly manifested at the injection site and 2–5 cm distal (Figs. 3 and 4). Such a well-localised hyperalgesia within the muscle is compatible with peripheral sensitization within the muscle. These findings are in agreement with previous animal studies in which the sensitizing mechanisms of 5-HT and BKN were shown for a single dorsal horn neurone processing input from the muscle nociceptors [19,39,40]. From the affected site (tissue injury or infusion of substances), 5-HT and BKN may be spread to a larger area of tissue due to the vasoactive effects of these substances [16,20,30,41,44]. The vasoeffect of 5-HT is important for the delivery and distribution of BKN to the adjacent areas. A spread of BKN in the tissue may be facilitated after infusion of 5-HT due to the synergism in vasoactive effects of these substances at the distance of 2–5 cm (into the muscle TA). A previous study with magnetic resonance imaging (MRI) has shown a spread of intramuscularly injected hypertonic saline for 8 cm [21].

Thus, we propose that the distributed hyperalgesic area, at least up to 5 cm away from the injection site, represented the area of peripheral sensitization (primary muscle hyperalgesia). A short-lasting decrease of PPT at 10 cm distal could reflect a distant activation (due to stretching-pressure of tissue) of the sensitized area (0–5 cm area around the injection site) at the time of the most decreased PPTs at that area (5 min after infusions). Only two subjects reported referred pain at the ankle after combined 5-HT + BKN infusions, and any subject had decreased PPTs at the control sites. These observations indicate that central events were not triggered to a significant degree. Hence, the involvement of central hyperexcitability was assumed marginal.

4.3. Peripheral vs. central aspects of muscle hyperalgesia

From the present study, it is not possible to determine clearly the contribution of peripheral and central mechanisms for the observed effects. Typically, sensitization of peripheral nociceptors associated with injury to the skin is restricted to about 5–10 mm of the site of injury [18]. In contrast, cutaneous hyperalgesia spreads as far as 10–20 cm beyond the site of injury [22,33]. In addition to the sensitization and prolonged excitation of dorsal horn neurones, noxious stimulation associated with tissue injury also produces an expansion of the receptive fields of dorsal horn neurones. Enlargement of the receptive fields (RFs) of the dorsal horn neurones following noxious stimulation could be a part of the mechanism of hyperalgesia [14,27,37,51]. The fact that pain and hyperalgesia can spread to areas distant from the injured region implies that central changes are involved in the spread of hyperalgesia [52]. In the present study, the nociceptive barrage was not specifically intense and may not have been sufficient to generate central changes. The decrease of PPT 10 cm away from the injection site may reflect expansion or formation of new receptive fields. Alternatively, insensitive peripheral branches have possibly been activated and contribute to the perceived pressure pain by spatial summation [43]. In clinical observations, the distribution of hyperalgesia and allodynia depends on the intensity and duration of noxious stimuli [7,23]. Early in the course of the disease when the stimulation is mild, the hyperalgesia and allodynia may be absent or present in the same anatomical deep structure. Bonica [7] suggested that strong and prolonged peripheral stimuli induce wide area of secondary hyperalgesia. Summation from the sensitized muscle nociceptors may recruit more dorsal horn neurones and could be important aspects for maintaining hyperalgesia or even manifestation of sensory changes at distant areas (referred pain with hyperalgesia) depending on summation. The use of pharmacological tools may be one of the possible methods to examine the contribution of peripheral and central mechanisms in muscle pain and soreness.

5. Conclusion

Serotonin seems to prime the nociceptors to subsequent bradykinin infusion. Combined intramuscular infusions of serotonin and bradykinin can produce muscle hyperalgesia up to 10 cm away from the injection site. This short-lasting distributed hyperalgesia may be predominantly due to peripheral sensitization of muscle nociceptors.

Acknowledgements

This study was supported by the Danish National Research Foundation.

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