Acta Odontologica Scandinavica, 2006; 64: 360–367

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ORIGINAL ARTICLE

Effects of isometric contraction on intramuscular level of neuropeptide Y and local pain perception

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Abstract

Objective. The release of neuropeptide Y (NPY) is reported to increase in ischemic conditions and may thus be involved in chronic myalgia. The purpose of this study was to investigate the effect of isometric contraction on intramuscular levels of NPY in relation to local pain development. ***Material and methods***. Intramuscular microdialysis was performed in the masseter and trapezius muscles to determine NPY levels before, during, and after isometric contraction in 16 healthy females. Pain intensity was assessed simultaneously with VAS. Repeated measures ANOVA, *t*-test, and Pearson correlation analysis were used for statistical analyses. ***Results***. The level of NPY in the trapezius muscle was increased during and after contraction, while there was no change in the masseter muscle. The level of NPY before contraction was higher in the masseter muscle than in the trapezius muscle, and the levels in the two muscles were correlated before and during contraction. Low-level pain in both muscles after probe insertion increased significantly during contraction, but the pain was not correlated to the NPY level. ***Conclusions***. Pain is developed in the trapezius and masseter muscles during repeated isometric contraction. The NPY level is increased in the trapezius muscle but is not associated with the pain development.

Key Words: *Isometric contraction, microdialysis, neuropeptide Y, pain, skeletal muscle*

Introduction

Chronic muscle pain (myalgia) is one of the most frequent causes of physical disability, and, as with many other pain conditions, is predominant in females. The etiology of myalgia is multiple, but there is evidence that physical overload due to low-level static contractions may be one factor [1,2]. Muscle biopsies from patients with work-related trapezius myalgia have shown reduced levels of adenosine triphosphate and phosphocreatine as well as a low capillary to fiber area ratio, suggesting that physical overload leads to ischemia [3]. Furthermore, patients with chronic myalgia have a lower intramuscular temperature than healthy individuals, indicating reduced microcirculation [4], and patients with trapezius myalgia show impaired regulation of microcirculation after static loading and repetitive exercise [5–7].

The pathophysiology behind myalgia is largely unknown, but both impaired microcirculation due to overload [8] and microtrauma [9] have been suggested as causing the release of algogenic substances such as neuropeptides, serotonin, histamine, and prostaglandins that may activate or sensitize muscle nociceptors and thus cause pain [10]. Several animal and human experimental studies support this hypothesis [9,11]. An association has previously been reported between the intramuscular level of prostaglandin E_2 (PGE_2) and serotonin versus pain level in patients with chronic myalgia [7,12,13]

Neuropeptide Y (NPY) is synthesized and produced along with noradrenaline in sympathetic nerve fibers that innervate the cardiovascular system [14] as well as in sensory afferents [15,16]. Severe or prolonged stress [17] and strenuous exercise [18,19] release NPY from its neuronal stores. NPY stores have recently been discovered in platelets, immune

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(Received 15 December 2005; accepted 12 June 2006)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.1080/00016350600856034

cells, and endothelial cells and extra-neuronal release may therefore also occur [20]. NPY has a strong vasoconstrictive effect on both the arterial and venous skeletal muscle vessels, an effect that is characterized by slow onset and long duration. The release of NPY is reported to increase in ischemic conditions [21], which leads to further impairment of tissue perfusion.

Owing to its effects on microcirculation, NPY could be involved in chronic myalgia. However, in the few studies that address this question the results are conflicting. Plasma NPY levels have been reported to be elevated in patients with fibromyalgia compared to healthy controls in one study [22], while in another study levels have been reported to be reduced [23]. Normal plasma levels have been reported in patients with tension-type headache and in patients with acute soft tissue injury, such as muscle tear and sprain [24,25]. From animal studies, too, it is reported that NPY release stimulates angiogenesis and increases blood flow [26], but the extent to which this is true for long-term sympathetic activation is not known.

The microdialysis technique is one method for studying biochemical changes in muscle tissue *in vivo*. Several articles have been published on the release of pain mediators in skeletal muscle tissue, pain mediators such as noradrenaline [27], serotonin [7,12], glutamate [7], and prostaglandin E_2 as well as leukotriene B_4 [13]. However, to our knowledge, the technique has not been used previously to study the concentration of NPY in human skeletal muscle.

Since the origin of local myalgia is unclear, it is important to investigate the influence of different mechanisms of pain development, e.g. microcirculatory disturbances including peripheral sympathetic nerve activity. It has been postulated that the sympathetic nervous system is involved in the sensitization of nociceptors and in amplification of pain in pathological pain conditions such as inflammation and neuropathic pain [28,29]. However, many studies indicate that sympathetic activation has little or no interaction with nociceptive neurons under physiological conditions [30,31]. We hypothesize that locally released NPY is involved in muscle pain conditions and use an experimental model to induce local myalgia and to sample NPY by microdialysis in healthy female volunteers. The specific aim of the study was to assess the level of NPY in the masseter and trapezius muscle before, during, and after isometric contraction and its relation to the development of muscle pain.

Material and methods

A group of 16 healthy females (mean (SD) age of 45 (10.9) years) participated in the study. None had pain from or impaired function in the masseter or trapezius muscles, but two had minor palpatory tenderness over the right trapezius muscle. None of the subjects took any analgesic medication.

The study was performed in accordance with the Helsinki Declaration of 1975 and was approved by the local ethics committee at Karolinska University Hospital in Huddinge, Sweden (5/00). The subjects were informed that they could refrain from participation in the study at any time without any consequences, and those who agreed to participate gave their verbal consent.

Experimental procedure

The subjects were first examined to determine whether they fulfilled the inclusion criteria and then a venous blood sample was taken for analysis of plasma NPY. Two experiments were performed. In the first, all individuals were subjected to intramuscular microdialysis before, during, and after simultaneous repetitive isometric contraction of the masseter and trapezius muscles. The right side was used primarily. However, two subjects had palpatory tenderness over the right trapezius muscle, so the muscles on the left side were used in these cases.

In order to determine the pain and NPY levels created by microdialysis without isometric contraction a second experiment was performed 5 months later. Five of the above individuals with a mean (SD) age of 48 (8.4) years were included. No blood sample was taken on this occasion.

Microdialysis

Unilateral intramuscular microdialysis was performed in order to sample *in vivo* NPY. The pars descendens at the anteriolateral margin of the trapezius muscle was used, and the most prominent central point of the masseter muscle (Figure 1). After skin surface anesthesia with EMLA® cream (Lidocain 25 mg, Prilocain 25 mg/g, AstraZeneca AB, Södertälje, Sweden) for 20 min, the selected points were punctured using a standard catheter with a diameter of 1.2 mm. The catheter was inserted at an angle of approximately 45° to the skin surface and to depths of 14 mm and 9 mm into the trapezius and masseter muscles, respectively. A flexible microdialysis probe with a membrane length of 10 mm, a diameter of 0.60 mm, and a molecular cut-off of 35 ku (MAB 3; Microbiotech AB, Stockholm, Sweden) was then inserted via the catheter to depths of 24 mm and 19 mm into the trapezius and masseter muscles, respectively. The probe thus protruded 10 mm outside the catheter in both muscles. A modified Krebs-Henseleit bicarbonate buffer [33] mixed with 0.5% human albumin [34] was used as perfusion medium. The probes were connected to a microinfusion pump (CMA/102 or CMA/100, Carnegie Medicine, Stockholm, Sweden) and perfused

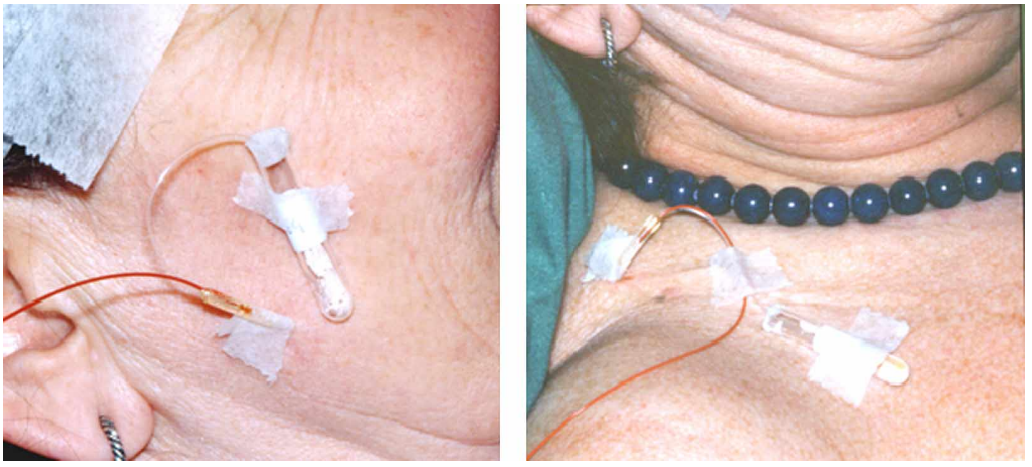

Figure 1. The sites used for intramuscular microdialysis. A. The superficial masseter muscle (the most prominent central point). B. The trapezius muscle (pars descendens at the anteriolateral margin).

at a flow rate of 4 μl/min. Three consecutive samples of 120 μl were collected from each muscle.

Before the experiments, the relative *in vitro* recovery of NPY was examined for eight microdialysis probes. The probes were put into Eppendorph vials containing standard solutions of NPY [35] and placed in a heating block (Termoblock, TC Instruments AB, Göteborg, Sweden) at a constant temperature of 36°C. The probes were connected to a microinfusion pump (CMA/102 or CMA/100, Carnegie Medicine, Stockholm, Sweden). During microdialysis, the dialysates were kept on ice and thereafter immediately frozen (−70°C). Samples of 240 ml were collected and analyzed in duplicate. The mean (SD) relative *in vitro* recovery of the 8 probes was 13 (4.8)%.

Isometric contraction

Isometric muscle contraction of the masseter muscle was performed by repetitive clenching on a force transducer connected to an oscilloscope (Gould 20 Hz Digital Storage, type 1425). The maximum voluntary bite force (MVB) between the second molars on the experimental side was determined before the experiment to provide feedback for the level of force to be produced by the subject. The highest value of triplicate readings was used. During isometric muscle contraction the subject was asked to clench for 30 s at a constant force of 50% of the MVB. To keep the force level constant during contraction the subject was instructed to view the oscilloscope screen curve displaying the bite-force level. Regarding the trapezius muscle, a weight cuff of 1 kg was attached to the middle part of the subject's upper arm on the experimental side. The subjects were then instructed to abduct the upper arm in a horizontal position for 1 min, as previously described [36]. The contractions were repeated at 1-min intervals in the trapezius muscle and at 1.5-min intervals in the masseter muscle for 30 min. In this manner, the contractions always started simultaneously in both muscles.

Assessment of pain

A 100-mm visual analog scale (VAS) with end-points marked "No pain" to the left and "Worst pain ever experienced" to the right was used to assess the degree of pain in the masseter and trapezius region. In the first experiment, pain was assessed immediately after probe insertion (VAS 0) and then every 30 min, i.e. before contraction (VAS 1), directly after contraction (VAS 2), as well as 30 min after contractions (VAS 3). In the second experiment, pain was assessed after each microdialysis sample (VAS 1–3).

Blood examination

Prior to the microdialysis and muscle contractions, a 10 ml venous blood sample was taken and immediately cooled and centrifuged (1700*g* for 10 min). The plasma was frozen (−70°C) and later analyzed for NPY.

Analysis of NPY

NPY in the dialysate and blood was analyzed in a single test by a competitive radioimmunoassay binding test. Intra- and interassay coefficients of variation were 7% and 14%, respectively, and the detection limit of NPY was 3.9 pmol/l [35,37]. The analyses were done, on average, 3 months after sampling.

Statistics

Two-way repeated measures ANOVA with the Student-Newman-Keuls method (SNK) as post-hoc test was used to test for changes in NPY concentration and pain intensity. The independent factors were condition (sample) at three levels (before,

during, and after contraction for NPY level; before, immediately after, and 30 min after contraction for VAS) and muscle at two levels (masseter and trapezius). Because of the low number of subjects in the second experiment, the Friedman repeated measures test was used to test the significance of the changes in NPY level and pain after probe insertion (alone). A dependent t-test was used to test the significance of the differences between plasma and precontraction dialysate levels of NPY. The significance of the correlation between the levels of NPY as well as between NPY level and VAS was tested with Pearson's product-moment correlation test. A probability level of <0.05 was considered as significant.

Results

Microdialysis with isometric contraction

The NPY level did not differ between samples and muscles, but there was a significant interaction between sample and muscle (RM ANOVA: $F=7.715$, $p=0.003$). The post-hoc test showed that the NPY level in the dialysate sample obtained during the initial 30-min rest period (Sample 1) was significantly higher in the masseter muscle compared to the trapezius muscle (SNK: $p=0.013$; Figure 2), while there was no difference between the levels in the masseter and trapezius muscles in the samples obtained during (Sample 2) and after contraction (Sample 3). In addition, the NPY level in the trapezius muscle was increased during isometric contraction (Sample 2; SNK: $p=0.010$) and remained increased 30 min after contraction (Sample 3; SNK: $p=0.038$) compared to before contraction (Sample 1; Figure 2B). The level of NPY in the masseter muscle was not significantly changed during or 30 min after contraction compared to before contraction (Figure 2A).

The NPY levels in the two muscles were correlated to each other before and during contraction (Figure 3).

The mean (SD) plasma level of NPY was 9.8 (3.7) pmol/l, which tended to be lower than the precontraction level in the masseter muscle (dependent t-test: $p=0.09$; Figure 2).

Pain developed during the experiments in both the trapezius and masseter muscles (Figure 2C, D) and reached its highest level directly after the isometric contractions (VAS 2), after which it decreased almost to precontraction levels. The difference between pain levels was significant (RM ANOVA: $F=5.150$, $p=0.004$). The post-hoc test showed that

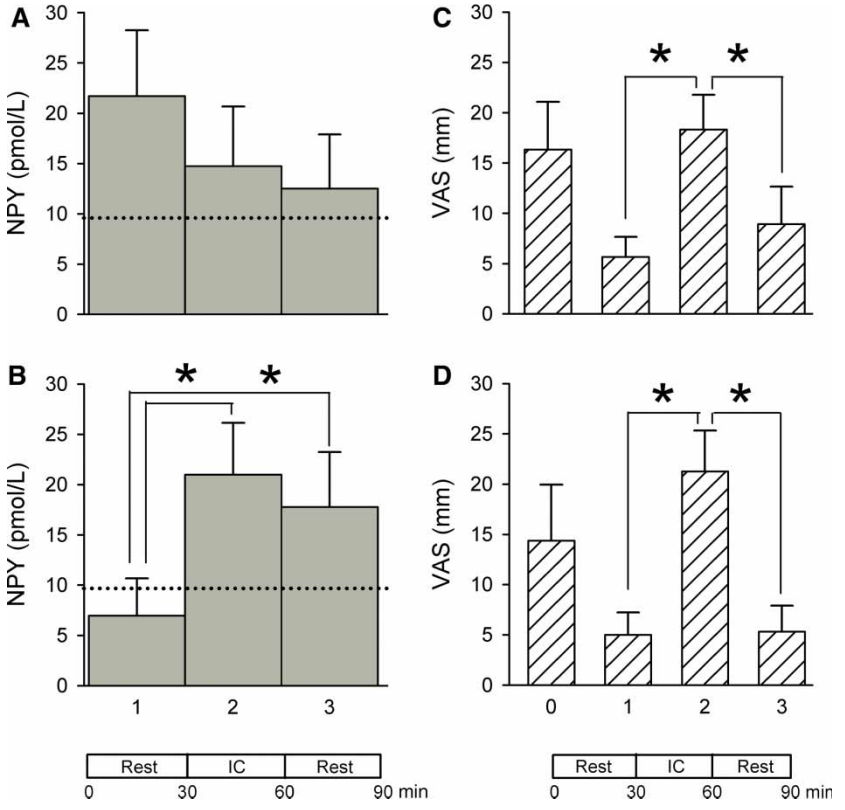

Figure 2. Bar graphs showing the mean (SEM) concentration of neuropeptide Y (NPY) in three consecutive intramuscular microdialysate samples and corresponding pain levels (VAS) in 16 healthy females. NPY levels in the masseter muscle (A) and in the trapezius muscle (B). The dialysates were collected during a 30-min rest period (1), during 30-min of repeated isometric contractions (IC) (2), and during a 30-min rest period after contraction (3). The dotted line shows the plasma level of NPY obtained immediately before dialysis. The pain level in the masseter muscle (C) and in the trapezius muscle (D). Pain was assessed immediately after probe insertion (0), after the initial 30-min rest period (1), immediately after contractions (2), and 30 min after contractions (3). *Significant difference ($p<0.05$).

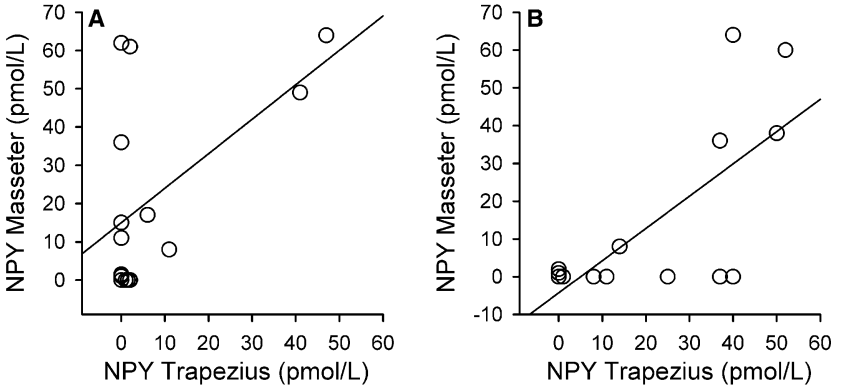

Figure 3. Scatter plot showing the correlation between masseter and trapezius muscle levels of neuropeptide Y (NPY) obtained before and during repeated isometric contractions in 14 healthy females. A. NPY levels during the 30-min rest period before contraction (Pearson: $r=0.54$, $p=0.037$, $n=15$). B. NPY levels during contraction ($r=0.71$; $n=14$; $p=0.004$).

the pain level assessed immediately after contraction (VAS 2) was higher compared to before contraction (VAS 1; SNK: $p=0.006$) and 30 min after contraction (VAS 3; SNK: $p=0.038$). There were no significant differences between muscles in regard to pain levels, and no interaction between pain level and muscle. There was no significant correlation between pain intensity and NPY level in any of the muscles.

Microdialysis without isometric contraction

Insertion of the microdialysis probe elicited pain in both muscles that lasted for 30 min. No changes in pain levels could be observed over the next 60 min. The NPY levels did not change significantly during the experimental period (Table I).

Discussion

This study investigated the level of NPY in skeletal muscle before and after isometric muscle contraction as well as in relation to the development of muscle pain. Our main finding was that isometric contraction increased the NPY level in the trapezius muscle only, whereas in the masseter muscle the NPY level remained stable during the experimental procedure. The precontraction level of NPY was higher in the resting masseter muscle than in the trapezius muscle. Despite the fact that the NPY level of the trapezius muscle increased during contraction it was not correlated to any increase of pain.

Table I. Intramuscular levels of NPY (pmol/l) and pain levels assessed by a visual analog scale (VAS) in five healthy females. Three consecutive samples of 30 min each were obtained by microdialysis from the masseter and trapezius muscles (sample 1–3)

	Masseter	Trapezius
Pain intensity (mm)		
Median (IQR)		
VAS 1	10 (15)	10 (10)
VAS 2	0 (5)	0 (5)
VAS 3	0 (10)	0 (5)
NPY (pmol/l)		
Median (IQR)		
Sample 1	10 (11)	0 (11)
Sample 2	16 (19)	6 (12)
Sample 3	11 (20)	10 (12)

IQR = Interquartile range.

The finding that the initial level of NPY 30 min after probe insertion and before contraction was higher in the masseter than in the trapezius muscle suggests that orofacial muscles differ from neck muscles in regard to NPY content. Neuronal differences between orofacial muscles and other skeletal muscles have been found regarding sympathetic fibers. Extremity nerves were found to contain 2.5 times more sympathetic neurons than the trigeminal nerve [38]. Accordingly, the higher NPY content in the masseter muscle is unlikely to be attributed to a higher density of sympathetic nerve fibers. However, the levels of NPY in the masseter and trapezius muscles were correlated, which indicates that these levels, although different, are related in the individual.

The NPY level was increased during isometric contraction in the trapezius, but not in the masseter muscle. Strenuous exercise is reported to increase sympathetic activity and to release NPY from its peripheral neural stores [18,19,39]. Since the level of NPY in the trapezius muscle exceeded the plasma level during and after contraction, the isometric muscle contraction probably elicits a peripheral release of NPY. No significant change in NPY levels was found in the trapezius muscle during microdialysis without contraction, which supports this. On the contrary, the isometric contraction does not seem to cause increased release of NPY in the masseter muscle, which might be due to its lower density of sympathetic fibers. In addition, the masseter muscle contains a relatively larger number

of type I muscle fibers, while type II fibers are more frequent in trunk and extremity muscles such as the trapezius muscle [40,41]. The longer endurance capability of type I fibers suggests that a longer period of contraction may be necessary to cause NPY release in the masseter compared to the trapezius muscle. Consequently, the intensity or duration of the isometric contraction of the masseter muscle may have been insufficient to cause NPY release. The influence during contraction of the microdialysis probe on the release of NPY in the muscle is unknown, but is unlikely to be different in the two muscles.

In this study, only spontaneously perceived muscle pain was measured, since digital palpation of tenderness or assessment of pressure pain threshold was excluded in the region where the microdialysis probe was inserted. Pain of low intensity developed in both muscles after insertion of the probe and decreased almost to zero during the first 30 min. Pain in both muscles then increased significantly after contraction. The pain response in the masseter muscle is in contrast to the results of a previous study in healthy females which reported difficulty eliciting pain and soreness in the jaw-closing muscles by clenching at 25% of the maximal bite force, even if clenching was repeated and long-lasting [42]. However, in the present study the subjects were instructed and encouraged to contract at a higher intensity (50%), which could explain this difference. Tissue displacement due to the presence of the probe during the contraction is an additional cause of pain.

The pain that developed in the trapezius muscle after contraction was not associated with the corresponding increase of NPY. This finding indicates that the primary function of the NPY released by this experimental contraction can be referred to microvascular regulation rather than pain development. Other mediators that may be released due to the contraction and be responsible for the pain development include bradykinin, glutamate, PGE_2, and serotonin, as has been shown in human experimental studies and in patients with chronic myalgia [7,12,13,43–45]. This might also explain why pain developed in the masseter muscle after contraction, where no increase of local NPY release was observed. However, in this study an acute experimental model was used and NPY may still be associated with pain development in chronic clinical pain conditions. This was not addressed in the present study. In order to further elucidate this question, an experiment administering agonists and antagonists of NPY intramuscularly should be performed.

Pain and soreness are increased in the trapezius muscle by isometric contractions in patients with chronic neck and shoulder myalgia [6] in association with reduced microcirculation [5,6]; static contraction therefore seems to cause impairment of blood flow in myalgic muscles. The impaired blood flow may be due to increased transmitter activity of neuropeptides such as NPY [25]. In this study, the intramuscular dialysate level of NPY in the trapezius muscle increased after isometric contractions, which concurs with previous results from animal studies [18,19,39].

There are certain methodological issues that need to be discussed. First, only women were included in the study – justified since sex differences in pain perception are reported of women being more prone to develop chronic muscle pain [46]. The subjects were in different phases of the menstrual cycle, which might have influenced our results, since pain perception varies during the cycle [46]. A local anesthetic cream was used to provide skin surface anesthesia. It could be argued that this might have influenced our results. However, it has previously been reported that topical anesthesia with EMLA cream does not penetrate the muscle [32]. The sample size might have been too small for a difference of NPY concentration to be detected in the masseter muscle before and after contraction. However, this is unlikely, since a significant increase was found in the trapezius muscle. In addition, similar sample sizes have been sufficient in detecting changes in studies of related topics [47,48].

In conclusion, pain is developed in the trapezius and masseter muscles during repeated isometric contraction. The NPY level is increased in the trapezius muscle but is not associated with pain development.

Acknowledgments

The study was supported financially through grants from the Institute of Odontology, Karolinska Institutet, and the Swedish Dental Association.

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