Possible mechanisms of action of nitric oxide synthase inhibitors in chronic tension-type headache

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Summary
It has been demonstrated recently that nitric oxide synthase (NOS) inhibition has an analgesic effect in patients with chronic tension-type headache. The aim of the present study was to investigate the influence of the NOS inhibitor, l-NG methyl arginine hydrochloride (l-NMMA), on two of the most prominent features of chronic tension-type headache, i.e. increased muscle hardness and increased myofascial tenderness. In a double blind, crossover designed trial, 16 patients with chronic tension-type headache were randomized to receive intravenous infusion of 6 mg/kg l-NMMA or placebo on 2 days separated by at least 1 week. Muscle hardness of the trapezius muscle was measured with a hardness meter. Myofascial tenderness in the pericranial region was evaluated by manual palpation with standardized and validated methodology. All parameters were recorded at baseline and at 60 and 120 min after start of infusion. Compared with baseline, muscle hardness, 107 ± 17 kPa/cm and tenderness, 18 ± 11 were significantly reduced at 60 and 120 min to: hardness, 101 ± 17 kPa/cm and 101 ± 17 kPa/cm, respectively; tenderness, 15 ± 11 and 14 ± 11, respectively, after treatment with l-NMMA (P < 0.05 and P < 0.01, respectively), while there was no significant reduction at any time after treatment with the placebo. Compared with the placebo, the summary score of muscle hardness was significantly reduced (P = 0.04), while tenderness showed a non-significant reduction (P = 0.11) following treatment with l-NMMA. Since increased muscle hardness in patients with chronic tension-type headache may reflect sensitization of second order neurons due to prolonged nociceptive input from myofascial tissues, we suggest that the decrease in muscle hardness following treatment with l-NMMA may be caused by reduction of central sensitization.

Keywords: tension-type headache; nitric oxide; central sensitization; myofascial tenderness, muscle hardness

Abbreviations: l-NMMA = l-NG methyl arginine hydrochloride; NO = nitric oxide; NOS = nitric oxide synthase; PPDT = pressure pain detection thresholds; TTS = total tenderness score; VAS = visual analogue scale

Introduction
Chronic tension-type headache represents a considerable health problem and results in large socio-economic costs (Rasmussen et al., 1992). Increased myofascial tenderness and muscle hardness are the most prominent abnormal findings in patients with chronic tension-type headache (Jensen et al., 1993; Sakai et al., 1995; Lipchik et al., 1997; Jensen et al., 1998; Ashina et al., 1999a). However, the mechanisms leading to the increased tenderness and muscle hardness are largely unknown. Progress in basic pain research has increased our knowledge about the mechanisms underlying chronic myofascial pain (Mense, 1993). Thus, substantial experimental evidence indicates that central sensitization, i.e. increased excitability of neurons in the CNS, generated by prolonged nociceptive input from the periphery plays an important role in the pathophysiology of chronic pain, particularly from myofascial tissues (Woolf, 1983; Hu et al., 1992; Woolf and Doubell, 1994; Bendtsen et al., 1996a). The freely diffusible gas nitric oxide (NO) is involved in the development of central sensitization (McMahon et al., 1993; Meller and Gebhart, 1993). Thus, nitric oxide synthase (NOS) inhibitors reduce central sensitization in animal models of persistent pain (Haley et al., 1992; Hao and Xu, 1996; Mao et al., 1997) and we demonstrated recently that NOS inhibition has an analgesic effect in patients with chronic tension-type headache (Ashina et al., 1999b). However, the mechanisms of this effect remain...
Table 1 Clinical data on patients

<table>
<thead>
<tr>
<th>Patients</th>
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<tbody>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Females/males</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Frequency of tension-type headache (days/4 weeks)</td>
</tr>
<tr>
<td>Amount of used analgesics (g/4 weeks)</td>
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<tr>
<td>Hamilton Depression Score</td>
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Mean values with range given in parentheses.

unknown. The aim of the present study was to investigate whether the NOS inhibitor L-N\textsuperscript{G} methyl arginine hydrochloride (L-NMMA), modulates muscle hardness and myofascial tenderness in patients with chronic tension-type headache.

**Material and method**

**Subjects**

Sixteen patients with a diagnosis of chronic tension-type headache according to the criteria of the International Headache Society (Headache Classification Committee, 1988) were included (Table 1). Five of the patients had coexisting infrequent migraine (≤4 days/year). The patients were recruited from the out-patient headache clinic at Glostrup University Hospital without respect to presence or absence of myofascial tenderness. All patients underwent a general physical and neurological examination and completed a diagnostic headache diary during a 4-week run-in period (Russell et al., 1992). Exclusion criteria were: coexisting infrequent migraine (>1 day/month); any kind of daily medication (including prophylactic headache therapy, but not oral contraceptives); excessive use of analgesics (corresponding to >2 g of aspirin/day); serious somatic or psychiatric diseases including depression (Hamilton Depression Score ≥17; Hamilton, 1960). Patients were examined and treated on a typical day of tension-type headache. All patients gave written consent to participate in the study, which was approved by the Danish Board of Health and the local ethics committee. The study was conducted in accordance with the Declaration of Helsinki.

**Apparatus**

**Muscle hardness**

The hardness of the trapezius muscle was measured with a hardness meter, which has been described in detail previously (Horikawa et al., 1993). In brief, the hardness meter consists of a laser distance sensor and a pressure terminal with a surface area of 1 cm\textsuperscript{2}. The muscle hardness is estimated by recording the relationship between the applied pressure and the displacement of the skin over the muscle. All calculations are performed by a purpose-made software-program in order to avoid observer bias. Hardness is expressed in kilopascals per centimetre. We have previously demonstrated that the hardness meter can measure muscle hardness reliably if the same observer is used throughout a study (Ashina et al., 1998).

**Pressure pain thresholds**

An electronic pressure algometer (Somedic AB, Stockholm, Sweden) was used to measure pressure pain thresholds. A circular stimulation probe (0.50 cm\textsuperscript{2}) and a pressure loading rate of 22 kPa/s (1 kPa = 10\textsuperscript{3} N/m\textsuperscript{2}) were used. The algometer has been described in detail elsewhere (Jensen et al., 1986).

**Methods**

The recordings were performed in a standardized manner by the same observer, a trained technician (H.A.), throughout the study. All parameters were recorded at baseline, and 60 and 120 min after the start of the infusion. The trial was designed as a double blind, placebo controlled, crossover study. The first part of the study examined the analgesic effect of L-NMMA and has previously been described in detail (Ashina et al., 1999b). Briefly, patients were randomized to receive 6 mg/kg L-NMMA (Clinalfa, Switzerland) or placebo (isotonic glucose) over 15 min into an antecubital vein on 2 days separated by at least 1 week. The patients were not allowed to take analgesics (including vasoactive drugs for migraine) or any other kind of medication 12 h prior to the examination. Headache intensity was measured on a 100 mm Visual Analogue Scale (VAS) (0, no headache and 100, worst imaginable headache) before, during and after start of infusion.

**Muscle hardness**

The muscle hardness was measured at a standard anatomical point on the trapezius muscle on the non-dominant side, as previously described (Ashina et al., 1998). Briefly, the point was located on the centre of the descending part of the trapezius muscle midway between the processus spinosus of the seventh cervical vertebra and the acromion. The muscle hardness was calculated as the mean of five consecutive determinations. All recordings were stored in the computer and they were not analysed before the study was completed.

**Total tenderness**

Tenderness of pericranial myofascial tissues was recorded according to the Total Tenderness Scoring system (Langemark and Olesen, 1987), which has previously proved to be reliable (Bendtsen et al., 1995). Eight pairs of muscles and tendon insertions (masseter, temporal, frontal, sternocleidomastoid and trapezius muscles, coronoid and mastoid processes, and neck muscle insertions) were palpated. Tenderness was scored on a four-point (0–3) scale at each location (local tenderness score) and values from left and right sides were summed to a total tenderness score (TTS) (maximum possible score = 48).
Nitric oxide in tension-type headache

Fig. 1 Percentage changes in muscle hardness. Muscle hardness was significantly more reduced following treatment with L-NMMA (circles) than with placebo (triangles) in patients with chronic myofascial pain. *P < 0.05 compared with baseline (time = 0). The plots represent mean scores.

Pressure pain thresholds
Pressure pain detection thresholds (PPDTs) were measured at the dorsum of the second finger (middle phalanx) and at a fixed point at the anterior part of the temporal muscle as previously described (Bendtsen et al., 1996b). Measurements were performed at the non-dominant side. The PPDT was defined as the pressure at which the sensation changed from pressure alone to pain. The subject indicated that the pain threshold was reached by pressing a handheld button. The algometer display was thereby ‘frozen’ and the pressure was immediately released. Each threshold was calculated as the mean of five consecutive determinations performed with intervals of ~30 s.

Data analysis and statistics
Results are presented as mean ± standard deviation. For each of the variables, the sum of the differences between the pre-treatment value and each of the post-treatment values was calculated in order to obtain a summary measure of effect for each treatment (Matthews et al., 1990). The summary scores calculated for active treatment and placebo were compared by use of the Wilcoxon Signed Ranks Test. Within each treatment, pre-treatment values were compared with values at 60 and 120 min post-dosing by use of the Wilcoxon Signed Ranks Test. Five percent was accepted as level of significance. All data were analysed with SPSS software®, version 7.5.1. (SPSS Inc., USA).

Results
Muscle hardness
The summary score of muscle hardness was reduced significantly more following treatment with L-NMMA than with placebo (P = 0.04) (Fig. 1). Compared with baseline, hardness was significantly reduced at 60 and 120 min after treatment with L-NMMA (P = 0.04 and P < 0.05, respectively). There was no significant reduction in muscle hardness at any time after treatment with placebo (Table 2).

Tenderness
The summary score of tenderness tended to be reduced more following treatment with L-NMMA than with placebo, but the difference was not statistically significant (P = 0.11) (Fig. 2). However, compared with baseline TTS was significantly reduced at 60 and 120 min after treatment with L-NMMA (P = 0.007 and P = 0.008, respectively). There was no

Table 2 Muscle hardness, TTS and PPDT in the finger and the temporal region recorded before and 60 and 120 min after start of the infusion of L-NMMA or placebo

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>60 min</th>
<th>120 min</th>
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<tbody>
<tr>
<td><strong>Muscle hardness</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>L-NMMA</td>
<td>107 ± 17</td>
<td>101 ± 17*</td>
<td>101 ± 17*</td>
</tr>
<tr>
<td>Placebo</td>
<td>106 ± 18</td>
<td>104 ± 17 (n.s.)</td>
<td>105 ± 22 (n.s.)</td>
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<tr>
<td><strong>TTS</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>L-NMMA</td>
<td>18 ± 11</td>
<td>15 ± 11**</td>
<td>14 ± 11**</td>
</tr>
<tr>
<td>Placebo</td>
<td>17 ± 12</td>
<td>16 ± 13 (n.s.)</td>
<td>15 ± 13 (n.s.)</td>
</tr>
<tr>
<td><strong>PPDT/finger</strong></td>
<td></td>
<td></td>
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<tr>
<td>L-NMMA</td>
<td>455 ± 155</td>
<td>436 ± 129 (n.s.)</td>
<td>449 ± 144 (n.s.)</td>
</tr>
<tr>
<td>Placebo</td>
<td>457 ± 141</td>
<td>435 ± 143 (n.s.)</td>
<td>420 ± 130 (n.s.)</td>
</tr>
<tr>
<td><strong>PPDT/temporal region</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-NMMA</td>
<td>279 ± 108</td>
<td>264 ± 86 (n.s.)</td>
<td>277 ± 95 (n.s.)</td>
</tr>
<tr>
<td>Placebo</td>
<td>274 ± 104</td>
<td>271 ± 109 (n.s.)</td>
<td>262 ± 95 (n.s.)</td>
</tr>
</tbody>
</table>

Mean values (± standard deviations) are given. Post-treatment values compared with pre-treatment values (Wilcoxon Signed Ranks test). *P < 0.05; **P < 0.009; n.s. = not significant.

Fig. 2 Percentage changes in TTS. The TTS tended to be reduced following treatment with L-NMMA (circles) compared with placebo (triangles) (P = 0.11). Within each treatment, the TTS was significantly reduced at 60 and 120 min after start of the infusion of L-NMMA, while there were no significant changes at any time after treatment with placebo. **P < 0.01 compared with baseline (time = 0). The plots represent mean scores.
significant reduction in TTS at any time after treatment with placebo (Table 2).

Pressure pain thresholds
There was no significant difference between PPDTs recorded during treatment with L-NMMA and placebo (finger: \(P = 0.78\) and temporal region: \(P = 0.77\)). There were also no changes in PPDTs at 60 and 120 min after treatment with L-NMMA compared with pre-treatment values in either the finger or in the temporal region (Table 2). Compared with baseline, PPDT decreased significantly in the finger (\(P = 0.04\)) but not in the temporal region following treatment with placebo (Table 2).

Pain intensity
Pain intensity was significantly more reduced following treatment with L-NMMA than following treatment with placebo (Fig. 3) as previously reported (Ashina et al., 1999b). Pain scores were significantly reduced at each time point after treatment with L-NMMA, while there was no significant reduction in pain intensity at any time point after treatment with placebo (Fig. 3).

Adverse events
Subjective symptoms in relation to the L-NMMA infusion were reported in seven patients (Ashina et al., 1999b). These were: tiredness (two); dryness of the mouth (three); drowsiness (one); exhaustion (one); nausea (one) and a feeling of tingling in one arm (one). Four patients reported subjective symptoms in relation to placebo treatment. These were: a feeling of tingling in the arms (two) and shoulders (two); dryness of the mouth (one); warm sensation in the body (one) and drowsiness (one). No patient withdrew from the study because of side-effects.

Discussion
Nociception from myofascial tissues probably plays a major role in the pathophysiology of chronic tension-type headache. Thus, several studies have reported consistently increased myofascial tenderness as the most prominent abnormal finding in patients with chronic tension-type headache (Langemark and Olesen, 1987; Jensen et al., 1993, 1998; Bendtsen et al., 1996b; Lipchik et al., 1997; Ashina et al., 1999a). Further support for myofascial involvement is the recent findings of increased muscle hardness (Sakai et al., 1995; Ashina et al., 1999a) and a positive correlation between muscle hardness and tenderness in chronic tension-type headache (Ashina et al., 1999a). Recently it has been suggested that the increased tenderness in patients with chronic tension-type headache may be due to sensitization of spinal dorsal horn neurons induced by prolonged noiceptive input from pericranial myofascial tissues (Bendtsen et al., 1996a; Jensen et al., 1998).

Animal experiments have suggested that NO is an important transmitter in pain pathways of the spinal cord and that sensitization of these pathways may be caused by or associated with activation of NOS and the generation of NO (Haley et al., 1992; Meller et al., 1992; Meller and Gebhart, 1993). In support for this, it has been shown recently, in animal models of persistent pain, that NOS inhibitors reduce spinal dorsal horn sensitization induced by continuous painful input from the periphery (Meller et al., 1994; Roche et al., 1996; Mao et al., 1997). In addition, we recently demonstrated that headache intensity in patients with chronic tension-type headache was significantly reduced following treatment with L-NMMA compared with placebo (Ashina et al., 1999b).

The present study provides important information about the mechanisms of the antinociceptive action of NOS inhibition in chronic tension-type headache. We found that both muscle hardness and tenderness were significantly reduced at each time point after treatment with L-NMMA, while there was no significant reduction in muscle hardness or tenderness at any time after treatment with placebo. The muscle hardness was significantly reduced following treatment with L-NMMA compared with placebo, while the reduction in tenderness following treatment with L-NMMA did not reach statistical significance compared with placebo. Although statistically significant, the reduction of hardness was small. This is understandable because the increased hardness is a rather stable feature that is present not only during, but in the absence of headache in patients who suffer chronic tension-type headache (Ashina et al., 1999a). Therefore muscle hardness may not be easy to change in an acute experiment with a drug of short half-life (Blitzer et al., 1996). Similar arguments apply to myofascial tenderness (Jensen et al., 1998). Long-term treatment could probably result in larger
changes. However, the importance of the present results lies
in the proof of the concept and not in the magnitude of the
effect. The PPDTs in the finger and temporal region were
largely unchanged following treatment with L-NMMA. This
indicates that L-NMMA did not significantly alter general
pain sensitivity and that it may exert a specific action in
myofascial pain pathways.

The question is how L-NMMA modulates muscle hardness
and tenderness and whether the effects of L-NMMA observed
in the present study are due to an action in muscle, peripheral
nerves or in the CNS? It has been shown that central
sensitization at the level of the spinal dorsal horn may
increase the drive to motor neurons both at the supraspinal
and the segmental level (Woolf, 1983). Thus, it is possible
that sustained muscle contraction due to central sensitization
contributes to the increased muscle hardness and tenderness
in chronic tension-type headache. This is supported by recent
findings of increased tenderness, muscle hardness and muscle
activity in patients with chronic tension-type headache not
only on days with headache, but also on days without
headache (Lipchik et al., 1997; Jensen et al., 1998; Ashina et al., 1999a). In addition, the search for peripheral
mechanisms responsible for muscle pain has largely been
negative (Langemark and Jensen, 1988; Yunus, 1993; Simms et al., 1994). Collectively, these results indicate that the
permanently altered muscle hardness, tenderness and muscle
activity may reflect sensitization of second order neurons at
the level of the spinal dorsal horn/trigeminal nucleus, and
that the effects of L-NMMA observed in the present study
are due to desensitization of these neurons.

However, it cannot be excluded that L-NMMA exerts its
effect in the periphery. L-NMMA inhibits all three types of
NOS (endothelial NOS, neuronal NOS and inducible NOS)
(Southan and Szabo, 1996) and rich sources of neuronal
NOS are present not only in nervous tissue but also in striated
muscles of mammals (Grozdanovic et al., 1995). In addition
to neuronal NOS, skeletal muscles also contain endothelial
NOS (Kobzik et al., 1995). However, a recent study
demonstrated that NO promotes relaxation in skeletal muscles
and that the contractile function of muscles was augmented
by blockers of NO synthase (Kobzik et al., 1994). Because
of this inverse correlation between contractile function and
neuronal NOS activity, one would expect that L-NMMA
acting in the muscle would induce muscle contraction with
subsequent increase of muscle hardness and tenderness.
However, in the present study we observed a reduction of
muscle hardness and tenderness following treatment with
L-NMMA. Thus, the effects of L-NMMA observed in the
present study cannot easily be related to the effects on muscle
contraction.

It is possible that L-NMMA has direct antinociceptive
effects in myofascial tissues. Thus, the ability of NOS
inhibitors to cause vasoconstriction (Rees et al., 1990) may
prevent inflammatory mediators and algogenic substances
involved in hardness and tenderness from reaching their site
of action (Haley et al., 1992). In addition, it has been
demonstrated that NOS inhibitors have antinociceptive effects
after peripheral administration (Haley et al., 1992; Kindgren-
Milles and Arndt, 1996; Nakamura et al., 1996). However,
the exact role of NO in the periphery is still far from
understood, and additional research is needed to clarify
whether NO may activate or sensitize nociceptors in
myofascial tissues.

The effect of L-NMMA on muscle hardness might be
secondary to reduction of headache caused by blocking
vasodilatation. Excessive vascular nociception may contribute
to a primary myofascial nociception in patients with tension-
type headache (Olesen, 1991). Thus, it has been demonstrated
that patients with tension-type headache are more susceptible
to NO donors such as histamine and nitroglycerin than
headache free controls (Krabbe and Olesen, 1980; Olesen et al., 1993). Furthermore, patients with chronic tension-type
headache often develop migraine-like episodes (Langemark et al., 1988) and it has been suggested that these episodes
may be due to the presence of moderate vascular input in
these patients (Olesen, 1991). Thus, it is possible that
L-NMMA exerts its action by blocking of moderate
vasodilatation of cephalic/extracranial arteries in patients
with chronic tension-type headache with subsequent modest
reduction of muscle hardness.

The antinociceptive effect of NOS inhibition might also
result from vascular and physiological effects elicited by
L-NMMA, such as changes in blood pressure, pulse rate and
subjective symptoms. Mean arterial blood pressure and pulse
rate were continuously monitored in the present study. We
found that the peak increase in mean arterial blood pressure
(12%) and maximum decrease in pulse rate (16%) occurred
15 and 10 min, respectively, after treatment with L-NMMA
(Ashina et al., 1999b). Patients were clinically unaffected by
these changes. The difference in the mean arterial blood
pressure and pulse rate between L-NMMA and placebo
disappeared 60 min after start of infusion. In contrast, the
antinociceptive effect on headache intensity and the reduction
of muscle hardness and tenderness lasted at least 120 min
after start of infusion. There were subjective symptoms in
seven patients after L-NMMA and in four patients after
placebo infusion. Most of the symptoms (dryness of the
mouth; drowsiness; a feeling of tingling in the arms) in
relation to the L-NMMA infusion were similar to those after
placebo infusion. It therefore seems unlikely that the observed
effects of L-NMMA were caused by a hypertensive effect of
the agent or influenced by the subjective symptoms reported
in relation to the L-NMMA infusion.

Finally, it could be argued that the effect of L-NMMA on
muscle hardness might be influenced by the presence of
coeexisting infrequent migraine in five patients. However, we
found that the reduction of muscle hardness after L-NMMA
infusion was of the same magnitude in patients without
coeexisting migraine (6%) and in patients with coexisting
migraine (7%). Moreover, regarding the effect of L-NMMA
on pain intensity, patients without coexisting migraine tended
to have an even higher reduction of pain intensity (35%)
than patients with coexisting migraine (23%) (Ashina et al., 1999b). This suggests that our results were not influenced by the inclusion of patients with coexisting infrequent migraine.

In conclusion, our data indicate that the NOS inhibitor L-NMMA elicits its antinociceptive effect in chronic tension-type headache by modulation of nociceptive information from myofascial tissues. We suggest that this antinociceptive effect may be due to reduction of central sensitization at the level of the spinal dorsal horn/trigeminal nucleus. Future studies with selective NOS inhibitors are needed in order to determine which type of NOS is involved and its exact site of action in chronic tension-type headache.

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References


