Kinin peptides in human trapezius muscle during sustained isometric contraction and their relation to pain

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Boix, Fernando, Cecilie Røe, Laila Rosenborg, and Stein Knardahl. Kinin peptides in human trapezius muscle during sustained isometric contraction and their relation to pain. J Appl Physiol 98: 534–540, 2005. First published October 8, 2004; doi:10.1152/japplphysiol.01340.2003.—To determine the muscular concentration of bradykinin and kallidin during static contraction, microdialysis probes were implanted bilaterally in the trapezius muscles of healthy women. Three hours after probe implantation, 200 μM of the angiotensin-converting enzyme (ACE) inhibitor enalaprilat were added to the perfusion solution in one of the sides for 30 min. Thirty minutes later, the subjects performed a sustained bilateral shoulder abduction at 10% of the maximal voluntary contraction until exhaustion. This protocol was repeated twice, with an interval of at least 17 days. High intersession repeatability was observed in the concentration of bradykinin but not of kallidin. Enalaprilat induced a significant increase in bradykinin levels in the dialysate, without affecting kallidin levels. The sustained contraction induced a significant increase in dialysate levels of both kinin peptides. The contraction also induced a significant increase in pain ratings, as measured by a visual analog scale. During contraction, positive correlations were found between pain ratings and levels of kinin peptides in dialysate, predominantly in the side previously perfused with enalaprilat. Subjects with the higher pain ratings also showed larger increases in kinin peptides in the side previously perfused with enalaprilat. The present results show that both plasma and tissue kinin-kallikrein are activated during muscle contraction, but that their metabolic pathways are differently regulated during rest and contraction, because they showed a different response to ACE inhibition. They also indicate that intramuscular kinin peptides levels, and ACE activity, may contribute to muscle pain.

Bradykinin; kallidin; muscle pain; angiotensin-converting enzyme; microdialysis

BRADYKININ IS A NONAPEPTIDE known to be released in muscle under contraction, both in humans (32) and animals (5, 28). Its liberation generates metabolic and physiological effects important for energy delivery to the working muscle (9, 24). This contraction-induced liberation of bradykinin is likely triggered by the rise in adenosine levels induced by muscle activity (5). Bradykinin is also a potent algogen and a key mediator of inflammatory hyperalgesia (10). The intramuscular administration of bradykinin can induce pain (1, 20, 23) and modify the receptive fields of dorsal horn neurons to noxious stimuli (19). Thus bradykinin may be an important factor in the appearance and development of muscular pain.

The kallikrein-kinin system liberates not only bradykinin but also the decapetide kallidin. Kallidin presents the same sequence as bradykinin but with an additional lysine residue at the NH2-terminal position. Bradykinin is liberated from high-molecular-weight kininogen, circulating in plasma, by the enzymatic action of the high-molecular-weight kininogen-bound plasma kallikrein (EC 3.4.21.34). Kallidin is liberated by tissue kallikrein (EC 3.4.21.35), acting preferentially on low-molecular-weight kininogen. Both kininogen varieties exhibit the sequences for bradykinin and kallidin, which are released by the differential cleavage of the sequence by the kallikrein enzymes. Possibly due to their comparable physiological actions, both act with a similar potency on the B2 receptors, and due to the lack of specific antibodies, most studies on kinin peptides have been confined to bradykinin or not discriminated between the two peptides. Some recent studies show that kallidin, but not bradykinin, can also activate the B1 receptor (16), a kinin receptor that is not normally present in the tissue but is induced by the presence of its agonists (25). In addition, both peptides appear to react differently (12) to inhibition of angiotensin-converting enzyme (ACE; EC 3.4.15.1), the main metabolizing pathway of the kinin peptides. It has been shown that both bradykinin and a kallidin-like peptide are released in rat muscle, but their release pattern differs (5). Thus the discrimination between the two kinin peptides can be essential for a complete understanding of their physiological function (14).

The aim of the present study was to characterize the release and metabolic regulation of the kinin peptides bradykinin and kallidin in humans under static muscle contraction and their relation to perceived pain. For this purpose, we used the microdialysis technique in trapezius muscles of healthy humans to monitor changes in the intramuscular bradykinin and kallidin levels under ACE inhibition (enalaprilat) and sustained isometric contraction. Because this method may be potentially very useful in assessing tissue levels of kinins, a second aim was to assess the test-retest reliability of the method.

MATERIAL AND METHODS

Subjects. Fourteen healthy, pain-free, female volunteers, aged between 21 and 31 yr, participated in the experiment. Persons with a medical history of high blood pressure, with muscular pain, or under current medication were excluded from the study. They also underwent a health check on the first day of the study. Participants were asked to avoid any medication and unusual physical activity during the preceding week and to abstain from caffeine or alcohol from the day before the experiment. Full information on the experimental protocol was given to the participants, who gave their signed consent. The experimental protocols used have been approved by the appropriate Norwegian Regional Committee for Medical Research Ethics.

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Microdialysis. Ethylene dioxide-sterilized microdialysis probes (CMA/20, CMA/Microdialysis, Stockholm, Sweden), with a 10-mm polyethylenesulfon membrane and a 90-mm shaft, were used. Two probes were placed bilaterally, one in each of the trapezius muscles. Under superficial local anesthesia with 1 ml lidocaine (10 mg/ml), a 20-gauge Teflon catheter was inserted midway between the processus spinosus at C7 and the acromion. The insertion was made from the upper side of the shoulder, at an angle of ~30° from the middle line to the side of the body. The localization of the catheter in the trapezius muscle was verified by ultrasound scanning equipment. If the catheter was correctly positioned inside the muscle, a microdialysis probe was introduced through the catheter, which was subsequently pulled back. Both the probe and the retracted catheter were fixed to the skin with tape. After insertion, the inlet tube of each probe was connected to a microdialysis pump (CMA/17, CMA/Microdialysis) for perfusion with a Ringer-acetate solution (B. Braun Melsungen, Melsungen, Germany), containing 0.5% human albumin (Octapharma, Hurdal, Norway), except in two of the subjects. Flow was set at 5 μl/min. Enalaprilat (Renitec, Merck Sharp & Dohme, Rahway, NJ) was added to the perfusion medium to a final concentration of 200 μM. To change the perfusion medium, the microdialysis pump was disconnected and replaced with another pump containing the syringe with the new buffer. To control for possible effects due to the disconnection of the pump, the same procedure was simultaneously performed on the control side with the regular perfusion buffer. Microdialysis samples were immediately frozen after collection for later analysis.

The in vitro recoveries, at 5 μl/min and room temperature, for bradykinin and kallidin were 10.2 ± 2.4% and 8.2 ± 3.9% (means ± SD), respectively. In vitro recovery for albumin (molecular mass in the region of 60 kDa) was ~0.3%, showing that, despite the relative high cutoff of the membrane used, no other components of the kinin-kallikrein system (kininogen, enzymes) could have crossed the membranes in physiologically significant amounts that could affect the results.

Isometric abduction. The bilateral isometric shoulder abduction was carried out with the subjects seated in a chair that allowed standardized positioning (6). The abduction was performed with both shoulders simultaneously, with the elbow flexed 90° and the shoulders abducted to 45° in the plane of the scapula. The contraction was monitored by measuring the force applied to two braces situated bilaterally just above the elbow. Each brace was connected to a force transducer (Sokki Kenkyujo, Tokyo, Japan), and the resulting signal was sampled by a computer. The subject received continuous feedback of the applied force through a graphic presentation on a computer monitor of the force exerted by each arm.

At the beginning of each experimental session, the subjects were asked to perform at least three maximal voluntary contractions (MVC), each ~3-s duration and with a 2-min rest in between. If the force applied to the braces in the last contraction was the highest, the procedure continued until one contraction was lower than the preceding one to ensure that the highest MVC was attained. From the highest force achieved, the 10% force intensity was calculated and used for the later sustained shoulder abduction.

Protocol. Once MVC was determined, the microdialysis probes were inserted, and afterward the subject remained comfortably seated in an upholstered armchair. Microdialysis samples were collected every 20 min for 3 h. After the ninth sample, the pumps were stopped and replaced, as explained above, on one side with one containing perfusion medium with 200 μM enalaprilat and the other side with one containing the normal perfusion medium. The side receiving enalaprilat was selected randomly. The experimental subject was blind to which side was perfused with the drug. After 30 min of sampling, the pumps were stopped again, they were replaced with ones containing normal perfusion medium, and the sampling was continued for a further 30 min. After this period, the experimental subjects moved to the chair for the shoulder abduction. Subjects were then asked to perform a bilateral, simultaneous, sustained shoulder abduction at 10% of the MVC force until exhaustion. Exhaustion was defined as the point at which the subjects could no longer keep the required forces on both arms concurrently, despite being encouraged by the experimenter to continue. After the exercise, the subject returned to the armchair for a last 30-min postcontraction sample.

During the experiment, mean arterial pressure (MAP) and heart rate were continuously recorded at the finger by a photoplethysmographic arterial/volume clamp method (Finapres 2300, Ohmeda, Louisville, CO, or Finometer, Finapres Medical Systems, Arnhem, The Netherlands). Pain in both shoulders was scored every 10 min (every 5 min during contraction) on a 100-mm electronic visual analog scale (VAS), with the lower end point marked as “no pain” and the upper end point as “most pain imaginable.” To avoid bias, the VAS questioning by the experimenter was standardized.

The whole procedure was carried out twice for each experimental subject, in two sessions separated by at least 17 days and a maximum of 42 days, except for two of the subjects, for whom a second session was not possible to arrange. In the second session, enalaprilat perfusion was applied in the shoulder opposite to the one used on the first session.

Data analysis. Abduction force, MAP, heart rate, and VAS were sampled in a computer by use of a LabView system (National Instruments, Austin, TX). Mean of MAP and heart rate and maximal VAS value were calculated for each time interval corresponding to each microdialysis sample.

Total amount (fmol) of kallidin and bradykinin was assessed by using a RIA analysis with specific antibodies developed by Hilgenfeldt et al. (18). An assessment of human microdialysis samples using HPLC before RIA confirmed that no other substances were detected with these antibodies, except for the already known 20% cross-reactivity of the bradykinin antibody with des-Arg⁵-bradykinin (18). The delivery rate (fmol/min) of the peptides was calculated to balance for the different sampling times. For samples from the first 3 h (first 9 samples), odd samples were used for measuring kallidin, and even samples were used for measuring bradykinin. For the rest of the experiment, samples were split in two, and each half was used for the analysis of one of the two peptides. Values from samples 8 and 9 were considered as baseline values for bradykinin and kallidin, respectively, and the peptide levels were recalculated as percentage of these baseline values.

The statistical analysis of data was done with nonparametric tests, Wilcoxon signed-rank test for repeated measures (within-subjects factors), and Mann-Whitney test for between-subjects factors, using the SPSS 11.0 for Windows statistical package. Test-retest repeatability was calculated using Spearman’s ρ correlations between sessions or sides, using only those cases available for both variables. Data were missed for some microdialysis samples, resulting in different number of cases (see Figs. 1–5) for control and enalaprilat-treated sides, as well as for kallidin and bradykinin measurements, precluding the comparison between these repeated measures. Therefore, statistical analyses were only applied between the microdialysis measurements and the respective baseline values.

RESULTS

Maximal voluntary force. No significant differences were observed between sides and sessions, either in the 10% MVC forces to be applied or in the duration of contraction (endurance), when the sustained shoulder abduction was performed during the experimental protocol (P > 0.05, 2-sided Wilcoxon signed-rank test; Table 1). The MVC forces showed a high repeatability (Spearman’s ρ ranging between 0.75 and 0.95; see Table 3). The intersession correlation for the duration of contraction was lower (Spearman’s ρ = 0.536; P = 0.073).

Kinin peptides. During the first 3 h after probe insertion, bradykinin and kallidin levels in the dialysate remained stable,
The shoulder abduction induced a significant increase of kallidin levels, either during or immediately after treatment (Fig. 2). Pain and hemodynamic variables. No significant changes were observed in heart rate during enalaprilat infusion, but MAP fell (P ≤ 0.05 vs. baseline, 2-sided Wilcoxon signed-rank test) during the second session (Fig. 3). Enalaprilat did not induce any significant change in maximal pain ratings during the perfusion (Fig. 3). However, higher pain ratings were observed during the sample after enalaprilat infusion, but this effect was only statistically significant on the first experimental session (P ≤ 0.05 vs. baseline, 2-sided Wilcoxon signed-rank test).

The shoulder abduction induced a significant increase in heart rate and MAP (P ≤ 0.05 vs. baseline, 2-sided Wilcoxon signed-rank test). After the contraction, MAP returned to baseline levels, whereas heart rate fell significantly below the baseline value (P ≤ 0.05, 2-side Wilcoxon signed-rank test).

Pain significantly increased during shoulder abduction, as can be observed by larger maximal pain scores (P ≤ 0.05 vs. baseline, 2-sided Wilcoxon signed-rank test), which usually were observed close before exhaustion. After the contraction, pain decreased but remained above baseline levels (P ≤ 0.05, 2-sided Wilcoxon signed-rank test).

During contraction, consistent positive correlations were observed between the maximal pain and kinin peptides recovered by microdialysis (Table 4), both for the total amount in the sample (total fmol) and for the delivery rate (fmol/min). In addition, consistent positive correlations, which were higher and significant during the second session, were found between duration of contraction and the bradykinin delivery rate (fmol/min) during and after contraction (Table 5). No such correlations were observed for kallidin (Table 5). However, one significant correlation on the second session was observed for this peptide, which could have appeared by chance. No correlations, across sessions and/or sides, were found between the exerted force or hemodynamic variables and kinin peptide levels (data not shown). No other significant correlations were observed.

To clarify the relation between pain and kinin peptides, the subjects were divided by median split into two equal subgroups by the maximal pain rating during contraction for each session: high pain [50% of subjects with highest pain scores; values without any significant differences between samples (data not shown). There were no statistically significant differences in the levels of kinin peptides in the baseline sample between left and right side in any of the experimental sessions (P > 0.05, 2-sided Wilcoxon signed-rank test; Table 2). No significant differences were observed in the levels of kinin peptides during baseline sampling with respect to the later enalaprilat or control treatment. The repeatability (between sides and between sessions), tested on baseline levels, was high for bradykinin but quite low for kallidin (Table 3).

Table 1. 10% of MVC applied by side and duration of contraction for the two experimental sessions

<table>
<thead>
<tr>
<th></th>
<th>Right Side, N</th>
<th>Left side, N</th>
<th>Time, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Session</td>
<td>14</td>
<td>16.4±1.0</td>
<td>15.6±1.1</td>
</tr>
<tr>
<td>2nd Session</td>
<td>12</td>
<td>16.0±1.0</td>
<td>15.9±1.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n; no. of cases. MVC, maximal voluntary contraction; Time, duration of contraction.

Table 2. Kinin peptide levels in dialysate in the baseline sample by side and sessions

<table>
<thead>
<tr>
<th></th>
<th>1st Session</th>
<th>2nd Session</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right side</td>
<td>Left side</td>
</tr>
<tr>
<td>Bradykinin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(sample 8)</td>
<td>0.33±0.10</td>
<td>0.37±0.12</td>
</tr>
<tr>
<td>Kallidin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(sample 9)</td>
<td>0.36±0.08</td>
<td>0.27±0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE given in fmol/min.

Table 3. Interside (right vs. left) and intersession (session 1 vs. session 2) Spearman’s ρ correlations for MVC and baseline measurements of kinin peptides

<table>
<thead>
<tr>
<th></th>
<th>1st Session</th>
<th>2nd Session</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right side</td>
<td>Left side</td>
</tr>
<tr>
<td>MVC</td>
<td>0.903*(14)</td>
<td>0.950*(12)</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>0.600 (12)</td>
<td>0.833*(10)</td>
</tr>
<tr>
<td>Kallidin</td>
<td>−0.024 (8)</td>
<td>0.333 (9)</td>
</tr>
</tbody>
</table>

In parentheses are the no. of cases. *P ≤ 0.05.
_means ± SE): 35.1 ± 8.9 mm for session 1 and 42.5 ± 14.8 mm for session 2] and low pain [50% of subjects with lowest pain score; values (means ± SE): 4.1 ± 1.5 mm for session 1 and 1.9 ± 1.0 mm for session 2]. When analyzed under this criterion, the total sample levels of both bradykinin and kallidin were persistently higher in the high-pain subgroup (Fig. 4), being statistically significant for bradykinin in the enalaprilat-treated side on the first session (P < 0.05; 2-sided Mann-Whitney test). A similar pattern was observed for the delivery rate (fmol/min) but with small and not significant differences between both subgroups (data not shown). When looking at the changes in peptide levels during contraction, the increase in the kinin peptides was similar for both groups in the control side. However, the increase was larger in the enalaprilat-treated side for the high-pain group (Fig. 5), this difference being statistically significant for bradykinin in the first experimental session (P < 0.05; 2-sided Mann-Whitney test).

**DISCUSSION**

The present results show that both bradykinin and kallidin are liberated in human trapezius muscle during contraction. They also show a relation between the pain triggered by the prolonged sustained contraction and the amount of the kinin peptides released.

At rest, bradykinin represented ~50–60% of the total kinin peptides. This is in contrast with what was observed in rat muscle, where bradykinin represented only ~25% of the two kinin peptides (5). Another striking difference between the two species is the different response of the kinin peptides to the insertion of the microdialysis probes. In the rat, the concentration of the kinin peptides in dialysate markedly declined during 1 h after insertion of the microdialysis probes in the hindlimb adductor muscle. This effect was interpreted as an acute response of the kinin system to the probe insertion, with a transient increase in production of these peptides and a return to preinsertion levels. We did not observe such a reaction in the present experiment. However, in a microdialysis study of the calf muscle in humans, Langberg et al. (22) found a significant decrease in kinin peptides levels from the initial measurements. Furthermore, in a pilot study performed in our laboratory with five subjects with microdialysis probes in the tibialis muscle, we observed a marked drop in the levels of kallidin, but not bradykinin, during the first 20 min. Taking these results together, these discrepancies may be due to a possible different response of the kinin system to tissue injury in the trapezius muscle compared with the limb muscles and not to an interspecies difference.

**Table 4.** *Spearman’s p* correlations between maximal pain and bradykinin, kallidin, or total kinin peptides (bradykinin + kallidin) levels in dialysate during contraction

<table>
<thead>
<tr>
<th></th>
<th>1st Session</th>
<th>2nd Session</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Enalapril</td>
</tr>
<tr>
<td>Bradykinin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total fmol in sample</td>
<td>0.564 (10)</td>
<td>0.685*(12)</td>
</tr>
<tr>
<td>fmol/min</td>
<td>0.588 (10)</td>
<td>0.615*(12)</td>
</tr>
<tr>
<td>Kallidin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total fmol in sample</td>
<td>0.527 (10)</td>
<td>0.291 (13)</td>
</tr>
<tr>
<td>fmol/min</td>
<td>0.370 (10)</td>
<td>0.401 (13)</td>
</tr>
<tr>
<td>Total kinin peptides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total fmol in sample</td>
<td>0.418 (10)</td>
<td>0.692*(12)</td>
</tr>
<tr>
<td>fmol/min</td>
<td>0.515 (10)</td>
<td>0.510 (12)</td>
</tr>
</tbody>
</table>

In parentheses are the no. of cases. *P ≤ 0.05.
The protocol chosen for the present study was also designed to evaluate the repeatability of bradykinin and kallidin measurement by microdialysis in skeletal muscle. Repeatability between sides and sessions was high for bradykinin but quite low for kallidin. Because all of the samples from the same subject were analyzed simultaneously, the low Spearman's \( r \) correlations observed for kallidin could be the consequence of a high intrasubject variability, perhaps due to fine local regulation of kallidin levels for adjustment to the physiological condition in the muscle. Despite the low repeatability of kallidin, both peptides showed a consistent response to physiological manipulation, both between sides and between sessions.

Several studies have already shown that bradykinin is liberated during muscle contraction in humans (3, 22, 29, 32). In these studies, kinin peptides were analysed by RIA using an antibody directed against the COOH terminus of bradykinin, with a 100% cross-reactivity with kallidin. In the present work, we used specific antibodies against both the NH\(_2\) and the COOH terminus (5, 18), which allow the individual determination of these peptides. The present results do not only corroborate these previous studies, but they show a differential pattern in the liberation of kinin peptides on the control side: the increase in kallidin levels is nearly twice as large as for bradykinin during contraction, but thereafter kallidin levels return to baseline values, whereas bradykinin levels descend more slowly, remaining elevated over baseline values after contraction. Given that the kinin peptides are potent vasodilators, this different release pattern can be reflected in the response of local blood flow to contraction. In addition to the immediate increase during muscle activity, local blood flow remains elevated during recovery even after low-level contraction (27). Because bradykinin, but not kallidin, levels after shoulder abduction were related to the duration of contraction (Table 5), this peptide is a good candidate for the agent mediating this circulatory response during recovery from muscular activity. Thus, whereas kallidin could be responsible for the immediate, acute physiological kinin effects, bradykinin could be important for more long-lasting effects related to muscle recovery from fatigue.

The present results show not only a different release pattern of bradykinin and kallidin during contraction but also a distinct regulation of their extracellular levels. Inhibition of ACE with enalaprilat increased the levels of bradykinin but not of kallidin. A similar response has also been observed in the blood (12). To explain this difference between the two kinin peptides, a separate compartmentalization of each peptide has been

### Table 5. Spearman's \( r \) correlations between duration of contraction and bradykinin and kallidin delivery rate (fmol/min) during and after contraction

<table>
<thead>
<tr>
<th></th>
<th>1st Session</th>
<th>2nd Session</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Enalaprilat</td>
<td>Control Enalaprilat</td>
</tr>
<tr>
<td>Bradykinin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contraction</td>
<td>0.518 (11)</td>
<td>0.300 (11)</td>
</tr>
<tr>
<td>Postcontraction</td>
<td>0.214 (13)</td>
<td>0.297 (14)</td>
</tr>
<tr>
<td>Kallidin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contraction</td>
<td>0.018 (10)</td>
<td>0.345 (10)</td>
</tr>
<tr>
<td>Postcontraction</td>
<td>0.376 (13)</td>
<td>0.187 (14)</td>
</tr>
</tbody>
</table>

In parentheses are the no. of cases. \(*P < 0.05\).

**Fig. 4.** Total bradykinin and kallidin amount in the dialysate sample (fmol; means \( \pm \) SE) during the sustained isometric abduction in the first and second sessions of the experimental protocol. Subjects were divided into 2 groups by the values of maximal pain ratings during contraction, with one-half of them in each group (low and high pain). \(*P < 0.05\) vs. low-pain subgroup (2-sided Mann-Whitney \( U \)-test).

**Fig. 5.** Changes (means \( \pm \) SE) in bradykinin and kallidin levels in dialysate (expressed as percentage of the baseline sample) during the sustained isometric abduction on the first and second sessions of the experimental protocol. Subjects were divided into 2 groups by the values of maximal pain ratings during contraction, with one-half of them in each group (low and high pain). \(*P < 0.05\) vs. low-pain subgroup (2-sided Mann-Whitney \( U \)-test).

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proposed (12). ACE-mediated bradykinin metabolism has been detected in rabbit muscle (31), but has not been observed in human myocytes, indicating that the ACE activity could be constrained to the muscle vascular endothelium (30). Considering the differential effect of ACE inhibition observed in the present study, we may presume that, whereas muscle bradykinin is confined to the muscle vascular system, and therefore accessible to ACE, kallidin may preferentially be present in the nonvascular compartment of the muscle.

During contraction, the increase in both bradykinin and kallidin levels tended to be larger in the enalaprilat-perfused side, accompanied by an increase in the sample variance, indicating that this tendency is due mostly to larger increases in some subjects. It is worth noting that, during shoulder abduction, the subjects showing the larger increases in the enalaprilat-perfused side for bradykinin also tended to show the larger increases in kallidin levels, something that does not happen in the control side (data not shown). Thus it appears that, during contraction, enalaprilat amplified the concentration of both kinin peptides in some subjects. Besides, enalaprilat prolonged the rise in kallidin levels over the end of the sustained abduction in the first session. These data point out that enalaprilat was able to block kallidin metabolism during contraction, at least in some subjects. This would imply a distinct regulation of local kallidin metabolism in the muscle during rest and activity; at rest, neutral endopeptidase or carboxypeptidase could be the main metabolic enzymes; during contraction, ACE would be activated to cope with the excess of kallidin. It has been shown that different enzymes can be activated to cope with kinin degradation when other enzymes are impaired (4, 11). On the other hand, it is also possible that, during contraction, the excess of kallidin can cross this apparent compartmentalization and be exposed to ACE action.

The different effect of enalaprilat and contraction on the kinin peptides also indicates that the present results are not the consequence of changes in probe recovery and/or in blood flow. If the increases in dialysate concentration of the peptides were due to an increase in probe recovery, a similar quantitative and time-response effect would be seen in both peptides, because they have a similar molecular mass. On the other side, an increase in local blood flow would be expected as response to contraction (27) and ACE inhibition (26). However, a pharmacologically induced increase in local blood flow reduced the dialysate concentration of kinin peptides in rats (5), probably due to a higher clearance from the tissue. Therefore, it can be assumed that the increase in total production of both kinin peptides may be underestimated in the current study. No attempt was made to calculate “true” interstitial concentrations by estimating in vivo probe recovery using the internal reference technique. The addition of radiolabeled bradykinin to the perfusion medium as internal reference can interfere with the RIA (22). Furthermore, when using radiolabeled compounds as reference, the radioactivity measured at the dialysis outlet would be affected both by the radiolabeled compound and by radiolabeled metabolites generated in the extracellular space. Therefore, the calculation of the recovery can be distorted, especially for a peptide with a relatively rapid metabolism and short half-life such as bradykinin. Probe recovery is dependent on delivery to and clearance from the interstitial space for each substance (7). Thus the use of another substance as reference requires that this substance behave similarly to the analyte with respect to these physiological processes, whose kinetics are mostly unknown. If they behave differently, the probe recovery obtained with the reference substance cannot be applied to the analyte. This can be even more problematic when studying changes after a pharmacological challenge affecting one of these processes, like inhibition of degradation, as is the case in the present study.

Muscle contraction induced an increase in MAP, heart rate, and subjective pain, which were not related to the exerted force or to endurance (duration of contraction). An association between the systemic hemodynamic variables and the levels of kinin peptides was not observed. However, enalaprilat infusion induced a fall in arterial blood pressure during the second session, probably reflecting its well-known hypotensive effect. This vascular action of enalaprilat is most probably mediated by the kinin peptides (13, 17).

A consistent positive relationship between the perceived pain during the sustained contraction and the levels of kinin peptides emerged. Intramuscular administration of bradykinin can change the receptive fields of dorsal horn neurons to noxious stimuli (19) and induce pain when administered in combination with serotonin (1, 20, 23). Furthermore, ischemia, which is well known to induce muscular pain, induces the liberation of bradykinin (28). The kallikrein-kinin system has been proposed as an important factor in the appearance and development of muscular pain (5, 21), and the present study is the first to show a relation between the intramuscular levels of kinin peptides during muscle activity and the perception of pain.

The correlations observed between kinin peptide levels and pain tended to be higher in the side treated with the ACE inhibitor enalaprilat. Besides, the subjects with the higher maximal pain ratings during contraction tended to show larger increases of peptides in the side previously perfused with enalaprilat. The lack of statistical significance is probably due to the small number of cases for each subgroup. Therefore, ACE activity could be a key factor in the determination of kinin peptide levels during contraction and, consequently, in the individual susceptibility for the appearance of muscular pain. In this context, the particular effect of ACE on kallidin levels can be of paramount importance because, unlike bradykinin, kallidin is also able to act on the bradykinin B1 receptors (16). The B1 receptor is not normally expressed in tissue, but it can be induced by the presence of agonists already after 0.5 h of incubation (25) and has been implicated in the mediation of inflammatory chronic pain states (10). In addition, a lower ACE activity could facilitate the activation of alternative, secondary metabolic pathways, like carboxypeptidase N, which liberates kinin metabolites that, like kallidin, are also active at the B1 receptor (8).

The increase in pain ratings during the sample immediately after enalaprilat perfusion would also support the probable relationship between kinin peptides in muscle and the appearance of muscular pain. However, no correlations were found between kinin levels and pain ratings during the postenalaprilat period. This increase in pain can have been a result of a direct interaction between enalaprilat and the bradykinin B2 receptor. ACE inhibitors not only increase bradykinin activity by their blockade of kinin metabolism but also enhance the receptor potency of kinin peptides by a direct interaction between the ACE inhibitor and the B2 receptor (15). The fact that pain
arises after, and not during, enalaprilat perfusion strengthens this possibility. Enalaprilat has a half-life of ~30 min. It is possible that, at the end of perfusion, enough ACE inhibitor has locally accumulated to achieve a potentiation of B₂ receptor activity sufficient to activate nociceptors.

In conclusion, trapezius muscle activity is able to activate both the plasma and tissue kallikrein-kinin system and, as a result, liberate bradykinin and kallidin, with a different time response, probably due to the different metabolic regulation of these peptides by ACE. The intramuscular levels of these peptides appear to contribute to the intensity of perceived pain during sustained contraction. ACE activity, due to its control of the intramuscular kinin levels during muscular activity, can be a significant factor in the development of muscular pain.

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GRANTS

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