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Release of algesic substances in human experimental muscle pain

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Abstract. *Objective:* We employed the 'delayed onset of muscle soreness' (DOMS) and the 'hypertonic saline' muscle pain models in combination with muscle microdialysis to evaluate the role of potentially algesic substances (lactate, glutamate, prostaglandin E_2 (PGE_2), nitric oxide (NO) and substance P (SP)) in the development of human muscle pain. *Methods:* DOMS was induced by 2 sets of 50 concentric/eccentric contractions of the calf muscles 24 h before the start of microdialysis. During microdialysis pain was stimulated through calf muscle contractions (dorsal and plantar flexions of the foot). Hypertonic saline was injected into the biceps muscle (5 × 200 µl 5.8% NaCl, 2 min interval) during dialysis. The calf (no treatment) and biceps (normal saline) of the other side was used as control.
Results: Both models reliably induced muscle pain with similar intensities as assessed by visual analog scale. The DOMS exercise caused an increase of lactate in serum and the calf muscles of the DOMS leg. In addition, glutamate, PGE_2 and substance P dialysate concentrations increased following contraction-induced pain stimulation (peak concentrations 125 ± 20 µM, 239 ± 45 pg/ml and 60 ± 11 pg/ml for glutamate, PGE_2 and SP, respectively). This increase did not occur in the control leg (peak concentrations 97 ± 12 µM, 114 ± 26 pg/ml and 46 ± 9 pg/ml for glutamate, PGE_2 and SP, respectively). Concentrations of nitric oxide were lower in the DOMS than control leg, particularly during the first 4h of microdialysis. Injection of hypertonic saline into the biceps muscle caused a significant increase of dialysate glutamate concentrations (peak 50 ± 3 µM) whereas glutamate remained constant after injection of normal saline (mean 26 ± 1 µM). Injection of hypertonic saline had no effect on lactate, PGE_2 or NO levels.
Conclusion: Our data support the notion that an inflammatory reaction may be involved in muscle soreness following eccentric exercise, whereas the injection of hypertonic saline into the muscle probably directly stimulates muscle nociceptors and causes glutamate release.

Key words: Muscle pain – Glutamate – Prostaglandin E_2 – Substance P – Microdialysis

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Introduction

Musculoskeletal pain is one of the most frequent symptoms for which medical assistance is sought [1]. It is a major contributor to widespread clinical pain syndromes such as low back pain, tension type headache and neck and shoulder pain. However, the majority of our knowledge regarding pain pathophysiology is based on studies in cutaneous tissue. Relatively little is known about the activation of muscle nociceptors possibly because of the complexity of inputs which can influence muscle sensation including muscle spindles, tendon-, joint- and skin receptors as well as central neurons involved in the processing of this input. Different types of muscle 'damage' such as overuse, muscle spasms, ischemia, trauma or inflammation may result in nociceptor activation and sensitization. The latter is defined by a lowering of the mechanical threshold into the innocuous range and may explain aspects of muscle tenderness and hyperalgesia [2, 3]. This process may also cause hyperexcitability of dorsal horn neurons, prolonged neuronal discharge, increased responses to noxious stimuli and expansion of receptive fields [4–6]. The peripheral component of mechanical muscle hyperalgesia is probably due to nociceptor sensitization by endogenous mediators [7]. Many substances have been suggested to be involved such as lactate, K^+ and H^+ ions [8, 9], glutamate [10], inflammatory mediators such as prostaglandins [11, 12], nitric oxide [13], histamine [14], serotonin [15], and cytokines [16, 17]. Furthermore neuropeptides such as substance P [18], bradykinin [7, 15], calcitonin gene related peptide [19] and nerve growth factor (NGF) [20] may also play important roles. However, the relative importance of the various substances is largely unknown. The identity and action of potential nociceptive substances is an important clinical issue because some may be specifically influenced by pharmacological agents.

The paucity of information concerning human muscle pain is caused in part by the paucity of well characterized experimental human muscle pain models. Two models have been repeatedly used: (i) the DOMS model (delayed onset of muscle soreness) which has been employed mainly in sports sciences and (ii) the injection of hypertonic saline into the muscle.

In the DOMS model, muscle pain is induced by exercise incorporating eccentric muscle contractions. Peak pain occurs 24–48 h post-exercise [21]. During this time, muscle fibers are swollen [16, 22], intramuscular fluid pressure is elevated [23] and muscle force is reduced [22, 24]. Although the exact mechanisms of DOMS are unclear [25, 26], it has been suggested to be associated with a myofibrillar disruption predominantly at the Z-lines [27–29] and subsequent inflammation [30–32].

With the hypertonic saline model, muscle pain can be induced in a specific muscle and the quality of the saline-induced muscle pain has been shown to mimic clinical muscle pain [10, 11, 33–38]. The mechanisms, which initiate the saline-induced pain, however, are not fully understood. A direct activation of nociceptors and the release of neuropeptides and other mediators has been suggested.

The aim of the present study was to evaluate further algesic substances that may be involved in human muscle pain. We therefore employed the DOMS model and the hypertonic saline model in combination with microdialysis to assess muscle concentrations of lactate, glutamate, prostaglandin E_2, nitric oxide and substance P.

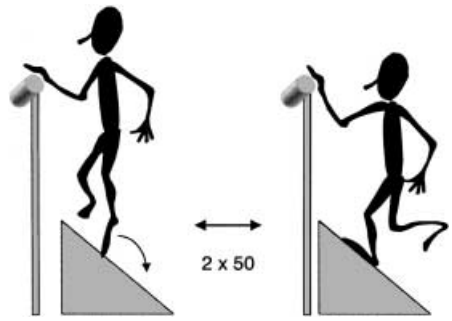

Fig. 1. Illustration of the exercise protocol causing delayed onset of muscle soreness. The subjects had to stand with one foot on a 45° sloped step looking to the upper side. They lifted on the tip of the toes (concentric contraction) and then slowly lowered the heel until the sole of the foot completely stood on the sloped surface of the step (eccentric contraction). Then they lifted on the tip of the toes again and repeated the contractions. The exercise consisted of 2 sets of 50 such concentric/eccentric contractions. The sets were separated by a 5 min rest. Before and after the exercise (directly after and at 24 h) pain intensity in the DOMS and control leg was assessed at rest, walking, going downstairs and tiptoeing.

Materials and methods

Subjects

10 healthy, non-obese subjects (9 males, 1 female; mean age 25.8 years, range 23–29) who were not taking any medication were enrolled in the study. None of the subjects was taking part in any regular training program in the last 6 month before the study. All subjects gave written informed consent to the study which was approved by the Ethics Committee of the medical faculty of the University of Frankfurt.

Muscle pain

The exercise protocol causing delayed onset of muscle soreness (DOMS) consisted of 2 sets of 50 concentric/eccentric contractions of the calf muscles as illustrated in Fig. 1. The sets were separated by a 5 min rest. The control leg did not perform any exercise. 24 h after the exercise microdialysis was started (time schedule Fig. 2). During microdialysis, pain was stimulated twice. Each stimulation consisted of 2 sets of 10 dorsal and plantar flexions of the foot with a rest of 10 min between both sets.

With hypertonic saline, muscle pain was induced by 5 sequential injections of 200 µl sterile hypertonic (5.8%) saline into the biceps muscle with an interval of 2 min. The saline solution was injected through a 27G intravenous catheter, which was inserted into the biceps muscle 2 h before the first injection. The distance between the tip of this catheter and the microdialysis membrane was as short as possible (0.5–1 cm). Hence, it was a compromise between the need to keep diffusion distances short and the risk to damage the microdialysis membrane. Normal saline (0.9%) was used for the control arm.

Treatments (DOMS vs control leg and hypertonic vs normal saline) were randomly allocated to the right and left side.

Assessment of muscle pain intensity

Pain intensity was assessed using a visual analog scale (VAS, extending from 'no pain' to 'intolerable pain'). The subjects were asked every 15 sec to estimate their current pain intensity by moving the curser of an electronically displayed VAS (length 10 cm, precision 1 mm). The curser was moved back to zero after each rating. Data were recorded using a self-designed data acquisition program. For statistical comparisons the area under the VAS versus time curves were calculated employing the linear trapezoidal rule.

Microdialysis

The technique is based on diffusion of small molecules through a semipermeable membrane which is located at the tip of a microdialysis catheter. The catheter is constantly perfused so that molecules entering the catheter through the membrane are transported outwards through the outlet tube and can be analyzed in the dialysate. Dialysate concentrations are proportional to tissue concentrations.

Twentyfour hours after the DOMS exercise microdialysis catheters (CMA 70 brain microdialysis catheter, membrane length 30 mm, membrane diameter 0.6 mm, molecular weight cut off 20kDa, CMA, Stockholm, Sweden) were inserted into the right and left biceps muscle and into the right and left medial gastrocnemius muscle as has been described previously [39]. The microdialysis catheters were perfused with Ringer solution at a flow rate of 2 µl/min using a microdialysis pump (CMA 102). Dialysates were collected in 20 min intervals. The flow rate of 2 µl/min was a compromise between the aim to get a high recovery and a high temporal resolution which requires short sampling intervals. According to literature data or information provided by the manufacturer the recovery of the analytes at this flow rate is about 50% for glutamate and PGE_2 [40–42], about 70% for lactate and 30% for substance P [43]. Dialysates were kept at –80 °C until analysis.

Study protocol

On the first day, the DOMS exercise was performed as described above. Before the exercise baseline values (CK, lactate, calf circumference, VAS) were recorded (time schedule Fig. 2). These parameters were measured again directly after the exercise and at 24 h after the DOMS exercise before starting microdialysis. The start of microdialysis is referred to as time '0'. Baseline dialysate samples were collected for 80 min (4 × 20 min). Muscle pain in the calves was then stimulated by contractions of the calf muscles as described above. For each leg, a 20 min dialysate sample was collected during this pain stimulation. Subse-

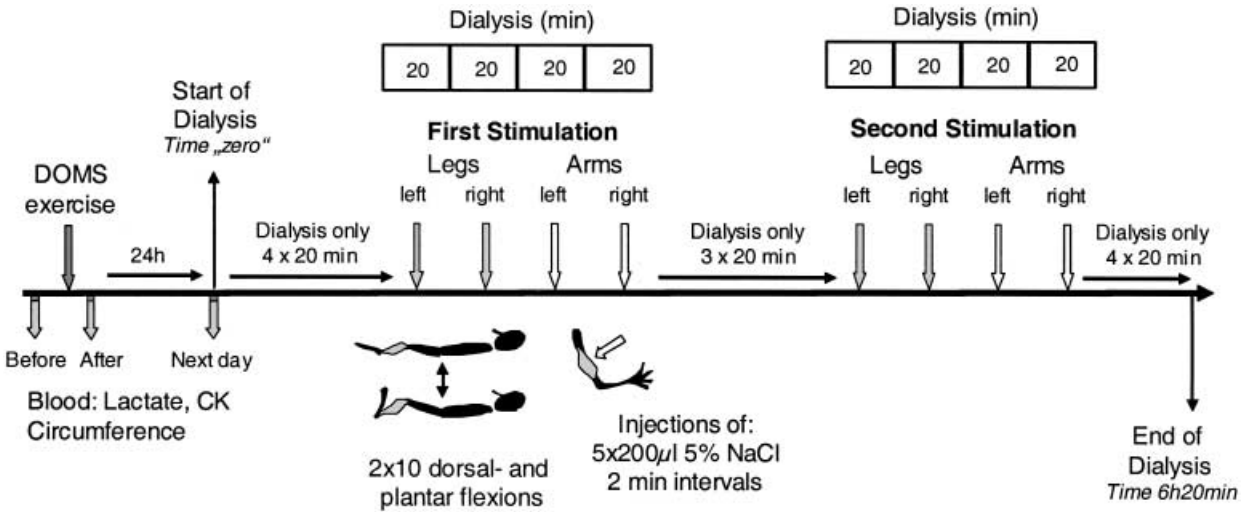

Fig. 2. Time schedule of microdialysis and pain stimulations. Treatments (DOMS, hypertonic saline) were randomly allocated to the right or left side. The pain stimulation during dialysis was always started on the left side.

quently, muscle pain in the biceps muscles was induced by injection of hypertonic or normal saline. For each arm, a 20 min dialysate sample was collected. The stimulation period was followed by a rest of 1 h (3 × 20 min dialysate sampling). After that, the stimulation was repeated which was again followed by a 'microdialysis-only' period (4 × 20 min). After removal of the catheters, the circumferences of the calves and upper arms were measured again.

Analysis

The dialysate concentrations of glutamate and lactate were measured with a CMA 600 Microdialysis Analyser (CMA, Stockholm, Sweden) according to the manufacturer's instructions. NO levels were assessed by measuring concentrations of its oxidation products, nitrite and nitrate, with commercially available kits (Biotrend, Germany). PGE_2 and substance P concentrations were determined with enzyme immuno assay kits (Biotrend and IBL-Hamburg, Germany, respectively). The reliable limit of quantification was 36 pg/ml, 7.8 pg/ml and 0.5 µmol/l for PGE_2, SP and NO_2^-/NO_3^-, respectively. The mean percentage deviation over the calibration range of 36–5000 pg/ml for PGE_2, 7.8–1000 pg/ml for SP and 0.5–100 µM for NO_2^-/NO_3^- was less than 18%. Because of the limited volume of the dialysate samples PGE_2 and NO were determined in only half of the subjects, i.e. PGE2 was measured in the dialysates of subjects 1–5 and NO in those of the subjects 6–10. For analysis of substance P two subsequent dialysate samples were pooled. SP was assessed only in muscle dialysates of the legs.

Serum creatine kinase (CK) activity and lactate concentrations were determined according to standard methods. The normal range for CK and lactate was 10–80 U/l and 5.7–22 mg/dl, respectively.

Data analysis

Data are presented as mean ± SEM. Mean dialysate concentrations were plotted versus time. The midpoints of the collection intervals were used as time points. They were corrected for the time needed to pass the dead space of the outlet tubing (6 min). For graphical presentation, dialysate concentration time courses were synchronized so that the respective stimulation periods occur at the same time in all subjects. For statistical comparisons, SPSS 9.0.1 for Windows was used. 'Before' and 'after' values (serum lactate, serum creatine kinase activity, calf and upper arm circumferences) were compared using t-tests for paired observations (2 consecutive observations) or analysis of variance (ANOVA) for repeated measurements (>2 consecutive observations). In case of a significant result of the ANOVA, groups were pairwise compared using t-tests with a Bonferroni alpha-correction for multiple comparisons. VAS-AUCs were submitted to ANOVA for repeated observations where the within and between subject factors were 'time' (i.e. first or second stimulation) and 'treatment' (i.e. DOMS vs. control and hypertonic saline vs. normal saline), respectively. Correlation between two parameters was assessed using linear regression analysis. Lactate and NO AUCs of the legs and arms (DOMS vs. control and hypertonic saline vs. normal saline) were compared using t-tests for independent observations. To assess whether the observed increase of glutamate, PGE_2 and SP was statistically significant baseline values (before stimulation) and peak values during or within 40 min after stimulation were submitted to ANOVA for repeated observations with the within subject factor 'stimulation' (i.e. baseline vs pain stimulation) and the between subject factor 'treatment' (i.e. DOMS vs. control and hypertonic saline vs. normal saline). α was set at 0.05.

Results

Serum lactate and creatine kinase activity

S-CK activity was significantly increased 24 h after the DOMS exercise (59.9 ± 7.7 U/l) as compared to the baseline value (45.1 ± 3.5 U/l; $p = 0.036$). Serum lactate concentrations were also significantly elevated directly after the DOMS exercise (32.5 ± 3.3) as compared to baseline (18.4 ± 2.2; $p = 0.016$). At 24 h, serum lactate had returned to the pre-exercise value (19.2 ± 1.3). There was no significant correlation between the percentage increase of CK activity and the percentage increase of serum lactate concentrations ($p = 0.543$).

Muscle swelling

The calf of the DOMS leg was significantly swollen directly after the DOMS exercise and at 24 h as compared to baseline ($p = 0.031$ and 0.006, respectively). The calf girth was

increased by 0.6 ± 0.2 cm directly after the exercise and by 0.5 ± 0.1 cm at 24 h. At the end of the dialysis period, it had returned to the pre-exercise value. There was no change of the calf circumference with the control leg. The upper arm circumference was not affected by hypertonic or normal saline.

Muscle pain – legs

24 h after the DOMS exercise all subjects reported calf muscle soreness of the DOMS but not the control leg. The VAS scores for the pain intensities at rest, walking, going downstairs and tiptoeing of the exercised and control leg are shown in Fig. 3. Except for the pain intensity at rest, the VAS scores of the exercised leg were significantly higher at 24 h as compared to those recorded directly after the exercise ($p = 0.005$, 0.003 and <0.001 for walking, going downstairs and tiptoeing). There was no change with the control leg.

The VAS scores following the pain stimulation during dialysis are shown in Fig. 4 (top). During both stimulations, the subjects reported considerable more pain in the DOMS leg than in the control leg. ANOVA comparing VAS-AUCs revealed significant differences between the DOMS and control leg ($F\{1; 18\} = 9.8$, $p = 0.006$). Interestingly, VAS scores following the second pain stimulation were significantly lower than those after the first stimulation ($F\{1; 18\} = 13.3$, $p = 0.002$). This decline was noted for the exercised as well as the control leg. However, the difference was only statistically significant for the DOMS leg ($p = 0.021$ and $p = 1$ for DOMS and control leg, respectively).

Muscle pain – arms

The VAS scores following injection of hypertonic and normal saline into the biceps muscles are shown in Fig. 4 (bottom). The injection of normal saline into the muscle caused no pain. The injection of hypertonic saline, however, reliably induced pain in all subjects with a maximum VAS score of 66 ± 7.1% and 62 ± 8.7% during the first and second series of injections, respectively. ANOVA revealed statistically significant differences between the VAS-AUCs of the hypertonic and the normal saline arm ($F\{1; 18\} = 20.95$, $p < 0.001$). In contrast to the DOMS protocol there was no statistically significant difference between the first and second pain stimulation ($F\{1; 18\} = 1.06$, $p = 0.316$). However, during the second series of injections VAS scores recorded after the 3rd, 4th and 5th injection gradually decreased.

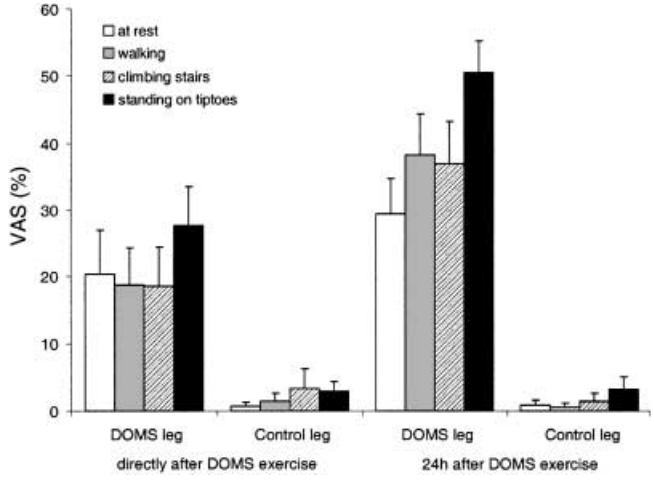

Fig. 3. Pain intensity (at rest, walking, going downstairs and tiptoeing) as assessed by visual analog scale (VAS; mean ± SEM) of the DOMS and control leg directly after the DOMS-inducing exercise and 24 h thereafter.

Muscle lactate

Dialysate concentrations of lactate were elevated in the DOMS leg as compared to control leg ($p = 0.44$; Fig. 5 left). In both legs lactate levels slightly increased during dialysis. This was also noted in the biceps muscles. However, there was no difference between the hypertonic saline and control arm (Fig. 5 right).

Glutamate

In the calf muscles, glutamate dialysate concentrations were considerably elevated during the first hour after insertion of the microdialysis catheters. They normalized after about 1 h (Fig. 5 left). The first glutamate value of the DOMS leg was higher than that of the control leg. However, this difference was not statistically significant. The first pain stimulation in the calf muscles caused a significant re-raise of glutamate dialysate concentrations in the DOMS leg, but not in the control leg ($F = 10.208$; $p = 0.005$ for the within subject factor 'stimulation'; $F = 4.553$; $p = 0.048$ for 'stimulation' * 'treatment'). The second pain stimulation caused only a slight glutamate increase in the DOMS leg.

Glutamate concentrations in the biceps muscles were lower than those in the calf muscles (Fig. 5 right). As in the calves they were elevated following the insertion of the microdialysis catheters. After normalization, the injection of hypertonic saline caused a significant increase of glutamate levels ($p = 0.003$). Glutamate levels in the hypertonic saline arm remained above those of the control arm up to the end of the dialysis period.

Prostaglandin E_2

The dialysate concentration time courses of PGE_2 and NO are shown in Fig. 6. The injection of hypertonic or normal saline did not affect PGE_2 or NO concentrations (Fig. 6 right). At the start of microdialysis, there was no difference in PGE_2 levels between DOMS and control leg (Fig. 6 left). Following pain stimulation however, PGE_2 levels increased in the DOMS but not the control leg. The PGE_2 raise in the DOMS leg was statistically significant during the second stimulation ($F = 6.175$, $p = 0.038$ for the within subject factor 'stimulation', $F = 12.099$, $p = 0.008$ for 'stimulation' * 'treatment').

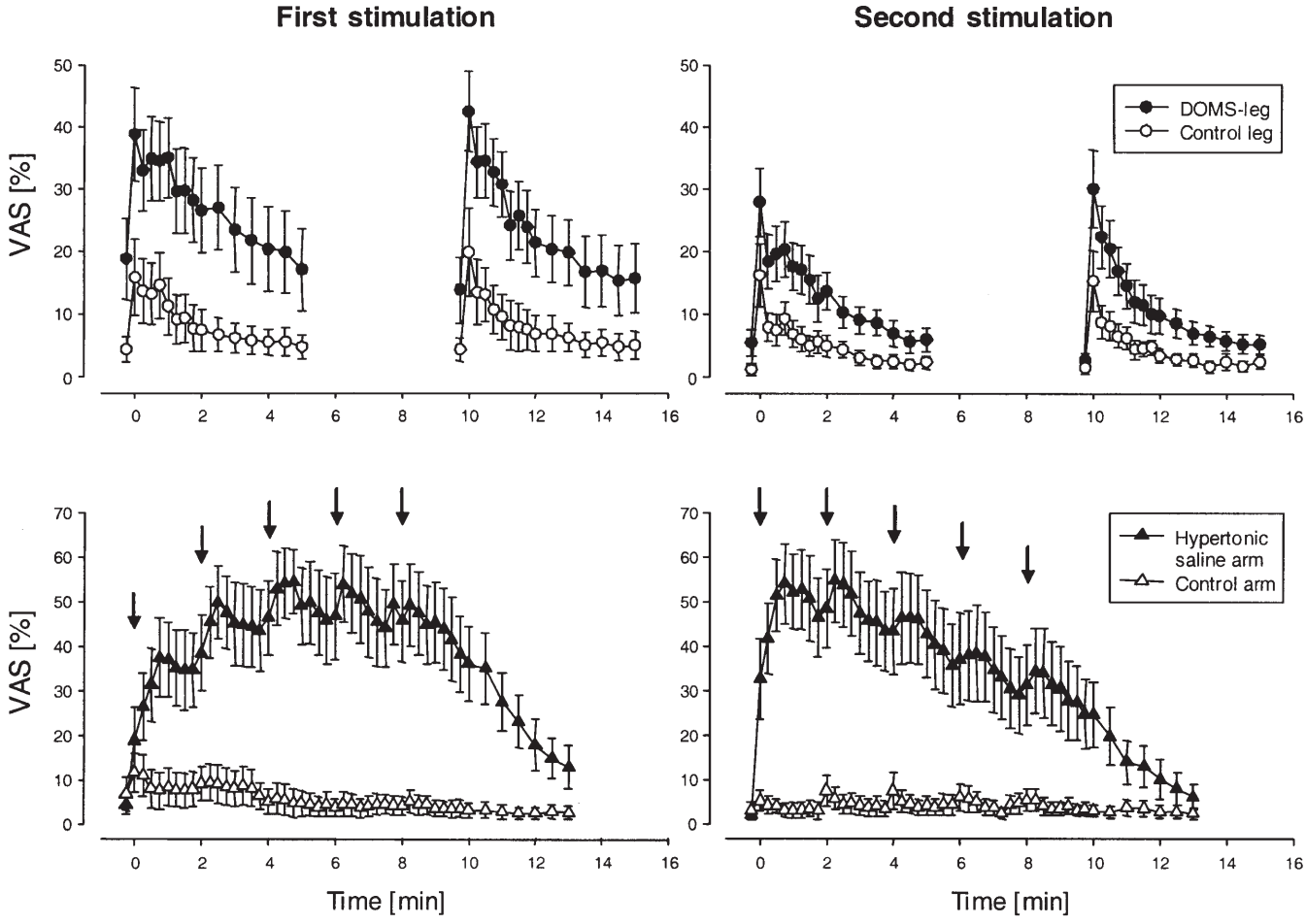

Fig. 4. Pain intensity as assessed by visual analog scale (VAS; mean ± SEM) of the DOMS and control leg (top) and the hypertonic and normal saline arm (bottom) during the first and second stimulation (for the start times in relation to microdialysis please see Fig. 2). The VAS scale was 10 cm. Pain in the DOMS and control leg was stimulated by 2 × 10 dorsal and plantar flexions of the respective foot with an interval of 10 min. The start of the stimulation is referred to as time *„zero"* for each leg. Pain in the biceps muscles was induced by 5 sequential injections of 5.8% hypertonic saline into the biceps muscle. The arrows indicate the injection times. Normal (0.9%) saline was used for the control arm. The start of the respective first 200 µl injection is referred to as time *„zero"* for each arm.

Nitric oxide

NO concentrations in the DOMS leg were considerably lower than in the control leg predominantly in the first 4 h of the dialysis. The difference between the DOMS and control leg was statistically significant as assessed by comparing NO-AUCs ($p = 0.02$).

Substance P

At the start of microdialysis, there was no difference in substance P concentrations between DOMS and control leg (Fig. 7). However, in the DOMS leg SP levels dropped following the first pain stimulation whereas they remained constant in the control leg. This drop was followed by a significant increase of SP during the second stimulation in the DOMS leg ($F = 29.023$, $p < 0.001$ for 'stimulation' and $F = 14.998$, $p = 0.002$ for 'stimulation' * 'treatment'). No increase occurred in the control leg.

Discussion

In the present study, the microdialysis technique was employed during experimentally induced human muscle pain to assess alterations of potentially algesic substances, which might contribute to the excitation and sensitization of muscle nociceptors. Two different human muscle pain models – the DOMS and the hypertonic saline model – were compared. In the DOMS model, muscle pain is induced by intensive and unaccustomed muscle use. Therefore, it may be regarded as a 'physiologic' muscle pain model. In contrast, the injection of hypertonic saline into the muscle causes high extracellular sodium concentrations [33] which do not normally occur in muscle tissue. With both models, spontaneous muscle pain was induced reliably in all subjects. In case of the DOMS model, muscle pain occurred 24 h after the DOMS inducing exercise. Microdialysis was performed during this painful state. Since there was only minor pain in resting muscles, slight muscle contractions were used to stimulate pain during dialysis. These contractions also caused some minor soreness

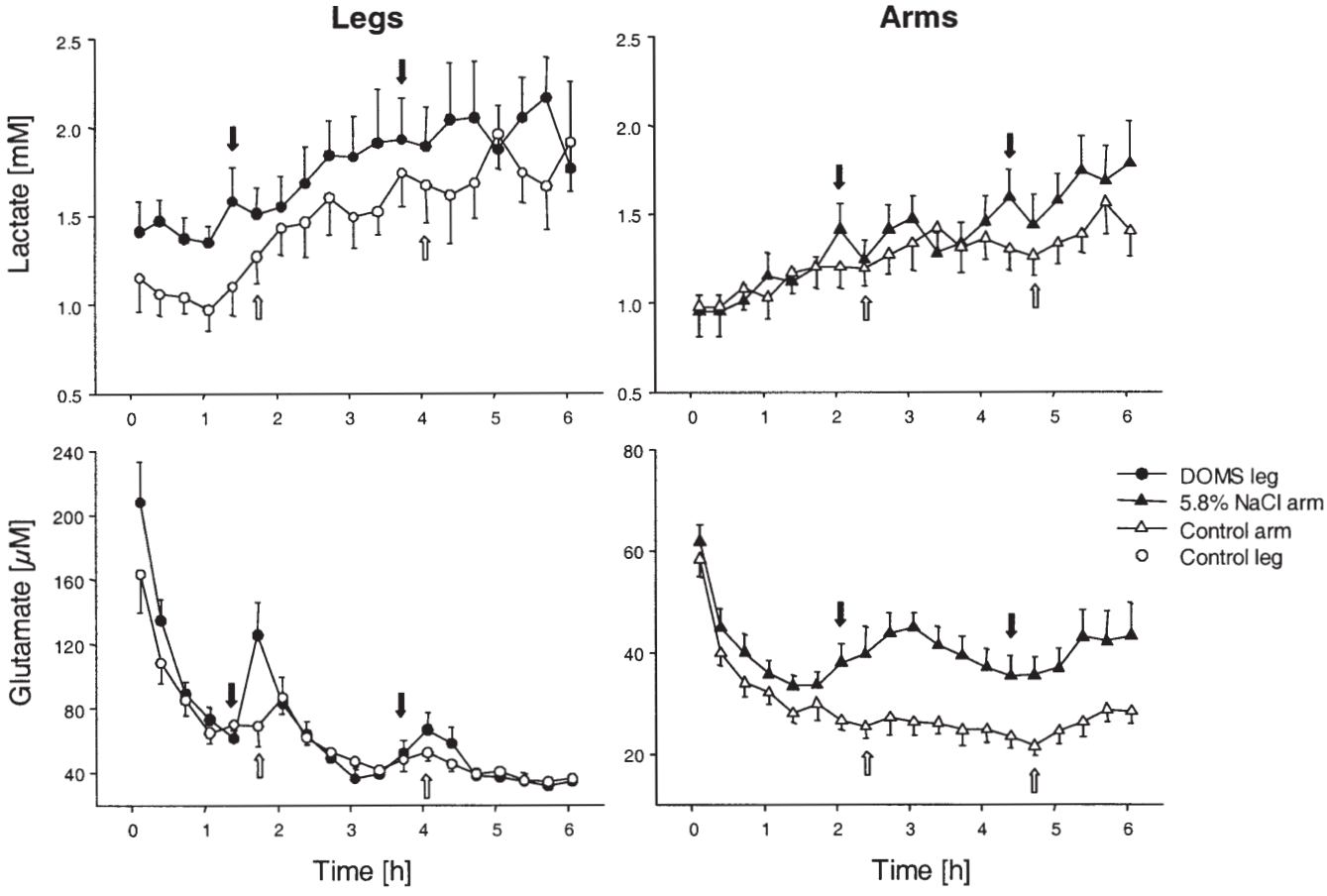

Fig. 5. Dialysate concentrations (mean ± SEM) of lactate (top) and glutamate (bottom) in calf (left) and biceps (right) muscles. The black arrows indicate the dialysates that were obtained during the pain stimulation of the DOMS leg and hypertonic saline arm. The white arrows indicate the dialysates obtained during the stimulation period of the control leg and control arm. (DOMS leg ●, control leg ○, hypertonic saline arm ▲ and control arm △).

in the control calf muscles, probably due to irritation caused by the intramuscular dialysis membrane. Interestingly, the stimulated increase in pain intensity was significantly more pronounced during the first than the second pain stimulation.

Following the injections of hypertonic saline into the biceps muscle there was also a decline in pain intensity during the second series of injections suggesting that some sort of 'adaptation' occurs in both muscle pain models. In animal studies, recordings from muscle afferents have shown that the firing rate after a second infusion of hypertonic saline was decreased as compared to the first one [44]. Although the mechanism for this suppression is not completely understood it has been suggested to be due to an increase in interstitial K^+ levels [11] which may result in an elevation of the resting potential of the nociceptor [45] and axon membrane [46]. A moderate increase in interstitial K^+ concentration was also observed during exercise in human muscles where the extent depended on the exercise intensity (up to 7 mmol/l) [47]. Although K^+ levels rapidly (15 min) returned to baseline (3–4 mmol/l) in that study [47] it is conceivable that a moderate increase of extracellular K^+ contributed to the rapid 'habituation' observed in the DOMS leg. In contrast, very high concentrations of extracellular K^+ (about 60 mM) enhance the excitability of muscle nociceptors [14] and injections of potassium chloride (100 mM) into the muscle cause muscle pain in humans [35]. However, such high levels are not achieved under physiological conditions and it has been demonstrated that the increase in extracellular K^+ is not responsible for muscle pain during or after exercise [48].

In addition to the rapid reduction of stimulated muscle pain with the DOMS model, it is well known that skeletal muscles adapt to eccentric exercise when it is repeated [21]. Hence, the muscle soreness, the increase in creatine kinase activity and muscle swelling in 'trained' subjects is considerably less intense [49]. This training effect has been suggested to be associated with an adaptive process in connective tissue proteins such as talin and vinculin [49]. Recently, a mechanical stimulation of muscle cells was shown to cause an increase of the expression of these two proteins. This depended on the activation of the nitric oxide/cGMP/protein kinase G pathway [50]. In addition, NO has been shown to regulate muscle contraction and metabolism. In particular, recent human data indicate that NO increases muscle glucose uptake during exercise [51–53] and modulates muscle contractility [54]. Exercise training in healthy individuals elevates NO bioavailability through a variety of mechanisms including increased NOS enzyme expression and activity [55]. It is conceivable that changes of the NO system induced

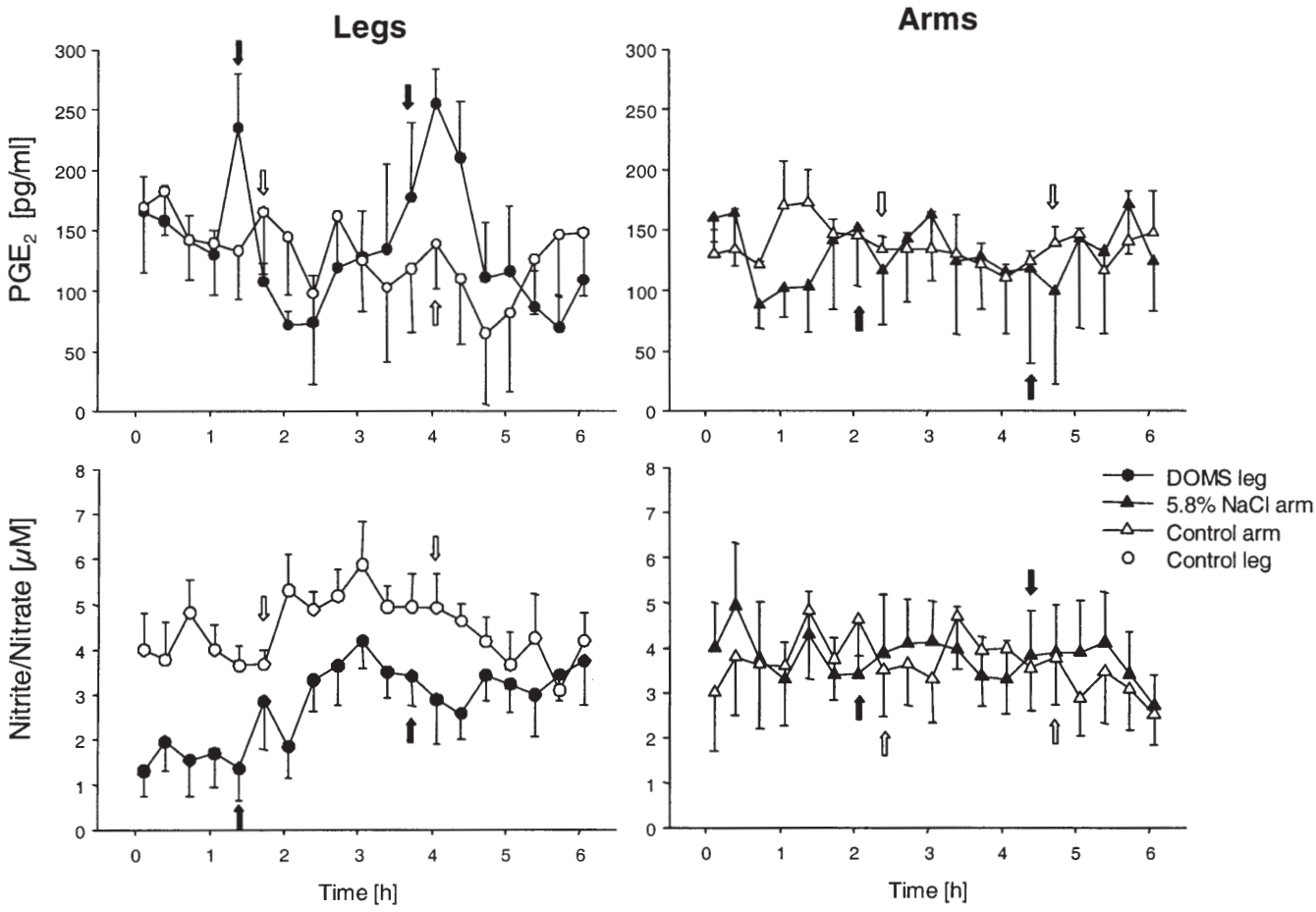

Fig. 6. Dialysate concentrations (mean ± SEM) of prostaglandin E_2 (PGE_2) and nitric oxide (NO) in the calf (left) and biceps muscles (right). The black arrows indicate the dialysates that were obtained during the pain stimulation of the DOMS leg and hypertonic saline arm. The white arrows indicate the dialysates obtained during the stimulation period of the control leg and control arm. (DOMS leg ●, control leg ○, hypertonic saline arm ▲ and control arm △).

by training are associated with the reduced muscle 'injury' and pain in trained muscles. The unaccustomed eccentric exercise in our 'untrained' subjects, however, was associated with a decrease of muscle NO concentrations. This may be due to reduced NOS activity or expression. Supporting this, a recent experimental study in rats revealed that a strong activation of ventilatory muscles (diaphragm, intercostal muscles) through exposure to a severe respiratory resistive load evoked a decline of muscle NOS activity [56]. Whether the reduced availability of NO contributed to the development of muscle soreness and whether NO levels are less affected in trained subjects remains to be investigated.

The eccentric exercise-induced muscle pain has been suggested to be caused by an inflammatory reaction evoked by minor myofibrillar and cytoskeletal damage [30]. This notion was motivated by the finding of cellular infiltrates, mainly macrophages, in muscles after eccentric exercise. These infiltrates were reported to resemble the morphological changes found in polymyositis [57, 58]. In addition, an increase of plasma cytokine levels such as IL-1 and IL-6 [16, 59, 60] and increased cytokine-immunoreactivity in muscle tissue [61–63] has been observed after eccentric exercise. The observed increase of CK activity and muscle swelling in

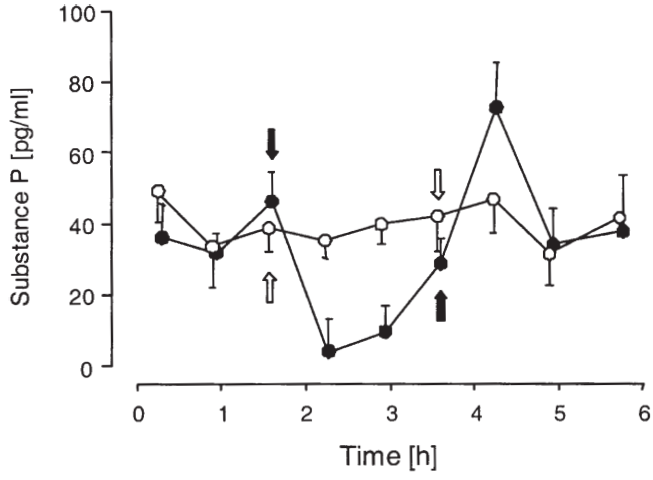

Fig. 7. Dialysate concentrations (mean ± SEM) of Substance P (SP) in the calf muscles. The black arrows indicate the dialysates obtained during pain stimulation in the DOMS leg; the white arrows indicate the respective dialysates of the control leg. (DOMS leg ●, control leg ○).

our subjects suggests that the DOMS exercise protocol employed in the present study was sufficient to evoke the previously described eccentric exercise-induced muscle 'injury'. At the start of microdialysis the inflammatory mediator PGE_2 was normal, i.e. there was no difference between DOMS and control leg. However, during the pain stimulation PGE_2 levels increased in the DOMS but not the control leg and then rapidly returned to baseline. It has been shown that PGE_2 concentrations increase during knee extensions in thigh muscles, suggesting that it is released during dynamic exercise [40]. In addition, the PGE_2 recovery was found to increase during exercise [40]. Both factors – dynamic contractions and increases of the recovery – might have contributed to the observed increase of PGE_2 during dialysis, because the pain was stimulated through albeit slight contractions. However, this is also true for the control muscles where we did not find an increase of PGE_2. We therefore suggest, that the facilitated PGE_2 release in the DOMS leg is caused by the underlying 'inflammatory' process in the sore muscles. Interestingly however, the PGE_2 release was more intense during the second stimulation, whereas the pain in the DOMS leg was more intense during the first stimulation. Hence, there was no direct correlation between PGE_2 levels and pain intensity, suggesting that PGE_2 may be one, but probably not the major algesic substance. This suggestion is supported by previous studies indicating that cyclooxygenase inhibitors administered orally [64, 65] or topically [49] can neither prevent nor alleviate eccentric exercise-induced muscle pain. Recently, the isoprostane, 8-epi-$PGF_2\alpha$ was found to be elevated in plasma and urine of patients with muscle pain due to statins [66]. Isoprostanes are generated from arachidonic acid by oxidation, thus independently of cyclooxygenase activity [67]. However, they probably also act at prostaglandin receptors [68] and augment nociception in animal models [69]. Thus, isoprostanes also have to be considered as potential substances involved in muscle pain.

In addition to PGE_2 we observed alterations of substance P levels in the DOMS leg. Particularly the second pain stimulation caused an increase of SP in DOMS dialysates. Again, a possible contraction-induced increase of muscle blood flow and recovery [40] does not fully explain the SP raise because SP levels did not increase in the control muscles. SP-immunoreactivity has been found to be increased in muscle tissue of patients with fibromyalgia and myofascial pain syndrome [70]. However, this finding was not unequivocally confirmed in other studies [71]. SP immunoreactivity was also increased in persistently inflamed muscles in rats, 12 days after injection of complete Freund's adjuvant [18]. However, it is still unclear whether the release of SP or other neuropeptides such as bradykinin contribute to muscle pain and hyperalgesia.

The acute muscle 'injury' caused by the insertion of the microdialysis catheters evoked a strong elevation of muscle glutamate levels in both calves and biceps muscles. After normalization there was a re-raise of glutamate levels in both painful muscles, i.e. the DOMS leg and the hypertonic saline arm but not in the respective control muscles suggesting that glutamate release may contribute to the sensation of muscle pain. This is supported by a clinical study in patients with fibromyalgia, where the NMDA receptor antagonist ketamine was found to reduce the muscle pain [10]. The authors suggested that ketamine reduces central hyperexcitability i.e. the site of action of ketamine was thought to be central. However recently, peripheral ionotropic [72] and metabotropic glutamate receptors [73] were found to be involved in nociceptive responses in animal experiments suggesting that a peripheral glutamate release might be involved in peripheral nociceptive mechanisms. In the biceps muscle glutamate levels remained elevated for about 2 h after injection of hypertonic saline. In contrast to glutamate lactate, PGE_2 and NO were not affected by the injection of hypertonic saline. This supports the hypothesis that the pain induced by injection of hypertonic saline is directly caused by an elevation of extracellular Na^+ concentrations, which may result in a depolarization of excitable membranes and glutamate release from activated nociceptors.

In summary, muscle 'injury' either due to the insertion of the microalysis catheters, contractions of the painful DOMS muscles or injections of hypertonic saline caused an increase of glutamate release in calf and biceps muscles. The glutamate release was strongly associated with muscle pain. In case of the DOMS model elevated lactate, PGE_2 and alterations of SP release may also contribute to the muscle pain.

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