Prostaglandin synthesis can be inhibited locally by infusion of NSAIDs through microdialysis catheters in human skeletal muscle

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Mikkelsen UR, Helmark IC, Kjær M, Langberg H. Prostaglandin synthesis can be inhibited locally by infusion of NSAIDS through microdialysis catheters in human skeletal muscle. J Appl Physiol 104: 534–537, 2008. First published December 13, 2007; doi:10.1152/japplphysiol.01016.2007.—Prostaglandins are known to be involved in the regulation of local blood flow within human skeletal muscles during exercise, and the concentration of prostaglandins increases locally and systemically in response to exercise. The systemic release of prostaglandins can be inhibited by oral intake of nonsteroidal anti-inflammatory drugs (NSAIDs). However, to study the specific role of prostaglandins, the formation of prostaglandins within the tissue must be controlled. Microdialysis enables determination of local concentrations of water-soluble substances within the tissue. In the present study, the microdialysis method was used to infuse NSAIDs locally into human skeletal muscles producing a local block of prostaglandin formation. In addition, the graded blockade at various distances from the infusion site within the muscle during rest, exercise and recovery was determined. Microdialysis was performed in thigh muscles (vastus lateralis muscle) in six healthy men. One of the microdialysis catheters was used to block prostaglandin synthesis by infusion of the NSAID indomethacin. Additional catheters were placed 1 and 4 cm away from the infusion and in the contralateral leg (working control). Following 2 h of rest, the subjects performed 200 maximal eccentric contractions with each leg followed by 3 h of rest. The study revealed that infusion of NSAID reduced local prostaglandin E2 concentration by ~30–50% (4 cm away from the infusion) and 85% (1 cm away from the infusion) compared with the contralateral (unblocked) thigh muscle. In conclusion, the present study shows that infusion of NSAIDs into human muscle via microdialysis catheters results in a graded blockade of prostaglandin synthesis.

graded blockade; prostaglandin E2; inflammation; eccentric exercise

PROSTAGLANDINS (PGS) ARE KNOWN TO be involved in the regulation of local blood flow within human skeletal muscles during exercise (1, 16), and the concentrations of circulating proinflammatory cytokines (12) and PGs (17) are found to increase in response to exercise. PGs are synthesized from arachidonic acid derived from cell membrane phospholipids by cyclooxygenase (COX) enzymes, and the systemic release of PGs can be inhibited by oral administration of COX inhibitors known as nonsteroidal anti-inflammatory drugs (NSAIDs). By use of NSAIDs to block overall PG synthesis, it has been shown that PGs play a role in exercise hyperemia when COX inhibitors are used in combination with nitric oxide (NO) (1, 16). However, to study the specific mechanisms of local muscle adaptation, it is important to be able to intervene not only systemically but also locally in the skeletal muscle. From such studies, it will be possible to determine the role of locally produced PGs in the control of muscle blood flow and to investigate whether other local agents are produced to participate in the control of the local perfusion of the exercising muscles. The microdialysis technique (18) has been developed to measure compounds within the extracellular space originally in the brain, but has in recent years successfully been used to study the interstitial environment in (mostly resting) human skeletal muscle, tendon and adipose tissue. Microdialysis makes it possible to determine the local concentration of water-soluble substances, e.g., PGs. In addition, the microdialysis technique can be used to introduce substances locally into human tissue (3, 9, 13), although this technique is not widely used yet. In the present study the microdialysis method was used to infuse NSAIDs locally within the human skeletal muscles to produce a local block of PG formation. In addition, it was investigated whether the microdialysis method could be used to generate a graded block in various distances from the infusion site within the muscle during rest, eccentric exercise, and recovery. This would allow for studies of the effect of various degrees of PG block within the same muscle.

The aim of the present study was to induce a local block of PG synthesis within the human skeletal muscle by the use of microdialysis, and to determine whether it was possible to block PG synthesis locally and whether this local infusion could be used to generate a graded blockade within the muscle both in the resting state and in response to eccentric exercise.

MATERIALS AND METHODS

Subjects. Six healthy men participated in this study [age 29 ± 2 yr (range 24–35 yr), height 185 ± 4 cm (range 176–201 cm), weight 85 ± 4 kg (range 76–105 kg), and body mass index (25 ± 0.5 kg/m²)]. All subjects were generally well trained (3 h training/wk), but they were unaccustomed to high-intensity eccentric exercise and had not performed leg resistance training within the last year.

The Ethics Committees of the Municipalities of Copenhagen and Frederiksberg approved this study (KF 01 306773); all procedures conformed to the Declaration of Helsinki; and written, informed consent was obtained from all subjects before the study.

Experimental protocol, overview. On the experimental day, subjects arrived in the morning and four microdialysis catheters (see below, 5 in 2 of the subjects) were positioned in the vastus lateralis muscle. The position was standardized based on the length of the thigh to minimize individual differences. Following 2 h of rest, each subject performed 200 maximal eccentric contractions with the quadriceps muscles of each leg in an isokinetic dynamometer (see below) followed by 3 h of rest. The microdialysis catheters remained in the muscles during the whole experiment.

Microdialysis and local blockade of PG synthesis. Microdialysis catheters were inserted under local anesthetic (1% lidocaine) in parallel to the muscle fibers in the vastus lateralis muscle as illustrated...
in Fig. 1. The microdialysis catheters were perfused at a rate of 2 μl/min with Ringer acetate or NSAID solution containing radioactively labeled prostaglandin E$_2$ (PGE$_2$) for relative recovery determination (14). Two different types of microdialysis catheters were used (both with a membrane length of 30 mm): custom-made, linear catheters with 3,000-kDa molecular mass cutoff (8) or commercially available CMA catheters (CMA 60; CMA/microdialysis, Solna, Sweden) with 20-kDa molecular mass cutoff. A custom-made catheter was used for infusion of the unspecific COX inhibitor indomethacin (Confortid, Dumex-Alpharma, Copenhagen, Denmark) to block local synthesis of PGs. Indomethacin has been shown to inhibit both COX-1 and -2 activity (6). Indomethacin was easily dissolved in its solvent (aqua ad injectabilia) at a concentration of 50 mg/ml according to the prescription for intramuscular injections. With a perfusion rate of 2 μl/min, and an assumed exchange rate over the membrane of 50–70% (based on previous pilot experiments and the fact that indomethacin has a molecular mass =356 Da), the amount of indomethacin infused into the muscle was calculated to be ~6 mg/h. In addition to this catheter for NSAID infusion, two more catheters (CMA 60) were inserted in the same leg at a distance of 4 cm proximal and distal to the NSAID infusion catheter, respectively. In the contralateral leg, a single catheter (CMA 60) was inserted serving as a working control leg. In two of the subjects, one additional catheter (CMA 60) was inserted 1 cm distal to the infusion catheter. All catheters, except the one for NSAID infusion, were perfused with Ringer acetate. The NSAID infusion catheter and the catheter in the unblocked leg were both inserted halfway between spina iliaca anterior superior and the base of patella.

Microdialysis samples (dialysate) were collected before (3 samples), during, and after the eccentric exercise in either 30 or 60 min pools.

**Exercise protocol.** The subjects performed an eccentric exercise protocol that has previously been used by our laboratory (2). It is important to note that both legs were exercising, and comparisons were made between the blocked (NSAID) and unblocked leg. Following warm-up (12–15 repetitions, load gradually increasing toward maximal) a total of 200 maximal isokinetic eccentric contractions were performed with the quadriceps femoris muscles of each leg. The exercise load consisted of two exercise protocols: 1) 100 maximal eccentric contractions (10 sets of 10 repetitions) at a slow contraction speed (knee joint angular velocity 30°/s) followed by 2) 100 maximal eccentric contractions (10 sets of 10 repetitions) at a fast contraction speed (knee joint angular velocity 120°/s) using an isokinetic dynamometer (KimCom KC125AP, Chattanooga Group, Harrison, TN) with a range of motion from 10 to 90° (0° = full extension). At the end of each eccentric contraction, the leg was immediately returned passively to the starting position (10°) by the dynamometer (angular velocity 60°/s), and the next eccentric contraction was initiated. A 1-min rest period was allowed after each set of contractions.

**PGE$_2$ concentration in dialysate.** The concentration of PGE$_2$ at each dialysate sample was measured by use of an enzyme immunoassay kit [Prostaglandin E$_2$ EIA Kit-Monoclonal, Cayman Chemical, catalog no. 514010; detection limit for PGE$_2$ of 15 pg/ml; intra-assay coefficient of variation (CV) of 5–12%; interassay CV of 8–11%]. All samples were analyzed in duplicate (dilutions 1:2, 1:4, and 1:5).

**Statistics.** Results are presented as means ± SE. The statistical significance of differences between groups was ascertained using a paired t-test. The level of significance was set at $p < 0.05$. Subjects served as their own controls; that is, they had the block in one leg with the contralateral leg as unblocked, working control.

**RESULTS**

The level of PGE$_2$ in the skeletal muscle interstitium during the first 30 min following insertion of microdialysis catheters was high with no significant difference between the groups (mean unblocked leg: 4,526 ± 936, mean 4 cm from NSAID infusion site: 3,532 ± 597, 1 cm from NSAID infusion site: 5,003 pg/ml; $p > 0.05$, data not shown). The level of PGE$_2$ was markedly reduced from the onset of exercise (2 h after insertion of the microdialysis catheters) in the leg with PG block compared with the unblocked leg both at a distance of 4 and 1 cm away from the infusion site of indomethacin (P = 0.02; Fig. 2). During exercise, the mean PGE$_2$ level in the unblocked leg and at 4 and 1 cm away from indomethacin infusion was 1,722 ± 259, 879 ± 183, and 400 pg/ml, respectively. Following exercise, the concentration of PGE$_2$ was reduced to 1,201 ± 204, 876 ± 168, and 318 pg/ml, respectively.

When expressing the PGE$_2$ level in each sample relative to the unblocked exercising leg (100%), a graded block of PGE$_2$ was found from the onset of exercise and in all subsequent samples (Fig. 3). During exercise, a graded block of released PG was found with the average PGE$_2$ level 4 and 1 cm away from infusion of indomethacin to be 49% (range 36–56%) and 13% (range 8–18%) of the level in the unblocked leg, respectively. Postexercise, the average PGE$_2$ level 4 and 1 cm away from indomethacin infusion was also reduced to 67% (range 63–75%) and 14% (range 13–14%) of the level in the unblocked leg, respectively.

No difference in the concentration of PGE$_2$ and thus response to indomethacin blockade of PG synthesis could be detected between the proximal compared with the more distal parts of the quadriceps muscle, both 4 cm away from the infusion catheter ($p > 0.05$; Fig. 4). This indicates that indomethacin can diffuse freely within the muscle tissue and that no heterogeneity existed in the response to COX blockade.

**DISCUSSION**

The main finding in the present study is that a local block of PG synthesis can be induced by infusion of the NSAID indomethacin into human skeletal muscle using the microdialysis technique. Furthermore, it can be shown that this reduction in local PG concentration is dependent on the distance from the infusion site, having an 85% blockade 1 cm and a 30–50% blockade 4 cm away from the infusion site. To our
knowledge, this is the first systematic evaluation of the effect of a local block of PG synthesis in human tissue. One previous study has used a local block of PG (and NO) synthesis (5); however, they did not report any data on PG concentrations.

Our data on interstitial PGE2 concentrations correspond very well with previous findings in humans where a systemic PG block was induced by oral administration of indomethacin (1). They reported PGE2 concentrations during exercise of 1,600–3,000 and 400 pg/ml in the control and blockade (indomethacin) trial, respectively. In the present study, PGE2 concentrations during exercise were 1,600–1,900 pg/ml and 300–500 pg/ml in the unblocked leg and 1 cm away from indomethacin infusion, respectively. This indicates that a local tissue infusion with indomethacin can create a similar blockade of PG synthesis in an area of ~1 cm from infusion, as can be obtained by systemic administration of indomethacin.

During the first 2 h following insertion of microdialysis catheters, local PGE2 concentrations were very high, most likely due to the trauma from insertion. This is in line with earlier observations using microdialysis in the peritendinous space around the Achilles tendon (7), and it illustrates that a resting and stable situation cannot be expected until at least 2 h after catheter insertion (7). Furthermore, the expected increase in PGE2 during exercise may still have been blunted by the effect of the trauma from insertion of microdialysis catheters, because this was in fact not fully resolved before the onset of exercise (2 h).

In the present study, we did not observe any ischemic pain during indomethacin infusion, but one has to take into account that indomethacin was infused at a very low dose: 6 mg/h, compared with the recommended dosage for analgesic of 50 mg (iv or im) at once. All the subjects were with no exceptions able to exercise maximally with the microdialysis catheters and simultaneous NSAID infusion.

No direct determination of plasma or dialysate concentrations of indomethacin was performed in the present study, making it impossible to ensure that indomethacin was actually infused to the tissue. However, we found a clear effect of the infusion on PG synthesis with a reduced concentration of interstitial PGE2. In addition, the present study indicates that the diffusion of indomethacin within the quadriceps muscle is homogeneous because no difference in PGE2 concentrations measured by the microdialysis catheter were found when comparing 4 cm proximal with 4 cm distal to the indomethacin infusion site (Fig. 4). Thus, in Figs. 2 and 3, the mean value from the proximal and distal microdialysis catheter was used for the 4-cm data points. These data may indicate that the quadriceps muscle tissue in proximal and distal direction 3–4 cm away from the infusions of NSAIDs could be seen as a homogenous tissue with respect to release of PG. Exercise, by its mechanical effect and by increasing blood flow, may increase diffusion of indomethacin within the tissue; however,
because the effect of the blockade is directly measured within the tissue via the PG concentration determination in the microdialysis catheters this is already reflected in the present study.

The local blockade demonstrated in the present study has several potential advantages compared with general blockade by oral intake (1). Compared with intra-arterial or intravenous infusions (4, 11, 15), local injections and oral intake of pharmacological substances (1), the presently used microdialysis technique with continuous infusion of NSAIDs allows for both a graded blockade as well as for maintaining steady-state situations over a long time period. In the present study an almost constant block was maintained from the end of exercise until 3 h postexercise (shown in Fig. 3). Compared with a systemic blockade, the local graded block used in the present study avoids potential confounding factors such as effects of the blocker on other organs or tissues influencing the local processes to be investigated (6).

Compared with repeated (muscle) biopsies, the obvious advantage of the present microdialysis method is that it is minimally invasive, allows for determinations over time, and in addition avoids the potential drawbacks of repeated biopsies. In general, the described technique could theoretically be used in any other accessible tissue as well (e.g., fat and tendon). This graded block will in the future allow for studying the role of PGs and inflammation in muscle adaptation, and it gives the opportunity to investigate the local effects of PGs. Furthermore, it will be useful for studying specific mechanisms of these effects, e.g., the potential involvement of different growth factors. This study has evaluated the effect of local indomethacin infusion on local, interstitial PGE2 concentration.

In conclusion, PG synthesis can be locally blocked by use of microdialysis for infusion of NSAIDs into human vastus lateralis muscle. The inhibition results in a local, graded block of PGE2 levels in the muscle. This method will be useful to study the role of inflammation in muscle recovery and adaptation.

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GRANTS

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