Increase in blood bradykinin concentration after eccentric weight-training exercise in men

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Blais, Charles, J. R., Albert Adam, Denis Massicotte, and François Péronnet. Increase in blood bradykinin concentration after eccentric weight-training exercise in men. J. Appl. Physiol. 87(3): 1197–1201, 1999.—The purpose of this study was to verify the possible appearance in the blood of bradykinin (BK) and des-Arg⁹-bradykinin (des-Arg⁹-BK) after eccentric exercise in 13 male subjects. Eccentric exercise (35 × 10 leg presses at 120% maximal voluntary concentric contraction) resulted in muscle damage and inflammation, as suggested by the significant increase in serum creatine kinase activity (from 204 ± 41 to 322 ± 63 U/l 12 h postexercise) and by severe lasting pain, which also peaked at 12 h postexercise. Blood BK and des-Arg⁹-BK concentrations were measured by competitive enzyme immunoassays using highly specific polyclonal rabbit IgG. Des-Arg⁹-BK concentration was not modified (preexercise: 44 ± 14 pmol/l; pooled postexercise: 47 ± 4 pmol/l). In contrast, BK concentration significantly increased immediately after the exercise session (68 ± 9 vs. 42 ± 3 pmol/l preexercise and returned to basal values at 12, 24, and 48 h (pooled value: 40 ± 4 pmol/l). This observation suggests that the inflammatory process due to eccentric exercise-induced muscle damage could be mediated in part by BK.

creatine kinase; delayed-onset muscle soreness; kinins; inflammation; des-Arg⁹-bradykinin

EXERCISE, particularly unaccustomed strenuous exercise, eccentric contractions, and contractions performed at long muscle length can result in delayed-onset muscle soreness (DOMS) (for review see, e.g., Refs. 1, 7, 14, 17, 21, 24, 33, and 37). Brendstrup [see Armstrong (1)] was apparently the first author to suggest that DOMS could be associated with an inflammatory process triggered by exercise-induced muscle damage. Damage to the muscle after various types of exercise has, indeed, been demonstrated from direct morphological evidence (16, 17) as well as from changes in a number of markers of tissue damage and repair. Serum creatine kinase (CK) concentration and/or activity are the most common markers used (e.g., see Refs. 4–6, 8, 18–20, 25, 28, 29, 36, and 37). Evidence of cell disruption and of increase in collagen and myosin turnover has also been shown by using other serum or urinary markers, e.g., aspartate (5, 29) and alanine aminotransferases (5), alkaline phosphatase (4, 5), lactate dehydrogenase (4, 5, 29), carbonic anhydrase (39), myoglobin (8, 19, 39), myosin heavy chain (25), galactosylhydroxylsyl glucosyltransferase (39), hydroxylsine and hydroxyproline (4), and pre-pro-collagens (39).

The hypothesis of an inflammatory process in the muscle as a consequence of this damage is consistent with the major signs and symptoms observed, such as muscle pain (i.e., DOMS), swelling, and the loss of functions, such as reduction in flexibility and strength (7, 24, 37) but also reduction in insulin sensitivity (19), the ability to store glycogen, and endurance (2). Data concerning specific histological and biochemical markers of inflammation are also in line with this hypothesis: changes in plasma white blood cell subpopulations (5, 6, 39) and infiltration of white blood cells in the muscle (16, 18, 23); increase in plasma (5, 18) and tissue cytokine concentrations (16); and activation of the complement pathway (6). The recent report (18) of an activation of xanthine oxidase in the microvascular endothelial cells of muscles and in infiltrating leukocytes after exercise is also consistent with the hypothesis of an inflammatory process. Finally, this hypothesis is also consistent with the observation that anti-inflammatory drugs could reduce DOMS and some of its associated signs (see, e.g., Ref. 22).

Several models have been proposed to explain how exercise could trigger an inflammatory response in the muscle (1, 14, 24, 33, 37). A common feature of these models is the involvement of various mediators of inflammation, including histamine, serotonin, nitric oxide, cytokines, prostaglandins, oxygen free radicals, and kinins. However, except for some cytokines (5, 16, 18), the involvement of these various putative factors in the response to exercise-induced muscle damage and in DOMS remains hypothetical.

The purpose of the present experiment was to verify the possible appearance of bradykinin (BK) and of its active metabolite des-Arg⁹-bradykinin (des-Arg⁹-BK) in the blood after muscle damage, triggered by eccentric exercise and associated with DOMS. BK and des-Arg⁹-BK are powerful mediators of inflammation (13) and, as suggested by Armstrong (1) and by Madnity et al. (24), could be involved in the processes linking exercise-induced muscle damage to inflammation and DOMS.

METHODS

Subjects. The experiment was conducted on 13 active and healthy male subjects who gave their informed written consent to participate in the study, which was approved by the Institutional Board on the Use of Human Subjects in Research. Their age, height, and weight were 22.7 ± 0.6 (SE) yr, 177 ± 1.7 cm, and 75.3 ± 1.8 kg, respectively. None of the subjects was taking part in any regular training program at the time of experiment.

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Exercise. The subjects performed a weight-training exercise session on a supine leg-press training apparatus (Trotter, Madway, MA). The exercise consisted of five sets of 10 eccentric contractions of the extensor muscles of the lower limbs at 120% of the maximal strength measured in concentric contraction, or 1.76 ± 0.07 kN. For this purpose, the maximal strength, which could be developed during a single eccentric contraction, was measured in a preliminary test session 1 wk before the experiment (1.47 ± 0.06 kN). The day of the experiment, two aides lifted the weight in the upper position on the leg-press apparatus while the subject extended his lower limbs against no resistance. The subject then lowered the charge as slowly as possible. Each eccentric contraction took ~3 s. A 5-s rest period was allowed between the contractions while the weight was lifted again by the aides to starting position. A 5-min rest period was allowed between two consecutive sets of contractions. The entire exercise session, which included 10 unaided concentric-eccentric contractions at 50% of the maximal concentric strength as a warm-up, lasted ~45 min.

Methods. The day of the experiment, observations and blood sampling from an antecubital vein (10 ml) were performed at rest before the beginning of the exercise session (between 6:00 and 7:00 PM) and immediately after the exercise period. Observations and blood samplings were also taken 12, 24, and 48 h after the end of the exercise session.

For the measurement of CK activity, 5-ml blood samples were centrifuged (2,000 \( g \), 10 min, 4°C), and the serum was stored at −20°C until analysis. Serum CK activity was assayed by using an automatic enzymatic method (Hitachi 717) with commercially available reagents (Boehringer-Mannheim).

For the measurement of BK and des-Arg \(^9\)-BK concentrations, 5-ml blood samples were immediately collected in polypropylene tubes containing 15 ml of ice-cold ethanol. The tubes were shaken vigorously and left to stand in crushed ice before centrifugation (2,000 g, 15 min, 4°C). The clear supernatant was collected and evaporated to dryness (Speed Vac Concentrator, Savant, Farmingdale, NY). The residues obtained were resuspended in 2 ml of trifluoroacetic acid (0.1% vol/vol) and purified on a 1-ml Waters Sep-Pak Vac C \(_{18}\)-silica cartridge (Millipore, Milford, MA). The eluates were then evaporated to dryness, and the residues were stored at −80°C until analysis. Immunoreactive BK was quantified in the residue resuspended in 400 µl of a Tris·HCl buffer (50 mM, pH 7.4), containing NaCl (100 mM) and Tween-20 (0.5 ml/l), by a competitive enzyme immunoassay (10). This assay uses highly specific polyclonal rabbit IgGs raised against the carboxy terminal end of BK, digoxigenin-labeled BK as tracer, and alkaline phosphatase-labeled Fab fragments anti-digoxigenin, with the substrat p-nitrophenyl phosphate to detect and quantify the immune complexes. Typical calibration curves for BK were characterized by a half-maximal saturation value of 0.78 pmol/ml (10). Immunoreactive des-Arg \(^9\)-BK was quantified by using a similar analytical approach to that described by Raymond et al. (34).

Muscle soreness was assessed for the right quadriceps muscle on a four-level scale: 0 = no pain; 1 = pain when palpated; 2 = pain when contracted; and 3 = painful at rest.

Statistics. Values are means ± SE. Comparisons were made by using analysis of variance for repeated measures and a Tukey’s post hoc test. Statistical significance was set at \( P < 0.05 \).

RESULTS

Serum CK activity and BK and des-Arg \(^9\)-BK concentrations in the blood before and after the exercise session are shown in Figs. 1 and 2, respectively. Immediately after the exercise session, serum CK activity was slightly (19 ± 3%) but significantly increased above basal value. The peak value was observed 12 h after the exercise session (64 ± 15%, with wide interindividual variations: range −20 to +170%). Serum CK activity decreased thereafter and was back to basal values 48 h after the exercise bout.

Blood BK concentration averaged 42 ± 3 pmol/l before the exercise session. A significant 68 ± 27% increase was observed immediately after the exercise session, also with wide interindividual variations (range: −47 to +330%). Blood BK concentration was back to basal values 12 h after the exercise session and remained unchanged thereafter.

The presence of des-Arg \(^9\)-BK was detectable in the blood in only 38 of the 65 samples analyzed (58%). No significant change from preexercise value (44 ± 15 pmol/l) was observed immediately after exercise or on the following days (pooled average postexercise value: 40 ± 5 pmol/l; \( n = 42 \) samples).

Severe pain developed in the quadriceps muscle immediately after the exercise session (Fig. 1) and further increased to reach a peak 12 h after the exercise session. Some of the subjects were still experiencing some pain 7 days after the exercise session, when a follow-up informal survey was made.

Fig. 1. Serum creatine kinase activity (A) and muscle soreness (B) on a 4-level scale immediately before (Pre) and after (Post) exercise session 12, 24, and 48 h after exercise session. Values are means ± SE; \( n = 13 \) men. *Significantly different from preexercise value, \( P < 0.05 \).
involved in the development of fatigue and DOMS. In ase in the muscle, suggesting that histamine could be exercise increases the activity of histidine decarboxyl-

evidence (see the introductory section), direct evidence that exercise-induced muscle damage triggers an inflammatory process (1, 24, 33, 37). Although this hypothesis is consistent with most of experimental evidence (see the introductory section), direct evidence of the appearance of the classic mediators of inflammation remains limited, except for some cytokines (5, 16, 18). Endo et al. (15) have recently reported in mice that exercise increases the activity of histidine decarboxylase in the muscle, suggesting that histamine could be involved in the development of fatigue and DOMS. In contrast, involvement of prostaglandins (8) and oxygen reactive species (8, 18, 36) has proven difficult to ascertain.

The present study was performed to verify the possible appearance of BK and des-Arg⁹-BK in the blood in response to eccentric exercise resulting in muscle damage and DOMS. BK is formed from kininogen precursors upon the action of tissue and plasma kininogenses, which are activated by various stimuli, including tissue damage and injury, anoxia, and acidosis, whereas its active metabolite des-Arg⁹-BK is produced upon the action of kininase I (see Ref. 13 for a review). BK and des-Arg⁹-BK, or kinins, acting on B₂ and B₁ kinin receptors (27), are powerful proinflammatory factors. They produce local edema and activate and sensitize sensory and sympathetic nerve endings, with the release of other mediators of inflammation (e.g., prostanoïds, substance P, neuropeptide A, and calcitonin gene-related peptide), which mediate some of the local action of kinins and amplify their proinflammatory, nociceptive, and algesic effects. Kinins also attract leukocytes, activate the phagocytic function, and increase production and release of inflammatory mediators from neutrophils and macrophages.

In experimental models of inflammation in rat, BK plays a major role in the development of the acute inflammatory process (9), whereas both BK and des-Arg⁹-BK are involved in the development of sustained inflammation (3). BK could also be generated in the exercising muscle (12, 31, 35, 38). We thus hypoth-
esized that BK could play a role in the acute develop-
ment of muscle inflammation after exercise-induced muscle damage, whereas both BK and des-Arg⁹-BK could play a role in the sustained inflammation observed the following days. We also hypothesized that the involvement of these peptides in the inflammatory process in the tissue could be reflected by changes in their concentration in the blood.

BK concentrations in blood sampled before the exercise session (42 ± 3 pmol/l) and at 12, 24, and 48 h after the exercise session (pooled average value 47 ± 4 pmol/l; n = 42 samples) were slightly higher but consistent with basal values observed by Pellarcani et al. (32), who used various methods of extraction and assays (17–36 pmol/l), and in our own laboratory (29 ± 9 pmol/l) (26). In hemodialyzed patients, despite the inflammatory process triggered by the dialysis mem-
brane, BK concentration in peripheral blood was not significantly modified 20 min after the start of dialysis (26). In contrast, Nussberger et al. (30) observed moderate (×2)-to-large increases (×35) in blood BK concentra-
tion in patients with angioedema. In the present experi-
ment, immediately after the exercise session, BK concentration in peripheral blood also increased above basal values observed before exercise or 12, 24, and 48 h after the end of exercise. The average response remained modest (64 ± 15%), and wide interindividual variations were observed (range of change: from −9 to 98 pmol/l), but the increase was statistically significant and was observed in 10 of the 13 subjects. This transient increase in blood BK concentration immediately
after exercise is a further support to the hypothesis that eccentric exercise triggers an acute inflammatory process that could be mediated, at least in part, by the generation of BK in the exercising muscles with a spillover in the blood.

In contrast to the transient change in blood BK concentration, no significant change from basal preexercise value was observed for des-Arg9-BK immediately after exercise. This observation is consistent with the results from our laboratory, suggesting that the conversion of BK into des-Arg9-BK in the blood is only a minor pathway in humans, contributing only ~3.5% to BK degradation (11).

Blood BK concentration had returned to the basal value 12 h after the exercise session and remained unchanged thereafter, and no significant changes in des-Arg9-BK concentration were observed. This could indicate that none of the kinins are involved in the sustained inflammatory process after eccentric exercise. An alternate and more likely hypothesis is that kinins are involved in this process but that their generation in the inﬂamed muscle does not translate into changes in their concentration in peripheral blood.

The present study does not allow us to ascertain the mechanisms by which the eccentric weight-training exercise performed acutely increased BK concentration in the blood, the source of BK found in the blood in higher amounts after than before exercise, and the possible role of BK in the development and maintenance of muscle inflammation and DOMS. Isometric muscle contraction for 30 s, which is unlikely to result in muscle damage, is associated with the generation of BK in the tissue (38). This suggests that the appearance of BK in the muscle could be a normal event, but the mechanism by which muscle contraction locally triggers the kininogenase-kinin system remains unknown. This mechanism could be responsible for an increase in tissue BK concentration with a spillover in the blood in response to the high-intensity eccentric exercise performed in the present study. Additionally, tissue kininogenases could have been activated by substances released from disrupted cells or extracellular structures as a consequence of exercise-induced muscle damage. The resulting buildup of BK in the tissue could explain that it escaped inactivation and leaked into the blood. Under this hypothesis, the increase in BK concentration in the tissue could play a role in the development of an inflammatory response, including pain. Alternately, BK could have been generated in organs and tissues other than the exercising muscles, from which it could have been released. Finally, it cannot be excluded that BK was also generated in the blood as a consequence of activation of plasma kininogenases by unidentified substances released from the muscle and/or other tissues.

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