Role of kallikrein-kininogen system in insulin-stimulated glucose transport after muscle contractions

C. L. DUMKE, J. KIM, E. B. ARIAS, AND G. D. CARTEE
Biodynamics Laboratory, Department of Kinesiology, University of Wisconsin, Madison, Wisconsin 53706

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Dumke, C. L., J. Kim, E. B. Arias, and G. D. Cartee. Role of kallikrein-kininogen system in insulin-stimulated glucose transport after muscle contractions. J Appl Physiol 92: 657–664, 2002; 10.1152/japplphysiol.00854.2001.—Serum proteins [molecular weight (MW) > 10,000] are essential for increased insulin-stimulated glucose transport after in vitro muscle contractions. We investigated the role of the kallikrein-kininogen system, including bradykinin, which is derived from kallikrein (MW > 10,000)-catalyzed degradation of serum protein kininogen (MW > 10,000), on this contraction effect. In vitro electrical stimulation of rat epitrochlearis muscles was performed in 1) rat serum + kallikrein inhibitors; 2) human plasma (normal or kallikrein-deficient); 3) rat serum + bradykinin receptor-2 inhibitors; or 4) serum-free buffer + bradykinin. 3-O-methylglucose transport (3-MGT) was measured 3.5 h later. Serum + kallikrein inhibitors tended (P = 0.08) to diminish postcontraction insulin-stimulated 3-MGT. Contractions in normal plasma enhanced insulin-stimulated 3-MGT vs. controls, but contractions in kallikrein-deficient plasma did not. Supplementing rat serum with bradykinin receptor antagonist HOE-140 during contraction did not alter insulin-stimulated 3-MGT. Muscles stimulated to contract in serum-free buffer plus bradykinin did not have enhanced insulin-stimulated 3-MGT. Bradykinin was insufficient for postcontraction-enhanced insulin sensitivity. However, results with kallikrein inhibitors and kallikrein-deficient plasma suggest kallikrein plays a role in this improved insulin action.

bradykinin; bradykinin receptor-2; insulin sensitivity; muscle contraction; exercise

Skeletal muscle glucose transport is stimulated both by insulin and exercise. Prior exercise increases glucose transport by two apparently separate mechanisms: 1) it enhances glucose transport in the absence of insulin (insulin independent) and 2) it enhances the ability of insulin to increase glucose transport (insulin dependent) (3, 29, 30, 40). The insulin-independent effect progressively declines after exercise (typically reversed ~3 h postexercise for rat muscle), at which time glucose transport in the presence of physiological insulin concentrations is enhanced, i.e., insulin sensitivity is improved (3, 4, 28, 30, 40).

Electrically stimulated contractile activity by isolated muscle in serum-free buffer also leads to a large increase in insulin-independent glucose transport, and this effect is lost after ~3 h of incubation, reminiscent of results after in vivo exercise (3, 9, 40). However, unlike in vivo exercise, the same contractile activity by isolated epitrochlearis muscle does not result in a subsequent increase in insulin-stimulated glucose transport measured ~3 h later (3). Gao et al. (9) demonstrated that a factor found in serum [protein(s) with molecular weight (MW) >10,000] must be present in order for muscle contraction to induce increased insulin sensitivity. Stimulating muscles to contract in vitro in the presence of serum (from nonexercised rats or humans), followed by a 3.5-h incubation in the absence of serum, resulted in greater insulin-dependent glucose transport compared with muscles treated identically, except for the absence of serum during the contractions (9). Gao et al. excluded several serum proteins, including insulin, insulin-like growth factor-I, albumin, or α-, β-, and γ-globulins, as being sufficient for the effect.

Bradykinin is released by contracting skeletal muscle (2, 27, 35, 36). At least under some conditions, bradykinin may play a role in enhanced insulin sensitivity (6, 10, 15, 17, 39). Kininases (expressed by most tissues, including skeletal muscle) rapidly degrade and inactivate bradykinin (7, 21). Inhibition of kininases results in sustained elevation of circulating bradykinin concentration and improved whole body insulin sensitivity (13, 38, 39). Bradykinin receptor antagonists can block this kininase effect on insulin action (13, 14, 18, 38, 39).

Although the MW of the nonapeptide bradykinin is <10,000, it is derived by enzymatic cleavage of large precursor proteins known as kininogens. A single gene encodes for both high-MW (88,000–120,000) and low-MW (50,000–68,000) kininogen (1, 21, 22). Enzymes known as kallikreins (MW >10,000) are found in blood (plasma kallikrein) and tissue (tissue kallikrein), including the smooth muscle of arteries and skeletal muscle cells themselves (1, 21, 34).

In vitro incubation with bradykinin does not stimulate glucose transport by isolated muscle in the ab-
sence of insulin (5, 34), but the potential role of bradykinin (or other aspects of the kallikrein-kininogen system) on the postcontraction-induced increase in insulin-stimulated glucose transport has not been studied. We hypothesized that supplementing serum with kallikrein inhibitors or bradykinin receptor antagonists during contractile activity would diminish the postcontraction increase in insulin action. We further hypothesized that isolated skeletal muscles performing in vitro contractions in serum-free buffer supplemented with bradykinin would subsequently exhibit enhanced insulin-stimulated glucose transport. We also hypothesized that in vitro contractions in kallikrein-deficient plasma would not enhance subsequent insulin sensitivity.

METHODS

Materials. 3-O-[3H]methyl-D-glucose was purchased from NEN (Boston, MA), and D-[1-14C]mannitol was from American Chemical (St. Louis, MO). HOE-140 was from RBI (Natick, MA). Soybean trypsin inhibitor (SBTI) (T9128; type II-S), aprotinin (A1153; from bovine lung), BSA, pyruvate, glucose, bradykinin receptor-2 (BK2) antagonist (B-6029; [N-adamantanecetyl-d-Arg0, Hyp3, Thi5,8, d-Phe7]-bradykinin), and bradykinin were all from Sigma (St. Louis, MO). KHB, Krebs-Henseleit buffer; 3-MG, 3-Methylglucose; KHB, Krebs-Henseleit buffer; 3-MG, 3-Methylglucose; KHB, Krebs-Henseleit buffer; 3-MG, 3-Methylglucose; KHB, Krebs-Henseleit buffer; 3-MG, 3-Methylglucose.

Animals. Male specific pathogen-free Wistar rats, dually housed in wire-bottom cages, were provided food (Harlan Teklad Laboratory Rodent Diet) and water ad libitum until the day before the experiment when food was removed to 1700. The procedures were approved by the Animal Care Committee of the University of Wisconsin-Madison. On the experimental day, rats (weighing 170 ± 30 g and 5 wk old) were anesthetized, between 1000 and 1400 h, by an intraperitoneal injection of pentobarbital sodium (50 mg/kg). On cessation of toe-pinch reflex, both epigastrocleftic muscles were removed.

Serum collection. Blood (collected via cardiac puncture or abdominal aorta from anesthetized rats as described above) was allowed to clot on ice and then centrifuged at 1,000 × g until thawed on day 3 of use and was not refrozen. These procedures are consistent with those described by Gao et al. (9).

Initial muscle incubation. Figure 1 illustrates the sequence of incubation steps used in experiments, unless otherwise noted. After dissection, muscles were incubated for 15 min (35°C) in the solution of interest (gassed with 95% O2 and 5% CO2 and supplemented appropriately): Krebs-Henseleit buffer (KHB) (19), rat serum, or human plasma. Paired muscles from the same rat were treated identically with the exception of one treatment factor (e.g., ±serum, ±contractions, ±inhibitors, ±bradykinin, etc.).

Electrical stimulation. In vitro electrical stimulation was performed by using the same protocol as Cartee and Holloszy (3) and Gao et al. (9). Briefly, the distal end of the muscle was attached by a modified muscle clip (Kent Scientific, Litchfield, CT) to a vertical glass rod suspended in a 5-ml bath containing two platinum electrodes (Radnoti, Monrovia, CA). The proximal end was attached by another modified muscle clip to an isometric force transducer (Radnoti). The muscle was then suspended in solution appropriate to the experiment (i.e., serum or KHB, supplemented as appropriate), and resting tension was adjusted to 0.4 g. The contralateral muscle was placed in an identical bath. Muscles in these two separate baths were then simultaneously stimulated with supramaximal square-wave pulses of 0.5 ms with a Grass S48 stimulator (Grass Instruments, Quincy, MA). A Stimu-Splitter II (Med-Lab Instruments, Loveland, CO) was used to ensure equal current in the two baths. Ten tetanic contractions were produced by stimulating the muscle at 100 Hz for 10 s at a rate of one contraction per minute for 10 min. Muscle contraction data were downloaded in graphical format to be analyzed by MP-100 software (BIOPAC Systems, Santa Barbara, CA).

The effect of muscle contractions was compared with resting muscles, which were incubated for 25 min (equal to 15-min initial incubation plus 10-min contraction protocol) in the appropriate solution.

3-MG transport. After electrical stimulation, muscles were placed in 25-ml Erlenmeyer flasks containing 3 ml of KHB and 0.1% BSA, 8 mM glucose, 32 mM mannitol, and continuously gassed with 95% O2-5% CO2 at 35°C with shaking. This incubation was for 3 h, which was long enough for the insulin-independent contraction-induced increase in glucose transport to wear off (3, 9). Muscles were then transferred to new flasks containing 3 ml of KHB and including 2 mM pyruvate, 36 mM mannitol, and 0.1% BSA with or without insulin (30 μU/ml), as in the preceding incubation, for 30 min. These flasks were also gassed and shaken as described above at 30°C.

Muscles were then transferred to a final incubation flask to measure glucose transport (10 min at 30°C). This flask in-

![Fig. 1. Muscle treatment protocol. KHB, Krebs-Henseleit buffer; 3-MG, 3-O-methylglucose; +, with; −, without.](https://www.jap.org)
cluded 2 ml KHB supplemented with 8 mM 3-O-methylglucose (3-MG) (including 3-[\(^{14}\)C]MG at 0.25 mCi/mmol), 32 mM mannitol (including D-[\(^{1-14}\)C]mannitol at 6.25 \(\mu\)Ci/mmol), 0.1% BSA, and the same insulin concentration as the preceding preincubation step. Muscles were rapidly blotted on ice-cold filter paper, trimmed, and rapidly frozen between aluminum tongs cooled to the temperature of liquid N\(_2\) and then stored at \(-80^\circ\)C until processed. Muscles were then homogenized in 0.3 M perchloric acid at 4°C and at the rate of 3-MG transport determined as previously described (41). Values are expressed as micromoles per gram per 10 min.

**Tension development.** Tension developed (total tension − baseline tension) was determined, and the following parameters were assessed: peak tension = highest tension developed during 10 min of stimulation; and fatigue index = lowest tension ÷ peak tension \(\times 100\).

**Experiments.** The first group of experiments replicated the design of Gao et al. (9) to confirm that muscles subjected to electrically stimulated contractions in rat serum would have a subsequent (3.5 h later) increase in glucose transport compared with controls: 1) one muscle from each pair was stimulated in KHB, and then both were exposed to insulin; 2) muscles were suspended in bath (one in KHB and one in serum, and then both were exposed to insulin) at resting tension but not stimulated to contract; 3) same as 2 but in the absence of insulin.

A second series of experiments was performed to determine if supplementing rat serum with inhibitors of kallikrein (which catalyzes bradykinin release from kininogen) or with bradykinin receptors would interfere with enhanced insulin sensitivity. Muscles were incubated with rat serum (15 min) with or without inhibitors [kallikrein inhibitors: 100 \(\mu\)g/ml SBTI, 1,000 kallikrein-inhibiting units (KIU)/ml aprotinin, or kallikrein receptor inhibitors: 10 or 42.5 \(\mu\)M B-6029 or 1.0 or 12.8 \(\mu\)M HOE-140] followed by muscle contraction in rat serum with identical supplementation in the preceding step. Paired muscles were treated identically thereafter (inhibitors and serum were absent in subsequent incubation steps for all muscles).

The third series of experiments was performed to determine if contractions in kallikrein-deficient plasma significantly altered the resulting insulin-stimulated glucose transport compared with normal plasma. The initial incubation (15 min) and electrical stimulation (10 min) were performed by using human plasma (kallikrein-deficient or normal plasma). Kallikrein-deficient and normal human plasma (George King Bio-Medical, Overland Park, KS) had been collected and treated identically (sodium citrate used as an anticoagulant). Paired muscles were treated identically after contractions (plasma was absent in subsequent incubation steps for all muscles).

The final experiment was performed to determine if the presence of bradykinin during contractions was sufficient for enhanced postcontraction insulin action. Paired muscles were incubated in KHB supplemented with or without bradykinin (10 ng/ml, 100 ng/ml, or 1,000 ng/ml) before and during electrical stimulation (total of 25 min). Paired muscles were treated identically thereafter (bradykinin was absent in subsequent incubation steps for all muscles).

**Statistics.** Data are presented as means \(\pm\) SE. A paired \(t\)-test was used to determine statistical significance when paired muscles from the same rat were compared (i.e., for all comparisons of glucose transport). An unpaired \(t\)-test was used only for comparison between unpaired muscles (i.e., to compare tension measurements of muscles contracting in normal plasma vs. kallikrein-deficient plasma). The relationship between glucose transport and fatigue index was assessed by using a Pearson product-moment correlation.

**RESULTS**

**In vitro contractions ± rat serum.** Consistent with data from Gao et al. (9), 3-MG transport by insulin-stimulated muscles determined 3.5 h after in vitro contractile activity was higher in muscles that performed contractions in the presence of rat serum compared with contralateral muscles treated identically, except that contractions were performed in KHB rather than in serum (Fig. 2). Serum in the absence of contractions did not have a significant effect on 3-MG transport in the presence (0.68 \(\pm\) 0.10 vs. 0.62 \(\pm\) 0.06 \(\mu\)mol·g\(^{-1}\)·10 min\(^{-1}\)), \(P = 0.53\), serum vs. KHB, respectively) or absence (\(P = 0.25\); Fig. 2) of insulin.

Peak tension did not differ between muscles contracting in rat serum compared with KHB (data not shown). Baseline tension (initially set at 0.4 g) was stable in muscles stimulated in serum, but it increased gradually in muscles stimulated in KHB. When baseline tension reached \(\sim\)0.8 g (typically after \(\sim\)7th tetanus), tension was readjusted to original baseline. Fatigue index was lower (indicating greater fatigue) for muscles stimulated to contract in KHB compared with muscles stimulated in serum \((P = 0.001)\). There was a significant correlation between fatigue index and glu-
cose transport when the analysis included combined data from muscles stimulated in KHB or serum ($r = 0.61, P = 0.003$). A significant correlation was also found for muscles stimulated in KHB alone ($r = 0.73, P = 0.01$) but not for muscles stimulated in serum alone ($r = 0.303, P = 0.365$).

**Rat serum ± kallikrein inhibitors.** The combination of kallikrein inhibitors [SBTI (100 μg/ml) and aprotinin (1,000 KIU/ml)] during contractions in serum tended ($P = 0.08$) to decrease insulin-stimulated 3-MG transport compared with paired controls treated identically except for absence of kallikrein inhibitors (Fig. 3). There was no effect with the combination of these inhibitors on insulin-stimulated 3-MG transport in muscles exposed to serum in the absence of contractions (data not shown). When present alone, neither SBTI nor aprotinin significantly altered insulin-stimulated 3-MG transport 3 h after contractions in serum (data not shown). Kallikrein inhibitors did not alter baseline tension, peak tension, or fatigue index.

**Human plasma.** Muscles stimulated to contract in normal human plasma had enhanced insulin-stimulated 3-MG transport compared with contralateral controls, which performed contractions in KHB (Fig. 4A), consistent with published results using normal human serum (9). However, insulin-stimulated 3-MG transport did not differ between muscles that performed contractions in kallikrein-deficient plasma and paired controls that performed contractions in KHB (Fig. 4B). As with rat serum, human plasma (kallikrein deficient or normal) prevented the progressive increase in baseline tension in contracting muscles. Unlike rat serum and compared with paired controls stimulated in KHB, muscles contracting in human plasma had a lower peak tension (kallikrein-deficient plasma was 44% lower than KHB control, $P = 0.002$; normal plasma was 35% lower than KHB control, $P = 0.078$). Also in contrast to results with rat serum, fatigue index was lower (indicating greater fatigue) in plasma-treated vs. KHB controls (normal plasma vs. KHB, $P = 0.01$; kallikrein deficient vs. KHB, $P = 0.002$). Neither peak tension nor fatigue index significantly differed for muscles contracting in kallikrein-deficient plasma compared with normal human plasma. There was not a significant correlation between fatigue index and glucose transport when data from plasma (normal or kallikrein deficient) and KHB controls were analyzed together ($r = -0.13, P = 0.48$). The correlation approached statistical significance when KHB controls were analyzed separately ($r = 0.44, P = 0.077$) but not for data from the normal plasma ($r = -0.05, P = 0.90$) or kallikrein-deficient plasma ($r = -0.32, P = 0.40$) groups.

**Rat serum ± BK2 antagonists.** Two compounds that block bradykinin binding to the BK2 receptor (HOE-140 and B-6029) were investigated. Exposure of muscle to BK2 antagonist HOE-140 (1.0 or 12.8 μM) during contractions in rat serum did not significantly change subsequent insulin-stimulated 3-MG transport (Fig. 5A). BK2-receptor antagonist B-6029 (10 μM) included during muscle contractions in serum also did not alter subsequent insulin-stimulated 3-MG transport (Fig. 5B). However, a higher concentration of B-6029 (42.5 μM) resulted in a significant ($P = 0.006$) attenuation of 3-MG transport. Neither BK2 antagonist, regardless of concentration, altered baseline tension, peak tension, or fatigue index.

**KHB ± bradykinin.** Supplementing KHB with 10, 100, or 1,000 ng/ml bradykinin during contractions did not result in significantly enhanced insulin-stimulated 3-MG transport compared with paired muscles contracting in KHB without added bradykinin (Fig. 6). Bradykinin had no effect on baseline tension, peak tension, or fatigue index.

**DISCUSSION**

The kallikrein-kininogen system has several characteristics that led us to believe that it might be involved in the postcontraction increase in insulin sensitivity, which has been shown to involve serum protein(s) of MW >10 kDa (9). I) The kallikrein-kininogen system is activated by muscle contractile activity (2, 27, 35, 36). 2) There is abundant evidence that activation of this system can enhance insulin sensitivity (6, 10, 15,
The kallikrein-kininogen system includes serum proteins with MW >10,000 (kininogen and kallikrein) (1). Therefore, our goal was to assess the possibility that the kallikrein-kininogen system plays a role in the serum-effect on the contraction-induced increase in insulin sensitivity for glucose transport.

We studied the effects of SBTI and aprotinin, which are commonly used kallikrein inhibitors (11, 12, 24, 32). SBTI is believed to more effectively inhibit plasma kallikrein, whereas aprotinin is considered a more effective inhibitor of tissue kallikrein (1, 12, 22, 24). Substrate preference is not absolute, as each compound has inhibitory properties against the alternate kallikrein. Plasma kallikrein and tissue kallikrein are distinct proteins that originate from different genes (1). In skeletal muscle, tissue kallikrein is localized in the endothelium and on the surface of the sarcolemma (1, 21, 34). Tissue kallikrein that has been released by cells can also be found in plasma. Purified plasma kallikrein activity has been shown to be completely inhibited by 25 μg/ml SBTI (37), well below the concentration (100 μg/ml) used in this and many other studies (11, 12, 24, 32). The concentration of aprotinin used in this study (1,000 KIU/ml) inhibited (98%) purified tissue kallikrein activity from rat vascular tissue (24). Both aprotinin (500 KIU/ml) and SBTI (100 μg/ml) reduced (70 and 35%, respectively) kinin release from rat hindlimb perfused with serum-free buffer, presumably reflecting the amount of tissue kallikrein inhibition (32). In the present study, the combination of these two kallikrein inhibitors during contractions tended to attenuate the subsequent insulin sensitivity. Neither SBTI nor aprotinin alone significantly changed the contraction-induced increase in insulin sensitivity, perhaps because of insufficient inhibition of kallikrein activity.

Gao et al. (9) found that contractions in the presence of human serum results in enhanced insulin-stimulated glucose transport similar to contractions in rat serum. Our results demonstrate that human plasma can yield a similar effect, and in addition we have shown that contractions in human kallikrein-deficient plasma does not. Kallikrein deficiency (also known as Fletcher trait) is a rare genetic disorder without obvious consequences other than abnormal blood clotting (31). The only known abnormality in plasma from individuals with congenital plasma kallikrein deficiency is a lack of kallikrein activity (e.g., kininogen concentration is present at normal levels). The results with kallikrein-deficient plasma implicate a role for plasma kallikrein in the postcontraction increase in insulin sensitivity, but it remains possible that tissue kallikrein can also influence this effect.

Fig. 4. Rate of 3-MG transport in paired muscles that differ by the presence (open bars) or absence (filled bars) of either normal human plasma (A) or human kallikrein-deficient plasma (B) during the first 25 min of incubation. All pairs of muscles were stimulated to contract in human plasma or KHB, followed by a 3-h incubation in plasma-free buffer, and then were insulin stimulated. Data are means ± SE (n = 8–9 paired muscles). *Significant difference for without normal human plasma vs. with normal human plasma for paired muscles that were stimulated to contract and insulin stimulated (P < 0.05).
lated glucose transport. HOE-140 was studied at a concentration (1 μM) that completely blocked specific bradykinin binding in dog skeletal muscle membrane preparations (25) and at a higher concentration (12.8 μM). In contrast to the wealth of information regarding HOE-140, published studies with B-6029, to our knowledge, have only described in vivo effects of the compound, providing information about infusion rates but not blood concentrations of the compound (18, 20). Including B-6029 during contractions in serum caused a reduction in the subsequent insulin-stimulated glucose transport at a higher (42.5 μM) but not lower (10 μM) concentration. If B-6029 and HOE-140 act solely by inhibition of BK2 receptors, they would be expected to similarly affect glucose transport. The divergent results with isolated muscles may reflect a nonspecific effect at the higher concentration of B-6029.

Fig. 5. Rate of 3-MG transport in paired muscles that differ by the presence (open bars) or absence (filled bars) of either bradykinin receptor-2 (BK2) antagonist (HOE-140, 1.0 or 12.8 μM; A) or BK2 antagonist (B-6029, 10.0 or 42.5 μM; B) during the first 25 min of incubation. All pairs of muscles were stimulated to contract in rat serum, followed by 3 h in serum-free buffer, and then were insulin stimulated. Data are means ± SE (n = 12–15 paired muscles).

*Significant difference for without BK2 antagonist vs. with BK2 antagonist for paired muscles.

Fig. 6. Rate of 3-MG transport in paired muscles that differ by the presence (open bars) or absence (filled bars) of bradykinin (BK) of varying concentrations during the first 25 min of incubation. All pairs of muscles were stimulated to contract in serum-free buffer, followed by a 3-h incubation in serum-free buffer, and then were insulin stimulated. Data are means ± SE (n = 10–17 paired muscles).
Supplementation of KHB with bradykinin during contractions did not alter the subsequent insulin-stimulated glucose transport. Bradykinin concentrations used in this investigation elicited biological effects in isolated cell preparations (15, 17, 23). Precise measurement of bradykinin levels in tissues is confounded by rapid degradation of bradykinin by tissue kininases: bradykinin half-life is ~10–30 s (1, 7, 21). Therefore, reported values of bradykinin are likely to underestimate tissue concentrations. Accordingly, a range of bradykinin concentrations was evaluated, including values similar to those reported in blood- or perfusate-draining contracting skeletal muscle (2, 32, 35, 36), as well as concentrations that were higher than the reported value levels. Our data indicate that increasing bradykinin across a wide range of bradykinin levels during isolated contractions was not sufficient for the contraction-induced increase in insulin sensitivity.

The negative results with bradykinin and HOE-140 raise the possibility that kallikrein may influence postcontraction insulin action by a bradykinin-independent mechanism. In most mammals, tissue kallikrein catalyzes the release of lys-bradykinin (called kallidin) from low-MW kininogens, its preferred substrate. However, in the rat, both tissue and plasma kallikrein catalyze the release of bradykinin (and not kallidin) from both high-MW and low-MW kininogens (1, 16). Kallikreins can also act on proteins other than kininogens (1). Perhaps the results we observed are related to this catalytic activity. In addition, although there was an apparent lack of effects of bradykinin or HOE-140 on postcontraction glucose transport in isolated muscles, it remains possible that these molecules can influence postexercise glucose uptake in the in vivo condition (36).

The underlying reason for the apparent relationship between fatigue index and glucose transport in muscles that had been stimulated to contract in KHB (higher glucose transport appeared to be associated with less fatigue) is uncertain, particularly because the mechanisms for fatigue itself are poorly understood. Regardless, enhanced postcontraction insulin sensitivity was found with either rat serum or normal human plasma, despite their differing effects on contractile performance (peak tension and fatigue). These findings suggest that the effects of serum and plasma on postcontraction glucose transport were not attributable to changes in peak tension or fatigue. Decreased peak tension in muscles contracting in plasma (both normal and kallikrein deficient) was likely caused by the anticoagulant sodium citrate, which can chelate extracellular cations and presumably influences ionic flux across the sarcolemma. Both serum and plasma prevented increases in baseline tension in contracting muscles, but this effect was also found in muscles that contracted in kallikrein-deficient plasma, and this treatment did not lead to subsequent improvement in insulin-stimulated glucose transport. None of the inhibitors studied had an effect on peak tension, fatigue index, or baseline tension.

In conclusion, supplementing serum with kallikrein inhibitors during contractions tended to attenuate the subsequent contraction-induced increase in insulin-stimulated glucose transport. Muscles stimulated to contract in normal human plasma, but not human plasma deficient in kallikrein, exhibited enhanced insulin action. These kallikrein-related effects were not attributable to changes in contractile performance. The addition of bradykinin during contractions did not enhance insulin action, and supplementing serum with the BK2-receptor antagonist HOE-140 did not diminish subsequent glucose transport, although a high concentration of another BK antagonist did. Taken together, these results suggest that kallikrein activity may play a role in the enhanced insulin action after in vitro contractions performed in serum or plasma.

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