Effect of chronic bradykinin administration on insulin action in an animal model of insulin resistance

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Henriksen, Erik J., Stephan Jacob, Donovan L. Fogt, and Guenther J. Dietze. Effect of chronic bradykinin administration on insulin action in an insulin resistance. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R40–R45, 1998.—The nonapeptide bradykinin (BK) has been implicated as the mediator of the beneficial effect of angiotensin-converting enzyme inhibitors on insulin-stimulated glucose transport in insulin-resistant skeletal muscle. In the present study, the effects of chronic in vivo BK treatment of obese Zucker (fa/fa) rats, a model of glucose intolerance and severe insulin resistance, on whole body glucose tolerance and skeletal muscle glucose transport activity stimulated by insulin or contractions were investigated. BK was administered subcutaneously (twice daily at 40 µg/kg body wt) for 14 consecutive days. Compared with a saline-treated obese group, the BK-treated obese animals had significantly (P < 0.05) lower fasting plasma levels of insulin (20%) and free fatty acids (26%), whereas plasma glucose was not different. During a 1 g/kg body wt oral glucose tolerance test, the glucose and insulin responses [incremental areas under the curve (AUC)] were 21 and 29% lower, respectively, in the BK-treated obese group. The glucose-insulin index, the product of the glucose and insulin AUCs and an indirect index of in vivo insulin action, was 52% lower in the BK-treated obese group compared with the obese control group. Moreover, 2-deoxyglucose uptake in the isolated epitrochlearis muscle stimulated by a maximally effective dose of insulin (2 mU/ml) was 52% greater in the BK-treated obese group. Contraction-stimulated (10 tetani) 2-deoxyglucose uptake was also enhanced by 35% as a result of the BK treatment. In conclusion, these findings indicate that in the severely insulin-resistant obese Zucker rat, chronic in vivo treatment with BK can significantly improve whole body glucose tolerance, possibly as a result of the enhanced insulin-stimulated skeletal muscle glucose transport activity observed in these animals.

Methods

Animals and treatments. Female obese Zucker rats (Hsd/ Ola:ZUCKER-fa; Harlan, Indianapolis, IN) and lean littermates (Fa−−) were received at 6–7 wk of age and were housed two per cage in a temperature-controlled room (20–22°C) at the Central Animal Facility of the University of Arizona. A 12:12-h light-dark cycle was maintained, and animals had free access to water and chow (Purina, St. Louis, MO). All procedures described below were approved by the University of Arizona Animal Use and Care Committee. Starting at 8 wk of age, lean and obese animals received subcutaneous injection of either 0.9% saline (vehicle-treated controls) or bradykinin (B3259; Sigma Chemical, St. Louis, MO) twice daily at 40 µg/kg body wt for 14 consecutive days. This is an effective acute glucose-lowering bradykinin dose in...
diabetic rodents (35). Although a single dose of bradykinin does acutely enhance insulin action in the obese Zucker rat (unpublished observations), it is unlikely that the effects on insulin action seen 20 h after the last chronic bradykinin treatment can be ascribed to a long-lasting acute effect of this last bradykinin administration, as bradykinin has a relatively short half-life (35).

Oral glucose tolerance tests. Animals were food-restricted (4 g of chow given at 5 PM, which was immediately consumed) the evening before the experiment. Between 8 and 10 AM, ~20 h after the most recent treatment, the animals underwent an oral glucose tolerance test (OGTT) using a 1 g/kg body wt glucose feeding by gavage (6). Blood was drawn from a cut at the tip of the tail at 0, 15, 30, and 60 min after the glucose feeding. This whole blood was thoroughly mixed with EDTA (18 mM final concentration) and centrifuged at 13,000 g to separate the plasma. Plasma samples were frozen at −70°C and subsequently analyzed for glucose (Sigma), insulin (Linco Research, St. Charles, MO), and free fatty acids (Wako, Richmond, VA). Immediately after the completion of the OGTT, all animals received 2 ml of sterile 0.9% saline subcutaneously to compensate for plasma loss, and vehicle or bradykinin treatments were resumed for 2 further days.

Glucose transport activity. At 8 AM, ~20 h after the final treatment and again having been restricted to 4 g of chow in the previous 15 h, animals were deeply anesthetized with pentobarbital sodium (Nembutal, 50 mg/kg ip). Both epitrochlearis muscles were surgically removed and prepared for in vitro incubation. Epitrochlearis muscles were initially incubated (without tension throughout) for 60 min in 3 ml of oxygenated Krebs-Henseleit buffer (KHB) containing 8 mM glucose, 32 mM mannitol, and 0.1% BSA (radioimmunoassay grade). In experiments investigating the effect of bradykinin treatment on insulin action, one muscle from each animal was incubated in the absence of insulin, while the contralateral muscle underwent the electrical stimulation protocol. For electrical stimulation of muscle contractions, the distal end of the epitrochlearis muscle was attached to a vertical Lucite rod containing two platinum electrodes. The proximal end was clipped to a jeweler’s chain and attached to a Grass model FTO3 isometric force transducer. The mounted muscle was immersed in 25 ml KHB containing 8 mM glucose and 32 mM mannitol and continuously oxygenated with 95% O2-5% CO2 at 37°C. The muscle was stimulated with supramaximal square wave pulses of 0.2-ms duration using a Grass S11 stimulator. Ten tetanic contractions were produced by stimulating at 50 Hz for 10 s at a rate of 1 contraction/min. Glucose transport activity was then assessed as described above.

Muscle biochemistry. The remaining muscle piece was homogenized in 40 volumes of ice-cold buffer containing 20 mM HEPES (pH 7.4), 1 mM EDTA, and 250 mM sucrose. The homogenates were then frozen at −70°C until analysis of total protein concentration (bicinchoninic acid method, Sigma), GLUT-4 protein (16), total hexokinase activity (33), and citrate synthase activity (29).

Statistical analysis. All data are presented as means ± SE. The significance of differences between two groups was determined by an unpaired Student’s t-test, whereas the significance of differences between multiple groups was assessed by analysis of variance with Duncan’s multiple-range post hoc tests. Statistical significance was set at the 0.05 probability level.

RESULTS

Plasma glucose, insulin, and free fatty acids. Final body weights, epitrochlearis muscle and heart wet weights, and plasma glucose values within the lean and obese groups were not significantly affected by the chronic bradykinin treatment (Table 1). In the lean group, chronic administration of bradykinin had no effect on plasma insulin or free fatty acids. In contrast, the chronic administration of bradykinin to obese animals resulted in a 20% decrease (P < 0.05) in plasma insulin and a 26% decline (P < 0.05) in plasma free fatty acids relative to the obese vehicle-treated control group.

OGTT responses. In the lean animals, treatment with bradykinin had no significant effect on disposal of the

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt, g</th>
<th>Epitrochlearis Wt, mg</th>
<th>Heart Wt, mg</th>
<th>Glucose, mg/dl</th>
<th>Insulin, µU/ml</th>
<th>Free Fatty Acids, mM</th>
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<tbody>
<tr>
<td>Lean vehicle treated</td>
<td>165 ± 2</td>
<td>35.6 ± 1.3</td>
<td>479 ± 14</td>
<td>130 ± 11</td>
<td>14 ± 1</td>
<td>0.52 ± 0.05</td>
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<tr>
<td>Lean chronic bradykinin treated</td>
<td>159 ± 2</td>
<td>31.4 ± 0.9</td>
<td>472 ± 5</td>
<td>125 ± 5</td>
<td>12 ± 1</td>
<td>0.52 ± 0.05</td>
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<tr>
<td>Obese vehicle treated</td>
<td>359 ± 7*</td>
<td>36.2 ± 0.6</td>
<td>694 ± 10</td>
<td>147 ± 5</td>
<td>183 ± 19*</td>
<td>1.53 ± 0.13*</td>
</tr>
<tr>
<td>Obese chronic bradykinin treated</td>
<td>360 ± 8*</td>
<td>36.6 ± 1.3</td>
<td>672 ± 6</td>
<td>136 ± 5</td>
<td>147 ± 13†</td>
<td>1.13 ± 0.05†</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5–10 animals (lean) or 10–15 animals (obese) per group. Bradykinin was administered subcutaneously twice daily at 40 µg/kg for 14 days. *P < 0.05 vs. lean vehicle-treated animals; †P < 0.05 vs. obese vehicle-treated animals.
glucose load during the OGTT (Fig. 1, top left; Fig. 2A, left). In addition, the insulin response to this glucose load was not substantially affected by chronic bradykinin treatment in the lean animals (Fig. 1, bottom left; Fig. 2A, middle). Chronic treatment of the obese animals with bradykinin resulted in uniformly lower (P < 0.05) plasma glucose values during the OGTT (Fig. 1, top right), and the incremental area under the glucose curve was 21% smaller (P < 0.05) compared with obese control values (Fig. 2B, left). Moreover, insulin values in plasma from the bradykinin-treated obese animals were also 20–31% less (P < 0.05) during the OGTT (Fig. 1, bottom right), and the incremental area under the insulin curve was 29% smaller (P < 0.05) in this group compared with obese controls (Fig. 2B, middle). The glucose-insulin index, the product of the glucose and insulin areas under the curve and an indirect index of in vivo peripheral insulin action (6), was 52% lower (P < 0.05) in the bradykinin-treated obese group compared with the obese control group (Fig. 2B, right).

Muscle glucose transport activity. No alteration in basal 2-deoxyglucose uptake due to prior chronic bradykinin treatment was observed in epitrochlearis muscles from either the lean animals (140 ± 19 vs. 160 ± 8 pmol·mg muscle⁻¹·20 min⁻¹) or the obese animals (123 ± 7 vs. 110 ± 11 pmol·mg muscle⁻¹·20 min⁻¹). Insulin-mediated (Fig. 3A) and contraction-mediated (Fig. 4A) increases in 2-deoxyglucose uptake above basal in the epitrochlearis muscle were not significantly different in the lean bradykinin-treated group compared with the lean control group. Following chronic administration of bradykinin to obese animals, insulin action on muscle glucose transport activity was 52% greater (P < 0.05) relative to the obese control group (Fig. 3B). In addition, glucose transport activity stimulated by muscle contractions was 35% greater (P < 0.05) in the bradykinin-treated obese group compared with obese controls.

Muscle biochemistry. There were no changes induced by bradykinin treatment of lean or obese animals in the epitrochlearis muscle activities for total hexokinase and citrate synthase, nor were total homogenate GLUT-4 protein levels altered by this intervention in epitrochlearis muscle from lean or obese animals (data not shown).

**Fig. 1.** Effects of chronic treatment with bradykinin on glucose and insulin responses to an oral glucose tolerance test in lean (left) and obese (right) Zucker rats. Animals were treated for 14 days with either vehicle or bradykinin (twice daily at 40 µg/kg), and the glucose and insulin values during a 1 g/kg oral glucose tolerance test were determined. Data are means ± SE for 5–10 animals (lean) or 10–15 animals (obese) per group. *P < 0.05 vs. vehicle-treated group at same time point.

**Fig. 2.** Effects of chronic treatment with bradykinin on incremental areas under the curves (AUC) for glucose and insulin during an oral glucose tolerance test and the glucose-insulin index in lean (A) and obese (B) Zucker rats. Values are means ± SE for vehicle-treated (open bars) and bradykinin-treated (solid bars) animals. Data for the AUCs were taken from Fig. 1. The glucose-insulin index is calculated as the product of the glucose AUC and the insulin AUC for each animal. *P < 0.05 vs. vehicle-treated group at same time point.
The beneficial effects of chronic treatment with bradykinin were observed only in the obese animals and not in the lean animals. These findings indicate that treatment with bradykinin overcomes a defect specific to the insulin-resistant animal. Although this specific defect remains unclear, it may involve systemic and/or local kallikrein-kinin systems.

Several animal model and human clinical investigations have demonstrated that chronic administration of ACE inhibitors can improve insulin-stimulated whole body and skeletal muscle glucose disposal in insulin-resistant subjects (13, 17, 19, 22, 24, 25, 28, 30, 31, 34). One contention from these investigations has been that the elevation in circulating bradykinin levels, resulting from the inhibition of bradykinin degradation (10), underlies the beneficial metabolic effects of ACE inhibitors (6, 17, 31). The present investigation, in which bradykinin was directly administered to insulin-resistant rats, supports this hypothesis that bradykinin can modulate the effect of insulin to stimulate skeletal muscle glucose transport and improve whole body glucose tolerance.

Additional evidence in the scientific literature supports a role of kinins, such as bradykinin or one of its metabolic products, in the regulation of insulin-dependent skeletal muscle glucose transport. For example, Wicklmayr and Dietze (35) showed that bradykinin, provided with an otherwise noneffective dose of insulin, had a marked blood glucose-lowering effect in alloxan-diabetic rats. Henriksen and Jacob (17) showed that acute and chronic oral treatment with the ACE inhibitor captopril causes a significant improvement in insulin-mediated glucose transport activity in skeletal muscle. Moreover, this acute ACE inhibitor-induced improvement in insulin action could be completely prevented by pretreatment with HOE-140, a selective B2-bradykinin receptor antagonist that blocks the formation and metabolism of bradykinin. Similar findings have been reported by Uehara et al. (31) using a diabetic dog model. Importantly, it has been shown recently that bradykinin B2 receptors are present in the sarcolemmal membrane of skeletal muscle (12), indicating that bradykinin can act directly on skeletal muscle. Indeed, several studies have demonstrated that direct bradykinin administration can improve insulin action on glucose uptake by muscle (9, 22, 23), although this is not a uniform finding (5).

Although the mechanism of action of bradykinin on muscle glucose transport is not directly addressed in the present study, several possibilities exist. Stimulation of the translocation of the glucose transporter isoform GLUT-4 is defective in muscle from the obese Zucker rat (11, 21), and recent investigations indicate that bradykinin administration can stimulate GLUT-4 translocation in skeletal muscle (32) and cardiac muscle (27). This stimulation of GLUT-4 translocation in muscle by bradykinin may be attributed to the interactions of the bradykinin and insulin intracellular signaling pathways. Carvalho et al. (3) recently demonstrated in insulin-resistant skeletal muscle from aged rats that bradykinin can enhance insulin-induced phosphorylation of insulin receptors and insulin receptor sub-
strate-1 (IRS-1), as well as the insulin-stimulated association of IRS-1 and phosphatidylinositol-3-kinase, all of which are essential for insulin-mediated GLUT-4 translocation and glucose transport (4). The potential interaction of bradykinin and the intracellular factors utilized in the contraction-dependent pathway for stimulation of GLUT-4 translocation and activation of glucose transport, which are still ill-defined, has yet to be addressed experimentally.

Insulin resistance of skeletal muscle glucose disposal may be in part related to elevated circulating free fatty acid levels (see recent review in Ref. 2). Indeed, compared with the lean control animals, the obese control animals displayed substantially elevated plasma free fatty acid levels (Table 1) and insulin resistance of whole-body glucose disposal (Fig. 2) and skeletal muscle glucose transport (Fig. 3). Moreover, chronic administration of bradykinin was associated with a significant diminution of circulating free fatty acids and an increase in insulin action on muscle glucose transport activity. These findings support the possibility that the increase in insulin action seen with chronic bradykinin treatment was secondary to the reduction in plasma free fatty acids elicited by this intervention.

We have found previously that chronic administration of the ACE inhibitortrandolapril is associated with increased muscle levels of GLUT-4 protein and hexokinase activity (19). Interestingly, bradykinin treatment did not increase either of these factors, indicating that the increased expression of these variables elicited by this ACE inhibitor is likely not mediated by kinins. In addition, the ACE inhibitor-induced regression of cardiac hypertrophy normally observed in the obese Zucker rat (17, 19) was not mimicked by the bradykinin treatment (Table 1), suggesting that kinins are not directly responsible for this cardiac remodeling following ACE Inhibitor treatment.

Perspectives

Insulin resistance and the compensatory hyperinsulinemia are often accompanied in the same individual by hypertension, dyslipidemia, and central adiposity, a condition collectively referred to as the insulin resistance syndrome or syndrome X. Although ACE inhibitors have been shown to be effective in reducing blood pressure as a primary effect and enhancing insulin action as a secondary effect in such individuals, it was not clear whether the metabolic improvements elicited by ACE inhibitors could be attributed, at least in part, to the nonapeptide bradykinin. We now have shown in the present investigation that the chronic in vivo administration of bradykinin to an animal model of insulin resistance, hyperinsulinemia, glucose intolerance, and dyslipidemia, the obese Zucker rat, can bring about significant metabolic improvements, including reductions in plasma insulin and free fatty acid levels and enhanced glucose tolerance and insulin action on whole body glucose disposal and on skeletal muscle glucose transport. In addition, we have demonstrated that bradykinin treatment can increase contraction-mediated glucose transport activity in skeletal muscle.

These data are therefore consistent with an important role of bradykinin as a mediator of the beneficial effects of ACE inhibitors on insulin resistance. Future investigations should focus on the molecular mechanisms, including the insulin signaling factors and GLUT-4 translocation, whereby bradykinin enhances skeletal muscle glucose transport in conditions of insulin resistance.

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