ACE inhibition and glucose transport in insulin-resistant muscle: roles of bradykinin and nitric oxide

ERIK J. HENRIKSEN,1 STEPHAN JACOB,2 TYSON R. KINNICK,1 ERIK B. YOUNGBLOOD,1 MELANIE B. SCHMIT, 1AND GUENTHER J. DIETZE2
1Muscle Metabolism Laboratory, Department of Physiology, University of Arizona College of Medicine, Tucson, Arizona 85721–0093; and 2Hypertension and Diabetes Research Unit, Max-Grundig-Klinik, 77815 Bühl, Germany

Henriksen, Erik J., Stephan Jacob, Tyson R. Kinnick, Erik B. Youngblood, Melanie B. Schmit, and Guenther J. Dietze. ACE inhibition and glucose transport in insulin-resistant muscle: roles of bradykinin and nitric oxide. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R332–R336, 1999.—Acute administration of the angiotensin-converting enzyme (ACE) inhibitor captopril enhances insulin-stimulated glucose transport activity in skeletal muscle of the insulin-resistant obese Zucker rat. The present study was designed to assess whether this effect is mediated by an increase in the nonapeptide bradykinin (BK), by a decrease in action of ANG II, or both. Obese Zucker rats (8–9 wk old) were treated for 2 h with either captopril (50 mg/kg orally), bradykinin (200 µg/kg ip), or the ANG II receptor (AT1 subtype) antagonist eprosartan (20 mg/kg orally). Captopril treatment enhanced in vitro insulin-stimulated (2 mM/l) 2-deoxyglucose uptake in the epitrochlears muscle by 22% (251 ± 7 vs. 205 ± 9 pmol·mg⁻¹·20 min⁻¹; P < 0.05), whereas BK treatment enhanced this variable by 18% (249 ± 15 vs. 215 ± 7 pmol·mg⁻¹·20 min⁻¹; P < 0.05). Eprosartan did not significantly modify insulin action. The BK-mediated increase in insulin action was completely abolished by pretreatment with either the specific BK-B1 receptor antagonist HOE 140 (200 µg/kg ip) or the nitric oxide synthase inhibitor N′-nitro-arginine methyl ester (50 mg/kg ip). Collectively, these results indicate that the modulation of insulin action by BK likely underlies the metabolic effects of ACE inhibitors in the insulin-resistant obese Zucker rat. Moreover, this modulation of insulin action by BK is likely mediated through BK-B2 receptors and by an increase in nitric oxide production and/or action in skeletal muscle tissue.

The majority of individuals with essential hypertension also display insulin resistance of peripheral glucose disposal and hyperinsulinemia (24). This clustering of several atherogenic risk factors, which can also include glucose intolerance, dyslipidemia, and central obesity, has been termed the “insulin resistance syndrome” (6). A frequently used intervention for the treatment of high blood pressure has been angiotensin-converting enzyme (ACE) inhibitors. This is an effective type of intervention for individuals with the insulin resistance syndrome, as ACE inhibitors not only reduce blood pressure, they also improve insulin action on peripheral glucose disposal, as shown in both animal model studies (13, 16, 28) and clinical investigations (17, 23, 24, 27–29).

Although several groups have documented the effectiveness of ACE inhibitors in enhancing insulin action in conditions of insulin resistance, the mechanism of action of these compounds in eliciting this beneficial metabolic effect is less well characterized. ACE inhibitors have at least two effects at the tissue level: they can inhibit the conversion of ANG I to ANG II, and they can also enhance bradykinin levels (28) by inhibiting the kininase II-mediated degradation of this nonapeptide (30). There is evidence that bradykinin itself may have an effect in enhancing insulin action and insulin signaling at the skeletal muscle level (4, 7, 14, 19, 20, 22). However, the role of the acute reduction in ANG II action in the metabolic effects of ACE inhibitor treatments in conditions of insulin resistance is currently less well described.

Bradykinin can bind to skeletal muscle cell surface BK-B1 kinin receptors (10, 22). Evidence to date indicates that one outcome of bradykinin administration to skeletal muscle tissue is an increase in activation of nitric oxide (NO) synthase and NO production (8, 21, 26). The role of NO in the modulation of skeletal muscle glucose transport and metabolism remains controversial. Whereas some studies have indicated that NO or NO donors, such as sodium nitroprusside, can enhance insulin-stimulated glucose oxidation (31, 32) or insulin-independent glucose transport (1, 2, 9, 25) in isolated rat skeletal muscle, others have reported that enhancement of muscle NO in vitro causes a decrease in insulin-stimulated skeletal muscle glucose transport (18). To date, no study has investigated the role of NO as a potential mediator of bradykinin action on glucose transport in insulin-resistant skeletal muscle.

In this context, the purpose of the present investigation was to assess and compare the respective roles of increased bradykinin and decreased ANG II action on...
insulin-stimulated glucose transport activity in skeletal muscle of the insulin-resistant obese Zucker rat.
Furthermore, using the NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME), we wished to
determine the possible role of NO in the modulation of
insulin action by bradykinin in this animal model of
insulin resistance.

MATERIALS AND METHODS

Animals and treatments. Female obese Zucker rats (Hsd/
Ola:ZUCKER-fa; Harlan, Indianapolis, IN) 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 were approved by the
University of Arizona Animal Care and Use Committee.

All experiments were performed when the animals were
8–9 wk of age and after an overnight period of food restriction
(4 g of chow was provided at 5:00 PM the evening before the
experiment). Starting at 8:00 AM, rats received hourly treat-
ments for 2 h of either vehicle (water for experiments
involving oral administration of a compound) or 0.9% saline
for intraperitoneal administration of a compound), the ACE
inhibitor captopril (50 mg/kg body wt orally; Sigma, St. Louis,
MO), the nonapeptide bradykinin (200 µg/kg ip, Sigma B3259),
or ANG II receptor (AT1 subtype) antagonist eprosartan (20
mg/kg orally; SmithKline Beecham, Munich, Germany).

In separate experiments, the animals were pretreated 1 h
before the commencement of bradykinin administration with
either the bradykinin B2 receptor antagonist HOE-140 (200
µg/kg ip, kindly provided by Hoechst-Roussel Pharmaceuticals,
Somerville, NJ) or the NO synthase inhibitor l-NAME
(50 mg/kg ip, Sigma). Treatments with HOE-140 and l-
NAME were also given concomitantly with the subsequent
bradykinin administrations.

Glucose transport activity. At the completion of the treat-
ment periods, animals were deeply anesthetized with pento-
barbital 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
Kreb-Henseleit buffer (KHB) containing 8 mM glucose, 32
mM mannitol, and 0.1% BSA (RIA grade). One muscle from
each animal was incubated in the absence of insulin, whereas
the contralateral muscle was incubated in medium contain-
ing a maximally effective concentration of insulin (2 mU/ml;
Humulin R, Eli Lilly, Indianapolis, IN). The flasks were
shaken in a Dubnoff incubator at 37°C and had a gas phase of
95% O2-5% CO2. After the initial treatments, all muscles were
rinsed for 10 min at 37°C in 3 ml of oxygenated KHB
containing 40 mM mannitol, 0.1% BSA, and, if present
previously, insulin. The muscles were then transferred to
flasks containing 2 ml of oxygenated KHB, 0.1% BSA, 1 mM
2-deoxy [1,2-3H]glucose (2-DG; 300 mCi/mmol), 39 mM
[U-14C]mannitol (0.8 mCi/mmol; ICN Radiochemicals, Irvine,
CA), and insulin, if present previously. After this final 20-min
incubation period at 37°C, muscles were trimmed of fat,
extraneous muscle, and connective tissue, frozen between
aluminum blocks cooled to the temperature of liquid N2, and
weighed. The frozen muscles were dissolved in 0.5 ml of 0.5 N
NaOH and used to determine glucose transport activity as
described by Henriksen and Ritter (15). Incubated epitrochle-
aris muscles of this size remain metabolically viable (11),
and this method for assessing glucose transport activity in the
epitrochlearis muscles has been validated (12).

Statistical analysis. The significance of differences between
two groups was determined by an unpaired Student’s t-test.
Differences between more than two groups were assessed by
ANOVA and Dunnett’s multiple range post hoc tests, with the
obese vehicle-treated control group being the reference group.
A P value of <0.05 was considered significant.

RESULTS

In all experiments described below, there were no
differences between groups for the final body weights
(300–320 g) or the incubated epitrochlearis weights
(32–35 mg).

Effects of acute treatment with captopril, bradykinin,
or eprosartan. As shown in Fig. 1, the acute treatment
with either captopril, bradykinin, or eprosartan did not
significantly affect basal 2-DG uptake in the epitrochle-
aris muscles of the obese Zucker rat. Captopril treatment
increased both the rate of insulin-stimulated 2-DG uptake above basal (33% P < 0.05).

Acute treatment with bradykinin increased the rate of
insulin-stimulated 2-DG uptake by 18% (P < 0.05) and the insulin-mediated
increase in 2-DG uptake above basal by 40% (P < 0.05). Acute treatment with eprosar-
tan had no significant effect on insulin action in the
epitrochlearis muscle.

Effects of pretreatment with HOE-140 or l-NAME
on bradykinin action. The effect of bradykinin B2 receptor

Fig. 1. Effects of acute treatment with captopril, bradykinin, or eprosartan on glucose transport activity in skeletal muscle of obese Zucker rat. 2-Deoxyglu-
cose (2-DG) uptake was determined in isolated epitrochlearis muscle in ab-
essence (open bars) or presence (filled bars) of insulin (2 mU/ml). Net in-
tcreases in 2-DG uptake above basal (Δ) caused by insulin are shown by shaded
bars. Data are means ± SE; n = 5–12
animals/group. * P < 0.05 vs. vehicle-
treated group.
antagonism with HOE-140 on the bradykinin-mediated increase in insulin action was assessed next (Fig. 2). Again, acute treatment with bradykinin led to a 19% increase \((P < 0.05)\) in the rate of insulin-stimulated 2-DG uptake and a 40% enhancement \((P < 0.05)\) in the insulin-mediated increase in 2-DG uptake above basal. Pretreatment with HOE-140 completely abolished the effect of bradykinin on insulin action on glucose transport activity in the epitrochlearis muscle of the obese Zucker rat. Treatment of obese Zucker rats with HOE-140 alone (without bradykinin administration) did not alter basal or insulin-stimulated muscle 2-DG uptake (data not shown).

Finally, the role of NO in the bradykinin-mediated increase in insulin action was investigated using the NO synthase inhibitor L-NAME. Treatment of obese Zucker rats with L-NAME alone led to a 21% decrease \((P < 0.05)\) in the basal rate of 2-DG uptake \((98 \pm 6 \text{ vs. } 77 \pm 5 \text{ pmol} \cdot \text{mg}^{-1} \cdot \text{20 min}^{-1})\), but did not diminish the insulin-mediated increase in 2-DG uptake above basal \((116 \pm 5 \text{ vs. } 111 \pm 4 \text{ pmol} \cdot \text{mg}^{-1} \cdot \text{20 min}^{-1}, \text{NS})\). L-NAME pretreatment of bradykinin-treated obese Zucker rats also tended to cause a 15% decrease in basal 2-DG uptake (Fig. 3). However, more striking in Fig. 3 is the observation that pretreatment with L-NAME completely prevented the enhanced insulin action normally seen after bradykinin administration.

DISCUSSION

In the present study, we have confirmed our previous observations \((13, 16)\) that the acute administration of ACE inhibitors enhances insulin-stimulated glucose transport activity in skeletal muscle of the markedly insulin-resistant obese Zucker rat (Fig. 1, left). Moreover, we have provided new information that this effect is likely mediated by the increased action of the nonapeptide bradykinin on this process (Fig. 1, middle) and that the effect of bradykinin on insulin action is mediated through its \(B_2\) receptors (Fig. 2). Importantly, we have also demonstrated that the inhibition of ANG II action by the use of the \(AT_1\) receptor antagonist eprosartan had no effect on insulin action in skeletal muscle of this animal model of insulin resistance (Fig. 1, right). These results are concordant with the interpretation that the acute ability of ACE inhibitors to augment insulin-stimulated glucose transport activity in insulin-resistant skeletal muscle is mediated primarily by the action of bradykinin, with little or no contribution from the decrease in ANG II action.

These results of the present investigation are consistent with and complement several recent findings. Henriksen and Jacob \((13)\) showed that the acute effect of the ACE inhibitor captopril on insulin-stimulated glucose transport in skeletal muscle of the obese Zucker rat could be completely prevented by the \(B_2\) receptor antagonist HOE-140, implicating an important role of bradykinin action in the effect of that ACE inhibitor. We now have more direct evidence supporting such a contention (Fig. 1). Carvalho et al. \((4)\) demonstrated that the acute administration of captopril to insulin-resistant aged rats augmented in skeletal muscle the early steps in the insulin-signaling cascade, such as insulin-induced phosphorylation of insulin receptors and insulin receptor substrate-1 \((IRS-1)\) and the insulin-stimulated association of IRS-1 and phosphotyrosylinositol-3-kinase. Moreover, this effect of captopril on insulin signaling in skeletal muscle was reproduced by acute treatment with bradykinin, but not after ANG II receptor antagonism with losartan \((4)\). Miyata et al. \((22)\) also showed that bradykinin administration can enhance insulin signaling, glucose transporter isoform \((GLUT)-4\) translocation, and insulin-stimulated glucose uptake in canine skeletal muscle. It is likely, therefore, that bradykinin can enhance insulin-stimu-

![Fig. 2. Effect of bradykinin \(B_2\) receptor antagonism by HOE-140 on bradykinin-mediated enhancement of insulin-stimulated glucose transport activity in skeletal muscle of obese Zucker rat. 2-DG uptake was determined in isolated epitrochlearis muscle in absence (open bars) or presence (filled bars) of insulin (2 mU/ml). Net increases in 2-DG uptake above basal caused by insulin are shown by shaded bars. Data are means ± SE; \(n = 5\) animals/group. *\(P < 0.05\) vs. vehicle-treated group.]

![Fig. 3. Effect of nitric oxide synthase inhibition by N\(^{-}\)nitro-L-arginine methyl ester on bradykinin-mediated enhancement of insulin-stimulated glucose transport activity in skeletal muscle of obese Zucker rat. 2-DG uptake was determined in isolated epitrochlearis muscle in absence (open bars) or presence (filled bars) of insulin (2 mU/ml). Net increases in 2-DG uptake above basal caused by insulin are shown by shaded bars. Data are means ± SE; \(n = 5\) or 6 animals/group. *\(P < 0.05\) vs. vehicle-treated group.]
lated glucose transport activity in insulin-resistant skeletal muscle by interacting with these early steps in insulin signaling, which are essential for the activation of insulin-mediated GLUT-4 translocation and glucose transport (5).

We have also addressed the potential role of NO as a mediator of bradykinin action on insulin-stimulated glucose transport in insulin-resistant skeletal muscle (Fig. 3). The acute effect of bradykinin on insulin-stimulated muscle glucose transport was completely prevented by pretreatment with the NO synthase inhibitor l-NAME. This strongly suggests that, in this animal model of insulin resistance, bradykinin brings about an increase in NO production or action that is essential for the increase in insulin-stimulated glucose transport. Whether this NO is of endothelial or myocellular origin could not be determined with this experimental design. In addition, based on the findings of Carvalho et al. (4), which indicated that bradykinin can upregulate the early steps in insulin signaling in skeletal muscle of insulin-resistant, aged rats, one might hypothesize that bradykinin-induced NO production can positively modify the early steps in insulin signaling in insulin-resistant skeletal muscle of the obese Zucker rat. We are currently testing this hypothesis in our laboratory.

NO can be synthesized by skeletal muscle (1). The majority of previous investigations have indicated that this molecule likely is important in modulating insulin-independent glucose transport (1, 2, 25, 31), although one report supports a unique role in skeletal muscle glucose transport modulation that is both insulin and contraction independent (9). Our finding that l-NAME alone caused a decrease in basal, insulin-independent glucose transport activity in muscle without any effect on insulin action (see RESULTS) is consistent with the concept that NO may be important as a regulatory factor in basal, insulin-independent glucose transport. However, it is important to emphasize that in the present study the inhibitory effect of l-NAME on the insulin-dependent pathway for stimulation of glucose transport was only observed when this process was augmented as a result of bradykinin treatment (Fig. 3). Collectively, these findings suggest that NO can indeed modulate insulin-independent glucose transport activity directly, but they also indicate that NO has an additional role as a mediator of the beneficial effect of bradykinin on the insulin-dependent glucose transport activity in skeletal muscle of the insulin-resistant obese Zucker rat.

In the present experimental design, the whole animal was acutely treated with either ACE inhibitor, bradykinin, ANG II receptor antagonist, or NO synthase inhibitor, and subsequently glucose transport activity in skeletal muscle was assessed in vitro to eliminate the known effects of these compounds on blood flow (reviewed in Refs. 3 and 8). Our results support the concept that ACE inhibitors or bradykinin can modulate the muscle glucose transport system itself, consistent with previous studies using in vitro treatment of skeletal muscle (19, 20) or L6 myocytes (22). However, several investigations using acute in vivo treatment with ACE inhibitors, bradykinin, or modulators of NO synthase activity have demonstrated a positive association between blood flow to skeletal muscle and insulin-stimulated muscle glucose uptake (reviewed in Refs. 3 and 8). On the basis of these observations, it is likely that the acute in vivo effects of ACE inhibitors and bradykinin on improving insulin-mediated skeletal muscle glucose disposal in conditions of insulin resistance involve both augmentation of blood flow and modulation of the muscle glucose transport system, possibly at the level of insulin signaling. Both effects are probably dependent on the existence of an intact, functional endothelium for responsiveness to bradykinin and production of NO.

Perspectives

Essential hypertension is associated with an increased incidence of insulin resistance of skeletal muscle glucose transport. Treatment of hypertensive individuals with ACE inhibitors is effective in lowering blood pressure and, in numerous studies, is also accompanied by increased insulin action on skeletal muscle glucose transport. The present results indicate that, in the insulin-resistant obese Zucker rat, the acute metabolic effects of ACE inhibitors on the skeletal muscle glucose transport process are mediated primarily via the action of bradykinin through its B2 receptor, with no substantial contribution via a reduction in ANG II action. Furthermore, the results support a critical role of nitric oxide in the enhancement of insulin-stimulated glucose transport brought about by the acute administration of bradykinin. Future investigations should focus on the interactions of bradykinin and nitric oxide with the early insulin signaling factors in insulin-resistant skeletal muscle, and should also address the important issue of whether these beneficial alterations in glucose transport are caused by an increase in GLUT-4 glucose transporter translocation and/or activity.

Wethank Donovan L. Fog for excellent technical assistance. This work was supported in part by grants from the Forschergruppe Hypertonie und Diabetes e.V., Baden-Baden, Germany, and SmithKline Beecham, Munich, Germany. Present address of S. Jacob: Dept. of Endocrinology, Eberhard-Karls-Univ., 72076 Tübingen, Germany. Address for reprint requests and other correspondence: E. J. Henriksen, Dept. of Physiology, Ina E. Gittings Bldg. #93, Univ. of Arizona, Tucson, AZ 85721–0093 (E-mail: ehenrik@u.arizona.edu).

Received 4 February 1999; accepted in final form 7 April 1999.

REFERENCES


