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

ERIK J. HENRIKSEN, 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 mU/ml) 2-deoxyglucose uptake in the epitrochlearis 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-B₂ receptor antagonist HOE 140 (200 µg/kg ip) or the nitric oxide synthase inhibitor N’-nitro-l-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 B₂ receptors and by an increase in nitric oxide production and/or action in skeletal muscle tissue.

Bradykinin can bind to skeletal muscle cell surface B₂ 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 treatments 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 treatment periods, 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 (RIA grade). One muscle from each animal was incubated in the absence of insulin, whereas the contralateral muscle was incubated in medium containing a maximally effective concentration of insulin (2 µU/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/mol), 39 mM [U-14C]mannitol (0.8 mCi/mol; 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 epitrochlearis 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 epitrochlearis muscle of the obese Zucker rat. Captopril treatment increased both the rate of insulin-stimulated 2-DG uptake (22%; P < 0.05) and the insulin-mediated increase in 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 eprosartan 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
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 ± 6 vs. 77 ± 5 pmol·mg⁻¹·20 min⁻¹), but did not diminish the insulin-mediated increase in 2-DG uptake above basal (116 ± 5 vs. 111 ± 4 pmol·mg⁻¹·20 min⁻¹, 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 B₂ receptors (Fig. 2). Importantly, we have also demonstrated that the inhibition of ANG II action by the use of the AT₁ 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 B₂ 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 phosphotidylinositol-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-
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.

We thank Donovan L. Fogt for excellent technical assistance. This work was supported in part by grants from the Forscherguppe 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: ejhenrik@u.arizona.edu).

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

REFERENCES


