Insulin Resistance in Adipocytes From Spontaneously Hypertensive Rats: Effect of Long-Term Treatment With Enalapril and Losartan

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Insulin responsiveness was studied in isolated adipocytes from the normotensive Wistar Kyoto (WKY) rat and the spontaneously hypertensive rat (SHR). The effect of insulin (0.1 to 5 nmol/L) on glucose uptake (glucose transport and lipogenesis) was measured, and the maximal effect of insulin (E_{max}) and the dose of insulin required to elicit 50% of the maximal response (EC_{50}) were calculated. A diminished E_{max} on lipogenesis without changes in the EC_{50} was detected in SHRs. The E_{max} was 0.49 ± 0.09 (SHR) and 1.16 ± 0.14 (WKY) μmol/10^5 cells ($P < .05$), and the EC_{50} was 0.13 ± 0.03 and 0.11 ± 0.02 nmol/L for WKY and SHR, respectively. Similar results were obtained when measuring insulin-stimulated glucose transport. A 30-day long-term treatment with enalapril (20 mg/kg/d) normalized insulin responsiveness in adipocytes from SHRs. The effect of enalapril was suppressed when SHRs were pretreated with enalapril and 150 μg/kg/d of the bradykinin (BK) B_2-receptor blocker Hoe 140. Pretreatment with losartan (40 mg/kg/d) did not improve insulin action in the SHR. Since these results were obtained with isolated cells in which glucose availability was not a function of blood flow, and the effect of insulin in the SHR was improved by pretreatment with an angiotensin-converting enzyme (ACE) inhibitor but not with the AT_1-receptor blocker, it appears that the insulin resistance linked to the hypertension is not related to changes in blood flow.

INSULIN RESISTANCE is a common feature in essential hypertension, but whether the increase in peripheral resistance is the cause or the consequence of insulin resistance is a controversial and unresolved matter.[1-3] In some studies, it has been proposed that vasoconstriction may induce insulin resistance by decreasing nutritional blood flow and, consequently, glucose delivery to peripheral tissues.[4-6] Although vascular resistance is almost invariably increased in hypertension, it does not necessarily follow that tissue blood flow is reduced. It has been suggested that a reduction in microvessel density and autoregulatory adjustments that increase peripheral vascular resistance may be compensatory responses to prevent overperfusion of the tissues in hypertension.[7,8] Moreover, Capaldo et al[9] have reported insulin resistance in skeletal muscle from hypertensive patients even though blood flow was not reduced. In addition, insulin resistance in isolated adipocytes from the spontaneously hypertensive rat (SHR) has also been reported.[10]

It is generally accepted that angiotensin-converting enzyme (ACE) inhibitors effectively prevent an increase in peripheral vascular resistance by inhibiting the formation of angiotensin II from angiotensin I. Angiotensin II, by interacting with AT_1 receptors, is a potent vasoconstrictive hormone.

To investigate further the link between insulin resistance and hypertension, the effect of insulin on glucose uptake was determined in adipocytes from SHRs and normotensive Wistar-Kyoto (WKY) rats. To study insulin responsiveness after decreasing the blood pressure (BP) in SHRs, insulin-stimulated glucose uptake was compared between isolated cells from both strains after long-term treatment with the ACE inhibitor enalapril and the AT_1-receptor blocker losartan.

MATERIALS AND METHODS

Animals and Materials

Male SHRs aged 4 to 5 months and age-matched normotensive WKY rats were used throughout the study. An osmotic minipump (Alzet, Palo Alto, CA) was subcutaneously implanted in the rats under anesthesia to administer the bradykinin (BK) B_2-receptor antagonist Hoe 140 (kindly provided by Dr Klaus J. Wirth, Hoechst, Frankfurt, Germany). Both WKY and SHR groups were treated for 30 days as follows: (1) without treatment (control group), (2) enalapril 20 mg/kg/d in the drinking water, (3) enalapril 20 mg/kg/d in the drinking water and Hoe 140 (150 μg/kg/d) by subcutaneous infusion, and (4) losartan 40 mg/kg/d in the drinking water. Animal body weight (BW) and blood pressure (BP) were determined before and after treatment. Systolic BP was measured with the indirect tail-cuff technique.[11] Rats were killed by decapitation, and the heart and epididymal fat pads were removed. The atria and all adjacent connective tissue were removed, and the remaining tissue was blotted and weighted to determine heart weight (HW). The ratio of HW (in milligrams) to BW (in grams) was used to determine the degree of hypertrophy. The epididymal fat pads were used to isolate adipocytes.

Adipocytes

Isolated adipocytes were obtained by digestion of epididymal fat pads with collagenase (Worthington, NJ) according to the procedure of Rodbell.[12] Krebs Ringer-MOPS (KRM) at pH 7.4 containing 4% bovine serum albumin ([BSA] Fraction V; GIBCO-BRL, Gaithersburg, MD) was used as the incubation buffer.

Glucose Transport

The adipocytes (10^6/mL) were suspended in KRM at pH 7.4 with 2% BSA and 2 mmol/L pyruvate. Aliquots (100 μL) of the cell suspension were pipetted into 75 × 12-mm polystyrene tubes and preincubated without shaking at 37°C for 30 minutes. This was followed by a 10-minute incubation with shaking, with and without insulin (0.1 to 5 nmol/L). Uptake measurements were initiated by addition of 3-*O*-[methyl-^{3}H]glucose (Du Pont-New England Nuclear, Boston, MA) to a final concentration of 0.1 mmol/L (42 mCi/mmol). After 8 seconds, uptake was stopped by the addition of 400 μL 1-mmol/L phloretin in phosphate-buffered saline. Aliquots (200 μL) of the cell suspension were pipetted into 400-μL microcentrifuge tubes containing 150 μL silicone oil (Thomas Scientific, Swedesboro, NJ) and centrifuged for 30 seconds at 10,000 × *g*. The tubes were cut through the oil layer, and

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radioactivity associated with the cells was measured by scintillation counting. Non–carrier-mediated uptake was assessed in parallel incubations containing 400 μmol/L phloretin, and the value was subtracted from each determination. The assay for each animal was performed in triplicate and the results were averaged. Glucose transport was expressed as nanomoles of 3-*O*-methyl-glucose per 10^5 cells per 8 seconds.

Lipogenesis

For the determination of glucose incorporation into total lipids, the adipocytes (5×10^5/mL) were suspended in KRM with 4% BSA at pH 7.4. Aliquots (200 μL) of the cell suspension were placed into 50-mL plastic flasks and diluted to 2 mL with the same incubation buffer containing [^{14}C]glucose (Du Pont-New England Nuclear) to produce a final concentration of 3 mmol/L (35 (Ci/mmol). The cells were incubated with and without insulin (0.1 to 5 nmol/L) at 37°C for 2 hours with shaking. In experiments to study the effect of enalapril in vitro, adipocytes were incubated in the absence and presence of 5 nmol/L insulin with and without 10^{-7}, 10^{-6}, and 10^{-5} mol/L enalaprilat. At the end of the incubation period, the cell suspensions were transferred to glass centrifuge tubes with stoppers and extracted with 5 mL Dole's extraction mixture.[13] After 15 minutes at room temperature, 1 mL water and 3 mL hexane were added and the phases were separated by centrifugation. Radioactivity incorporated into total lipids was measured in 1 mL of the upper phase by liquid scintillation counting. The assay for each animal was performed in triplicate and the results were averaged. Lipogenesis was expressed as micromoles of glucose incorporated into lipids per 10^5 cells per 2 hours.

The maximal effect of insulin (E_{max}) and the dose of insulin required to elicit 50% of the maximal response (EC_{50}) value were obtained from a double-reciprocal plot in which the ordinate is glucose transport (expressed as nanomoles of 3-*O*-methyl-glucose per 10^5 cells per 8 seconds) or lipogenesis (expressed as micromoles of glucose incorporated into lipids per 10^5 cells per 2 hours) and the abscissa is insulin (nanomolars). The E_{max} and EC_{50} from each experiment were calculated, and the mean ± SE for each experimental group were obtained.

Statistics

The data from each experimental group were normally distributed. Statistical analysis of the results was performed by one-way ANOVA for multiple comparisons (basal lipogenesis, the data in Table 1, E_{max}, and EC_{50}) or two-way ANOVA (dose-response curves). A P value less than .05 was considered statistically significant.

RESULTS

General characteristics of the WKY and SHR groups without and with long-term treatment with enalapril, enalapril plus Hoe 140, and losartan are shown in Table 1. Systolic BP and cardiac hypertrophy, as indicated by the ratio of HW to BW (HW/BW), were significantly elevated in SHRs compared with age-matched WKY rats ($P < .05$). BP and cardiac hypertrophy returned to normal values in SHRs after pretreatment with enalapril and losartan. Pretreatment with enalapril and the BK B_2-receptor antagonist Hoe 140 completely reversed the changes in HW and HW/BW, but the BP remained slightly elevated in SHRs. The different treatments did not affect BP, HW, and cardiac hypertrophy in WKY rats. BP and BW were comparable among groups before treatment. BW (~300 g) was not affected by the different treatments. The mean daily intake of water (30 ± 3 mL) for the 30-day treatment period was not affected by the different pharmacological interventions.

Basal and insulin-stimulated glucose transport values by isolated adipocytes from SHRs and WKY rats are shown in Fig 1. Glucose uptake in the presence of insulin was significantly impaired in adipocytes from SHRs. Maximal glucose uptake (E_{max}) was 0.59 ± 0.05 in WKY rats and 0.32 ± 0.08 nmol/10^5 cells in SHRs ($P < .05$). The EC_{50} did not show significant differences between isolated adipocytes from either strain (EC_{50}, 0.09 ± 0.02 and 0.12 ± 0.03 nmol/L for WKY and SHR, respectively). Basal transport was not statistically different between adipocytes from WKY and SHR groups (Fig 1).

Table 1. General Characteristics of the Rat Groups

Treatment Group	BP (mm Hg)	BW (g)	HW (mg)	HW/BW (mg/g)
Untreated (control)				
WKY (n = 14)	113 ± 5	293 ± 7	832 ± 25	2.84 ± 0.10
SHR (n = 20)	180 ± 5*	314 ± 11	1,074 ± 60*	3.42 ± 0.10*
Enalapril				
WKY (n = 10)	110 ± 8	290 ± 8	792 ± 50	2.72 ± 0.12
SHR (n = 10)	125 ± 6	288 ± 13	822 ± 30	2.85 ± 0.11
Enalapril + Hoe 140				
WKY (n = 8)	103 ± 1	290 ± 10	768 ± 31	2.65 ± 0.07
SHR (n = 8)	143 ± 1†	317 ± 5	888 ± 27	2.78 ± 0.08
Losartan				
WKY (n = 6)	99 ± 4	276 ± 26	743 ± 40	2.78 ± 0.11
SHR (n = 6)	138 ± 2	304 ± 8	765 ± 48	2.50 ± 0.21

NOTE. Values are the mean ± SE from WKY.
*$P < .01$ *v* all other groups.
†$P < .05$ *v* WKY (ANOVA).

The conversion of glucose to lipids by isolated adipocytes accounts for approximately 65% of glucose uptake, and there is an excellent correlation between insulin-stimulated glucose uptake and lipogenesis.[12,14] Therefore, the effect of insulin on lipogenesis, ie, ^{14}C-glucose conversion into total lipids, was studied in the following experiments.

Insulin-stimulated lipogenesis from glucose by isolated adipocytes from WKY and SHR groups is shown in Fig 2. As shown for glucose transport, the effect of insulin was significantly less in SHRs ($P < .05$). E_{max} values in adipocytes from WKY and SHR groups were 1.16 ± 0.14 and 0.49 ± 0.09

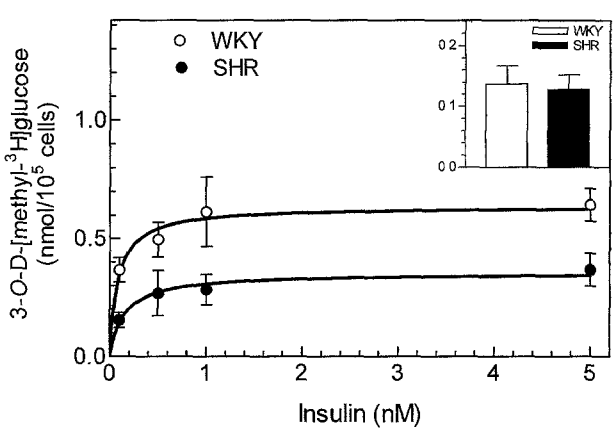

Fig 1. Effect of insulin on glucose transport in isolated adipocytes from SHRs (n = 6) and normotensive WKY rats (n = 6). Basal glucose transport (inset) was subtracted from each value obtained in the presence of the indicated concentration of insulin. Results are the mean ± SE. Insulin-stimulated glucose transport was lower in SHRs. The curves were analyzed by ANOVA.

μmol/10^5 cells, respectively ($P < .05$), with no change in the EC_{50} (WKY, 0.13 ± 0.03 nmol/L; SHR, 0.11 ± 0.02). Figure 2 shows that basal lipogenesis was also lower in SHRs compared with WKY rats ($P < .05$). To study whether the normalization of BP in SHRs was also accompanied by an impairment of insulin responsiveness, additional experiments were performed in isolated adipocytes from WKY and SHR groups after long-term treatment with enalapril for 30 days.

Insulin-stimulated lipogenesis in adipocytes from WKY and SHR groups treated with enalapril is shown in Fig 3 (data from WKY and SHR groups without treatment are also included for comparison). Compared with untreated SHRs, enalapril treatment increased insulin-stimulated glucose uptake in adipocytes from SHRs to values similar to those obtained with normotensive animals (E_{max}, 1.01 ± 0.16 μmol/10^5 cells; EC_{50}, 0.13 ± 0.02 nmol/L; both values not statistically different compared with untreated WKY). Although it did not reach statistical significance, basal lipogenesis seemed slightly improved in adipocytes from SHRs treated long-term with enalapril. Figure 3 shows the insulin responsiveness in adipocytes from WKY rats. Compared with untreated WKY rats, no significant changes were detected in either the E_{max} or the EC_{50} (E_{max}, 1.08 ± 0.07 μmol/10^5 cells; EC_{50}, 0.12 ± 0.03 nmol/L) after enalapril treatment in the normotensive animals. Enalapril did not affect basal lipogenesis in adipocytes from WKY rats.

To study the effect on insulin-stimulated lipogenesis of enalaprilat directly added to adipocytes, 10^{-7} to 10^{-5} mol/L enalaprilat was used in adipocytes from WKY and SHR groups incubated with and without 5 nmol/L insulin. Results obtained from three experiments showed that the addition of enalaprilat in vitro did not improve insulin-stimulated lipogenesis in adipocytes from SHRs (not shown).

The effect of blocking BK B_2 receptors during ACE inhibition was studied in adipocytes from WKY and SHR groups pretreated with Hoe 140 plus enalapril. Figure 4 shows that Hoe 140 suppressed the improvement of insulin resistance induced by pretreatment of the SHR group with enalapril (Fig 3). The

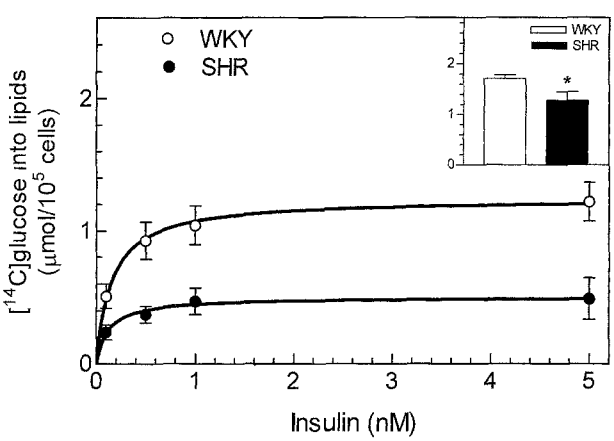

Fig 2. Effect of insulin on lipogenesis from glucose in isolated adipocytes from SHRs (n = 6) and normotensive WKY rats (n = 6). Basal lipogenesis was subtracted from each value obtained in the presence of the indicated concentration of insulin. Results are the mean ± SE. Basal lipogenesis (inset) and insulin-stimulated lipogenesis were lower in adipocytes from SHRs. The curves were analyzed by ANOVA. *$P < .05$.

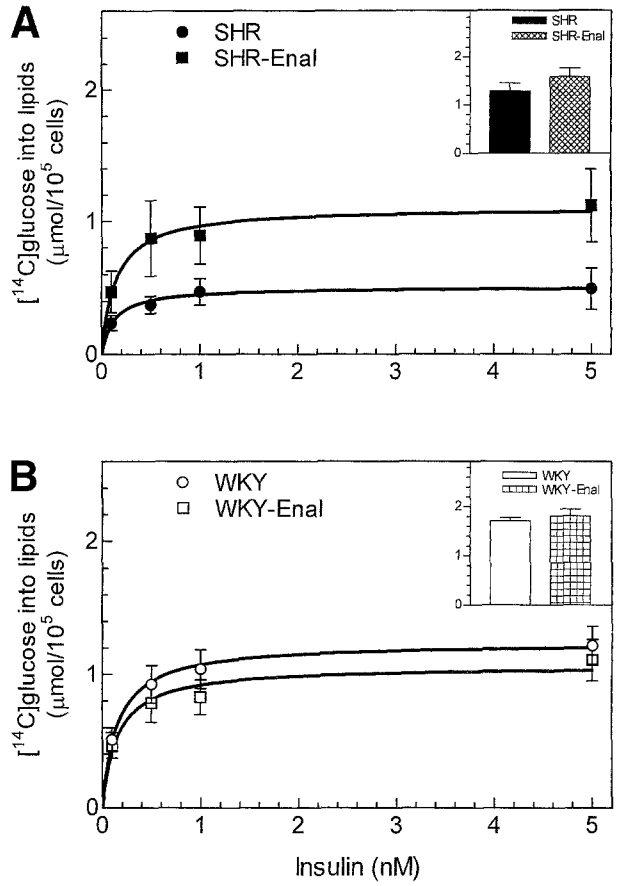

Fig 3. Effect of long-term treatment with enalapril on insulin-stimulated lipogenesis from glucose in adipocytes from SHRs and WKY rats. (A) Results in adipocytes from SHRs treated with enalapril (SHR-Enal, n = 7) and, for comparison, the results from Fig 2 in untreated SHRs (n = 6). Compared with adipocytes from untreated SHRs, enalapril increased insulin-stimulated lipogenesis in SHR-Enal. The curves were analyzed by ANOVA. (B) Results obtained in adipocytes from WKY rats treated with enalapril (WKY-Enal, n = 7) compared with the untreated WKY rats (n = 6) in Fig 2. Basal lipogenesis (inset) was subtracted from each value obtained in the presence of insulin. Results are the mean ± SE. Treatment with enalapril did not change basal or insulin-stimulated responsiveness in normotensive rats.

E_{max} of 0.349 ± 0.02 μmol/10^5 cells and the K_d of 0.11 ± 0.02 nmol/L were not different from the values shown for SHRs without treatment (Fig 2). The same treatment did not change the E_{max} of 1.028 ± 0.05 μmol/10^5 cells and the EC_{50} of 0.29 ± 0.08 nmol/L in WKY compared with untreated WKY rats.

To evaluate insulin resistance in SHRs treated with another antihypertensive agent, experiments were performed with adipocytes from WKY and SHR groups pretreated with the AT_1 angiotensin II receptor antagonist losartan. The results for insulin-stimulated lipogenesis are shown in Fig 5. Pretreatment with losartan did not improve insulin responsiveness in adipocytes from SHRs. Values for the E_{max} (0.407 ± 0.08 μmol/10^5 cells) and EC_{50} (0.14 ± 0.04 nmol/L) were not different from those obtained with untreated SHRs.

Compared with untreated WKY rats, a similar insulin responsiveness was obtained in adipocytes from WKY rats treated

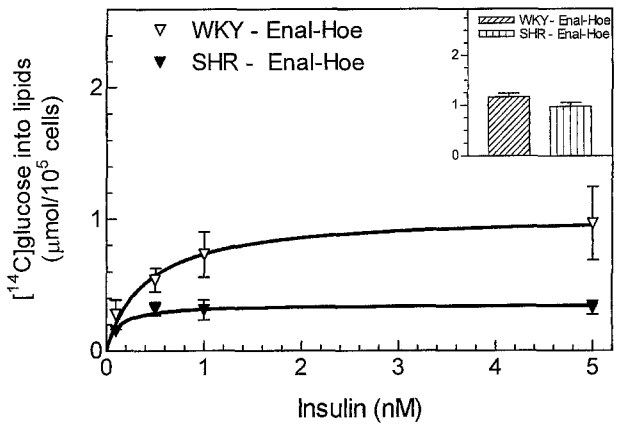

Fig 4. Effect of long-term treatment with enalapril and Hoe 140 on insulin-stimulated lipogenesis in adipocytes from SHRs (SHR-Enal-Hoe, n = 8) and WKY rats (WKY-Enal-Hoe, n = 8). Basal lipogenesis (inset) was subtracted from each value in the presence of insulin. Results are the mean ± SE. The BK B_2-receptor antagonist suppressed the improvement of insulin resistance induced by enalapril (see Fig 3).

with losartan (E_{max}, 0.94 ± 0.13 µmol/10^5 cells; EC_{50}, 0.19 ± 0.04 nmol/L). Figure 5 shows that basal lipogenesis did not change in WKY and SHR groups after losartan treatment compared with untreated WKY and SHR groups, respectively (Fig 2). In other words, adipocytes from WKY and SHR groups pretreated with losartan responded comparably to those from untreated animals.

DISCUSSION

Our results demonstrate that the decrease of insulin responsiveness in adipocytes from SHRs is due to a decrease in the maximal response to insulin without changes in sensitivity to the hormone. Insulin resistance without changes in sensitivity in

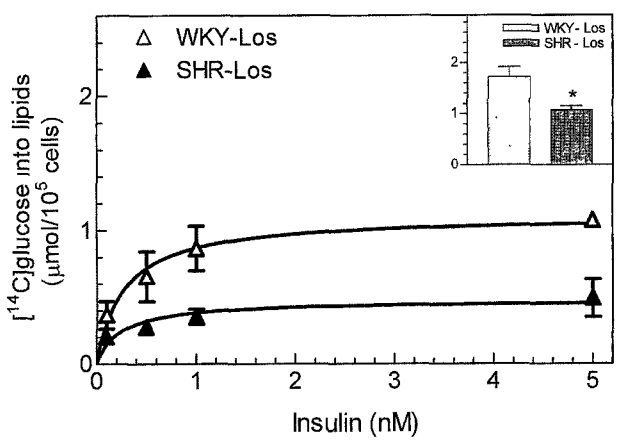

Fig 5. Effect of long-term treatment with losartan on insulin-stimulated lipogenesis from glucose in adipocytes from SHRs (SHR-Los, n = 6) and WKY rats (WKY-Los, n = 6). Basal lipogenesis (inset) was subtracted from each value obtained in the presence of the indicated concentration of insulin. Compared with untreated animals (Fig 2), treatment with losartan did not change basal or insulin-stimulated values in adipocytes from SHRs and WKY rats. Results are the mean ± SE.

isolated cells from hypertensive animals was detected by measuring both glucose uptake and incorporation of glucose into lipids. These data confirm previous results from Reaven et al[10] with regard to insulin resistance detected in adipocytes from the SHR. However, in addition to a decrease in the maximal response to insulin in adipocytes from hypertensive rats, they also reported a greater EC_{50}.[10]

Insulin resistance can be due to a decrease in the maximal response and/or sensitivity to insulin. At the cellular level, a decrease in the maximal response can be explained by intracellular defects and/or alterations at the insulin receptor itself that impair the effects of insulin.[15] Since the insulin receptor number and affinity, as well as receptor tyrosine kinase activity, have been found to be unchanged in adipocytes from SHRs,[10] it can be suggested from our results that intracellular defects are the cause of insulin resistance. Although we cannot completely rule out the possibility of changes at the receptor level with our data, the fact that the insulin resistance of isolated cells from hypertensive animals was not accompanied by alterations in insulin sensitivity is consistent with a postreceptor alteration.

Our findings are not in agreement with the proposed hypothesis that the decrease in glucose availability induced by a decrease in nutritional blood flow is the cause of insulin resistance, although such a decrease is frequently observed in hypertension.[4-6] In connection with this, it has been reported recently that vasodilation with sodium nitroprusside does not improve insulin action in essential hypertension.[16]

The present results show that decreasing the BP and cardiac hypertrophy by long-term treatment with enalapril is effective for normalizing the diminished insulin responsiveness in adipocytes from hypertensive animals. However, pretreatment with the AT_1-receptor blocker losartan failed to improve insulin action in adipocytes from the SHR. These results suggest that a mechanism other than a decrease in peripheral resistance is involved in the improvement of insulin resistance produced by ACE inhibition.

The improvement of insulin resistance in SHRs during treatment with the ACE inhibitor enalapril has already been described by Tomiyama et al[17] with the euglycemic clamp technique. This study also showed that losartan failed to produce any significant improvement of insulin resistance. Studies in humans also indicate that treatment with an ACE inhibitor can improve insulin resistance in hypertension.[18] Regarding the lack of effect of losartan to improve insulin responsiveness in the SHR, similar results were reported in hypertensive patients.[19] In addition to confirming these studies, our data also show that the effect can be detected at the cellular level without the possible changes in blood flow induced by pharmacological intervention. However, Santoro et al[20] reported that a 3-month period of ACE inhibition did not affect insulin resistance in hypertensive patients. Since ACE has a kininase II–like effect, apart from inhibiting the degradation of angiotensin I to angiotensin II, it acts by catabolizing several kinins, including BK. Thus, ACE inhibitors decrease angiotensin II levels and increase kinin levels. In addition to its depressor effect, BK has been reported to potentiate insulin-stimulated glucose uptake by itself[21-23] or to increase glucose uptake through stimulation of prostaglandin synthesis.[24,25] In

connection with this, studies in vivo have shown that the improvement of insulin responsiveness produced by treatment with enalapril is blunted in the presence of the BK B_2-receptor antagonist Hoe 140.[17] The effect of blocking BK B_2 receptors would suppress the vasodilator effect of BK and, consequently, the increase in blood flow required to supply glucose and allow insulin to have a maximal effect. On the other hand, a contribution of prostaglandins, but not of kinins, in the prolonged antihypertensive effect of the ACE inhibitor ramipril has been suggested.[26] Moreover, BK stimulates the synthesis of prostaglandins in isolated adipocytes.[27] In our experiments, the method for isolating adipocytes includes several washes of the cell preparation. This makes the presence of residual binding of either BK or prostaglandins at their respective receptors unlikely. The addition of enalapril directly to the adipocytes did not improve insulin-stimulated lipogenesis, which eliminates a direct effect of the ACE inhibitor on glucose uptake. This suggests that the improvement of the insulin response induced by pretreatment with enalapril is due to a lasting effect of intracellular changes occurring in the whole animal. These changes could be attributed to BKs, since our results show that the improvement of insulin resistance induced by enalapril is abolished by Hoe 140.

Independently of changes in blood flow and/or glucose availability, it is possible that intracellular alterations inducing hypertension also induce insulin resistance. Accordingly, it has been suggested that elevations in the intracellular calcium concentration ($[Ca^{2+}]_i$), frequently observed in hypertension,[28-31] may be the cause of insulin resistance.[32] Glucose transport in adipocytes is $[Ca^{2+}]_i$ dependent.[33,34] However, it has been shown that insulin-mediated glucose transport effectively functions only within an optimal range of $[Ca^{2+}]_i$ and that elevated basal $[Ca^{2+}]_i$ can produce insulin resistance.[35] Since it is premature to speculate based on our data, experiments to study the mechanisms involved in the improvement of insulin resistance in adipocytes from SHRs pretreated with enalapril, and to analyze if this pharmacological effect is shared with other antihypertensive treatments, are under way.

The major contribution of our study is to show for the first time that the decreased responsiveness to insulin detected in isolated adipocytes from the SHR can be normalized when systolic BP and cardiac hypertrophy are normalized by long-term treatment with enalapril, but not with losartan.

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REFERENCES

1. Hall JE, Brands MW, Zappe DH, et al: Insulin resistance, hyperinsulinemia, and hypertension: Causes, consequences, or merely correlations? Proc Soc Exp Biol Med 208:317-329, 1995

2. Julius S, Kenneth J: Sympathetics, insulin resistance and coronary risk in hypertension: The "chicken-and-egg" question. J Hyperten 12:495-502, 1994

3. Reaven GM, Lithel J, Landsberg L: Hypertension and associated metabolic abnormalities—The role of insulin resistance and the sympathoadrenal system. N Engl J Med 334:376-381, 1996

4. Baron AD, Brechtel-Hook G, Johnson A, et al: Skeletal muscle blood flow. A possible link between resistance and blood pressure. Hypertension 21:129-135, 1993

5. Egan BM: Neurohumoral, hemodynamic and microvascular changes as mechanisms of insulin resistance in hypertension: A provocative but partial picture. Int J Obes 15:133-139, 1991

6. Julius S, Gudbrandsson T, Jamerson K, et al. The hemodynamic link between insulin resistance and hypertension (hypothesis). J Hypertens 9:983-986, 1991

7. Cowley AW Jr: Long-term control of arterial blood pressure. Physiol Rev 72:231-300, 1992

8. Guyton AC: Circulatory Physiology, vol 3. Arterial Pressure and Hypertension. Philadelphia, PA, Saunders, 1980, pp 958-970

9. Capaldo B, Lembo G, Napoli R, et al: Skeletal muscle is the primary site of insulin resistance in hypertension. Metabolism 40:1320-1322, 1991

10. Reaven GM, Chang H, Hoffman BB, et al: Resistance to insulin-stimulated glucose uptake in adipocytes isolated from spontaneously hypertensive rats. Diabetes 38:1155-1160, 1989

11. Buñag RD: Validation in awake rats of a tail-cuff method for measuring systolic pressure. J Appl Physiol 34:279-282, 1973

12. Rodbell M: Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J Biol Chem 239:375-380, 1964

13. Dole VP: A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J Clin Invest 35:150-154, 1956

14. Gliemann J. Glucose metabolism and response to insulin of isolated fat cells and epididymal fat pads. Acta Physiol Scand 72,481-485, 1968

15. Hall JE, Summers RL, Brands MW, et al: Resistance to metabolic actions of insulin and its role in hypertension. Am J Hypertens 7:772-788, 1994

16. Natali A, Quiñones Galván A, Pecori N, et al: Vasodilation with nitroprusside does not improve insulin action in essential hypertension. Hypertension 31:632-636, 1998

17. Tomiyama H, Kushiro T, Abeta H, et al: Kinins contribute to the improvement of insulin sensitivity during treatment with angiotensin converting enzyme inhibitor. Hypertension 23:450-455, 1994

18. Pollare T, Lithel H, Berne C: A comparison of the effects of hydrochlorothiazide and captopril on glucose and lipid metabolism in patients with hypertension. N Engl J Med 321:868-873, 1989

19. Laakso M, Karjalainen L, Lempiäinen-Kuosa P: Effects of losartan on insulin sensitivity in hypertensive subjects. Hypertension 28:392-396, 1996

20. Santoro D, Natali A, Palombo C, et al: Effects of chronic angiotensin converting enzyme inhibition on glucose tolerance and insulin sensitivity in essential hypertension. Hypertension 20:181-191, 1992

21. Goldman J, Pfister D, Vukmirovich R: Potentiation of insulin stimulation of hexose transport by kallikrein and bradykinin in isolated rat adipocytes. Mol Cell Endocrinol 50:183-191, 1987

22. Isami S, Shichini M, Miyamura N, et al: Bradykinin enhances GLUT4 translocation through the increase of insulin receptor tyrosine kinase in primary adipocytes: Evidence that bradykinin stimulates the insulin signaling pathway. Diabetologia 39:412-420, 1996

23. Kishi K, Ebina Y, Hayashi H, et al: Bradykinin directly triggers GLUT4 translocation via an insulin-independent pathway. Diabetes 47:550-558, 1998

24. Olefky JM: Interaction between insulin receptors and glucose transport: Effect of prostaglandin E2. Biochem Biophys Res Commun 75:271-276, 1977

25. Simpson NT, Cushman SW: Hormonal regulation of mammalian glucose transport. Annu Rev Biochem 55:1059-1089, 1986

26. Cachoeiro V, Maeso R, Rodrigo E, et al: Nitric oxide and prostaglandins in the effects of losartan and ramipril in hypertension. Hypertension 26:236-243, 1995

27. Axelrod L, Minnich AK, Ryan CA: Stimulation of prostacyclin production in isolated rat adipocytes by angiotensin II, vasopressin, and bradykinin: Evidence for two separate mechanisms of prostaglandins synthesis. Endocrinology 116:2548-2553, 1985

28. Erne P, Bolli P, Burgisser E, et al: Correlation of platelet calcium with blood pressure: Effect of antihypertensive therapy. N Engl J Med 310:1084-1088, 1984

29. Le Quan Sang KH, Devynck MA: Increased cytosolic free calcium in essential hypertension. J Hypertens 9:567-574, 1984

30. Bruschi G, Bruschi ME, Caroppo M, et al: Cytoplasmic free [Ca^{2+}] is increased in the platelets of spontaneously hypertensive rats and essential hypertensive patients. Clin Sci 68:179-184, 1985

31. Sugiyama T, Yoshizumi M, Takaku F, et al: Abnormal calcium handling in vascular smooth muscle cells of spontaneously hypertensive rats. J Hypertens 8:369-375, 1990

32. Draznin B, Reusch J, Begum N, et al: Calcium, insulin action and insulin resistance, in Smith U, Bruun NE, Hedner T, et al (eds): Hypertension as an Insulin-Resistant Disorder. Genetic Factors and Cellular Mechanisms. New York, NY, Elsevier Science, 1991, pp 225-245

33. Taylor WM, Hau L, Halperin ML: Stimulation of glucose transport in rat adipocytes by calcium. Can J Biochem 57:692-699, 1979

34. Bonne D, Belhadj O, Cohen P: Modulation by calcium of the insulin action and of the insulin-like effect of oxytocin in isolated rat lipocytes. J Biochem 75:101-105, 1977

35. Draznin B, Sussman K, Kao M, et al: The existence of an optimal range of cytosolic free calcium for insulin-stimulated glucose transport in rat adipocytes. J Biol Chem 262:14385-14388, 1987