Mechanisms

Chronic Estrogen Treatment Modifies Insulin-Induced Insulin Resistance and Hypertension in Ovariectomized Rats

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Background: Gender differences have been found in the development of hypertension. The role of estrogen in the association between hyperinsulinemia/insulin resistance and hypertension was investigated in an insulin-induced, insulin-resistant, and hypertensive model.

Methods: Ovariectomized or sham operated female Wistar rats were chronically treated with insulin and/or estrogen via subcutaneous implants (insulin, 2 U/day; 17β-estradiol 0.5 mg/pellet, 60-day release). Systolic blood pressure was monitored at weeks 0, 3, and 6. At week 7, an oral glucose tolerance test was performed.

Results: Ovariectomy resulted in the development of insulin resistance and blood pressure elevation in chronically insulin-treated female rats. Chronic estrogen treatment prevented the elevation in blood pressure and the development of insulin resistance.

Conclusion: The results indicate that chronic estrogen treatment modifies the insulin-induced hypertension by increasing insulin sensitivity in ovariectomized rats. Am J Hypertens 2005;18:1189–1194 © 2005 American Journal of Hypertension, Ltd.

Key Words: Estrogen, ovariectomy, insulin resistance, hypertension.

Elevated plasma levels of insulin (hyperinsulinemia) and abnormally reduced insulin-stimulated glucose uptake (insulin resistance), combined with vascular endothelial dysfunction, are key features of the metabolic syndrome and cardiovascular disorders including diabetes and hypertension.[1] Direct proof for the causal relationship of hyperinsulinemia/insulin resistance and hypertension remains elusive. There is evidence to suggest that hyperinsulinemia/insulin resistance can lead to hypertension in humans and in animal models.[2,3] Our previous studies demonstrated that drugs that improve insulin sensitivity and decrease insulin levels prevented the development of hypertension in spontaneously hypertensive rats (SHR) and fructose-induced hypertensive rats (FHR).[4,5] This strongly suggests that hyperinsulinemia and insulin resistance are intrinsically linked to the pathogenesis of hypertension.

The incidence of hypertension and hypertension-related diseases is known to be lower in women in the childbearing years compared with the postmenopausal years or with the incidence in men.[6] Ovarian hormones, particularly estrogen, may protect premenopausal women against hypertension. In animal studies a reduction in plasma estrogen levels caused by aging or ovariectomy is shown to worsen hypertension in female SHR.[7,8] High fructose feeding, which induces hypertension in male rats, caused an elevation in blood pressure (BP) in ovariectomized female rats but not in ovary-intact female rats.[9] Chronic insulin treatment impairs insulin sensitivity in both male and female rats, although this occurred to a lesser degree in female rats; in addition, only male rats developed hypertension, whereas female rats did not.[10] These data suggest that the link between hyperinsulinemia/insulin resistance and hypertension is dependent on gender. It is not clear how estrogen affects the previously mentioned relationship in female rats. In this study we investigated the influence of estrogen on the inter-relationship among hyperinsulinemia, insulin resistance, and hypertension in chronically insulin-treated female rats.

Received December 7, 2004. First decision March 30, 2005. Accepted April 11, 2005.

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This work was supported by the Heart and Stroke Foundation of British Columbia and the Yukon (HSFBCY). E.A. was a research trainee of the Heart and Stroke Foundation of Canada, and J.Y. received funding through a summer studentship from the Heart and Stroke Foundation of British Columbia and the Yukon.

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0895-7061/05/$30.00
doi:10.1016/j.amjhyper.2005.04.003

Methods

Animals and Research Design

A total of 48 Wistar female rats, 6 weeks of age, were obtained from Charles River Laboratories Inc. (St. Constant, PQ, Canada). Before shipment, 24 animals were ovariectomized and the other 24 were sham-operated at 5 weeks of age. The rats were divided into six experimental groups: sham-operated (C, $n = 8$); sham-operated + insulin (CI, $n = 8$); sham-operated + insulin + estrogen (CIE, $n = 8$); ovariectomized (O, $n = 8$); ovariectomized + insulin (OI, $n = 8$); and ovariectomized + insulin + estrogen (OIE, $n = 8$). Before insulin and estrogen treatment, basal systolic BP, plasma glucose and plasma insulin (5-h fasted) were measured in all groups. After baseline measurements, chronic hyperinsulinemia was induced by placement of insulin implants (Linplant, Linshin, Toronto, ON, Canada), designed to release 2 U/day of regular bovine insulin) subcutaneously at the back of the neck. This dosage regimen has been previously shown to produce comparable levels of hyperinsulinemia in male and female rats and to elevate BP and insulin resistance.[10–12] Estrogen therapy was initiated on the same day via subcutaneous estrogen implants (Innovative Research of America, Sarasota, FL; 0.5 mg/pellet, designed to release 17β-estradiol at a constant rate over 60 days). To prevent hypoglycemia after the insulin implants, the rats received 10% glucose in their drinking water for the first 4 days of treatment. The rats were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care. The rats were housed on a 12-h light–dark cycle and received normal rat chow and water ad libitum.

Measurement of BP

Systolic BP was measured at weeks 0, 3, and 6 in conscious rats using the indirect tail-cuff method without external preheating as previously described.[4] This method has been validated in our laboratory and closely approximates direct intra-arterial BP values (within 5 mm Hg).[4]

Oral Glucose Tolerance Test

Seven weeks after placement of implants, the rats were fasted overnight and an oral glucose tolerance test (OGTT) performed using 1 g of glucose, administered by oral gavage, per kilogram of body weight. Blood samples were taken before and 10, 20, 30, 60, and 90 min after gavage for the measurement of plasma glucose and insulin levels. Three animals in the OIE group could not tolerate the fast and died during the experiment.

Insulin Sensitivity Index

The insulin sensitivity index (ISI) was calculated from the plasma glucose and insulin data obtained from the OGTT, using the formula of Matsuda and DeFronzo[13] but using a constant of 100 instead of 10,000. Matsuda and DeFronzo showed that this index correlated well with the results obtained from the euglycemic hyperinsulinemic clamp technique.

Biochemical Analyses

Plasma glucose levels were determined using the Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Plasma insulin and 17β-estradiol levels were determined by radioimmunoassay using kits from Linco Research Inc (St. Charles, MO) and MP Biomedical (Irvine, CA).

Statistical Analysis

All data are presented as means ± SEM. Statistical analyses were performed using a one-way ANOVA or general linear models of ANOVA using the Number Cruncher Statistical System software (Kaysville, UT). Mean values were considered significant at $P < .05$. When a mean difference was detected, a Newman-Keuls test was applied.

Results

General Characteristics

The general characteristics of the six groups are depicted in Table 1. The animals were ovariectomized or sham-operated at 5 weeks of age, 1 week before shipment. Before initiation of treatment protocols, the ovariectomized animals had a greater body weight than the sham-operated controls (body weight, in grams): C 198 ± 6, CI 194 ± 2, CIE 199 ± 3, O 222 ± 7, OI 229 ± 2, OIE 239 ± 8; values for O, OI, and OIE different from control groups, $P < .05$). After 7 weeks of treatment the C, CI, CIE, and OIE groups had a lower body weight than the O and OI groups (Table 1). The raw data food intake value in the CIE group is lower than that of the O group at week 7. When corrected for differences in body weight, the food consumption for the OI group was lower than all the other groups at week 7 of treatment. The fluid consumption was similar for all groups during the study with the exception of the OI group as compared to the CIE group at week 7 only. At the termination of the study, OIE rats had a lower plasma glucose level than all other groups. The plasma insulin levels at week 7 were higher in OI and OIE rats when compared with C, CI, CIE, and O groups, and the value for the OI group was greater than that for the OIE group.

We did not control for the estrus stage of the rats. Therefore the plasma estradiol levels in each group had a high degree of variability (C, 312 ± 40 pmol/L at week 7). Consequently, when all six groups are compared using analysis of variance, the only significant difference is that the OI group is lower than the CIE group at week 7 (Table 1). However when we used a nonparametric t test to compare the ovariectomized group (O, 223 ± 11 pmol/L) with the control group, the O group had significantly less estradiol ($P = .035$). Furthermore, when we examine the three ovariectomized groups by one-way

analysis of variance (O, 223 ± 11; OI, 209 ± 14; OIE, 278 ± 32) the values for the OIE group was greater than those for the O or OI groups ($P = .049$). As shown in Table 1, the insulin values for the OI and OIE groups were each different from all other groups at week 7. The changes in the insulin levels during the study are shown in Fig. 1 along with the area under the curve for the insulin values over the 7 weeks of the study. The insulin levels were clearly elevated by the implants in the OI and OIE groups. The same implants failed to elevate the levels of insulin in the CI and CIE groups, which surprised us because we previously found elevated insulin in intact females.[10]

Blood Pressure

There was no difference in BP among the 6 groups at the beginning of the experiment (C, 101 ± 2 mm Hg; CI, 102 ± 2 mm Hg; CIE, 107 ± 2mm Hg; O, 100 ± 1 mm Hg; OI, 99 ± 1 mm Hg; OIE, 98 ± 2 mm Hg; $P > .05$) (Fig. 2). At week 3, the systolic BP was higher in the OI group when compared with the other groups. The OIE group did not display any significant increase in systolic BP. At 6 weeks of treatment, the OIE group had a systolic BP measurement (BP, 103 ± 2 mm Hg) that was lower than the OI group (BP, 112 ± 2 mm Hg). The area under the BP curve was larger in OI group compared with all the other groups except the CI group (Fig. 2).

Results of OGTT and ISI

Plasma insulin and glucose data from OGTT are plotted in Figs. 3A and B, respectively. Plasma insulin levels in response to an oral glucose load were higher in OI and OIE animals when compared with C, CI, CIE, and O groups (area under curve of OGTT plasma insulin [nmol/L/90 min]: C, 28.8 ± 2.6; CI, 26.5 ± 1.4; CI, 22.4 ± 1.4; O, 32.5 ± 2.2; OI, 52.6 ± 2.8; OIE, 46.2 ± 6.2; $P < .05$ for OI and OIE groups versus C, CI, CIE, and O groups) (Fig. 3A). The OIE rats exhibited greater glucose tolerance than the other groups, as demonstrated by the lower plasma glucose levels of OIE during OGTT (area under curve of OGTT plasma glucose [mmol/L/90 min]: C, 848.6 ± 21.8; CI, 855.0 ± 10.2; CIE, 808.9 ± 37.7; O, 879.2 ± 13.0; OI, 846.6 ± 33.5; OIE, 631.4 ± 104.3; $P <$ .05, OIE versus all other groups) (Fig. 3B). A comparison of ISI values calculated from the OGTT data showed that insulin sensitivity of the OI group was impaired compared with the sensitivities of the other groups (Fig. 4). Chronic treatment with estrogen prevented insulin-induced impairment in insulin sensitivity (Fig. 4).

Discussion

Previous experiments in our laboratory have shown that female rats are protected against the development of hypertension in rodent models of insulin resistance, unlike their male counterparts.[9,10] We have shown that chronic insulin treatment causes insulin resistance in both male

Table 1. General characteristics of the six treatment groups at study termination

Characteristic	C ($n = 8$)	CI ($n = 8$)	CIE ($n = 8$)	O ($n = 8$)	OI ($n = 8$)	OIE ($n = 5$)
Body weight (g)	272 ± 7	270 ± 3	251 ± 4	344 ± 1*	370 ± 8*	279 ± 8
Raw food intake (g/day)	20.5 ± 1.0	18.7 ± 0.7	18.3 ± 1.0†	23.3 ± 1.5	20.6 ± 1.0	21 ± 1.2
Food intake (g/kg/day)	76 ± 2	69 ± 2	73 ± 3	68 ± 3	56 ± 5*	75 ± 3
Fluid intake (mL/day)	43 ± 3	40 ± 4	31 ± 2	38 ± 4	46 ± 3‡	42 ± 5
Plasma glucose (mmol/L)	7.8 ± 0.1	8.0 ± 0.2	7.8 ± 0.2	7.5 ± 0.4	8.1 ± 0.2	6.2 ± 0.8*
Plasma insulin (pmol/L)	248.3 ± 15.6	274.0 ± 11.2	233.9 ± 28	245.3 ± 16.0	498.0 ± 34.0*	353.5 ± 26.4*
Plasma 17β-estradiol (pmol/L)	312 ± 40	313 ± 17	330 ± 34	223 ± 11	209 ± 14‡	278 ± 32§

C = sham-operated group; CI = sham-operated + insulin; CIE = sham-operated + insulin + estrogen; O = ovariectomized; OI = ovariectomized + insulin; OIE = ovariectomized + insulin + estrogen.

* $P < .05$ different from all other groups; † $P < .05$ different from O; ‡ $P < .05$ different from CIE; § $P < .05$ different from O when ovariectomized groups are compared.

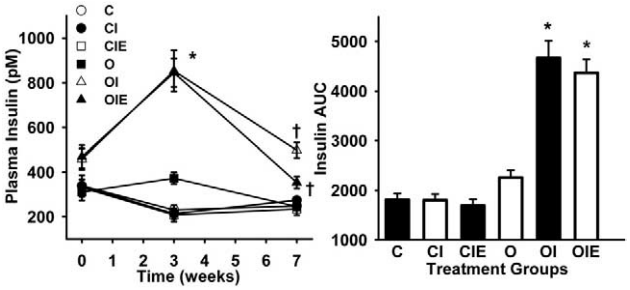

FIG. 1. Plasma insulin levels in sham-operated control (C, $n = 8$), sham-operated insulin treated (CI, $n = 8$), sham-operated insulin + estrogen-treated (CIE, $n = 8$), ovariectomized (O, $n = 8$), ovariectomized insulin-treated (OI, $n = 8$) and ovariectomized insulin + estrogen-treated (OIE, $n = 5$) groups after 0, 3, and 7 weeks of insulin/estrogen pellet implantation in CI, CIE, OI, and OIE groups. Values are presented as mean ± SEM. *$P < .05$, different from C, CI, CIE, and O; †$P < .05$ versus all other groups. The inserts show the areas under the blood pressure curves (AUC), *$P < .05$ different from C, CI, CIE, and O groups.

and female rats; however, hypertension develops only in the male rats, suggesting that the presence of estrogen and/or other female sex hormones prevents the increase in BP in insulin-resistant female rats. In the present study we confirmed this hypothesis by treating ovariectomized female rats with insulin and reversing the insulin-induced insulin resistance and hypertension with chronic estrogen treatment.

These results are consistent with our previous findings and provide an explanation for the gender-related differences that we have observed in insulin sensitivity.[10] In previous experiments, insulin-treated female rats developed insulin resistance but not to the same extent as male rats, nor did they subsequently develop hypertension. Because a decrease in estrogen after ovariectomy in the presence of chronic hyperinsulinemia induced insulin resistance and an elevation in BP, the mechanisms that link hyperinsulinemia/insulin resistance to hypertension are

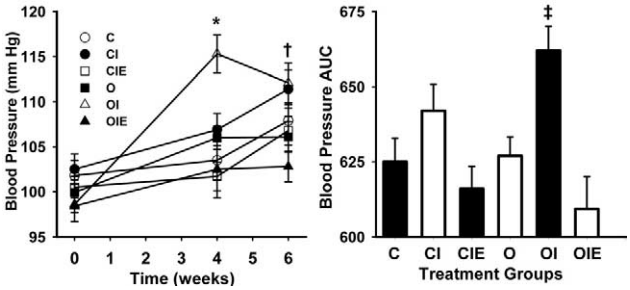

FIG. 2. Blood pressure in sham-operated control (C, $n = 8$), sham-operated insulin treated (CI, $n = 8$), sham-operated insulin + estrogen-treated (CIE, $n = 8$), ovariectomized (O, $n = 8$), ovariectomized insulin-treated (OI, $n = 8$) and ovariectomized insulin + estrogen-treated (OIE, $n = 5$) groups after 3 and 6 weeks of insulin/estrogen pellet implantation in CI, CIE, OI, and OIE groups. Values are presented as mean ± SEM. *$P < .05$, different from all other groups, †$P < .05$ different from C, CIE, O, and OIE groups. The inserts show the areas under the blood pressure curves (AUC), ‡$P < .05$ different from C, CIE, O, and OIE groups.

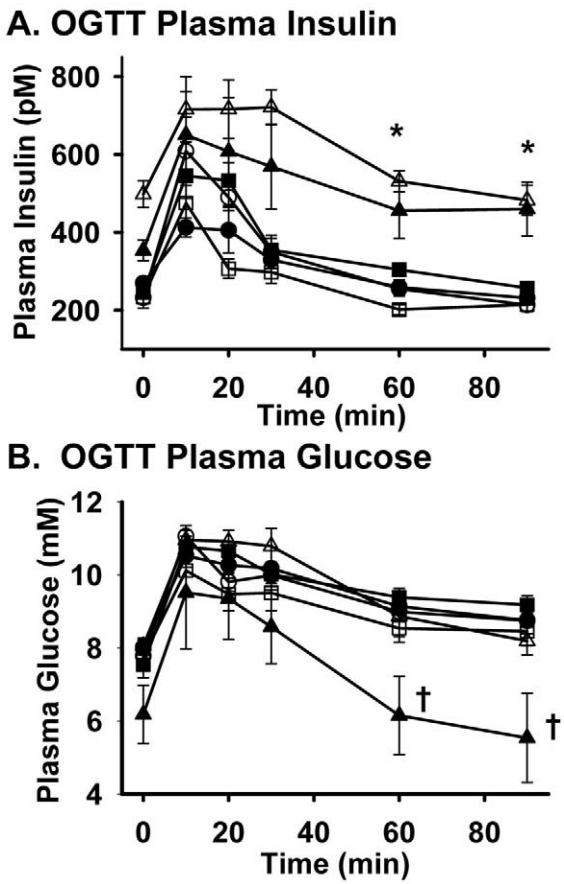

FIG. 3. Plasma insulin (**A**) and glucose (**B**) levels during the oral glucose tolerance test (OGGT) in sham-operated control (**C**, $n = 8$, ○), sham-operated insulin treated (CI, $n = 8$, ●), sham-operated insulin + estrogen-treated (CIE, $n = 8$, □), ovariectomized (O, $n = 8$, ■), ovariectomized insulin-treated (OI, $n = 8$, △) and ovariectomized insulin+estrogen-treated (OIE, $n = 5$, ▲) groups after 7 weeks of insulin/estrogen pellet implantation in CI, CIE, OI, and OIE groups. Values are presented as mean ± SEM. *OI, OIE different from C, CI, CIE, and O groups; †$P < 0.05$ different from all other groups.

present in female rats as well as in males. Replacement of 17β-estradiol in the OIE group benefited this complex interrelationship.

It is important to point out that insulin levels were not increased by chronic insulin treatment in CI and CIE groups as we expected in the present study. It is possible that in intact females the higher levels of estrogen prevented the increase of insulin levels by decreasing the endogenous release of insulin. Because chronic estrogen treatment significantly lowered insulin-induced high BP in ovariectomized rats, the data do suggest a protective effect of chronic estrogen treatment against insulin-induced hypertension in ovariectomized female rats.

The results of this study are also consistent with the findings of other laboratories that show that estradiol lowers BP in various animal models such as SHR,[8] spontaneously hypertensive, heart failure–prone rats,[14] deoxycorticosterone acetate-salt–induced hypertensive rats,[15] and Dahl salt-sensitive rats.[16] This correlates with human studies that demonstrate a shielding effect of estrogen against

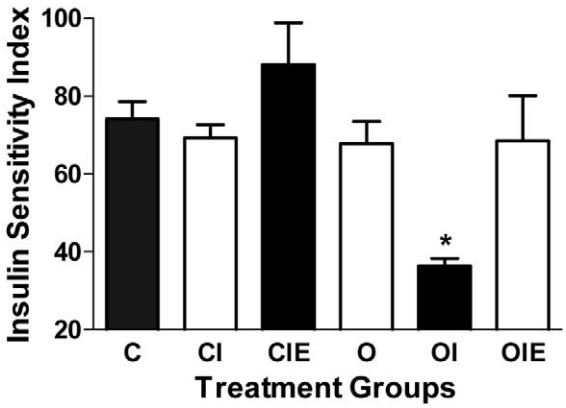

FIG. 4. Insulin sensitivity index (ISI) in sham-operated control (C, $n = 8$), sham-operated insulin treated (CI, $n = 8$), sham-operated insulin + estrogen-treated (CIE, $n = 8$), ovariectomized (O, $n = 8$), ovariectomized insulin-treated (OI, $n = 8$) and ovariectomized insulin + estrogen-treated (OIE, $n = 5$) groups after 7 weeks of insulin/estrogen pellet implantation in CI, CIE, OI, and OIE groups. Values are mean ± SEM. $*P < .05$, OI versus all other groups.

hypertension. During the menstrual cycle, BP is lower in the luteal phase (ie, when estradiol levels peak) than during the follicular phase,[17,18] and BP increases after the onset of menopause (a time when estrogen drastically decreases).[19] In addition, it has been found that estrogen levels increase 50- to 180-fold during pregnancy[20] and that these increases are associated with substantial reductions in BP.[21]

Several possibilities may explain the mechanism or mechanisms for these observations. 17β-Estradiol has direct effects on the vasculature that favor vasorelaxation and lower BP.[22] Also, estrogen may have obviated hypertension by beneficial effects on insulin sensitivity. 17β-Estradiol has been shown to cause vasodilation and to increase blood flow to various organs,[23,24] which can potentially enhance glucose uptake and increase insulin sensitivity. Estrogen has been found to increase glycogen deposition[25] and glucose uptake during exercise in rats.[26] Recent studies in mice suggest that estrone sulfate reduces hepatic glucose production by inhibiting hepatic glucose-6-phosphatase activity.[27] Estrogen may contribute to improvements in insulin sensitivity by independently reducing catecholamine levels, either by increasing norepinephrine degradation in the brain (and thereby reducing sympathetic drive)[28] or by decreasing secretion from the adrenal medulla.[29] Because the sympathetic nervous system is involved in insulin resistance,[30] these negative modulatory effects on the sympathetic nervous system could indirectly preserve insulin sensitivity. In the retina estrogen may have neuroprotective effects by the activation of insulin receptor β-subunit/PI3 K/Akt pathway; thus it may improve insulin sensitivity by directly promoting insulin signaling activity.[31]

Obesity is another factor known to have a negative influence on insulin resistance and BP. In our study, at week 7 of treatment, the OI group had a greater body weight than the OIE and C groups, so that the impairments in insulin sensitivity and elevations in BP observed in the OI group could be related to obesity. However, the body weight of the O group was similar to the OI group, and this group maintained normal insulin sensitivity and BP.

Furthermore, it has been shown that endothelin and angiotensin II levels were up-regulated in female rats after ovariectomy and estrogen replacement was able to reverse those changes.[32–34] An indirect effect of ovariectomy and estrogens on endothelin and angiotensin II system could play a role in modifying BP and insulin resistance in estrogen-treated ovariectomized female rats.

Ovariectomy decreases the levels of progesterone as well as estrogen. However, because progesterone has been found to antagonize the effect of estrogen on insulin sensitivity,[35] glycogen deposition,[25] and glucose uptake[26] and to reduce the endothelium-dependent vasodilatory action of estrogens, it is unlikely that the lack of progesterone was a contributory factor to the development of insulin resistance and hypertension in rats of the OI group in this study.

Acknowledgment

We sincerely thank Dr. Linfu Yao for his technical advice and assistance.

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