Exogenous Hyperinsulinemia Causes Insulin Resistance, Hyperendothelinemia, and Subsequent Hypertension in Rats

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In many clinical and animal studies, hypertension and insulin resistance coexist, but their mechanistic relationship is unclear. We explored the causal link between these two parameters in a rat model with chronic hyperinsulinemia induced with human insulin (1 U/d) released from subcutaneously implanted minipumps. Rats with saline minipumps served as a control. During the first experiment, plasma levels of insulin and glucose and the systolic blood pressure of the two groups were continuously monitored for 17 days. In the subsequent four experiments, rats were killed on days 10 and 13 to measure plasma endothelin-1 (ET-1) levels and the glucose transport into and insulin and ET-1 binding of isolated adipocytes. In one experiment, rats were tested for oral glucose tolerance on days 10 and 13. In another experiment, ET-1 binding to the aortic plasma membrane was also determined. The results showed that rats became hyperinsulinemic throughout the experimental period by the instillation of exogenous insulin. Hyperinsulinemic rats were consistently hypoglycemic during the first day, but they became euglycemic thereafter, indicating an insulin-resistant state. Glucose intolerance was obvious by day 10, but significant hypertension was not detected until the 11th day on insulin infusion. Compared with the saline controls, insulin-infused rats had an increase of plasma ET-1 levels but a decrease of both basal and insulin-stimulated glucose transport into adipocytes. ET-1 binding to adipocytes of the insulin-infused group was elevated significantly from day 10 through day 13. ET-1 binding to the aortic membranes, supposedly downregulated by the increased plasma ET-1 and hypertension, was similar to that found in the controls on day 13. These results imply that hyperinsulinemia in rats could lead to hypertension via the elevation of plasma ET-1 levels together with an unaltered vascular binding of ET-1, which was probably unrelated to the insulin resistance.

EPIDEMIOLOGICAL STUDIES have revealed a significantly higher prevalence of hyperinsulinemia and insulin resistance in treated or untreated hypertensive patients versus normotensive cohorts.[1,2] In patients with a clinical diagnosis of non–insulin-dependent diabetes mellitus (NIDDM), obesity, hypertension, dyslipidemia, or atherosclerotic cardiovascular disease, and also in healthy aged persons a multifaceted syndrome (syndrome X), are often present, the latter consisting of hyperinsulinemia, insulin resistance, very–low-density lipoprotein hypertriglyceridemia, decreased plasma high-density lipoprotein cholesterol, hyperuricemia, high plasminogen activator inhibitor-1, high postprandial lipemia, and high blood pressure.[3,4] Nevertheless, subjects with insulin resistance do not always develop hypertension, and hypertensive patients are not necessarily insulin-resistant and hyperinsulinemic.[5] A recent prospective study with a large population and a study period of 11.5 years concluded that impaired glucose tolerance and hyperinsulinemia per se do not play an etiologic role in the development of hypertension.[6]

To demonstrate the interrelationship between hypertension and insulin resistance, several rat models have been used. Sprague-Dawley rats fed with a rat chow substituting fructose, glucose, or sucrose for all carbohydrates manifested insulin resistance, hyperinsulinemia, and subsequently elevated blood pressure.[7,8] The fructose-induced hypertension was not secondary to an increase in sympathetic nervous system[7] and renin-angiotensin-aldosterone[9] activity, but was attenuated by exercise training,[10] significantly reduced by somatostatin infusion,[11] and prevented by vanadyl treatment.[12] All of these studies indicate a possible hemodynamic effect of insulin resistance and ambient hyperinsulinemia in the blood pressure elevation.

Insulin resistance was found in rats with genetic hypertension. The spontaneously hypertensive rat (SHR) strain is hyperinsulinemic and resistant to insulin compared with its normal cohort, the Wistar-Kyoto rat.[13,14] And compared with normal Sprague-Dawley rats, the hypertensive Dahl salt-sensitive strain is more insulin-resistant than the Dahl salt-resistant strain.[15] Milan hypertensive rats are also insulin-resistant.[16] However, using high- and low-dose insulin clamps to study glucose uptake and suppression of hepatic glucose production in two rat models with genetic hypertension (including the SHR), the results showed that high blood pressure per se did not lead to the onset of insulin resistance.[17] When male SHRs were treated with troglitazone, the hyperinsulinemia and insulin resistance, but not the high systolic blood pressure, could be alleviated. Such a finding goes against the suggestion that hyperinsulinemia and insulin resistance are involved in the development of sustained hypertension.[18] However, in diabetic hypertensives, troglitazone enhanced insulin sensitivity together with a reduction in blood pressure.[19]

In view of these controversies in human and animal observations, we undertook a series of experiments examining the causal role of insulin resistance in the incidence of hypertension in a rat model rendered chronically hyperinsulinemic by exogenously administered insulin, and also attempted to elucidate the mechanism underlying the development of hypertension in hyperinsulinemic rats.

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MATERIALS AND METHODS

Animal Model and Experimental Design

Male Sprague-Dawley rats weighing 250 to 300 g (purchased from a local breeder) were housed four per cage in a temperature (20° to 22°C)- and light-controlled room with an alternating 12-hour light/dark cycle (lights on 7 AM). The animals had access to regular rat chow—except rats scheduled for the oral glucose tolerance test and for death—and water ad libitum. In a pilot study of two groups of rats, we found that the blood pressure increased significantly between day 11 and day 13 after the implantation of insulin minipumps. In subsequent experiments, therefore, we designated alternate dates to measure blood pressure or to collect blood samples for plasma glucose and insulin measurements. In four experiments, eight rats from each group were killed on day 10 and day 13 for plasma and adipocyte preparations. In one of the experiments, oral glucose tolerance tests were performed on day 10 and day 13. In another one of the experiments, aortic membranes were prepared for the endothelin-1 (ET-1) binding study. All procedures were performed in accordance with the guidelines of the Taiwan Government Guide for the Care and Use of Laboratory Animals, and the protocol was approved by the animal welfare committees of Veterans General Hospital-Taipei and National Yang-Ming University.

Minipump Implantation

Mini-osmotic pumps (Alzet model 2002) were purchased from Alza (Palo Alto, CA). A regular human insulin injection (100 U/mL) was diluted with saline to 1.2 mL from 1.0 mL and then injected into each pump to a volume of 190 to 210 μL, to ensure an insulin release rate of 0.5 μL/h or 1 U/d in accordance with the product specifications. For controls, minipumps were filled with equal volumes of normal saline. After 1 week of acclimatization, the rats were anesthetized with sodium pentobarbital (30 mg/mL/kg body weight intraperitoneally). Under sterile conditions, a minipump was surgically inserted subcutaneously into the lower back of each rat. Experimentation started as soon as the minipump was implanted.

Blood Pressure Measurement

We used Narco Bio-Systems (Houston, TX) physiograph equipment to measure rat blood pressure by the tail-cuff method. The small-animal study unit of the system has a rat-holder base with a built-in warming element to increase the ambient temperature to 37°C and maintain an adequate circulation in the rat's tail for indirect systolic blood pressure measurement. The animal was positioned in the Lucite (DuPont, Wilmington, DE) housing with its tail firmly held outside. With the occluding metal tubular cuff (11.1 mm, ID) and the pneumatic-pulse sensor-transducer placed on the rat's tail and connected to the electrosphygmograph (PE 300), a preadjusted inflation-deflation rate controlled the occluding cuff pressure until the first pulse was recorded, which represented the systolic blood pressure. Since normal blood pressure has intrinsic diurnal variations and may be disturbed by environmental conditions, all measurements were performed in a quiet room during afternoon hours, alternating the cages of four control and four insulin-treated rats. An experienced technician could repeat three to five measurements per rat in 20 to 30 minutes and finish the measurements before 7 PM. Blood pressure was measured on days when the rats were not scheduled for blood specimen collection.

Blood Sample Collection

Blood samples were collected by the tail-bleeding method. After the tail tip was severed with a sharp razor blade, approximately 600 μL free-flowing blood was collected into a 1.5-mL polyethylene heparin-coated microfuge tube kept on ice. Normally, the tail cut stopped bleeding by itself, even with repeated bleedings within a short period. On rare occasions, unceasing bleeding required heat cautery. For ET-1 determination, a minimum of 5 mL blood was collected from each rat by decapitation. The plasma was separated by centrifugation and stored at −20°C until assayed for glucose and insulin or ET-1.

Oral Glucose Tolerance Test

On days 10 and 13 after minipump implantation, eight randomly selected rats in each group were subjected to an oral glucose tolerance test performed without anesthesia after a 24-hour fasting period as described by Whittington et al.[20] Immediately after a tail-vein blood sample was collected, a glucose solution (2 g/mL/kg body weight) was administered by gavage. Following glucose administration, four more blood samples were collected at 30, 60, 90, and 120 minutes.

Measurement of Plasma Glucose, Insulin, and ET-1

The plasma insulin concentration was determined by a radioimmunoassay (RIA) technique developed in our laboratory.[21] The anti–porcine insulin antiserum produced in guinea pigs cross-reacts 100% with human and rat insulin. Each plasma sample (100 μL) was assayed in duplicate with a mean variation of 4% (range, 1% to 8%). The plasma glucose level in 25 μL sample was measured by a glucose analyzer (model 23A; Yellow Springs Instrument, Yellow Springs, OH). Plasma ET-1 in 2 mL plasma was extracted with a Sep-Pak C18 cartridge (Millipore, Milford, MA) in accordance with the procedure described by Xuan et al,[22] and then assayed using an ET-1 RIA kit.

Isolation of Adipocytes

On days 10 and 13 after minipump implantation when the rats were killed by decapitation, the epididymal fat pads were excised and processed into control and insulin-infused groups. Adipocytes were isolated from fat pads using the Rodbell method with minor modifications.[23] Briefly, the fat pads were finely minced with scissors, and for each gram of tissue, 10 mL Krebs-Ringer bicarbonate (KRB) buffer containing 1 mmol/L pyruvate, 1% bovine serum albumin (BSA), and 0.1% collagenase was used to digest the tissue using gentle shaking at 37°C for 1 hour. The cell suspension was then filtered through nylon mesh (400 μm) and centrifuged at 100 rpm for 1 minute. The supernatant layer of cells was harvested and washed twice with 50 mL KRB buffer containing 1 mmol/L pyruvate and 1% BSA. The cell number was counted after an aliquot of diluted cell suspension was fixed in a collidine buffer containing 2% osmium tetroxide.[24]

Preparation of Rat Aortic Membranes

In one experiment, the aortas from dead animals were excised, frozen immediately in liquid nitrogen, pooled into control and insulin-treated groups, and then stored at −80°C until measurement of ET-1 binding. Aortic plasma membranes were prepared using a published procedure.[25] After thawing on ice, the aortic tissues were minced into small pieces (~1 mm) and homogenized in a solution containing 20 mmol/L $NaHCO_3$ and 0.1 mmol/L phenylmethylsulfonyl fluoride (PMSF), pH 7.4, with two 15-second bursts of a polytron (PT 10-35; Kinematica, Switzerland) at one third maximal speed. The resultant homogenate was diluted with 0.1 mmol/L PMSF, pH 7.4, and centrifuged at 1,500 × *g* for 15 minutes at 4°C. The supernatant was transferred to a fresh tube and centrifuged again at 48,000 × *g* for 20 minutes at 4°C. The resultant pellet was suspended in an ice-cold buffer of 50 mmol/L Tris and 0.1 mmol/L PMSF, pH 7.4, and centrifuged again at 48,000 × *g* for 15 minutes at 4°C. The pellet was resuspended in a 50-mmol/L Tris/0.1-mmol/L PMSF buffer, pH 7.4, to a concentration of 0.4 to 0.5 mg protein/mL, as assayed by the Lowry method.

Measurement of Glucose Transport Into Adipocytes

Adipocyte glucose uptake was determined by measuring the transport of 2-deoxy-glucose (2-DG) into the cells, as described by Garvey et al

with minor modifications.[23] Briefly, aliquots (400 μL) of isolated adipocytes at a predetermined cell number were mixed with increasing concentrations of insulin (0 to 50,000 pmol/L) in 100 μL KRB buffer containing 1 mmol/L pyruvate and 1% BSA. After the mixtures were incubated at 37°C for 30 minutes, 50 μL [^{3}H]2-DG (to a final concentration of 50 mmol/L) was added and the incubation continued for 3 more minutes. The incubation was terminated by adding 200 μL unlabeled 2-DG in KRB solution (500 mmol/L). After thoroughly mixing, 300 μL of the mixture was transferred to a microfuge tube containing 200 μL silicone and the cellular layer was separated by mild centrifugation (100 rpm for 1 minute). The radioactivity retained by the adipocytes was measured by a liquid scintillation counter.

Measurement of Insulin Binding to Adipocytes

Insulin binding to the adipocytes was determined according to a procedure previously described.[26] In aliquots of fat cells (2×10^5/400 μL), a fixed amount of [^{125}I]insulin (to a final concentration of 0.25 nmol/L, $\sim 5 \times 10^5$ cpm/tube) and an increasing concentration of unlabeled insulin (5 pmol/L to 500 nmol/L) were added. The cells were incubated in an oxygen-rich chamber (5% CO_2:95% O_2) at 37°C with gentle rotation at 40 rpm, for 30 minutes. Then, 300 μL cell suspension was transferred to a fresh centrifuge tube containing 200 μL silicone, and the mixture was centrifuged at 1,000 × *g* for 1 minute. The cellular layer was transferred to a counting vial for measurement of radioactivity. The nonspecific-binding tube contained 1 μmol/L unlabeled insulin.

Measurement of ET-1 Binding to Adipocytes and Aortic Membranes

ET-1 binding to fat cells was determined in the same manner used for the insulin binding study,[26] with [^{125}I]ET-1 as a tracer and unlabeled ET-1 as a competitive binding ligand. ET-1 binding to aortic membranes was determined according to procedures previously described.[25] The reaction mixture (250 μL) in duplicate, containing membranes (with a final protein concentration of 0.2 mg/mL) in 50-mmol/L Tris/0.1-mmol/L PMSF (pH 7.4), [^{125}I]ET-1 (1×10^4 cpm), and various amounts of unlabeled ET-1, was incubated at 37°C for 60 minutes. The mixture was diluted with 3 mL 10% polyethylene glycol (PEG) in 50 mmol/L Tris, pH 7.4. Free [^{125}I]ET-1 was separated from the bound fraction by vacuum filtration through Whatman (Maidstone, England) GF/C filters. After two washes with the same Tris-PEG buffer, radioactivity retained by the filters was measured with a gamma counter. Nonspecific binding was determined using 1 μmol/L unlabeled ET-1 in the reaction mixture, and was about 2% of the total binding.

Statistical Analysis

Results are expressed as the group mean ± SD or mean ± SEM. Differences between the two groups were analyzed by either *t* test or two-way ANOVA when multiple measurements were applied. Statistical significance was defined at a *P* level less than .05.

RESULTS

Changes in Plasma Insulin and Glucose, and Systolic Blood Pressure

Figure 1 shows plasma insulin and glucose levels in rats after implantation of the insulin minipumps compared with the saline-control rats in a representative experiment. Using insulin instillation via the minipump, plasma insulin levels were doubled within the first day. A steady but significantly higher plasma insulin level versus the controls was maintained throughout the remaining days of the experiment. Thus, a chronic state of hyperinsulinemia was achieved in this model. Compared with the normal control group, plasma glucose levels in

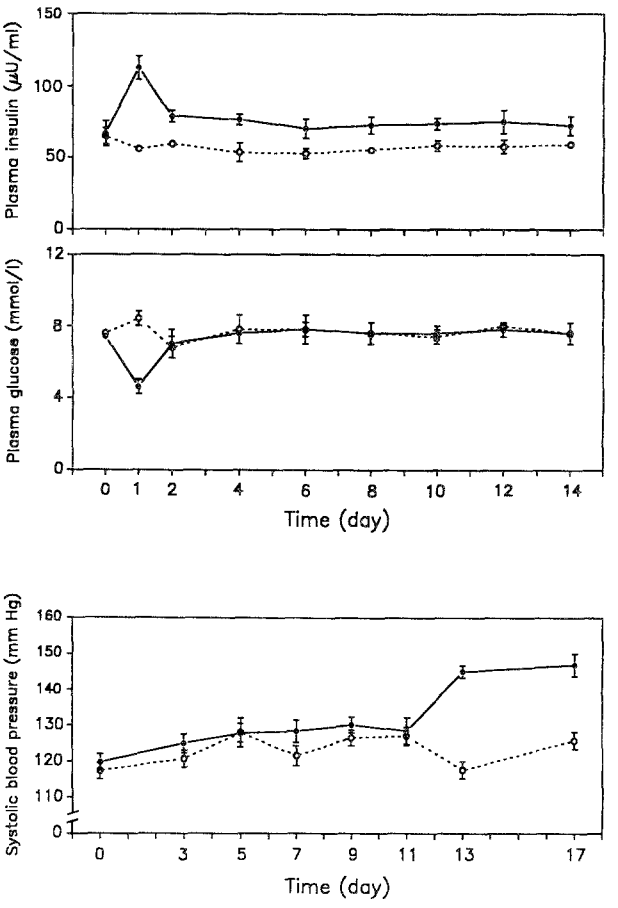

Fig 1. Plasma insulin, glucose, and systolic blood pressure in rats after osmotic minipumps containing insulin (●) or saline (○) were subcutaneously implanted on day 0. Differences between the 2 groups were significant ($P < .05$) from day 1 through day 14 for insulin, on day 1 for glucose, and after day 11 for blood pressure. Data are the mean ± SD for insulin and glucose and the mean ± SEM for blood pressure; n = 20 before day 10, 12 after day 10, and 4 after day 13.

insulin-infused rats were significantly decreased during the first day and then normalized to the control levels thereafter. These results clearly indicate that the hyperinsulinemic rats adapted to an insulin-resistant state promptly after the first day on the insulin minipump. However, blood pressure did not differ in hyperinsulinemic rats versus the controls up to day 11, and then increased significantly throughout the ensuing days of the experiment. This hypertensive state must continue, since high blood pressure was still evident on day 17 when the experiment was terminated (Fig 1).

Oral Glucose Tolerance

The results of oral glucose tolerance tests on day 10 and 13 in eight rats from each group are shown in Fig 2. Although there was no difference in baseline plasma glucose levels between the two groups, hyperinsulinemic rats had significantly higher baseline plasma insulin than controls on both days. In response to a challenge dose of glucose given orally, hyperinsulinemic rats responded with a further increase in plasma insulin due to unaffected insulin secretion, just like normal control rats, but

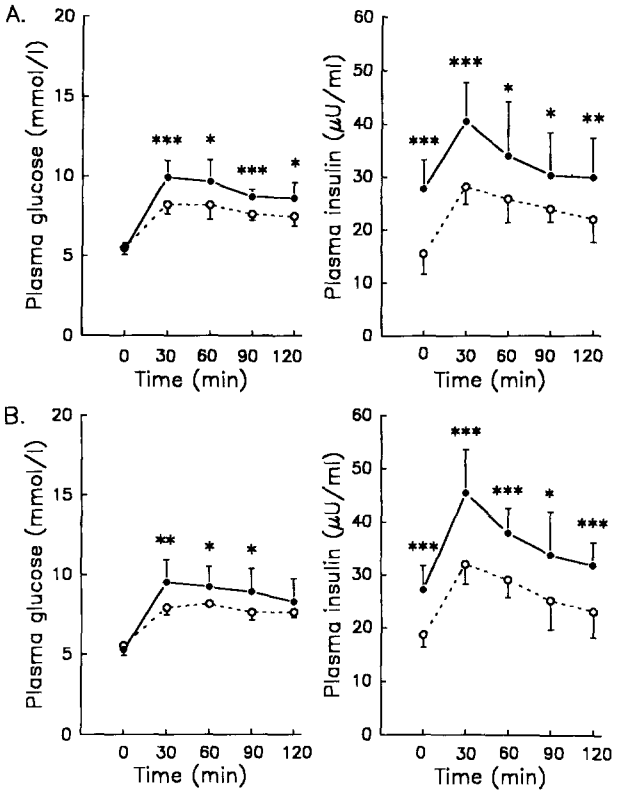

Fig 2. Oral glucose tolerance test results for hyperinsulinemic rats (●) and normal controls (○) on day 10 (A) and day 13 (B) after implantation of minipumps. Data are the mean ± SD; n = 8. *$P < .05$, **$P < .01$, ***$P < .005$.

simultaneously, they also manifested significantly higher plasma glucose than the controls, which did not return to pretest levels at the end of the 120-minute period. These results attest to a characteristic tissue resistance to insulin action on glucose disposal. This glucose-intolerant response in hyperinsulinemic rats occurred on both occasions, namely on day 10 when they were still normotensive and on day 13 when they became hypertensive.

Glucose Transport Into Adipocytes

Glucose transport studies on 2 different days—before and after the rats were hypertensive—produced similar results (Fig 3). On day 10 after minipump implantation, fat cells isolated from hyperinsulinemic rats showed significantly reduced baseline glucose transport compared with the controls (48.6 ± 7.0 *v* 72.6 ± 7.4 fmol/10^5 cells/3 minutes, mean ± SD, $P < .005$). Adipocytes from hyperinsulinemic rats also responded to insulin with much less stimulation on glucose transport versus the control rats. Baseline glucose transport in adipocytes prepared on day 13 showed the same type of difference between hyperinsulinemic and control rats (33.1 ± 12.2 *v* 60.0 ± 19.6 fmol/10^5 cells/3 minutes, mean ± SD, $P < .005$). Upon in vitro insulin stimulation, adipocytes from hyperinsulinemic rats again had a much lower response than fat cells from the normal controls. Glucose uptake comparisons between day 10 and day 13 showed no significant alterations during this 3-day interval in either group.

Insulin Binding to Adipocytes

Baseline insulin binding to adipocytes was not changed, and was 3.0% ± 0.1% of the total radioactive insulin added to preparations of adipocytes isolated from hyperinsulinemic and control rats on days 10 and 13. Table 1 summarizes the results for competitive insulin binding in four experiments. Scatchard plots revealed the high- and low-affinity binding sites. For high-affinity binding sites, there were no differences in the dissociation constants (K_d) and maximal binding (Bmax) between fat cells originating from the two groups of rats and between the preparations from the 2 different days. For low-affinity binding sites, the dissociation of bound insulin from the cells tended to increase from day 10 to day 13, as the K_d of adipocytes isolated from hyperinsulinemic rats was significantly higher on day 13 versus day 10, indicating a deterioration of insulin-binding affinity. Nevertheless, the Bmax increased significantly in both groups from day 10 to day 13, indicating a shifted prevalence of binding sites toward a reduced affinity.

Plasma ET-1 Concentration

Figure 4 shows that on day 10, plasma ET-1 levels of hyperinsulinemic rats were similar to those of normal controls. On day 13, plasma ET-1 levels were significantly higher in the hyperinsulinemic group, in contrast to the unchanged ET-1 levels in the normal control group.

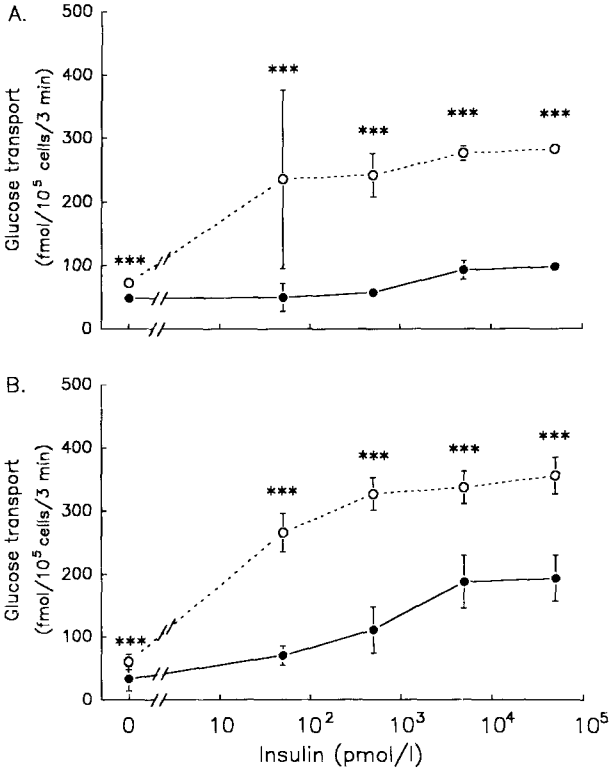

Fig 3. Glucose uptake of adipocytes from hyperinsulinemic rats (●, n = 8) and normal controls (○, n = 8) on day 10 (A) and day 13 (B) after implantation of minipumps. Glucose transport at each insulin concentration was the average measured in duplicate. Data are the mean ± SD of 4 experiments. ***$P < .005$.

Table 1. Insulin Binding to Adipocytes (four experiments, mean ± SD)

Binding Parameter	Control Rats	Hyperinsulinemic Rats	P
High-affinity binding sites			
K_d (nmol/L)			
Day 10	0.18 ± 0.08	0.13 ± 0.05	NS
Day 13	0.10 ± 0.05	0.10 ± 0.04	NS
P	NS	NS	
Bmax (fmol/2 × 10^5 cells)			
Day 10	5.53 ± 2.57	4.27 ± 0.85	NS
Day 13	3.11 ± 0.97	3.52 ± 1.88	NS
P	NS	NS	
Low-affinity binding sites			
K_d (nmol/L)			
Day 10	14.29 ± 13.98	14.93 ± 2.94	NS
Day 13	34.48 ± 17.15	52.63 ± 28.92	NS
P	NS	<.05	
Bmax (fmol/2 × 10^5 cells)			
Day 10	119.42 ± 108.08	124.74 ± 23.70	NS
Day 13	327.93 ± 118.97	480.30 ± 142.31	NS
P	<.05	<.005	

Abbreviation: NS, not significant.

ET-1 Binding to Adipocytes and Aortic Membranes

The results for ET-1 binding to adipocytes are summarized in Table 2. In the insulin-infused group, increases in the K_d and Bmax for ET-1 binding to adipocytes were observed on day 13. The results for ET-1 binding to aortic membranes in one experiment are shown in Fig 5. The basal binding (B0), as retained on the filters, was about 3×10^3 cpm, or 30.0% of the total radioactivity added to each tube. The binding-displacement curves for [^{125}I]ET-1 produced by increasing concentrations of unlabeled ET-1 and the related Scatchard plots do not show any significant differences in binding kinetics between the two groups on days 10 or 13. However, from day 10 to day 13, there was a tendency for an increase in the ET-1 binding site and a decrease in affinity, as Bmax values increased from 29.1 to 44.8 and from 21.8 to 47.8 fmol/50 mg protein, while K_d values increased from 69.5 to 87.1 and from 74.4 to 89.0 pmol/L ET-1

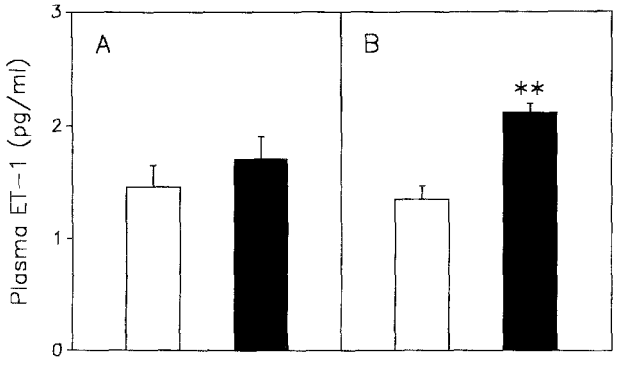

Fig 4. Plasma ET-1 of hyperinsulinemic rats (■) and normal controls (□) on day 10 (A) and day 13 (B) after implantation of minipumps. Data are the mean ± SD of 1 representative experiment; n = 8. **P < .01.

Table 2. ET-1 Binding to Adipocytes (four experiments, mean ± SD)

Binding Parameter	Control Rats	Hyperinsulinemic Rats	P
K_d (pmol/L)			
Day 10	330 0 ± 73.3	398.2 ± 113.1	NS
Day 13	392.2 ± 53.6	532.0 ± 197.3	NS
P	NS	<.05	
Bmax (fmol/2 × 10^5 cells)			
Day 10	8 3 ± 1 5	11.6 ± 2.4	NS
Day 13	11.7 ± 3.0	18.5 ± 3.7	<.05
P	NS	<.02	

Abbreviation: NS, not significant.

for aortic membranes isolated from control and hyperinsulinemic rats, respectively.

DISCUSSION

We induced chronic primary hyperinsulinemia in rats simply by continuous instillation of insulin via implanted minipumps without any carbohydrate supplementation, and confirmed tissue resistance to insulin on several well-defined parameters. In addition, we found that the insulin-infused rats developed hypertension after day 11. Thus, this technique provided a convenient and reliable animal model to study the relationship between insulin resistance and the development of hypertension.

Despite the persistent hyperinsulinemia originating from the insulin minipump, the rats maintained euglycemia after day 2 of the experiment (Fig 1). One could suspect that these rats may have a nullified blood glucose–lowering effect of hyperinsulinemia by mobilization of glucose-counterregulatory factors, including glucagon. However, based on our results, we believe these hyperinsulinemic rats developed insulin resistance very quickly. The hypoglycemic reaction to excessive insulin—observed on day 2 only—resulted primarily from hormonal stimulation on glucose uptake and utilization by the muscles and other insulin-sensitive tissues together with hormonal inhibition on gluconeogenesis in the liver.[27] With the glucose-counterregulatory mechanism alone, the rats were unable to maintain a euglycemic status when blood insulin was continuously supplied in excess.[27] Should glucose counterregulation play a critical role in the observation, we would expect to observe a euglycemic state in advance of sustained hypoglycemia. Therefore, the lack of glucose responsiveness to a sustained abnormally high level of insulin has been considered the most important evidence of insulin insensitivity, and serves as a gold standard for defining insulin resistance in clinical medicine and animal research. Thus, with glucose intolerance, a clinical indicator commonly used for diagnosing NIDDM, insulin resistance is recognized as the fundamental defect in glucose metabolism. In our study, we demonstrated that hyperinsulinemic rats were glucose-intolerant on day 10 or possibly earlier. Our results (Fig 2) also show that the pancreas of these animals was functionally intact, as it responded to the oral glucose load by secreting an additional amount of insulin superimposing the preexisting hyperinsulinemia—a scenario mimicking NIDDM.

Since we previously found that ET-1 inhibited insulin-stimulated glucose uptake in isolated rat adipocytes,[23] we

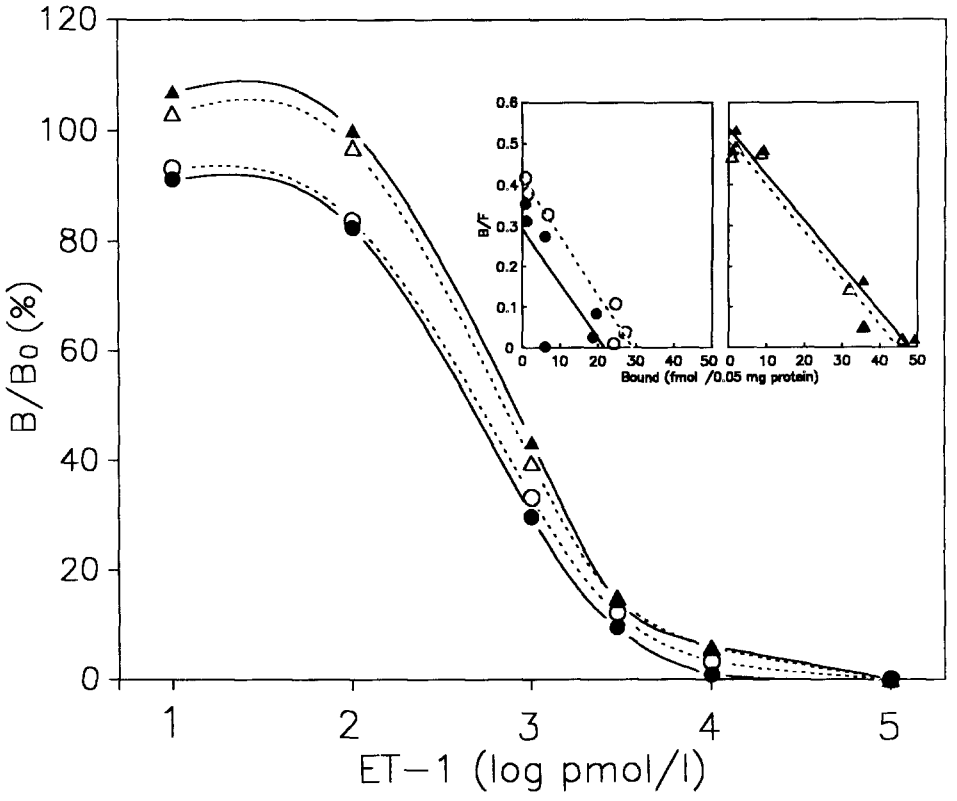

Fig 5. Competitive binding of ET-1 to pooled aortic membranes from hyperinsulinemic rats (—) and normal controls (----) on day 10 (●, ○) and day 13 (▲, △). B0, basal binding without ET-1, designated as 100%. Insert, Scatchard plot.

performed three types of in vitro tests (glucose transport, insulin binding, and ET-1 binding) using isolated fat cells to exemplify the role of ET-1 in mediating insulin resistance. Compared with the normal controls, hyperinsulinemic rats showed reduced glucose uptake on day 10 but did not show altered insulin binding until day 13 (Fig 3)—additional evidence of insulin resistance. Even on day 13, only the low-affinity binding site showed increased K_d and Bmax (Table 1). Thus, the adipocytes of hyperinsulinemic rats showed insulin resistance at least 3 days prior to the detection of hypertension on day 13 (Fig 1). ET-1 binding to adipocytes changed significantly from day 10 to day 13 by increasing the K_d and Bmax in hyperinsulinemic rats only, such that they had a significantly higher Bmax for ET-1 binding to adipocytes than the controls on day 13 (Table 2).

Whereas insulin resistance in various tissues occurred prior to hypertension, the concomitance of changes in plasma ET-1 levels (Fig 4), adipocyte ET-1 binding, and systolic blood pressure provided a clue to explore the mediator role of ET-1 in the development of hypertension with insulin within the vascular system. The aorta was used for ET-1 binding studies because it is a vascular tissue in relative abundance. The results showed that ET-1 binding to aortic membranes was altered similarly from day 10 to day 13 among hyperinsulinemic and control rats (Fig 5). However, an increased plasma ET-1 level usually downregulates vascular ET receptors.[28] Furthermore, the expected downregulation of ET-1 binding sites in the aorta by the elevated blood pressure, as observed in the SHR[29] and in deoxycorticosterone acetate-salt hypertensive rats,[30] did not occur in the hyperinsulinemic state. There was likely a derangement of the regulatory feedback mechanisms between ET-1 and ET receptors and also between ET receptors and the blood pressure elevation relating to a chronic hyperinsulinemic state. The increased plasma ET-1 level without downregulation of vascular ET-1 binding in hyperinsulinemic rats would result in no other alternative but a sustained hypertension.

Insulin has been found to stimulate ET-1 mRNA expression in endothelial cells.[31] Insulin also stimulates ET-1 production and secretion from endothelial cells in culture.[32] Furthermore, insulin induces a twofold increase in ETA receptors of normal vascular smooth muscle cells, and insulin together with ET-1 potentiates the proliferation of these cells in culture.[33] The biochemical evidence accumulated thus far supports the proposition that the hypertensive effect of hyperinsulinemia is likely mediated by the enhanced ET-1 action, and ET-1 is involved in developing and maintaining hypertension in some experimental models and in human essential hypertension.[34]

Insulin resistance—an omnibus term[35]—is a global concept in clinical applications. The coexistence of hypertension and insulin resistance, as described in syndrome X, merely offers circumstantial evidence for the correlation between the two symptoms. Theoretically, insulin resistance occurs by either downregulation of insulin receptors or signal impairment at postreceptor levels. Neither pathway can adequately explain the insulin effect on blood pressure. On the contrary, insulin at an unphysiological dose is a vasodilator, increasing the skeletal muscle blood flow.[36] Acute hyperinsulinemia does not increase arterial pressure in dogs and humans.[37] Thus, if chronic hyperinsulinemia has anything to do with blood pressure elevation, the effect of insulin should be mediated by other factors closely affecting the vascular system rather than any

speculative hemodynamic action of insulin itself. A study by Meehan et al[38] suggested a sympathetically mediated mechanism for rat hypertension induced by long-term administration of insulin. In fructose-hypertensive rats, Verma et al[39] found that long-term oral bosentan treatment (for endothelin blockade) decreased the hyperinsulinemia-related development of high blood pressure. Our results also suggest ET-1 as a mediator for the hypertensive effect of insulin. When hyperinsulinemia lasts as long as 13 days in rats, insulin resistance occurs in tissues and coexists with hypertension. Thus, hypertension and insulin resistance appear to be two collateral sequelae of prolonged hyperinsulinemia, and related by hyperendothelinemia.

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