Evidence for Increased Functional Vascular Na\(^+\)/K\(^+\) Pump Activity in the Obese Zucker Rat

Michael S. Golub, Chwen-Tzuei Chang*, Michael L. Tuck, and Morris E. Berger

Insulin is known to stimulate Na\(^+\)/K\(^+\) ATPase and to relax vascular smooth muscle. We hypothesized that vascular tone in the obese Zucker (fa/fa) rat, a hyperinsulinemic model in which hypertension can develop, may be influenced by insulin's ability to stimulate Na\(^+\)/K\(^+\) ATPase at the vascular level. We studied isometric preparations of tail and femoral arteries from 10-wk-old, male obese Zucker rats, which were hyperinsulinemic but still normotensive vs. lean controls. Sensitivity to potassium-induced relaxations, an index of vascular Na\(^+\)/K\(^+\) ATPase activity was significantly greater in the obese Zucker rat than control. Sensitivity to transmural-nerve-stimulation-induced contractions was decreased in the femoral and tail arteries from obese rats as compared with lean controls. Insulin (50 to 200 mU/ml) mimicked potassium-induced relaxations in the femoral artery, an effect that was significantly greater in the obese group. These data suggest that in the young hyperinsulinemic Zucker rat, insulin has a stimulatory effect on the vascular Na\(^+\)/K\(^+\) pump, which may be associated with a decreased presynaptic adrenergic influence on vascular tone. Development of resistance to these vascular relaxant effects of insulin with advancing age might contribute to the onset of hypertension in this model. (Hypertens Res 1998; 21: 283-288)

Key Words: adenosine triphosphatase, sodium, potassium, hyperinsulinism, hypertension, diabetes, blood vessels

It has been postulated that insulin resistance associated with hyperinsulinemia is an etiologic factor in the development of essential hypertension in some human populations (1). Several animal models that become hypertensive also display insulin resistance associated with hyperinsulinemia (2, 3). The Zucker fatty rat strain (fa/fa) has a recessively inherited propensity to obesity, which metabolically is characterized by hyperinsulinemia, insulin resistance, and hyperlipidemia (4). Mild hypertension is found in about half of previously reported studies (5). In addition to variable blood pressures, studies of vascular responsiveness in isolated blood vessels have yielded conflicting results (6, 7). Abnormalities in the obese Zucker rat's autonomic nervous system, described as both central hypothalamic lesions and blunted peripheral sympathetic catecholamine responses (8), may contribute to the variable incidence of hypertension. It is apparent from the literature that factors such as the age of the rat, method of blood pressure measurement, use of anesthesia, and choice of in vitro tissue preparation may also be important.

Insulin is reported to stimulate Na\(^+\)/K\(^+\) ATPase (sodium pump) activity in several tissues (9), either directly or secondary to changes in intracellular sodium (10). Activity of the sodium pump is an important determinant of vascular responsiveness (11). There is evidence that insulin reduces agonist-induced contraction by stimulating the sodium pump, hyperpolarizing vascular smooth muscle cells, and decreasing calcium influx (12). Although vascular tissue is heterogeneous and difficult to evaluate directly, evidence for altered sodium pump activity has been reported in several animal models of hypertension (13, 14). We recently reported evidence for increased functional Na\(^+\)/K\(^+\) ATPase activity in the vasculature of fructose-fed hyperinsulinemic and hypertensive rats (15).

In the young Zucker rat (10 to 12 wk of age), before the onset of significant renal injury (24 wk), we hypothesized that insulin's vasodilatory effects, mediated by increased Na\(^+\)/K\(^+\) ATPase activity, contribute to the maintenance of normotension. In contrast to demonstrations of increased sympathetic activity (16) and vascular responsiveness (17-19), there are also reports of decreased sympathetic activity in the Zucker rat (20). We speculated that...
in addition to a pump-related peripheral vasodilatory effect there might be an abnormal sympathetic neurogenic response to insulin in this model.

Methods

Characterization of Animal Model

Studies were performed in 10- to 12-wk-old male Zucker fatty (fa/fa) or lean (FA/FA) rats, obtained from the Vassar College Animal Facility (N.I.H. Obesity Research Center, Poughkeepsie, New York) and fed normal sodium (4 g/kg of diet) Purina (No. 5001) laboratory chow ad libitum. Body weight and insulin levels were significantly (p < 0.01) higher in the obese male Zucker rats (Table 1). Serum glucose values were not significantly different, and tail cuff blood pressures were also comparable (Table 1).

Contractile Responses to Electrical Nerve Stimulation in Isolated Blood Vessels

Contraction studies in isolated blood vessels

Femoral and tail arteries were excised, cleaned of loose fat and connective tissue, and placed in oxygenated Krebs bicarbonate buffer of the following composition (mM): Na, 144.2; K, 4.9; Ca, 1.3; Mg, 1.2; Cl, 126.7; HCO3, 25; SO4, 1.2; PO4, 1.2; glucose, 11.1; ascorbic acid, 0.11; and EDTA, 0.025. Care was taken to maintain an intact endothelium. Stainless steel wires were inserted through the lumen of 4-mm ring segments, which were then mounted in specially constructed water-jacketed (37.5°C) tissue baths, as described previously (21). Each bath was oxygenated (5% CO2, 95% O2) and equipped with an isometric force transducer (UC-2, Gould-Statham, Oxnard, CA) coupled to an eight-channel recording system (8800, Gould, Cleveland, OH). Femoral and tail arteries were equilibrated for 2 h at optimum passive tension, as determined in previous studies (0.6 and 2.0 g, respectively). Femoral and tail arteries from Zucker rats, incubated in a potassium (0.6 mM) solution and precontracted with norepinephrine (5 x 10^-7 M) while in low potassium (0.6 mM) and normal potassium (4.9 mM) solutions.

Potassium-Induced Relaxation Bioassay for Na+/K+ ATPase Pump Activity

For measurement of potassium-induced relaxations as an assessment of Na+/K+ ATPase pump activity (23), femoral ring segments were incubated in low potassium (0.6 mM) buffer for 15 min with or without added ouabain (10^-4 M). The tissues were then contracted with 5 x 10^-7 M norepinephrine. Five minutes later, the contractile response had stabilized and successive doses of potassium chloride (0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mM) were added to produce cumulative stepwise increases in potassium concentration (i.e., 0.4, 0.8, 1.4, 2.2, 3.2, and 4.4 mM). The relaxation responses to potassium were recorded as a percent of the norepinephrine-induced contraction. The direct effect of porcine insulin (Sigma, St. Louis, MO) added cumulatively (1, 10, 25, 50, 100, and 200 mU/ml) at 6-min intervals was evaluated in the femoral artery from rats, precontracted with norepinephrine (5 x 10^-7 M) while in low potassium (0.6 mM) and normal potassium (4.9 mM) solutions.

Statistical Analysis

Comparisons between obese and lean rats utilized Student’s unpaired t-test after two-way analysis of variance (ANOVA) when appropriate. A personal computer program (Statpak, Northwest Analytical, Inc., Portland, OR) was used for these calculations. Data are expressed as the means ± SEM.
obtained when the tissues were incubated in a low-potassium buffer (Fig. 2, ANOVA, p < 0.007). The relaxation responses were significantly greater in the vessel segments obtained from the obese rats at 50 to 200 mU/ml (p < 0.05). No relaxation responses were noted in obese or lean vessels incubated in a normal potassium Krebs solution.

Contractile Responses to Nerve Stimulation
Femoral arteries from obese rats showed reduced responsiveness to transmural nerve stimulation as compared with lean controls (Fig. 3, ANOVA, F = 16.9, p < 0.005). The relaxation responses were significantly greater in the vessel segments obtained from the obese rats at 50 to 200 mU/ml (p < 0.05). No relaxation responses were noted in obese or lean vessels incubated in a normal potassium Krebs solution.

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Table 1. Physiological Characteristics of 10- to 12-Week-Old Male Zucker Rats

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Weight (g)</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (mU/ml)</th>
<th>Blood Pressure (systolic, mmHg)</th>
</tr>
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<tbody>
<tr>
<td>Obese</td>
<td>8</td>
<td>356 ± 7**</td>
<td>121 ± 4</td>
<td>75 ± 7**</td>
<td>112 ± 3</td>
</tr>
<tr>
<td>Lean</td>
<td>7</td>
<td>250 ± 9</td>
<td>107 ± 7</td>
<td>30 ± 4</td>
<td>112 ± 1</td>
</tr>
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**p < 0.01 vs. lean; (n), number of rats

![Graph of relaxation responses to added potassium in femoral artery segments from obese and lean rats, incubated in a low potassium buffer (0.6 mM) and precontracted with norepinephrine (5 X 10^-7 M) with and without added ouabain (10^-4 M). Final potassium concentration (logarithmic scale) is plotted against percent relaxation (as a percent of contraction induced by norepinephrine). Significant differences (p<0.05) at a given dose between obese and lean groups are indicated by *. All data points were evaluated by two-way analysis of variance and differed significantly between the obese and lean groups without the addition of ouabain (F=10.1, p<0.02).](image1)

![Graph of relaxation responses to added insulin in femoral artery segments from obese and lean rats, preincubated in a low potassium buffer (0.6 mM) and precontracted with norepinephrine (5 X 10^-7 M). The dose of insulin (logarithmic scale) is plotted against the relaxation response (as a percent of the contraction induced by norepinephrine). Significant differences (p<0.05) between the obese and lean groups at individual doses are indicated by *. Two-way analysis of variance demonstrated significant differences for all data points (F=17.1, p<0.007).](image2)

![Graph of transmural nerve stimulation (TNS) responses in femoral artery segments from obese and lean rats. Nerve stimulation (in Hz, logarithmic scale) is plotted against percent of maximal contraction to TNS. Significant differences (p<0.01) for a given stimulation rate are indicated by **. Two-way analysis of variance demonstrated a significant difference between obese and lean rat arteries (F=16.9, p<0.005).](image3)

![Graph of maximal contractile responses to nerve stimulation in femoral arteries from obese and lean animals (obese ED50, 13.8 ± 0.9 Hz vs. lean ED50, 9.1 ± 1.2 Hz, n = 8, p < 0.01). The maximal contractile responses to nerve stimulation (40 Hz) were not significantly different (obese, 2.47 ± 0.46 g vs. lean, 2.89 ± 0.34 g). Treatment of the femoral arteries with insulin (12 mU/ml for 30 min had no effect on TNS responses. In parallel studies, we found that similar to femoral arteries, tail arteries from the obese Zucker rat showed decreased responsiveness to TNS as compared with lean controls (obese ED50, 6.2 ± 0.5 Hz, vs. lean ED50, 3.8 ± 0.6 Hz, n = 7 for both, p < 0.01). The maximal gram responses to TNS were not significantly different (obese, 2.56 ± 0.39 g vs. lean, 3.53 ± 0.38 g), although lean tail arteries had greater maximum responses to exogenous NE (6 × 10^-6 M).](image4)
Na+/K+-ATPase activity (30) in femoral arteries pump activity in vitro in vessels from the Zucker fatty rat could also be a secondary response to internal reabsorption of sodium, while increasing vascular tone or removal of the effect of a circulating Na+/K+ ATPase inhibitor reduces reuptake of norepinephrine from nerve terminals that were pretreated with reserpine to deplete the vesicles (36). Thus, both the release and reuptake of norepinephrine may be influenced by increased Na+/K+ ATPase activity and contribute to the decrease in vascular responsiveness to TNS seen in the obese Zucker rat. We recently found that isolated arteries from hyperinsulinemic and hypertensive, fructose-fed rats also have increased vascular Na+/K+ ATPase pump activity and decreased sensitivity to norepinephrine in vitro (15) and in vivo (37).

Increased sympathetic activity is often associated with obesity-related hypertension, and hyperinsulinemia has been implicated as a factor in this increased neural activity (38). Zemel et al., however, reported that sympathetic blockade did not eliminate the blood pressure difference between obese and lean Zucker rats (39). Increased sympathetic activity in the Zucker fatty rat in vivo might result in neurotransmitter depletion or adrenergic desensitization in vascular segment responses to transmural nerve stimulation in vitro. Although desensitization to norepinephrine’s contractile effects was not directly tested in our study, others (17–19) have reported increased sensitivity to adrenergic agonists in the aorta from obese rats of similar age. The increased neurotransmitter reuptake resulting from Na+/K+ ATPase stimulation may eventually lead to such post-synaptic potentiation.

The effects of insulin on vascular reactivity are complex. Acute insulin infusions generally have a vasoconstrictive effect. However, more chronic insulin infusions can raise blood pressure in the rat (3) but not the dog (40). In vitro, insulin antagonizes con-
tractile responses to norepinephrine and angiotensin in a dose-dependent manner in rabbit arterial and venous segments (41). Kahn et al. (12) recently showed that insulin antagonized angiotensin-induced contractions in individual vascular smooth muscle cells, an effect that was prevented by ouabain, suggesting that the vasodilatory effect was directly mediated by Na+/K+ ATPase. Insulin may also have a direct inhibitory effect on smooth-muscle-cell intracellular signaling mechanisms induced by contractile agonists. We have demonstrated that insulin decreases angiotensin-induced mobilization of intracellular calcium in rat aortic vascular smooth muscle cells (42). Recent studies with vasopressin (43) and serotonin (12) have also shown an attenuating effect of insulin on the vascular smooth muscle cell calcium signal. However, insulin attenuated the vasopressin-induced calcium signal to a similar extent in cultured vascular smooth muscle cells from obese and lean Zucker rats (43). Wambach and Liu (44) could only elicit attenuation of mesenteric artery contractions in the rat with insulin concentrations of more than 100 mU. We could not elicit direct vasorelaxant effects to insulin unless the tissues were incubated with supraphysiologic doses in a low-potassium medium. Under these artificial conditions, the effect of insulin was actually somewhat greater in the femoral vessels from the obese strain.

The Zucker rat has been studied extensively as an example of genetic obesity and hyperlipidemia. Recent interest in this model has developed because of the similarity of this inbred animal strain to the syndrome of obesity, hyperlipidemia, hyperinsulinemia, and hypertension in human populations (1). However, as compared with the consistent metabolic changes in this model, hypertension as measured by tail cuff has been a variable finding. Several studies demonstrating elevated blood pressure used older animals, which may have had concomitant renal injury (45). Reddy and Kotchen (46) could only demonstrate elevated blood pressure when the animals were fed a high sodium intake, but others (47) could not find significant changes in blood pressure during normal or high sodium intake. On the other hand, careful investigations with indwelling arterial catheters have revealed small, consistent differences in blood pressure between obese and lean animals (7, 17), independent of sodium intake (48). Cox and Kikta studied the Zucker model at several ages and found that tail-cuff blood pressures rose with age at a greater rate in the obese animals, achieving statistically higher levels at weeks 33 to 36. Until 24 wk the obese animals had lower blood pressures than the lean controls (18). In agreement with these results, the present study’s tail-cuff measurements did not show differences in blood pressure between obese and lean Zucker rats 10 to 12 wk of age.

In summary, this study provides evidence of increased insulin-related stimulation of vascular Na+/K+ pump activity in the Zucker obese rat. Increased levels of insulin may be associated with increased activation of this enzyme. This action of insulin may be an important factor in altering sympathetic and vascular reactivity in vivo and in vitro. Chronic hyperinsulinemia could produce down-regulation of the vascular relaxant effects of insulin and contribute to the later rise in blood pressure in this model.

References