Increased Functional Na⁺-K⁺ Pump Activity in the Vasculature of Fructose-Fed Hyperinsulinemic and Hypertensive Rats

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We hypothesized that hyperinsulinemia may alter insulin's ability to stimulate vascular Na⁺/K⁺-ATPase pump activity and modulate changes in vascular responsiveness associated with hypertension. We measured potassium-induced relaxation as an indicator of Na⁺/K⁺-ATPase pump activity in isolated femoral arteries from fructose-fed (FF) hyperinsulinemic, Sprague-Dawley rats. FF rats had higher mean arterial blood pressures than did normal diet-fed (NF) rats (FF, 125 ± 2.2, n = 20, vs. NF, 113.5 ± 2.5 mmHg, n = 20, p < 0.05) and were hyperinsulinemic (FF, 64 ± 4 vs. NF, 37 ± 2, μU/ml insulin, p < 0.01). FF rats were more sensitive to KCl in the Na⁺/K⁺ pump bioassay (FF, 0.86 ± 0.07, n = 21 vs. NF, 1.18 ± 0.08, n = 17, p < 0.05, expressed as ED50 in mmol/l KCl). Exogenous insulin (100 mU/ml) increased Na⁺/K⁺ pump sensitivity in FF rats as compared with a boiled insulin control (insulin 45 ± 6%, n = 11, vs. control, 11 ± 7%, n = 13, p < 0.01, expressed as percent increase in sensitivity, i.e., ED50). There were no significant differences in Na⁺/K⁺ pump sensitivity between insulin and control responses in the NF animals (insulin 29 ± 6%, n = 11, vs. control 46 ± 5%, n = 10, NS). Dose-response curves were obtained in tail and femoral arteries from the same animals to norepinephrine and acetylcholine, basally and after exogenous insulin. FF vessels had reduced sensitivity to norepinephrine as compared with the NF group. Insulin increased sensitivity to acetylcholine-induced relaxations and increased All-induced contractions in FF-rat vessels. These data suggest that in the FF rat insulin's influence on the vascular Na⁺/K⁺ pump is enhanced and may modulate the changes in vascular responsiveness seen in this model. (Hypertens Res 1998; 21: 73-80)

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

Hypertension has been found to coexist with hyperinsulinemia and insulin resistance in both humans (1) and various animal models of hypertension, including the obese Zucker rat (2), the fructose-fed rat (3), the Dahl salt-sensitive rat (4), and the spontaneously hypertensive rat (5). In these models, increases in adenosinetriphosphatase (Na⁺/K⁺-ATPase) activity have been proposed to contribute to alterations in vascular responsiveness. Insulin has been reported to stimulate the active transport of Na⁺ and K⁺ ions across the plasma membrane via Na⁺/K⁺-ATPase in various tissues (6). This translocation of sodium and other ions has also been related to changes in vascular reactivity associated with hypertension (7). Insulin has been reported to have a direct vasodilatory effect in vitro (8-11) and in vivo (12-14). We evaluated the effect of insulin on the Na⁺/K⁺ pump in diet-induced hyperinsulinemia with the use of a bioassay method (15) in isolated blood vessels in which K⁺-induced relaxations are related to Na⁺/K⁺ pump activity. We also compared in vitro vascular responses to exogenous norepinephrine, transmural nerve stimulation, angiotensin II (All), and acetylcholine to determine if increases in pump activity correlated with alterations of vascular responsiveness. We hypothesized that increased vascular responsiveness and hypertension in this model would be promoted by down-regulation of the stimulatory effect of insulin on Na⁺/K⁺-ATPase activity. Conversely, if the sodium pump response was enhanced there would be a greater compensatory vasodilatory mechanism.
Methods

Experimental Animals

Fifty, male, 9 wk-old Sprague-Dawley rats (200 to 230 g) were purchased from Taconic Farms Inc. (Germantown, NY). The rats were housed in a vivarium (approved by the American Association for the Accreditation of Laboratory Animal Care) on a 12 h light/dark cycle at 75°F. For three weeks, the rats were fed standard, 501 Purina Laboratory Chow (Richmond, IN) ad libitum, and acclimated to routine blood pressure measurement via indirect tail cuff electromyographiometry (Narco Biosystems, Houston, TX). Rats were randomly divided into two groups, a high fructose diet (TD 89054, Harlan Teklad, Madison, WI) in which 60% (g/kg) of the diet consisted of fructose and a group remaining on standard laboratory chow (normal diet-fed = NF). All rats had free access to water. Studies were staggered over a 3 mo period in order to accommodate the in vivo blood pressure studies and the in vitro tissue bath contraction studies.

Blood Pressure Measurement

On each study day between week 16 (after starting the diet) and week 28, direct basal and agonist-induced blood pressure responses were measured in a FF and a NF rats. Twenty-four hours before study, two rats were catheterized for direct blood pressure measurement. The rats were anesthesized with a combination of ketamine hydrochloride (90 mg/kg) and xylazine hydrochloride (10 mg/kg). A heparin (1,000 U/ml)-filled polyethylene catheter (PE 50) was directed toward the heart through the carotid artery and subsequently sutured in place, tunneled subcutaneously between the scapulae, and exteriorized. Mean arterial blood pressure (MAP) was continuously recorded the following day in conscious, unrestrained animals with the use of a Statham P23ID pressure transducer coupled to a recording system (Model 411, Beckman Instruments, Fullerton, CA).

Biochemical Measurements

Biochemical Measurements

On the following day, the rats were exsanguinated (following the guidelines of the Guide for the Care and Use of Laboratory Animals, NIH85-23, 1985) for in vitro vascular activity studies, and blood samples were collected for determination of plasma insulin by radioimmunoassay (ICN Diagnostics, Costa Mesa, CA) and for determination of glucose by a glucose oxidase-oxygen rate method (Glucose Analyzer 2, Beckman Instruments, Fullerton, CA).

Vascular Reactivity

Twenty-four hours after blood pressure studies, the rats were sacrificed, and the femoral and tail arteries were rapidly removed and transferred to an oxygenated, Krebs-bicarbonate solution, (mmol/l composition: Na+ 144.2, K+ 4.9, Ca2+ 1.3, Mg2+ 1.2, Cl– 26.7, HCO3– 25.0, SO42– 1.2, PO42– 1.2, glucose 11.1, EDTA 0.024). Methods for preparing isolated blood vessels for contraction studies have been described previously (16). Briefly, 4-mm ring segments were mounted isometrically in heated (37.5°C) and oxygenen (5% CO2, 95% O2) tissue baths. To produce neurogenic contractions via release of endogenous norepinephrine, each bath was equipped with a pair of platinum wire electrodes straddling each ring segment. Electrodes delivered 17-volt, square wave, monophasic pulses (duration 0.3 ms) for 30 s intervals at pre-set frequencies (0.5 to 50 pulses/s) via a specially designed 8-channel stimulator. In previous experiments, these transmural nerve stimulation (TNS)-induced contractions were completely inhibited by tetrodotoxin (1 X 10–6 mol/l), confirming the lack of artifacts due to direct electrical stimulation (17). Force in grams was measured from 12 tissue baths simultaneously via UC-2 transducers (UTC Inc., Camarillo, CA) and amplifiers (Gould Electronics, Cleveland, OH) connected to a data acquisition board, using a software program customized for tissue bath studies (Buxco Electronics, Troy, NY). Tail and femoral arteries were stretched to an optimal passive tension (0.6 g and 2.0 g, respectively) for 2 h. Optimal tension was previously determined as the passive force producing the greatest contraction to an ED50 dose (mean effective dose) of norepinephrine (NE). After the equilibration period, stable responses to NE (3 X 10–7 mol/l femoral and 6 X 10–7 mol/l tail) were obtained in both arteries. To assess the quality of endothelium, NE contracted vessels were then exposed to acetylcholine (1 X 10–4 M). Vessels that relaxed less than 40% of the NE contraction were not used. In addition, tail artery preparations were stimulated via transmural nerve stimulation (TNS, 8 pulses/s) to assess the quality of neurogenic contractile responses. Vessels were discarded if they yielded variable or low (less than 0.5 g) contractile responses to TNS.

Adjacent 4-mm ring segments from each rat within the two diet groups were then assigned to subgroups, which were studied after incubation with either exogenous insulin (100 mU/ml) or a similar dose of heat-inactivated insulin. Initially, basal vascular responsiveness was measured in both subgroups. Basal responses were pooled to describe the effects of diet on vascular responsiveness.

AII Responsiveness

The femoral arteries were exposed to repeated single doses of AII (because significant immediate tachyphylaxis makes femoral arteries unresponsive to cumulative addition of AII) and then used as a bioassay tissue for Na+ K+-ATPase activity. Three AII (5 X 10–8 mol/l) contractions were obtained in normal K+-Krebs at 30 min intervals. Significant tachyphylaxis was apparent between the first and second dose, but decreased after the second dose.

Potassium-Induced Relaxation

Femoral arteries were incubated for 30 min in a low potassium chloride (KCl, 0.6 mmol/l) Krebs buffer and then contracted with NE (3 X 10–7 mol/l). After 5 min a stable NE plateau was obtained, and
KCl was added in cumulative doses to yield 0.2, 0.4, 0.8, 1.2, 1.8, 2.6, 3.6, and 4.8 mmol/l concentrations, which caused stepwise relaxations. Following maximal relaxation, the vessels were washed with normal (4.9 mmol/l) KCl Krebs and allowed to reequilibrate for 30 min. Insulin (100 mU/ml, Iletin II, Eli Lilly, Indianapolis, IN) or heat-inactivated insulin (100°C for 10 min.) was added for 30 min, and the AII and the K+ relaxation protocols were repeated.

Acetylcholine-Induced Relaxations and Contraction to Electrical Stimulation
Tail artery preparations were contracted with NE (6 × 10^{-7} mol/l) until a stable plateau was reached. Acetylcholine was added cumulatively in 10-fold increasing dose steps (10^{-8}-10^{-4} mol/l) to yield maximal relaxations at each dose. Tissues were washed and reequilibrated in normal Krebs for 30 min. Contractions were then obtained in response to TNS by stimulating these vessels every 10 min for 30-s intervals with square wave electrical pulses of increasing frequency (0.5, 1, 2, 4, 8, 16, 32, and 50 pulses/s). The acetylcholine and TNS protocol was then repeated after incubating each vessel in insulin (100 mU/ml) for 30 min.

NE Dose Response Curves
In separate tail artery segments, NE was added in increasing doses (10^{-9} to 6 × 10^{-5} mol/l), producing stepwise contractions until a maximal response was obtained. Vessels were repeatedly washed over the next hour until they reached baseline, followed by addition of insulin (100 mU/ml) for 30 min and repetition of the NE dose-response curve. An adjacent segment of tail artery from the same rat served as a parallel control in both protocols and received heat-inactivated insulin. NE, acetylcholine, and AII (Sigma Chemical, St Louis, MO) solutions were prepared fresh daily.

Data Analysis
ED50s for cumulative relaxation responses (KCl and acetylcholine) and contraction responses (NE and TNS) were calculated by regression analysis of the responses that fell between 15% and 85% of the maximal response to that agonist. The ED50 for KCl is an index of the sensitivity of the Na+/K+ pump for K+ and is the calculated concentration of K+ that relaxed precontracted vessels to 50% of maximal relaxation. Statistical analysis was performed with paired t-tests when the same treatment group was being compared or unpaired t-tests when NF rats were compared with FF rats. P values less than 0.05 were considered to indicate statistical significance, and the data are expressed as the means ± SEM.

Results
Physiological and Biochemical Measurements
Blood pressure and plasma insulin levels were elevated at 16 to 28 wk of fructose feeding in rats with patent catheters and reliable direct blood pressure measurements (Table 1). There was no significant effect of the fructose diet on body weight or blood glucose. The insulin/glucose ratio was higher in the FF group (FF, 0.53 ± 0.04, n = 20, vs. NF, 0.32 ± 0.02, n = 20, p < 0.01). There were no significant differences in basal tail cuff, systolic blood pressures between the groups before beginning the diets (FF-fed, 116 ± 1 vs. NF, 113 ± 2 mmHg, NS).

Potassium-Induced Relaxation, Femoral Artery
Femoral arteries from the FF group, incubated in low KCl Krebs and contracted with NE, were significantly more sensitive to the relaxing effect of KCl than were the arteries from the NF group (Fig. 1). In these vessels, the contractions induced by the fixed dose of NE (3 × 10^{-7} mol/l) were significantly decreased in vessels from FF as compared with the NF rat arteries (FF, 1.64 ± 0.14, vs. NF, 2.31 ± 0.23 g, n = 22, p < 0.05). The readdition of KCl relaxed both FF and NF femoral arteries to the baseline of the NE-induced contractions. Thus, the total cumulative relaxations to the readdition of KCl were less in the FF femoral arteries than in the NF arteries (FF, 1.64 ± 0.17, n = 27, vs. NF, 2.51 ± 0.24 g, n = 24, p < 0.01).

The effects of a 30-min exposure to insulin (100 mU/ml) on the relaxation response to the cumulative addition of KCl are shown in Fig. 2 for both the FF and NF groups. Exogenous insulin resulted in a greater sensitivity to the relaxing effect of KCl in the FF animals as compared with the addition of a boiled insulin control (Fig. 2A). There was no significant difference between the relaxation curves after incubation with insulin and boiled insulin in the NF group (Fig. 2B). The FF vessels treated with insulin increased their sensitivity (expressed as a percent change in ED50) to KCl by 45 ± 6%, (n = 11), whereas heat-inactivated insulin increased sensitivity by 11 ± 7% (n = 13, p < 0.01). In the NF vessels, there was no significant difference in the percent change in ED50s between insulin and heat-inactivated insulin controls (29 ± 6, n = 11, vs. 46 ± 5%, n = 10, NS).

AII Responsiveness, Femoral Artery
There was no effect of diet on the magnitude of AII

Table 1. Effects of High Simple Carbohydrate Diets in Sprague-Dawley Rats

<table>
<thead>
<tr>
<th>Diet Group</th>
<th>(N)</th>
<th>MAP (mmHg)</th>
<th>Insulin (mU/ml)</th>
<th>Glucose (mg/dl)</th>
<th>Body wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>19</td>
<td>113.5±2.5</td>
<td>37±2</td>
<td>118.1±2.0</td>
<td>498±9</td>
</tr>
<tr>
<td>Fructose</td>
<td>19</td>
<td>125.2±2.2*</td>
<td>64±4**</td>
<td>126.0±5.2</td>
<td>476±5</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, vs. normal diet.
(5 × 10^{-8} \text{ mol/l}) contractile responses in the femoral artery, but exogenous insulin (100 \text{ mU/ml}) potentiated AII contractions in the FF group. All contractions increased from 0.38 ± 0.04 g to 0.50 ± 0.07 g, \( n = 12 \), after insulin incubation (\( p < 0.05 \)). Heat-inactivated insulin was without significant effect (basal, 0.35 ± 0.05 g vs. heat-inactivated insulin, 0.40 ± 0.07 g, \( n = 11 \), NS). No significant effects of insulin or heat-inactivated insulin were seen on AII contractions in vessels from NF rats.

Dose Response Curves to NE, Electrical Stimulation, and Acetylcholine, Tail Artery

The ED50 values to exogenous NE in the tail artery were greater in the FF group than in the NF group, while sensitivities to TNS and acetylcholine were similar (Fig. 3, Table 2). There were no significant differences between the diet groups in maximal gram responses to NE (FF, 5.58 ± 0.49, \( n = 20 \) vs. NF, 4.71 ± 0.51 g, \( n = 18 \), NS) or TNS (FF, 4.23 ± 0.54, \( n = 20 \) vs. NF, 4.40 ± 0.67 g, \( n = 21 \), NS). These latter maximal responses were similar to those obtained after treatment with insulin.

The NE contractions before the addition of acetylcholine in the FF and NF animals were similar (FF, 1.59 ± 0.20, \( n = 12 \), vs. NF, 1.86 ± 0.22 g, \( n = 15 \)). The maximal relaxations to acetylcholine were also similar (FF 0.56 ± 0.10, \( n = 9 \), vs. NF, 0.78 ± 0.11 g, \( n = 6 \)). However, following exogenous insulin there was increased sensitivity (reduction of the ED50 value) to acetylcholine in the FF group (insulin, 0.62 ± 0.21 × 10^{-6}, vs. basal, 1.37 ± 0.35 × 10^{-6} \text{ mol/l}, \( n = 7 \), \( p < 0.05 \)). There was no significant effect of the heat-inactivated insulin control.
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on sensitivity to acetylcholine in the FF group (heat-inactivated insulin, 1.12 ± 0.23 × 10⁻⁶ mol/l, vs. basal, 1.46 ± 0.32 × 10⁻⁶ mol/l, n = 7, NS). In the NF rats, ED50s for acetylcholine-induced relaxation were similar before and after insulin and before and after the heat-inactivated insulin control.

There was no significant effect of exogenous insulin on the sensitivity to norepinephrine and no difference in the effect of insulin between tail arteries from FF and NF rats. Heat-inactivated insulin also had similar, insignificant effects on norepinephrine sensitivity in FF and NF rats. The ED50s to TNS after insulin pretreatment for 30 min were also similar in the FF and NF rats.

Discussion

The high simple carbohydrate fructose diet used in this study had effects similar to those reported by others, including elevated blood pressure, hyperinsulinemia, and insulin resistance (3, 18). Our primary finding was that femoral arteries from rats made hyperinsulinemic by fructose feeding showed a functional equivalent of increased Na⁺,K⁺-ATPase pump activity. This was demonstrated as increased sensitivity to the relaxing effect of K⁺ in NE-contracted femoral arteries incubated in low K⁺ (15). The ability of exogenous insulin to further increase sensitivity of potassium-induced relaxation in blood vessels from FF rats also suggests than insulin has a direct effect on the pump. This vascular effect was accompanied by reduced responsiveness to norepinephrine in tail arteries from FF rats. Furthermore, we recently reported decreased blood pressure responses in vivo to norepinephrine infusion in the same FF rats (19). Together these results suggest that decreased adrenergic responsiveness in FF hyperinsulinemic rats may originate in the blood vessel. Thus, it is unlikely that the elevated blood pressure in this model can be explained by resistance to insulin's vasodilatory effects at the vascular level.

Blood vessels incubated in K⁺-deficient Krebs solution and contracted with various agonists relax in response to the readdition of K⁺, which is a functional index of the activity of the electrogenic Na⁺/K⁺-ATPase pump (10). The mechanism of K⁺-induced relaxations (20) has been related to Na⁺/K⁺ pump activity because of the similar effects of intracellular sodium concentration, ouabain administration, magnesium, temperature, and potassium concentration (15). Furthermore, in femoral arteries from deoxycorticosterone acetate (DOCA)-salt hypertensive rats (21), increased K⁺-induced relaxation correlated with increased ouabain-sensitive rubidium⁸⁶ (⁸⁶Rb) uptake, the latter a measurement of K⁺ influx and an independent measure of Na⁺/K⁺ pump activity (22). Increased ouabain sensitive ⁸⁶Rb uptake was also increased in aortic tissue from DOCA-salt and one-kidney Grollman renal hypertensive rats (23). Increased sensitivity to potassium is also directly correlated with increased affinity of pump sites for ouabain in aortas from renal hypertensive rabbits (24). K⁺-induced relaxations in the femoral arteries from rats (21), dogs (25), and pigs (26) have been a useful tool for relating vascular pump activity to contraction in various hypertensive models. Increased sensitivity to the readdition of K⁺ under similar bioassay conditions as ours has been demonstrated in blood vessels from several hypertensive animal models, including the SHR (27, 28), stroke prone SHR (29), two-kidney, one-clip renal hypertensive rat (30), DOCA hypertensive pig (26), Dahl salt-sensitive rat (31), and DOCA rat

Table 2. Vascular Responsiveness of in vitro Arteries from Diet-Fed Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fructose</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine contraction (×10⁻⁶ mol/l)</td>
<td>2.53±0.43* (17)</td>
<td>1.63±0.25 (18)</td>
</tr>
<tr>
<td>Transmural nerve stimulation (pulses/s)</td>
<td>6.46±0.45 (20)</td>
<td>7.47±0.68 (21)</td>
</tr>
<tr>
<td>Acetylcholine relaxation (×10⁻⁶ mol/l)</td>
<td>1.13±0.17 (20)</td>
<td>1.77±0.35 (24)</td>
</tr>
</tbody>
</table>

*p<0.05, vs. normal diet, mean±SEM (n per group).

1: Treatment ED50 is the dose calculated by regression analysis that produced a response equivalent to 50% of the maximum response to that treatment.

Fig. 3. Dose response curves to norepinephrine in tail arteries from FF and NF rats. Data represent the means±SEM of contractions in response to the cumulative addition of norepinephrine in isolated tail arteries from FF rats (n = 8) and NF rats (n = 7). ED50s calculated from these curves were significantly different (p<0.05).
In the present study, the increased sensitivity to the relaxing effect of potassium in femoral arteries incubated in low K⁺ suggests that Na⁺/K⁺ pump activity is increased as in other hypertensive models. Several lines of evidence suggest that this effect may be mediated by insulin (32).

Gene expression studies support insulin's ability to stimulate vascular Na⁺/K⁺-ATPase. Insulin increased the mRNA for the α2 isoform of Na⁺/K⁺-ATPase and ouabain-inhibitable ATPase activity in cultured aorta cells, a vascular smooth muscle cell line of embryonic origin (33). Increased expression of the α1 isoform of the enzyme in response to insulin and other vasoactive agonists was demonstrated in vascular smooth muscle (34). Insulin has also been found to increase Na⁺/K⁺-ATPase in muscle by stimulating Na⁺/H⁺ exchange, resulting in an increase in Na⁺ influx and intracellular Na⁺ concentration (35). The sodium gradient maintained by Na⁺/K⁺-ATPase can also influence intracellular calcium. A decrease in the Na⁺ gradient with ouabain or low sodium may also inhibit Na⁺/Ca²⁺ exchange and increase intracellular Ca²⁺ tension in resistance arteries (36). Decreased Na⁺/Ca²⁺-ATPase has been reported in the obese, hypertensive, and hyperinsulinemic Zucker rat (2).

The Na⁺/K⁺ pump and the regulation of intracellular sodium has been proposed to be an important mechanism for the regulation of vascular tone (7). In vascular smooth muscle, the extrusion of Na⁺ via a membrane bound Na⁺/K⁺-ATPase causes a hyperpolarization that leads to relaxation. Insulin has been demonstrated to stimulate the active transport of Na⁺ and K⁺ ions across the plasma membrane via Na⁺/K⁺-ATPase in various tissues (6) and to cause hyperpolarization in isolated skeletal muscle, adipocytes, and cardiocytes (37). Fujita and Ito, using an in vivo version of the in vitro potassium-induced relaxation Na⁺/K⁺-pump bioassay, proposed that an increase in an endogenous Na⁺/K⁺ pump inhibitor during sodium loading attenuated the potassium-induced vasodilatation in human forearm vasculature (38).

It has been demonstrated that hyperinsulinemia, although associated with hypertension, is not necessarily an epiphenomenon of elevated blood pressure (3). It has been postulated that hypertension in hyperinsulinemic states is promoted by desensitization of the direct vasodilatory effects of insulin rather than by the pro-hypertensive effects of insulin on sympathetic activity and renal sodium absorption (39). Thus, Zemel reported that the obese Zucker rat may be an insulin-resistant model in which increased sensitivity of aortic contractions to phenylephrine are the result of a failure of insulin to attenuate vascular responses (40).

However, our results suggest that insulin stimulation of potassium-induced relaxation is associated with a vascular vasodilatory effect. This must be reconciled with other vasoactive mechanisms of insulin action. Notably, increased sympathetic activity, associated with hyperinsulinemia and hypertension, has been reported in rats fed diets high in simple carbohydrates (41, 42). In insulin infusion studies, both the direct effects of insulin on sympathetic activity and peripheral vasodilatory effects can be demonstrated. Chronic-insulin-infusion-induced hyperinsulinemia increases blood pressure in conscious rats (8, 43). However, Edwards found that insulin infusions in the conscious Sprague-Dawley rat elevated blood pressure only if the sympathetic nervous system was intact (8). In the same study, insulin depressed blood pressure responses to norepinephrine in vivo and decreased the sensitivity of isolated aortic strips to norepinephrine in vitro (8).

In the present study, the decreased responses to exogenous norepinephrine and the lack of differences in sensitivity and maximal responses of tail arteries to TNS suggest that sensitivity to peripheral sympatho-metabolic and renal effects can influence blood pressure and mask a peripheral vasodilator effect of insulin. For example, Brands found that hyperinsulinemia in dogs associated with hypoglycemia resulted in renal sodium retention and a decrease in blood pressure (13). Liang reported that when euglycemia was maintained in dogs, the blood pressure increased, an effect probably mediated by an increase in NE (14). Liang concluded that insulin had specific effects on regional circulations, influenced by adrenergic stimulation in the splanchic bed but independent of sympathetic activity in the skeletal muscle and renal circulations.

In support of a vasodilator insulin action, our laboratory (44) and others (45) have demonstrated that insulin reduces AII-induced mobilization of intracellular calcium in cultured vascular smooth muscle cells, including unpassaged cells from spontaneously hypertensive rats that were insulin resistant and hyperinsulinemic (46). Kahn et al. reported that physiologic concentrations of insulin inhibited agonist-induced contractions of individual cultured vascular smooth muscle cells, an effect blocked by ouabain, an inhibitor of Na⁺/K⁺-ATPase (45).

In addition to reduced responsiveness to exogenous NE, we also found that insulin may have a vasodilatory action by stimulating endothelial-derived relaxing factor (EDRF). Endothelial-derived relaxing factor has also been proposed to be a mediator of insulin's vasodilator effect (39). In the present in vitro studies, sensitivity to acetylcholine-induced relaxations tended to be increased in FF rats. Furthermore, exogenous insulin significantly increased sensitivity to acetylcholine-induced relaxations in FF rats.

In the present in vitro studies, exogenous insulin increased contractile responses to AII in the femoral artery of FF rats. Iyer et al. also recently reported increased responses to AII in vivo and in vitro but only within the first 2 wk of fructose-induced
hypothesis (47). It is possible that the development of hyperinsulinemia is associated with potentiation of AVI vascular responses, although this was not seen in vivo in our study after 16 wk of fructose feeding (19). However, Iyer et al. also reported changes in AVI type 1 receptor density in rat aortas after 4 wk of fructose feeding (44). In humans, Gaboury et al. found that hyperinsulinemic, hypertensive subjects had greater blood pressure responses to insulin, which were correlated with decreased insulin sensitivity as measured by the glucose disposal rate (49). Angiotensin has been reported to stimulate the a1 and not the a2 isoform of Na+/K+ -ATPase in vascular smooth muscle cells (34) and it increases Na+ influx and Na+/K+ pump activity in rat aortas (50). Additional studies are required to reconcile a role of AVI as both a mediator of Na+/K+ -ATPase activity and as a prohypertensive factor in the insulin resistant FF rat.

Insulin-like peptides such as IGF-1 have receptors in vascular smooth muscle and have been reported to stimulate growth and development, increase glucose transport, and stimulate Na+/K+ -ATPase pump activity (51). In the present study, the effect of fructose feeding on IGF-1 levels was not measured. Although we have shown that insulin’s effect on intracellular calcium mobilization in cultured vascular smooth muscle cells was not mediated by IGF-1 or IGF-2 receptors (44), it is possible that the responses to the supraphysiologic insulin doses in this study were mediated by both IGF and insulin receptors.

In summary, in this hypertensive and hyperinsulinemic animal model, our primary finding of increased Na+/K+ pump activity, as measured by potassium-induced relaxation in isolated blood vessels, was associated with decreased vasoconstrictor sensitivity and increased endothelium-derived vaso-dilator activity. Whether central adrenergic or renal (including renin-angiotensin system) effects of insulin mediate the elevation of blood pressure seen in these models requires further investigation. However, our results in this chronic hyperinsulinemic model suggest that the stimulatory effect of insulin on Na+/K+ -ATPase activity at the vascular level was not down-regulated, but enhanced, resulting in compensatory vaso-dilator mechanisms opposing blood pressure elevation.

References