Vanadyl Sulfate Lowers Plasma Insulin and Blood Pressure in Spontaneously Hypertensive Rats

Sanjay Bhanot, John H. McNeill

Abstract  Spontaneously hypertensive rats (SHR) are hyperinsulinemic compared with their Wistar-Kyoto (WKY) controls. Since previous studies have demonstrated that vanadyl sulfate lowers insulin levels in nondiabetic rats, we used vanadyl to explore the relation between hyperinsulinemia and hypertension. In a prevention study, 5-week-old SHR and WKY rats were started on long-term vanadyl sulfate treatment. Vanadyl in doses of 0.4 to 0.6 mmol/kg per day lowered plasma insulin (252±22.8 versus 336±12.6 pmol/L, treated versus untreated, P<.01) and systolic blood pressure (158±2 versus 189±1 mm Hg, P<.001) in SHR without causing any change in plasma glucose. No changes were seen in the treated WKY rats. At 11 weeks of age, a group of untreated rats from the prevention study was started on vanadyl treatment as before. Again, vanadyl caused significant and sustained decreases in plasma insulin (264±12.6 versus 342±6.6 pmol/L, treated versus untreated, P<.001) and blood pressure (161±1 versus 188±1 mm Hg, P<.001) in SHR but had no effect in the normotensive WKY controls. Furthermore, restoration of plasma insulin in the vanadyl-treated SHR to pretreatment levels (subcutaneous insulin, 14,000 pmol/kg per day) reversed the effects of vanadyl on blood pressure (vanadyl with insulin, 190±3.0 mm Hg versus vanadyl without insulin, 152±3.0 mm Hg, P<.001). Since vanadyl treatment resulted in decreased weight gain, treated SHR were compared with a corresponding pair-fed group. The pair-fed rats remained as hypertensive as the untreated group, thus excluding any contribution of weight loss toward the antihypertensive effects observed with vanadyl. These results support the notion that hyperinsulinemia may contribute to the development of high blood pressure in SHR. (Hypertension. 1994;24:625-632.)

Key Words • hyperinsulinemia • hypertension, spontaneous • vanadyl • blood pressure

Essential hypertension is associated with multiple metabolic defects in carbohydrate and lipoprotein metabolism1-3 that include insulin resistance, hyperinsulinemia, and dyslipidemia.4,5 Insulin resistance in hypertension is often accompanied by hyperinsulinemia,6,7 and these metabolic defects persist when blood pressure (BP) is reduced by conventional antihypertensive drugs.8,9 Hyperinsulinemia in hypertension is probably a reflection of the resistance to the peripheral uptake and utilization of glucose, with high levels of insulin needed to maintain and/or sustain euglycemia in the presence of insulin resistance.9,10 However, the precise nature of this relation remains unexplained. The primary question that needs resolution is whether or not these defects in carbohydrate metabolism are causally related to hypertension.

Insulin resistance and hyperinsulinemia have also been documented in three models of experimental hypertension11-14 including the genetically predisposed spontaneously hypertensive rat (SHR).15 Furthermore, SHR exhibit a decreased insulin clearance, which may also result in increased plasma insulin levels.15 Although a few recent studies do not support the presence of insulin resistance in the SHR,16,17 the presence of hyperinsulinemia was confirmed even in those studies.16 It has been suggested that nutrient-stimulated hyperinsulinemia may play a role in the development of high BP in the SHR.16 If this hypothesis is valid, then a drug intervention that decreases plasma insulin levels in the SHR should also lower BP. Previous studies from our laboratory have shown that vanadyl, the (+IV) form of the trace element vanadium, exhibits effects on carbohydrate metabolism that are very similar to those of insulin.18 Besides decreasing plasma glucose in diabetic animals (without any increase in plasma insulin), vanadyl also decreases insulin levels in nondiabetic rats,18 probably by enhancing in vivo sensitivity to insulin.19 In the present study, we used vanadyl sulfate as an experimental intervention to elucidate the relation between hyperinsulinemia and hypertension. This was done in two phases: a prevention study, in which vanadyl was started before the SHR became hypertensive, and a reversal study, in which treatment was started after the SHR were fully hypertensive.

Methods

General Protocol

Prevention Study

Twenty-three SHR and 18 Wistar-Kyoto (WKY) rats (all male) were procured at 4 weeks of age from Charles River (Montreal, Canada) and randomly assigned to four experimental groups: SHR (untreated, n=15), SHR (vanadyl-treated, n=8), WKY (untreated, n=12), and WKY (vanadyl-treated, n=6). Systolic BP in all groups was measured before vanadyl treatment was started. Subsequently, long-
term vanadyl sulfate treatment (VOSO₄·nH₂O, Fisher Scientific) was begun on 5-week-old SHRV and WKYY rats. Vanadyl sulfate was administered at a concentration of 0.75 mol/L, dissolved in the drinking water. Starting at week 8, weekly measurements of systolic BP, plasma glucose, and plasma insulin were done on all the groups. Food and fluid intakes and body weight were measured once a week. In addition, fluid intake of rats on vanadyl was measured 5 times a week for calculation of the vanadyl dose consumed.

Reversal Study

At the start of week 11 (weeks denote the age of rats), the untreated SHR and WKY rats from the prevention study were further grouped as follows: SHR (untreated, n=9), SHRV₄ (vanadyl-treated, n=6), WKY (untreated, n=6), and WKYYV₄ (vanadyl-treated, n=6). The treated groups were started on vanadyl sulfate (0.75 mg/mL) in the drinking water at the beginning of week 11. Weekly measurements of BP, plasma glucose, and plasma insulin were continued for the next 3 weeks. At 15 weeks of age (termination), direct and indirect systolic BP measurements were done to validate the indirect BP readings obtained in the preceding weeks.

Vanadyl sulfate caused a decrease in plasma insulin levels and BP in the SHR (see “Results”). To examine this issue further, we initiated a separate study in which 9-week-old SHR and WKY rats (n=12 in each group) were treated with vanadyl as before. Three weeks after vanadyl treatment was started, 6 vanadyl-treated rats in each group were administered exogenous insulin (14,000 pmol/kg per day as a subcutaneous insulin implant, Linshin Inc), and the other 6 were continued on vanadyl as before. This was done in an effort to restore plasma insulin levels in the vanadyl-treated rats to those that existed before vanadyl treatment and to then observe the resultant change in BP. During the postimplant period, plasma insulin, plasma glucose, and BP were measured 1 and 3 weeks after putting in the insulin implants. In addition, the rats were sampled for plasma catecholamines and glucagon before and 3 weeks after putting in the insulin implants. Plasma catecholamine samples were collected in an identical fashion in all rats. The rats were frequently handled for a few days before sampling. The samples were collected within 2 minutes of touching the individual rat in an undisturbed environment, and the same procedure was followed for every animal. Thus, the catecholamine values do not represent the absolute stress-free basal values. The main purpose was to look for major changes in catecholamine concentrations that could occur secondary to the hypoglycemia (in rats with insulin implants), and therefore, the rats were not chronically cannulated for catecholamine sampling.

All experimental procedures followed were approved by the University of British Columbia Animal Care Committee.

Pair-Feeding Study

Since vanadyl sulfate decreased food and fluid consumptions and body weight in treated rats (see “Results”), a separate study was initiated in which one group of rats (SHR and WKY rats) was pair-fed with the corresponding vanadyl-treated group but was not given vanadyl. This was done to observe whether a decrease in food and fluid intake per se contributed to the amelioration of hypertension in the vanadyl-treated rats. Twenty-three SHR and 24 WKY rats (all male) were procured at 5 weeks of age from Charles River and used as follows: SHR: untreated (control, n=8), treated (vanadyl-treated since the start of week 6, n=8), and pair-fed (pair-fed with treated rats for food and fluid consumed, n=7); WKY: untreated (control, n=8), treated (vanadyl-treated since the start of week 6, n=8), and pair-fed (pair-fed with treated rats, n=8). At weeks 5, 9, 10, 12, 15, and 16, systolic BP was measured by the indirect tail-cuff method, which had already been validated by direct arterial cannulation in the previous experiment. During the weeks mentioned above, 5-hour fasting plasma samples were also collected via the tail vein and later analyzed for glucose and insulin. At 15 weeks of age (after 10 weeks of vanadyl treatment), the rats were fasted overnight and plasma was collected and later analyzed for urea nitrogen, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and vanadium levels.

Blood Pressure

Indirect BP measurements (systolic) were done using the tail-cuff method. Rats were removed from the animal room and taken to a quiet room at 8 AM; they were allowed ad libitum access to food and water. The rats were prewarmed for 10 minutes in a rat holder placed on a hot plate with a surface temperature of 32°C. Since the rats had been preconditioned to the BP measurement procedure, they became sedate within 2 minutes of being restrained in the rat holder. The cuff used was 35 mm in length and was placed at the base of the tail. A pneumatic pulse sensor was taped to the tail and connected to a pneumatic pulse transducer (Narco BioSystems). A programmed electrocardiogram (PE-300, Narco) was used to keep the various parameters such as inflation and deflation rates and cycling interval constant. The reappearance of pulsations (on gradual deflation) signified the systolic BP. In each rat, three consecutive readings were taken and averaged to obtain the individual BP.

Direct BP was measured at termination. Rats were anesthetized with a short-acting barbiturate (sodium methohexital, Brietal sodium, Eli Lilly), which was given at a dose of 60 mg/kg body wt IP. Anesthesia was maintained with nitrous oxide, and a catheter (1 m PE-50 tubing joined to 7 cm PE-10 tubing) was introduced through the caudal artery and placed into the abdominal aorta of each rat. Plasma catecholamines (catecholamines and glucagon) were sampled for rats used as controls to determine the cardiovascular status and baroreflex sensitivities of rats were similar 5 and 72 hours after anesthesia and surgery. Therefore, at least 5 hours after surgery, BP was recorded continuously by connecting the catheter via a Gould p23db pressure transducer to a polygraph (Gould TA 2000). At the time of BP recording, rats were fully conscious, freely moving, and had recovered from the effects of anesthesia.

Plasma Analyses

Plasma glucose was determined by the glucose oxidase method using kits purchased from Boehringer Mannheim. Plasma insulin and glucagon were determined using a doubleantibody radioimmunoassay with kits procured from Immuno Corp. In the pair-feeding study, GOT, GPT, and urea nitrogen levels were assessed by colorimetric determinations with kits obtained from Sigma Diagnostics. Plasma vanadium analysis was done by electrochemical atomic absorption spectrophotometry according to the method used by Mongold et al with minor modifications. Plasma catecholamines were measured by a radioenzymatic method (Amersham).

Statistical Analyses

All data are presented as mean±SEM. All data were analyzed by a multivariate analysis of variance (MANOVA) procedure followed by a Newman-Keuls test using the Number Cruncher Statistical System (NCSS). A value of P<.05 was taken to indicate a significant difference between means. Differences in various parameters before and after administration of exogenous insulin (in rats given insulin implants) were compared by using the paired t test procedure on the NCSS statistical package. BP and plasma insulin values (reported below) represent the average values from weeks 10 to 12 in the prevention study and weeks 11 to 13 in the reversal study.
Results

Body Weight

Representative results from the pair-feeding study are outlined below; similar results were obtained in the prevention and reversal studies. In agreement with earlier studies, the untreated WKY rats gained weight more quickly than the untreated SHR (Fig 1). Although vanadyl consumption resulted in reduced weight gain in the treated rats, they continued to gain weight throughout the study period. The weights of the pair-fed SHR and WKY rats remained similar to weights of the corresponding vanadyl-treated groups throughout the study.

Plasma Insulin Levels

Prevention and Reversal Studies

SHR were hyperinsulinemic compared with WKY rats. Vanadyl lowered plasma insulin levels in SHR in both the prevention study (252±22.8 versus 336±12.6 pmol/L, treated versus untreated, P<.01) and reversal study (264±12.6 versus 342±6.6 pmol/L, P<.001; Figs 2A and 3A) to control WKY values (264±22.8 pmol/L, P>.05). This decrease in insulin was maintained throughout the study periods. The weights of the pair-fed SHR and WKY rats remained similar to weights of the corresponding vanadyl-treated groups throughout the study.

Blood Pressure

Prevention and Reversal Studies

Vanadyl produced a marked and sustained decrease in systolic BP in the SHR, which was noticed at all time points starting at week 9 (158±2 versus 189±1 mm Hg, treated versus untreated, P<.001; Fig 2B). BP remained unchanged in the normotensive, treated WKY rats (127±1 versus 135±1 mm Hg, P>.05). Direct systolic BP measurements done at termination confirmed the previous indirect readings, reflecting on the validity of both the techniques used (Fig 2). There was a difference of approximately 8 mm Hg between the direct and indirect systolic BP readings, which was consistent in all groups except the untreated SHR. During the indirect BP measurements, the pulsations in the caudal arteries in the SHR appeared earlier than in the other groups, probably because of the other two studies. Untreated SHR were hyperinsulinemic compared with their WKY controls even as early as 5 weeks of age (Fig 4A). Vanadyl reduced fasting plasma insulin values in SHR by approximately 35%, and this decrease persisted throughout the study. No change in plasma insulin values was observed between control, treated, or pair-fed WKY rats. However, as the WKY rats grew older and heavier, their plasma insulin levels increased, which probably signified the combined (negative) effect of increasing weight and age on insulin sensitivity in these rats.

Plasma insulin values in the pair-feeding study represented 5-hour fasted values as opposed to fed values in the prevention and reversal studies. Untreated SHR were hyperinsulinemic compared with their WKY controls even as early as 5 weeks of age (Fig 4A). Vanadyl reduced fasting plasma insulin values in SHR by approximately 35%, and this decrease persisted throughout the study. No change in plasma insulin values was observed between control, treated, or pair-fed WKY rats. However, as the WKY rats grew older and heavier, their plasma insulin levels increased, which probably signified the combined (negative) effect of increasing weight and age on insulin sensitivity in these rats.

Blood Pressure

Prevention and Reversal Studies

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Fig. 3. Line graphs show plasma insulin levels (A) and systolic blood pressure (B) in the four rat groups in the reversal study. Vanadyl treatment was started at week 11. WKYV, indicates vanadyl-treated Wistar-Kyoto rats (n=6); WKY, untreated WKY rats (n=6); SHR, treated spontaneously hypertensive rats (n=6); and SHR, untreated SHR (n=9). Values are mean±SEM.

*P<.05, SHR different from SHRV, after week 11; blood pressure of both SHR and SHRV, different from WKY and WKYV, (the latter two not different from each other).

increased pulse pressure and hyperdynamic state associated with hypertension. Consequently, the untreated SHR had to be restrained for a relatively shorter time (approximately 3 minutes after the initial 10-minute warming period, as opposed to 5 to 10 minutes in the other groups), which shortened the period of restraint and external heating in that group. This may be the reason why the modest increase in the indirect BP readings (compared with the direct measurements) that occurred in the other three groups was not observed in the untreated SHR. Similar results were obtained in the reversal study (161±1 versus 188±1 mm Hg, treated versus untreated, P<.001; Fig 3B).

Pair-Feeding Study

The decrease in BP in the vanadyl-treated SHR was similar in magnitude to that seen in the prevention and reversal studies (Fig 4B). However, the pair-fed SHR remained as hypertensive as the untreated control rats, and their BP remained unchanged. Also, no change in BP was noticed between the normotensive control, treated, or pair-fed WKY rats (Fig 4B).

Restoration of plasma insulin in the vanadyl-treated SHR (Table) reversed the effects of vanadyl sulfate on BP, and this reversal was observed as early as 1 week after putting in the insulin implants (190±3.0 versus 152±3.0 mm Hg, vanadyl with insulin versus vanadyl without insulin, P<.001). No change in BP was observed in the vanadyl-treated WKY rats treated with exogenous insulin. Catecholamine levels in the vanadyl-treated SHR remained unchanged compared with those seen in the untreated SHR (2470±203 versus 2284±377 pg/mL, treated versus untreated, P>.05), suggesting that the antihypertensive effects of vanadyl are independent of changes in sympathetic activity.

Plasma Glucose

The average plasma glucose values in the various groups during the different study protocols ranged from 6.4 to 7.6 mmol/L. All the groups in the prevention, reversal, and pair-feeding studies remained euglycemic (<8.5 mmol/L) throughout the respective study periods. No changes in plasma glucose were observed after vanadyl treatment in either the fed (prevention and reversal studies) or the 5-hour fasted (pair-feeding study) states. Furthermore, plasma glucose remained unchanged in the vanadyl-treated SHR 1 week after putting in the insulin implants (6±0.2 versus 6±0.1 mmol/L, after versus before implant, P>.05). However, a decrease in plasma glucose was observed 3 weeks after implant (4±0.9 versus 6±0.1 mmol/L, P<.05), which was not accompanied by any change in plasma glucagon levels (Table). Plasma catecholamines in the vanadyl-treated SHR given insulin implants showed an increase compared with their preimplant values (4940±662 versus 2073±245 pg/mL, 3 weeks after versus before im-
Various Parameters From the Experimental Groups in the Exogenous Insulin Study

<table>
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<td></td>
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<td>A1</td>
<td>A3</td>
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<tr>
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<td>191±5</td>
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<td>WKYI*</td>
<td>135±4†</td>
<td>144±2†</td>
<td>148±3†</td>
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SHR indicates untreated spontaneously hypertensive rats (n=4); SHRV, vanadyl-treated SHR (n=6); SHRI, vanadyl-treated SHR plus subcutaneous insulin (n=6); WKY, untreated Wistar-Kyoto rats (n=4); WKYV, vanadyl-treated WKY rats (n=6); and WKYI, vanadyl-treated WKY rats plus subcutaneous insulin (n=6). Values are mean±SEM. Shown are various parameters before (B), 1 week after (A1), and 3 weeks after (A3) insulin was implanted in the experimental groups.

*Insulin dose from the insulin implants was 14,000 pmol/kg per day; implants were given only in the SHRI and WKYI groups.

†P<.05, different from SHR.

‡P<.05, different from respective preimplant values.

§P<.05, SHRI different from SHRV.

plant), which may indicate either a compensatory response to a fall in plasma glucose or a direct effect of sustained hyperinsulinemia on the sympathetic nervous system.

### Food and Fluid Intakes

Food and fluid intakes in the treated groups decreased compared with the untreated groups. This was seen in all the treated groups to a similar extent and was noticed in the prevention, reversal, and pair-feeding studies. The average decrease in food intake ranged from 10% to 15%, and the decrease in fluid intake ranged from 30% to 40%. The pair-fed SHR and WKY rats were given food and fluid in amounts that were similar to those in the corresponding vanadyl-treated groups, which was reflected as similar weight gain patterns between the pair-fed and vanadyl-treated rats (Fig 1).

### Dose of Vanadyl Consumed

Daily vanadyl dose calculated over the study periods showed an overall downtrend with time caused by the increasing body weights of the rats rather than a reduction in their fluid intake. The dose consumed ranged from 0.4 to 0.6 mmol/kg per day and was not different among the various treated groups.

### Hepatic and Renal Function

Vanadyl did not cause any changes in plasma GPT (SHR: untreated, 69.9±3.9 units/L; treated, 65.5±6.6 units/L; pair-fed, 70.2±4.4 units/L; WKY: untreated, 56.0±3.7 units/L; treated, 56.7±4.3 units/L; pair-fed, 59.9±3.2 units/L) or GOT (SHR: untreated, 18.1±0.4 units/L; treated, 15.2±0.4 units/L; pair-fed, 17.3±0.8 units/L; WKY: untreated, 16.6±1.3 units/L; treated, 16.3±0.9 units/L; pair-fed, 14.3±2.4 units/L) values. Plasma urea nitrogen values also remained unchanged in the treated SHR and WKY rats (SHR, 10.0±0.8 mmol/L; WKY, 10.0±0.9 mmol/L) compared with the respective untreated (SHR, 12.6±0.9 mmol/L; WKY, 10.0±0.8 mmol/L) or pair-fed (SHR, 11.0±0.8 mmol/L; WKY, 9.0±1.3 mmol/L) groups. Thus, 10 weeks of vanadyl treatment did not cause any impairment of hepatic or renal function in either the SHR or WKY rats. In addition, none of the treated rats died or exhibited any signs of gastrointestinal disturbance throughout the experimental period.

### Discussion

The results confirm previous observations that SHR are hyperinsulinemic compared with their genetic WKY controls. In this study, we used the vanadyl form of vanadium because previous reports from our laboratory suggested that it was better tolerated than other forms of vanadium. Vanadyl sulfate in doses of 0.4 to 0.6 mmol/kg per day lowered both plasma insulin level and systolic BP in SHR. The decrease in plasma insulin observed in the prevention, reversal, and pair-feeding studies was quite marked and was accompanied by concurrent decreases in systolic BP. Furthermore, the effects of vanadyl were independent of changes in plasma glucagon or catecholamines, suggesting that the effects were not mediated by a change in sympathetic activity. Although these findings do not prove that these events are causally related, they do provide indirect support for such a link. The observation that the pair-fed SHR remained as hypertensive as the untreated SHR indicates that the antihypertensive effect observed was specific to vanadyl and that the decrease in BP was independent of any changes in food and fluid consumptions or body weight. Vanadyl did not lower BP in the normotensive WKY rats, nor did it have any significant effect on their plasma insulin values.

Some of the well-recognized insulin-like effects of vanadium include activation of both glucose transport and glycogen synthesis in rat adipocytes and skeletal muscle, inhibition of lipolysis, and stimulation of lipogenesis. Fantus et al recently reported that the vanadate form of vanadium caused marked increases in insulin-stimulated receptor kinase activity and prolonged insulin-stimulated lipogenesis in rat adipocytes. Other studies suggest that the glucoregulatory effect of vanadium is mediated by either an insulin-independent cascade or via its action at a site distal to the insulin receptor. Whatever the precise mechanism or mechanisms of action of the drug may be, they get translated in vivo as an improvement in glucose utilization. Not only does vanadyl lower glucose levels
in diabetic rats (without any increase in plasma insulin), it also causes a decrease in insulin levels in nondiabetic rats without any change in plasma glucose concentrations.\textsuperscript{39} Furthermore, studies conducted in the isolated perfused pancreas of vanadyl-treated rats indicate that there is no direct effect of the drug on pancreatic insulin secretion or content.\textsuperscript{34} By performing euglycemic clamps in conscious rats, we recently demonstrated that vanadyl improved insulin sensitivity in both SHR and fructose hypertensive rats.\textsuperscript{32,33} Thus, it seems that by either replacing or potentiating the actions of endogenous insulin, vanadyl causes a feedback inhibition of insulin release in nondiabetic rats.

It was recently reported that the short-term insulin responses to a glucose load were twofold to threefold higher in SHR compared with WKY rats, and these responses were accompanied by an increased glucose disappearance rate in the SHR.\textsuperscript{16} Furthermore, this hypersecretion of insulin seemed to be primary and not related to insulin resistance, since the 3-O-methylglucoside rates increased in skeletal muscle (isolated from the SHR and WKY rats) were similar at physiological and pharmacological concentrations, an issue that has been discussed in detail in the article cited above.\textsuperscript{16} Thus, it appears that in the SHR, the increase in plasma insulin may not be related to insulin resistance.

Previous studies have shown that in hyperinsulinemic rats, experimental interventions that decrease plasma insulin levels also attenuate increases in BP.\textsuperscript{34,35} We recently demonstrated that long-term metformin treatment in SHR causes decreases in insulin levels and BP that are very similar to those observed with vanadyl.\textsuperscript{36} Furthermore, the decrease in BP in the metformin-treated rats was reversed when insulin levels in the treated SHR were restored to those that existed before treatment. In the present study, replacement of plasma insulin levels in the vanadyl-treated SHR to those that existed before treatment also caused a corresponding increase in BP. This effect was evident as early as 1 week after putting the insulin implants, when postimplant plasma insulin and glucose values in the rats were similar to those seen in the untreated SHR. These findings, along with the observation that sustained, physiological increases in plasma insulin cause an increase in BP in conscious rats,\textsuperscript{37} suggest that hyperinsulinemia may increase BP in rats. This view is further supported by studies documenting that hyperinsulinemia can elicit many hypertensinogenic mechanisms such as activation of the sympathetic nervous system, increase in renal sodium and water reabsorption, and proliferation of vascular smooth muscle tissue (for review, see Reference 1).

Implicit in the point of view outlined above is the assumption that vanadyl does not exhibit any other antihypertensive properties and that it selectively improves insulin action. Although the vanadate (+V) form of vanadium has been shown to affect the activities of various intracellular enzymes in vitro (mostly at pharmacological concentrations), vanadate is reduced intracellularly to the vanadyl (+IV) state.\textsuperscript{8,39} Vanadyl in turn is a very poor inhibitor of cellular enzyme systems.\textsuperscript{38,40} Since it was not possible to alter the rate of insulin release with the type of implants used in this study, plasma insulin values in the vanadyl-treated SHR given insulin implants exceeded those seen in the untreated SHR 3 weeks post-implant (causing a decrease in plasma glucose and an increase in plasma catecholamines). However, it is perhaps important that a reversal of the antihypertensive effects of vanadyl was evident even 1 week after implant, when plasma glucose and insulin levels in the rats with implants were similar to those of the untreated SHR. Although we cannot unequivocally explain why plasma insulin levels in the rats increased 3 weeks after implant compared with those observed 1 week after implant, it is probable that the insulin implants were not fully functional by the end of the first week.

The present study revealed some other interesting observations briefly mentioned below. Although vanadyl lowered plasma insulin in the SHR to control WKY levels, BP in the SHR did not decline to normotensive values. This suggests that hyperinsulinemia may be only one of several factors causing high BP in SHR. Also, although vanadyl sulfate decreased plasma insulin levels in the SHR, it had no effect on these metabolic parameters in the WKY rats. Recent studies in our laboratory indicate that although vanadium compounds lower plasma insulin levels in nondiabetic Wistar and Sprague-Dawley rats,\textsuperscript{41,42} they do not affect insulin levels in WKY rats.\textsuperscript{43} Furthermore, WKY rats show remarkably different effects to other metabolic insults compared with other rat strains. For example, WKY rats are resistant to the effects of streptozotocin-induced diabetes\textsuperscript{44} and show marked differences in heart rate, cardiac function, and plasma triglycerides compared with Wistar and Sprague-Dawley diabetic rats.\textsuperscript{45,46} We recently completed another study using vanadyl sulfate as the experimental intervention and the insulin-resistant, hyperinsulinemic fructose hypertensive rat as the experimental model and found that although vanadyl sulfate caused a modest decrease (approximately 10% to 14%) in plasma insulin in control, untreated Sprague-Dawley rats, it had no effect on their BP.\textsuperscript{33} Interestingly, vanadyl improved insulin sensitivity, decreased insulin levels, and prevented the increase in BP in the fructose-fed rats. In the present study, exogenous insulin infusion in WKY rats did not cause an increase in BP. This suggests that although hyperinsulinemia may cause hypertension in rats, a modest decrease in insulin levels in control, normoinsulinemic rats does not affect BP. To the extent that hyperinsulinemia in the SHR may be interacting with other genetic factors or organ systems, the results of our study do not allow us to rule out such an interaction.

Some investigators have reported toxicity with vanadyl administration at levels much lower than those administered in the present study,\textsuperscript{47} whereas others have not documented toxic effects even at higher doses.\textsuperscript{23} In some of the studies reporting toxicity at lower vanadyl concentrations, the rats were made extremely diabetic;\textsuperscript{47} no control treated rats were included in the study, and the effects of diabetes per se (as opposed to those of vanadium) were not excluded. In the present series of experiments, none of the rats died in any of the three studies conducted, and no gastrointestinal, hepatic, or renal toxicity was ob-
erved after 10 weeks of vanadyl treatment. Not only did the vanadyl-treated rats continue to gain weight throughout the experimental period, the pair-fed rats also gained weight at rates that were similar to those of the treated rats. This suggests that the reduced weight gain caused by vanadyl administration is due to the reduced food and fluid intake in the treated rats rather than to any additional toxic effect of vanadyl. Plasma vanadium levels were not detectable in the untreated and pair-fed SHR and WKY rats. Plasma vanadium levels in the treated animals ranged from 0.48 to 1.07 µg/mL, which corresponds to the levels at which vanadyl exhibits antidiabetic effects.

In conclusion, this study confirms the presence of hyperinsulinemia in SHR compared with their WKY controls. Vanadyl sulfate caused concurrent and sustained decreases in both plasma insulin and BP in the SHR. Increasing plasma insulin levels in the treated SHR to those that existed before treatment reversed the effects of vanadyl on BP. This suggests that hyperinsulinemia may contribute to the development of high BP in the SHR or, if hyperinsulinemia is not causally related to hypertension, the underlying mechanism may be closely related to the expression of both disorders.

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