Bis(maltolato)oxovanadium(IV) Attenuates Hyperinsulinemia and Hypertension in Spontaneously Hypertensive Rats

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We previously reported that bis(maltolato)oxovanadium(IV) (BMOV), an organic vanadium complex, decreased plasma insulin concentrations in nondiabetic rats without affecting plasma glucose levels (McNeill JH, Yuen VG, Hoveyda HR, Orvig C: Bis(maltolato)oxovanadium(IV) is a potent insulin mimic. *J Med Chem* 35:1489–1491, 1992). In this study, chronic oral BMOV treatment was started in 6-week-old spontaneously hypertensive (SH) rats and their Wistar-Kyoto (WKY) controls, and the effects of the drug on insulin sensitivity, plasma insulin, and blood pressure (BP) were studied. BMOV (0.35–0.45 mmol · kg^{-1} · day^{-1}) caused a sustained reduction in plasma insulin (198 ± 6 vs. untreated 366 ± 13.2 pM, $P < 0.0001$) and systolic BP (149 ± 3 vs. untreated 184 ± 3 mmHg, $P < 0.0001$) in SH rats. No changes were seen in WKY rats (plasma insulin: treated 228 ± 4.8 vs. untreated 222.6 ± 3.6 pM, $P > 0.05$; BP: treated 134 ± 3 vs. untreated 134 ± 5 mmHg, $P > 0.05$). At 13 weeks of age, euglycemic clamps were performed in fasted, conscious, mobile rats. During low-dose insulin infusions (14 pmol · kg^{-1} · min^{-1}) with concomitant somatostatin administration, neither hepatic glucose output nor total body glucose uptake differed between the untreated SH and WKY rats. Insulin sensitivity, expressed as steady-state glucose clearance per unit of plasma insulin, was higher in the untreated SH compared with the untreated WKY rats (2.1 ± 0.2 vs. 1.2 ± 0.1 ml · kg^{-1} · h^{-1} · pM^{-1}, $P < 0.002$). BMOV further enhanced insulin sensitivity in SH rats (3.6 ± 0.4, $P < 0.002$ vs. untreated SH rats). In conclusion, *1*) SH rats are hyperinsulinemic but not insulin resistant compared with WKY rats; and *2*) BMOV caused concurrent decreases in plasma insulin and BP in SH rats, which suggests that hyperinsulinemia may contribute toward the development of high BP in the SH rat. *Diabetes* 43:857–861, 1994

Although insulin resistance and hyperinsulinemia are common findings in both untreated and drug-treated essential hypertensive patients, current data linking these metabolic defects with hypertension is associative rather than causal (1). Insulin resistance and hyperinsulinemia have also been documented in several models of experimental hypertension (2,3), including spontaneously hypertensive (SH) rats. However, conflicting data have emerged between euglycemic clamp studies done in anesthetized SH rats (3,4) and those conducted in conscious, unstressed rats (5,6). Euglycemic clamps in anesthetized SH rats demonstrate impaired insulin sensitivity compared with Wistar-Kyoto (WKY) controls (3), whereas those done in conscious SH rats do not support these findings (6). Therefore, the precise nature of the relationship between these metabolic abnormalities and hypertension in the SH rat remains unexplained.

We previously reported that bis(maltolato)oxovanadium(IV) (BMOV), an organic vanadium complex, decreased plasma insulin levels in nondiabetic rats without changing plasma glucose levels (7), suggesting that the drug either replaced or potentiated the action of endogenous insulin. If insulin resistance and/or hyperinsulinemia were causally related to the development of high blood pressure (BP) in SH rats, then a drug intervention that improved these metabolic defects should also attenuate the hypertension. Therefore, this study was initiated with the following objectives: *1*) to assess insulin sensitivity in SHR and WKY rats by performing euglycemic hyperinsulinemic clamps in conscious, minimally restrained rats and *2*) to administer BMOV to SH and WKY rats and examine the effects of the drug on insulin sensitivity, plasma insulin levels, and systolic blood pressure (sBP).

RESEARCH DESIGN AND METHODS

Five-week-old male SH and WKY rats were procured from Charles River (Montreal, Canada) and were randomly assigned to four experimental groups: S (SH rats, untreated, $n = 9$), SO (SH rats, BMOV-treated, $n = 11$), W (WKY rats, untreated, $n = 11$), and WO (WKY rats, BMOV-treated, $n = 9$). BMOV was synthesized in our laboratory by complexing one molecule of vanadyl sulfate with two molecules of the common food additive maltol (7). Chronic BMOV treatment (0.75 mg/ml ad libitum in the drinking water) was initiated on 6-week-old rats. A concentration of 0.75 mg/ml was chosen because previous results indicated that it lowered plasma insulin in nondiabetic rats while allowing them to gain weight at rates that were comparable to untreated controls (7). Once at week 5 and again starting at week 8 (weeks denote the age of the rats), sBP, plasma insulin (5-h fasting) and plasma glucose were measured weekly for the next 4 weeks. At termination (14 weeks of age), the rats

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SH, spontaneously hypertensive; WKY, Wistar-Kyoto; BMOV, bis(maltolato)oxovanadium(IV); BP, blood pressure; sBP, systolic blood pressure; R_d, rate of glucose disposal; R_a, rate of glucose appearance.

were fasted overnight and hyperinsulinemic clamps were performed in conscious rats. All the experimental procedures followed were approved by the University of British Columbia Animal Care Committee.

BP measurement. Indirect sBP was measured in conscious rats using the indirect tail cuff method (Model 179 semi-automatic BP analyzer, IITC, Woodland Hills, CA) without external preheating (8). The animals were conditioned to the experimental procedure before actual measurements were conducted. In this method, the reappearance of pulsations (on gradual deflation of the BP cuff) are detected by a photoelectric sensor and are amplified and recorded digitally as the sBP. The measurements were performed in a blinded fashion with respect to the various treatment groups, and the average of five such readings was taken as the individual sBP. The major advantage of this method is that the recordings are carried out at a temperature of 26–27°C, thus eliminating the heat stress typical of other methods. In a preliminary study, we validated readings obtained by this method by comparing them with those obtained by direct intra-arterial cannulation. Recorded pressures were similar (within 5 mmHg) to those obtained by direct cannulation; similar results have also been reported by other laboratories (9,10).

Euglycemic hyperinsulinemic clamp technique. Rats were conditioned to tail restraint by a modification of the approach of Buchanan et al. (5). In brief, the tail was passed through a hole (~1.5 cm in diameter) in the cage, after which it was immobilized at a point halfway along its length by passing it through a soft cork and taping it distally. The rats had free access to food and water and were conditioned for increasing periods of time over 3 days before the clamp study. The rats were weighed daily, and weight gain was comparable to that observed during the pre-conditioning period. The rats were fasted overnight (20 h) before the clamp studies. Four hours before the start of the insulin infusions, each animal was placed in a specially designed foam rubber jacket, which allowed free movement of all four limbs and forward vision. Subsequently, the rat was placed on a board with a belt positioning system that allowed it to be immobilized in the left or right lateral and supine positions. Lidocaine 1% was then infiltrated into the tissue on the ventral aspect of the tail. A 0.5-cm incision was made in the tail and the tail artery was cannulated with fine-bore polyethylene tubing (PE 10) and flushed with heparin 50 U/ml of 0.9% saline. The tail vein was then cannulated percutaneously with a 24G Intracath (Jelco, Tampa, FL) attached to PE 50 tubing. The animal was returned to the cage and was allowed to recover with free access to water. Fifty microliters of blood was withdrawn immediately after surgery and at 15, 30, 60, and 120 min after surgery (but before the start of the insulin infusion) for determination of catecholamine levels. During the first 15 min of the clamp, baseline plasma glucose measurements were obtained. Thereafter, the following were infused: *1*) 14 pmol · kg^{-1} · min^{-1} of insulin from 0 to 120 min (preceded by a loading dose of 3×, 2×, and 1× for 1 min each); *2*) 920 pmol · kg^{-1} · min^{-1} of somatostatin from 0 to 120 min; and *3*) D-[3-^{3}H]glucose at a rate of 0.10 μCi/min from −60 to 120 min, after an initial square-wave bolus over 1 min. This was done for isotopic determination of glucose turnover as described by Steele (11). Where cold glucose infusion rate exceeded the isotopically calculated rate of glucose appearance (R_a), the former figure was used to calculate the total rate of glucose disposal (R_d). During the clamps, 20% D-glucose was infused as needed to maintain plasma glucose at the preinfusion level. Thirty microliters of arterial blood was sampled at 5-min intervals for determination of plasma glucose. At 100, 110, and 120 minutes, 200 μl of blood was withdrawn for measurement of steady-state plasma insulin levels and tracer dilution, and the animals were then killed by an intravenous injection of pentobarbital (250 mg/kg). For each animal, a steady-state value for plasma glucose, insulin, glucose production, and glucose clearance was obtained by averaging the data recorded during the final 30-min period; glucose clearance was calculated by dividing the R_d by the steady-state glucose concentration. Insulin sensitivity was then expressed as the ratio of the steady-state glucose clearance to the steady-state plasma insulin value (12).

Biochemical measurements. Plasma glucose was measured by the glucose oxidase method in a YSI 23A glucose analyzer (YSI, Yellow Springs, OH). Plasma insulin was assayed by a double antibody radioimmunoassay technique using human insulin standards (ICN Biomedicals, Costa Mesa, CA). For determination of D-[3-^{3}H]glucose concentrations, serum was diluted 1:4 with water and then added to an equal volume of perchloric acid, with the final concentration at 2.5%. Proteins were precipitated by centrifugation at 2,000 *g* for 10 min. Aliquots of supernatant were dehydrated for 6 h at 55°C and counted in a β-scintillation counter. Plasma catecholamines were measured by a radioenzymatic method (Amersham, Arlington Heights, IL).

Statistical analysis. All data are presented as means ± SE and were analyzed by a multivariate analysis of variance procedure followed by a Newman-Keul's test, using the Number Cruncher Statistical System. Changes within each group over time were analyzed by an analysis of variance followed by a Newman-Keul's test. A probability of $P < 0.05$ was taken to indicate a significant difference between means.

TABLE 1
Characteristics of animals at weeks 9–11

	Group			
	S	SO	W	WO
n	9	11	11	9
Weight (g)	253 ± 10	233 ± 10	248 ± 8	225 ± 8
Plasma glucose (mM) (5-h fasting)	6.2 ± 0.1	6.0 ± 0.1	6.3 ± 0.1	5.9 ± 0.1
Plasma insulin (pM)	366 ± 13.2*	198 ± 6†	222.6 ± 3.6	228 ± 4.8
sBP (mmHg)	184 ± 3*	149 ± 3†	134 ± 5	134 ± 3

Data are means ± SE. All data represent the average of values from weeks 9–11. S, untreated SH rats; SO, BMOV-treated SH rats; W, untreated WKY rats; WO, BMOV-treated WKY rats. *$P < 0.0001$, S different from W; †$P < 0.0001$, SO different from S.

RESULTS

BP, plasma insulin, and plasma glucose. Five-hour fasted SH rats were hyperinsulinemic compared with the WKY rats (Table 1). BMOV caused a sustained decrease in plasma insulin in the SH rats (mean of plasma insulin at 9–11 weeks: treated 198 ± 6.0 vs. untreated SH 366 ± 13.2 pM, $P < 0.0001$) but did not have any effect in the WKY rats (mean plasma insulin at 9–11 weeks: treated 228 ± 4.8 vs. untreated (WKY) 222.6 ± 3.6 pM rats, $P > 0.05$, Table 1). Interestingly, BMOV also caused a marked decrease in sBP in the SH rats (treated 149 ± 3 vs. untreated 184 ± 3 mmHg, $P < 0.0001$, Table 1), but had no effect on the WKY rats. Five-hour fasting glucose in all groups remained normal (<7.0 mM), and no changes in plasma glucose were observed after BMOV treatment in either SH or WKY rats (Table 1). Body weight in the BMOV-treated SH and WKY rats was ~8–9% lower than in their respective untreated groups, but did not attain statistical significance ($P > 0.1$ for treated versus the respective untreated groups for both SH and WKY rats).

Euglycemic clamp studies. During the 3-day conditioning period, weight gain in the rats was normal and similar to that seen during the previous weeks (data not shown), and none of the rats lost weight. Plasma catecholamine levels declined within the first 30 min of surgery in both the untreated SH rats (plasma levels at 30 min were 5.25 ± 0.31 vs. 8.03 ± 0.62 nM immediately after surgery) and WKY rats (plasma levels at 30 min were 5.49 ± 1.19 vs. 8.36 ± 1.37 nM immediately after surgery). Furthermore, no difference in plasma catecholamine concentrations was found between the four experimental groups at the start of the clamp infusions (data not shown). Steady-state plasma glucose levels during clamps were similar in the four experimental groups (Table 2) and were well matched to their corresponding basal concentrations. Mean plasma insulin levels during the final 30 min of the clamp were consistently higher in the SH rats compared with the WKY rats, although they did not attain statistical significance. Hepatic glucose production was similarly suppressed in all groups. Negative values were obtained for hepatic glucose production because cold glucose infusate was not "spiked" with D-[3-^{3}H]glucose during these

TABLE 2
Results of glucose clamp studies

	Group			
	S	SO	W	WO
n	11	8	9	9
Basal glucose (mM) (20-h fasted)	3.2 ± 0.1	3.3 ± 0.1	3.2 ± 0.1	2.8 ± 0.1
Basal insulin (pM) (20-h fasted)	93.8 ± 7.7	62 ± 10.7	89.9 ± 20.1	61.1 ± 6.3
Clamp glucose (mM)	3.3 ± 0.1	3.0 ± 0.2	3.4 ± 0.1	3.0 ± 0.2
Clamp insulin (pM)	495 ± 51	427 ± 40	363 ± 66	381 ± 66
Clamp R_d ($mmol \cdot kg^{-1} \cdot h^{-1}$)	3.2 ± 0.2	4.1± 0.3	1.5 ± 0.3	2.2 ± 0.3
Clamp hepatic glucose output ($mmol \cdot kg^{-1} \cdot h^{-1}$)	−1.4 ± 0.2	−1.6 ± 0.3	−0.4 ± 0.2	−0.6 ± 0.2
Clamp R_d/[G]/[I] ($ml \cdot kg^{-1} \cdot h^{-1} \cdot pM^{-1}$)	2.1 ± 0.2*	3.6 ± 0.4†	1.2 ± 0.1	2.4 ± 0.5

Data are means ± SE. Clamp R_d/[G]/[I] is steady-state glucose clearance/steady-state plasma insulin. S, untreated SH rats; SO, BMOV-treated SH rats; W, untreated WKY rats; WO, BMOV-treated WKY rats. *$P < 0.002$, S different from W; †$P < 0.01$, SO different from S.

studies. When insulin sensitivity was expressed as the steady-state glucose clearance per unit of steady-state insulin (12), the untreated SH rats were found to be more insulin sensitive than the untreated WKY rats (Table 2). BMOV treatment caused an enhancement in the insulin sensitivity index in the SO group ($P < 0.002$ vs. untreated SH rats). Although the WO group also demonstrated a similar increase in insulin sensitivity, the difference did not attain statistical significance ($P = 0.052$ vs. untreated WKY rats, Table 2).

DISCUSSION

This study was initiated to examine the relationship between insulin resistance, hyperinsulinemia, and BP in a well-established genetic model of hypertension. In view of reported alterations in glucose metabolism induced by general anesthesia (13,14), hyperinsulinemic clamp studies were performed in conscious rats. During euglycemic clamps, the total R_d value at a given insulin concentration represents the sum of insulin-dependent and insulin-independent glucose disposal, both of which are influenced by the plasma glucose levels. Therefore, to exclude any underestimation of insulin action (because of minor differences in steady-state plasma glucose levels between groups), we calculated insulin sensitivity by dividing the steady-state glucose clearance by the steady-state plasma insulin. Results from our study demonstrate that SH rats are not insulin resistant (but rather more insulin sensitive) compared with their WKY controls. These results are in agreement with those from studies where clamps were performed in conscious rats (5,6) but are in conflict with those obtained after clamping in anesthetized rats (3,4). This apparent conflict between results from studies conducted in conscious and anesthetized rats has been attributed to an exaggerated stress response to anesthesia in SH rats, which could result in secondary insulin resistance (5). Rao (15) recently reported that part of this discrepancy could be explained by the variability in insulin clearance that occurs during hyperinsulinemic clamp studies in rats. He suggested that such variability in insulin clearance could result in an increased risk of a type (II) statistical error (especially when small sample sizes were used), which could mask the differences in insulin sensitivity between SH and WKY rats. However, in that study, clamps were performed in anesthetized rats and catecholamine levels were not measured; therefore, the possibility of an increased stress response in the SH rats cannot be excluded.

Buchanan et al. (5) reported that catecholamine concentrations returned to normal within 4 h of cannulation (of the tail artery and vein) in animals preconditioned to partial restraint by the tail. In a modification of his method, we have reported that catecholamine concentrations return to normal within 30 min of line placement in both Sprague Dawley and SH rats (S.B., J.H.M., and M.B.-A., unpublished observations). We also measured plasma catecholamine concentrations before starting the clamp infusions and found them to be similar in the untreated SH and WKY groups. Steady-state plasma insulin levels during the clamps were consistently higher in the SH rats compared with the WKY rats (36% higher in the untreated SH rats than in the untreated WKY rats and 21% higher in the BMOV-treated SH rats than in the treated WKY rats), although they did not reach the desired statistical significance. These results are consistent with those obtained in other laboratories (5), and suggest a reduced insulin clearance in the SH rat. Although SH rats do not appear to be insulin resistant compared with WKY rats, SH rats have been shown to exhibit postabsorptive hyperinsulinemia (6). Buchanan et al. (16) have demonstrated that, compared with WKY rats, SH rats have an exaggerated insulin response to an intravenously administered glucose load, an enhanced glucose tolerance, and similar insulin-mediated glucose transport into skeletal muscle. Briefly, they studied both 4-h and 12-h fasted rats and observed that, whereas 4-h fasted rats were hyperinsulinemic compared with their controls, 12-h fasted SH rats had insulin levels that were similar to those seen in WKY rats. Furthermore, in the 4-h fasted SH rats, the decrease in plasma glucose did not attain statistical significance (although a trend toward lower plasma glucose levels was observed), whereas in the 12-h fasted SH rats, plasma glucose values were lower than those seen in the WKY rats. Results from our study are similar to those of Buchanan et al. in that glucose levels after a 5-h fast were similar between SH and WKY rats and were, therefore, reported as being normal.

Perhaps more important is the observation that the acute insulin responses to a glucose load (after both a 4-h and a 12-h fast) were two- to threefold higher in the SH rats compared with the WKY rats, which was accompanied by an

increased R_d in the SH rats (16). Thus, the primary reason for postabsorptive hyperinsulinemia in SH rats seems to be hypersecretion of insulin (in response to a glucose load) compared with their controls. Furthermore, this hypersecretion of insulin does not seem to be related to insulin resistance, because the 3-*O*-methylglucose transport rates into the skeletal muscle isolated from SH and WKY rats were similar at physiological as well as pharmacological concentrations (16). Although a few studies done in vitro have demonstrated that there is a decrease in basal and insulin-stimulated glucose transport in isolated adipocytes from SH rats compared with WKY rats (17,18), results from two studies (6,16) that directly examined glucose metabolism in skeletal muscle (which is the primary site of glucose utilization) indicate that SH rats are not insulin resistant compared with WKY rats. Therefore, results from studies conducted in isolated adipocytes (17,18) do not contradict the observation that SH rats show normal insulin sensitivity, but rather suggest a differential regulation of carbohydrate metabolism in the muscle and adipose tissue in SH rats.

If hyperinsulinemia contributed to an increase in BP in the SH rats, then a decrease in plasma insulin levels should also attenuate the hypertension. The results of this study support this hypothesis, because BMOV improved insulin sensitivity, decreased insulin levels, and caused concurrent decreases in BP in the SH rats. We have recently shown that vanadium compounds lower BP and plasma insulin levels in SH rats and that the effect on BP is reversed by artificially raising insulin levels in the treated SH rats to those that are seen in the untreated SH rats (19,20). Interestingly, several recent reports indicate that compounds that enhance insulin sensitivity and thereby lower insulin levels also decrease BP in rats (21–23). In addition, it has been reported that exogenous insulin infusion in normotensive rats caused an increase in BP (24). Hyperinsulinemia can stimulate 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 (1). It can be argued that BMOV, an organic vanadyl complex, may also affect factors other than insulin, i.e., it may decrease BP through a direct vascular effect. Although such an effect cannot be excluded, we have considered several possibilities. Vanadyl is a very poor inhibitor of cellular enzyme systems (25,26), and we are not aware of any study that indicates any direct antihypertensive effect of BMOV or vanadyl in vivo at concentrations used in this study. Furthermore, if the antihypertensive effect of BMOV were because of a direct vascular effect, the drug should also have lowered BP in the treated WKY rats (which was not observed), unless the SH rats responded differently than the WKY rats toward the direct effect(s) of BMOV. BMOV caused an 8–9% decrease in body weight in both the SO and WO groups, and the contribution of such a decrease in weight toward the improvement in insulin sensitivity (in both the SO and WO groups) cannot be excluded. Taken together, these data suggest that either hyperinsulinemia contributes to the development of high BP in rats or that the underlying mechanism is closely related to the expression of both these disorders. It is important to mention, however, that, although the SH rats were not insulin resistant compared with their controls, BMOV further improved insulin sensitivity in the SH rats. Such an improvement in insulin sensitivity could also affect BP in an independent manner (1) that may not be related to hyperinsulinemia; results from our study, however, do not allow us to rule out such an effect.

Another observation that stems from the current data is that there was a definite trend toward an improvement in insulin sensitivity in the treated WKY rats ($P = 0.052$, such that the possibility of a type II statistical error cannot be excluded) and that the absolute increase in insulin sensitivity in the treated WKY rats was comparable to that seen in the treated SH rats. However, no difference in the 5-h fasted plasma insulin levels was observed between the treated and untreated WKY rats. Interestingly, several recent studies in our laboratory indicate that, although vanadium compounds lower plasma insulin levels in nondiabetic Wistar and Sprague Dawley rats (7 and S.B., J.H.M., M.B.-A., unpublished observations), they do not affect insulin levels in the WKY rats (19,20). Furthermore, the WKY rats show remarkably different effects to other metabolic insults when compared with other strains of rats. For example, the WKY rats are resistant to the effects of streptozotocin-induced diabetes (27) and show marked differences in heart rate, cardiac function, and plasma triglycerides compared with Wistar and Sprague Dawley diabetic rats (28,29). Thus, the WKY rats seem to exhibit certain metabolic differences when compared with other rat strains, which is an issue worthy of investigation.

In summary, although the SH rats are not insulin resistant compared with their WKY controls, they exhibit higher postabsorptive insulin levels. Furthermore, BMOV, a drug that improved insulin sensitivity and decreased insulin levels in SH rats, also caused sustained decreases in BP in SH rats. Although these findings do not establish causality, they support the notion that hyperinsulinemia may be a contributing factor to the increase in BP in this animal model of hypertension. Further studies are needed to identify the mechanisms underlying the association between insulin and BP in spontaneously hypertensive rats.

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