ELSEVIER

European Journal of Pharmacology 357 (1998) 61–65

ejp

Short communication

Vanadate treatment normalizes exaggerated vascular smooth muscle responses in the obese Zucker rat

Rob L. Hopfner, J. Robert McNeill, Venkat Gopalakrishnan *

The Cardiovascular Risk Factor Reduction Unit and the Department of Pharmacology, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada S7N 5E5

Received 14 July 1998; accepted 17 July 1998

Abstract

The effect of oral vanadate treatment on isometric tension responses were examined in aortic rings isolated from obese and lean Zucker rats. Rats from both strains that were either maintained on food ad libitum or pair-fed were included to serve as controls. Higher plasma insulin and glucose levels and exaggerated aortic tension responses to endothelin-1, methoxamine, and KCl observed in obese Zucker rats were normalized in vanadate treated, but not pair-fed, rats. These data suggest that abnormal vascular responses in obese Zucker rats can be normalized by vanadate treatment in a manner at least partly independent of food intake.

Keywords: Vanadate; Obese Zucker rat; Aorta; Tension response; Plasma insulin

1. Introduction

The obese Zucker rat is an appropriate model of the human 'syndrome X'—often exhibiting hyperglycemia, hyperphagia, hyperlipoproteinemia, hyperinsulinemia, insulin resistance, and hypertension (Kasiske et al., 1992). Cardiovascular abnormalities occur in this strain as studies have shown that these rats are hypertensive (Kasiske et al., 1992) and exhibit exaggerated vascular smooth muscle responses to vasoactive agonists (Zemel et al., 1990; Ouchi et al., 1996).

Vanadium agents are well known insulinomimetics and they have been shown to have beneficial effects on both metabolic abnormalities and cardiovascular function in various models of metabolic dysregulation, including the obese Zucker rat (Bhanot and McNeill, 1994; Ozcelikay et al., 1994; Brichard and Henquin, 1995; Yuen et al., 1996). However, the effect of vanadium treatment on vascular smooth muscle function ex vivo in states of insulin resistance associated with metabolic dysregulation has not yet been investigated. Therefore, we set out to determine the effect of sodium orthovanadate treatment on vascular reactivity in the hyperinsulinemic/insulin-resistant obese Zucker rat.

2. Materials and methods

2.1. Animals and treatment

Twelve week old obese and lean Zucker rats were separated into 6 groups: lean–control, lean–vanadate, lean–pair fed with vanadate, obese–control, obese–vanadate, and obese–pair fed with vanadate. Animals were housed in individual metabolic cages to allow for accurate measurement of food and water intake. Sodium orthovanadate (vanadate; 0.5 mg/ml) was administered to the treatment groups for a period of 25 days. The dose was increased progressively in the week preceding the study to allow the rats to become accustomed to its taste. Since vanadate is known to decrease body weight and food intake (Malabu et al., 1994; Brichard and Henquin, 1995), pair-fed groups were included in which the level of food intake remained restricted to the same as that consumed by vanadate treatment groups. Vanadate intake was determined by multiplying the daily water intake with vana-

* Corresponding author. Tel.: +1-306-966-6294; Fax: +1-306-966-6220; E-mail: gopal@sask.usask.ca

0014-2999/98/$19.00
PII: S0014-2999(98)00535-4

dium concentration and was 11.6 ± 1.4 mg rat^{-1} day^{-1} for LZR and 14.0 ± 1.3 mg rat^{-1} day^{-1} for OZR treatment groups.

2.2. Metabolic parameters

Body weight was recorded before and after vanadate treatment; food and water intake was recorded on a daily basis throughout. On the 25th day, rats were killed and blood samples were collected immediately in tubes containing EDTA, centrifuged at $3000 \times g$ for 10 min at 3°C, and plasma was collected and immediately frozen at −20°C. Blood glucose was determined at the time of sacrifice by a glucose oxidase method (One Touch Basic, Lifescan Canada, Vancouver, BC, Canada). Plasma insulin was detected using a rat insulin radioimmunoassay kit (Amersham Life Sciences, Oakville, ON, Canada).

2.3. Tension response measurements

Tension responses were recorded as described in detail earlier (Chen et al., 1995). Immediately after sacrifice, the thoracic aorta was excised and placed in Krebs'-bicarbonate buffer (in mmol/l: NaCl 118, $NaHCO_3$ 25, KCl 4.8, $MgCl_2$ 1.2, $CaCl_2$ 1.8, KH_2PO_4 1.0, and D-glucose 11), cleared of fat and connective tissue, and cut into ~5 mm segments. For experiments requiring endothelial denudation, tissues were rubbed gently between thumb and forefinger to facilitate endothelial removal. Tissues were then suspended in 20 ml tissue baths containing oxygenated (95% O_2/5% CO_2 at 37°C) Krebs'-bicarbonate buffer, and connected to a FTO.3 isometric force transducers (Grass Instruments) under a resting tension of 2 g. The rings were allowed to equilibrate for 90 min while changing the buffer solution every 20 min after which a single methoxamine (10^{-5} mol/l) challenge was used to wake-up the tissue. Endothelial integrity was assessed by acetylcholine (10^{-5} mol/l) evoked relaxation at the peak of the methoxamine evoked response. After extensive washout allowing the baseline to return to the 2 g preload tension, cumulative concentration–response (C–R) curves to either methoxamine (10^{-7}–10^{-4} mol/l), endothelin-1 (10^{-10}–10^{-7} mol/l) or KCl (10–60 mmol/l) were determined in both endothelium denuded and intact preparations. Only one agonist was used per segment of aortic tissue. After cumulative dose–response curves were complete, the tissues were allowed to dry overnight and then weighed. In order to normalize for potential differences in wall thickness and length amongst individual aortic segments, the data were expressed as increase in tension development per cross-sectional area of tissue (g/mm^2) as described earlier (Chen et al., 1995; Wyse, 1980).

2.4. Materials

Acetylcholine chloride, methoxamine hydrochloride and sodium orthovanadate were from Sigma (St. Louis, MO, USA). Endothelin-1 was from American Peptide (Sunnyvale, CA, USA).

2.5. Statistical methods

The concentration of agonist required to produce 50% of the maximal response (EC_{50}) and the maximal isometric tension response (E_{max}) developed to each agonist was assessed for each individual C–R curve and pooled in order to attain mean + S.E.M. Statistical differences between means were evaluated using analysis of variance (Superanova-Biosoft) and simultaneous multiple comparisons were examined using Scheffe's F test.

3. Results

Metabolic parameters after the treatment period are shown in Table 1. In contrast to untreated groups, both

Table 1
Metabolic parameters in lean Zucker rat (LZR) and obese Zucker rat (OZR) subjected to either 0.5 mg/ml p.o. sodium orthovanadate (vanadate) administration, pair feeding, or no treatment (control)

Group	Body weight before (g)	Body weight after (g)	Food intake ($g.rat^{-1}$ day^{-1})	Blood glucoses (mmol/l)	Plasma [insulin] (pmol/l)
LZR-control	303 ± 6	372 ± 6^{b}	26 ± 1	5.7 ± 0.3	808 ± 81
LZR-pair fed	306 ± 8	334 ± 10^{a}	20 ± 1^{d}	5.3 ± 0.3	589 ± 42
LZR-vanadate	291 ± 6	303 ± 6	20 ± 1^{d}	5.8 ± 0.2	560 ± 84
OZR-control	489 ± 16	575 ± 13^{a}	36 ± 1^{f}	9.5 ± 1.8^{e}	2536 ± 280^{e}
OZR-pair fed	479 ± 13	500 ± 13	23 ± 1^{d}	6.2 ± 0.4^{c}	2896 ± 784^{e}
OZR-vanadate	489 ± 11	507 ± 13	23 ± 1^{d}	5.4 ± 0.2^{c}	1306 ± 198^{c}

Values shown above are mean ± S.E.M. from 8 separate rats for each group.
[a] $P < 0.01$ vs. respective weight before group.
[b] $P < 0.001$ vs. respective weight before group.
[c] $P < 0.01$ vs. respective untreated group.
[d] $P < 0.001$ vs. respective untreated group.
[e] $P < 0.01$ vs. respective LZR group.
[f] $P < 0.001$ vs. respective LZR group.

lean and obese rats treated with vanadate failed to exhibit significant weight gain. Pair-fed rats exhibited decreased weight gain in the lean group, and failed to exhibit significant weight gain in the obese group. Blood glucose levels were significantly higher on average in the obese–control group than in all other groups, yet were only mildly elevated. Plasma insulin levels were 3 to 4 fold higher in untreated obese Zucker rats compared to lean controls. Vanadate treatment, but not pair feeding, significantly attenuated elevated plasma insulin levels in the obese group.

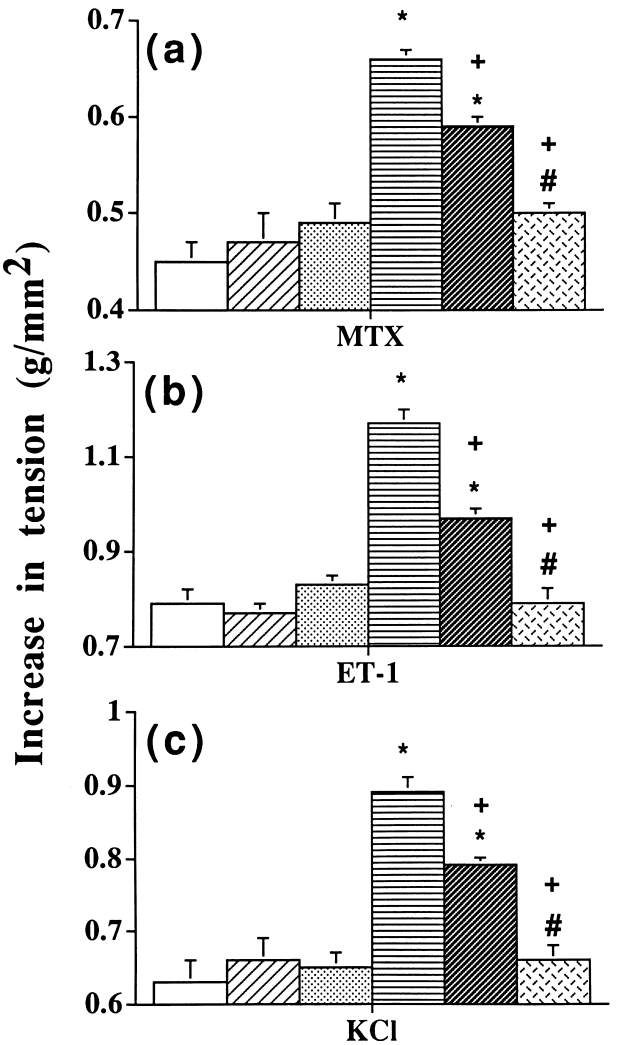

Fig. 1. Maximal isometric tension responses (E_{max}) to (a) methoxamine (MTX), (b) endothelin-1 (ET-1) and (c) KCl in aortic rings isolated from the various groups. These are represented by: lean–control (open bar); lean–pair fed (cross hatch light); lean–vanadate (spotted bar); obese–control (side hatch); obese–pair fed (cross hatch dark); and obese–vanadate (mixed hatch). Maximal tension development to all three stimuli was measured as grams tension developed per cross-sectional area of tissue (g/mm^2). Concentration–response curves to all stimuli were assessed in individual aortic ring segments, with only one agonist tested per segment. The data derived for each agonist from different rats in each group were pooled together and subjected to analysis of variance. Mean ± S.E.M. values were obtained using aorta derived from $n = 12$ rats from each group. ($* P < 0.05$ vs. lean–control group; $+ P < 0.05$ vs. obese–control group; $\# P < 0.05$ vs. pair-fed group).

E_{max} values to methoxamine, endothelin-1, and KCl in endothelium intact aortic rings were significantly greater in untreated obese compared to lean Zucker rats (Fig. 1). There were no significant differences in EC_{50} values between obese and lean groups (data not shown). Elevated maximal responses to all stimuli were attenuated in the obese–vanadate group to near the level of that of lean–controls (Fig. 1). Pair feeding of obese rats led to a partial attenuation in E_{max} for all three stimuli, but the fall in E_{max} did not reach the level of that evoked by vanadate treatment. Endothelium denudation of aortic segments had no effect on E_{max} values for any vasoconstrictor stimulus, thus leaving the differential E_{max} relationship between obese and lean rats intact in all groups (data not shown). However, denudation resulted in a universal and consistently highly significant ($P < 0.01$) left-ward shift in the C–R curves with a reduction in EC_{50} values for both methoxamine (endo intact: 2.4 to 2.7 μM, endo denuded: 0.5 to 0.6 μM) and endothelin-1 (endo intact: 2.4 to 2.8 nM; endo denuded: 0.6 to 0.7 nM), but not to KCl (endo intact: 25 to 28 mM; endo denuded: 24 to 26 mM) in all groups. While decreases in EC_{50} values after endothelium denudation were consistently seen in pair-fed and vanadate treated groups as well as the untreated groups, there were no differences in the degree of shift for each agonist amongst the various groups. This shift likely occurs secondary to loss of endothelial derived factors such as nitric oxide and other hyperpolarizing factors that are released upon methoxamine and endothelin-1 stimulation that function to dampen agonist evoked vasoconstrictor responses.

4. Discussion

We have demonstrated that obese Zucker rats exhibit a non-specific, endothelium independent, increase in vascular smooth muscle responses to both agonist evoked and depolarizing stimuli as reported earlier (Ouchi et al., 1996; Zemel et al., 1990). The mechanism of this alteration is unclear, but may be related to accelerated atherosclerosis, increased vascular rigidity, and/or altered vascular smooth muscle cation transport resulting from genetic and/or metabolic factors present in the obese Zucker rat (Kasiske et al., 1992; Epstein and Sowers, 1992). Oral vanadate treatment corrected mild hyperglycemia, and attenuated hyperinsulinemia, food intake and body weight gain in obese rats over the treatment period. In addition, we have shown for the first time that exaggerated vascular responses in obese Zucker rats can be corrected by vanadate treatment, and significantly attenuated by caloric restriction.

Vanadium compounds such as sodium orthovanadate, vanadyl sulphate, peroxovanadium and bismaltolatooxovanadium have all been demonstrated to promote normal-

ization of hyperglycemia, dyslipidemia and insulin dysregulation both in vitro and in vivo, through either direct insulinomimetic effects or through enhancement of insulin action via inhibition of protein tyrosine phosphatases (Brichard and Henquin, 1995). They may also confer beneficial cardiovascular effects as these agents have been shown to normalize exaggerated vascular responses in streptozotocin diabetic rats (Ozcelikay et al., 1994), lower blood pressure in spontaneously hypertensive rats (Bhanot and McNeill, 1994), and prevent development of hypertension in obese Zucker rats (Yuen et al., 1996).

The mechanism of vanadate evoked correction of exaggerated vascular smooth muscle responses in the present study is speculative. We have observed that vanadate treatment lowers both plasma insulin and glucose levels in the obese Zucker rat model—likely mediated by increases in insulin sensitivity as previously demonstrated in this strain (Brichard and Henquin, 1995). The profound ability of vanadium compounds to correct such metabolic abnormalities, even in conditions of insulin resistance, may be linked to its ability to correct vascular dysfunction. Support for this concept can be found in recent work from ours (Hopfner et al., 1998), and other (Ozcelikay et al., 1994; MacLeod, 1985) laboratories demonstrating that both vanadate and insulin treatment normalize exaggerated vascular responses in streptozotocin diabetic rats (a model of type I diabetes) in conjunction with correction of most, if not all, of its metabolic abnormalities. However, direct effects of vanadate cannot be ruled out, since some of its potential cellular effects per se, including effects on Na^+/K^+ ATPases and protein tyrosine phosphatases (Brichard and Henquin, 1995), could have relevance to vascular smooth muscle function. Indeed, the observation that insulin sensitizers, such as the thiazolidenediones, lower blood pressure in different models of insulin resistance in conjunction with beneficial systemic metabolic as well as direct effects on vascular smooth muscle function (Buchanan et al., 1995; Pershadsingh et al., 1993) warrants a wider perspective in interpretation of the present results. It can be assumed that correction of abnormal vascular responses by vanadate does not involve alterations in endothelial function, since endothelium denudation resulted in similar effects on vasoconstrictor evoked E_{max} and EC_{50} values across all of the treatment and control groups. In addition, since caloric restriction only led to a partial attenuation of exaggerated vascular responses, it is reasonable to state that the vascular effects of vanadate are mediated by a mechanism at least partly distinct from its effects on food intake.

5. Conclusion

Our data demonstrates that vanadate treatment may normalize exaggerated vascular smooth muscle responses to vasoconstrictor stimuli in the obese Zucker rat, a model of metabolic dysfunction associated with insulin resistance and hypertension. This correction occurs in conjunction with beneficial metabolic effects and in a manner at least partly independent of its effects on food intake. Since central artery distensibility is thought to be a major contributor to increases in systolic blood pressure in hypertensive and diabetic states (Megnien et al., 1992), it is possible that vanadate treatment may beneficially affect the hypertensive state in the obese Zucker rat.

Acknowledgements

This work was supported by a grant (grant # MT-14377) from the Medical Research Council of Canada to V.G. Rob Hopfner is grateful to the Medical Research Council of Canada for the Doctoral Research Traineeship Award.

References

Bhanot, S., McNeill, J.H., 1994. Vanadyl sulphate lowers plasma insulin and blood pressure in spontaneously hypertensive rats. Hypertension 24, 625–632.

Brichard, S.M., Henquin, J.C., 1995. The role of vanadium in the management of diabetes. Trends Pharmacol. Sci. 15, 265–270.

Buchanan, T.A., Meehan, W.P., Jeng, Y.Y., Yang, D., Chan, T.M., Nadler, J.L., Scott, S., Rude, R.K., Hsueh, W.A., 1995. Blood pressure lowering by pioglitazone—evidence for a direct vascular effect. J. Clin. Invest. 96, 354–360.

Chen, L., McNeill, J.R., Wilson, T.W., Gopalakrishnan, V., 1995. Differential effects of phosphoramidon on contractile responses to angiotensin-II in rat blood vessels. Br. J. Pharmacol. 114, 1599–1604.

Epstein, M., Sowers, J.R., 1992. Diabetes Mellitus and hypertension. Hypertension 19, 403–418.

Hopfner, R.L., Misurski, D., Wilson, T.W., McNeill, J.R., Gopalakrishnan, V., 1998. Insulin and vanadate normalize decreased plasma endothelin-1 levels and exaggerated vascular responses in the streptozotocin diabetic rat. Diabetologia 41, in press.

Kasiske, B.L., O'Donnell, M.P., Keane, W.F., 1992. The Zucker rat model of obesity, insulin resistance, hyperlipidemia and renal injury. Hypertension 19 (Suppl. I), I10–I15.

MacLeod, K.M., 1985. The effect of insulin treatment on changes in vascular reactivity in chronic, experimental diabetes. Diabetes 34, 1160–1167.

Malabu, U.H., Dryden, S., McCarthy, H.D., Kilpatrick, A., Williams, G., 1994. Effects of chronic vanadate administration in the STZ-induced diabetic rat. The antihyperglycemic action of vanadate is attributable entirely to its suppression of feeding. Diabetes 43, 9–15.

Megnien, J.L., Simon, A., Valensi, P., Flaud, P., Merli, I., Levenson, J., 1992. Comparative effects of diabetes mellitus and hypertension on physical properties of human large arteries. J. Am. Coll. Cardiol. 20, 1562–1568.

Ouchi, Y., Han, S., Kim, S., Akishita, M., Kozaki, K., Toba, K., Orimo, H., 1996. Augmented contractile function and abnormal calcium handling in the aorta of Zucker obese rats with insulin resistance. Diabetes 45, S55–S58.

Ozcelikay, A.T., Pekiner, C., Ari, N., Ozturk, Y., Ozuari, A., Altan, V.M., 1994. The effect of vanadyl treatment on vascular responsiveness of streptozotocin diabetic rats. Diabetologia 37, 572–578.

Pershadsingh, H.A., Szollosi, J., Benson, S., Hyun, W.C., Feuerstein, B.G., Kurtz, T.W., 1993. Effects of ciglitazone on blood pressure and intracellular calcium metabolism. Hypertension 21, 1020–1023.

Wyse, D.G., 1980. On 'the normalization' of active developed force of isolated helical strips of muscular and elastic arteries for variation in wall thickness. J. Pharmacol. Methods 4, 313–326.

Yuen, V.G., Pederson, R.A., Dai, S., Orvig, C., McNeill, J.H., 1996. Effects of low and high dose administration of bis(maltolato) oxovanadium (IV) on fa/fa zucker rats. Can. J. Physiol. Pharmacol. 74, 1001–1009.

Zemel, B., Sowers, J.R., Shehin, S., Walsh, F., Levy, J., 1990. Impaired calcium metabolism associated with hypertension in zucker obese rats. Metabolism 39, 704–708.