Metabolism
Clinical and Experimental

VOL 45, NO 9 SEPTEMBER 1996

PRELIMINARY REPORT

Decreased Vascular Reactivity in Metformin-Treated Fructose-Hypertensive Rats

Subodh Verma, Sanjay Bhanot, and John H. McNeill

We have previously demonstrated that long-term metformin treatment prevents the development of hyperinsulinemia and hypertension in fructose-hypertensive (FH) rats; however, the exact nature of its antihypertensive effects remains elusive. Since hyperinsulinemia has been proposed to be a strong stimulus for norepinephrine (NE) release, the present study examined the effects of long-term metformin treatment (500 mg/kg/d for 10 weeks) on the reactivity of superior mesenteric arteries to NE in FH rats. Metformin treatment prevented the development of hyperinsulinemia and hypertension in FH rats. Mesenteric arteries from FH rats exhibited an increased cross-sectional area ([CSA] 0.45 ± 0.07 mm^2 *v* 0.32 ± 0.05 in controls, $P < .05$), which was prevented by long-term metformin treatment (0.34 ± 0.04 mm^2, $P > .05$ *v* untreated FH). Interestingly, mesenteric arteries from metformin-treated fructose and control rats exhibited a reduction in maximum responsiveness to NE both with and without the endothelium. These data suggest that metformin directly reduces catecholamine constrictor responses in resistance arteries of rats, which may contribute to its antihypertensive effects in rats.

ALTHOUGH DRUGS that improve insulin sensitivity and decrease plasma insulin levels have been shown to exert antihypertensive effects,[1-5] the mechanism(s) underlying this link remain elusive. Accumulating evidence suggests that insulin exerts important effects on the sympathetic nervous system (SNS) and vascular smooth muscle (VSM).[6] Although insulin causes a marked increase in sympathetic nerve activity, it causes vasodilation of the peripheral and systemic circulation.[6] Thus, it has been postulated that, on one hand, hyperinsulinemia may serve as a continual stimulus for SNS activation, and on the other hand, resistance to the vasodilatory actions of insulin may result in increased sympathetic-mediated vasoconstriction and an increase in blood pressure (BP).[6]

We have previously demonstrated that long-term treatment with the widely used antihyperglycemic agent, metformin, causes sustained decreases in plasma insulin levels and systolic BP in hyperinsulinemic fructose-hypertensive (FH) rats.[4] Although the exact mechanism underlying this effect remains elusive, of particular interest are recent studies demonstrating that metformin exerts acute sympathoinhibitory effects in spontaneously hypertensive rats.[7] Hence, it is possible that long-term metformin treatment may decrease SNS activity (independently or secondarily to attenuation of hyperinsulinemia), which in turn could lead to decreased sympathetic-mediated vasoconstriction and a decrease in BP. At the other end of the spectrum are recent studies demonstrating the inhibitory effects of metformin on agonist-stimulated calcium transients in VSM cells, suggesting a direct action of metformin at the level of VSM.[8] Although the antihypertensive effects of metformin have been documented in human and animal models of hypertension, we are not aware of any study that has evaluated the effect of long-term metformin treatment on the reactivity of resistance vessels to the major sympathetic neurotransmitter, norepinephrine (NE). We report herein that long-term metformin treatment of FH rats is associated with both prevention of structural alterations and a reduction in the reactivity of mesenteric arteries to NE, suggesting that the reduction in catecholamine constrictor responses may contribute to the antihypertensive effects of this drug.

From the Division of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, Canada.

Submitted January 5, 1996; accepted March 29, 1996.

Supported by a grant from the B.C. and Yukon Heart and Stroke Foundation, and in part by fellowships from the Medical Research Council of Canada (S.V.) and the Heart and Stroke Foundation of Canada (S.B.).

Address reprint requests to John H. McNeill, PhD, Professor and Dean, Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, B.C. V6T 1Z3, Canada.

0026-0495/96/4509-0001$03.00/0

MATERIALS AND METHODS

Male Sprague-Dawley rats were procured at 5 weeks of age (University of British Columbia animal care unit) and assigned to four experimental groups: control (C, n = 10), control metformin-treated (CM, n = 10), fructose (F, n = 10), and fructose metformin-treated (FM, n = 10). Groups C and F served as controls for two studies performed in parallel. At week 6 (weeks signify the age of the rats), systolic BP, plasma glucose, and plasma insulin (5-hour fasted) were measured in all groups. Blood samples for plasma glucose and insulin measurements were collected from the tail vein. Starting at week 6, long-term oral metformin treatment was initiated in CM and FM groups (500 mg/kg/d dissolved in drinking water). Starting at week 7, rats in the F and FM groups were started on a 66% fructose diet for 10 weeks. At week 17, superior mesenteric arteries from all the groups were removed, and NE concentration-response curves were constructed (10^{-9} to 10^{-4} mol/L) in the presence and absence of the endothelium as described previously.[9,10] At completion of each experiment, the cross-sectional area (CSA) of the arteries was determined, and the tension responses were corrected for both CSA and length and expressed as g/mm^3. The CSA of each tissue was calculated using the formula, CSA (millimeters squared) = weight (milligrams)/length (millimeters) × density (milligrams per cubic millimeter). The density of the arteries was assumed to be 1.05 mg/mm^3. Agonist pD_2 values (-log 50% effective dose) were calculated by nonlinear regression analysis of the dose-response curves. Systolic BP was measured in conscious rats using the indirect tail-cuff method without external preheating as described previously.[1-4] NE and metformin were obtained from Sigma (St Louis, MO). The fructose diet was obtained from Harlan Teklad Laboratories (Madison, WI). Plasma glucose and insulin levels were measured as described previously.[1-4]

Results were compared using a one-way ANOVA followed by Newman-Keuls test. P less than .05 indicated a significant difference between means.

RESULTS

As previously demonstrated,[4] long-term metformin treatment prevented the development of hyperinsulinemia and hypertension in the FM group (plasma insulin, FM 3.6 ± 0.4 *v* F 5.8 ± 0.3 ng/mL, $P < .05$; systolic BP, FM 140 ± 3 *v* F 157 ± 5 mm Hg, $P < .05$) and had no effect in the CM group (plasma insulin, CM 3.0 ± 0.5 *v* C 3.2 ± 0.5 ng/mL, $P > .05$; systolic BP, 132 ± 4 *v* C 130 ± 4 mm Hg, $P > .05$). Superior mesenteric arteries from F rats were characterized by an increased CSA when compared with those from the C group (F 0.45 ± 0.07 *v* C 0.32 ± 0.05 mm^2, $P < .05$). Long-term metformin treatment prevented the increase in CSA in the FM group (0.34 ± 0.04 mm^2, $P < .05$ *v* F) and had no effect in the CM group (0.33 ± 0.04 mm^2, $P > .05$ *v* C). Metformin treatment had no effect on plasma glucose levels in any group (C 5.5 ± 0.5, CM 4.8 ± 0.5, F 6.0 ± 0.6, and FM 5.3 ± 0.4 mmol/L, P = NS), an observation we have previously documented.[4]

The intriguing observation in this study is that although the maximum tension that developed in response to NE was similar between the C and F groups (with endothelium, C 0.89 ± 0.05 *v* F 0.82 ± 0.05 g/mm^3, $P > .05$; without endothelium, C 0.95 ± 0.03 *v* F 0.99 ± 0.05 g/mm^3, $P > .05$; Fig 1), long-term metformin treatment reduced the tension that developed in both the FM and CM groups (with endothelium, CM 0.72 ± 0.03 and FM 0.70 ± 0.03, $P < .05$ *v* C and F; Fig 1). This reduction in the catecholamine constrictor response in FM and CM arteries also persisted after removal of the endothelium (CM 0.85 ± 0.04 and FM 0.83 ± 0.04, $P < .05$ *v* C and F without endothelium; Fig 1). Metformin treatment did not affect the sensitivity of the mesenteric arteries to NE (pD_2 values: with endothelium, C

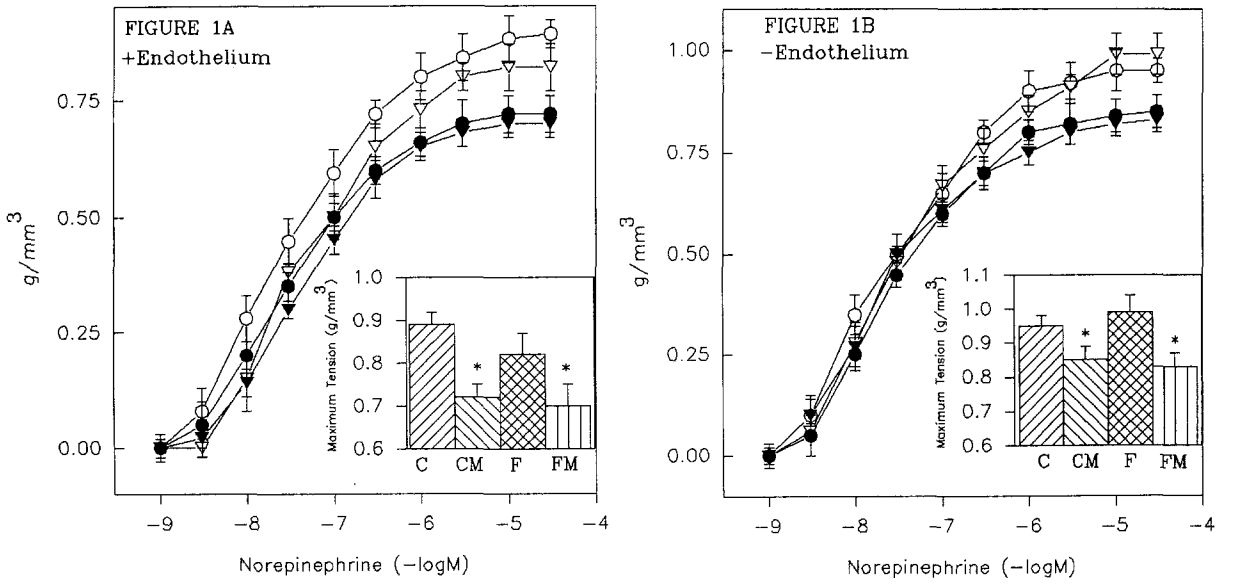

Fig 1. Cumulative NE dose-response curves of mesenteric arteries from C (○), CM (●), F (▽), and FM (▼) groups in the presence (A) and absence (B) of the endothelium. Each point is the mean ± SE and is the average of all curves. Tension responses have been corrected for CSA and length and are expressed as g/mm^3. Insert, maximum contraction (g/mm^3) elicited in response to NE in the 4 groups. *$P < .05$ *v* C and F.

7.26 ± 0.12, CM 7.10 ± 0.11, F 6.95 ± 0.26, and FM 7.00 ± 0.13, $P > .05$; without endothelium, C 7.46 ± 0.08, CM 7.40 ± 0.15, F 7.34 ± 0.86, and FM 7.36 ± 0.05, $P > .05$).

DISCUSSION

The FH rat represents an acquired model of hypertension wherein feeding normal rats a fructose-enriched diet leads to hyperinsulinemia, insulin resistance, and an elevation in BP.[1,4,9,11] Although evidence suggests that insulin resistance and hyperinsulinemia are closely related to the development of high BP in FH rats,[1,4,9,11] the underlying mechanisms remain undefined. We previously demonstrated that long-term metformin treatment (at doses similar to those used in this study) completely prevented the development of hyperinsulinemia and hypertension in FH rats.[4] The main finding of the present study is that long-term metformin treatment prevents the development of structural alterations in arteries from FM rats and reduces the catecholamine constrictor responses in both FM and CM rats. These findings suggest that metformin has direct sympathoinhibitory properties, which may contribute to its antihypertensive effects (independent of any change in plasma insulin concentration) in FH rats.

Recent studies indicate that insulin exerts potent hemodynamic effects at the level of the SNS and VSM.[6] At the level of the SNS insulin causes a marked increase in muscle sympathetic nerve activity, and at the level of the VSM it blunts the effects of several vasoactive agents,[12] causing vasodilation of the peripheral and systemic circulation.[6] Thus, a pathogenic model has been postulated such that, on one hand, hyperinsulinemia may serve as a continual stimulus for SNS activation, and on the other hand, resistance to the vasodilatory actions of insulin may result in increased sympathetic-mediated vasoconstriction and an increase in BP.[6] Hence, it could be hypothesized that the antihypertensive effect of metformin may result from a correction of this scheme of events, secondary to amelioration of hyperinsulinemia. Another possibility is that metformin may affect a third factor, which in turn closely modulates both hyperinsulinemia and BP. One such factor appears to be the SNS, and preliminary observations from our laboratory indicate that chemical sympathectomy completely prevents the development of both hyperinsulinemia and hypertension in FH rats (S. Bhanot, S. Verma, L. Yao, and J.H. McNeill, unpublished observations, February 1996). Therefore, by exerting an inhibitory influence on the SNS system, metformin could lead to a decrease in both plasma insulin levels and BP.

Although this preliminary study cannot answer this question unequivocally, there are two possibilities. Either metformin could exert a direct effect at the level of VSM (independently of the SNS and insulin), or it could exert sympathoinhibitory effects, which in turn negatively modulate vascular resistance, BP, and insulin levels (as discussed earlier). The observation that metformin treatment also reduced the response in arteries from CM rats (which neither are hyperinsulinemic or hypertensive nor exhibit SNS overactivity) suggests a direct effect of the drug on resistance arteries, and raises the compelling question as to whether decreased NE-mediated contraction underlies the antihypertensive effects of the drug. Clearly, further studies are warranted to delineate the direct versus indirect effects of metformin on VSM reactivity, and our research team is currently attempting to explore these avenues. What is perhaps more important is the observation that a widely used clinical drug decreases the constrictor responses to the main sympathetic neurotransmitter, NE, in both C and FH rats.

ACKNOWLEDGMENT

The assistance of Linfu Yao is greatly appreciated. We thank Mary Battell and Sylvia Chan for technical and secretarial assistance, respectively.

REFERENCES

1. Bhanot S, McNeill JH, Bryer-Ash M: Vanadyl sulfate prevents fructose-induced hyperinsulinemia and hypertension in rats. Hypertension 23:308-312, 1994

2. Bhanot S, Bryer-Ash M, Cheung A, et al: Bis(maltolato)oxovanadium (IV) attenuates hyperinsulinemia and hypertension in spontaneously hypertensive rats. Diabetes 43:857-861, 1994

3. Verma S, Bhanot S, McNeill JH: Metformin decreases plasma insulin levels and blood pressure in spontaneously hypertensive rats. Am J Physiol 36:H1250-H1253, 1994

4. Verma S, Bhanot S, McNeill JH: Antihypertensive effects of metformin in fructose-fed hyperinsulinemic, hypertensive rats. J Pharmacol Exp Ther 271:1334-1337, 1994

5. Landin K, Tengborn L, Smith M: Treating insulin resistance in hypertension with metformin reduces both blood pressure and metabolic risk factors. J Int Med 229:181-187, 1992

6. Baron AD: Hemodynamic actions of insulin. Am J Physiol 267:E187-E202, 1994

7. Petersen J, Dibona GF: Sympathoinhibitory actions of metformin in spontaneously hypertensive rats. Hypertension 26:559, 1995 (abstr)

8. Sharma RV, Bhalla RC: Metformin attenuates agonist-stimulated calcium transients in vascular smooth muscle cells. Clin Exp Hypertens 17:913-929, 1995

9. Verma S, Bhanot S, Yao L, et al: Defective endothelium-dependent relaxation in fructose hypertensive rats. Am J Hypertens 9:370-376, 1996

10. Agrawal DK, McNeill JH: Vascular responses to agonists in rat mesenteric artery from diabetic rats. Can J Physiol Pharmacol 65:1484-1490, 1987

11. Hwang I, Ho H, Hoffman BB, et al: Fructose-induced insulin resistance and hypertension in rats. Hypertension 10:512-516, 1987

12. Wambach GK, Liu D: Insulin attenuates vasoconstriction by noradrenaline, serotonin and potassium chloride in rat mesenteric arterioles. Clin Exp Hypertens [A] 14:733-740, 1992