Troglitazone and Vascular Reactivity: Role of Glucose and Calcium

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We sought to determine whether insulin/insulin-like growth factor-1 (IGF-1) and an insulin-sensitizing agent, troglitazone, have additive vasodilatory effects and the possible involvement of intracellular Ca^{2+} ($[Ca^{2+}]_i$) and/or glucose utilization in these effects. Contractile responses to norepinephrine (NE) and potassium chloride (KCl), as well as relaxation to endothelium-dependent (acetylcholine [Ach]) and -independent (sodium nitroprusside [NaNP]) agents, were examined in rat tail artery rings in the presence of insulin/IGF-1 and/or troglitazone. Endothelium-intact tail artery rings stretched to 1 g tension were preincubated with troglitazone (3 μmol/L) and/or insulin/IGF-1 (100 nmol/L) prior to addition of graded doses of NE and KCl. A 90-minute exposure to troglitazone attenuated the maximal contraction to graded doses of NE and KCl ($P < .0001$). Incubation in glucose-free medium decreased the responses only to NE; troglitazone further attenuated the NE-induced contraction ($P = .001$). In submaximally precontracted endothelium-intact rings, troglitazone increased the relaxation both to NaNP ($P < .0001$) and to Ach ($P = .001$). Contraction experiments in depolarizing KCl (25 mmol/L) or Ca^{2+}-free buffer showed that troglitazone and insulin have a similar Ca^{2+} dependency. In conclusion, troglitazone, like insulin/IGF-1, attenuates responses to vasoactive agonists through a Ca^{2+}-dependent mechanism that may require the presence of glucose but is independent of insulin action and nitric oxide (NO) production.

AUGMENTED RESPONSIVENESS to constriction, impaired vascular relaxation, and alterations in blood flow in individuals with impaired carbohydrate tolerance probably reflect resistance to the vasodilatory actions of insulin and insulin-like growth factor-1 (IGF-1).[1-9] In vivo, treatment of carbohydrate intolerance with insulin-sensitizing agents such as troglitazone improves vascular relaxation and organ perfusion.[10-12] In vitro, insulin, IGF-1, and insulin-sensitizing agents may induce vasodilation through a number of mechanisms. Insulin/IGF-1[6,13-16] and thiazolidinediones[12,17,18] may decrease vasoconstriction, in part, by decreasing vascular smooth muscle cell (VSMC) intracellular calcium ($[Ca^{2+}]_i$) responses to various vasoagonists. However, while insulin/IGF-1 also stimulate endothelial cell production of nitric oxide (NO),[3,6,19-22] it is not clear whether all thiazolidinediones act via this mechanism,[12,21,23] suggesting that these compounds may enhance relaxation further in the presence of hormones.

Insulin/IGF-1 and troglitazone increase glucose uptake in cardiovascular tissue.[6,23-25] In normoglycemic states, troglitazone requires the presence of insulin for its hypoglycemic effects, indicating that it acts through sensitization of tissues to insulin action.[24] Since intermediary glucose metabolism plays a role in excitation-contraction coupling responses in VSMCs,[23,26,27] enhanced glucose utilization may contribute to the vascular actions of these agents. Accordingly, we have investigated the relationship between insulin/IGF-1 and the insulin-sensitizing agent troglitazone and the roles of glucose and Ca^{2+} in mediating the vascular relaxation. Norepinephrine (NE) increases VSMC $[Ca^{2+}]_i$, in part as a result of influx through voltage-dependent Ca^{2+} channels[28] and in part via release from internal stores; NE-induced contractions appear to be especially dependent on glucose availability.[26,27] In contrast, potassium chloride (KCl)-induced contractions are less sensitive to changes in intermediary metabolism and totally dependent on Ca^{2+} entry through voltage-operated channels.[16] Thus, we have used these contractile agonists, as well as the endothelium-dependent and -independent relaxing agents acetylcholine (Ach) and sodium nitroprusside (NaNP), respectively, to examine the mechanisms whereby insulin and/or troglitazone attenuate contractility and enhance vascular dilation.

MATERIALS AND METHODS

Artery Rings and Muscle Bath Preparation

Male Wistar rats (200 to 300 g) were purchased from Harlan (Indianapolis, IN), housed two to four per cage, and given food and water ad libitum. On the day of the experiment, the rats were anesthetized with sodium pentobarbital (30 mg/kg intraperitoneally) and the tail artery was carefully dissected out. (All procedures for animal handling received institutional approval.) The artery was immediately placed into ice-chilled buffer containing the following (in mmol/L): 130 NaCl, 15 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 1.6 $CaCl_2$, 1.2 $MgSO_4$, 0.03 EDTA, and 10 glucose. The buffer solutions were devoid of glucose and Ca^{2+} in the glucose dependency and Ca^{2+} readdition experiments, respectively. The artery was further cleaned of fat and connective tissue, and 3-mm artery rings were mounted on stirrups and suspended from isometric force transducers (Gould Instruments, Cleveland, OH) in muscle baths containing the buffer at 37°C aerated with 95% O_2/5% CO_2 to maintain pH at 7.35 to 7.40. All rings were stretched to 1 g tension and allowed to equilibrate for 30 minutes before the start of the experiments.[29] Vascular contractility was assessed by stepwise addition of KCl (25 to 150 mmol/L) and NE (10^{-9} to 10^{-5} mol/L). The presence of endothelium was confirmed with a single dose (10^{-5} mol/L) of Ach. Preincubation times and troglitazone, insulin, and IGF-1 concentrations were previously optimized in our laboratory.[12,22,29]

Glucose Dependency

To assess the glucose dependency of the vascular effects of troglitazone (Regulin; Parke-Davis, Ann Arbor, MI), experiments were performed in the presence and absence of glucose. Artery rings were preincubated with 3 μmol/L troglitazone (in ethanol) for 90 minutes or vehicle for 30 minutes with and without glucose before evaluating the dose-response to KCl and NE.

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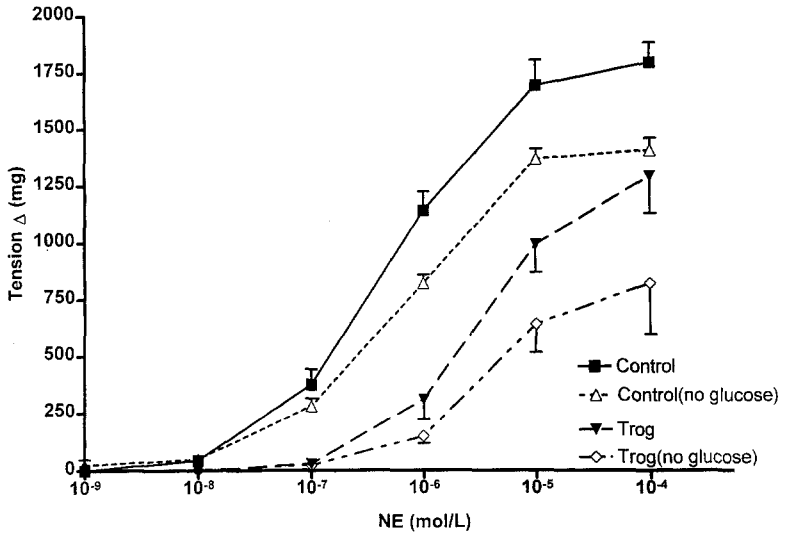

Fig 1. NE dose-response in glucose (10 mmol/L)-containing and glucose-free buffer ($P < .0001$, n = 4). Preincubation (90 minutes) with 3 μmol/L troglitazone [Trog] decreased the contractility; this was decreased further in glucose-free buffer ($P < .005$ *v* Trog alone, n = 7).

Insulin/IGF-1–Troglitazone Interactions and Contractility

To determine whether insulin/IGF-1 and troglitazone have additive effects, the artery rings were preincubated with (1) insulin (porcine; Sigma, St Louis, MO; dissolved in acidulated water) at a low (100 nmol/L) or high (700 nmol/L) dose, (2) 3 μmol/L troglitazone, (3) 3 μmol/L troglitazone and 100 nmol/L insulin, or (4) 3 μmol/L troglitazone and 100 nmol/L IGF-1 (human recombinant; gift from Genentech, San Francisco, CA) for 90 minutes before addition of KCl and NE to measure contractile responses.

Insulin-Troglitazone Interactions and Vascular Relaxation

After a 90-minute preincubation with 3 μmol/L troglitazone, 100 nmol/L insulin, or both, artery rings were precontracted to 60% to 80% of the maximal response with the addition of submaximal concentrations of KCl (for Ach experiments) or NE (for NaNP experiments). The dose-response to the endothelium-dependent and -independent agonists was established after stabilization of the initial contraction.

Ca^{2+} Dependency

Ca^{2+} dependency was examined by two different methods: (1) Arterial rings preincubated with 3 μmol/L troglitazone were exposed to a depolarizing concentration of KCl (25 mmol/L) before addition of graded doses of NE. (2) Rings were stretched as before but incubated in Ca^{2+}-free buffer containing 3 μmol/L troglitazone and/or 100 nmol/L insulin. After 90 minutes, a single dose of NE (10^{-5} mol/L) was added before reintroduction of Ca^{2+} (0.5 to 2.0 mmol/L) to assess the accentuation of the contractile response to NE. Statistical Analysis

Two-way (with Fisher's protected least-significant difference) and repeated-measures ANOVAs were used to compare changes in contractility and relaxation between groups. EC_{50} values were calculated with the Pharm C Program (Springer-Verlag, New York, NY); statistical differences between paired experiments were assessed by Student's *t* test. Results are presented as the mean ± SEM, and *P* values less than .05 were considered significant.

RESULTS

Glucose Dependency

The absence of glucose resulted in a markedly attenuated contractile response to NE (maximal tension, 1,400 ± 54 *v* 1,788 ± 88 mg in controls with normal glucose, $P < .0001$ by ANOVA). Troglitazone alone at a dose of 3 μmol/L significantly decreased the maximal contractility to 1,286 ± 161 mg (Fig 1). There was further attenuation of the contractile response following incubation in a glucose-free medium (818 ± 225 mg

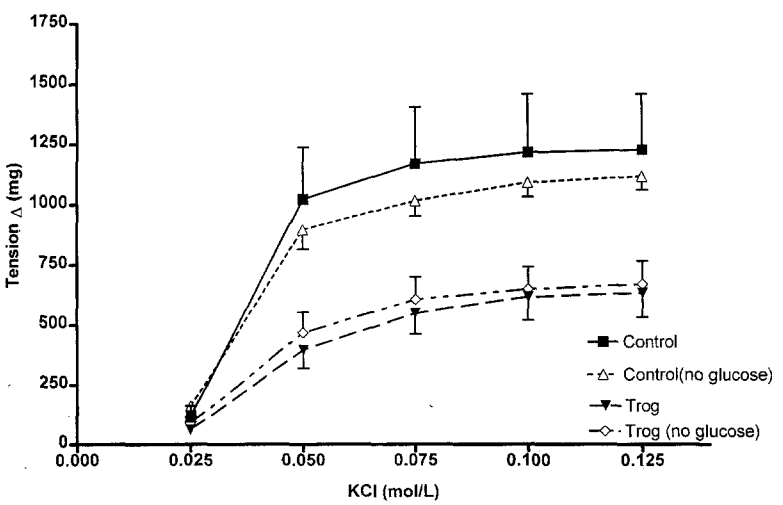

Fig 2. Preincubation (90 minutes) with 3 μmol/L Trog significantly ($P < .0001$, n = 7) attenuated the contractile response to KCl. There was no additive effect of glucose removal (n = 4 for control experiments).

Table 1. EC_{50} Values for NE- and KCl-Induced Contraction in Rat Tail Artery Rings Incubated With Troglitazone in the Presence and Absence of Glucose

Condition	KCl (mmol/L)	NE (μmol/L)
Control	39 ± 2	0.43 ± 0.08
Control without glucose	39 ± 5	0.37 ± 0.06
Troglitazone 3 μmol/L	40 ± 3	1.54 ± 0.54*
Troglitazone without glucose	42 ± 3	1.72 ± 0.58†

*P = .082 *v* control.
†P = .001 *v* control without glucose.

tension, $P < .005$ *v* troglitazone alone). However, although contractile responses to KCl in rings preincubated with 3 μmol/L troglitazone were significantly decreased (630 ± 99 *v* 1,223 ± 234 mg tension in control rings, $P < .0001$ by ANOVA), removal of glucose from the buffer had no significant effect in either the control (1,113 ± 55 mg tension) or troglitazone-treated rings (667 ± 97 mg), suggesting that glucose plays a role in NE- but not KCl-induced contractions (Fig 2).

EC_{50} calculations showed no difference in sensitivity between 10-mmol/L glucose and nonglucose controls in response to NE. Although troglitazone alone decreased the sensitivity to NE, the rightward shift of the EC_{50} was significant (P = .001) only when troglitazone was combined with the buffer containing no glucose. Sensitivity in the KCl experiments was unaffected by either troglitazone or glucose removal (Table 1).

Insulin/IGF-1–Troglitazone Interactions and Contractility

IGF-1 and troglitazone have been shown to independently and significantly attenuate vasoconstrictor responses to agonists in previous experiments in our laboratory,[12,22] and the same was shown for insulin in some but not all prior studies.[30] Troglitazone significantly reduced contractile responses to both NE and KCl (Fig 1). Experiments using 100 nmol/L insulin did not show any inhibition of contractility to either KCl or NE, but a higher dose of insulin (700 nmol/L) did attenuate vasoconstrictor responses to these agents. Additionally, coincubation with either insulin or IGF-1 did not significantly enhance the effects of troglitazone on the contractility to either KCl or NE (Table 2). Thus, in the rat tail vasculature, neither insulin nor IGF-1 had an additive effect on the troglitazone attenuation of contractility.

Table 2. Maximal Achieved Tension (mg) in Rat Tail Artery Rings Exposed to Troglitazone and Insulin/IGF-1

Condition	No.	KCl	*P*	NE	*P*
Control	5	990 ± 40		1,400 ± 85	
Insulin 100 nmol/L	5	1,180 ± 111		1,480 ± 80	
Control	5	1,188 ± 165		1,560 ± 201	
Insulin 700 nmol/L	5	1,012 ± 152	.072	1,330 ± 147	.062
Troglitazone 3 μmol/L	8	1,165 ± 115		1,856 ± 103	
Troglitazone + insulin 100 nmol/L	8	1,104 ± 121		1,837 ± 135	
Troglitazone 3 μmol/L	5	316 ± 95		805 ± 205	
Troglitazone + IGF-1 100 nmol/L	5	308 ± 126		758 ± 305	

NOTE. All results are for paired experiments on rings from the same artery.

Insulin-Troglitazone Interactions and Vascular Relaxation

There was a significant enhancement of relaxation to NaNP and Ach in rings preincubated with 3 μmol/L troglitazone (Fig 3). Results are presented as tension changes (relaxation) in response to graded doses of vasorelaxants. The maximum relaxation to NaNP was 327 ± 79 mg in control rings and 432 ± 46 mg in troglitazone-treated rings ($P < .0001$; Fig 3A), while the maximum relaxation to Ach was 251 ± 45 mg in control rings and 370 ± 70 mg in troglitazone-treated rings (P = .001; Fig 3B). Addition of 100 nmol/L insulin either to control rings or in coincubation with troglitazone had no significant additional effects on relaxation responses to NaNP or Ach (Fig 3). Troglitazone significantly (P = .025) reduced the sensitivity to NaNP but not to Ach (Table 3). As with contractility, no additional changes in EC_{50} values were observed in the presence of insulin.

Ca^{2+} Dependency

When rings were preincubated in 25 mmol/L KCl to depolarize the membrane and open calcium channels, NE-induced

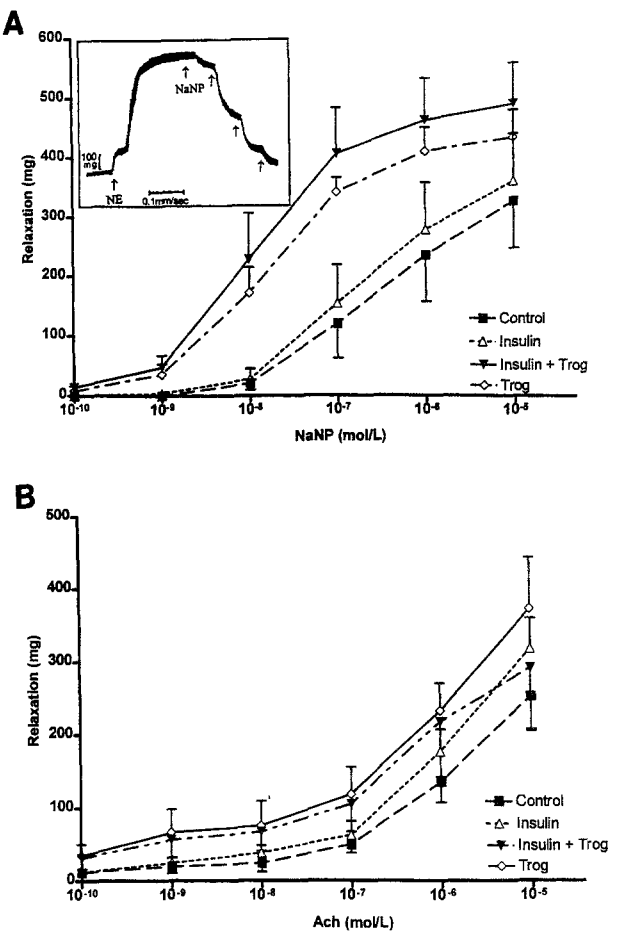

Fig 3. (A) Rat tail artery rings precontracted with NE (10^{-5} mol/L) were relaxed with cumulative doses of NaNP (inset). In vitro relaxation to NaNP was significantly increased ($P < .0001$, n = 5) by preincubation with 3 μmol/L Trog. (B) Similarly, Ach relaxation was potentiated by Trog (P = .001, n = 5). Coincubation with 100 nmol/L insulin had no additional effect.

Table 3. EC_{50} Values for NaNP and Ach Relaxation in Rings Preincubated With Troglitazone and/or Insulin

Condition	NaNP (μmol/L)	*P*	Ach (μmol/L)	*P*
Control	0.21 ± 0.06		0.28 ± 0.13	
Troglitazone alone	0.03 ± 0.01	.025*	0.19 ± 0.09	NS
Troglitazone + insulin	0.02 ± 0.007	.020*	0.16 ± 0.06	NS
Insulin alone	0.18 ± 0.05	NS	0.27 ± 0.18	NS

Abbreviation: NS, nonsignificant.
**P v* control.

contractility was attenuated ($P < .0008$) by troglitazone (maximum tension, 1,184 ± 191 *v* 698 ± 150 mg in control and troglitazone-treated rings, respectively; Fig 4).

Figure 5 illustrates the results of Ca^{2+} readdition experiments following incubation in Ca^{2+}-free buffer and initial contraction with NE (10^{-5} mol/L). Significant differences in contractility ($P = .001$ by ANOVA) were observed between the control (maximal tension, 846 ± 106 mg) and the other groups. However, there were no significant differences among these three groups (maximal tension, 540 ± 119 mg in troglitazone, 582 ± 103 in insulin, and 524 ± 142 in troglitazone + insulin groups, $P = .87$), again suggesting that troglitazone and insulin have no additive effects. In contrast, only the troglitazone-treated rings showed a significant change in sensitivity versus the controls (EC_{50}, 0.46 ± 0.03 Ca^{2+} mmol/L *v* 0.37 ± 0.02 mmol/L in controls, $P = .0186$).

Regional differences in contractile/relaxation properties exist in different vascular beds. Although specific characteristics of the rat tail artery may not be generally translated, the actions of troglitazone on VSMC Ca^{2+} may be relevant to overall vascular contractility.

DISCUSSION

The results of this investigation indicate that tail artery contractile responses to NE, but not to KCl, are modulated by glucose availability. Specifically, the contractile response to NE was attenuated in glucose-free medium, an effect exacerbated by the concurrent presence of troglitazone. It was previously reported that the contractile response of porcine carotid artery to NE, but not to KCl, was specifically dependent on the presence of glucose.[26] In another study,[27] resting tension in porcine carotid arteries was abnormally increased under glucose-free conditions, suggesting that the impaired Ca^{2+} extrusion and uptake by the plasmalemmal and sarcoplasmic reticulum Ca^{2+} pumps resulted from a lack of a normal energy source via the glycolytic pathway. Our experiments were not designed to measure basal resting tension, but increased basal tension in the absence of glucose may have offset the lower maximal contraction, thus explaining the lack of difference in EC_{50} values in control rings in the presence or absence of glucose in our experiments. We have previously shown that troglitazone and pioglitazone decrease Ca^{2+} entry via voltage-dependent L-type channels in VSMCs.[12,18] Ca^{2+} binding and/or transport is facilitated by glycolytic production of adenosine triphosphate[31] in muscular arteries such as the carotid,[26,27] the mesenteric,[32] and the rat tail artery.[26] Thus, our observation in this study showing additional attenuation of vascular contractility to NE in troglitazone-pretreated rat tail artery rings under glucose-free conditions likely represents the involvement of the above-mentioned mechanism. Levels of glucose in the muscle bath had no effect on KCl-induced contractility. This observation is in concert with a previous report suggesting that vascular attenuation achieved with pioglitazone is independent of glucose.[33] However, the present results showing not only decreased maximal tension but also significantly decreased sensitivity to NE when rings were coincubated with troglitazone in glucose-free medium indicate that glucose is important in the drug's modulation of contractile responses to this agonist.

The fact that troglitazone decreased NE-induced contractility under KCl depolarizing conditions substantiates its action on voltage-induced Ca^{2+} channels.[31,34] Results of Ca^{2+} readdition experiments showing decreased contractile responses in the presence of both troglitazone and insulin are consistent with prior data showing that both block Ca^{2+} channel currents in VSMCs.[12-18] However, in these experiments, the initial contrac-

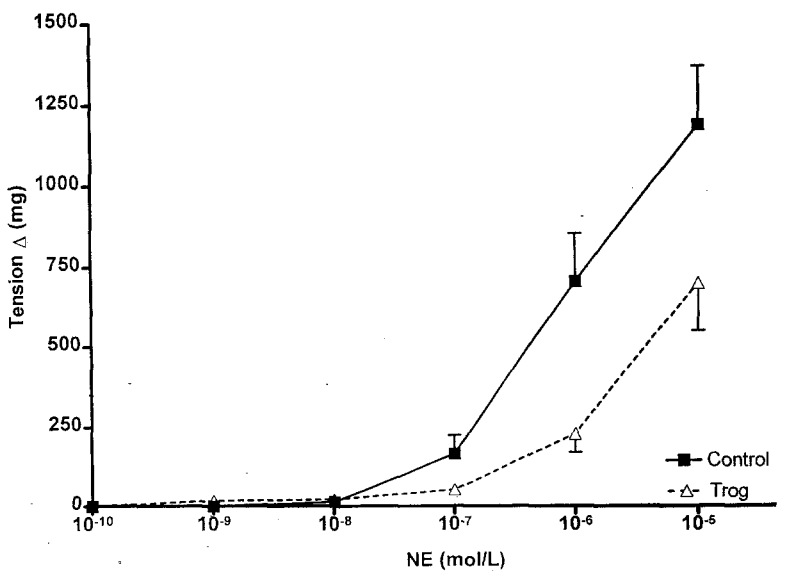

Fig 4. Preincubation with a depolarizing concentration of KCl (25 mmol/L) significantly ($P < .0008$) reduced the contractile response to NE in tail artery rings exposed to 3 μmol/L Trog (n = 6).

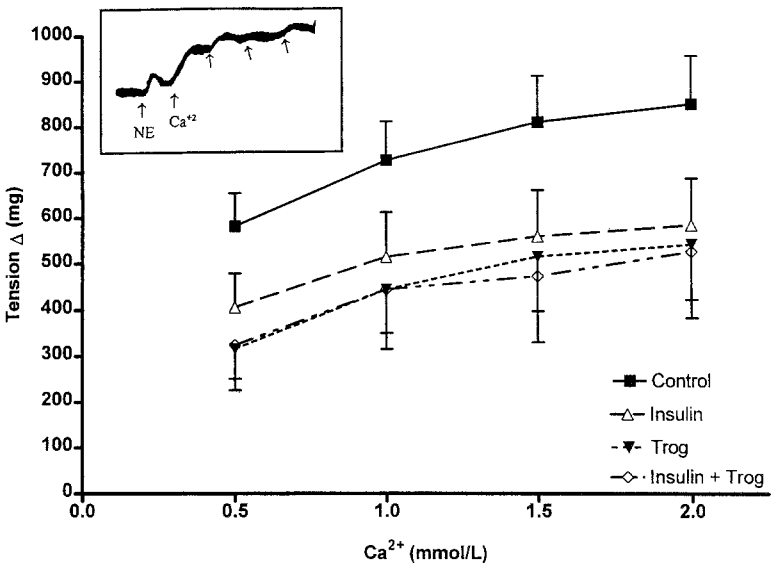

Fig 5. Rat tail artery rings were preincubated in Ca^{2+}-free buffer and precontracted with NE (10^{-5} mol/L) (inset) before incremental readdition of Ca^{2+}. Insulin (100 nmol/L), Trog (3 μmol/L), and insulin + Trog rings all responded less than control ($P = .001$ by ANOVA, n = 5), but there was no difference among the 3 groups ($P = .87$).

tion to NE was significantly lower in troglitazone-treated rings versus either control or insulin-incubated rings (data not shown), suggesting that troglitazone may decrease intracellular Ca^{2+} release as well. In this study, we also found no additive effects of troglitazone and insulin/IGF-1 in attenuating the contraction to either KCl or NE. Similarly, increased relaxation to NaNP and Ach in troglitazone-treated rings was unaffected by coincubation with insulin. While we have reported that troglitazone decreases Ca^{2+} currents at the level of the plasma membrane[12] and may also affect intracellular Ca^{2+} release (as already mentioned), both endothelium-dependent and -independent NO donors increase Ca^{2+} sequestration into the sarcoplasmic reticulum and reduce the $[Ca^{2+}]_i$ sensitivity of myosin phosphorylation/contraction.[35,36] These mechanisms may explain the additional relaxation observed with troglitazone and further support the notion that troglitazone action is independent of NO release. Since insulin/IGF-1 modulation of vascular tone is partly dependent on NO production,[19-22] collectively, these observations suggest that all three agents share at least one common mechanism, inhibition of Ca^{2+} entry, so that additive effects may not be expected, particularly if this action is greater in magnitude and may overwhelm any potential synergistic effect such as NO-mediated vascular relaxation.

Previous investigation in our laboratory has shown that metformin, like troglitazone, has vascular insulin-sensitizing properties[13] and attenuates the VSMC $[Ca^{2+}]_i$ response to vasoconstrictor agonists. The present data indicate that pharmacologic agents that share vascular-sensitizing properties, like insulin, attenuate Ca^{2+} responses to vasoconstrictive agents and may require the presence of glucose. However, the concomitant presence of insulin is neither necessary nor enhancing in this regard.

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REFERENCES

1. Baron AD, Brechtel-Hook G, Johnson A, et al: Skeletal muscle blood flow: A possible link between insulin resistance and blood pressure. Hypertension 21:129-135, 1993

2. Hall JE, Brands MW, Dixon WN, et al: Obesity-induced hypertension: Renal function and systemic hemodynamics. Hypertension 22:292-299, 1993

3. Johnstone MR, Craeger SJ, Scales KM, et al: Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. Circulation 88:2510-2516, 1993

4. Kahn A, Seidel CL, Allen JC, et al: Insulin reduces contraction and intracellular calcium concentration in vascular smooth muscle. Hypertension 22:735-742, 1993

5. Reaven GM, Lithell H, Landsberg L: Hypertension and associated metabolic abnormalities: The role of insulin resistance and the sympathetic adrenal system. N Engl J Med 334:374-381, 1996

6. Sowers JR: Insulin and insulin-like growth factor in normal and pathological cardiovascular physiology. Hypertension 29:691-699, 1997

7. Sowers JR, Epstein M: Diabetes mellitus and associated hypertension, vascular disease, and nephropathy: An update. Hypertension 26:869-879, 1995

8. Sowers JR, Sowers PS, Peuler JD: Role of insulin resistance and hyperinsulinemia in development of hypertension and atherosclerosis. J Lab Clin Med 123:647-652, 1994

9. Standley PR, Ram JL, Sowers JR: Insulin attenuation of vascular smooth muscle calcium responses in Zucker lean and obese rats. Endocrinology 133:1693-1699, 1993

10. Ghazzi MN, Perez JE, Antonucci TK, et al: Cardiac and glycemic benefits of troglitazone treatment in NIDDM. Diabetes 46:433-439, 1997

11. Ogihara T, Rakugi H, Ikegami H, et al: Enhancement of insulin sensitivity by troglitazone lowers blood pressure in diabetic hypertensives. Am J Hypertens 8:316-320, 1995

12. Song J, Walsh MF, Igwe R, et al: Troglitazone reduces contraction by inhibition of vascular smooth muscle cell Ca^{2+} currents and not endothelial nitric oxide production. Diabetes 46:659-664, 1997

13. Dominguez LJ, Davidoff AJ, Srinivas PR, et al: Effects of

metformin on tyrosine kinase activity, glucose transport, and intracellular calcium in rat vascular smooth muscle. Endocrinology 137:113-121, 1996

14. Kahn AM, Song T: Effects of insulin on vascular smooth muscle contraction, in Sowers JR (ed): Contemporary Endocrinology: Endocrinology of the Vasculature. Totowa, NJ, Humana, 1996, pp 215-223

15. Saito F, Hori MT, Fittingott M, et al: Insulin attenuates agonist-mediated calcium mobilization in cultured rat vascular smooth muscle cells. J Clin Invest 92:1161-1168, 1993

16. Standley PR, Zhang F, Ram JL, et al: Insulin attenuates vasopressin-induced calcium transient and voltage-dependent calcium response in rat vascular smooth muscle cells. J Clin Invest 88:1230-1236, 1991

17. Pershadsingh HA, Szollosi J, Benson S, et al: Effects of ciglitazone on blood pressure and intracellular calcium metabolism. Hypertension 21:1020-1023, 1993

18. Zhang F, Sowers JR, Ram JL, et al: Effects of pioglitazone on L-type calcium channels in vascular smooth muscle. Hypertension 24:170-175, 1994

19. Baron AD, Steinberg HO: Vascular actions of insulin in health and disease, in Sowers JR (ed): Contemporary Endocrinology: Endocrinology of the Vasculature. Totowa, NJ, Humana, 1996, pp 95-107

20. Scherrer U, Randin D, Vollenweider P, et al: Nitric oxide release accounts for insulin's vascular effects in humans. J Clin Invest 94:2511-2515, 1994

21. Steinberg HO, Brechtel G, Johnson A, et al: Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent: A novel action of insulin to increase nitric oxide release. J Clin Invest 94:1172-1179, 1994

22. Walsh MF, Barazi M, Pete G, et al: Insulin-like growth factor 1 diminishes in vivo and in vitro vascular contractility: Role of vascular nitric oxide. Endocrinology 137:1798-1803, 1996

23. Dominguez LJ, Peuler JD, Sowers JR: Endocrine regulation of vascular smooth muscle intermediary metabolism, in Sowers JR (ed): Contemporary Endocrinology: Endocrinology of the Vasculature. Totowa, NJ, Humana, 1996, pp 325-339

24. Saltiel AR, Olefsky JM: Thiazolidinediones in the treatment of insulin resistance and type II diabetes. Diabetes 45:1661-1669, 1996

25. Sowers JR: Effects of insulin and IGF-1 on vascular smooth muscle glucose and cation metabolism. Diabetes 45:47-51, 1996

26. Adams GR, Dillon PF: Glucose dependence of sequential norepinephrine contractions of vascular smooth muscle. Blood Vessels 26:77-83, 1989

27. Zhang C, Paul RJ: Excitation-contraction coupling and relaxation in porcine carotid arteries are specifically dependent on glucose. Am J Physiol 267:H1996-H2004, 1994

28. Saida K, van Breeman C: Characteristics of norepinephrine-sensitive Ca^{2+} store in vascular smooth muscle. Blood Vessels 21:43-52, 1984

29. Peuler JD, Johnson BA, Phare SM, et al: Sex-specific effects of an insulin secretagogue in stroke-prone hypertensive rats. Hypertension 22:214-220, 1993

30. Kotchen TA, Zhang HY, Reddy S, et al: Effect of pioglitazone on vascular reactivity in vivo and in vitro. Am J Physiol 270:R660-R666, 1996

31. Khan SA, Mathews WR, Meisheri KD: Role of calcium-activated K^+ channels in vasodilation induced by nitroglycerin, acetylcholine and nitric oxide. J Pharmacol Exp Ther 267:1327-1335, 1993

32. Lundholm L, Mohme-Lundholm E: Dissociation of contraction and stimulation of lactic acid production in experiments on smooth muscle under anaerobic conditions. Acta Physiol Scand 57:111-124, 1963

33. Buchanan TA, Meehan WP, Jeng YY, et al: Blood pressure lowering by pioglitazone. Evidence for a direct vascular effect. J Clin Invest 96:354-360, 1995

34. Clapp LH, Gurney AM: Modulation of calcium movements by nitroprusside in isolated vascular smooth muscle cells. Pflugers Arch 418:462-470, 1991

35. Chen XL, Rembold CM: Nitroglycerin relaxes rat artery primarily by lowering Ca^{2+} sensitivity and partially by repolarization. Am J Physiol 271:H962-H968, 1996

36. McDaniel NL, Chen XL, Singer HA, et al: Nitrovasodilators relax arterial smooth muscle by decreasing $[Ca^{2+}]^i$ and uncoupling stress from myosin phosphorylation. Am J Physiol 263:C461-C467, 1992