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PRELIMINARY REPORT

Effects of an Intracellular Ca^{2+} Chelator on Insulin Resistance and Hypertension in High–Fat-Fed Rats and Spontaneously Hypertensive Rats

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We explored the possibility that a sustained elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) may be a cellular abnormality common to both insulin resistance and hypertension. In high-fat diet (HFD) fed rats, the steady-state glucose infusion rate (GIR) during the euglycemic hyperinsulinemic clamp was reduced by 40% ($P < .05$) and mean arterial pressure (MAP) was elevated by 20 mm Hg ($P < .01$) in comparison to the normal chow-fed rats. Intravenous injection of 5,5′-dimethyl derivative of bis(*o*-aminophenoxy)ethane-N,N,N′,N′ tetraacetic acetoxymethyl ester (dimethyl-BAPTA/AM), an effective intracellular Ca^{2+} chelator, 90 minutes before the clamp not only restored about 50% of the reduced GIR, but also normalized MAP in the HFD rats. The chelator injection also significantly increased GIR by 25% ($P < .01$) and reduced MAP about 30 mm Hg ($P < .01$) in the spontaneously hypertensive rats (SHR). In addition, we have recently shown in the HFD rats that an injection of dimethyl-BAPTA/AM normalizes elevated $[Ca^{2+}]_i$ in adipocytes. These results together demonstrate that lowering $[Ca^{2+}]_i$ simultaneously ameliorates both insulin resistance and hypertension and provide presumptive evidence that sustained high levels of $[Ca^{2+}]_i$ may play a common pathophysiologic role in these 2 diseases.

THERE IS A frequent association of insulin resistance and hypertension.[1] Moreover, a direct correlation between the degree of insulin resistance and the severity of hypertension has been reported.[2] Thus, it has been postulated that insulin resistance and hypertension are merely different tissue manifestations of the same underlying cellular abnormality and elevated intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is a pathophysiologic link between these 2 morbid conditions.[3-6]

Intracellular Ca^{2+} plays a key role in signal transduction to control diverse cellular processes and thus disturbed intracellular Ca^{2+} homeostasis can readily impair many cellular functions. In fact, the results implicating a sustained elevation in $[Ca^{2+}]_i$ as a major cause of cellular dysfunction have been reported in a variety of the cells, including aged neurons[7] and glucose-unresponsive β cells.[8] Recently, we have demonstrated that $[Ca^{2+}]_i$ is significantly increased in adipocytes of the rats with insulin resistance induced by HFD.[9] In these rats, injection of dimethyl-BAPTA/AM, an effective intracellular Ca^{2+} chelator, normalizes elevated $[Ca^{2+}]_i$ and ameliorates insulin resistance. In an extension of this result, the present study was planned to test the hypothesis that a sustained elevation of $[Ca^{2+}]_i$ may be a common cellular abnormality causing frequent clinical coexistence of insulin resistance and hypertension.[3-6] To address this possibility, we investigated whether an injection of dimethyl-BAPTA/AM could ameliorate both insulin resistance and hypertension in 2 different animal models, the high-fat diet (HFD) rats and the spontaneously hypertensive rats (SHR).

MATERIALS AND METHODS

Male Sprague-Dawley (SD) rats and SHR from Harlan Sprague Dawley (Indianapolis, IN), were bred and maintained in a specific pathogen-free environment. Six-week-old SD rats were fed either HFD formulated as previously described[9] or the standard rat chow for 3 to 4 weeks before experiments. The SHRs used in the experiments were aged 12 to 14 weeks, a period when the rats have fixed hypertension.

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Table 1. Effects of Dimethyl-BAPTA/AM in the HFD- and Chow-Fed Rats

	HFD + Chelator	HFD + Vehicle	Chow + Chelator	Chow + Vehicle
No.	25	26	7	15
BW (g)	384 ± 8	378 ± 6	340 ± 20	356 ± 14
Basal glucose (mg/dL)	97 ± 2	98 ± 2	92 ± 2	98 ± 2
Basal insulin (μU/mL)	34 ± 3	33 ± 4	17 ± 2	31 ± 6
SS insulin (μU/mL)	168 ± 11	150 ± 10	201 ± 18	148 ± 10
GIR (mg · kg^{-1} · min^{-1})	16.1 ± 0.3*†	12.1 ± 0.3*	20.7 ± 0.5	20.5 ± 0.8
Basal MAP (mm Hg)	124 ± 2*	121 ± 2*	101 ± 4	100 ± 1
Postclamp MAP (mm Hg)	105 ± 2‡	118 ± 1*	89 ± 3*‡	101 ± 1

NOTE. Dimethyl-BAPTA/AM (6 mg/kg in 250 μL DMSO 1:3 diluted with distilled water) or the same volume of the vehicle DMSO was injected 90 minutes prior to the start of euglycemic hyperinsulinemic (6 mU · kg^{-1} · min^{-1}) clamps. The whole body insulin sensitivity was represented by the steady state GIR during the clamps. MAP was measured at basal state and immediately after the clamp.

Abbreviation: SS, steady state.

*$P < .05$ *v* chow + vehicle group; †$P < .05$ *v* HFD + vehicle group; ‡$P < .05$ *v* basal MAP in the same group.

Euglycemic hyperinsulinemic clamps were performed on conscious rats as previously described.[9] Briefly, after an overnight fast, each animal underwent placement of a tail artery catheter and 2 tail vein catheters and were then stabilized for at least 2 hours. After basal blood sampling, mean arterial pressure (MAP) (ie, basal MAP) was measured directly through a tail artery catheter using a pressure transducer and physiograph (Harvard Apparatus, Kent, England). At −90 minutes, dimethyl-BAPTA/AM (TEFLABS, Austin, TX; 6 mg/kg in 250 μL dimethyl sulfoxide (DMSO) 1:3 diluted with distilled water) or the same volume of vehicle DMSO was injected through a tail vein catheter. Human insulin (Humulin, regular; Daewoong Lilly, Korea) was infused from time 0 to 120 minutes at 6 mU/kg/min in the HFD and the chow rats or at 4 mU/kg/min in the SHR. Plasma blood glucose concentration was measured every 10 minutes and clamped at 100 mg/dL by a variable glucose infusion. Average glucose infusion rate (GIR) during the final 30 minutes of the glucose clamp (steady state) was used as an estimate of whole-body insulin sensitivity. Blood samples for the steady-state plasma insulin concentration were taken at time 90, 100, 110, and 120 minutes. Immediately after the last blood sampling, MAP (ie, postclamp MAP) was measured while insulin and glucose infusions were continued.

To examine the dose-dependent effects of dimethyl-BAPTA/AM on insulin resistance and hypertension, in addition to 6 mg/kg, 3 mg/kg or 9 mg/kg of the chelator was injected in the HFD rats.

Plasma glucose concentrations were measured by a glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Basal and steady-state plasma insulin concentrations were measured using rat (Linco Research, St Charles, MO) and human insulin (DPC, Los Angeles, CA) radioimmunoassay kits.

Values are presented as mean ± SE. Student's paired and unpaired *t* tests were performed as indicated when 2 groups were compared. Analysis of variance followed by the Bonferroni test was performed to compare more than 2 groups. Differences were considered significant at $P < .05$.

RESULTS

Effects of Dimethyl-BAPTA/AM in the HFD Rats

Basal concentrations of plasma glucose ($P > .6$) and insulin ($P > .6$) and body weight (BW) ($P > .1$) were not significantly different between the HFD rats and the chow rats (Table 1). During the hyperinsulinemic clamps, there was no statistically significant difference in the steady-state insulin concentrations among 4 groups of the HFD rats and the chow rats injected with dimethyl-BAPTA/AM (6 mg/kg) or the vehicle ($P > .07$). However, the steady-state GIR was significantly lower by 40% in the vehicle-injected HFD rats than the vehicle-injected chow rats ($P < .05$). In the HFD rats, the chelator injection restored approximately 50% of this reduction in GIR ($P < .01$), whereas it did not affect GIR in the chow rats ($P > .8$). We have previously demonstrated that basal $[Ca^{2+}]_i$ of adipocytes is significantly elevated in the HFD rats in comparison to that in the normal chow rats (145 ± 11 *v* 112 ± 9 nmol/L; $P < .05$), and it is reduced by the same maneuver as used in the present study to the level, which is indistinguishable from the normal level (127 ± 11 *v* 112 ± 9 nmol/L; $P > .2$).[9]

Mild degree hypertension was induced by the HFD feeding: basal MAP was significantly higher by about 20 mm Hg in the HFD rats than the chow rats ($P < .01$; Table 1). In the vehicle-injected groups, postclamp MAP was almost identical to basal MAP ($P > .1$), indicating that the hyperinsulinemic clamp itself did not affect MAP. On the contrary, an injection of dimethyl-BAPTA/AM significantly reduced MAP from 124 ± 2 to 105 ± 2 mm Hg ($P < .01$) in the HFD rats and from 101 ± 4 to 89 ± 3 mm Hg ($P < .01$) in the chow rats.

Dose-dependent effects of dimethyl-BAPTA/AM on the HFD-induced insulin resistance and hypertension are shown in Table 2. At a dose of 3 mg/kg, the chelator did not affect the steady-state GIR, but significantly reduced MAP; at 6 mg/kg, it significantly improved GIR and normalized MAP; increasing the dose to 9 mg/kg changed neither GIR nor MAP any further.

Effects of Dimethyl-BAPTA/AM in the SHR

In the SHR, there was no significant difference in BW ($P > .7$), basal plasma glucose ($P > .5$) and insulin ($P > .9$) concentrations, and basal MAP ($P > .2$) between the vehicle- and the chelator-injected groups (Table 3). Although insulin infusion increased plasma insulin concentrations to a similar level ($P > .9$) in these 2 groups, the steady-state GIR was significantly higher by 25% in the chelator-injected than the vehicle-injected SHR ($P < .01$). In the chelator-injected SHR, postclamp MAP was significantly lower than basal MAP

Table 2. Dose-Dependent Effects of Dimethyl-BAPTA/AM on Insulin Resistance and Hypertension in the HFD Rats

Dose (mg/kg)	No.	GIR ($mg \cdot kg^{-1} min^{-1}$)	Basal MAP (mm Hg)	Postclamp MAP (mm Hg)
0	26	12.1 ± 0.3	121 ± 2	118 ± 1
3	7	12.1 ± 0.6	120 ± 2	109 ± 4*
6	25	16.1 ± 0.3*	124 ± 2	105 ± 2*
9	8	16.2 ± 0.4*	123 ± 1	107 ± 2*

NOTE. The whole body insulin sensitivity is represented by the steady state GIR during euglycemic hyperinsulinemic (6 $mU \cdot kg^{-1} \cdot min^{-1}$) clamps. Dimethyl-BAPTA/AM (3, 6, or 9 mg/kg in 250 μL DMSO 1:3 diluted with distilled water) or the same volume of the vehicle DMSO was injected 90 minutes prior to the start of glucose clamp. MAP was measured at basal state and immediately after the clamp.

*$P < .05$ *v* the vehicle (0 mg/kg) group.

(124 ± 4 *v* 154 ± 5 mm Hg; $P < .01$), whereas the 2 measurements were almost identical ($P > .9$) in the vehicle-injected SHR.

DISCUSSION

Dimethyl-BAPTA/AM, as well as other BAPTA/AM analogues, which have been widely applied to buffer $[Ca^{2+}]_i$,[10-12] readily enters into the cells and is hydrolyzed by cellular esterases to yield Ca^{2+} binding forms.[11] Among them, dimethyl-BAPTA binds to Ca^{2+} with the highest affinity (k_d = 40 nmol/L).[11] Hence, it can function as an intracellular Ca^{2+} buffer, in addition to endogenous buffer, such as calmodulin (k_d = 10 μmol/L), and stabilize $[Ca^{2+}]_i$ at or lower than the physiologic level. In our previous study, an injection of dimethyl-BAPTA/AM in the HFD rats normalizes elevated $[Ca^{2+}]_i$ in adipocytes.[9] Thus, although $[Ca^{2+}]_i$ was not directly measured in the SHR, the same maneuver might also reduce $[Ca^{2+}]_i$ in these rats.

The high levels of $[Ca^{2+}]_i$ have been observed in the skeletal muscle cells and adipocytes of insulin-resistant subjects.[13,14] suggesting that a sustained elevation of $[Ca^{2+}]_i$ in insulin target cells may diminish cellular responsiveness to insulin. In the current study, pretreatment with dimethyl-BAPTA/AM increased the steady-state GIR during glucose clamps in the HFD rats and SHR. We have previously shown in the HFD rats that the increment in steady-state GIR by the chelator injection is mainly, if not totally, due to enhancement of the insulin-mediated glucose uptake by peripheral tissues, and this effect is not associated with augmentation of blood flow to these tissues.[9] The same results were obtained in the assessments of the blood flow rate and glucose uptake by peripheral tissues during the glucose clamp in the SHR (data not shown). Therefore, the increased steady-state GIR by the chelator in the HFD rats (Table 1) and the SHR (Table 3) was likely due to amelioration of insulin resistance in peripheral insulin target cells.

Table 3. Effects of Dimethyl-BAPTA/AM in the SHR

	Chelator	Vehicle
No.	10	8
BW (g)	298 ± 8	302 ± 10
Basal glucose (mg/dL)	86 ± 2	89 ± 4
Basal insulin (μU/mL)	26 ± 4	26 ± 5
SS insulin (μU/mL)	120 ± 11	118 ± 12
GIR ($mg \cdot kg^{-1} \cdot min^{-1}$)	25.0 ± 1.0*	19.9 ± 1.1
Basal MAP (mm Hg)	154 ± 5	142 ± 8
Postclamp MAP (mm Hg)	124 ± 4†	143 ± 8

NOTE. Dimethyl-BAPTA/AM (6 mg/kg in 250 μL DMSO 1:3 diluted with distilled water) or the same volume of the vehicle DMSO was injected 90 minutes prior to the start of euglycemic hyperinsulinemic (4 $mU \cdot kg^{-1} \cdot min^{-1}$) clamps. The whole body insulin sensitivity was represented by the steady state GIR during the clamps. MAP was measured at basal state and immediately after the clamp.

Abbreviation: SS, steady-state.

*$P < .05$ *v* vehicle group, †$P < .05$ *v* basal MAP in the same group.

In the vascular smooth muscle cells (VSMC), $[Ca^{2+}]_i$ is a major determinant of tension development with increased $[Ca^{2+}]_i$ leading to an elevation of arteriolar resistance. An elevated $[Ca^{2+}]_i$ has been observed in hypertensive VSMC.[15,16] Therefore, it is now relatively well recognized that increased $[Ca^{2+}]_i$ in VSMC leads to high vascular resistance and hence plays a primary role in development of hypertension. This notion is further supported by reduction of MAP with dimethyl-BAPTA/AM shown in Tables 1 and 3.

In the dose-dependent effects of dimethyl-BAPTA/AM on GIR and MAP (Table 2), increasing the dose above 6 mg/kg did not change either GIR or MAP any further: there might be a limitation upon the maximum intracellular concentration of Ca^{2+} chelator achieved, which may be due to finite intracellular esterase activity, active extrusion of the compound, and/or its metabolism into inactive form(s).[10,12] However, the reason the MAP was modulated at lower doses of the chelator than the GIR was not clear in the present study. It is possibly by the differences among tissues in distribution of the chelator, esterase activities, and/or threshold $[Ca^{2+}]_i$ maintaining insulin resistance and hypertension. With respect to the last possibility, it seems noteworthy that whereas dimethyl-BAPTA/AM increases GIR, as well as reduces MAP in the SHR, the same dose of BAPTA/AM, which has lower Ca^{2+} affinity (k_d = 107 nmol/L)[11] than that of dimethyl-BAPTA, only reduces MAP without affecting GIR.[17]

Several studies[3-6] have suggested the possibility that a sustained elevation of $[Ca^{2+}]_i$ may be a common cellular abnormality causing insulin resistance and hypertension. In the present study, a single injection of intracellular Ca^{2+} chelator simultaneously ameliorates both insulin resistance and hypertension in the HFD rats and the SHR. Although other possibilities cannot be excluded, sustained high levels of $[Ca^{2+}]_i$ may play a common pathophysiologic role in these 2 diseases.

REFERENCES

1. Reaven GM: Insulin resistance, hyperinsulinemia and hypertriglyceridemia in the etiology and clinical course of hypertension. Am J Med 90:7-12S, 1991

2. Defronzo R, Ferrannini E: Insulin resistance: A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia and atherosclerotic cardiovascular disease. Diabetes Care 14:173-194, 1991

3. Zemel MB: Nutritional and endocrine modulation of intracellular calcium: Implications in obesity, insulin resistance and hypertension. Mol Cell Biochem 188:129-136, 1998

4. Resnick LM: Cellular ions in hypertension, insulin resistance, obesity and diabetes: A unifying theme. J Am Soc Nephrol 3:S78-S85, 1992

5. Resnick LM: Calcium metabolism in hypertension and allied metabolic disorders. Diabetes Care 14:505-520, 1991

6. Levy J, Gavin JR, Sowers JR: Diabetes mellitus: A disease of abnormal cellular calcium metabolism? Am J Med 96:260-273, 1994

7. Verkhratsky A, Toescu EC: Calcium and neuronal ageing. Trends Neurosci 21:2-7, 1998

8. Minami K, Yokokura M, Ishizuka N, et al: Normalization of intracellular Ca^{2+} induces a glucose-responsive state in glucose-unresponsive β-cells. J Biol Chem 277:25277-25282, 2002

9. Jang YJ, Ryu HJ, Choi YD, et al: Improvement of insulin sensitivity by chelation of intracellular Ca^{2+} in high-fat fed rats. Metabolism 51:912-918, 2002

10. Pertusa JAG, Sanchez-Anderes JV, Martin F, et al: Effects of calcium buffering on glucose-induced insulin release in mouse pancreatic islets: An approximation to the calcium sensor. J Physiol 520:473-483, 1999

11. Tsien RY: New calcium indicators and buffers with high selectivity against magnesium and protons: Design, synthesis, and properties of prototype structures. Biochemistry 19:2396-2402, 1990

12. Tsien RY: A non-disruptive technique for loading calcium buffers and indicators into cells. Nature 290:527-528, 1981

13. Draznin B, Sussman KE, Eckel RH, et al: Possible role of cytosolic free calcium concentrations in mediating insulin resistance of obesity and hyperinsulinemia. J Clin Invest 82:1848-1852, 1988

14. Zemel MB, Kim JH, Woychik RP, et al: Agouti regulation of intracellular calcium: Role in the insulin resistance of viable yellow mice. Proc Natl Acad Sci USA 92:4733-4737, 1995

15. Touyz RM, Schiffrin EL: Insulin-induced Ca^{2+} transport is altered in vascular smooth muscle cells of spontaneously hypertensive rats. Hypertension 23:931-935, 1994

16. Shehin SE, Sowers JR, Zemel MB: Impaired vacular smooth muscle ^{45}Ca efflux and hypertension in Zucker obese rats. J Vasc Med Biol 1:278-282, 1989

17. Jang YJ, Ryu HJ, Leem CH, et al: Chelation of intracellular calcium improves both insulin sensitivity and blood pressure in spontaneously hypertensive rats. Diabetes 49:A284-A285, 2000 (suppl 1)