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ORIGINAL ARTICLE

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Inhibitory effect of insulin on vasopressin-induced intracellular calcium response is blunted in hyperinsulinemic hypertensive patients: role of membrane fatty acid composition

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Abstract Impaired insulin-mediated vasodilation has been implicated in hypertension that is associated with the metabolic syndrome. The aim of this study was to determine whether an abnormality in membrane fatty acid composition was related to a weakening of insulin's inhibitory effect on agonist-stimulated intracellular free calcium elevation. Mild to moderate hypertensive patients ($n = 27$) and normotensive controls ($n = 11$) were studied. Hypertensive patients were divided into normoinsulinemic patients ($n = 14$) and hyperinsulinemic patients ($n = 13$) according to the area under the curve of plasma insulin concentrations during a 75-g oral glucose tolerance test. Nonstimulated and arginine-vasopressin (AVP) (1 μmol/l)-stimulated intraplatelet free calcium concentrations ($p[Ca^{2+}]_i$) were measured with or without insulin (100 μU/ml) preincubation. Platelet membrane fatty acid composition, intraerythrocyte sodium content, and the ouabain-sensitive sodium efflux rate constant (K_{os}) of erythrocytes were also determined. Insulin preincubation reduced AVP-stimulated $p[Ca^{2+}]_i$ elevation in both normotensive controls and hypertensive patients. The inhibitory effect of insulin on AVP-stimulated elevation of $p[Ca^{2+}]_i$ (%Inhibition) was significantly ($P < 0.05$) blunted in hyperinsulinemic hypertensive patients (9.7% ± 2.4%) as compared to normoinsulinemic hypertensive patients (17.4% ± 2.7%) and normotensive controls (16.9% ± 1.7%). In hypertensive patients, the %Inhibition was correlated negatively with saturated fatty acids (SFA) ($r = –0.51$, $P < 0.05$) and systolic blood pressure ($r = –0.44$, $P < 0.05$), and correlated positively with membrane polyunsaturated fatty acids (PUFA) ($r = 0.53$, $P < 0.01$) and K_{os} ($r = 0.53$, $P < 0.005$). Multiple regression analysis showed that SFA, PUFA, and K_{os} were the significant variables for %Inhibition. These findings indicate that an increase in SFA and a decrease in PUFA may cause insulin insensitivity in cellular calcium and sodium handling in hypertension with hyperinsulinemia.

Key words Hypertension · Insulin resistance · Membrane fatty acid · Intracellular calcium · Stearic acid

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Introduction

Epidemiological data indicate that there is an association between obesity, dyslipidemia, glucose intolerance, and hypertension.[1,2] Insulin resistance and hyperinsulinemia have been implicated in the pathogenesis of this multiple atherogenic risk factor syndrome, which has recently been labeled "the metabolic syndrome."[3,4]

Previous studies have shown that insulin causes vasodilation by endothelium-dependent and independent mechanisms.[5–8] For insulin-mediated and endothelium-independent vasodilation, the inhibition of agonist-stimulated contraction and intracellular calcium responses by insulin has been suggested as playing an important role.[9–13] Insulin is thought to lower intracellular free calcium by several mechanisms. One mechanism involves activation of Ca^{2+}-ATPase.[14] Another acts through activation of Na^+/K^+-ATPase, which decreases intracellular sodium content.[10,15,16] Reduced intracellular sodium content suppresses sodium–calcium exchange, leading to membrane hyperpolarization, inactivation of the voltage-dependent calcium channel and, finally, to decreased intracellular calcium. In hypertensive patients, insulin's inhibitory effects on the agonist-induced calcium, pH, and aggregatory responses have been shown to be blunted.[17] These findings suggest that insulin resistance in intracellular free calcium regulation might be one of the mechanisms that fosters hypertension in patients with the metabolic syndrome.

Modifications in the membrane fatty acid composition can alter many cellular function.[18] The properties of the

insulin receptor are also affected by the membrane lipid environment in several cell types.[19–25] Moreover, the fatty acid composition of skeletal muscle membrane phospholipid has been reported to be associated with insulin sensitivity in humans.[26,27] These findings suggest that an abnormality in membrane lipid composition may be one of the underlying mechanisms of insulin resistance. However, the exact relationship between the alteration of membrane fatty acid composition and the decreased intracellular calcium-lowering effect of insulin has yet to be elucidated. The present study was undertaken to test whether insulin's inhibitory effect on agonist-stimulated intraplatelet free calcium responses is related to membrane fatty acid composition in patients with essential hypertension. The relationship between the insulin-mediated intracellular calcium-lowering effect and Na^+/K^+-ATPase activity was also studied.

Materials and methods

Subjects

Twenty-seven outpatients, aged 38–68 years (mean age 52.2 years) with mild to moderate essential hypertension and 11 normotensive controls (mostly medical staff) were studied at the Kobe University Hospital. The blood pressures of the subjects in the sitting position were measured twice using a standard mercury sphygmomanometer. The diagnosis of hypertension was confirmed if the office systolic blood pressure was greater than 140 mmHg or the diastolic blood pressure was greater than 90 mmHg on at least two separate visits. The patients had to have been either never treated or had to have ceased taking their antihypertensive and antihyperlipidemic medications at least 4 weeks prior to study entry. Secondary causes of hypertension were ruled out by history, physical examination, and the usual screening tests, including urinalysis and blood chemistry. Blood samples were taken at 09:00 h after an overnight fast, to determine serum blood chemistry, intraplatelet free calcium concentration, platelet fatty acid composition, intraerythrocyte sodium content, and the erythrocyte ouabain-sensitive sodium efflux rate constant. After initial blood samples were drawn, a 75-g oral glucose tolerance test (OGTT) was done to examine insulin sensitivity and secretion. Area under the curve for insulin (AUC insulin: μU/ml per hour) was determined by the trapezium rule for plasma insulin concentrations at 0, 30, 60, and 120 min, and was used to evaluate hyperinsulinemia. The difference in the plasma insulin concentration divided by the difference in glucose concentration at 30 min (Δinsulin/Δglucose) during the 75-g OGTT was the calculation used for insulin secretion at the early stage. Using the homeostasis model assessment (HOMA),[28] insulin resistance (HOMA-R) was calculated as fasting plasma glucose (mg/dl) × fasting insulin (μU/ml)/405, and insulin secretion (HOMA β-cell) was calculated as 360 × fasting insulin (μU/ml)/[fasting plasma glucose (mg/dl) – 63]. The insulin sensitivity index during 75-g OGTT was used to evaluate peripheral insulin sensitivity and was calculated as [glucose uptake rate/mean glucose concentration/log(mean insulin concentration)]. The glucose uptake rate was calculated as [75 000 mg/120 + (fasting plasma glucose concentration – plasma glucose at 120 min) × 0.19 × body weight/120].[29] All subjects gave their informed consent to participate in the study. The study protocol was approved by the Human Ethics Committee of Kobe University Graduate School of Medicine.

Platelet cytosolic free calcium concentration

The cytosolic free calcium concentrations in the platelets ($p[Ca^{2+}]_i$) were measured as previously described.[30] Briefly, 2 ml of platelet-rich plasma was incubated with 5 μM Fura-2/acetoxy-methylester (AM) (Dojin, Kumamoto, Japan) for 30 min, and the platelets were gel-filtered. Fluorescence was measured at an emission wavelength of 510 nm with a sequential excitation wavelength of 340 and 385 nm on a Hitachi fluorescent spectrophotometer (F-2000; Tokyo, Japan). The $p[Ca^{2+}]_i$ was calculated as previously described.[30,31] Nonstimulated and arginine-vasopressin (AVP; Sigma-Aldrich Japan, Tokyo, Japan) (1 μmol/l)-stimulated intraplatelet free calcium concentrations were measured with and without human insulin (Penfill R; Novo Nordisk Phama, Tokyo, Japan) (100 μU/ml) preincubation for 5 min. We selected AVP as an agonist because AVP plays an important role in the regulation of blood pressure[32] and AVP-stimulated $p[Ca^{2+}]_i$ showed the most stable responses in a preliminary study. The effect of various concentrations of AVP on stimulating intraplatelet free calcium responses had been examined in a preliminary study, and the concentration of AVP with a submaximal response was selected. One μmol/l of AVP was also used in a previous study from another laboratory.[9] The percentage of the change in the AVP-stimulated calcium response with insulin to the AVP-stimulated calcium response without insulin was calculated and called %Inhibition (Fig. 1).

Intraerythrocyte sodium concentration and Na^+/K^+-ATPase activity

Intraerythrocyte sodium concentration (R-Na) was determined by a previously reported method with a slight modification.[33] Briefly, an aliquot of venous blood (60 μl) was injected into a microhematocrit capillary tube (75 mm length) and centrifuged at 11 000 rpm for 5 min at room temperature. After determining the hematocrit, the tube was cut at the boundary between the packed cells and the plasma. The packed erythrocytes were then diluted in 1.5 ml of diluted lithium solution, and the sodium concentration of the hemolysate was determined by flame photometry.

Na^+/K^+-ATPase activity was evaluated by the erythrocyte ouabain-sensitive sodium efflux rate constant (K_{os}).[33] The rate of ouabain-sensitive sodium efflux of sodium ($mmol/l \cdot cells \cdot h^{-1}$) was measured as the increase in the R-Na of whole blood during incubation with or without 10^{-4} mol/l of ouabain for 120 min at 37°C, which totally in-

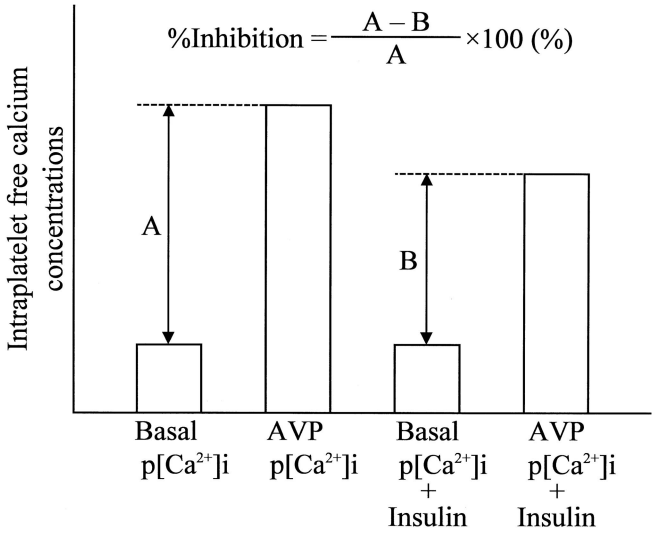

Fig. 1. Effect of insulin preincubation on basal and arginine-vasopressin (*AVP*)-stimulated intraplatelet free calcium concentration. *A* indicates AVP-induced increae in intraplatelet free calcium concentration without insulin preincubation. *B* indicates AVP-induced increase in intraplatelet free calcium concentration with insulin preincubation. A percentage of the change in the AVP-stimulated calcium response with insulin preincubation to the AVP-stimulated calcium response without insulin preincubation was calculated as above, and called %Inhibition. *Basal* $p[Ca^{2+}]_i$, nonstimulated intraplatelet free calcium concentration without insulin preincubation; *AVP* $p[Ca^{2+}]_i$, arginine-vasopressin (AVP: 1 μmol/l)-stimulated intraplatelet free calcium concentration without insulin preincubation; *Basal* $p[Ca^{2+}]_i$ + *insulin*, intraplatelet free calcium concentration with insulin preincubation (100 μU/ml for 5 min); *AVP* $p[Ca^{2+}]_i$ + *insulin*, AVP-stimulated intraplatelet free calcium concentration with insulin preincubation

hibits the ouabain-sensitive sodium efflux. The K_{os} (/h) was calculated as the ratio of the efflux to the R-Na before the addition of the ouabain.

Platelet membrane fatty acid composition

To measure the platelet fatty acid composition, platelet-rich plasma was washed three times with 0.9% NaCl. All solvents used for the extraction and derivatization of the fatty acid components of platelets contained 0.01% butylated hydroxytoluene as an antioxidant. Platelet membrane total lipids were extracted from the washed platelets according to the method of Bligh and Dyer.[34] After drying by evaporation under a nitrogen stream, the samples were hydrolyzed and methylesterified with a 5% hydrogen chloride methanol solution for 1 h at 120°C. Fatty acid analysis was performed by a gas chromatography (QP-1000EX; Shimazu, Kyoto, Japan) fitted with a capillary column as previously described.[35] Peaks were identified in comparison with the known standards and quantitative determinations were carried out by measuring the mass chromatogram peak areas. Fatty acid fractions, including total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), and total polyunsaturated fatty acids (PUFA), were expressed as a percentage compared with the total fatty acids.

Statistical analysis

Values are expressed as mean ± SEM. Differences between the mean values were tested by unpaired Student's *t*-test or analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD). Correlations of the data were determined with a linear regression analysis or multiple regression analysis (StatView, ver. 4, Abacus Concepts, Berkeley, CA, USA). A P level of less than 0.05 was considered statistically significant.

Results

The clinical features of the normotensive controls (NT group) and the hypertensive patients (HT group) are presented in Table 1. Body weight, body mass index, systolic and diastolic blood pressure, fasting plasma insulin concentration, and HOMA-R were significantly higher in the HT group than in the NT group. The HT group was divided into normoinsulinemic patients (NI/HT group: n = 14; AUC insulin was 42.2 ± 2.8 μU/ml per hour) and hyperinsulinemic patients (HI/HT group: n = 13; AUC insulin was 132.9 ± 24.3 μU/ml per hour), depending on the median AUC insulin (54.0 μU/ml per hour). The characteristics of the NI/HT group and the HI/HT group are presented in Table 1. Body weight, body mass index, systolic and diastolic blood pressure, fasting plasma insulin concentration, AUC insulin, HOMA-R, and the insulin sensitivity index were significantly higher in the HI/HT group than in the NT group and the NI/HT group.

Basal $p[Ca^{2+}]_i$ and AVP-stimulated $p[Ca^{2+}]_i$ were not significantly different between the NT group and the HT group (Table 2). Insulin preincubation reduced the AVP-stimulated $p[Ca^{2+}]_i$ response from 280.6 ± 22.2 to 244.2 ± 18.7 nmol/l in the NT group ($P < 0.0001$), and from 351.7 ± 21.6 to 316.0 ± 19.8 nmol/l in the HT group ($P < 0.0001$).

The inhibitory effect of insulin on AVP-stimulated elevation of $p[Ca^{2+}]_i$ (%Inhibition) was not significantly different between the NT group and the HT group. However, the %Inhibition was significantly blunted in the HI/HT group as compared to the NI/HT group and the NT group (Table 2).

Analysis of the membrane fatty acid composition revealed that SFA were increased and PUFA were decreased in the HI/HT group as compared to both the NI/HT group and the NT group (Table 3). We explored the factors that influence the %Inhibition in hypertensive patients. The %Inhibition was negatively correlated with SFA ($r = –0.51$, $P < 0.05$) and positively correlated with the membrane fatty acid fraction of PUFA ($r = 0.53$, $P < 0.01$) (Fig. 2A,B) (n = 23). Among the SFA, C18:0 (stearic acid) was negatively correlated with the %Inhibition ($r = –0.47$, $P < 0.05$) (Table 4). %Inhibition was also correlated positively with K_{os} ($r = 0.53$, $P < 0.005$) (Fig. 2C) (n = 27). The %Inhibition was significantly negatively correlated with systolic blood pressure ($r = –0.44$, $P < 0.05$), but no statistically significant

Table 1. Clinical features of the normotensive controls and hypertensive patients

	NT	HT	HT	
			NI	HI
n	11	27	14	13
Age (years)	49.8 ± 4.5	52.2 ± 1.5	53.3 ± 2.0	51.0 ± 2.2
Body weight (kg)	58.6 ± 3.5	67.7 ± 1.8*	62.8 ± 1.5	73.1 ± 2.7***†
Body mass index (kg/m^2)	22.7 ± 0.7	25.0 ± 0.5*	23.6 ± 0.5	26.5 ± 0.6***††
Systolic blood pressure (mmHg)	118.5 ± 2.9	155.4 ± 2.5***	150.6 ± 3.6***	160.6 ± 2.9***†
Diastolic blood pressure (mmHg)	71.1 ± 2.9	95.6 ± 1.8***	91.4 ± 2.8***	100.2 ± 1.7***†
Pulse rate (beats/min)	76.0 ± 2.5	72.0 ± 1.8	70.3 ± 2.9	73.8 ± 2.1
Total cholesterol (mg/dl)	191.1 ± 6.5	203.4 ± 5.9	211.7 ± 8.5	194.4 ± 7.8
HDL cholesterol (mg/dl)	55.2 ± 4.3	56.2 ± 2.3	60.0 ± 3.1	52.1 ± 3.2
Triglycerides (mg/dl)	105.1 ± 27.2	125.3 ± 16.7	95.8 ± 5.3	157.0 ± 32.7
Glucose (mg/dl)	91.9 ± 2.8	91.6 ± 1.7	89.5 ± 2.6	93.8 ± 2.0
Insulin (μU/ml)	4.9 ± 0.3	6.8 ± 0.5*	5.7 ± 0.7	8.0 ± 0.7**†
R-Na (mEq/l/cells)	10.1 ± 0.5	10.4 ± 0.3	10.1 ± 0.3	10.6 ± 0.4
K_{os} (/h)	0.202 ± 0.015	0.204 ± 0.011	0.206 ± 0.017	0.202 ± 0.013
OGTT				
AUC insulin (μU/ml/h)	40.6 ± 5.4	85.8 ± 14.6	42.2 ± 2.8	132.9 ± 24.3**†††
HOMA-R	1.1 ± 0.1	1.6 ± 0.1*	1.3 ± 0.2	1.8 ± 0.2**†
HOMA βcell	65.0 ± 5.9	93.8 ± 9.0	88.0 ± 12.9	100.1 ± 13.0
Δinsulin/Δglucose (30 min)	0.49 ± 0.23	0.91 ± 0.29	0.99 ± 0.55	0.84 ± 0.16
insulin sensitivity index (mg·l^2/mmol·mU·min)	79.7 ± 5.7	67.4 ± 3.5	81.4 ± 3.1	52.3 ± 2.9***†††

Values are mean ± SEM

NT, normotensive controls; HT, hypertensive patients; NI, normoinsulinemic hypertensive patients; HI, hyperinsulinemic hypertensive patients; HDL cholesterol, high-density lipoprotein cholesterol; R-Na, intraerythrocyte sodium concentration; K_{os}, erythrocyte ouabain-sensitive sodium efflux rate constant; OGTT, 75-g oral glucose tolerance test; AUC insulin, area under the insulin curve; HOMA-R, homeostasis model assessment of insulin resistance; HOMA βcell, homeostasis model assessment of insulin secretion

* $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$ vs normotensive controls; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.005$, $^{\dagger\dagger\dagger}P < 0.0005$ vs normoinsulinemic hypertensive patients (unpaired *t*-test)

Table 2. Nonstimulated and AVP-stimulated intraplatelet free calcium concentrations with or without preincubation of insulin

	NT	HT	HT	
			NI	HI
Basal p[Ca^{2+}]$_i$	61.3 ± 3.5	70.4 ± 3.4	65.8 ± 4.6	75.5 ± 4.9
AVP p[Ca^{2+}]$_i$	280.6 ± 22.2	351.7 ± 21.6	345.1 ± 33.2	358.9 ± 28.4
Basal p[Ca^{2+}]$_i$ + insulin	62.1 ± 4.4	72.5 ± 2.6*	68.1 ± 3.2	77.2 ± 3.9*
AVP p[Ca^{2+}]$_i$ + insulin	244.2 ± 18.7††	316.0 ± 19.8*††	299.6 ± 29.4†	333.8 ± 26.6†
%Inhibition	16.9 ± 1.7	13.7 ± 1.9	17.4 ± 2.7	9.7 ± 2.4*$^{\#}$

Values are mean ± SEM

NT, normotensive controls; HT, hypertensive patients; NI, normoinsulinemic hypertensive patietns; HI, hyperinsulinemic hypertensive patients; Basal p[Ca^{2+}]$_i$ nonstimulated intraplatelet free calcium concentration without insulin preincubation; AVP p[Ca^{2+}]$_i$, arginine-vasopressin (AVP)-stimulated intraplatelet free calcium concentration without insulin preincubation; Basal p[Ca^{2+}]$_i$ + insulin, intraplatelet free calcium concentration with insulin preincubation; AVP p[Ca^{2+}]$_i$ + insulin, AVP-stimulated intraplatelet free calcium concentration with insulin preincubation

* $P < 0.05$ vs normotensive controls; $^{\#}P < 0.05$ vs normoinsulinemic hypertensive patients; $^{\dagger}P < 0.005$, $^{\dagger\dagger}P < 0.0001$ vs AVP-stimulated intraplatelet free calcium concentration without insulin preincubation (ANOVA followed by Fisher's PLSD)

correlation was found with diastolic blood pressure ($r = -0.23$, $P = 0.24$) ($n = 27$). Also, the %Inhibition was not significantly correlated with the insulin sensitivity index ($r = 0.22$, $P = 0.26$), HOMA-R ($r = 0.05$, $P = 0.79$), and R-Na ($r = -0.04$, $P = 0.83$) ($n = 27$).

K_{os} was not significantly correlated with any of the fractions of fatty acid composition (data not shown). There were no statistically significant correlations between K_{os} and R-Na ($r = -0.37$, $P = 0.06$), systolic blood pressure ($r = -0.24$, $P = 0.24$), and diastolic blood pressure ($r = -0.26$, $P = 0.20$).

The %Inhibition was correlated with SFA, PUFA, K_{os}, and systolic blood pressure. Because these factors and indexes of metabolic syndrome correlate with each other, multiple regression analysis with these relationships together with age, gender, body mass index, fasting glucose, fasting insulin, high-density lipoprotein cholesterol, and triglycerides as independent variables were done. Saturated

Table 3. Fatty acid composition of platelet membrane

Fatty acid	NT	HT	HT	
			NI	HI
C14:0	0.56 ± 0.05	0.58 ± 0.10	0.53 ± 0.08	0.63 ± 0.20
C16:0	27.6 ± 0.6	26.9 ± 0.6	26.1 ± 0.7	27.8 ± 1.0
C18:0	16.5 ± 1.5	16.4 ± 0.8	15.3 ± 0.9	17.5 ± 1.2
C18:1	22.8 ± 0.9	22.4 ± 0.6	23.3 ± 0.5	21.5 ± 1.2
C18:2	14.1 ± 1.6	13.7 ± 0.9	15.3 ± 1.4	12.0 ± 1.1
C18:3	ND	ND	ND	ND
C20:0	ND	ND	ND	ND
C20:2	0.53 ± 0.09	0.52 ± 0.08	0.51 ± 0.10	0.53 ± 0.14
C20:4	13.7 ± 0.8	11.5 ± 0.7	12.5 ± 0.7	10.4 ± 1.3
C20:5	0.79 ± 0.34	0.68 ± 0.15	0.45 ± 0.15	0.94 ± 0.26
C22:0	0.97 ± 0.15	1.05 ± 0.14	1.1 ± 0.2	1.0 ± 0.2
C22:2	0.14 ± 0.05	0.26 ± 0.07	0.28 ± 0.12	0.25 ± 0.07
C22:4&6	1.1 ± 0.3	1.8 ± 0.3	1.5 ± 0.3	2.1 ± 0.6
C24:0	ND	ND	ND	ND
C24:2	0.53 ± 0.15	0.86 ± 0.27	0.50 ± 0.16	1.25 ± 0.53
SFA	46.2 ± 1.2	47.9 ± 0.9	45.5 ± 1.1	50.5 ± 1.1*††
MUFA	22.8 ± 0.9	22.4 ± 0.6	23.3 ± 0.5	21.5 ± 1.2
PUFA	31.0 ± 0.9	29.4 ± 0.7	31.1 ± 0.9	27.5 ± 1.0*†

Values are mean ± SEM
NT, normotensive controls; HT, hypertensive patients; NI, normoinsulinemic hypertensive patients; HI, hyperinsulinemic hypertensive patients; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids
* $P < 0.05$ vs normotensive controls; † $P < 0.05$, †† $P < 0.005$ vs normoinsulinemic hypertensive patients (ANOVA followed by Fisher's PLSD)

Table 4. Correlations between membrane fatty acid components and %Inhibition

	r	*p*
C14:0	–0.08	0.73
C16:0	–0.21	0.35
C18:0	–0.47	0.02*
C18:1	0.25	0.25
C18:2	0.21	0.33
C18:3	ND	ND
C20:0	ND	ND
C20:2	–0.021	0.92
C20:4	0.15	0.49
C20:5	–0.09	0.68
C22:0	0.24	0.27
C22:2	0.21	0.34
C22:4&6	0.37	0.08
C24:0	ND	ND
C24:2	–0.1	0.64

ND, not determined
* Indicates statistical significance

Table 5. Multiple regression analysis with %Inhibition as a dependent variable and SFA, PUFA, K_{os}, and systolic blood pressure together with indexes of metabolic syndrome as independent variables

Variable	Correlation coefficient	SE	*t*	*p*
SFA	–0.931	0.369	–2.522	0.0284
PUFA	0.991	0.351	2.823	0.0166
K_{os}	114.289	32.763	3.488	0.0033
SBP	–0.254	0.164	–1.547	0.1414

Adjusted for age, gender, body mass index, fasting glucose, fasting insulin, high-density lipoprotein cholesterol, and triglycerides
SBP, systolic blood pressure; SE, standard error of correlation coefficient

fatty acids, PUFA, and K_{os} were shown to be the significant variables for %Inhibition, while systolic blood pressure was not significant (Table 5).

Discussion

The present study demonstrates that the insulin-related inhibitory effect on platelet calcium responsiveness to AVP was blunted in hypertensive patients with hyperinsulinemia. In this study, platelets were used because they have been developed as a model of vascular smooth muscle cells and can thus help elucidate the pathophysiology of hypertension.[36–38] Platelets are known to possess both insulin receptor and vasopressin receptor, and their agonists induce similar changes in second messengers in both platelets and vascular smooth muscle cells.[38] In addition, we and other groups have previously reported that $p[Ca^{2+}]_i$ is increased in hypertensive patients.[30,39] In this study population, $p[Ca^{2+}]_i$ in hypertensives tended to be higher than that in normotensives, but the difference did not reach statistical significance probably due to the small number of subjects.

It has been reported that insulin attenuates various agonist-induced responses in several cell types. These agonists include angiotensin II (Ang II),[10–12,15,17] endothelin-1 (ET-1),[11,17] norepinephrine,[13] platelet activating factor,[11] serotonin,[10,15] thrombin,[17] phenylephrine,[14] and AVP.[9] Insulin induces vasodilation, and attenuates agonist-induced contraction and the intracellular free calcium response in

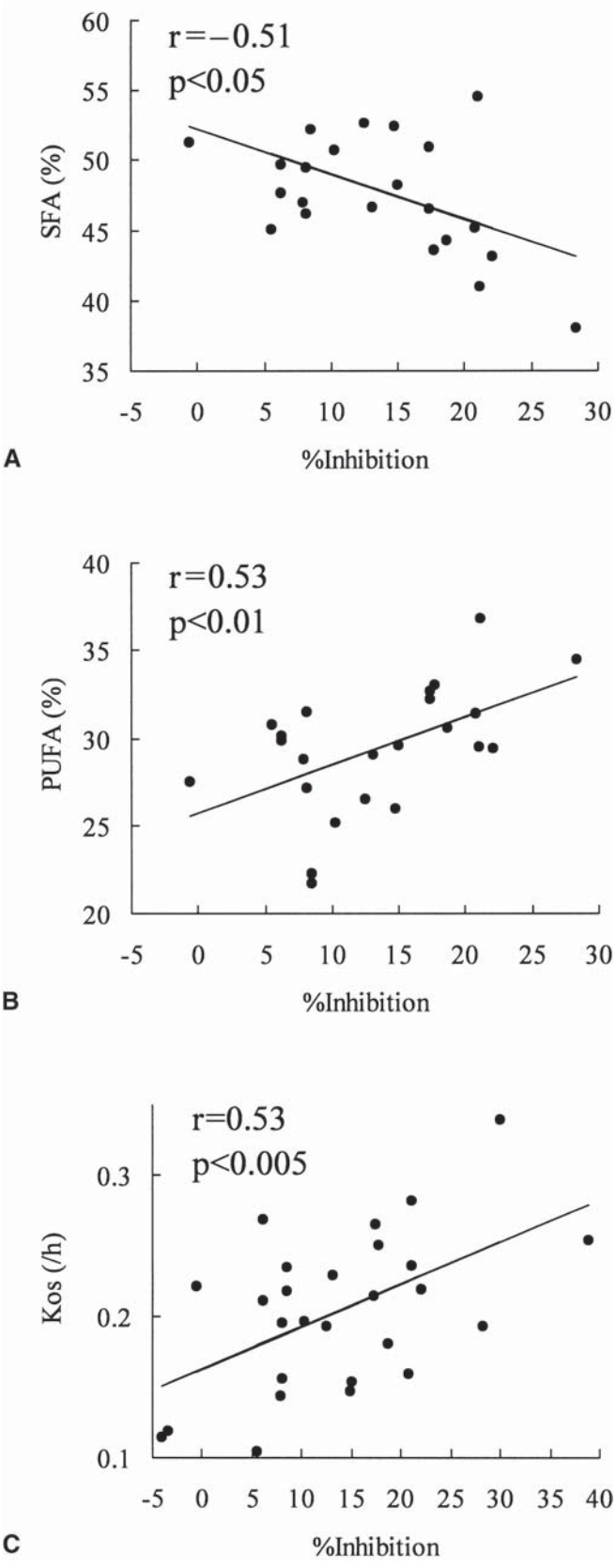

Fig. 2. The relationships between the %Inhibition and platelet membrane saturated fatty acids (*SFA*) (*A*) (n = 23), platelet membrane polyunsaturated fatty acids (*PUFA*) (*B*) (n = 23), and erythrocyte ouabain-sensitive sodium efflux rate constant (K_{os}) (*C*) (n = 27) in hypertensive patients

vascular smooth muscle cells.[9,10,13–15] Touyz and Schiffrin reported that the inhibitory effect of insulin on Ang II- and ET-1-stimulated elevation of $p[Ca^{2+}]_i$ was blunted in hypertensive patients.[17] In our study, insulin's inhibitory effect on AVP-stimulated elevation of $p[Ca^{2+}]_i$ was reduced only in those hypertensive patients whose plasma insulin concentration was relatively high. In fact, in the study by Touyz and Schiffrin the body mass index of hypertensive subjects was $27 \pm 0.16\,kg/m^2$, which was comparable to the body mass index of the hyperinsulinemic hypertensive patients in our study. These findings suggest that blunting of insulin's inhibition of agonist-induced intracellular free calcium elevation may be one of the mechanisms that exacerbate hypertension in patients with hyperinsulinemia. In our study, %Inhibition did not significantly correlate with blood pressure when the relationship was tested with multiple regression analysis. This means that the blunted insulin inhibitory effect on calcium responsiveness is not the major determinant of blood pressure, but it does not negate the possibility that blunted insulin inhibitory effect on calcium responsiveness is one of the exacerbating factors of hypertension.

Changes in membrane fatty acid composition can modulate certain cellular functions, such as carrier-mediated transport, membrane-bound enzymes, and receptors.[18] The present study demonstrated that an increase in membrane SFA and a decrease in membrane PUFA correlated with a blunted insulin inhibitory effect on calcium responsiveness. Our findings are consistent with a previous report showing that decreased insulin sensitivity was associated with decreased C20–C22 PUFA in human skeletal muscle phospholipids.[26,27] Furthermore, we have shown that improving insulin sensitivity with bezafibrate was accompanied by decreased SFA and increased PUFA in erythrocyte membrane fatty acid composition.[40] We have also reported that oral eicosapentaenoic acid supplementation increased membrane eicosapentaenoic acid content and reduced blood pressure in patients with essential hypertension.[35] Combined with these previous findings, our present data suggest that increased SFA and decreased PUFA in membrane fatty acid composition may be causally related to elevated blood pressure and the blunting of insulin's inhibitory effect on agonist-induced intracellular free calcium responses.

The membrane fluidity is a physicochemical feature of biomembranes, and plays an important role in regulating cell functions.[41] Increase in membrane SFA and decrease in PUFA decrease membrane fluidity.[18] Tong et al. reported that decreased membrane fluidity was correlated with insulin resistance in diabetes mellitus.[42] Decreased membrane fluidity has also been shown to be correlated with increased intracellular free calcium[43] and hyperinsulinemia in hypertensive patients.[44] An increase in the membrane SFA/PUFA ratio may blunt insulin's inhibitory effect on agonist-induced intracellular free calcium responses by decreasing membrane fluidity.

Increased sodium content in erythrocytes was shown in hypertensive patients.[33,45] In the present study, R-Na in hypertensives tended to be higher than that in normoten-

sives, but the difference did not reach statistical significance probably due to the small number of subjects. As the mechanism for increased intracellular sodium content, decreased Na^+/K^+-ATPase activity has been suggested in hypertensive patients,[46,47] though these findings remain controversial.[48] The present study showed that insulin's inhibitory effect was positively correlated with the ouabain-sensitive sodium efflux rate constant. This finding suggests that Na^+/K^+-ATPase is involved in the mechanisms of insulin-mediated $p[Ca^{2+}]_i$ lowering, though the data were obtained from different cells. Thus, insulin insensitivity of Na^+/K^+-ATPase might cause a reduction of its activity and lead to intracellular sodium accumulation. This increased intracellular sodium concentration might increase intracellular calcium by activating Na^+/Ca^{2+}-exchange and/or activating the voltage-dependent calcium channel, which might in turn contribute to increased vascular resistance and blood pressure elevation. Although it has been reported that membrane lipid alterations can be related to Na^+/K^+-ATPase activity,[49] we found no such association between its activity and membrane fatty acid composition. Thus, Na^+/K^+-ATPase activity might not be directly regulated by membrane fatty acid. We used red blood cells for evaluating sodium metabolism because we did not have the technique to measure it in platelets. The membrane fatty acid composition of red blood cells might be different from that of platelets. We do not have any evidence that there is a correlation between erythrocyte Na^+ metabolism and platelet Ca^{2+} metabolism, and this is the limitation of our study.

In summary, insulin's inhibitory effect on agonist-induced intracellular calcium elevation was inversely correlated with membrane SFA and blood pressure, and was positively correlated with membrane PUFA and Na^+/K^+-ATPase activity in hypertensive patients. Abnormal membrane fatty acid composition may cause insulin insensitivity in cellular calcium and sodium handling in hypertension with hyperinsulinemia.

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