Inhibitory Effects of Insulin on Intracellular Calcium and Aggregatory Response of Platelets Are Impaired in Hypertensive Subjects with Insulin Resistance

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To determine the effects of insulin on intracellular calcium and platelet aggregatory responses in hypertensive subjects with insulin resistance, we measured insulin sensitivity in terms of glucose disposal rate (GDR) by the hyperinsulinemic euglycemic clamp technique (GC) in 14 non-diabetic untreated hypertensive subjects, and determined basal ($[Ca^{2+}]_i$) and thrombin-stimulated ($T-[Ca^{2+}]_i$) platelet-free calcium concentrations and thrombin-stimulated platelet aggregatory response (AG) before (PRE $[Ca^{2+}]_i$, T-PRE $[Ca^{2+}]_i$, and PRE AG, respectively) and during (POST $[Ca^{2+}]_i$, T-POST $[Ca^{2+}]_i$, and POST AG, respectively) GC. As a control for GC, vehicle (normal saline) was infused on another day. No significant difference was observed between PRE $[Ca^{2+}]_i$ and POST $[Ca^{2+}]_i$, T-PRE $[Ca^{2+}]_i$ and T-POST $[Ca^{2+}]_i$, or PRE AG and POST AG. GDR inversely correlated with $\Delta[Ca^{2+}]_i$ (POST $[Ca^{2+}]_i$ - PRE $[Ca^{2+}]_i$, $r = -0.75$, $p < 0.02$), $\Delta T-[Ca^{2+}]_i$ (T-POST $[Ca^{2+}]_i$ - T-PRE $[Ca^{2+}]_i$, $r = -0.63$, $p < 0.02$) and ΔAG (POST AG - PRE AG, $r = -0.67$, $p < 0.01$). No significant changes were observed in these variables during vehicle infusion. $[Ca^{2+}]_i$, $T-[Ca^{2+}]_i$, and AG decreased during GC as compared with baseline in hypertensive subjects with normal insulin sensitivity, but were unchanged in those with insulin resistance, suggesting that the vasodilatory and anti-platelet aggregatory effects of insulin are impaired in patients with insulin-resistant hypertension. (*Hypertens Res* 1997; 20: 225-231)

Key Words: insulin resistance, calcium, platelet aggregation, glucose clamp, hypertension

The association between hypertension and insulin resistance is well recognized (1). Possible mechanisms by which insulin participates in the pathogenesis of hypertension include increased sodium reabsorption in the kidney (2, 3), activation of the sympathetic nervous system (4), and proliferation of vascular smooth muscle cells (5, 6). On the other hand, evidence against an association between hypertension and insulin resistance includes the following findings: hypertension is not observed in patients with insulinoma despite the presence of hyperinsulinemia (7), continuous infusion of high-dose insulin for 7 days does not increase blood pressure in dogs (8), and acute increases in plasma insulin within the physiological range increase sympathetic neural outflow but produce forearm vasodilatation and do not elevate arterial pressure in humans (9). Insulin has dual pressor and depressor (vasodilatory) effects (10). Also, insulin attenuates both agonist-stimulated vasoconstriction (11) and aggregatory response of platelets (12, 13).

If the vasodilatory and antiaggregatory effects of

insulin were impaired in hypertensive subjects with insulin resistance, an association between hypertension and insulin resistance would be supported. We therefore measured insulin sensitivity in peripheral glucose utilization as determined by glucose clamp, intracellular calcium concentration in platelets as a marker of the vasodilatory effect of insulin on vascular smooth muscle cells, and platelet aggregation before and during glucose clamp, and examined the relationship between insulin resistance and the effects of insulin on intracellular calcium concentration and platelet aggregation in hypertensive subjects.

Methods

The subjects were 14 men with non-diabetic untreated essential hypertension. Insulin sensitivity in terms of glucose disposal rate (GDR) was determined by an artificial endocrine pancreas using the hyperinsulinemic euglycemic clamp technique (GC). Venous blood was drawn from subjects to measure

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basal ($[Ca^{2+}]_i$) and thrombin-stimulated ($T-[Ca^{2+}]_i$) platelet free calcium concentrations as well as thrombin-stimulated platelet aggregatory responses (AG) before (PRE $[Ca^{2+}]_i$, T-PRE $[Ca^{2+}]_i$, and PRE AG, respectively) and after 115 min of GC (POST $[Ca^{2+}]_i$, T-POST $[Ca^{2+}]_i$, and POST AG, respectively). Before venous blood sampling, systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured with a sphygmomanometer with the patient in the supine position both before (PRE-SBP, PRE-DBP, and PRE-HR, respectively) and during (POST-SBP, POST-DBP, and POST-HR, respectively) GC. Serum immunoreactive insulin level (IRI) was measured at 115 min of GC. As a control for GC, an infusion of vehicle (normal saline) was given to all subjects on another day. The subjects were free from other diseases as determined by case history, physical examination, and routine laboratory screening. This study was approved by the Medical Research Review Board of Jikei University, and informed written consent for participation was obtained from each subject.

Glucose Clamp Studies

GC was performed to assess insulin sensitivity with an artificial endocrine pancreas (Biostator, Life Science Instruments, Indiana) after an overnight fast (14, 15). On the study day, the subjects arrived at the artificial endocrine pancreas room by 8:30 AM. A cannula was inserted into a left antecubital vein to aspirate blood (2 ml/h) for continuous blood glucose monitoring by the artificial endocrine pancreas. A cannula was inserted into a superficial vein at the ankle joint to infuse insulin and glucose. To maintain catheter patency, saline was infused continuously at a rate of 0.5 ml/min from 9 AM to noon. From 9 AM to 10 AM, blood glucose was continuously monitored, and insulin was not infused. From 10 AM to noon, GC was performed.

Insulin (Novolin R, Novo-Nordisk, Denmark) infusate was prepared in normal saline, to which 4 ml of the subject's blood per 100 ml infusate was added to prevent adsorption of insulin onto plastic surfaces (14, 16). Insulin (300 mU/ml) was administered as a priming dose followed by continuous infusion. In the first 10-min priming period, the total amount of insulin administered was twice that administered during the subsequent 110 min, when insulin was infused at a rate of 40 mU/m² body surface area per min. In the first 5 min of the priming period, two-thirds of the priming dose was infused, and in the next 5 min of the priming period the remaining one-third of the priming dose was infused to mimic a logarithmically falling manner of priming (16). Blood glucose was maintained at the fasting level by infusing 20% glucose according to mode 9:1 of the Biostator (17). GC was continued for 120 min. GDR during the last 30 min of GC was used as an index of insulin sensitivity. The subjects remained lying quietly on a bed throughout the entire course of the study.

Table 1. Clinical Characteristics of Subjects

Characteristic	Means $\pm$ SD
Age (yr)	44 $\pm$ 6
Body mass index (kg/m ²)	24.0 $\pm$ 2.6
Systolic blood pressure (mmHg)	146 $\pm$ 10
Diastolic blood pressure (mmHg)	95 $\pm$ 7
Pulse rate (/min)	68 $\pm$ 10
Total cholesterol (mg/dl)	200 $\pm$ 33
HDL cholesterol (mg/dl)	53 $\pm$ 13
Triglyceride (mg/dl)	89 $\pm$ 44
Fasting plasma glucose (mg/dl)	99 $\pm$ 8
F-IRI (μ U/ml)	6.7 $\pm$ 3.8

F-IRI, fasting serum immunoreactive insulin.

Measurement of Intracellular Calcium Concentration and Platelet Aggregation

Blood was mixed with 1/10 volume of 3.8% trisodium citrate and centrifuged at 1,200 rpm for 10 min at room temperature. The upper phase platelet-rich plasma (PRP) was incubated with 1 mM fura 2-AM in dimethyl sulfoxide, adjusted to a final concentration of 1 μ M, for 45 min at 37°C.

Fura 2-loaded PRP was mixed with 1/5 volume of acid-citrate-dextrose solution (ACD, 0.80 w/v% citrate, 2.20 w/v% sodium citrate, and 2.20 w/v% dextrose) and centrifuged at 2,500 rpm for 10 min at room temperature. The pellet obtained was suspended in washing buffer (134 mM NaCl, 5 mM dextrose, 1 mM EDTA, 15 mM Tris, pH 6.3) and re-centrifuged at 2,500 rpm for 10 min at room temperature. The resulting pellet was resuspended in Hepes-Tyrode buffer (129 mM NaCl, 2.8 mM KCl, 0.8 mM KH₂PO₄, 8.9 mM NaHCO₃, 0.8 mM MgCl₂, 5.6 mM dextrose, 10 mM Hepes, pH 7.4), adjusted to a final concentration of 3×10^8 platelets/ml.

$[Ca^{2+}]_i$ was measured in a fura 2-AM-loaded platelet suspension that had been incubated with CaCl₂ (final concentration: 1 mM). The platelets were stirred at 37°C in a CAF-100 Ca²⁺ analyzer (Jasco, Japan Spectroscopic Co., Ltd., Tokyo). The instrument was set at excitation wavelengths of 340 and 380 nm and an emission wavelength of 500 nm. We measured both resting and thrombin (final concentration: 0.5 U/ml)-stimulated fluorescence of the sample at 340 nm (F_1) and fluorescence at 380 nm (F_2). Maximum fluorescence (F_{1max} , F_{2max}) was achieved by adding Triton X-100 (final concentration: 0.2%). This corresponded to complete complexation of fura 2 with Ca²⁺. Minimum fluorescence (F_{1min} , F_{2min}) was obtained in the presence of EGTA (final concentration, 10 mM). $[Ca^{2+}]_i$ was calculated according to the formula of Grynkiewicz *et al.* (18):

$[Ca^{2+}]_i = Kd(R - R_{min}) / (R_{max} - R) \times b$ (nM), where Kd is the binding constant, R is the ratio of fluorescences of the sample at 340 and 380 nm (F_1/F_2), R_{max} and R_{min} are the ratios of fura 2 free acid at 340 and 380 nm in the presence of saturating calcium (with Triton X-100) and EGTA, respectively,

Table 2. Comparison between Insulin Resistant Group and Normal Insulin Sensitivity Group with Respect to Blood Pressure and Heart-Rate-Related Variables during Glucose Clamp

	Insulin resistant (n=8)	Insulin sensitive (n=6)
PRE-SBP (mmHg)	148 ± 11	143 ± 8
POST-SBP (mmHg)	147 ± 8	149 ± 12
ΔSBP (mmHg)	-6 ± 12	5 ± 12
PRE-DBP (mmHg)	94 ± 11	98 ± 5
POST-DBP (mmHg)	97 ± 12	94 ± 5
ΔDBP (mmHg)	3 ± 7	-4 ± 5
PRE-HR (/min)	72 ± 13	63 ± 9
POST-HR (/min)	77 ± 15	67 ± 8
ΔHR (/min)	5 ± 8	4 ± 5

Values are expressed as means ± SD. Insulin resistant, GDR < 7 mg/kg per min; Insulin sensitive, GDR > 7 mg/kg per min.

and b is the ratio of fluorescence at 380 nm in the presence of EGTA to that at 380 nm in the presence of Triton X-100 (F_{2min}/F_{2max}). The dissociation constant of fura 2 for calcium was taken to be 224 nmol/l at 37°C.

For aggregation studies, the change in optical density in plasma was measured using the same instrument as for $[Ca^{2+}]_i$, but with the setting adjusted for aggregation measurements. Calibration was performed with PRP as zero aggregation, and platelet-poor plasma (obtained by centrifugation of an aliquot of the same blood sample for another 10 min at 2,500 rpm) as maximal aggregation. The instrument was recalibrated after each experiment. Aggregation was measured in continuously stirred (1,000 rpm) thrombin-stimulated platelets at 37°C, and expressed as a percentage of maximal aggregation.

Vehicle Infusion

As a control for GC, an infusion of vehicle (normal saline) was given to all subjects on another day. The protocol used was the same, except that GC was not performed. Saline solution equal in volume to the vehicle for insulin was infused for 2 h by a syringe pump (Atom, Tokyo, Japan), and venous blood was drawn from all subjects before and after 115 min of vehicle infusion.

Statistics

Values are expressed as means ± SD. Statistical comparisons were performed with the paired two-tailed Student's *t*-test. Correlations were assessed by standard linear regression. *P* values less than 0.05 were considered to indicate statistical significance.

Results

The clinical characteristics of the subjects are shown in Table 1. The steady-state serum immunoreactive insulin level after 115 min of GC was 82.0 ± 11.6 μ U/ml.

Insulin Sensitivity and the Effects of Insulin on SBP, DBP, and HR

Although no significant difference was observed between PRE-SBP and POST-SBP (146 ± 10 vs. 148 ± 10 mmHg, n.s.) or between PRE-DBP and POST-DBP (96 ± 9 vs. 95 ± 10 mmHg), POST-HR was significantly higher than PRE-HR (68 ± 12 vs. 72 ± 13 , $p < 0.04$). There was no correlation between GDR and PRE-SBP ($r = -0.33$, n.s.), PRE-DBP ($r = 0.30$, n.s.), PRE-HR ($r = -0.27$, n.s.), POST-SBP ($r = 0.01$, n.s.), POST-DBP ($r = -0.06$, n.s.) or POST-HR ($r = -0.25$, n.s.), or between GDR and ΔSBP (POST-SBP - PRE-SBP, $r = 0.28$, n.s.), ΔDBP (POST-DBP - PRE-DBP, $r = -0.47$, n.s.), or ΔHR (POST-HR - PRE-HR, $r = -0.01$, n.s.). In order to investigate significant differences in these variables between insulin resistant subjects and insulin sensitive subjects, we divided them into two groups: those with GDR being < 7 mg/kg per min, and those with GDR being > 7 mg/kg per min. However, there was no statistically significant difference in these variables between the two groups (Table 2).

Insulin Sensitivity and the Effect of Insulin on $[Ca^{2+}]_i$

Although no significant difference was observed between PRE $[Ca^{2+}]_i$ and POST $[Ca^{2+}]_i$ (61.2 ± 9.8 vs. 59.8 ± 10.9 nM) and no significant correlation was observed between GDR and PRE $[Ca^{2+}]_i$ ($r = 0.37$, n.s.) (Fig. 1-a), GDR was inversely correlated with both POST $[Ca^{2+}]_i$ ($r = -0.62$, $p < 0.02$) (Fig. 1-b) and the change in $[Ca^{2+}]_i$ from before to during GC ($\Delta[Ca^{2+}]_i$: POST $[Ca^{2+}]_i$ - PRE $[Ca^{2+}]_i$) ($r = -0.75$, $p < 0.02$) (Fig. 1-c). In the comparison of the two groups, the change in $[Ca^{2+}]_i$ was significantly less in the insulin resistant group than in the insulin sensitive group (Table 3).

Insulin Sensitivity and the Effect of Insulin on Thrombin-Stimulated $[Ca^{2+}]_i$

Although GDR positively correlated with T-PRE $[Ca^{2+}]_i$ ($r = 0.71$, $p < 0.01$) (Fig. 2-a), no significant correlation was observed between GDR and T-POST $[Ca^{2+}]_i$ ($r = 0.01$, n.s.) (Fig. 2-b).

Fig. 1. Effects of insulin on basal platelet cytosolic free calcium concentration ($[Ca^{2+}]_i$ in hypertensive subjects. a) Relationship between glucose disposal rate (GDR) and $[Ca^{2+}]_i$ before glucose clamp (GC) (PRE $[Ca^{2+}]_i$ ($r=0.37$, n.s.). b) Relationship between GDR and $[Ca^{2+}]_i$ during GC (POST $[Ca^{2+}]_i$ ($r=-0.62$, $p<0.02$). c) Relationship between glucose disposal rate (GDR) and $\Delta [Ca^{2+}]_i$ (POST $[Ca^{2+}]_i$ - PRE $[Ca^{2+}]_i$) by GC ($r=-0.75$, $p<0.02$).

Although no significant difference was observed between T-PRE $[Ca^{2+}]_i$ and T-POST $[Ca^{2+}]_i$ (670 ± 151 vs. 646 ± 200 nM), GDR inversely correlated with the change in T- $[Ca^{2+}]_i$ from before to during GC ($\Delta T-[Ca^{2+}]_i$: T-POST $[Ca^{2+}]_i$ - T-PRE $[Ca^{2+}]_i$) ($r=-0.63$, $p<0.02$) (Fig. 2-c). In the comparison of the two groups, T-PRE $[Ca^{2+}]_i$ was significantly less in the insulin resistant group than in the insulin

Fig. 2. Effects of insulin on thrombin-stimulated platelet cytosolic free calcium concentration (T- $[Ca^{2+}]_i$ in hypertensive subjects. a) Relationship between glucose disposal rate (GDR) and T- $[Ca^{2+}]_i$ before glucose clamp (GC) (T-PRE $[Ca^{2+}]_i$ ($r=0.71$, $p<0.01$). b) Relationship between GDR and T- $[Ca^{2+}]_i$ during GC (T-POST $[Ca^{2+}]_i$) ($r=-0.01$, n.s.). c) Relationship between GDR and $\Delta T-[Ca^{2+}]_i$ (T-POST $[Ca^{2+}]_i$ - T-PRE $[Ca^{2+}]_i$) by GC ($r=-0.63$, $p<0.02$).

sensitive group and the change in T- $[Ca^{2+}]_i$ was significantly less in the insulin resistant group than in the insulin sensitive group (Table 3).

Insulin Sensitivity and the Effect of Insulin on Platelet Aggregation

Although no significant difference was observed between PRE-AG and POST-AG (81 ± 8 vs. $76 \pm$

Table 3. Comparison between Insulin Resistant Group and Normal Insulin Sensitivity Group with Respect to $[Ca^{2+}]_i$ and Aggregation-Related Variables during Glucose Clamp

	Insulin resistant (n=8)	Insulin sensitive (n=6)
PRE $[Ca^{2+}]_i$	60.4 ± 12.1	62.1 ± 6.6
POST $[Ca^{2+}]_i$	63.7 ± 10.5	54.5 ± 9.9
$\Delta[Ca^{2+}]_i$	3.3 ± 6.0*	-7.6 ± 10.1
T-PRE $[Ca^{2+}]_i$	583 ± 123**	785 ± 103
T-POST $[Ca^{2+}]_i$	642 ± 256	651 ± 113
$\Delta T-[Ca^{2+}]_i$	59 ± 163*	-134 ± 115
PRE-AG	78 ± 8	84 ± 5
POST-AG	79 ± 10	73 ± 4
ΔAG	1 ± 8**	-13 ± 6

Values are expressed as means ± SD. Insulin resistant, GDR < 7 mg/kg per min; Insulin sensitive, GDR > 7 mg/kg per min. * p < 0.05, ** p < 0.01.

8%) and GDR did not correlate with either PRE-AG or POST-AG ($r = 0.32$, n.s. vs. $r = -0.42$, n.s.) (Fig. 3-a, 3-b), GDR inversely correlated with the change in AG from before to during GC (ΔAG : POST-AG - PRE-AG) ($r = -0.67$, $p < 0.01$) (Fig. 3-c).

A positive correlation was observed between the change in thrombin-stimulated $[Ca^{2+}]_i$ and the change in aggregation from before to during GC ($r = 0.55$, $p < 0.05$) (Fig. 4). In the comparison of the two groups, the change in AG was significantly less in the insulin resistant group than in the insulin sensitive group (Table 3).

Vehicle Infusion

No significant difference was observed between PRE-SBP and POST-SBP (146 ± 10 vs. 146 ± 10 mmHg, n.s.), PRE-DBP and POST-DBP (94 ± 9 vs. 96 ± 10 mmHg), or PRE-HR and POST-HR (68 ± 12 vs. 70 ± 13 , n.s.). There was no correlation between GDR and PRE-SBP ($r = -0.23$, n.s.), PRE-DBP ($r = 0.28$, n.s.), PRE-HR ($r = -0.32$, n.s.), POST-SBP ($r = 0.14$, n.s.), POST-DBP ($r = -0.03$, n.s.) or POST-HR ($r = -0.38$, n.s.), or between GDR and ΔSBP ($r = 0.21$, n.s.), ΔDBP ($r = -0.34$, n.s.), or ΔHR ($r = -0.22$, n.s.) during vehicle infusion.

No significant difference was observed between PRE $[Ca^{2+}]_i$ and POST $[Ca^{2+}]_i$ (64.9 ± 11.6 vs. 64.0 ± 11.1 nM), T-PRE $[Ca^{2+}]_i$ and T-POST $[Ca^{2+}]_i$ (703 ± 139 vs. 721 ± 146 nM), or between PRE AG and POST AG (81 ± 9 vs. $80 \pm 7\%$). There was no correlation between GDR and PRE $[Ca^{2+}]_i$ ($r = -0.54$, n.s.), T-PRE $[Ca^{2+}]_i$ ($r = 0.50$, n.s.), PRE AG ($r = -0.20$, n.s.), POST $[Ca^{2+}]_i$ ($r = -0.15$, n.s.), T-POST $[Ca^{2+}]_i$ ($r = 0.37$, n.s.), or POST AG ($r = -0.35$, n.s.), or between GDR and $\Delta[Ca^{2+}]_i$ ($r = -0.37$, n.s.), $\Delta T-[Ca^{2+}]_i$ ($r = 0.09$, n.s.), or ΔAG ($r = -0.21$, n.s.) during vehicle infusion.

Discussion

The difference between baseline and insulin-stimulated $[Ca^{2+}]_i$ values was found to be inversely correlated with insulin sensitivity in terms of GDR.

Thus, $[Ca^{2+}]_i$ decreased after insulin infusion in hypertensive subjects with normal insulin sensitivity, but was unchanged or increased slightly in those with insulin resistance. Results of studies of the effects of insulin on $[Ca^{2+}]_i$ metabolism have been inconsistent. In animals, insulin has been reported to increase $[Ca^{2+}]_i$ (19, 20), decrease $[Ca^{2+}]_i$ (21) or have no effect on it (22, 23). In a human study, insulin has been reported not to change platelet $[Ca^{2+}]_i$ of normotensive subjects *in vitro* and *in vivo* (24). The above discrepancies may reflect differences in experimental conditions, such as kind of cells, insulin concentration and duration of co-incubation time. However, a possible reason for this variability in results is lack of determination of insulin sensitivity in these studies. Consistent with the findings of these previous studies, platelet $[Ca^{2+}]_i$ did not change from before to during glucose clamp in the present study either, unless insulin sensitivity is considered. Baseline $[Ca^{2+}]_i$ did not correlate with insulin sensitivity in the present study. Low insulin concentrations of fasting level do not seem to affect $[Ca^{2+}]_i$.

Since the reduction in $[Ca^{2+}]_i$ is thought to be caused by stimulation of Ca^{2+} -ATPase (25) or Na^+ , K^+ -ATPase (11, 26), or both, by insulin, our findings show that insulin resistance occurs not only in its main action of glucose uptake, but also in other actions. In an animal model of insulin-resistant hypertension, Zucker obese rats, Ca^{2+} -ATPase activity is lower than in lean controls, although there is no difference in Na^+ , K^+ -ATPase activity between the two (27). These findings suggest that resistance in Ca^{2+} -ATPase activity following stimulation by insulin might explain the lack of reduction in platelet $[Ca^{2+}]_i$ in hypertensive subjects with insulin resistance.

We also demonstrated that the changes in thrombin-stimulated $[Ca^{2+}]_i$ and aggregatory responses from before to during GC inversely correlated with insulin sensitivity. These findings indicate that thrombin-evoked $[Ca^{2+}]_i$ and aggregatory responses are inhibited by insulin in hypertensive subjects with normal insulin sensitivity, and that these inhibitory effects are blunted in hypertensive sub-

Fig. 3. Effects of insulin on thrombin-stimulated aggregation (AG) in hypertensive subjects. a) Relationship between glucose disposal rate (GDR) and AG before glucose clamp (GC) (PRE AG) ($r=0.32$, n.s.) b) Relationship between GDR and AG during GC (POST AG) ($r=-0.42$, n.s.). c) Relationship between GDR and ΔAG (POST AG - PRE AG) by GC ($r=-0.67$, $p<0.01$).

jects with insulin resistance. A mechanism by which insulin might attenuate platelet aggregation is inhibition of thrombin-evoked $[Ca^{2+}]_i$, since a positive correlation was observed between the change in thrombin-stimulated $[Ca^{2+}]_i$ and the change in aggregation from before to during glucose clamp.

Insulin signal transduction has branches on its pathway. Therefore, it may be possible that certain actions of insulin are impaired and others are not.

Fig. 4. Relationship between the change in thrombin-stimulated $[Ca^{2+}]_i$ ($\Delta T-[Ca^{2+}]_i$) and the change in aggregation (ΔAG) from before to during GC ($r=0.55$, $p<0.05$).

Among insulin's pressor mechanisms, insulin resistance has been reported not to exist with respect to stimulation of sodium reabsorption in the kidney (28) and sympathetic activation (29). Insulin has been reported to attenuate vasoactive agonist-stimulated vasoconstriction in vascular smooth muscle by inhibiting agonist-evoked increase in $[Ca^{2+}]_i$ (11, 21). These effects of insulin have been shown to be impaired in animals with insulin resistance (21). The present study revealed that insulin attenuates platelet aggregation by the same mechanism as in vascular smooth muscle, and that these effects of insulin are also impaired in patients with insulin resistance. However, whether or not resistance develops to the depressor effects of insulin in humans has yet to be clearly determined. If the same phenomenon as observed in platelet Ca^{2+} metabolism also occurred in vascular smooth muscle cells, this would show that insulin induces vasodilation by decreasing $[Ca^{2+}]_i$ in hypertensive subjects with normal insulin sensitivity, but that the vasodilatory effect of insulin is attenuated in those with insulin resistance.

A negative correlation has been reported between blood pressure and thrombin-evoked $[Ca^{2+}]_i$ because subjects with higher blood pressures have impaired Ca^{2+} influx by thrombin stimulation (30). The positive correlation that we observed between insulin sensitivity and baseline thrombin-evoked $[Ca^{2+}]_i$ may therefore imply that hypertensive subjects with insulin resistance have impaired Ca^{2+} influx by thrombin stimulation.

Heart rate during GC was significantly higher than the baseline value in our subjects. No significant correlation was observed between the change in heart rate and insulin sensitivity. Blood pressure did not change during GC, either. We measured blood pressure only before and at the end of GC to avoid activation of platelet aggregation by cuff inflation. Further investigations of the relationships between heart rate and blood pressure change and insulin sensitivity are needed.

In conclusion, insulin-induced reduction in $[Ca^{2+}]_i$ in platelets was impaired in hypertensive patients with insulin resistance. Thrombin-evoked increases in $[Ca^{2+}]_i$ and platelet aggregation were inhibited by insulin in hypertensive subjects with normal insulin sensitivity, whereas these effects were blunted in hypertensive subjects with insulin resistance. These findings suggest that insulin resistance exists not only in glucose uptake but also in Ca^{2+} metabolism in hypertensive subjects. This fact implies that insulin acts as a pressor hormone in subjects with insulin resistance and may thereby contribute to the development of hypertension.

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