Activation of the Sodium-Potassium Pump Contributes to Insulin-Induced Vasodilation in Humans

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Abstract Systemic hyperinsulinemia induces vasodilation in human skeletal muscle. This vasodilation contributes to insulin-stimulated glucose uptake and has been found to be reduced in various insulin-resistant states. The mechanism of the effect of insulin on vascular tone is not completely understood. We hypothesized that activation of the sodium-potassium pump (Na^+, K^+ -ATPase) located in endothelial or smooth muscle cells would be involved in the insulin-mediated vasodilation. Therefore, in 24 healthy, nonsmoking, nonobese, normotensive volunteers, we infused ouabain, a specific inhibitor of Na^+, K^+ -ATPase, into the brachial artery before and during euglycemic hyperinsulinemia. As expected, insulin (systemic concentrations, approximately 700 [low] and 1400 [high] $\text{pmol} \cdot \text{L}^{-1}$) induced vasodilation in the control arm (forearm blood flow [FBF, plethysmography] from 1.6 ± 0.2 to $2.1 \pm 0.4 \text{ mL} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$ [low insulin] and from 1.6 ± 0.2 to 2.1 ± 0.2 [high insulin], $P < .05$ for both), but the increase in FBF was abolished in the ouabain-infused forearm (from 1.3 ± 0.1 to $1.4 \pm 0.2 \text{ mL} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$

[low] and from 1.3 ± 0.1 to 1.3 ± 0.1 [high], $P = \text{NS}$). Ouabain-induced increases in forearm potassium release were partly reversed by insulin. To investigate whether the mechanism of action could be at the endothelial level, we infused *N*^G-monomethyl-L-arginine (L-NMMA), an inhibitor of endothelial nitric oxide synthase ($0.05, 0.1$, and $0.2 \text{ mg} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$) intra-arterially in 12 subjects and induced a clear dose-dependent decrease of FBF from 1.7 ± 0.2 to $1.2 \pm 0.1 \text{ mL} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$ ($P < .01$). In contrast, after ouabain (and continued insulin) infusion, L-NMMA had no effect on FBF (from 1.6 ± 0.4 to $1.5 \pm 0.3 \text{ mL} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$, $n = 6$, $P = .66$). These results demonstrate that insulin induces vasodilation by stimulation of Na^+, K^+ -ATPase. This activation of Na^+, K^+ -ATPase could occur at the level of the endothelium rather than that of vascular smooth muscle and contributes to the endothelium-dependent vasodilator response to insulin. (*Hypertension*. 1996;28:426-432.)

Key Words • insulin • vasodilation • sodium-potassium pump • ouabain • nitric oxide • endothelium

Various investigators, including ourselves, have shown that in acute experiments in humans, systemic insulin infusion with maintenance of euglycemia has a vasodilator effect in skeletal muscle.¹⁻³ This insulin-induced vasodilation has been found to be reduced in disorders characterized by insulin resistance, such as obesity,¹ hypertension,⁴ and non-insulin-dependent diabetes mellitus.^{5,6} Since insulin-mediated skeletal muscle vasodilation significantly contributes to glucose disposal, a diminished vasodilator capacity in itself explains part of the decreased insulin sensitivity in insulin-resistant states.^{1,4} Therefore, further research on the mechanism of action of insulin-induced vasodilation may be of clinical importance with respect to the issue of insulin resistance.

One of the well-described cellular actions of insulin is an effect on ion transport in general and on stimulation of the Na^+, K^+ -ATPase activity in particular.^{7,8} Based on the exchange of three intracellular potassium ions with two extracellular sodium ions, stimulation of

the Na^+, K^+ -ATPase will result in hyperpolarization of the cell membrane. In vascular smooth muscle cells, this hyperpolarization will trigger closure of voltage-dependent calcium channels, resulting in a decrease of intracellular Ca^{2+} concentration.⁹ This will lead to relaxation of the smooth muscle cell and hence in the vascular bed to vasodilation.¹⁰ This means that insulin-induced vasodilation may in theory be explained by stimulation of the Na^+, K^+ -ATPase in vascular smooth muscle cells. However, Na^+, K^+ -ATPase is also located in endothelial cells,¹¹ in which hyperpolarization will lead to an influx of calcium into the cell because of the increased electrogenic driving force (calcium channels in endothelial cells are voltage independent¹²). The increase in cytosolic Ca^{2+} concentration will stimulate endothelial synthesis and release of NO.¹³ Therefore, an alternative explanation for the mechanism of insulin-induced vasodilation could be insulin-mediated stimulation of endothelial Na^+, K^+ -ATPase, giving rise to NO-dependent vasodilation. Recent observations that insulin-induced vasodilation can be blocked by inhibition of NO synthase^{14,15} are in accord with this latter theory. Furthermore, stimulation of Na^+, K^+ -ATPase probably occurs by translocation of intracellular Na^+, K^+ -ATPase molecules to the cellular membrane.^{8,16} This process needs some time for its full effect, which fits with the observation that insulin-mediated vasodilation is slow in onset.^{1,2,17-19}

Therefore, in the present human in vivo study, we investigated the role of Na^+, K^+ -ATPase activation in the vascular response to insulin by evaluating the effects of

Received December 4, 1995; first decision February 2, 1996; revision accepted April 23, 1996.

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Selected Abbreviations and Acronyms

AU	= arbitrary units
FBF	= forearm blood flow
FVR	= forearm vascular resistance
L-NMMA	= <i>N</i> ^G -monomethyl-L-arginine
NO	= nitric oxide

ouabain, a specific inhibitor of the sodium-potassium pump. Moreover, we tried to establish whether the putative mechanism of action could be at the level of the vascular endothelium by use of the specific NO synthase inhibitor L-NMMA.

Methods

Subjects

The study group consisted of 36 healthy volunteers. All met the following inclusion criteria: age between 18 and 35 years, nonsmoking, normotensive (office blood pressure <140/90 mm Hg measured after 5 minutes of rest in the supine position), and body mass index less than 25 kg·m⁻². Participants used no medication, with the exception of oral contraceptives. The subjects were selected by advertisement and received payment. All subjects had a negative family history of diabetes and hypertension. They all gave written informed consent before participation. The experimental protocol was approved by the hospital ethics committee.

Procedures

The experiments were performed with the subjects in a supine position after an overnight fast and in a quiet, temperature-controlled room (23°C to 24°C). Under local anesthesia (0.3 to 0.4 mL lidocaine HCl, 20 mg/mL), a 20-gauge catheter (Angiocath, Becton Dickinson) was inserted into the left brachial artery and connected with an arterial pressure monitoring line (Viggo Spectramed, 5269-129) to a monitor (Hewlett-Packard 78353B). Mean arterial pressure was determined by the electronically integrated area under the brachial arterial pulse-wave curve. The arterial line was kept patent with saline infusion (3 mL/h with 2 U heparin/mL added). In the same arm, a catheter (Venflon, 20-gauge, 32 mm) was inserted retrogradely into a deep forearm vein so venous blood samples could be obtained. On the contralateral side, an identical catheter was inserted into a large forearm vein for infusion.

Forearm volume was measured with the water displacement method, and all drugs were dosed per 100 mL forearm tissue. FBF was measured with mercury-in-Silastic strain-gauge venous occlusion plethysmography as previously described.²⁰ One minute before the start of measurements, a wrist cuff was inflated to 100 mm Hg above systolic pressure. The collecting cuff around the upper arm was inflated to 40 mm Hg during eight heart cycles with a rapid cuff inflator (DE Hokanson E20). The strain gauges were connected to the plethysmographs (Hokanson EC4).

For calculation of net uptake or release of glucose and potassium, arterial and venous blood was sampled simultaneously at relevant times (see Fig 1 and calculations). Venous blood was sampled with inflated wrist cuffs.

Protocols

Effect of Ouabain Alone

After complete instrumentation, at least 30 minutes of rest were allowed for subjects (n=6) to obtain a steady state, after which baseline measurements were performed and repeated after 15 minutes. When FBF was stable, ouabain was infused into the brachial artery in a dose of 0.2 µg·dL⁻¹·min⁻¹ (approximately 0.3 nmol·dL⁻¹·min⁻¹; volume rate, 50 µL·dL⁻¹·min⁻¹) for 20 minutes. It was calculated that with this dose, local ouabain con-

centrations of 10⁻⁷ to 10⁻⁶ mol·L⁻¹ would be reached, a concentration shown to inhibit Na⁺,K⁺-ATPase activity in vitro.²¹ Earlier studies in which ouabain was infused indicated that the vascular effect of ouabain was maximal after 10 to 15 minutes²²⁻²⁶ and lasted for at least 30 minutes after administration was interrupted.^{23,24} Pilot studies in our laboratory in which ouabain was infused for more than 60 minutes confirmed that vasoconstriction was maximal after 15 minutes and did not increase over time. To obtain effective Na⁺,K⁺-ATPase inhibition throughout the study, we repeated the 20-minute ouabain infusion twice at 45 and 90 minutes after the first administration was started. With this intermittent dosage schedule, the cumulative dose of ouabain was only 128±25 µg, minimizing the risk of systemic effects.²⁷

Effects of Ouabain on Insulin-Induced Vasodilation

In these protocols, we used the hyperinsulinemic, euglycemic clamp technique. In six individuals, insulin (Actrapid, Novo-Nordisk) was systemically infused in a dose of 430 pmol·m⁻²·min⁻¹ IV (60 mU·dL⁻¹·min⁻¹) during 90 minutes. Insulin (50 U/mL) was diluted in 47.5 mL of 0.9% NaCl with the addition of 2 mL of 20% human albumin (Central Laboratory of Blood Transfusion, Amsterdam, Netherlands) to a concentration of 1 U/mL. Euglycemia was maintained by a variable infusion of glucose (20% solution) adjusted by arterial glucose measurements at 5-minute intervals.²⁸ Before and during systemic hyperinsulinemia, ouabain was infused into the left brachial artery (see Fig 1 for a protocol schedule). With the use of this experimental set-up, it was possible for us to study the vasodilator effect of insulin alone (control arm) and insulin plus ouabain (experimental arm) simultaneously within each subject.

In another six individuals, exactly the same study was performed, but insulin was infused at a higher dose (860 pmol·m⁻²·min⁻¹ [120 mU·m⁻²·min⁻¹]).

Effects of L-NMMA Alone and With Ouabain Plus Insulin

To quantify the baseline endothelial production of NO in the forearm vascular bed, we first measured the vasoconstrictor response to three sequential intra-arterial infusions of L-NMMA (0.05, 0.1, and 0.2 mg·dL⁻¹·min⁻¹, 5 minutes per dose, n=12).

Using the same technique, two independent investigators have recently shown that the vasoconstrictor response to L-NMMA was augmented during insulin administration,^{14,15} suggesting an increased endothelial NO release. Since these experiments showed convincing results, we decided not to repeat these particular tests. However, to elucidate the role of Na⁺,K⁺-ATPase activation on this insulin-mediated stimulation of baseline NO release, we recorded the vasoconstrictor response to L-NMMA during insulin infusion after previous ouabain administration. Therefore, again in six subjects, insulin was systemically infused at a dose of 430 pmol·m⁻²·min⁻¹ (60 mU·m⁻²·min⁻¹) during 90 minutes with intra-arterial ouabain infusion before and during systemic hyperinsulinemia, identical to the second protocol. Now

Fig 1. Time schedule of the protocol. Ouabain was infused in three 20-minute periods. Insulin (plus glucose [G]) was infused during 90 minutes. In six individuals, this protocol was extended: After the last ouabain infusion, L-NMMA was given in three increasing doses, while systemic insulin and glucose infusions were continued. See text (protocol 3) for further explanation.

the three doses of L-NMMA were infused after the last ouabain infusion, with continuation of insulin (and glucose) infusion.

During the low-dose insulin experiments, no potassium supplementation was given; during high-dose insulin, 8 mmol KCl was infused during the last 60 minutes of the clamp. In women, experiments were planned during the luteal phase of the menstrual cycle.

Drugs

L-NMMA was obtained from Sigma Chemical Co and stored as a dry powder after manufacturing. The solution was freshly diluted with 0.9% NaCl to its final concentration just before the experiment. Ouabain (0.025%) was obtained from Pharmachemie BV.

Analytic Methods

Plasma glucose was measured in duplicate with the glucose oxidation method (Glucose Analyzer 2, Beckman Instruments Inc). Plasma insulin was measured with a conventional double-antibody radioimmunoassay (interassay coefficient of variation, 6.2%). Potassium was measured by a standard procedure with a K⁺ ion-selective electrode and Hitachi 747 Auto-analyzer (Boehringer Mannheim).

Calculations and Data Analysis

FVR was calculated by dividing mean arterial pressure with FBF and is expressed as AU. Assuming that Whole Blood Glucose=(1-0.3×Hematocrit)×Plasma Blood Glucose,²⁹ glucose uptake was calculated as ΔGlucose_{Arterial-Venous}×(1-0.3×Hematocrit)×FBF. Potassium uptake was calculated as ΔK⁺_{Arterial-Venous}×Forearm Plasma Flow (=FBF×[1-Hematocrit]).

During euglycemic clamp, one coefficient of plasma glucose variation was calculated. Whole-body glucose uptake was defined as the glucose infusion rate during the last 30 minutes of the clamp and expressed as micromoles per kilogram per minute. Effects of insulin on hemodynamic parameters were analyzed by one-way repeated measures ANOVA, with insulin as the dependent factor. Percent changes in flow did not meet the criteria for a gaussian distribution and were analyzed by a nonparametric test (Wilcoxon). All other data were statistically analyzed by Student's *t* test. All statistical analyses were performed with the SPSS personal computer software package.

Results in the tables and figures are expressed as mean±SE, unless otherwise indicated. Statistical significance was set at a value less than .05 (two-sided).

Results

Baseline Characteristics

Baseline characteristics of the three study groups are given in Table 1. Participants were lean, young, and normotensive and had a strictly normal fasting glucose level.

Responses to Ouabain Infusion Alone

Initial administration of ouabain alone was part of the protocol in a total of 24 subjects. Ouabain infusion induced vasoconstriction in the infused arm (FBF from 1.6±0.1 to

FIG 2. Time course of FBF in control arm and experimental (ouabain-infused) arm during three sequential 20-minute periods of ouabain (oua) infusion.

1.2±0.1 mL·dL⁻¹·min⁻¹, *P*<.001, with an increase in FVR from 56±4 to 73±5 AU, *P*<.001). FBF in the control arm did not change (from 1.6±0.1 to 1.6±0.1 mL·dL⁻¹·min⁻¹, *P*=.67; FVR from 60±5 to 60±5 AU, *P*=.93). Mean arterial pressure remained stable (from 81±1 to 82±1 mm Hg, *P*=.36).

Ouabain induced a clear increase of venous potassium levels (from 4.2±0.1 to 4.8±0.1 mmol·L⁻¹, *P*<.001), whereas arterial levels changed slightly (4.0±0.0 to 4.2±0.1, *P*<.01), indicating an obvious increase of net forearm potassium release (from 0.18±0.06 to 0.36±0.08 μmol·dL⁻¹·min⁻¹, *P*<.01).

Response to Repeated Ouabain Administration

During prolonged intermittent ouabain infusion alone (three periods of 20-minute infusions, *n*=6), FBF in the infused and control arms remained stable throughout the study (ouabain-infused arm: from 1.1±0.1 mL·dL⁻¹·min⁻¹ at the end of the first ouabain infusion to 1.2±0.2 at the end of the third ouabain infusion 90 minutes later, *P*=.78; control arm: from 1.5±0.2 mL·dL⁻¹·min⁻¹ at the end of the first ouabain infusion to 1.7±0.2 at the end of the third ouabain infusion, *P*=.13) (see Fig 2).

During prolonged intermittent ouabain infusion alone, arterial potassium concentration did not change (from 4.0±0.1 mmol·L⁻¹ before to 4.0±0.1 at the end of the third ouabain infusion); venous potassium increased after the first infusion but remained stable thereafter (from 4.1±0.2 mmol·L⁻¹ to 4.6±0.3, 4.7±0.3, and 4.6±0.2 before and at the end of the first, second, and third ouabain infusions, respectively). The net potassium release (see response to ouabain infusion alone) remained stable (Table 2). Ouabain did not affect baseline forearm glucose uptake (from 0.41±0.09 μmol·dL⁻¹·min⁻¹ before to 0.41±0.15 during ouabain).

TABLE 1. Clinical Characteristics of Study Participants

	Ouabain Alone	Low Insulin+Ouabain	High Insulin+Ouabain	L-NMMA Alone	Low Insulin+Ouabain+L-NMMA
n (M/F)	6 (2/4)	6 (4/2)	6 (4/2)	12 (7/5)	6 (4/2)
Age, y	24.5±2.3	23.7±2.3	21.8±2.1	25.7±5.7	22.7±1.7
BMI, kg·m ⁻²	21.8±1.3	21.8±1.2	22.9±1.7	21.3±1.8	22.6±2.6
SBP, mm Hg	125±12	126±6	132±8	123±9	126±11
DBP, mm Hg	74±5	72±5	76±9	71±6	77±7
Fasting glucose, mmol·L ⁻¹	4.96±0.29	5.03±0.20	5.22±0.30	5.12±0.32	5.01±0.27

BMI indicates body mass index; SBP, systolic blood pressure; and DBP, diastolic blood pressure. Values are mean±SD.

TABLE 2. Forearm Potassium Release During Baseline, Ouabain, and Ouabain Plus Insulin

	Ouabain Alone (n=6)	Low Insulin (n=6)	High Insulin (n=6)	Low Insulin (n=6)*
Baseline	0.09±0.07	0.25±0.13	0.16±0.14	0.17±0.11
Ouabain	0.27±0.09	0.39±0.20	0.44±0.11	0.33±0.15
Insulin (45 minutes)+ouabain	0.36±0.13	0.24±0.10	0.19±0.04	0.12±0.15
Insulin (90 minutes)+ouabain	0.36±0.09	0.03±0.09	-0.03±0.06	0.18±0.05

Values are expressed as $\mu\text{mol} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$. Note that ouabain induced an increase in net potassium release, which remained stable during continued ouabain administration (ouabain alone), but was partly reversed by additional insulin administration. For *P* values, see text.

*These individuals were subsequently given L-NMMA, see "Methods," third protocol.

Vascular Response to Insulin Plus Ouabain

Low-dose insulin infusion (n=6) increased arterial (systemic) insulin concentrations from 43 ± 7 to 695 ± 7 and $688 \pm 29 \text{ pmol} \cdot \text{L}^{-1}$ after 60 and 90 minutes, respectively. As can be seen in Fig 3 (top), insulin induced a gradual vasodilation in the control arm (FBF from 1.2 ± 0.2 to $1.8 \pm 0.2 \text{ mL} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$ after 90 minutes, $P < .05$; FVR from 78 ± 12 to $54 \pm 7 \text{ AU}$, $P < .01$), but FBF in the ouabain-infused arm did not change (from 1.1 ± 0.1 to $1.1 \pm 0.2 \text{ mL} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$, $P = .70$; FVR from 91 ± 16 to $90 \pm 13 \text{ AU}$, $P = .96$). After 90 minutes of insulin infusion, FBF had not reached a steady state but still seemed to increase. Mean arterial pressure did not change significantly (from 86 ± 2 to $88 \pm 3 \text{ mm Hg}$, $P = .46$).

High-dose insulin infusion (n=6) increased arterial (systemic) insulin concentrations from 57 ± 7 to 1477 ± 165 and $1506 \pm 86 \text{ pmol} \cdot \text{L}^{-1}$ after 60 and 90 minutes, respectively. Again, insulin induced a gradual vasodilation in the control arm (FBF from 1.6 ± 0.2 to $2.1 \pm 0.2 \text{ mL} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$ after 90 minutes, $P < .05$; FVR from 57 ± 9 to $44 \pm 4 \text{ AU}$, $P = .15$), but FBF in the ouabain-infused arm remained unchanged (from 1.3 ± 0.1 to $1.3 \pm 0.1 \text{ mL} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$, $P = .69$; FVR

from 62 ± 5 to $67 \pm 3 \text{ AU}$, $P = .64$) (Fig 3, bottom). Percent flow changes were not different ($P = .53$) between the insulin experiments (see Fig 4).

Metabolic Responses to Insulin Plus Ouabain

Arterial forearm glucose values were clamped at fasting levels (coefficient of variation, $5.1 \pm 0.4\%$; n=18); forearm deep venous glucose levels decreased to trough levels at 45 minutes and remained at this level throughout the study. Therefore, despite ouabain infusion, insulin thus induced a clear increase in forearm skeletal muscle glucose uptake from 0.35 ± 0.06 to $2.35 \pm 0.52 \mu\text{mol} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$ ($P = .01$) (low insulin) and from 0.23 ± 0.08 to $2.48 \pm 0.48 \mu\text{mol} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$ ($P = .003$) (high insulin). As can be seen in Fig 5, forearm glucose uptake was already near maximal after 45 minutes. Maximal forearm glucose uptake did not differ significantly between the low- and high-dose insulin experiments ($P = .86$). Also, whole-body glucose uptake during the last 30 minutes of the clamp was not different (51 ± 7 [low insulin] and $42 \pm 4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ [high insulin]), $P = .31$).

Insulin decreased ouabain-induced forearm potassium release from 0.39 ± 0.20 to $0.03 \pm 0.09 \mu\text{mol} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$ ($P = .18$) (low insulin) and from 0.44 ± 0.11 to -0.03 ± 0.06 ($P < .01$) (high insulin) (see also Table 2).

Response to L-NMMA

The single infusion of three doses of L-NMMA into the brachial artery induced a dose-dependent decrease in FBF from 1.7 ± 0.2 to $1.2 \pm 0.1 \text{ mL} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$ (n=12, $P < .01$).

In six individuals, L-NMMA was administered after insulin was infused (systemic insulin concentrations from

Fig 3. Time course of FBF in both control arm (insulin) and experimental arm (ouabain+insulin) during low-dose ($430 \text{ pmol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$, top) and high-dose ($860 \text{ pmol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$, bottom) insulin infusions. oua indicates ouabain.

Fig 4. Percent change in FBF after 90 minutes of low-dose ($430 \text{ pmol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) and high-dose ($860 \text{ pmol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) insulin infusions in control arm (insulin) and experimental arm (ouabain+insulin).

FIG 5. Time course of forearm glucose uptake during low-dose ($430 \text{ pmol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) and high-dose ($860 \text{ pmol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) insulin infusions. oua indicates ouabain.

43 ± 7 to $645 \pm 50 \text{ pmol} \cdot \text{L}^{-1}$) during 90 minutes, identical to the low-insulin dose protocol. However, after the previous administration of ouabain, L-NMMA induced no vasoconstriction (FBF from 1.6 ± 0.4 to $1.5 \pm 0.3 \text{ mL} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$ at the highest L-NMMA dose) ($P = .66$, ANOVA), despite the concomitant continued administration of insulin. A significant difference between the percent change of FBF was established between the two groups ($P < .03$, see Fig 6).

Again, the prior administration of insulin induced vasodilation at the control side (FBF from 1.9 ± 0.3 to $2.4 \pm 0.8 \text{ mL} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$) but did not change FBF at the ouabain-infused side (from 1.5 ± 0.2 to $1.6 \pm 0.4 \text{ mL} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$). Furthermore, ouabain induced potassium release, which was counteracted by insulin (see Table 2), but ouabain did not affect insulin-induced forearm glucose uptake (from 0.42 ± 0.16 to $2.24 \pm 0.93 \text{ } \mu\text{mol} \cdot \text{dL}^{-1} \cdot \text{min}^{-1}$).

Discussion

The major new observation of the present study is that ouabain largely inhibits insulin-induced vasodilation, which indicates that activation of Na^+, K^+ -ATPase is a key factor in the mechanism of insulin-mediated vasodilation.

In recent years, the vasodilator capacity of insulin has again been recognized¹⁻³ and considered to be important with respect to a further increase of glucose disposal in target tissues. Interestingly, in various related disorders, such as obesity, hypertension, and non-insulin-dependent diabetes mellitus, in which resistance to the metabolic effect of insulin has been described,³⁰ a decreased insulin-induced vasodilation has also been reported.^{1,4,5} Therefore, unraveling the mechanism of the effect of insulin on vascular tone could be of great pathophysiological importance. In this study, as in many others,^{1,2,17-19} systemic insulin infusion induced a gradual slow-onset vasodilation (control arm) that after 90 minutes did not seem to have reached its maximal effect. Forearm glucose uptake, being maximal after 45 minutes, precedes vasodilation¹ and was similar during the low- and high-dose insulin experiments, confirming that the arteriovenous glucose difference is already maximal at an insulin concentration of approximately $700 \text{ pmol} \cdot \text{L}^{-1}$.¹⁸

Interactions Between Na^+, K^+ -ATPase and Insulin

It has long been described that insulin induces Na^+, K^+ -ATPase activity,^{7,8} but the underlying signals and mechanisms are not yet fully clear.

Possible reported mechanisms are an increase in skeletal muscle mRNA levels,³¹ translocation of Na^+, K^+ -ATPase molecules (especially α_2 -isoforms) from intracellular stores to the plasma membrane,^{8,16} or activation of protein kinase C.³² Na^+, K^+ -ATPase is a ubiquitous enzyme located in nearly every cell type, and when involved in insulin-induced vasodilation, it theoretically could be located at the level of the smooth muscle cell, the endothelium, or both.

Ouabain infusion alone induced vasoconstriction in our study, an effect that was maximal after 10 to 15 minutes and has been reported before.^{22,24,25} Apparently, baseline activity of Na^+, K^+ -ATPase contributes to resting vascular tone. Ouabain induced a clear forearm release of potassium, which was stable throughout the study, indicating an effective inhibition of Na^+, K^+ -ATPase activity. The absence of systemic effects (no change in blood pressure or pulse rate; no change in arterial potassium levels) and the unchanged FBF at the contralateral side make it reasonable to conclude that ouabain had significant effects only at the experimental side, of course as a result of the small cumulative dose. Ouabain did not affect baseline glucose uptake, and the insulin-induced increase in forearm glucose uptake appeared normal, confirming that ouabain does not inhibit insulin-induced glucose uptake.²⁵

Hyperinsulinemia was still capable of reversing (gradually) the ouabain-induced forearm potassium release, although this did not result in a net uptake during the experiment. This could indicate that the Na^+, K^+ -ATPase inhibition by ouabain was not complete or alternatively, that insulin-induced potassium uptake might be mediated by mechanisms other than activation of Na^+, K^+ -ATPase. We also noticed that opposite to the effect on glucose uptake, the stimulation of potassium uptake seemed more pronounced during the high-dose insulin infusion.

Mechanism of Effect

Ouabain was clearly capable of preventing the insulin-induced vasodilation. This seems to be a specific effect, as it has been demonstrated quite convincingly that ouabain does not inhibit the effects of various other vasodilator substances, such as verapamil, sodium nitroprusside,²⁴ nifedipine, phentolamine, prazosin,³³ or histamine.²⁶

FIG 6. Percent change in FBF from baseline during three subsequent intra-arterial L-NMMA infusions alone (control) or after ouabain (and continued hyperinsulinemia) (ouabain+insulin).

The exact level at which insulin stimulates an increase in Na^+, K^+ -ATPase activity was not determined. Na^+, K^+ -ATPase could be stimulated at the level of the smooth muscle cell, as shown by data from in vivo experiments.^{16,32} This concept, in which the inhibition of insulin-induced vasodilation by ouabain is well explained, seems to conflict with the fact that insulin-induced vasodilation can also be inhibited by L-NMMA.^{14,15}

In the current study, we report that compared with subjects not treated with ouabain, subjects treated with ouabain showed blunted L-NMMA-induced vasoconstriction, whereas the responses to L-NMMA would be expected to be more intense than normal because of the previous 90 minutes of hyperinsulinemia.^{14,15} This may be explained by recent in vitro data providing some evidence that part of endothelium-dependent NO-induced vasodilation is mediated through stimulation of Na^+, K^+ -ATPase and thus inhibited by ouabain,^{34,35} but of course, also by L-NMMA.

Another explanation could be that insulin activates Na^+, K^+ -ATPase also at the level of the endothelial cell. It has been shown that endothelial cells contain Na^+, K^+ -ATPase molecules.^{11,36} Activation of the sodium-potassium pump and subsequent hyperpolarization have different effects on intracellular calcium in endothelial cells ($[\text{Ca}^{2+}]_i$ decrease) compared with smooth muscle cells ($[\text{Ca}^{2+}]_i$ increase).¹² An increase of intracellular calcium in the endothelial cell would induce the release of NO.¹² In this concept, insulin-induced vasodilation could be inhibited by both L-NMMA and ouabain. Some reports, in which acetylcholine-induced vasodilation and insulin-mediated potentiation of this response were partly inhibited in vivo by ouabain,^{21,31,37} seem to support this concept. Further research will be needed to identify the exact mechanism underlying the ouabain-related inhibition of insulin-induced vasodilation.

In conclusion, we report that inhibition of Na^+, K^+ -ATPase by ouabain largely inhibited in vivo insulin-induced vasodilation in humans, which suggests that activation of this enzyme must be involved in the effects of insulin on vascular tone. We hypothesize that insulin could activate Na^+, K^+ -ATPase at the level of the endothelial cell, which implies that Na^+, K^+ -ATPase activation will contribute to the endothelium-dependent vasodilator response to insulin. Clearly, the interaction between insulin and Na^+, K^+ -ATPase should be investigated further at different cellular levels.

Acknowledgment

This work was supported by grants from the Dutch Diabetes Research Foundation.

References

- Laakso M, Edelman SV, Brechtel G, Baron AD. Decreased effect of insulin to stimulate skeletal blood flow in obese man: a novel mechanism for insulin resistance. *J Clin Invest*. 1990;85:1844-1852.
- Tack CJJ, Smits P, Willemsen JJ, Lenders JWM, Thien T, Lutterman JA. Effects of insulin on vascular tone and sympathetic nervous system in NIDDM. *Diabetes*. 1996;45:15-22.
- Anderson EA, Hoffmann RP, Balon TW, Sinkey CA, Mark AL. Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. *J Clin Invest*. 1991;87:2246-2252.
- Baron AD, Brechtel-Hook G, Johnson AD, Hardin D. Skeletal muscle blood flow: a possible link between insulin resistance and blood pressure. *Hypertension*. 1993;21:129-135.
- Laakso M, Edelman SV, Brechtel G, Baron AD. Impaired insulin-mediated skeletal muscle blood flow in patients with NIDDM. *Diabetes*. 1992;41:1076-1083.
- Baron AD, Laakso M, Brechtel G, Edelman SV. Reduced capacity and affinity of skeletal muscle for insulin-mediated glucose uptake in non-insulin-dependent diabetic subjects: effects of insulin therapy. *J Clin Invest*. 1991;87:1186-1194.
- Moore RD. Effects of insulin upon ion transport. *Biochim Biophys Acta*. 1983;737:1-49.
- Ewart HS, Klip A. Hormonal regulation of the Na^+-K^+ -ATPase: mechanisms underlying rapid and sustained changes in pump activity. *Am J Physiol*. 1995;269:C295-C311.
- Kahn AM, Song T. Insulin inhibits dog vascular smooth muscle contraction and lowers Ca^{2+}_i by inhibiting Ca^{2+} influx. *J Nutr*. 1995;125:1732S-1737S.
- Kahn AM, Seidel CL, Allen JC, O'Neil G, Shelat H, Song T. Insulin reduces contraction and intracellular calcium concentration in vascular smooth muscle. *Hypertension*. 1993;22:735-742.
- Meharg JV, McGowan-Jordan J, Charles A, Parmelee JT, Cutaia MV, Rounds S. Hydrogen peroxide stimulates sodium-potassium pump activity in cultured pulmonary arterial endothelial cells. *Am J Physiol*. 1993;265:L613-L621.
- Lückhoff A, Busse R. Activators of potassium channels enhance calcium influx into endothelial cells as a consequence of potassium currents. *Naunyn-Schmiedeberg's Arch Pharmacol*. 1990;342:94-99.
- Moncada S, Palmer RMJ. The L-arginine nitric oxide pathway in the vessel wall. In: Moncada S, Higgs, eds. *Nitric Oxide From L-Arginine: A Bioregulatory System*. Amsterdam, Netherlands: Elsevier; 1990:19-33.
- Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P. Nitric oxide release accounts for insulin's effects in humans. *J Clin Invest*. 1995;94:2511-2515.
- Steinberg HM, Brechtel G, Johnson A, Fineberg N, Baron AD. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent: a novel action of insulin to increase nitric-oxide release. *J Clin Invest*. 1994;94:1172-1179.
- Omatsu-Kanbe M, Kitasato H. Insulin stimulates the translocation of Na^+-K^+ dependent ATPase molecules from intracellular stores to the plasma membrane in frog skeletal muscle. *Biochem J*. 1990;272:727-733.
- Randin D, Vollenweider P, Tappy L, Jequier E, Nicod P, Scherrer U. Effects of adrenergic and cholinergic blockade on insulin-induced stimulation of calf blood flow in humans. *Am J Physiol*. 1994;247:R809-R816.
- Utriainen T, Malmström R, Mäkimattila S, Yki-Järvinen H. Methodological aspects, dose-response characteristics and causes of interindividual variation in insulin stimulation of limb blood flow in normal subjects. *Diabetologia*. 1995;38:555-564.
- Lundgren F, Edén E, Arfvidsson B, Lundholm K. Insulin time-dependent effects on the leg exchange of glucose and amino acids in man. *Eur J Clin Invest*. 1991;21:421-429.
- Brakkee AJM, Vendrik AJH. Strain gauge plethysmography: theoretical and practical notes on a new design. *J Appl Physiol*. 1966;21:701-704.
- Woolfson RG, Poston L. Effect of ouabain on endothelium-dependent relaxation of human resistance arteries. *Hypertension*. 1991;17:619-625.
- Mason DT, Braunwald E. Studies on digitalis, X: effects of ouabain on forearm vascular resistance and venous tone in normal subjects and in patients with heart failure. *J Clin Invest*. 1964;43:532-543.
- Pedrinelli R, Taddei S, Graziadei L, Salvetti A. Vascular responses to ouabain and norepinephrine in low and normal renin hypertension. *Hypertension*. 1986;8:786-792.
- Robinson BF, Phillips RJW, Wilson PN, Chiodini PL. Effect of local infusion of ouabain on human forearm vascular resistance and on response to potassium, verapamil and sodium nitroprusside. *J Hypertens*. 1983;1:165-169.
- Ferrannini E, Taddei S, Santoro D, Natali A, Boni C, Del Chiaro D, Buzzigoli G. Independent stimulation of glucose metabolism and Na^+-K^+ exchange by insulin in the human forearm. *Am J Physiol*. 1988;255:E953-E958.
- Pedrinelli R, Taddei S, Salvetti A. Sympathetic vasoconstriction as a mechanism of action of ouabain in forearm arterioles of hypertensive patients. *Clin Sci*. 1989;77:541-545.
- Pidgeon GB, Richards AM, Nicholls MG, Lewis LK, Yandle TG. Acute effects of intravenous ouabain in healthy volunteers. *Clin Sci*. 1994;86:391-397.
- DeFronze RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol*. 1979;237:E214-E223.
- Dillon RS. Importance of hematocrit in interpretation of blood sugar. *Diabetes*. 1965;14:672-674.

30. DeFronzo RA, Ferrannini E. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia and atherosclerotic cardiovascular disease. *Diabetes Care*. 1991;14:173-194.
31. Tirupattur PR, Ram JL, Standley PR, Sowers JR. Regulation of Na⁺-K⁺-ATPase gene expression by insulin in vascular smooth muscle cells. *Am J Hypertens*. 1993;6:626-629.
32. Sampson SR, Brodie C, Alboim SV. Role of protein kinase C in insulin activation of the Na-K pump in cultured skeletal muscle. *Am J Physiol*. 1994;266:C751-C758.
33. Schulte K, Van Gemmeren D, Thiede H, Meyer-Sabellek W, Gotzen R, Distler A. Ouabain-induced elevation of forearm vascular resistance, calcium entry and alpha-adrenoceptor blockade and release and removal of noradrenaline. *J Hypertens*. 1987;5(S):S215-S218.
34. Gupta S, McArthur C, Grady C, Ruderman NB. Stimulation of vascular Na⁺-K⁺-ATPase activity by nitric oxide: a cGMP-independent effect. *Am J Physiol*. 1994;266:H2146-H2151.
35. Gupta S, Sussman I, McArthur CS, Tornheim K, Cohen RA, Ruderman NB. Endothelium-dependent inhibition of Na⁺-K⁺-ATPase activity in rabbit aorta by hyperglycemia: possible role of endothelium-derived nitric oxide. *J Clin Invest*. 1992;90:727-732.
36. Elliott SJ, Schilling WP. Oxidant stress alters Na⁺-pump and Na⁺-K⁺-Cl⁻-cotransporter activities in vascular endothelial cells. *Am J Physiol*. 1992;263:H96-H102.
37. Taddei S, Virdis A, Mattei P, Natali A, Ferrannini E, Salvetti A. Effect of insulin on acetylcholine-induced vasodilation in normotensive subjects and patients with essential hypertension. *Circulation*. 1995;92:2911-2918.