Insulin Blunts Sympathetic Vasoconstriction Through the $\alpha_2$-Adrenergic Pathway in Humans

Giuseppe Lembo, Guido Iaccarino, Virgilio Rendina, Massimo Volpe, Bruno Trimarco

Abstract  We investigated the mechanisms underlying the insulin-induced attenuation of sympathetic forearm vasoconstriction in healthy humans. In 5 subjects, we applied 20 mm Hg lower body negative pressure for 30 minutes in control conditions and during a 60-minute infusion of insulin (0.05 mU/kg per minute) in the brachial artery and measured forearm norepinephrine kinetics and hemodynamics. In 11 subjects, we applied graded lower body negative pressure at 5, 10, 15, and 20 mm Hg for 5 minutes each in control conditions and with simultaneous intrabrachial administration of insulin (0.05 mU/kg per minute) (5 subjects) or insulin plus ouabain (3.5 $\mu$g/min per liter) (6 subjects) to investigate whether insulin acts through a potentiation of the vascular smooth muscle Na$^+$,K$^+$-ATPase. To assess a possible effect of insulin on a specific adrenergic receptor pathway, in a further study group we evaluated (1) the forearm vascular response to intrabrachial infusion of the $\alpha_2$-adrenergic receptor agonist phenylephrine (0.5, 1, 2 $\mu$g/kg per minute; n=7) and of the $\alpha_2$-adrenergic receptor agonist BHT-933 (0.5, 1, 2, and 4 $\mu$g/kg per minute; n=9), and (2) the effects of intra-arterial infusion of prazosin (0.5 $\mu$g/100 mL per minute) alone or combined with insulin on the forearm vascular response to graded lower body negative pressure (7 subjects). Insulin blunted the peak increase in forearm vascular resistance (from 13±2 to 6±2 U, $P<.05$) but not the rise in forearm norepinephrine spillover induced by 20 mm Hg lower body negative pressure (from 8.3±1.8 to 11.1±3.5 pmol/min per liter, $P=NS$). Ouabain administration did not prevent the insulin-induced attenuation of the forearm vasoconstrictive response to graded lower body negative pressure. Insulin infusion in the brachial artery did not modify the forearm vasoconstriction induced by intra-arterial infusion of phenylephrine but significantly reduced the increase in forearm vascular resistance induced by BHT-933 (F=6.111, $P<.001$). Finally, intra-arterial infusion of prazosin significantly attenuated the forearm vasoconstriction induced by graded lower body negative pressure. The residual vasoconstrictive response was abolished by insulin infusion. Taken together, these findings suggest that insulin interacts with the sympathetic nervous system at the vascular level predominantly through the $\alpha_2$-adrenergic vasoconstrictive pathway. (Hypertension. 1994;24:429-438.)

Key Words  • vascular resistance  • lower body negative pressure  • Na$^+$,K$^+$-transporting ATPase  • norepinephrine  • ouabain

It is now clearly demonstrated that an important cross talk exists between insulin and the sympathetic nervous system. In humans, physiological hyperinsulinemia evokes a net reflex increase in both muscle sympathetic nervous activity and norepinephrine release. The interplay between insulin and the sympathetic nervous system occurs at different levels. In the skeletal muscle the sympathetic nervous system modulates insulin action on glucose metabolism and at the vascular level insulin attenuates the vasoconstrictive effects of reflex sympathetic activation. This latter observation, obtained in an in vivo study, extends the results of in vitro studies showing that insulin is able to blunt vascular reactivity. The mechanisms through which insulin exerts this short-term and direct effect on sympathetic-induced vasoconstriction still remain unclear.

On the basis of in vitro studies it is possible to suggest several hypotheses: (1) Insulin may act at the neuro-junctional level, causing a decrease in the amount of norepinephrine reaching the adrenergic receptors; (2) insulin may act at the vascular smooth muscle level, which increases Na$^+$,K$^+$-ATPase activity and consequently cell membrane polarization, resulting in attenuation of the cellular response to vasoconstrictive stimuli; and (3) insulin may act at the adrenergic receptor level, which causes a heterologous interaction between insulin and a specific adrenergic receptor population or a downstream modulation of the receptor effector coupling mechanism, resulting in a reduced ability to transduce the vasoconstrictive norepinephrine signal.

The purpose of the present study was to challenge these hypotheses in humans to clarify the potential site of functional attenuation of sympathetic vasoconstriction during a short-term increase in plasma insulin within the physiological range.

Methods

Subjects

The study group consisted of 32 healthy volunteers (29 men and 3 women) whose ages ranged from 19 to 40 years (average, 29±2 years). Health was determined by a careful history, physical examination, and laboratory analyses. Renal, liver, and endocrine functions were normal. No subject had recent changes in body weight or dietary habits. All subjects had a normal tolerance to a 75 g oral glucose load (according to the criteria of the National Diabetes Data Group). No subject was engaged in competitive sports or did intense physical activity during the days preceding the study. Written informed consent was obtained from all participants. The experimental protocol was in accordance with the institutional guidelines for...
human research and approved by the Ethics Committee of the University of Naples School of Medicine.

**Procedures**

The study began at 8 AM in a quiet room with a constant temperature of 22° to 24°C. All subjects were studied in a postabsorptive state in the supine position after a 12- to 15-hour overnight fast. No premedication was administered. On a subject's arrival at the laboratory, forearm volume was measured by water displacement. The forearm perfusion technique was performed as previously described. A plastic cannula was introduced in a retrograde manner into a large antecubital vein and threaded as deeply as possible. In the same arm, a second double-lumen catheter with the distal hole separated by approximately 3 cm from the proximal one (Arrow International Inc) was introduced into the brachial artery. The distal hole was used for the infusion of insulin and other test substances, and the proximal lumen was used for sampling of arterial blood entering the forearm, uncontaminated by solutions infused downstream, and measurement of arterial blood pressure, by means of a Statham P23DD pressure transducer. Systolic and diastolic blood pressures were recorded on a multichannel polygraph (Gould Instruments). FBF was measured at a rate determined from a simultaneously obtained electrocardiographic signal and calculated from the R-R interval. Bilateral blood flow (expressed in milliliters per 100 mL of tissue per minute) was measured by strain-gauge plethysmography using a Digimatic DM2000 (Medimatic) with a calibrated mercury-in-Silastic strain gauge applied on each arm approximately 5 cm below the antecubital crease. Both arms were supported above heart level. Forearm blood flow (FBF) was measured simultaneously in both arms from the rate of increase in forearm volume while venous return from the forearm was prevented by inflating a cuff around the upper arm. Forearm vascular resistance (FVR) was calculated as the ratio of mean arterial pressure (diastolic pressure plus one third pulse pressure) to FBF and expressed as arbitrary units reflecting millimeters of mercury per milliliter per 100 mL of tissue per minute. The intrasubject coefficient of variation was 7% based on two consecutive measurements taken at 1-minute intervals.

Lower body negative pressure (LBNP) was used to cause forearm reflex sympathetic vasoconstriction. An airfilled chamber, similar to that described by Mark and Kerber, was placed over the lower portion of each subject's body, from the iliac crest down. Negative pressure applied into the chamber was monitored by a pressure transducer. FBF was measured in both arms, at the last 90 seconds of each level of LBNP.

Forearm norepinephrine kinetics were measured with a tracer technique based on a primed constant infusion of tritiated norepinephrine. Briefly, 40 minutes before the study was started, the subjects received intravenously a priming dose (27 μCi of l-(2,5,6)-[3H]norepinephrine (New England Nuclear; specific activity 43.7 Ci/mmol) diluted in 0.9% saline containing ascorbic acid (2 mg/mL), followed by a constant infusion (0.63 μCi/min), which was continued throughout the study period.

**Protocols**

The doses of all the substances infused into the brachial artery were chosen to act selectively in the experimental forearm without causing systemic effects. After complete instrumentation, all subjects rested at least 30 minutes for a stable baseline to be established before data collection.

**Series 1: Effects of Insulin on Forearm Norepinephrine Kinetics During Neurogenic Vasoconstriction**

In five subjects, forearm norepinephrine kinetics responses to 20 mm Hg LBNP were assessed during continuous infusion of 0.9% saline into the brachial artery at a flow rate of 0.3 mL/min and during the second half of a 60-minute intraarterial infusion of human regular insulin. The insulin delivery rate was 0.05 mU/kg per minute and was aimed at producing an increase in brachial artery plasma insulin level to approximately 400 pmol/L. We applied the LBNP stimulus for 30 minutes to obtain a steady-state evaluation of forearm norepinephrine kinetics at 20, 25, and 30 minutes.

To simplify the experimental protocol, we did not measure the changes in intracardiac pressure induced by LBNP, which represent the stimulus for reflex sympathetic activation. The simultaneous assessment of FBF in the arm used to rule out the possibility that the changes in the vascular response in the experimental arm could result from a different fall in cardiac filling pressure during LBNP. Comparison of the vascular responses observed in the contralateral arm during vehicle or insulin infusion allowed us to assess the stability and reproducibility of the LBNP stimulus.

**Series 2: Effects of Ouabain on Forearm Neurogenic Vasoconstriction During Local Hyperinsulinemia**

To test the hypothesis that an increase of vascular smooth muscle cell Na⁺/K⁺-ATPase activity mediates the effects of insulin on neurogenic vasoconstriction, in five subjects we evaluated the forearm vasoconstrictor response to LBNP, applied incrementally at 5, 10, 15, and 20 mm Hg for 5 minutes each, during infusion of 0.9% saline in the brachial artery and then during administration of regular insulin at the delivery rate specified above. In another group of six subjects we assessed the forearm vasoconstrictive response to LBNP applied as indicated above first during infusion of 0.9% saline and then during the simultaneous intrabrachial administration of insulin and ouabain. This latter agent was infused at a rate of 3.5 μg/min per liter to expose forearm tissue to ouabain levels close to those that have been shown to counteract the insulin effect on Na⁺/K⁺-ATPase activity in tissues incubated in vitro.

**Series 3: Effects of Insulin on Forearm Vasoconstrictive Responses Evoked by Selective α₁- and α₂-Adrenergic Receptor Agonists**

To investigate whether the insulin effect on sympathetic vasoconstriction could be caused by the action of the hormone on specific α-adrenergic receptors, we assessed the responses to intrabrachial infusion of phenylephrine (α₁-selective agonist, seven subjects) and BHT-933 (α₂-selective agonist, nine subjects) before and during intrabrachial infusion of insulin at the same rate used in series 1 and 2. Dose-response curves were generated for each drug infusion; phenylephrine was infused at 0.5, 1, and 2 μg/kg per minute and BHT-933 at 0.5, 1, and 2 μg/kg per minute. Each drug dose was maintained for 10 minutes to analyze the vascular steady-state response. To rule out the possibility of a downregulation of α-adrenergic receptors induced by the first infusion of multiple doses of each α-adrenergic agonist, a 90-minute period elapsed before the second dose-response curves were recorded. The duration of this interval was chosen according to the results of pilot experiments showing that no differences could be detected between two consecutive infusions of these drugs performed with such an interval.

**Series 4: Effects of Intra-arterial Prazosin or Prazosin Plus Insulin on Forearm Neurogenic Vasoconstriction**

To further investigate the possibility that hyperinsulinemia blunts forearm reflex sympathetic vasoconstriction through an interference with the α₁-adrenergic pathway, in seven additional subjects we evaluated the forearm vasoconstrictive response to graded LBNP, applied as specified for series 2, first during the infusion of 0.9% saline in the brachial artery and then during the administration of prazosin at the delivery rate of 0.5 μg/100 mL of forearm tissue per minute.
after 60 minutes of recovery, graded LBNP was applied during simultaneous intra-arterial infusion of prazosin and insulin delivered at the rate specified above.

The effectiveness of the pharmacological blockade of α-adrenergic receptors induced by prazosin administration was documented by the observation that the vasoconstrictive response elicited by the intra-arterial administration of phenylephrine (2 μg/kg per minute for 5 minutes) was abolished. The prazosin dose was chosen according to the results of pilot experiments showing that such a dose was able to induce a complete blockade of the vasoconstrictive response elicited by the administration of increasing doses of phenylephrine. In particular, in four subjects the intra-arterial injection of phenylephrine at 0.5, 1, 2, 4, 6, and 8 μg/kg per minute did not modify arterial blood pressure but induced a progressive fall in FBF that reached its nadir at 6 μg/kg per minute and then remained stable (from a basal value of 3.1±0.2 to 2.4±0.2, 2.0±0.1, 1.6±0.1, 1.3±0.1, 1.1±0.1, and 1.1±0.1 mL/100 mL per minute, all P<.05 versus baseline). Simultaneously, FVR increased from a basal value of 31±3 to 41±4, 48±5, 57±5, 70±6, 85±9, and 86±9 U (all P<0.05 versus baseline). Also in this case, the peak response was reached with the dose of 6 μg/kg per minute and then remained stable. During prazosin infusion (0.5 μg/100 mL of forearm tissue per minute) in the brachial artery, the hemodynamic response to increasing doses of phenylephrine was abolished (FBF: from a basal value of 6.6±2 to 6.7±1, 6.7±2, 6.7±2, 6.9±1, 7±1, and 7±2 mL/100 mL per minute, P=NS; FVR: from a basal value of 15±2 to 17±4, 15±3, 16±4, 14±2, 14±2, and 15±3 U, P=NS).

Methodological Analysis

Plasma insulin was measured by radioimmunoassay.23 Serum potassium concentrations were determined on an electrode electrolyte analyzer (EA2, Beckman Instruments Inc). Plasma catecholamines were partially purified by batch alumina extraction,24 separated using ion-pair reversed phase high-performance liquid chromatography (μBondapak C18 column, Powerline 600A chromatography system, and WISP 700 autoinjector, Waters Chromatography Division), and quantified by a current produced on exposure of the column effluent to oxidizing and then reducing potentials connected in series (Coulochem II, ESA).25 Recovery through the alumina extraction step, calculated using dihydroxybenzylamine as an internal standard, ranged 60% to 70%, and each sample was corrected for its recovery. The detection limit was 3 pg injected for norepinephrine. Intra-assay and interassay variation coefficients for norepinephrine were 4.1% and 9.8%, respectively.

The amount of [3H]norepinephrine in plasma and infusate was measured by batch alumina extraction,24 separated using ion-pair reversed phase high-performance liquid chromatography (μBondapak C18 column, Powerline 600A chromatography system, and WISP 700 autoinjector, Waters Chromatography Division), and quantified by a current produced on exposure of the column effluent to oxidizing and then reducing potentials connected in series (Coulochem II, ESA).25 Recovery through the alumina extraction step, calculated using dihydroxybenzylamine as an internal standard, ranged 60% to 70%, and each sample was corrected for its recovery. The detection limit was 3 pg injected for norepinephrine. Intra-assay and interassay variation coefficients for norepinephrine were 4.1% and 9.8%, respectively.

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Calculation and Data Analysis

Forearm potassium balance was calculated by the formula (K arterial − K venous) · FBF, where K arterial and K venous are the arterial and deep venous concentrations of serum potassium, respectively. FBF was converted to plasma flow by correcting for the hematocrit value. Forearm extraction fraction and forearm norepinephrine uptake and spillover were quantified from measurements of forearm plasma flow and arterial and forearm deep venous concentrations of radioactive and endogenous norepinephrine during systemic intravenous infusion of trace amounts of radiolabeled norepinephrine ([3H]NE) according to the following formulas:

\[
\text{Forearm Norepinephrine Uptake (NE}_{\text{upt}} = \frac{[\text{3H}]\text{NE}}{\text{A}} - \frac{[\text{3H}]\text{NE}}{\text{V}} \cdot \frac{\text{FFP}}{\text{A}} - \frac{\text{FFP}}{\text{V}}
\]

\[
\text{Forearm Norepinephrine Spillover (NE}_{\text{sp}} = \frac{[\text{3H}]\text{NE}}{\text{A}} - \frac{[\text{3H}]\text{NE}}{\text{V}} \cdot \frac{\text{FFP}}{\text{A}} - \frac{\text{FFP}}{\text{V}}
\]

where [3H]NEa and [3H]NEv are the arterial and deep venous concentrations of tritiated norepinephrine (disintegrations per minute per milliliter), FFP is the forearm plasma flow (milliliters per 100 mL per minute), and NEa and NEv are the arterial and deep venous norepinephrine concentrations (nanomoles per liter). Norepinephrine uptake and spillover were normalized for differences in forearm volume.

Results

Series 1: Effects of Insulin on Forearm Norepinephrine Kinetics

In control conditions, the application of 20 mm Hg LBNP did not modify mean blood pressure (from 88±1 to 87±3 mm Hg, P=NS) or heart rate (from 17±4 to 15±3 U, P=NS).

Table 1. Effects of Lower Body Negative Pressure on Forearm Norepinephrine Kinetics During Intrabrachial Infusion of Saline (Vehicle) or Insulin

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline (−20 mm Hg)</th>
<th>LBNP (−20 mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>NEx, nmol/L</td>
<td>1.06±0.07</td>
</tr>
<tr>
<td></td>
<td>NExv, nmol/L</td>
<td>-0.13±0.09</td>
</tr>
<tr>
<td></td>
<td>[3H]NEa, dpm/mL</td>
<td>637±44</td>
</tr>
<tr>
<td></td>
<td>NEsp, (pmol/min)/L</td>
<td>10.1±1.9</td>
</tr>
<tr>
<td></td>
<td>NEsp, (pmol/min)/L</td>
<td>12.1±2.6</td>
</tr>
<tr>
<td>Insulin</td>
<td>NEx, nmol/L</td>
<td>1.07±0.12</td>
</tr>
<tr>
<td></td>
<td>NExv, nmol/L</td>
<td>-0.19±0.09</td>
</tr>
<tr>
<td></td>
<td>[3H]NEa, dpm/mL</td>
<td>577±64</td>
</tr>
<tr>
<td></td>
<td>NEsp, (pmol/min)/L</td>
<td>0.49±0.06</td>
</tr>
<tr>
<td></td>
<td>NEsp, (pmol/min)/L</td>
<td>9.9±1.8</td>
</tr>
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</table>

LBNP indicates lower body negative pressure; NEx, arterial plasma norepinephrine; NExv, arterial plasma norepinephrine difference; [3H]NEa, arterial plasma tritiated norepinephrine; NEsp, forearm norepinephrine extraction fraction; NEsp, forearm norepinephrine uptake; and NEsp, forearm norepinephrine spillover. Data are mean±SEM. LBNP values represent the mean of three observations performed after 20, 25, and 30 minutes of −20 mm Hg LBNP.

*P<.05 compared with baseline.
Series 2: Effects of Ouabain on Forearm Neurogenic Vasoconstriction During Local Hyperinsulinemia

In both study groups during vehicle infusion the application of graded LBNP did not modify mean arterial pressure and heart rate (data not shown) but induced a progressive increase in FVR in both the experimental (group A: F=28.013, P<.005; group B: F=11.436, P<.005) and contralateral (group A: F=20.143, P<.005; group B: F=5.629, P<.005) arms (Fig 1). No significant difference could be detected between the vasoconstrictive responses observed in the two arms in each group (group A: F=0.536, P=NS; group B: F=0.170, P=NS).

During infusion of insulin alone (group A) or combined with ouabain (group B) in the brachial artery of the experimental arm, upstream arterial insulin concentration remained unchanged (group A: from 47±14 to 49±11 pmol/L, P=NS), whereas insulin level in the venous blood from the experimental arm increased to 370±19 pmol/L (P<.01). Simultaneously, there were no changes in mean blood pressure (87±2 mm Hg) and heart rate (69±3 beats per minute) or in FBF (experimental arm: 2.7±0.1 mL/100 mL per minute; contralateral arm: 2.8±0.1 mL/100 mL per minute) and FVR (experimental arm: 34±4 U; contralateral arm: 31±3 U). The application of 20 mm Hg LBNP induced hemodynamic responses comparable to those observed during saline infusion both at the systemic level and in the contralateral arm (ΔFVR: 18±3 U). In contrast, forearm vasoconstriction in the experimental arm was significantly attenuated (ΔFFBF: -0.76±0.09 versus -0.25±0.08 mL/100 mL per minute, P<.01; ΔFVR: +13±2 versus +6±2 U, P<.01). Simultaneously, NE_A, NE_V, and norepinephrine spillover from the experimental arm increased to levels similar to those achieved when LBNP was applied before insulin infusion (Table 1).
simultaneously in the contralateral arm (F=3.510, P<.05) (Fig 1, top). Similarly, in group B the application of graded LBNP during insulin plus ouabain infusion elicited a forearm vasoconstrictive response that was significantly smaller compared with that recorded in the contralateral arm (F=2.887, P<.05) (Fig 1, bottom).

Series 3: Effects of Insulin on Forearm Vasconstrictive Response Evoked by Selective $\alpha_1$- and $\alpha_2$-Adrenergic Receptor Agonists

In control conditions the infusion of increasing amounts of phenylephrine did not modify blood pressure and heart rate but induced a dose-dependent decrease in FBF in the experimental arm (F=16.423, n=7, P<.01) (Table 2). Similarly, the infusion of increasing doses of BHT-933 in the brachial artery of the experimental arm did not modify systemic hemodynamics but both of these responses were significantly smaller compared with those produced by the infusion of the same agent in control conditions (A\text{FBF} -0.81±0.16 versus -1.37±0.16 mL/100 mL per minute, P<.05; A\text{FVR}: +11±3 versus +30±3 U, P<.01).

The changes in arterial and venous insulin plasma concentrations observed in these subjects during insulin infusion in the brachial artery of the experimental arm were strictly comparable to those observed in the remaining study groups (arterial concentration: from 40±2 to 53±6 pmol/L, P=NS; venous concentration: from 38±6 to 41±103 pmol/L, P<.01), and there were no significant hemodynamic changes (Table 2).

The administration of increasing doses of phenylephrine during insulin infusion still induced a significant fall in FBF (F=7.045, n=7, P<.01; Table 2). Furthermore, the maximal fall in FBF was similar to that recorded in control conditions ($\Delta_{\text{max}}$ 1.16±0.31 versus 1.16±0.49 mL/100 mL per minute, P=NS). Similarly, the FVR response in the experimental arm (F=5.622, n=7, P<.01) was comparable in magnitude to that obtained in control conditions ($\Delta_{\text{max}}$ 22±5 versus 20±8 U, P=NS).

Finally, the intra-arterial infusion of increasing doses of BHT-933 during insulin administration was still able to induce a dose-dependent decrease in FBF (F=5.989, n=9, P<.001; Table 2) and an increase in FVR (F=8.627, n=9, P<.01), but both of these responses were significantly smaller compared with those produced by the infusion of the same agent in control conditions ($\Delta_{\text{max}}$ FBF -0.81±0.16 versus -1.37±0.16 mL/100 mL per minute, P<.05; $\Delta_{\text{max}}$ FVR: +11±3 versus +30±3 U, P<.01).

Because there is evidence that the magnitude of vascular response ($\Delta\text{FVR}$) to vasoactive agents is a function of the basal FVR,26,27 we calculated linear regression, plotting basal FVR versus $\Delta\text{FVR}$ for each dose of phenylephrine and BHT-933. At an infusion rate of 0.5 $\mu$g/kg per minute of phenylephrine, we failed to detect any significant correlation between basal FVR and $\Delta\text{FVR}$. On the contrary, significant correlations between basal FVR and $\Delta\text{FVR}$ were found for the vascular responses to all the remaining doses of phenylephrine and BHT-933 (Figs 2 and 3). We also compared by covariance analysis the corresponding regression-adjusted responses obtained during vehicle infusion and insulin infusion. We failed to detect any significant difference for phenylephrine responses. On the contrary, insulin infusion consistently reduced the slope of the linear regression of vascular responses to BHT-933 administration (0.5 $\mu$g/kg per minute: F=5.111; 1 $\mu$g/kg per minute: F=5.817; 2 $\mu$g/kg per

Table 2: Effects of Intrabrachial Infusion of Phenylephrine (n=7) and BHT-933 (n=9) During Intrabrachial Saline (Vehicle) and insulin Infusion on Arterial Pressure and Experimental Forearm Blood Flow

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Baseline Infusion</th>
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<td>Phenylephrine</td>
<td></td>
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<tr>
<td>MAP</td>
<td>83±2</td>
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<td>86±2</td>
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<tr>
<td>FBF</td>
<td>3.12±0.34</td>
<td>3.12±0.34</td>
<td>2.45±0.22*</td>
<td>1.93±0.19*</td>
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<td>Vehicle MAP</td>
<td>81±3</td>
<td>80±2</td>
<td>82±3</td>
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<td>87±3</td>
</tr>
<tr>
<td>FBF</td>
<td>3.68±0.63</td>
<td>3.84±0.59</td>
<td>2.96±0.57*</td>
<td>2.63±0.59*</td>
<td>2.69±0.56*</td>
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<tr>
<td>Insulin MAP</td>
<td>88±2</td>
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<tr>
<td>FBF</td>
<td>3.02±0.22</td>
<td>3.02±0.22</td>
<td>2.28±0.24*</td>
<td>2.08±0.19*</td>
<td>1.90±0.19*</td>
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<td>BHT-933 MAP</td>
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<tr>
<td>FBF</td>
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<td>3.37±0.42</td>
<td>2.92±0.32*</td>
<td>2.78±0.29*</td>
<td>2.64±0.33*</td>
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</table>

MAP Indicates mean arterial pressure (millimeters of mercury); FBF, forearm blood flow (milliliters per 100 mL per minute). Data are mean±SEM.

*P<.05 compared with baseline infusion.
AFVR

Fig 2. Plots show forearm vascular resistance (FVR) responses to intrabrachial arterial infusions of phenylephrine (A, 0.5 μg/kg per minute; B, 1 μg/kg per minute; C, 2 μg/kg per minute) plotted against level of basal FVR in individual subjects. Open and closed squares indicate responses obtained during infusion of vehicle and insulin, respectively. Solid and dotted lines indicate regression lines obtained during infusion of vehicle and insulin, respectively. B, Vehicle: r=.746, slope=1.23; insulin: r=.959, slope=1.93. C, Vehicle: r=.852, slope=1.44; insulin, r=.937, slope=1.96.

Series 4: Effects of Intra-arterial Infusion of Prazosin Alone or Combined With Insulin on Forearm Neurogenic Vasoconstriction

The application of graded LBNP did not modify mean arterial pressure and heart rate either in control conditions or during the intra-arterial infusion of prazosin alone or combined with insulin (data not shown). On the contrary, in control conditions LBNP induced significant opposite changes of FBF and FVR in both the experimental and contralateral arms (Table 3). During the infusion of prazosin alone the reflex forearm response elicited by graded LBNP was significantly attenuated in the experimental arm (FBF: F=2.984, P<.05 versus vehicle; FVR: F=10.548, P<.01 versus vehicle) but not in the contralateral arm (Table 3). In particular, the maximal change in FVR induced by LBNP during prazosin infusion in the experimental arm was significantly smaller than during vehicle administration (Fig 4). Finally, the simultaneous intra-arterial administration of prazosin and insulin, associated with changes in deep venous insulin concentration comparable to those observed in the previous series of studies, was able to abolish the forearm reflex vascular response in the experimental arm (F=1.018, P:NS), and the neurogenic vasoconstriction in the contralateral arm remained unchanged (Fig 4, Table 3).

Discussion

Despite the recent progress in the understanding of the relations between insulin and the sympathetic nervous system in humans,¹ the mechanisms by which the hormone attenuates sympathetic-induced vasoconstriction are still unclear. The results of the present study demonstrate that (1) local hyperinsulinemia per se has no detectable effect on peripheral sympathetic nervous activity and in particular does not affect the release or metabolism of norepinephrine during reflex sympathetic activation; (2) local infusion of ouabain is able to antagonize the insulin effect on Na⁺,K⁺-ATPase but does not restore the normal vascular responsiveness to the adrenergic vasoconstrictive stimuli; and (3) dissecting the vasoconstrictive adrenergic signal in α₂-mediated vasoconstriction, local hyperinsulinemia antagonizes selectively the α₂-adrenergic receptor-mediated vasoconstriction.
There is much evidence in humans that insulin stimulates peripheral sympathetic efferent outflow,\(^1,3\) probably through its action on the central nervous system,\(^3\) and simultaneously is able to attenuate the sympathetic effects at the vascular level.\(^6,9\) One of the potential mechanisms by which insulin modulates the vascular effects of sympathetic nervous system activation could be a direct action on norepinephrine release from nerve endings or on tissue-norepinephrine metabolism. In vitro studies may support this hypothesis because it has been shown that insulin reduces norepinephrine concentration in the perfusate from the mesenteric vascular preparation\(^11\) and increases tissue norepinephrine uptake in atrial strips.\(^10\) To test the possibility that insulin blunts sympathetic vasoconstriction through a direct peripheral effect on the amount of sympathetic neurotransmitter reaching the receptor sites, we infused insulin intrabrachially to raise the hormone concentration only locally in the forearm tissue and measured forearm norepinephrine kinetics in basal conditions and during reflex sympathetic activation induced by 20 mm Hg LBNP. The validity of the model used is supported by the finding that during local insulin infusion the systemic insulin concentration remained unchanged while the forearm deep venous plasma insulin level was similar to that commonly observed after oral glucose load, suggesting that the insulin levels achieved in the experimental arm in our study increased within the physiological range.\(^28\) With this approach, we did not detect any appreciable change in norepinephrine uptake or release during forearm hyperinsulinemia both in basal conditions and during reflex sympathetic activation, despite the simultaneous attenuation of forearm sympathetic vasoconstriction. This may appear to contrast with previous studies,\(^10,11\) although the insulin concentrations achieved in those studies were four to six orders of magnitude higher than those reported in the present study, and insulin doses threefold higher than ours had no effect on the stimulated norepinephrine overflow from peripheral sympathetic nerves.\(^11\) This observation supports the conclusion that the insulin effect on sympathetic vasoconstriction is not mediated through an interference with the release of norepinephrine or through the mechanisms responsible for the inactivation of the amine.

A second possibility that needs to be considered is that insulin could stimulate the Na\(^+\),K\(^+\)-ATPase in vascular smooth muscle, as it does in adipocytes and skeletal muscle tissue,\(^29,32\) causing cell hyperpolarization and thus making the vascular tissue less reactive to

### Table 3. Effects of Graded Lower Body Negative Pressure During Intrabrachial Infusion of Vehicle, Prazosin, and Prazosin Plus Insulin

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Baseline Infusion</th>
<th>−5 mm Hg</th>
<th>−10 mm Hg</th>
<th>−15 mm Hg</th>
<th>−20 mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle MAP</td>
<td>86±3</td>
<td>86±3</td>
<td>88±5</td>
<td>91±4</td>
<td>87±3</td>
<td>85±3</td>
</tr>
<tr>
<td>FBF(_{\text{EXP}})</td>
<td>2.84±0.17</td>
<td>2.84±0.17</td>
<td>2.57±0.17*</td>
<td>2.39±0.17*</td>
<td>2.14±0.14*</td>
<td>1.93±0.13*</td>
</tr>
<tr>
<td>FBF(_{\text{CONTRA}})</td>
<td>3.07±0.18</td>
<td>2.79±0.19</td>
<td>2.62±0.16*</td>
<td>2.52±0.16*</td>
<td>2.23±0.15*</td>
<td>2.03±0.09*</td>
</tr>
<tr>
<td>Prazosin MAP</td>
<td>86±3</td>
<td>84±1</td>
<td>86±2</td>
<td>87±2</td>
<td>87±2</td>
<td>85±2</td>
</tr>
<tr>
<td>FBF(_{\text{EXP}})</td>
<td>2.53±0.18</td>
<td>4.27±0.14†</td>
<td>4.05±0.14*</td>
<td>3.89±0.17*</td>
<td>3.74±0.14*</td>
<td>3.57±0.09*</td>
</tr>
<tr>
<td>FBF(_{\text{CONTRA}})</td>
<td>2.84±0.21</td>
<td>2.76±0.244</td>
<td>2.59±0.22*</td>
<td>2.54±0.21*</td>
<td>2.16±0.99*</td>
<td>2.04±0.12*</td>
</tr>
<tr>
<td>Prazosin plus insulin MAP</td>
<td>86±3</td>
<td>88±3</td>
<td>88±4</td>
<td>86±5</td>
<td>87±4</td>
<td>86±5</td>
</tr>
<tr>
<td>FBF(_{\text{EXP}})</td>
<td>3.08±0.30</td>
<td>5.16±0.13†</td>
<td>5.33±0.19</td>
<td>5.54±0.22</td>
<td>5.66±0.08*</td>
<td>5.46±0.22</td>
</tr>
<tr>
<td>FBF(_{\text{CONTRA}})</td>
<td>2.81±0.15</td>
<td>2.89±0.19</td>
<td>2.63±0.18</td>
<td>2.43±0.18*</td>
<td>2.18±0.13*</td>
<td>2.03±0.30*</td>
</tr>
</tbody>
</table>

LBNP indicates lower body negative pressure; MAP, mean arterial pressure (millimeters of mercury); FBF\(_{\text{EXP}}\), forearm blood flow in experimental arm (milliliters per 100 mL per minute); and FBF\(_{\text{CONTRA}}\), in contralateral arm (milliliters per 100 mL per minute). Data are mean±SEM.

*\(P<.05\) compared with baseline infusion.
†\(P<.05\) compared with baseline.

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**Figure 4.** Bar graph shows maximal change in forearm vascular resistance induced by lower body negative pressure in experimental (closed bars) and contralateral (open bars) arms during intra-arterial infusion of vehicle, prazosin, and prazosin plus insulin. Each point represents mean±SEM; \(n=7\). *\(P<.05\) compared with vehicle; †\(P<.01\) compared with prazosin.
the vasoconstrictive stimuli. For this reason, we investigated whether in our model of insulin-attenuated reflex sympathetic vasoconstriction the concomitant intrabrachial infusion of ouabain, countering the insulin effect on cellular cation exchange, was able to prevent the insulin-induced attenuation of reflex sympathetic vasoconstriction. Although we could not measure directly the specific effects on the forearm vascular Na⁺-K⁺ pump induced by insulin and ouabain, we could demonstrate that the ouabain dose used in our experiments prevented the insulin effect on forearm potassium uptake. This observation suggests that in such conditions ouabain counteracts the insulin action on potassium metabolism in forearm tissues. The simultaneous infusion of ouabain, however, failed to abolish the attenuating effect of hyperinsulinemia on the forearm sympathetic-mediated vasoconstriction. These results are in keeping with recent experimental evidence in rabbit aorta showing that physiological levels of insulin do not stimulate vascular smooth muscle Na⁺,K⁺-ATPase, and only supraphysiological concentrations of insulin cause increases in Na⁺,K⁺-ATPase activity, probably through the insulin-like growth factor 1 receptor.

With regard to a possible direct vasodilator effect of insulin, the available evidence is controversial. In the present study no significant changes in baseline FVR were detected during forearm hyperinsulinemia within the physiological range. This observation is consistent with the results reported in our previous studies with both local and systemic insulin infusions. Interestingly, similar results were obtained with the use of two approaches different from the plethysmographic and indocyanine green dye dilution techniques for the measurement of FBF. Our data, however, are in contrast with some reports describing the vasodilatory property of insulin, although they are in keeping with several studies showing minimal or no effects of insulin on FBF. Moreover, our finding that hyperinsulinemia does not modify FVR in control conditions but blunts its increase during reflex sympathetic activation is strongly supported by the study of Porcellati et al., who have recently demonstrated that therapeutic hyperinsulinemia markedly blunts the vasoconstrictive responses secondary to the sympathetic nervous system activation in the standing position but has no effect on supine vascular tone in subjects with insulin-dependent diabetes mellitus but without autonomic neuropathy. These observations may be accounted for by the recent report by Saito et al. that insulin alone does not alter basal Ca²⁺ levels but only blunts Ca²⁺-stimulated responses at the vascular smooth muscle level.

Another potential mechanism by which insulin may attenuate sympathetic vasoconstriction could be an action on the complex sequence of molecular events that mediate the vasoconstrictive response of vascular smooth muscle to extracellular signals. Actually, the response of an organ to norepinephrine depends not only on the amount of neurotransmitter that reaches the receptor sites but also on the number of adrenergic receptors on the cellular membrane, on the sensitivity of these receptors, on the activity of the signal transduction pathway, and finally on the release of Ca²⁺, which represents the final molecular mechanism mediating the vascular smooth muscle contraction. Recent studies have shown that insulin is able to inhibit cytosolic calcium-stimulated signaling in cultured vascular smooth muscle cells. However, this effect on the cellular Ca²⁺ response is common to many transmembrane signal transduction pathways, and it is not clear whether physiological hyperinsulinemia antagonizes specific receptor-propagated vasoconstrictive signals.

To address this issue, we dissected the reflex sympathetic vasoconstrictive response in α₁ and α₂-mediated vasoconstriction by using selective pharmacological interventions. Our finding that insulin, at plasma levels comparable to those observed in the postprandial state, attenuates selectively the increase in FVR induced by an α₁-adrenergic agonist could indicate that the cross talk between the hormone and the sympathetic nervous system at the vascular level occurs through a well-identified vasoconstrictive signal transduction pathway. The observation that insulin infusion is able to abolish the reflex forearm vasoconstriction induced by graded LBNP during intra-arterial prazosin treatment supports this conclusion and may be in keeping with the hypothesis that α₂-adrenergic receptors participate in the mediation of vasoconstriction elicited by increased endogenous catecholamine levels. Taken together, our findings suggest that insulin blunts the reflex neurogenic vasoconstriction predominantly through an interaction with α₂-adrenergic receptors. This possibility may appear in contrast to recent data showing attenuation of forearm vascular reactivity to phenylephrine or angiotensin II in humans receiving intrabrachial insulin infusion. However, a careful perusal of those data reveals that the amount of insulin infused is threefold higher than that reported in our study (0.15 versus 0.05 mU/kg per minute), with a consequent increase of insulin levels in the blood draining the forearm that is approximately threefold that observed in the current study. Furthermore, Buchanan et al., analyzing the pressor response to angiotensin II in humans during physiological and pharmacological hyperinsulinemia, recently clarified the hypothesis that only supraphysiological insulin levels appear to have a depressor effect on the vascular target for angiotensin II. On the other hand, biochemical studies have already demonstrated that insulin interferes with the α₂-adrenergic receptor-mediated effect. α₂-Adrenergic receptors have their signal transduction pathway coupled to G protein, and the functional mechanism to transduce the molecular signal from receptors depends on a cyclic conformational shift in the α-subunit of this G protein. Using pertussis toxin-catalyzed [2P]ADP ribosylation of Gα as an index of G protein α-subunit conformation, Rothenberg and Kahn have clearly demonstrated that insulin alters the ability of Gα protein to undergo pertussis toxin-catalyzed ADP ribosylation, suggesting a functional interaction between insulin receptors and the G protein transduction system. Finally, during α₂-adrenergic receptor stimulation, only very low levels of Ca²⁺ are released, whereas α₂-adrenergic receptor stimulation causes large sustained increases in Ca²⁺ release. Therefore, our results may indirectly support the evidence obtained at the vascular smooth muscle level that insulin attenuates the vasoconstrictive signals through an interference with calcium mo-
It is also of interest that upregulation of α2-adrenergic receptors has been reported in animal models of hypertension.56 In addition, in human essential hypertension an inability to desensitize the α2-adrenergic receptor pathway has been described.57 Finally, we have recently shown that in hypertensive patients insulin is not able to modulate reflex forearm sympathetic vasoconstriction.58

The physiological implication of our study is that in healthy subjects the ability of insulin to modulate at the peripheral level the adrenergic-mediated reflex vasoconstriction represents a mechanism that buffers the consequences of the reflex activation of the sympathetic system induced by hyperinsulinemia. In turn, in insulin-resistant hypertensive patients the lack of the modulatory action of insulin might result in an impaired balance of the sympathetic control on peripheral vascular resistance.

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