

Insulin Modulation of an Endothelial Nitric Oxide Component Present in the α_2 - and β -Adrenergic Responses in Human Forearm

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Abstract

We explored in 51 normal subjects, distributed in various series of experiments, whether endothelium nitric oxide may play a role in insulin modulation of α_2 - and β -adrenergic-evoked vascular responses. In particular, we examined the forearm blood flow response (FBF, $\text{ml} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$) to intrabrachial infusion of BHT-933 (0.5, 1, and 2 $\mu\text{g} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$) or isoproterenol (1, 3, and 6 $\text{ng} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$) in control conditions, during intrabrachial infusion of insulin alone (0.05 $\text{mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and associated with L-N-monomethylarginine (L-NMMA) (0.05 $\mu\text{g} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$), a nitric oxide synthase inhibitor. In control conditions both BHT-933 and isoproterenol induced a dose-dependent vascular response. Local hyperinsulinemia (deep venous plasma insulin $68.5 \pm 4 \mu\text{U/ml}$) did not change basal FBF whereas attenuated BHT-933 vasoconstriction and enhanced isoproterenol vasodilation. L-NMMA reduced basal FBF and abolished the insulin effect on BHT-933 and isoproterenol response. To clarify whether a nitric oxide component is included in α_2 - and β -adrenergic response and may be responsible for insulin vascular effect, we further examined BHT-933 and isoproterenol responses during nitric oxide inhibition. Interestingly, L-NMMA potentiated the BHT-933 vasoconstriction and attenuated the isoproterenol vasodilation and, in these conditions, insulin was no more able to exhibit its vascular effects. Finally, to rule out the possibility that the counteracting effect of L-NMMA may not be specifically related to insulin action, dose-response curves to phenylephrine (0.5, 1, and 2 $\mu\text{g} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$) or sodium nitroprusside (1, 2, and 4 $\mu\text{g} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$) were also performed. Both insulin and L-NMMA were unable to alter the phenylephrine-induced vasoconstriction and the sodium nitroprusside vasodilation. In conclusion, our data demonstrate an endothelial nitric oxide component in the α_2 - and β -adrenergic vascular responses which is the target of the insulin vascular action. (*J.*

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Introduction

Among the broad variety of effects evoked by insulin, its ability to modulate the vascular tone has recently been the focus of a growing body of studies. The attention for this issue comes from the evidence that essential hypertension and other vascular diseases show a strong association with resistance to insulin action (1–6). Thus, the accurate definition of mechanisms by which insulin exerts its vascular effects could help to unveil the meaning of such association.

Actually, it has been clearly evidenced that insulin, at level physiologically reached postprandially, induces a reflex increase in sympathetic skeletal muscle outflow (7–9). However, interestingly, in presence of insulin, the vasoconstriction typically generated by the sympathetic activation is markedly blunted. The analysis of this phenomenon has been the object of many recent studies, which have attempted to dissect the mechanisms by which insulin is able to exert its vascular effects.

Using the euglycemic hyperinsulinemic clamp technique, which raises peripheral insulin levels in the physiological range, maintaining the blood glucose concentration at its basal value, several groups have observed an unequivocal vasodilator effect of insulin, which is abolished by nitric oxide inhibition (10, 11). Nevertheless, this technique prevents only the hypoglycemia, while a number of other hormonal and substrate changes systemically occur that may be actually responsible for the vascular effects ascribed to insulin.

In contrast, another approach is to deliver insulin directly in the brachial artery limiting the increase of plasma levels of the hormone to the forearm. This setting circumvents the perturbations induced by systemic insulin administration, reflecting solely the direct influence of insulin per se on vascular function. In these experimental conditions, we and others were not able to disclose any direct vasodilator effect of insulin, at least when the hormone was raised in the physiological range (12–16). However, even in this setting, we were able to reveal that insulin itself is able to attenuate the sympathetic-evoked vasoconstriction, through a crosstalk with α_2 and the β -adrenergic signal transduction pathways (16–18).

Such conclusions were also supported by studies performed in more elementary models of vascular function, such as rat aortic rings, where it is possible to evaluate the direct vascular effect of test substances. In particular, this kind of study has further attested that insulin is able to attenuate the norepinephrine-induced contractile response interacting both with the α_2 and the β -adrenergic vascular response (19, 20) in humans. Furthermore, such an approach has also revealed that

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insulin vascular effects on these adrenergic responses were endothelium nitric oxide dependent, since endothelium removal as well as nitric oxide inhibition is able to abolish them (20).

There exist indications, also in humans, that the endothelium may account for the insulin vascular effects. In particular, it has been recently reported that intraarterial infusion of insulin, although incapable to evoke a direct vasodilation, can enhance the endothelial response to increasing doses of acetylcholine (21). However, it is still not clear how insulin can interfere with the α_2 and the β -adrenergic vascular responses in humans.

To address this issue, we first studied in the forearm of healthy subjects the effect of nitric oxide inhibition on the insulin modulation of α_2 and the β -adrenergic vascular responses. Furthermore, since *in vitro* studies had demonstrated α_2 and β -adrenergic receptors also on the endothelium (22, 23), where they activate nitric oxide production, we extended our analysis to clarify whether the nitric oxide inhibition may unveil in the α_2 and β -adrenergic vascular response an endothelial component which may be the target of the vascular effect of insulin.

Methods

Subjects

The study group consisted of 51 normal volunteers (35 males and 16 females) whose ages ranged from 20 to 38 yr, average 27 ± 1 yr. Medical history, physical examination, and laboratory analyses were performed to evaluate subjects' normalcy. Renal, liver, and endocrine functions were normal. Body weight and body mass index were 72 ± 2 kg and 24.1 ± 0.4 kg/m², respectively. No subject had recent changes in body weight or dietary habits, nor was engaged in competitive sports or did intense physical activity during the days before the study. Written informed consent was obtained from all participants. The experimental protocol was in accordance with the institutional guidelines for human research.

Experimental procedures

The study began at 8:00 a.m. 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 12–15-h 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 (24). 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 (Arrow International Inc., Reading, PA), with the distal hole separated by ~ 3 cm from the proximal one was introduced into the brachial artery. The distal hole was used for the infusion of test substances, and the proximal lumen was used to sample arterial blood entering the forearm, uncontaminated by solutions infused downstream, and to measure arterial blood pressure, by means of a Statham P23Db pressure transducer (Statham Instruments, Cleveland, OH). Systolic and diastolic blood pressure were recorded on a multichannel polygraph (Gould Instruments, Oxford, CA). Heart rate was determined from a simultaneously obtained electrocardiographic signal and calculated from R–R interval. Forearm blood flow (FBF,¹ expressed in ml·min⁻¹·dl⁻¹ of forearm tissue) was measured by strain gauge plethysmography (25), using a Digimatic DM2000 (Medimatic, Copenhagen, Denmark) with a calibrated mercury-in-Silastic strain-gauge, applied on the arm ~ 5 cm below the antecubital crease. Both arms were supported above heart level. FBF

was measured from the rate of the increase in forearm volume while venous return from the forearm was prevented by inflating a cuff around the upper arm. The intrasubject coefficient of variation was 7%, based on two consecutive measurements taken at a one minute interval. After complete instrumentation, all subjects rested at least 30 min to establish a stable baseline before data collection.

All the test substances were dissolved in NaCl 0.9% on the day of the study. Infusion rates of drugs were normalized to decaliter of forearm tissue and were chosen to act selectively in the experimental forearm without causing systemic effects.

The α_2 -selective agonist BHT 933 (infusion rate of 0.5, 1.0, and 2.0 $\mu\text{g}\cdot\text{min}^{-1}\cdot\text{dl}^{-1}$ forearm tissue; Research Biochemical Inc., Natick, MA), the selective β -adrenergic agonist isoproterenol (1, 3, and 6 ng·min⁻¹·dl⁻¹ forearm tissue; Sigma Chemical Co., St. Louis, MO), the α_1 selective agonist phenylephrine (0.5, 1, and 2 $\mu\text{g}\cdot\text{min}^{-1}\cdot\text{dl}^{-1}$ forearm tissue; Sigma Chemical Co.), the vasodilator sodium nitroprusside (1, 2, and 4 $\mu\text{g}\cdot\text{min}^{-1}\cdot\text{dl}^{-1}$ forearm tissue; Malesci, Florence, Italy) and the sympathetic neurotransmitter norepinephrine (140, 280, and 560 ng·min⁻¹·dl⁻¹ forearm tissue; Sigma Chemical Co.) were utilized as test substances to produce dose–response curves. Each drug dose was maintained for 10 min to analyze the vascular response at the steady state. At the end of each dose–response curve a recovery period of at least 50 min was allowed. The intrabrachial infusion of insulin was performed at a rate of 0.05 mU·kg⁻¹·min⁻¹ able to reach increments in the forearm of ~ 60 mU/mL and was started 30 min before performing the dose–response curves of the test substances. For each insulin infusion, simultaneous arterial and venous samples were obtained before the start and at the end of the 30-min prime for the measurement of plasma insulin concentration. Insulin infusion was continued throughout the drug response curves. Intrabrachial infusion of the nitric oxide synthase competitive inhibitor L-N-monomethylarginine (L-NMMA; Sigma Chemical Co.; 0.05 $\mu\text{g}\cdot\text{min}^{-1}\cdot\text{dl}^{-1}$) was aimed to obtain constant nitric oxide inhibition in the forearm, throughout the dose–response curves. In particular, we started L-NMMA infusion 15 min before each drug response curve and continued throughout. This dose of L-NMMA has been shown to effectively blunt endothelium-dependent vasodilator response to acetylcholine in the human vasculature (26, 27) and such effect was confirmed in our pilot studies.

Experimental design

Series 1: Effects of L-NMMA on insulin modulation of forearm α_2 - and β -adrenergic response. Fig. 1 shows flow diagrams of the protocol. To verify whether the inhibition of nitric oxide may influence the insulin effect on α_2 - and β -adrenergic responses, in two groups of six subjects each, we assessed a dose–response curve to BHT-933 or isoproterenol in control conditions (i.e., during intrabrachial infusion of saline), during intrabrachial infusion of insulin and, finally, during concomitant infusion of insulin plus L-NMMA.

Series 2: Effects of L-NMMA on forearm α_2 - and β -adrenergic response. To clarify whether in both α_2 and β -adrenergic-evoked vascular response is present a vasorelaxant component, nitric oxide dependent, which may interact with insulin accounting for its effect on the overall α_2 - and β -adrenergic vascular response, we studied two groups of healthy subjects: seven with BHT-933 and five with isoproterenol. Dose–response curves to these drugs were performed in control conditions, during infusion of L-NMMA and, finally, during concomitant infusion of insulin plus L-NMMA.

Series 3: Effects of insulin plus L-NMMA on forearm α_2 - and β -adrenergic response. To examine the effect of insulin plus L-NMMA by eliminating other potential sources of noise represented by preliminary interventions such as insulin or L-NMMA alone, in two other groups of five normal subjects each, we tested dose–response curves to BHT-933 or to isoproterenol in control conditions and during insulin plus L-NMMA intrabrachial infusion.

Series 4: Effects of insulin or L-NMMA on forearm phenylephrine and sodium nitroprusside response. To rule out that L-NMMA could not specifically interact with the α_2 - and β -adrenergic vascular re-

1. Abbreviations used in this paper: FBF, forearm blood flow; L-NMMA, L-N-monomethylarginine.

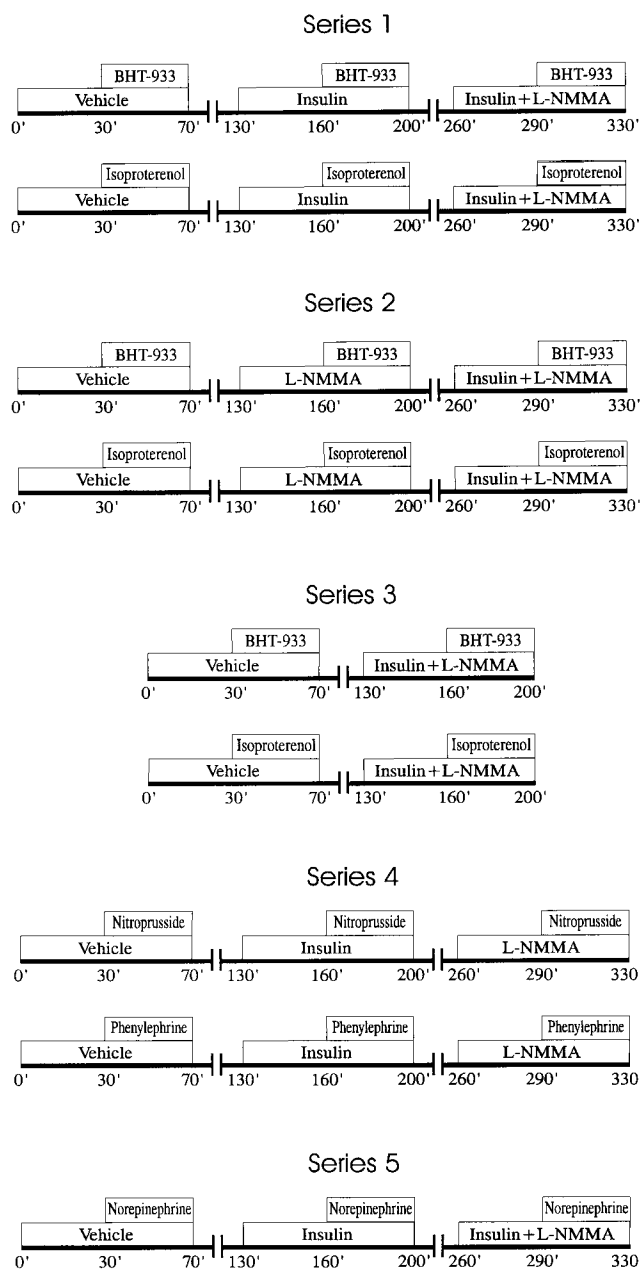


Figure 1. Flow diagrams of the protocol.

sponses, in two other groups of six normal subjects each, we tested dose-response curves to phenylephrine, a vasoconstrictive α_1 -adrenergic agonist, or to sodium nitroprusside, a vasodilator agent acting directly on smooth muscle, in control conditions, during insulin and finally, during L-NMMA intrabrachial infusion.

Series 5: Effects of insulin and insulin plus L-NMMA on forearm norepinephrine response. To confirm that insulin modulatory effect on α_2 - and β -adrenergic response is also physiologically relevant, we tested further in five normal subjects the effect of insulin alone and insulin plus L-NMMA on the forearm response evoked by norepinephrine, which represents the physiological neurotransmitter of the sympathetic nervous system.

Analytical procedures. Insulin was measured in plasma by a standard radioimmunoassay method (INCSTAR Corp., Stillwater, MN).

Data analysis. Because mean arterial blood pressure and heart rate did not change significantly throughout the study, data were ana-

lyzed in terms of changes in FBF. Because L-NMMA altered resting FBF, data were analyzed as percent change from baseline. Contralateral FBF did not change in the diverse experimental series.

Comparison of plasma insulin levels obtained in basal state and during intraarterial infusion of the hormone was accomplished by paired *t* test. Two-way analysis of variance was performed to estimate the changes on FBF responses to BHT-933, isoproterenol, phenylephrine, and sodium nitroprusside. Post hoc simultaneous multiple comparisons were done by Bonferroni's analysis. Results are presented as mean \pm SE.

Results

Mean blood pressure and heart rate of all subjects were 91 ± 2 mmHg and 70 ± 2 bpm, respectively. No differences in these parameters could be detected between different study groups nor in the same group throughout the experimental protocol. In all series of experiments intrabrachial insulin infusion induced an increase in deep venous insulin concentration (from 4.8 ± 1 to 65.3 ± 3 μ U/ml, $n = 51$, $P < 0.01$) without affecting the systemic levels of the hormone (from 6.2 ± 1 to 7.9 ± 1 μ U/ml, NS). Furthermore, pooling all experiments where insulin or L-NMMA were administered alone, we observed that intrabrachial insulin administration did not significantly modify basal FBF in the experimental arm (2.54 ± 0.12 vs. 2.66 ± 0.15 ml·min⁻¹·dl⁻¹, $n = 29$, NS) whereas this latter was significantly reduced by L-NMMA infusion (1.89 ± 0.16 vs. 2.29 ± 0.15 ml·min⁻¹·dl⁻¹, $n = 24$, $P < 0.05$). The combined infusion of insulin plus L-NMMA was able to provoke a decrease in FBF similar to that observed with L-NMMA alone ($-18 \pm 2\%$ vs. $-19 \pm 4\%$, $n = 12$, NS).

Series 1: Effects of L-NMMA on insulin modulation of forearm α_2 - and β -adrenergic response. In control conditions the infusion of increasing amounts of BHT-933 in the brachial artery induced a dose-dependent vascular response in the experimental arm (FBF decreased from 2.64 ± 0.14 to a minimum of 1.96 ± 0.16 ml·min⁻¹·dl⁻¹; Fig. 2). As previously observed, insulin infusion blunted significantly the FBF response elicited by BHT-933 (from 2.80 ± 0.27 to a minimum of 2.59 ± 0.23 ml·min⁻¹·dl⁻¹; $P < 0.01$ when compared to control conditions). The administration of L-NMMA in the brachial artery during insulin infusion was able to completely restore the full forearm response to BHT-933, which was no longer different in magnitude from that observed in control conditions (from 2.64 ± 0.29 to a minimum of 2.04 ± 0.26 ml·min⁻¹·dl⁻¹).

Similarly, the infusion of increasing amounts of isoproterenol in the brachial artery induced a dose-dependent vascular response in the experimental arm (FBF increased from 2.61 ± 0.19 to a maximum of 6.27 ± 0.44 ml·min⁻¹·dl⁻¹; Fig. 2). As expected, the isoproterenol-induced vasodilator response was significantly increased in presence of insulin (from 2.92 ± 0.25 to a maximum of 8.82 ± 0.70 ml·min⁻¹·dl⁻¹; $P < 0.01$). However, even in these conditions, the simultaneous infusion of L-NMMA and insulin was capable to restore the response to isoproterenol to that observed in control conditions (from 2.27 ± 0.16 to a maximum of 5.06 ± 0.46 ml·min⁻¹·dl⁻¹; NS when compared to control conditions).

Fig. 3 shows that the reversal of insulin influence on α_2 - and β -adrenergic response by L-NMMA was a common denominator of all subjects.

Series 2: Effects of L-NMMA on forearm α_2 - and β -adrenergic responses. In these subjects both BHT-933 and isoproterenol intrabrachial infusion induced in the experimental arm

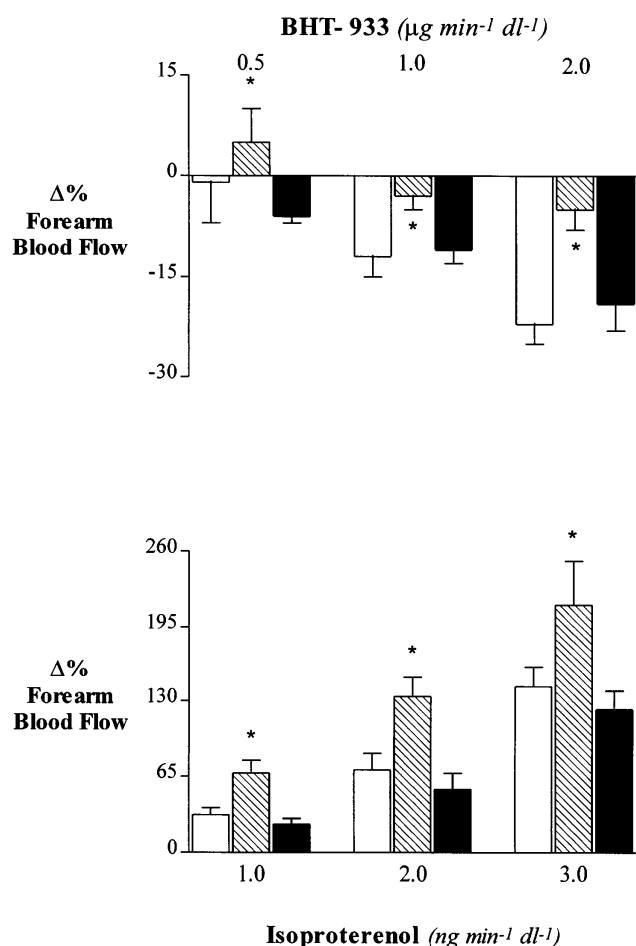


Figure 2. Changes in forearm blood flow response to intrabrachial infusions of BHT-933 (top) and isoproterenol (bottom) in control conditions (open bars) during intraarterial (i.a.) infusion of insulin (striped bars) and during i.a. infusion of insulin plus L-NMMA (solid bars). Each point represents mean \pm SEM. * $P < 0.05$ as compared to control conditions.

dose-dependent vascular responses similar to those observed in the previous series (BHT-933: from 2.43 ± 0.22 to a minimum of $1.84 \pm 0.20 \text{ ml} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$; isoproterenol: from 2.56 ± 0.46 to a maximum of $6.37 \pm 0.11 \text{ ml} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$; Fig. 4). However, in presence of L-NMMA, the BHT-933-evoked vasoconstrictive response was significantly potentiated (from 2.05 ± 0.39 to a minimum of $1.27 \pm 0.19 \text{ ml} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$, $P < 0.05$ when compared to control conditions) while, the isoproterenol-evoked vasodilator response was significantly attenuated (from 1.95 ± 0.27 to a maximum of $4.06 \pm 0.71 \text{ ml} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$, $P < 0.05$ when compared to control conditions). Finally, in these series of experiments the intrabrachial infusion of L-NMMA was able to restrain the insulin vascular effect on α_2 - and β -adrenergic elicited response (BHT-933: from 2.31 ± 0.42 to a minimum of $1.40 \pm 0.17 \text{ ml} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$; isoproterenol: from 1.95 ± 0.25 to a maximum of $4.12 \pm 0.61 \text{ ml} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$; $P < 0.05$ when compared to control conditions for both responses).

Series 3: Effects of insulin plus L-NMMA on forearm α_2 - and β -adrenergic response. As previously observed in series 1 and 2, both BHT-933 and isoproterenol intrabrachial infusion induced in the experimental arm a dose-dependent vaso-

lar response (BHT-933: from 3.19 ± 0.32 to a minimum of $2.31 \pm 0.26 \text{ ml} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$; isoproterenol: from 3.07 ± 0.14 to a maximum of $8.04 \pm 0.25 \text{ ml} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$; Fig. 5). The exposure to insulin plus L-NMMA, without preliminary interventions, confirmed the previous results obtained in series 2. In particular, the BHT-933-evoked vasoconstrictive response was significantly potentiated (from 2.29 ± 0.03 to a minimum of $1.42 \pm 0.19 \text{ ml} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$, $P < 0.05$ when compared to control conditions) while, the isoproterenol-evoked vasodilator response was significantly attenuated (from 2.35 ± 0.11 to a maximum of $4.53 \pm 0.28 \text{ ml} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$, $P < 0.05$ when compared to control conditions).

Series 4: Effects of insulin or L-NMMA on forearm phenylephrine- and sodium nitroprusside-evoked responses. As expected, both phenylephrine and sodium nitroprusside intra-brachial infusions induced dose-dependent vascular responses in the experimental arm (phenylephrine: from 2.27 ± 0.24 to a minimum of $1.14 \pm 0.25 \text{ ml} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$; sodium nitroprusside: from 2.01 ± 0.12 to a maximum of $5.48 \pm 0.69 \text{ ml} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$; Fig. 6). However, unlikely to that previously observed with BHT-933 and isoproterenol, insulin was unable to modify such vascular responses (phenylephrine: from 2.45 ± 0.23 to a minimum of $1.28 \pm 0.20 \text{ ml} \cdot \text{min}^{-1} \cdot \text{dl}^{-1}$; sodium nitroprusside: from

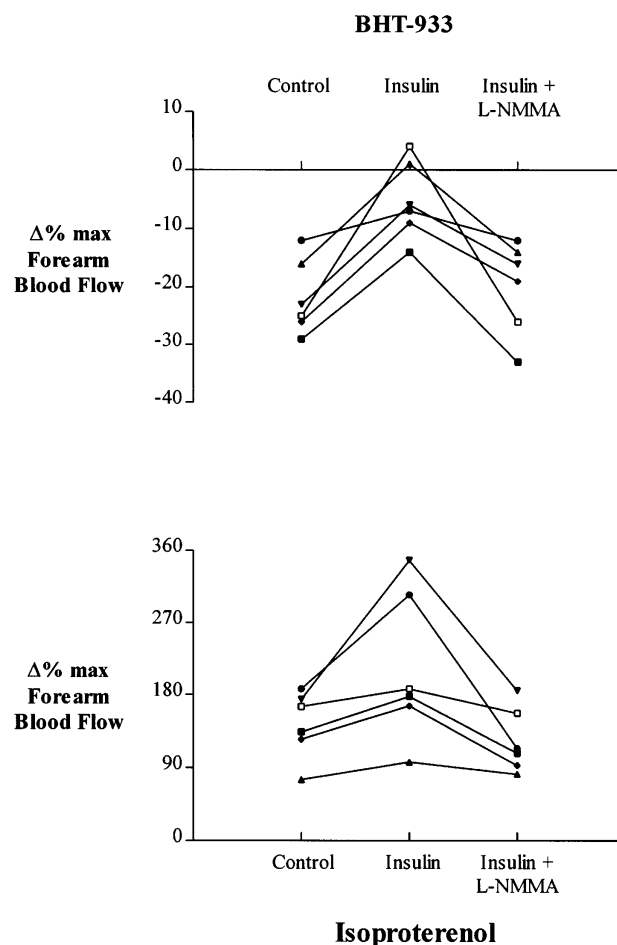


Figure 3. Changes in maximal forearm blood flow response to intrabrachial infusions of BHT-933 (top) and isoproterenol (bottom) in control conditions during i.a. infusion of insulin and during i.a. infusion of insulin plus L-NMMA in each subject.

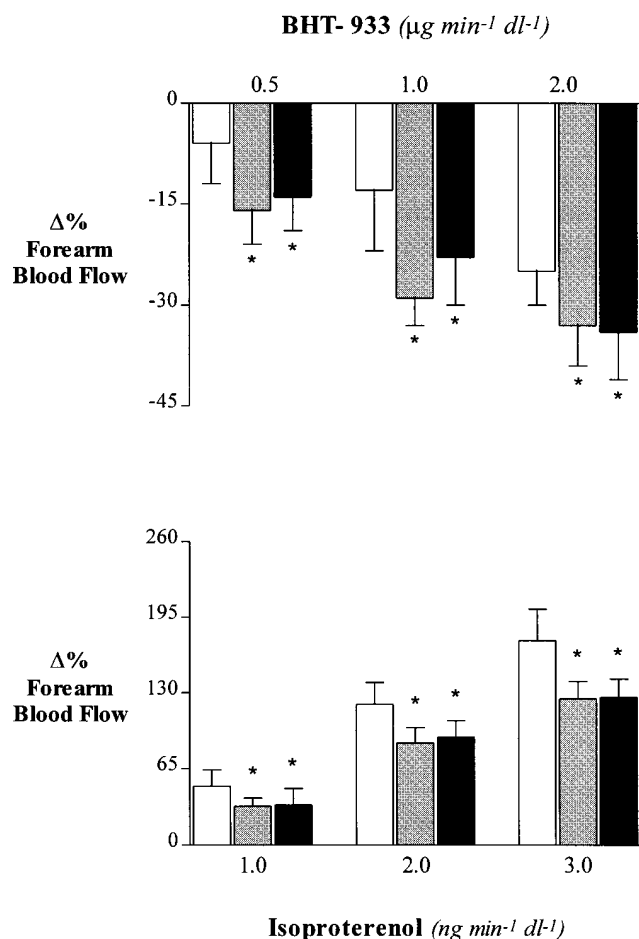


Figure 4. Changes in forearm blood flow response to intrabrachial infusions of BHT-933 (top) and isoproterenol (bottom) in control conditions (open bars) during i.a. infusion of L-NMMA (shaded bars) and during i.a. infusion of insulin plus L-NMMA (solid bars). Each point represents mean \pm SEM. * $P < 0.05$ as compared to control conditions.

1.99 \pm 0.11 to a maximum of 5.56 \pm 0.64 ml \cdot min $^{-1}$ \cdot dl $^{-1}$; NS when compared to control conditions). Furthermore, the dose-response curves obtained both with phenylephrine and sodium nitroprusside remained unmodified also during L-NMMA intrabrachial administration (phenylephrine: from 1.95 \pm 0.22 to a minimum of 1.01 \pm 0.22 ml \cdot min $^{-1}$ \cdot dl $^{-1}$; sodium nitroprusside: from 1.59 \pm 0.1 to a maximum of 4.45 \pm 0.57 ml \cdot min $^{-1}$ \cdot dl $^{-1}$; NS when compared to control conditions).

Series 5: Effects of insulin and insulin plus L-NMMA on forearm norepinephrine response. As expected norepinephrine intrabrachial infusion induced a dose-dependent vascular response in the experimental arm (from 2.96 \pm 0.16 to a minimum of 1.33 \pm 0.16 ml \cdot min $^{-1}$ \cdot dl $^{-1}$; Fig. 7). Insulin intrabrachial infusion was able to blunt norepinephrine-induced vasoconstriction (from 2.90 \pm 0.28 to a minimum of 1.89 \pm 0.22 ml \cdot min $^{-1}$ \cdot dl $^{-1}$; $P < 0.05$ when compared to control conditions). Finally, when the experimental arm was exposed to insulin in combination with L-NMMA the norepinephrine-induced vasoconstriction was no longer different from that observed in control conditions (from 2.17 \pm 0.21 to a minimum of 0.97 \pm 0.14 ml \cdot min $^{-1}$ \cdot dl $^{-1}$; NS when compared to control conditions).

Discussion

In this study we investigated in human forearm whether endothelium nitric oxide plays a role in the direct insulin vascular effect on α_2 - and β -adrenergic-evoked responses. Two major observations were noted. First, insulin modulation of α_2 and β -adrenergic vascular response is suppressed by nitric oxide inhibition. Second, the dose-response curves produced both with α_2 - or β -adrenergic selective agonists are significantly altered in presence of a competitive antagonist of nitric oxide synthase, suggesting the existence of an endothelium nitric oxide component in their overall vascular responses. More important, this nitric oxide component is essential for the insulin modulation of α_2 - and β -adrenergic responses.

Several recent studies have investigated the hemodynamic effects of insulin. In particular, we and others have examined the direct hemodynamic effect of insulin by intrabrachial infusion of the hormone, which realizes no systemic perturbation, and, therefore, the response measured in the forearm must reflect the net effect of insulin per se. In these conditions, insulin does not exhibit a direct vasodilator effect (12–15) whereas it does blunt the sympathetic-evoked vasoconstriction (16). This

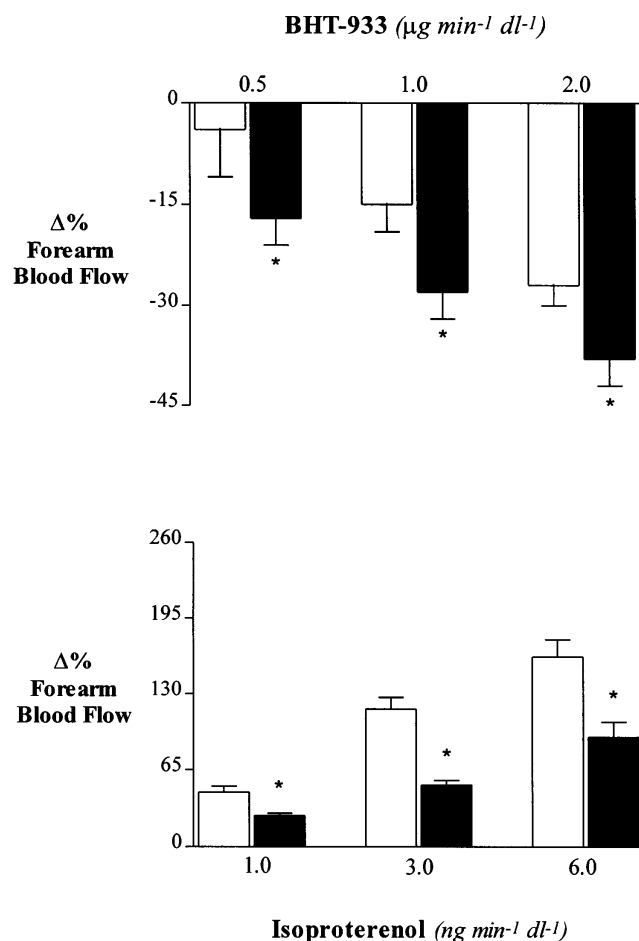


Figure 5. Changes in forearm blood flow response to intrabrachial infusions of BHT-933 (top) and isoproterenol (bottom) in control conditions (open bars) and during i.a. infusion of insulin plus L-NMMA (solid bars). Each point represents mean \pm SEM. * $P < 0.05$ as compared to control conditions.

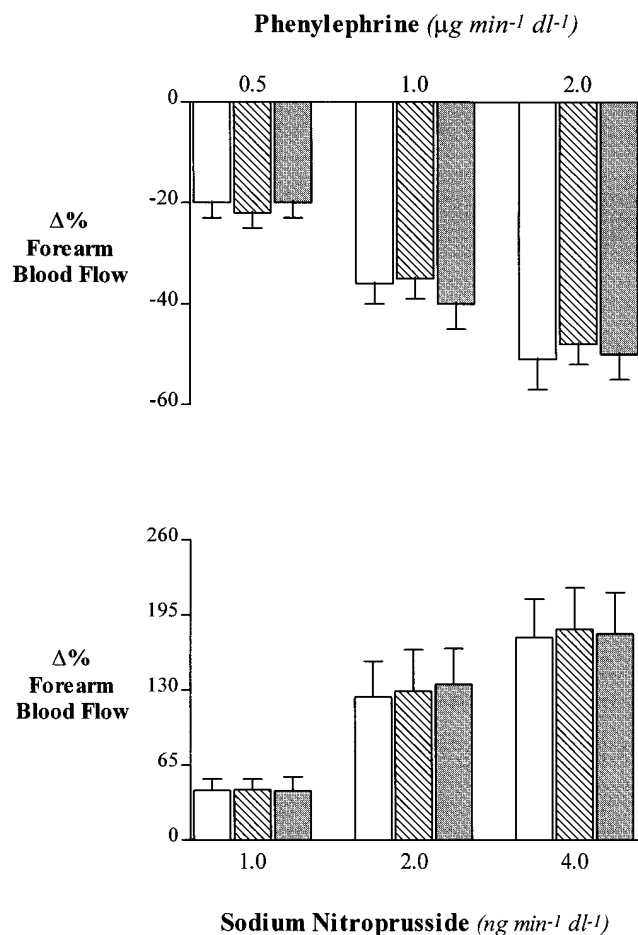


Figure 6. Changes in forearm blood flow response to intrabrachial infusions of phenylephrine (top) and sodium nitroprusside (bottom) in control conditions (open bars) during i.a. infusion of insulin (striped bars) and during i.a. infusion of L-NMMA (shaded bars). Each point represents mean \pm SEM.

phenomenon can be explained by the ability of insulin to selectively interact with α_2 - and β -adrenergic vascular responses (17, 18). In particular, insulin is able to attenuate the α_2 -adrenergic vasoconstriction and to potentiate the β -adrenergic vasodilation, whereas no significant effects are realized by insulin on the α_1 -adrenergic evoked response. Furthermore, in vitro studies support this evidence, suggesting that the insulin interaction with α_2 - and β -adrenergic response may occur at the endothelial level (19, 20). On this issue, Gros et al. have clearly demonstrated in rat aortic rings that insulin-mediated enhancement of vascular β -adrenergic responsiveness is completely abolished by endothelial removal (19). Additionally, in the same experimental model, we have recently observed that insulin interacts with the α_2 -adrenergic endothelium dependent vasorelaxation (20).

Recent data in humans have depicted a facilitating action of insulin on endothelium-dependent vasodilation mediated by activation of the L-arginine nitric oxide pathway, when the hormone is infused locally (21). Thus, we decided to verify in human forearm whether the interaction among insulin and the α_2 and β -adrenergic vascular responses might be mediated by an endothelial nitric oxide production. In particular, we hy-

pothesized the presence of an endothelial nitric oxide component which could be positively modulated by insulin and could account for both the attenuation of α_2 -evoked vasoconstriction and the potentiation of β -adrenergic vasodilation.

Our results confirm previous observations. In particular (a) forearm exposure to insulin, raising the plasma levels of the hormone within the physiological range, does not significantly affect forearm blood flow; (b) the hormone significantly alters the responses to α_2 and β -adrenergic stimulation but not those to phenylephrine or sodium nitroprusside.

A novel observation was that L-NMMA, which inhibits the endothelial formation of nitric oxide in a stereospecific manner, completely abolishes both the facilitating effect of insulin on the β -adrenergic evoked vasodilation and the negative effect of the hormone on the α_2 -evoked vasoconstriction. This suggests that a common endothelial nitric oxide-dependent mechanism may be involved in the insulin effect on α_2 and β -adrenergic receptors mediated vascular response.

However, the counteracting effect of L-NMMA on insulin modulation of α_2 and β -adrenergic responses may be also a merely pharmacological effect. Actually, any perturbation of vascular tone may trigger the release of nitric oxide from endothelium and, consequently, the neutralizing effect of L-NMMA on the insulin vascular action may be not specific. On this issue, our data clearly indicate that only the α_2 - and β -adrenergic-evoked responses are modulated by endothelial nitric oxide release, whereas the vasoconstriction obtained by α_1 -adrenergic selective agonist and the vasodilation induced by sodium nitroprusside are entirely unaffected by the inhibition of nitric oxide production. Thus, insulin effect on the selective adrenergic receptor-mediated responses becomes evident only for those responses which include an endothelial nitric oxide component in their overall vascular response.

A careful perusal of the results obtained in the first two series revealed slight differences exhibited by simultaneous infusion of insulin and L-NMMA compared with control conditions. In particular, while in series 2 insulin in presence of L-NMMA significantly modified the α_2 - and β -adrenergic-evoked responses, the same intervention of insulin plus L-NMMA was unable to determine a significant change of the α_2 - and

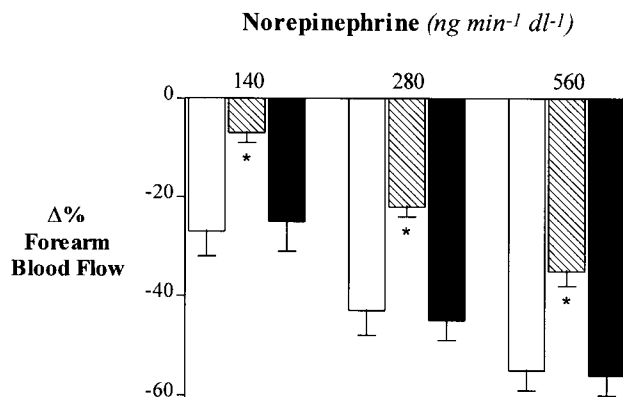


Figure 7. Changes in forearm blood flow response to intrabrachial infusions of norepinephrine in control conditions (open bars) during i.a. infusion of insulin (striped bars) and during i.a. infusion of insulin plus L-NMMA (solid bars). Each point represents mean \pm SEM. * $P < 0.05$ as compared to control conditions.

β -adrenergic responses in comparison with control conditions in series 1. It is important to note that such discrepancy is realized after a previous intervention that was different between the two series. In fact, in series 1, insulin was administered twice: the first time alone and, subsequently, in addition to L-NMMA. In contrast, in series 2, insulin was administered only once in conjunction with L-NMMA. The results of series 3, where no preliminary interventions were performed, confirms that during insulin plus L-NMMA exposure, the α_2 - and β -adrenergic responses are significantly modified in comparison to the same adrenergic-mediated responses obtained in control conditions, suggesting that the increased variability between the first two series may be the price of a double closed insulin exposure accomplished in series 1. On the other hand, the administration of the hormone activates molecular events which may be not easily removed even if a recovery period is allowed.

The effect of insulin is not limited to the pharmacological adrenergic agonists but is also clearly detectable on the vascular effects evoked by norepinephrine, the major sympathetic neurotransmitter, which realizes α_1 , α_2 , and β receptor activation, thus indicating the physiological relevance of our findings. On this issue, we have also to consider that in spite of the insulin modulatory effect on norepinephrine-induced vasoconstriction when this latter is infused in the brachial artery, we were not able to disclose any direct effect of insulin on the basal vascular tone which is indubitably also adrenergic dependent. However, it is likely that in resting conditions the main adrenergic influences on vascular tone are mediated through a signaling of α_1 adrenergic pathway on which insulin is unable to exert any modulatory influence. In contrast, α_2 and β adrenergic pathway may have a marginal role in the control of the resting vascular tone and, therefore, it is necessary to challenge them to reveal the whole insulin effect.

It is important to emphasize that our results represent the first evidence in humans of an endothelial nitric oxide component in the α_2 - and the β -adrenergic-evoked vascular responses. In particular, the overall vascular responses evoked by the activation of these specific adrenergic receptors have to be considered as the result of influences both on the smooth muscle and on endothelial cells. In fact, isoproterenol-induced vasodilation is obtained with a contribution of both a direct relaxing action on the smooth muscle and an indirect relaxing action mediated through the release of nitric oxide from endothelium. Analogously, the vascular response obtained with a selective α_2 -adrenergic agonist, such as BHT-933, is the net result of a direct vasoconstrictive action on the smooth muscle and of an indirect endothelial nitric oxide relaxing action. Our data are strongly supported by the evidence attained in endothelial cells which have clearly revealed that endothelium contains both α_2 - and β -adrenergic receptors (22, 23). Moreover, on isolated vessels Miller and Vanhoutte have demonstrated that removal of the endothelium causes a significant potentiation of the concentration-response curve to the selective α_2 -adrenergic agonist UK14,304 but not of that to the selective α_1 -adrenergic vasoconstrictor phenylephrine (28). In the same experimental model, it has been shown that the vasorelaxation induced by isoproterenol is attenuated by endothelium removal (29).

It should be noted that in the smooth muscle the stimulation of α_2 and β -adrenergic signals are able to provoke opposite effects, while in endothelial cells these adrenergic signals

converge positively on the release of nitric oxide. This suggests that the intracellular molecular events generated by α_2 and β -adrenergic receptor activation may have peculiar features in endothelial cells which allow to couple these two distinct adrenergic receptors to a common pathway stimulating the release of nitric oxide. Insulin is likely able to sensitize this endothelial pathway modifying the overall vascular response evoked by α_2 and β -adrenergic agonists. More important, since the vasoconstriction resulting from the sympathetic nervous system activation represents the balance of opposite actions on the various adrenergic receptors present on smooth muscle and endothelium, it is reasonable to speculate that the attenuation of sympathetic vasoconstriction induced by insulin may be also the result of this sensitizing action of the hormone on the endothelial adrenergic component which realizes a new equilibrium among the opposite actions evoked by adrenergic receptors stimulation.

In summary, this study demonstrates that both α_2 and β -adrenergic receptors include in their overall vascular response an endothelial nitric oxide component which is the target of insulin action. The definition of such a mechanism could be very important just in diseases like essential hypertension where both an exaggerated sympathetic nerve activity in response to various stimuli such as insulin itself (9, 30) and a generalized impairment of endothelial nitric oxide vasodilator function have been clearly demonstrated (31). Actually, in insulin-resistant hypertensive patients the lack of insulin sensitization of the endothelial component existing in the α_2 and β -adrenergic signals may result in an impairment of the equilibrium between endothelial and vascular smooth muscle adrenergic signaling, thus contributing to the increase of vascular resistance, a pivotal phenotypical trait of essential hypertension.

References

1. Ferrannini, E., G. Buzzigoli, R. Bonadonna, M.A. Giorico, M. Oleggini, L. Graziadei, R. Pedrinelli, L. Brandi, and S. Bevilacqua. 1987. Insulin resistance in essential hypertension. *N. Engl. J. Med.* 317:350-357.
2. Modan, M., H. Halkin, S. Almog, A. Lusky, A. Eshkol, M. Shefi, A. Shitrit, and Z. Fuchs. 1985. Hyperinsulinemia. A link between hypertension, obesity and glucose intolerance. *J. Clin. Invest.* 75:809-817.
3. Reaven, G.M. 1991. Insulin resistance, hyperinsulinemia, and hypertriglyceridemia in the etiology and clinical course of hypertension. *Am. J. Med.* 90(Suppl. 2):7-12.
4. Ferrari, P., P. Weidmann, S. Shaw, D. Giachino, W. Riesen, Y. Allemann, and G. Heynen. 1991. Altered insulin sensitivity, hyperinsulinemia, and dyslipidemia in individuals with a hypertensive parent. *Am. J. Med.* 91:589-596.
5. Botker, H.E., N. Moller, P. Ovesen, A. Mengel, O. Schmitz, H. Orskov, and J.P. Bagger. 1993. Insulin resistance in microvascular angina (syndrome X). *Lancet.* 342:136-140.
6. Suzuki, M., K. Shinozaki, A. Kanazawa, Y. Hara, Y. Hattori, M. Tsushima, and Y. Harano. 1996. Insulin resistance as an independent risk factor for carotid wall thickening. *Hypertension (Dallas)*. 28:593-598.
7. Anderson, E.A., R.P. Hoffman, T.W. Balon, C.A. Sinkey, and A.L. Mark. 1991. Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. *J. Clin. Invest.* 87:2246-2252.
8. Berne, C., J. Fagius, T. Pollare, and P. Hjendahl. 1992. Sympathetic response to euglycaemic hyperinsulinemia. *Diabetologia.* 35:873-879.
9. Lembo, G., R. Napoli, B. Capaldo, V. Rendina, G. Iaccarino, M. Volpe, B. Trimarco, and L. Saccà. 1992. Abnormal sympathetic overactivity evoked by insulin in the skeletal muscle of patients with essential hypertension. *J. Clin. Invest.* 90:24-29.
10. Steinberg, H.O., G. Brechtel, A. Johnson, N. Fineberg, and A.D. Baron. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. 1994. A novel action of insulin to increase nitric oxide release. *J. Clin. Invest.* 94:1172-1179.
11. Scherrer, U., D. Randin, P. Vollenweider, L. Vollenweider, and P. Nicod. 1994. Nitric oxide release accounts for insulin's vascular effects in humans. *J. Clin. Invest.* 94:2511-2515.

12. Louard, R.J., D.A. Fryburg, R.A. Gelfand, and E.J. Barrett. 1992. Insulin sensitivity on protein and glucose metabolism in human forearm skeletal muscle. *J. Clin. Invest.* 90:2348–2352.
13. Chisholm, D.J., G.A. Klassen, J. Dupre, and T. Pozefsky. 1975. Interaction of secretin and insulin on human forearm metabolism. *Eur. J. Clin. Invest.* 5:487–494.
14. Natali, A., G. Buzzigoli, S. Taddei, D. Santoro, M. Cerri, R. Pedrinelli, and E. Ferrannini. 1990. Effects of insulin on hemodynamics and metabolism in human forearm. *Diabetes.* 39:490–500.
15. Capaldo, B., R. Napoli, P. Di Bonito, G. Albano, and L. Saccà. 1991. Dual mechanism of insulin action on human skeletal muscle: identification of an indirect component not mediated by FFA. *Am. J. Physiol.* 260:E389–E394.
16. Lembo, G., V. Rendina, G. Iaccarino, F. Lamenza, M. Volpe, and B. Trimarco. 1993. Insulin reduces reflex forearm sympathetic vasoconstriction in healthy humans. *Hypertension (Dallas)*. 21:1015–1019.
17. Lembo, G., G. Iaccarino, V. Rendina, M. Volpe, and B. Trimarco. 1994. Insulin blunts sympathetic vasoconstriction through the α_2 -adrenergic pathway in humans. *Hypertension (Dallas)*. 24:429–438.
18. Lembo, G., G. Iaccarino, C. Vecchione, V. Rendina, L. Parrella, and B. Trimarco. 1996. Insulin modulation of β -adrenergic pathway in human forearm. *Circulation*. 93:1403–1410.
19. Lembo, G., G. Iaccarino, C. Vecchione, V. Rendina, and B. Trimarco. 1995. Insulin selectively enhances endothelial α_2 -adrenergic vasorelaxation. *Hypertension (Dallas)*. 26:578a. (Abstr.)
20. Gros, R., K.R. Borkowski, and R.D. Feldman. 1994. Human insulin-mediated enhancement of vascular β -adrenergic responsiveness. *Hypertension (Dallas)*. 23:551–555.
21. Taddei, S., A. Virdis, P. Mattei, A. Natali, E. Ferrannini, and A. Salvetti. 1995. Effect of insulin on acetylcholine-induced vasodilation in normotensive subjects and patients with essential hypertension. *Circulation*. 92:2911–2918.
22. Angus, J.A., T.M. Cocks, and K. Satoh. 1986. The α_2 -adrenoceptors on endothelial cells. *Fed. Proc.* 45:2355–2359.
23. Graf, K., M. Grafe, U. Dummmler, A. O'Connor, V. Regitz-Zagrosek, G. Kunkel, R. Auch-Schwellk, and E. Fleck. 1993. Regulation of β -adrenergic receptors on endothelial cells in culture. *Eur. Heart J.* 14:173–176.
24. Lembo, G., B. Capaldo, V. Rendina, G. Iaccarino, R. Napoli, R. Guida, B. Trimarco, and L. Saccà. 1994. Acute noradrenergic activation induces insulin resistance in human skeletal muscle. *Am. J. Physiol.* 266:E242–E247.
25. Trimarco, B., G. Lembo, B. Ricciardelli, N. De Luca, V. Rendina, G.L. Condorelli, and M. Volpe. 1991. Salt-induced plasticity in cardiopulmonary baroreflexes in salt-resistant hypertensives. *Hypertension (Dallas)*. 18:483–493.
26. Vallance, P., J. Collier, and S. Moncada. 1989. Effects of endothelium-derived nitric oxide on peripheral arterial tone in man. *Lancet*. ii:997–1000.
27. Panza, J.A., P.R. Casino, C.M. Kilcoyne, and A.A. Quyyumi. 1993. Role of nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. *Circulation*. 87:1468–1474.
28. Miller, V.M., and P.M. Vanhoutte. 1985. Endothelial α_2 -adrenoceptors in canine pulmonary and systemic blood vessels. *Eur. J. Pharmacol.* 118:123–129.
29. Gray, D.W., and T. Marshall. 1992. Novel signal transduction pathway mediating endothelium-dependent β -adrenoceptor vasorelaxation in rat thoracic aorta. *Br. J. Pharmacol.* 107:684–690.
30. Mark, A. L. 1990. Regulation of sympathetic nerve activity in mild human hypertension. *J. Hypertens.* 8:S67–S75.
31. Panza, J.A., C.E. Garcia, C.M. Kilcoyne, A.A. Quyyumi, and R.O. Cannon. 1995. Impaired endothelium-dependent vasodilation in patients with essential hypertension. Evidence that nitric oxide abnormality is not localized to a single signal transduction pathway. *Circulation*. 91:1732–1738.