Regional Hyperinsulinemia Induces Vasodilation But Does Not Modulate Adrenergic Responsiveness in Humans

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Summary: The relation between insulin resistance/hyperinsulinemia and cardiovascular disease may be related to one of the cardiovascular effects of insulin. In acute experiments in humans, systemic euglycemic hyperinsulinemia induced vasodilation in skeletal muscle. Furthermore, the sympathetic nervous system is activated, although this does not lead to increase in blood pressure (BP). We hypothesized that insulin could induce vasodilation either by reduction of α- or by augmentation of β-adrenergic responsiveness. The effect of insulin infusion into the brachial artery (regional forearm hyperinsulinemia; venous insulin concentration ~500 pM) on forearm blood flow (FBF: plethysmography) was studied. Responses to the α-adrenoceptor-mediated vasoconstrictor norepinephrine (NE: once with and once without the β-adrenoceptor antagonist propranolol, 2 × n = 12; 9 participated in both), and to the β-adrenoceptor-mediated vasodilator isoproterenol (n = 12) were measured before and during local hyperinsulinemia. Time/control studies (n = 6) were performed. Insulin alone induced vasodilation, as indicated by an increase in FBF-ratio (infused/control arm) from 1.2 ± 0.1 to 1.6 ± 0.2, p = 0.009. Increasing dosages of NE (1.25 to 240 ng ⋅ dl⁻¹ ⋅ ml⁻¹) induced vasoconstriction that was more pronounced during concomitant propranolol infusion (p < 0.001), indicating a dose-dependent vasodilatory component in the effect of NE. Isoproterenol (ISO 0.03 to 10 ng ⋅ dl⁻¹ ⋅ ml⁻¹), a pure β-adrenoceptor agonist, induced vasodilation. The percentage changes of FBF-ratio during NE + propranolol were similar and not significantly different before and during hyperinsulinemia. The same was true of the response to NE alone and the response to ISO. Neither was the intrinsic β-agonist component of NE influenced by insulin. Repeated NE infusion showed no time- or vehicle effect. We conclude that regional hyperinsulinemia in the physiological range induces local vasodilation in the skeletal muscle vascular bed, but this vasodilation is not mediated through modulation of α- or β-adrenergic responsiveness. Key Words: Insulin—α-Adrenoceptor—β-Adrenoceptor—Vasodilation.

Hyperinsulinemia as a counterpart of insulin resistance is a prominent feature of associated cardiovascular risk factors as hypertension, obesity, dyslipidemia, and non-insulin-dependent diabetes mellitus (1,2). The nature and significance of this association, an issue of considerable pathophysiological importance, is still unclear but may be related to the cardiovascular effects of insulin. Besides its key role in the regulation of carbohydrate metabolism (3), insulin has important cardiovascular effects (4,5). Despite earlier reports that insulin was in itself capable of inducing hypertension (6), most recent reports have failed to confirm this (7,8). Indeed, in acute experiments in healthy subjects (9,10) as well as in patients with hypertension (11), insulin has been shown to induce a vasodilator effect and not to increase systemic blood pressure (BP). Furthermore, several groups of researchers, including ourselves, have shown that during acute hyperinsulinemia the sympathetic nervous system is activated (9,11–13).

The mechanism of insulin's effect on vascular tone has not yet been clarified (4), but an interaction with the autonomic nervous system seems obvious because insulin-induced stimulation of the sympathetic nervous system does not lead to increases in BP, at least not in acute experiments. Thus, insulin-induced vasodilation could be explained by a de-
creased sensitivity of the vascular bed to α-adrenergic stimuli or an increased responsiveness to β-adrenoceptor agonists. Indeed, various investigators have reported a change in responses to adrenergic stimuli deemed to be a modulatory effect of insulin. The reported results, however, are extremely controversial and show various differences in design: Studies in humans versus in animal (14,15), studies with systemic hyperinsulinemia (euglycemic clamp technique) versus studies with regional hyperinsulinemia (16,17), systemic administration of vasoactive drugs versus local administration (18,19), and great variety in drugs administered: norepinephrine (NE) (14-17,20-22), phenylephrine (PE) (18,23), angiotensin II (AI) (15,16,19), isoproterenol (ISO) (24,25), and epinephrine (26). Furthermore, studies are performed in healthy humans as well as in disease states characterized by insulin resistance (20,27-29). In the presence of hyperinsulinemia, attenuated (15,18,22,23,30) un­changed (14,20), and exaggerated (16,19,21), responses to the various vasoconstrictors have been reported, as have increased (24) and decreased (25) responses to the β-adrenergic vasodilator ISO.

In the present study, we used the perfused forearm technique to investigate whether acute hyperinsulinemia affected vascular α- or β-adrenoceptor responsiveness in humans. Studying these two aspects together may be advantageous, because at different levels significant interactions and cross-talks do exist: Both endogenous catecholamines NE and epinephrine exhibit α- and β-adrenoceptor affinity. Furthermore, stimulation of presynaptic α- and β-adrenoceptors inhibits and stimulates NE release from sympathetic nerve endings, respectively (31). Finally, an eventual effect of insulin modulating the α-adrenoceptor-mediated vasoconstriction could be counterbalanced by a change in β-adrenoceptor sensitivity. Our results convincingly show that insulin has local vasodilator properties itself but does not affect the responsiveness to α- or β-adrenocep­tor stimulation.

METHODS

Subjects
In all, 42 studies were performed in 33 healthy subjects (16 women, 17 men), aged 19–32 years (mean ± SD, 23 ± 3 years). All were normotensive [mean office BP after 5 min rest in the supine position: systolic BP (SBP) 125 ± 11 mm Hg and diastolic BP (DBP) 75 ± 8 mm Hg, and normoglycemic and had a normal weight [body mass index (BMI) 22.5 ± 1.7 kg · m⁻²]. All subjects had a negative family history of diabetes mellitus and hypertension. The participants were recruited by advertisement and received a small remuneration. All gave written in­formed consent. The experimental protocol was ap­proved by the local ethics committee.

Measurements
Forearm volume was measured by water displacement. Forearm blood flow (FBF) was measured simultaneously in both forearms by mercury-in-silastic strain-gauge ve­nous occlusion plethysmography. The elbows and wrist were supported at or just above heart level. Strain gauges were attached around the forearm at the level of the max­imal diameter. One minute before the start of the FBF measurements, a pediatric cuff around the wrists was inflated to 100 mm Hg above the SBP level to ensure that the measurements referred only to the skeletal muscle vascular bed of the forearm. The collecting cuff around the upper arm was inflated to a supravenous pressure of 40 mm Hg during eight heart cycles with the Hokanson E20 rapid cuff inflator (ECG-triggered). This cycle was repeated three to four times each minute. The strain gauges were connected with Hokanson’s EC4 plethysmog­raphs, and FBF was determined in mm/100 ml forearm volume per minute from the mean vertical deflection per minute divided by a 1% electrical calibration signal. FBF was expressed as milliliters per minute per deciliter of forearm volume. In addition, the ratio of the FBF in the infused arm to that in the control arm was calculated for each measurement (FBF ratio).

All experiments were performed in a temperature-controlled room (22°–24°C). Before, after 30 min, and after 60 min of local hyperinsulinemia, arterial and venous blood samples were taken for determination of glucose and insulin. Plasma glucose was measured by the glucose oxidation method (BeckmannR Glucose analyzer 2, Beckman Instruments, Fullerton, CA, U.S.A.); plasma insulin concentration was measured by a double-antibody in-house radioimmunoassay (RIA) with an interassay coefficient of variation of 6.2%.

Protocols
After a 12-h overnight fast, subjects came to the laboratory. Under local anesthesia [0.3–0.4 ml lidocaine HCl 20 mg/ml (Xylocaine 2%, Astra)], a 20-gauge, 2-inch catheter (Angiocath, Becton Dickinson, Sandy, UT, U.S.A.) was inserted in the brachial artery and connected with an arterial pressure monitoring line (Viggo Spectramed, 5269-129) to a Hewlett Packard 78353B monitor. Mean arterial pressure (MAP) was determined by an electronic­ally integrated area under the brachial arterial pulse­wave form. The line was kept patent with saline infusion (3 ml/h with 2 U heparin/ml added). The dosages of drugs that administered were calculated per deciliter of forearm volume (ng · dl⁻¹ · min⁻¹). Moreover, a catheter (Ven­flon, 20 gauge, 32 mm) was retrogradely inserted in a deep ipsilateral forearm vein to obtain venous blood samples.

Effect of insulin on α-adrenergic responsiveness. First, after 30-min equilibration, baseline measurements were performed during concomitant intraarterial infusion of placebo (NaCl 0.9%, 50 µl · dl⁻¹ · min⁻¹). In 12 sub­jects, NE (Centrapharm) was infused in six sequential ascending doses into the brachial artery. Each concentra­tion was prepared separately so that infusion volume did not change. The doses were 1.25, 5, 20, 80, 160, and 240 ng · dl⁻¹ · min⁻¹ (17). Each infusion lasted 4 min; in the third and fourth minute, MAP and FBF were measured. Between the first and second series of three dosage lev­els, 15 min of rest were included to restrict the occlusion time of the hand circulation. To avoid eventual β-adreno­ceptor-mediated effects of the intravenous NE infu­sion (32), the nonselective β-blocking drug proprano­lol was infused concomitantly in a dose of 1.0 µl · dl⁻¹ · min⁻¹ from 2 min before until discontinuation.
of the NE infusion. After the final dose of NE, baseline measurements were repeated after 30-min equilibration. Subsequently, instead of NaCl 0.9%, insulin (Actrapid, Novo-Nordisk, Denmark) dissolved in an equal volume was infused intraarterially in a dose of 0.3 mU \cdot dl^{-1} \cdot min^{-1}. After 30 min of insulin infusion, measurements were repeated, during ongoing insulin infusion. Insulin was diluted twice by addition of human albumin 20% (CBR Amsterdam) or autologous plasma. Subsequently, six increasing dosages of NE were infused, with addition of propranolol, exactly as before the insulin administration.

Second, after an interval of at least 1 month, the protocol was repeated in 9 of the 12 subjects, but without the concomitant infusion of propranolol, to study the effects of NE alone. Unfortunately, 3 subjects were not measured a second time: 1 refused a second measurement, 1 could not be cannulated again, and 1 could not be measured again due to lack of time. Because of these 3 drop-outs, 3 other subjects were included in the second protocol to obtain a similar statistical power as in the first series.

Third, time control studies were made of \( \alpha \)-adrenergic responsiveness. To exclude the possibility of down regulation of \( \alpha \)-adrenoceptor induced by the first infusion of multiple doses of NE, to correct for eventual systemic effects, and to exclude an effect of the time course and vehicle, control experiments were performed in 6 subjects: NE was administered in six doses (similar to series I and II), first with placebo (NaCl 0.9%) and followed by a time period of saline + addition of autologous plasma (but not insulin); NE dose–response measurements were repeated, during ongoing insulin infusion. After the final dose of NE, baseline blood flow in the infused arm as compared with that in the non-infused arm was determined percentage change was used for comparison. Often, FBF at the experimental side is also indicated to illustrate obtained flows. Differences between baseline flow parameters and flows during local hyperinsulinaemia were calculated by Student's paired \( t \) test.

The NE (\( \pm \) propranolol)/ISO dose–response curves expressed in percentage change in FBF ratio from baseline, before and during hyperinsulinemia, were evaluated by a two-factor repeated-measures analysis of variance (ANOVA) to assess the effects of insulin, NE (\( \pm \) propranolol)/ISO and the interaction of these agents. In the 9 subjects in whom two experiments were performed (once with and once without propranolol), the differences in the individual responses were used to calculate a \( \beta \)-adrenoceptor–mediated vasodilator component of NE infusion. When data were pooled (effect of insulin/time), only the first obtained data set of subjects who were studied twice was used for evaluation.

A \( p \)-value <0.05 was considered statistically significant. All statistical analyses were performed with the SPSS personal computer package. All results are mean \( \pm \) SEM unless otherwise indicated.

**RESULTS**

**Insulin and glucose concentrations during the study**

During intraarterial infusion of insulin, deep venous forearm insulin concentration increased from 65 ± 22 to 498 ± 48 \( \mu \)M, indicating regional physiological hyperinsulinaemia. In contrast, arterial insulin levels did not change. After 30 min of local insulin administration, venous plasma glucose concentration was decreased from 4.6 ± 0.3 to 3.8 ± 0.6 mM, whereas arterial levels did not change (from 4.8 ± 0.3 to 4.7 ± 0.3 mM).

**Effects of adrenergic stimulation on skeletal muscle blood flow**

Regional infusion of propranolol in itself did not affect baseline flow (FBF ratio 1.08 ± 0.08 vs. 1.07 ± 0.09, \( p = NS \)). NE in combination with propranolol caused a dose-dependent decrease in flow (FBF ratio from 1.07 ± 0.09 to 0.22 ± 0.04, FBF from 1.7 ± 0.2 to 0.4 ± 0.1 \( \mu \)l \cdot dl^{-1} \cdot min^{-1}, \( p < 0.001 \) ) (Fig. 1). NE alone again induced a forearm vasoconstrictor response (FBF ratio from 1.12 ± 0.07 to 0.45 ± 0.07, FBF from 1.8 ± 0.2 to 0.8 ± 0.2 \( \mu \)l \cdot dl^{-1} \cdot min^{-1}, \( p < 0.001 \) for both) that was initially dose dependent, but the response to the high-
Effects of insulin on adrenergic responsiveness

Due to the previous insulin-induced vasodilator response, baseline parameters before the two sets of experiments were not equal. To correct for these differences and for possible non-specific systemic changes, we calculated percentage changes of FBF ratios, according to the literature (28) (described in the Materials and Methods section).

Percentage changes in FBF ratio during NE + propranolol were similar and not significantly different before and during hyperinsulinemia (maximum percentage decrease 59.5 ± 3.0% before insulin vs. 75.5 ± 4.0% during hyperinsulinemia, p = 0.25 (Fig. 3)).

Similar results were observed for the percentage changes of the FBF ratios during insulin and NE administration alone (maximum percentage decrease 55.7 ± 8.7% before insulin vs. 57.4 ± 3.8% during insulin, p = 0.81). In time control studies, as shown in Fig. 4, repeated infusion of NE induced a reproducible degree of vasoconstriction.

In 9 subjects, experiments with both NE alone and with NE + propranolol were performed. Vasocostriction with NE + propranolol was more intense than vasoconstriction with NE alone. Subtraction of the individual vasoconstrictor responses to NE from the first and the second study showed a vasodilator component of the NE administration (described in the Materials and Methods section).

The results of this subtraction procedure for all six NE dosages showed a dose relation before as well as during hyperinsulinemia. The difference in percentage change in FBF ratio between NE + propranolol and NE alone was maximal 27 ± 9% before insulin vs. maximal 18 ± 3% during insulin, p = 0.41. Again therefore, this β-adrenoceptor-mediated vasodilator component was not significantly altered by regional hyperinsulinemia. Finally, the responses of FBF ratio during ISO infusion were identical before and during hyperinsulinemia (max-
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2.0
1.5
1.0
0.5
0.0
— v — second series
— A *-■ first series

FIG. 4. Effect of repeated administration of six increasing doses of norepinephrine (+ propranolol) on forearm blood flow (FBF) ratio.

minimum percentage increase 524 ± 88% before insulin vs. 441 ± 63% during insulin, p = 0.19) (Fig. 5).

DISCUSSION

We confirmed that insulin administered locally in physiological concentrations induces vasodilation in forearm skeletal muscle. Our results also show that hyperinsulinemia in the physiological range does not attenuate the forearm vasoconstrictor response to NE or the vasodilatory response to ISO. This conclusion is based on the comparable percentage responses of FBF ratio before as compared with during insulin administration. Indeed, evaluation of the absolute changes after drug infusion would have shown a more pronounced response during hyperinsulinemia. However, as reported previously (28), the absolute decrease in FBF during administration of a vasoconstrictor is significantly correlated to the level of the baseline FBF (correlations in our study between mean decrease in FBF during combined NE/propranolol and baseline flow: r = 0.76 before insulin and r = 0.93 during insulin). Because insulin increased the baseline FBF significantly, the subsequent absolute NE-induced decrease in FBF was also increased, but nonspecifically. This view is further supported by the study of Neahring and colleagues (20), who showed that the absolute but not the relative response to intrabrachial NE infusion was increased by regional infusion of the vasodilator drug sodium nitroprusside.

Neither were the responses to the β-adrenoceptor-mediated vasodilator ISO affected by local hyperinsulinemia. With all these considerations taken into account, our data suggest that insulin in physiological concentrations shows no specific interaction with α- or β-adrenergic-stimulating agents at the level of the forearm vascular bed.

Vasodilatory effect of insulin

In acute experiments in humans, systemic insulin infusion with maintenance of euglycemia exerted a vasodilator effect in skeletal muscle (9,12,35). Although controversial results have been reported after local insulin administration, recent reports mostly show a local vasodilatory effect as well (10,36). Two important aspects could explain part of the controversial findings. First, insulin-induced vasodilation apparently is not an acute effect, but instead is one of slow onset. Steinberg and associates infused insulin into the femoral artery and reported a significant increase in femoral blood flow after 20 min but not after 10 min of infusion (37). We noted a clear increase in FBF after 30 min. In the group receiving ISO, insulin was infused for 60 min, but FBF did not increase from 30 to 60 min. Second, the individual vasodilator response to insulin shows a high variability, in our study ranging from −23 to +158%, as has been reported by other investigators (10). This indicates that in studies with small sample sizes the effect could be missed due to a type 2 statistical error. Because of the many participants, the insulin-induced vasodilation was highly significant in the current study.

Effect of insulin on α-adrenoceptor-mediated vasoconstriction

Several reports have shown very controversial findings with regard to the effect of insulin on α-adrenoceptor-mediated vasoconstriction. Results of several recent studies in vivo in humans, comparable to ours, appear to be in contrast to our findings. Sakai and co-workers (18) did not observe a significant vasodilator effect of insulin infusion but reported an attenuated α-adrenoceptor stimulation by

FIG. 5. Effect of six increasing doses isoproterenol on percentage increase in forearm blood flow (FBF) ratio before and during hyperinsulinemia.

We believe that the contrasting results may be explained by slight but relevant differences in the design of the studies. Sakai and co-workers started the infusion of PE after 20 min of insulin infusion and evaluated the effect of insulin alone after 10 min. Therefore, because the insulin-induced vasodilation can be slow in onset, Sakai and co-workers (18) may have missed this effect. Assuming that the vasodilator effect occurred later, the attenuated response to PE could be explained simply by the additive effects of vasodilation by insulin and vasoconstriction by PE. The same is true of the results of Lembo (30). On the contrary, results of other investigators, although in studies with a slightly different design, are in complete accord with ours (14,20).

Effect of insulin on β-adrenoceptor-mediated vasodilation

Recent in vitro experiments have shown an insulin-mediated enhancement of vascular β-adrenergic responsiveness to ISO (24). On the other hand, impaired forearm β-adrenoceptor-mediated vasodilation to isoproterenol has been described in patients with hypertension (25). Our experiments quite convincingly show that acute local physiological hyperinsulinemia in the human forearm vascular bed does not influence the sensitivity to the β-adrenoceptor agonist isoproterenol. In addition, we provide further indirect evidence for these findings: Our experiments were performed with the endogenous neurotransmitter NE instead of PE or related α-adrenoceptor agonists, especially because it is the most physiological method of investigating the effect of insulin on α-adrenoceptor sensitivity. However NE can induce β-adrenoceptor-mediated vasodilation and also has vasoconstrictor properties (34,38). To study the pure α-effect, we performed the study twice, once with and once without a β-adrenoceptor blocking agent. The last study was also performed to exclude an effect of insulin which could have been β-adrenoceptor mediated, an effect that has been reported previously (59). By comparing the paired studies with and without propranolol, we were able to confirm the dose-dependent (β-adrenoceptor-mediated) vasodilator component of NE and were also able to confirm that this vasodilator component was not influenced by regional hyperinsulinemia.

This finding further supports our conclusion that the vascular effects of the endogenous neurotransmitter NE are not altered by increased insulin concentrations. Moreover, our results further show that the addition of a β-adrenoceptor blocking agent is essential when NE is used to study pure α-adrenoceptor-mediated effects. Therefore, our results appear to be significant because most studies of the interaction between insulin and NE did not correct for eventual β-adrenoceptor-mediated effects of NE (14-17,20,21).

We confirmed that regional infusion of insulin induced a unilateral increase in FBF, indicating a local mechanism. The mechanism of action of this direct vasodilator effect of insulin is not completely clear (40), but our results indicate that it is not related to modulation of α- or β-adrenergic responsiveness. Other mechanisms probably are involved; recent reports indicate a nitric oxide-dependent pathway (37,41).

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REFERENCES

15. Yagi S, Takata S, Kiyokawa H, Yamamoto M, Noto Y,...


